



# Breeding for Salinity Tolerant Crops

*A Technique Package for the Regional Training Course on “Mutation Breeding Approaches to Improving Tolerance to Salinity, Drought and Heat Stress in Crop Plants”, IAEA/RCA Project RAS5045  
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## FOREWORD

Soil salinity is a major increasing worldwide problem, which has affected sustainable agricultural production. New varieties with improved resistance to such stress give the farmer added value and a competitive market advantage, which in turn will result in improved human welfare and increased farm income. Breeding for salt tolerance in a sustainable manner requires understanding of the genetic and physiological basis of salt tolerance for identifying salt tolerant germplasm. In the past, breeding for salt tolerance has been limited due to lack of physiological information and the complex genetic nature of salt tolerance processes thus lack of efficient selection procedures.

A project of the Regional Cooperative Agreement for Research, Development and Training (RCA) of International Atomic Energy Agency(IAEA), i.e. RAS/5/045 Improvement of Crop Quality and Stress Tolerance for Sustainable Crop Production Using Mutation Techniques and Biotechnology, was approved for 2007-2010. Under this project, a Regional Training Course on Mutation Breeding Approaches to Improving Salinity, Drought and Heat Stress Tolerance, which is jointly sponsored by IAEA and China Atomic Energy Authority (CAEA) through the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, was held on 13-22 October 2008 in Beijing, China.

This technique package was prepared for the trainees of above training course. In this book, all the commonly used and newly coined terminologies related to research on soil salinity and crop salt tolerance were introduced; The effects of salts on both soils and plants, mechanisms of plant tolerance to salinity, approaches and strategies, and progress on salinity tolerance breeding in major crop plants were extensively reviewed. The commonly used laboratory and field methods and protocols for plant salt tolerance were compiled. The ample literatures related were also listed in each chapter for further reference.

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# PART I INTRODUCTION

## CHAPTER 1 CONCEPTS GOVERNING SALINITY RESEARCH

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### 1. INTRODUCTION

Salinization has been identified as a world wide major process of soil degradation particularly in arid and semi-arid regions both in irrigated and dry land agriculture. The causes of salinity build up are usually associated either with irrigation activity or due to sea water intrusion in the coastal areas (Al-Ghawas, 1997; Al-Ghanem, 1997; Al-Gabri, 1997). The salt-affected soils may be of primary or secondary origin. While salt-affected soils of primary origin are formed as a result of long term influence of natural processes leading to an accumulation of salts in the region, secondary salt-affected soils are the result of the salts stored in the soil profile and/or ground water being mobilized by extra water provided by human activities such as irrigation or land clearing. Although the extent of secondary salinity is smaller than primary salinity, it is a huge socioeconomic problem particularly in developing countries and contributes to social unrest because of crop failures. In several developing countries, secondary salinity is resulting in dislocation of populations. This phenomenon is not new; in the past, this process has forced people to shift to other locations which, in turn became salinized in many cases (Szabolcs, 1989).

History reveals that civilization began in an environment of irrigation agriculture. History also reveals that salinity has been the major and ever-present world wide threat for permanence of irrigation agriculture. Downfall of several civilizations has been attributed to the final blow by water logging and salinity, although many (and varied) detrimental factors were also at work. One notable example is from Mesopotamia, where an early great civilization developed in the valley formed by the Tigris and Euphrates Rivers. Agriculture was the main stay of the economy. With time, water logging, the precursor of salinization, appeared, and salinity problem followed. Wheat was gradually replaced by salt-tolerant barley. Eventually salt reached such concentrations that even the substitute barley would not grow. This process took almost two millennia to devastate a well flourished agriculture (Boyden, 1987). Historic evidence from other parts of the world, such as Indus River Basin (Pakistan), Nile Valley in Egypt, China South America and Arizona also indicates that salinity problem permeated our civilizations (Casey, 1972).

The salinity problem has been viewed as cancer or a divine curse by some people. For others it was a problem for which a cure may be found. In-fact, for thousands of years, Human beings have sought solutions that they hoped would help mitigate salinity effects. They employed different

on-farm and off-farm interventions and miscellaneous soil additives in a quest to maintain farm productivity. Different plant species and crops/crop varieties were grown. In general, these interventions were employed on the principle that what works, works and it was not always necessary to know why. Some of these interventions were simply shots in the dark and were abandoned after trial and error. To avoid the agony of long process of trial and error, scientific information on soil salinization is needed to understand the processes, establish the cause-effect relationship, and conceive/develop appropriate science based solutions.

The search for dealing with the salinity problem continued today with the only difference that human beings are more informed now. Advancement in knowledge has improved the ability of human beings to plan better. With more cumulative knowledge, concepts, about salinity problems are sharpened and diverse nature of the problem is better known. This has made it possible to develop and apply more site-specific and effective solutions. Now scientists /engineers and farmers are working on a long list of intervention in the context of their improved concepts or perceptions. Such concepts or perceptions/ perceived solutions, mostly in the context of irrigated agriculture/secondary salinity, are briefly described below.

## **2. SALT-AFFECTED SOILS**

The term “salt-affected” soil is being used commonly to include saline, saline-sodic and sodic soils. So a salt-affected soil may be defined as a soil which contains sufficient soluble salts or exchangeable sodium to interfere with the growth of plants.

### **2.1 Saline soil**

A soil, which contains sufficient soluble salts in the root-zone to impair the growth of crop plants, is defined as “saline”. But, because salt injury depends on species, variety, growth stage, environmental factors, and nature of the salts, it is very difficult to define a saline soil precisely. The most widely accepted definition of a saline soil is one that gives an electrical conductivity (ECe) exceeding  $4 \text{ dSm}^{-1}$  at  $25^\circ\text{C}$ , in the soil saturation extract. Saline soils have pHs usually less than 8.5,  $\text{ECe} > 4 \text{ dSm}^{-1}$  and  $\text{ESP} < 15$ . The high ECe with low ESP tends to flocculate soil particles into aggregates. Permeability is either greater or equal to those of similar normal soils.

Saline soils are formed due to the accumulation of soluble salts (including chlorides, bicarbonates, carbonates, sulphates, sodium, potassium, calcium and magnesium), in soil or water. They are usually recognized by the presence of white salt-crust during some part of the year.

### **2.2 Sodic soil**

Sodicity is a measure of sodium ions on the soil exchange complex or water relative to calcium and magnesium ions. It is expressed either as Exchangeable Sodium Percentage (ESP) or as Sodium Adsorption Ratio (SAR). Sodic soils contain  $\text{ESP} > 15$  and  $\text{ECe} < 4 \text{ dSm}^{-1}$  and pHs generally range between 8.5 and 10 and may be even as high as 11. The low ECe and high ESP tends to deflocculates soil aggregates and hence lower their permeability.

### **2.3 Saline-sodic soil**

Saline-sodic soils contain sufficient soluble salts ( $EC_e > 4 \text{ dSm}^{-1}$ ) to interfere with the growth of most crop plants and sufficient ESP ( $>15$ ) to affect the soil properties and plant growth adversely by the degradation of soil structure. The pHs may be less or more than 8.5.

Sometimes it is advantageous to further classify salt-affected soils to distinguish the intensity of the problem as: class 0 (non-saline  $0-2 \text{ dSm}^{-1}$ ); class 1 (very slightly saline  $2-4 \text{ dSm}^{-1}$ ), class 2 (slightly saline  $4-8 \text{ dSm}^{-1}$ ), class 3 (moderately saline  $8-16 \text{ dSm}^{-1}$ ) and class 4 (strongly saline  $> 16 \text{ dSm}^{-1}$ ). The class 0 shows no visible salts on the soil surface and plant growth is not affected by salinity/sodicity. In class 1 & 2 soils the plant growth may be uneven or patchy. Salts are generally present in small sized patches which do not cover more than 25% area collectively. In class 3 the plant growth on these soils is very patchy and the salts are fairly visible on the soil surface. The area in class 4 lies unused and may support some salt-tolerant plants.

### **3. EXTENT OF SALT-AFFECTED SOILS**

As a general estimate about 7% of the total soil surface of the world is covered by salt-affected soils and no continent is free from salt-affected soils (Szabolcs, 1989): Australia 38%, South America 14%, Africa 8.4%, North America 1.7%, Central America 0.5% and Europe 5.4%. On a global level, the extent of natural or primary salt-affected soils is 955 Mha. Other estimates show that the world's irrigated land are damaged by the salinity up to 25 % (Postel, 1990), and up to 50% (Adam and Hughes, 1990).

There are other estimates, which show that about 77 million ha of cultivated lands are salt-affected by human induced processes (Oldeman et al, 1991; Ghassemi et al, 1995) and approximately more than 30 million hectare (Mha) can be attributed to secondary salinization of non-irrigated lands. The secondary salinization is predominantly located in the arid and semi-arid regions including Egypt, Iran, Iraq, India, China, Chile, Argentina, Central Asia, Russia, Spain, Thailand, Pakistan, Syria, Turkey, Algeria, Tunisia, Sudan and the Gulf States.

The deleterious effect of increased salinity in the soil is to impair the plant growth. With increase in salt concentration of the soil, the osmotic pressure of the soil solution increases and plants are not able to extract waters as easily as they can from a relatively non-saline soil (Al-Mehrizi, 1997). Soluble salts exert this potential over and above the matrix potential already existing in the soil. Thus, as the salt concentration increases the water becomes less available to the plant even though the soil may contain water and appears quite moist. There are however cases when plants, particularly, stone fruits are damaged at so low salt concentrations that osmotic effects of salts appear irrelevant. In such cases internal salt concentration seem to damage the plants. Hence, a high concentration of salts in the root-zone may reduce plant growth by a complex process broadly termed as water deficit or ion excess (Greenway and Munns, 1980).

In sodic soils, the main damage to plants is due to its poor physical properties. The effect of  $\text{Na}^+$  is to disperse the fine clay particles and clog the soil pores. The soil tends to swell and its aggregates to slake down, desirable crumb structure to collapse creating a less permeable condition. The resulting poor physical conditions of the soil restricts water penetration and aeration (Hillel, 1990), badly affecting the growth and development of plants.

## **4. ECONOMIC, ENVIRONMENTAL AND SOCIAL LOSSES DUE TO SOIL SALINITY**

### **4.1 Economic losses**

Economic losses may be due to the following reasons;

- saline water tables can cause productive land to become barren, causing loss of agricultural production
- soil salinity also enhances “erosion” and loss of farm income
- salinity can deteriorate the quality of drinking water
- in salt-affected areas, roads and building foundations are weakened by high salty water tables
- high water table also affect biological activity in the soil
- costs associated with land rehabilitation for public utilities

### **4.2 Environmental losses**

Environmental losses are introduced through land degradation by physical, chemical and biological changes in the soils and waterways, loss of vegetation and change to the landscape.

### **4.3 Social losses**

Social losses include an increased production costs on farms and reduced value of land as a result of soil salinization. The people from the salinized area move away and the social set up are disturbed.

## **5. SOIL DEGRADATION DUE TO IRRIGATION PRACTICES**

Irrigation is the major cause of secondary salinity. In arid and semi-arid regions, irrigation is a must for permanent agriculture since rainfall is low. It is irrigation which provides control over soil moisture to meet the requirement of different crops. Therefore, irrigation leads to phenomenal increase in land productivity. Irrigated land, although, small in size, accounts for large proportion of crop production in the world. There are many issues associated with irrigation that, if not properly understood, planned and managed, can cause build up of soil salinization and may create other serious hazards to soil environment.

There are at least two ways in which irrigation leads to salinization process, if it is mismanaged. Firstly, under conditions of insufficient precipitation, salts formed *in situ* by weathering of soil minerals or salt deposited from inadequately applied water tends to accumulate in the soil profile. In some areas the general shortage of good quality irrigation water necessitates the use of underlain brackish/saline water having a range of salinity and sodicity levels. This intensifies the problem pertaining to salinity and sodicity, and may cause perched layer if drainage is restricted. To prevent salts build up, a net removal of the salts out of the root zone is mandatory.

Secondly, when too much water is applied, in addition to the losses and inefficiency in water use, the extra water raises water table or increases the pressure of confined aquifers, creating an upward leakage of water. When the water table becomes close to the soil surface, it is water logging. Water is evaporated leaving salts behind, causing soil salinization and degradation. It is wise to state that water logging and soil salinization of agricultural lands is a symptom of misuse and mismanagement that jeopardizes the integrity of soil's self regulatory capacity. Prolonged mismanagement of irrigation practices will cause irrigated soils to be more affected by excess soluble salts. Thus, when irrigation water is applied, there is a two-sided question: whether sufficient supplemental water is given to provide the required leaching and whether the drainage network has the capacity to remove sufficient water with its dissolved salts.

Associated with soil salinization may be the process of sodication, whereby the clay fraction of the soil becomes saturated with  $\text{Na}^+$ , and the soils become sodic. Therefore, one of the major concerns of irrigated agriculture is the development of salinity and sodicity both in the soils and in the water resources. Once the water logging and soil salinity are developed, they undermine the resource base by decreasing soil quality.

## **6. SALINE SEEP**

In several countries saline seeps are instrumental in the development of a salinity problem. A saline seep is primarily the result of discharge of a saline groundwater system. The process is often accelerated by dry land farming which allows water to move through salt-laden substrata below the root zone. It refers to intermittent or continuous saline water discharge at or near the soil surface down slope from recharge areas under dry land conditions (Brown et al., 1983). It reduces or eliminates the growth of crops in the discharge area due to increased soluble concentration of salt in the root zone. The characteristics and causes of saline seeps are similar everywhere. Typically, native vegetation, which may include grasses, has been replaced with agricultural fields and cropping systems with lower potential evapotranspiration requirements.

The water balance of a catchment can be manipulated to some extent by altering agronomic practices. Varying agronomic practices should result in different amounts of water being removed from the landscape via evapotranspiration. Moreover, the recharge rate under different crops depends, among other things, on soil type, irrigation and the average annual rainfall and its seasonal distribution. There is a range of agricultural practices which can reduce the rate of recharge to the groundwater system. Specific techniques include the replacement of shallow-rooted agricultural species with deep-rooted ones such as alfalfa, lucerne and perennials (Schofield et al., 1989).

## **7. MANAGEMENT OF SALINITY PROBLEMS**

As mentioned earlier, secondary salinity is not a recent phenomenon. It is as old as irrigation. Salinization forced early settlers in Mesopotamia, the Indus River Basin and china to abandon their land and move to non-salinized land. Although, many irrigation systems around the world are faced with serious problems of secondary salinity, the abandonment option is no longer possible. Development of the world's land and water resources has required a very big investment and



massive engineering efforts. The gigantic irrigation system in Indus Basin of Pakistan on 16 Mha of irrigated land with the world's largest infrastructural works is a case in point. Development of new land and water resources will be much more difficult and much more expensive than in the past. Therefore, efficient use of developed irrigation and land resources together with reclamation/improvement and sustainable utilization of saline land and water resources are better long-term options than the development of new resources.

A wide range of management options is available for preventing salinization and for management of irrigated and dry land (non-irrigated) salinity and salinization of watercourses. However, implementation of any option depends on particular circumstances. While some of the options are more suitable for irrigated land salinity, others are suitable for dry land salinity or reduction of salinity levels in watercourses and may be highly site specific. Technical, economic, social and political considerations are the major influences on the implementation of management options.

### **7.1 Salinity control and methods of irrigation**

In Arid and semi arid zones the major constraints are: 1) limited quantities of good quality water, and to increase its efficiency; and 2) exploitation of unsuitable saline water for irrigation. The method of irrigation affects both the efficiency of water use and the way salt accumulates. Therefore, there is a clear need to adopt suitable irrigation techniques for eliminating salinity hazards to the soil. Each irrigation method has certain advantages and disadvantages and all known factors should be considered before attempting to improve salinity control by changing the method (Ayers and Westcot, 1989).

#### **7.1.1 Irrigation channel lining or piping**

Eliminating channel seepage can have some effect on the problems of shallow water tables and salinity. Hawkins (1978) describes different types of channel lining which include: concrete lining; masonry-type lining; synthetic membrane lining; bentonite membrane lining; and compacted earth lining. Channels can also be replaced by pipelines. Piping is usually considered an economic proposition in areas with permeable soils and high seepage losses. In Pakistan, at present a mega project is in progress for masonry-type lining of water channels.

#### **7.1.2 Surface Irrigation**

Flood, basin, border and furrow are known surface irrigation methods. At the end of each irrigation cycle, soil dries out and concentrates the salt, which adversely affects the crop yield. More frequent irrigations may reduce the salinity but may waste water. With surface irrigation methods, depth of applied water entering the soil varies with location in the field and depends on the infiltration rate and time available for infiltration. Surface irrigation methods are usually not sufficiently flexible to apply less than 80 to 100 mm depth per irrigation. Irrigating more frequently to reduce possible water stress may also waste water and increases waterlogging and drainage problems.

Differences in the rate of infiltration may be caused by land slope, degree of compaction, textural changes and soil chemistry. The time during which infiltration can take place also varies; the upper end of the field nearest the water source usually has water on its surface for a much longer time than does the lower end. High spots in the field receive less water because they are covered by less

water and for a shorter period. There may also be areas of more dense soil where water does not infiltrate sufficiently to accomplish leaching. Isolated pockets of accumulated salt frequently result in such cases. Surface flooding may apply a uniform depth of water across the entire field, thus salt accumulation increases with depth. Moreover in case of furrow irrigation, which applies water to only part of the field surface, salt also accumulates in the ridges between furrows.

### **7.1.3 Pressurized irrigation system**

The alternatives to improve the efficiency of water are the pressurized irrigation systems, drip or sprinkler irrigation. Such a good system must meet the requirements of the crop for water evapotranspiration (ET) of the soil, since it may be easier to increase the frequency of irrigation to relieve water stress with such a system rather than with surface flooding.. They often allow efficient and economic use of water, and reduce deep percolation losses. If water application through pressurized irrigation systems is in close agreement with crop needs (ET and leaching), drainage and high water table problem can be greatly reduced, which in turn should improve salinity control. However, pressurized irrigation systems have their problems too and are not adapted to all conditions of water, soil, climate or type of crop (Ayers and Westcot, 1989). The change over to pressurized irrigation systems in developing countries may be too expensive and will require justification, and better crop adaptability.

#### **7.1.3.1 Drip (Trickle) irrigation**

It is a method that supplies the required quantity of water to the crop almost on a daily basis. Drip irrigation has the potential to sustainable utilize saline and / or sodic waters. The poor quality water used in drip irrigation may yield better due to continuous high moisture contents and daily replenishment of water loss by ET. Salts may accumulate with drip irrigation at surface, within the soil, outside edges of the area wetted by emitters but the daily irrigation continuously move the moisture down to keep the salts under control. Therefore, frequent applications in small quantities could be an effective way to manage salinity and sodicity hazard of irrigation water.

In case the water is saline, frequent applications of water would keep the salts in the root-zone of the plants close to that in irrigation water and salt would move and accumulate at the periphery of the wet zone preferably away from the roots. In case the water is sodic it would increase hardness of soil reducing its permeability. It would cause temporary water logging when wet, which affect most crops adversely and reduces flow of water towards roots. When at field capacity frequent application of water in small amounts, would also keep the soil around optimum moisture level reducing possibilities of both temporary water logging and soil hardness. The possibility of sodication and thus increased soil hardness of soil has been further overcome by acidification of sodic ground water in our recent experiments (our unpublished data). Moreover, frequent irrigations with drip irrigation could also reduce the precipitation of  $\text{Ca}^{2+}$  as  $\text{CaCO}_3$  by keeping the soils wet.

#### **7.1.3.2 Sprinkler irrigation**

It is a method of making rain over the canopy of plants. Sprinkler irrigation, applies a uniform depth of water across the entire field, thus salt accumulation increases with depth. It is not advisable in most cases to use saline water with sprinkler irrigation as it may cause leaf burn, defoliation of sensitive species.

#### **7.1.4 Irrigation scheduling**

Irrigation scheduling is the management practice of determining how much water to apply during irrigation and the timing of the application. Irrigation scheduling requires knowledge of the crop water requirement for particular growing conditions. The applied irrigation water depends on crop evapotranspiration, the cropping pattern, and type of planting (density or spacing), leaching requirement, irrigation management and effective precipitation. Correct irrigation restores the soil water deficit and avoids the application of a wasteful and potentially harmful excess. Several methods are used for determining the time when a crop requires water; the soil moisture status and the plant stress indicators are among the most frequently used.

Irrigation scheduling by soil moisture measurement is probably the oldest method in existence (Campbell and Campbell, 1982). Several devices and procedures have been used for obtaining soil moisture measurements. The most frequently used are the tensiometers and the neutron probe. Tensiometers consist of a metal or plastic shaft, the lower end of which is connected to a porous ceramic cell and the upper end to a pressure measuring device. Through the contact of the water-saturated porous cell with the soil, the soil water pressure is transmitted via the ceramic cell and the tensiometer fluid to the pressure indicator (Albert et al., 1987). The tensiometers have the advantage that they are relatively simple and inexpensive. Their disadvantage is that they measure the soil moisture only in the immediate vicinity of the unit, so that several tensiometers are needed to give a reliable spatial average.

The neutron probe operates by producing fast neutrons from a radioactive source. The emitted neutrons are scattered and moderated by the water in the soil. The probe is inserted into the soil through an access tube, and the water content is directly related to the number of neutrons scattered back to a detector. The measurement is therefore an average for the soil volume surrounding the access tube, so a single measurement gives the same information that several measurements from tensiometers would give. The calibration for the probe is relatively constant from soil to soil and, once it is known, it should not change with time. Readings are rapid and the instruments are reasonably portable (Campbell and Campbell, 1982).

Irrigation scheduling does not require knowledge of the field average water content or potential for a field. Since the field is irrigated as a unit, a single representative monitoring site can be used to indicate the water status for the entire field. It is desirable to select a site for convenient access that is more than 10-20 m from any edge of the field and that is among healthy, vigorous plants, in soil of above-average water capacity for the field, and in a location exposed to normal climatic and irrigation variations. For irrigation scheduling it is necessary to determine the full and refill points of the field. The full point is the field capacity for the soil, while the refill point is the potential below which crop production is measurably reduced (Campbell and Campbell, 1982).

Since soil moisture measurements are generally not read on a continuous basis, moisture measurements should be plotted as a function of time such that soil moisture can be extrapolated to the point in time when irrigation is required. Irrigation should be scheduled so that the soil water content stays between the refill and full point values. If it goes above the full point, leaching will occur. If it goes below the refill point, production will be reduced (Campbell and Campbell, 1982; Boyle Engineering Corporation, 1986).

Plant stress indicators can be used for irrigation scheduling. Several observational methods can be used to determine plant stress, including changes in plant color or wilting. Generally, when plant stress is observed, irrigation will be too late to prevent some suppression of plant growth and yield. Recent techniques such as the pressure chamber technique or the measurement of plant temperature with an infrared thermometer can be used to determine the plant stress (Boyle Engineering Corporation, 1986).

Leaf and canopy temperatures may be either warmer or cooler than the air, depending upon environmental factors. In humid climates canopy temperatures will be near to or higher than air temperature, with only a small range of temperatures. In arid areas, however, canopy temperatures may be more than 10°C below air temperature and have a range of perhaps 15°C (Jackson, 1982). It is in the arid areas where irrigation is practiced that temperature techniques can work best and are most needed.

The tremendous advance in infrared (IR) technology allowed the production of lightweight hand-held IR thermometers that can be used to measure plant canopy temperature rapidly (Jackson, 1982). The plant temperature, the temperature of the air and the wet and dry bulb temperature are all used together to determine when a plant is stressed. The measurements take place inside the field where the temperature will not be affected by the field edge, which is drier and hence warmer. Measuring is best done between noon and 2 pm when the sun is higher overhead, shadows are at a minimum and transpiration is at its peak.

## **7.2 Reclamation of Salt-Affected Soils**

In order to reclaim the existing salt-affected area for its sustainable use in future and to avoid other areas to become barren, there is a need to determine the best practices of soil reclamation and management with particular relevance to the associated problems of the salt-affected soils. Four reclamation methods are well known and are described below.

### **7.2.1 Physical**

It includes land leveling, surface flushing, salts scraping, deep plowing and land forming or tillage, sub soiling and sanding. With land leveling, existing land topography is changed by mechanical shifting of soil to create a defined slope. It is the process of smoothing the land to achieve a uniform surface. This can occur without significant land-forming. In irrigated areas both processes are now being aided by laser technology which improves accuracy and precision. The overall result of land-forming and grading is that more efficient irrigation is possible. The application of water to the land surface is much more precise and the land can be drained more effectively. Land leveling is a pre-requisite for an efficient leaching and/or flushing the salts from a salt-affected soil. Accessions to the groundwater are thereby reduced by the elimination of waterlogged areas, resulting in a lowering of the water table and greater control of salinity.

Surface flushing allows the surface accumulated salts to be washed away. Particularly this technique is useful where surface salt crusts are present in arid and semiarid areas. The flushed saline water then enters the drainage system, which becomes concentrated with salts. The surface salt crusts can also be removed by mechanical means such as scraping. This practice minimizes the salts temporarily, they can be accumulated again if there is a continuous subsurface movement of saline water to the surface.

Low salinity in the root zone can be achieved by manipulating the soil surface condition i.e., bed shape and irrigation management. It is very well recognized that salts tend to accumulate on the ridges away from the wet zone when furrow irrigation is adopted. Placing the seeds on off-center slope of the single row will put the seed in minimum salinity and optimum moisture condition. Sub soiling is particularly important for disrupting the dense layers at depth to enhance permeability. Particularly this is important while reclaiming sodic soils after the addition of suitable amendment such as gypsum and watering the field. This will enhance the removal of exchangeable sodium already exchanged by  $\text{Ca}^{2+}$  to move in the lower layers before it is put into main drainage system. Sand mixing with heavy textured soil can change the texture permanently, and the soils become more permeable and easy to reclaim. Sanding also provides favorable environment for plant growth compared to the original clayey soil without sanding.

### 7.2.2 Chemical

Chemical approaches are commonly used to reclaim saline-sodic or sodic soils. To have successful crops on these soils, ESP of the soil must be lowered to safer limits, which can be achieved by adding suitable amendments. The main aim is to replace exchangeable sodium by calcium ions. Abrol et al. (1988) describe the chemical amendments suitable for the reclamation of sodic soils. These chemicals consist of gypsum ( $\text{CaSO}_4$ ), calcium chloride ( $\text{CaCl}_2$ ), iron sulphates ( $\text{FeSO}_4$ ), aluminum sulphates ( $\text{Al}_2(\text{SO}_4)_3$ ), sulfuric acid ( $\text{H}_2\text{SO}_4$ ), hydrochloric acid ( $\text{HCl}$ ), sulfur(S) and pyrite ( $\text{FeS}_2$ ).

The chemical reaction between the amendment and soils leads to formation of the leachable sodium components such as sodium sulfate ( $\text{Na}_2\text{SO}_4$ ) and sodium chloride ( $\text{NaCl}$ ). The choice of an amendment depends on its effectiveness, relative cost and the time required for the amendment to react with the soil. Calcium chloride, iron sulphates and aluminum sulphates are usually expensive and have not been used for large-scale improvement of sodic soils. Amendments like sulfur and pyrite must first be oxidized to sulfuric acid by soil micro-organisms before being available for reaction.

Gypsum is the most widely used amendment world over because it is the cheapest and the most abundantly available. It comes both from mines and as a by-product of the phosphate fertilizer industry. Gypsum is soluble in water to the extent of about 0.25 per cent at 25°C and is a source of soluble calcium. Gypsum reacts with both sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) and absorbed sodium and produces sodium sulfate ( $\text{Na}_2\text{SO}_4$ ), which is leachable. The gypsum requirement is the amount of gypsum required to reduce the sodium saturation to some acceptable level for a given quantity of soil. For each milliequivalent of exchangeable sodium per 100 g of soil, 0.086 g of gypsum is required. Amendments like gypsum are normally applied on the soil surface and then incorporated with the soil by disking or plowing. Gypsum mixed with the surface 15 cm of soil is more effective in the removal of exchangeable sodium than gypsum applied on the soil surface.

Mined gypsum requires grinding before application in sodic soil reclamation. The fineness to which gypsum must be ground is an economic consideration. Very fine grinding entails higher cost. Although it is often maintained that finer gypsum particles are more effective, Abrol et al. (1988) demonstrate that gypsum passed through a 2 mm sieve and with a wide particle size distribution is likely to be more efficient for the reclamation of sodic soils having appreciable quantities of sodium carbonate.

Gypsum is used on sodic and saline-sodic soils, while sulfur, sulfuric and hydrochloric acid to only calcareous sodic and/or calcareous saline-sodic soils. These chemicals lower the soil reaction (pH), react with carbonates and replace exchangeable sodium with calcium. The long-term objective is to replace ES with calcium, and use organic matter to bind the soil and improve its structure. The calcium causes particles to form clusters (flocculates), forming a very clear puddle of water.

Prior to gypsum application to sodic soils soluble salts e.g., carbonates and bicarbonates should be leached out. Otherwise gypsum equivalent to the quantities of soluble carbonates and bicarbonates will be inactivated and reclamation process will be affected. Gypsum should be broadcasted and incorporated in the upper 10 cm soil. Water is to be ponded in soil (up to 80% gypsum get dissolved in 7-10 days ponding water). In wheat/barley or other crops 304 irrigation of 7.5 cm each is recommended to dissolve gypsum and leach salts.

Equivalent amount (in tons) of various amendments (equivalent to 1 ton of gypsum) for supplying Ca in terms of 100 % pure gypsum is; calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) 0.85 tons; calcium nitrate [ $\text{Ca}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ ] 1.06 tons; press-mud (lime-sulfur 9% Ca 4% S) 0.78 tons; sulfuric acid ( $\text{H}_2\text{SO}_4$ ) 0.61 tons; iron sulfate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) 1.62 tons; ferric sulfate [ $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ ] 1.09 tons; aluminum sulfate [ $\text{Al}_2(\text{SO}_4)_3 \cdot 18\text{H}_2\text{O}$ ] 1.29 tons; Sulfur (S) 0.19 tons; pyrites ( $\text{FeS}_2$ , 30% S) 0.63 tons; and limestone ( $\text{CaCO}_3$ ) 0.58 tons. The amendments alternate to gypsum should be applied with care and should be based on other soil properties.

### **7.2.3 Biological**

Addition of organic residues (preferably of legumes) is beneficial in terms of improving soil structure, supplying plant nutrients, prevent soil erosion and hasten reclamation. Most of the organic amendments lower the soil pH. Care should be taken in selecting the organic amendments, they may cause N deficiency (high C: N ratio amendment), and increase salinity (cow dung slurry). Growing legumes improve the structure, and the physical properties like water holding capacity, infiltration rate is improved. Green manuring have better chance than farm yard manure (FYM) of being integrated into a reclamation management package.

Reclamation can also be triggered by growing plants on problem soils. Along with leaf litter, they add a considerable amount of organic matter as leaf and root litter to the soil, release carbon dioxide by respiration and decomposition of organic materials, which converts the insoluble calcium carbonate to bicarbonate. Calcium thus solubilised would exchange with sodium, producing sodium bicarbonate, which is soluble in water and can be easily leached down. Exchange of  $\text{Na}^+$  with  $\text{Ca}^{2+}$  along with action by organic matter results in production of soil aggregates and restores the soil structure. This phenomenon and mechanical action of roots increases soil permeability and help to open up the impermeable sodic soils enhancing downward movement of water and leaching of salts.

Kallar grass roots are profuse and deep. Therefore, the growth of the grass is known in Pakistan to improve soil conditions to the extent that have enabled some moderately salt-tolerant crops, and a few selected varieties of barley and some other plant species, to grow on the ameliorated soils.

Salt removal by agronomic crops is insufficient to maintain salt balance at intermediate levels. Under saline condition alfalfa contains 3-4% salts (Francois, 1981). Oster (1984) reported if 2000

mm of irrigation water with an EC  $1 \text{ dSm}^{-1}$  was applied annually in order to grow alfalfa, the total salt applied would be about  $13 \text{ Mg ha}^{-1}$ , salt uptake by alfalfa would represent 6% of the amount applied. Similar calculations with halophytes also substantiate this point although halophytes would remove more salts than halophytes.

#### **7.2.4 Leaching and drainage (Hydro-Engineering)**

All irrigation projects need leaching and drainage, if they are to be sustained over time. In irrigated arid/semi-arid areas, the objective of the drainage is to avoid salinization. In humid areas it is to provide aeration for plant roots.

##### **7.2.4.1 Leaching**

Leaching is a traditional method for reclaiming saline soils. It is effected by flooding or by ponding water and to avoid re-salinization, a downward flux of water is maintained through and beyond the root zone. When more water is applied than is used during a crop season and adequate drainage exists, net water movement is downward and salts are leached from the root zone. The salt concentration in the root zone will increase if the net downward movement of salts is lesser than the salt input from irrigation. Therefore the salt balance must be kept under control/maintained during the cropping season in order to avoid salinity build up. Crop water requirement and irrigation water quality are two primary parameters that have to be considered in order to ensure proper water management for salinity control. Over time, the amount of salt removed by leaching must suffice to prevent the build up of salinity beyond the level the crop can tolerate. Therefore, it is very important to know the leaching requirement (LR) for a particular soil in order to effectively leach the salts.

The LR is the calculated fraction (depth) or quantity of water that must pass through the root zone to maintain the EC of the drainage water at or below some specified level. Calculation of leaching requirement (LR) requires some understanding of how plant growth responds to a variable salinity with depth. In general, the depth of soil leached is roughly equal to the depth of water infiltrated during leaching. The recent trend is to minimize the leaching requirements in order to prevent rising of groundwater and minimize the load in drainage system. The LR requirement to avoid salt damage to crop yield can be obtained and also threshold level of crops (Maas, 1990). Estimates of the average leaching fraction for a field based on salinity measurements (Rhoades, 1982) would require repeated measurements at several locations. As a general rule, more the plant is salt tolerant, the lower would be the LR (Aslam, 1985).

Timing of leaching does not appear to be critical, provided crop salinity tolerance limit is not exceeded for extended periods of time or critical stage of plant growth. The leaching can be effected at each irrigation time. However in a soil with low permeability and for crops sensitive to excess moisture in the root zone, leaching at each irrigation time may not be possible. Leaching should be done when soil moisture is low and water table is deep and it should precede the critical growth stage of the crop. Moreover, leaching is recommended when demand for evapotranspiration is low, i.e. at night, during high humidity, or in cooler weather. Better, the leaching should be done before or at the end of cropping season.

The salinity data obtained during several field experiments (US Salinity Laboratory Staff 1981) indicate that salinity changes during a crop season do provide useful information for water

management. Where water and salt have moved upward into a soil profile over a period of time, one expects to find an “inverted” salinity profile, or a soil profile in which the salinity decreases with depth; where the dominant movement of water has downward one normally encounters a “normal” salinity profile (Shahid and Jenkins, 1992). It is emphasized that an excellent on farm leaching/drainage system has no value unless there is an adequate outlet for disposal of the drainage water.

#### **7.2.4.2 Drainage for salinity control**

Drainage implies the removal of salts in the drainage water. Presumably drainage is required to remove water applied in excess of crop use, so that it does not transport salts to soil surface by capillary action. Not all the water requiring removal by drainage may originate from on-farm irrigation. In fact, it is common that a significant part of the water input to the system is the result of seepage from irrigation canals or from areas irrigated some distance above the area under consideration.

The traditional drainage system lowers the water table, minimizes upward flow and thus minimizes builds up of salts due to capillary rise, and also provides adequate leaching. Under condition where underlying layers are permeable and relief is adequate, natural drainage may work, these ideal conditions generally do not exist in saline areas and therefore, a drainage system is always required. Depending upon the site condition and nature of the problem different types of drainage systems can be used: 1) surface drainage - to runoff excess water before entering to soil; 2) subsurface drainage - to control water table at safer depth, consisting of open ditches or tile drains or perforated plastic pipes, mole drainage, and vertical drainage (pumping water) when the deep horizons have an adequate hydraulic conductivity.

##### **7.2.4.2.1 Surface drainage**

It is the removal of excess water by shaping the land so as to make the water flow over the surface to furrows, ditches or waterways. Excess water on farmlands may be caused by excess irrigation, rain or poor land grading. Surface drainages are used to overcome these conditions. Surface drains may be deep enough to intercept groundwater, which will then enter the drain. The rate of groundwater flow into the drain depends on soil permeability and height of the water table. The deeper a drain, the greater is the width of adjacent land affected by the drawdown of the groundwater. Although surface drains can serve to control the water table, their use is being superseded by subsurface drains. Disadvantages of surface drains include loss of land, hindrance to farming operations and overland traffic and heavy maintenance requirements due to prolific weed growth or instability' of banks (Zijlstra and van Someren, 1980).

##### **7.2.4.2.2 Seepage interceptor drain**

It may be a suitable intervention, when a seep or spring is fed by a distinct water source. Interceptor drains would cut off this excess groundwater before it reaches the problem area. Interceptor drains are usually a single tube or open drains placed between the water source and the problem area. Accurate location of the source of excess groundwater and proper placement of the drain are critical to the success of this form of drainage. However, investigation of subsoil conditions and groundwater flow is essential before drainage work begins (George, 1985).



#### **7.2.4.2.3 Horizontal subsurface drainage**

It is the technique of controlling the water table and salinization by installation of horizontal drains at a certain depth (about 1.5 to 2.5 m) below the surface. The pattern of drains allows the land to drain to collectors which remove the water from the land. The drains are installed at an appropriate depth and are spaced at intervals designed to ensure that the water table in the intervening space does not rise above a given height. (Please see McWhorter (1977), FAO (1980), Euroconsult (1989) and Amer (1990) for the design of subsurface drainage systems). Flow into drains is induced by the lateral hydraulic gradient.

A mole drain is a simple subsurface drain. For its construction, a small mole (25 mm to 100 mm) is drawn through the ground at a predetermined level (0.4 to 0.7 m and spacing of 2 m to 5 m). In the right soil this mole then creates a cavity similar to the inside of a pipe that will allow the passage of water. Since no pipe or refill material is required, this is the lowest priced of all drainage techniques; likewise, it is expected to have the shortest length life and possibly the lowest efficiency (Hawkins, 1978). The practical application of a mole drain is limited to heavy clay soils. Mole drains have limited stability and the moling operation has to be repeated every few years (Castle et al., 1984).

#### **7.2.4.2.4 Vertical drainage systems**

Vertical drainage systems are basically water wells spaced on a grid which, like horizontal drainage, bring the water table down to a predetermined level. Ground water pumping is the most effective method of lowering water table and removing ground water. Tube-well drainage may be an alternative to horizontal drainage in areas with productive aquifers at moderate depth. Tube-well drainage offers the following advantages (Euroconsult, 1989): it can be applied on undulating land without extensive earthmoving and leveling for the installation of pipelines or main drain channels to interconnect the wells; it diminishes maintenance costs because of the smaller network of canals and/or drains that are necessary; the water table can be drawn down to a much greater depth, reducing the risk of salinization of the soil; it diminishes artesian pressure of aquifers underlying the top layer, which may reverse the direction of flow so that downward percolation in areas where groundwater is saline, the effluent of drainage wells is likely to be much more saline than that of horizontal drains, which makes vertical drainage less attractive or not acceptable for those areas.

Hydro-geological investigations are necessary for the feasibility study and design of pumping wells or well-point systems. It must be aimed at the determination of the extent, depth and magnitude of the aquifer, its transmissivity and the vertical hydraulic conductivity of the covering layers and the effect of pumping from deeper aquifers on shallow water tables Driscoll (1989). To be able to pump groundwater, there needs to be a pocket of very coarse sand or gravel (an aquifer) below the surface into which a slotted pipe ("well-point" or "spear") can be installed. Water drains into pipes through the slots and can then be pumped to the surface. These systems are also sometimes referred to as "bores". Apart from salinity and water table control, pumping groundwater can provide extra water to supplement irrigation supplies (depending on the salinity of the groundwater). Pumping groundwater from shallow aquifers (<25 meters) is the most effective way to alleviate salinity effects near the surface.

Tile drainage is a very effective way of controlling water tables and reducing water logging in areas with no pumpable aquifers. It involves the installation of slotted PVC pipe (or other material) at about 1 meter below the surface. Soil water enters the pipe through the slots and is carried to a central well pit where it can then be pumped away either via irrigation or drainage systems. Tile drainage is very expensive. If the above targets are met, the whole community will feel the benefits.

#### **7.2.4.3 Disposal of drainage water**

Besides the benefits to the land being drained, one needs to consider disposal of the drainage water. Several methods have been tried in some countries with complete or partial success.

##### **7.2.4.3.1 Disposal to rivers**

This option is widely in practice. Disposal can be; controlled or uncontrolled. In the case of controlled disposal, the volume, salinity and time of disposal are controlled. Effluents from the drainage facilities are collected in holding basins or evaporation basins and released to the river during periods of river flow. The *common* practice of uncontrolled disposal of the drainage effluent merely serves to salinize the vital water supply for the less fortunate users who happen to be located downstream from the point of discharge. If they also drain their fields in a similar fashion, the river will undergo progressive salinization and its lower reaches may become unfit as a water source for either human use or irrigation. The river then turns into a saline stream, with consequent effect upon its associated aquifer and estuary, or upon the lake or bay into which the river flows. If, in addition to agricultural drainage, domestic, municipal and industrial effluents are also discharged into the river, it can become in effect an open sewer which can endanger the entire population of the region (Hillel, 1990).

##### **7.2.4.3.2 Disposal to seas and lakes**

Disposal to open seas, inland seas and lakes is an attractive option, but mainly for economic reasons cannot be considered as a feasible option for areas far from the sea. With this option, saline effluents are disposed of after being concentrated in evaporation basins to reduce their volume or without concentration. Effects of the nutrients, pesticides and chemical components of the disposed waters on the aquatic life and algae growth should be carefully investigated during the period of feasibility study and environmental impact assessment. Several countries are known to dispose saline effluents by this method. For example, Egypt and Pakistan practice the disposal of its agricultural saline effluents to the Mediterranean and Arabian Sea respectively.

##### **7.2.4.3.3 Disposal to evaporation basins**

Drainage water can be disposed to evaporation basins (ponds). These can be formed from natural/constructed depression or saline lakes (Evans 1989). They must be designed and operated with care since drainage water with high levels of trace elements can be considered hazardous or attain toxic levels in evaporation basins. Drainage water may be treated to remove toxic constituents before ponding, but this is costly.

Evaporation basins vary in size from a few hectares serving individual farms to very large basins (thousands of hectares) serving large areas. Their advantages include: effectiveness, if they are properly designed, operated and maintained; salt harvesting potential; and the possibility of

providing a habitat for a diversity of bird life. Their disadvantages consist of adverse effects due to lateral seepage causing salinity and waterlogging in adjoining areas; their finite life; their land requirements; and possible adverse environmental impacts. One of the major problems associated with the operation of evaporation basins is leakage. There are two broad types: lateral and vertical leakage.

Much of the previous discussion concerned regional or sub-regional basins. The use of on-farm evaporation basins to dispose of saline water from tile drainage or groundwater pumping on individual farms also has several problems: effectiveness of basins located on the more permeable soils will be limited unless they are lined, and the potential to adversely affect neighboring properties would be higher than that of large, public-scale basins which are usually sited on lower-permeability soils. However, the on-farm basins may be well used as short-term holding basins with release permitted during high flow periods.

#### **7.2.4.3.4 Deep-well disposal**

Injection of brine into isolated deep aquifers can be a convenient and economically feasible disposal method in particular areas with adequate geologic and hydro-geologic characteristics. Problems, failures and environmental consequences of poor deep-well injection are mostly related to errors in well construction, undetected pathways for fluid migration, operational errors and natural events such as earthquakes and specific problems arise in the injection of brine requiring compromise (Gutteridge Haskins et al., 1983). Deep-well injection schemes should have adequate monitoring facilities to provide evidence that the injection well is operating correctly and that the injected fluids are being contained to avoid environmental problems.

#### **7.2.4.3.5 Desalination**

Desalination is the process of separating the dissolved salt content of saline or brackish water, to render it suitable for domestic, industrial or other purposes. Various desalination processes are: reverse osmosis (RO), electro-dialysis (ED), ion exchange (IE), multistage flash distillation (MSF), vapor compression (VC) and solar distillation. In reverse osmosis process, ions are transported through a membrane from one solution to another under the influence of a direct current electrical potential. Ion exchange involves passing the saline water through two beds of resins, one of which removes the anions and the other the cations. Pure water emerges from the second bed. After a time the beds become exhausted and are regenerated. In MSF, the heated saline water is passed through a series of chambers under a gradually increasing vacuum. The water thereby 'flashes' into a steam-water mixture and the steam is separated and condensed to pure water. In VC, steam produced from boiling water is compressed mechanically to produce freshwater. Solar distillation in its simplest form consists of a basin with a heat-absorbing surface (usually colored black) containing the saline water. The basin is covered by a sloping transparent glass or clear plastic which transmits sunlight and condenses vapor evolved from the saline water. The condensate trickles down the slope of the cover to the edge and is collected in a trough. All desalination processes produce, as well as freshwater, a by-product with high salt content which needs to be disposed of. Plants on the seaboard can conveniently discharge their by-product to the sea, but for inland plants disposal is a problem. A possible solution could be disposal to evaporation ponds or salt lakes.

#### **7.2.4.3.6 Conjunctive/cyclic use of surface and groundwater**

Irrigation wells are installed primarily to provide additional water for irrigation, either as insurance against drought or to provide a long-term supplementary source of water to the channel-supplied surface water. There is also a growing awareness of the salinity benefits of groundwater pumping and the need for a fully integrated groundwater and surface water allocation to deal with salinity issues (Evans and Nolan, 1989).

However, groundwater extraction wells can be installed to control conjunctive use of surface and groundwater to control salinity and waterlogging.

If the groundwater quality is not suitable, good quality water can be mixed to make it suitable for irrigation or cyclic use of good and bad quality water can be practiced. Currently the conjunctive use of surface and groundwater is practiced widely in Pakistan.

#### **7.2.4.3.7 Reuse of Drainage water**

Depending on its quality, drainage water may be a total or partial substitute source for irrigation water, brackish water can sometimes be used to irrigate salt-tolerant crops. According to Rhoades (1984), drainage water can be used again for irrigation to the extent that it still has value for use by a crop of higher salt tolerance. This could be achieved by a 'cyclic' drainage water reuse strategy (successive irrigations of a sequence of crops of increasing salt tolerance). The strategy is to irrigate moderately sensitive crops (lettuce, alfalfa, etc.) in rotation with river water and salt-tolerant crops (cotton, sugar beet, wheat, etc.) with drainage water. When the quality of drainage water deteriorates to the extent that its potential for reuse is exhausted, the drainage water should be disposed to evaporation ponds or by any other convenient means. For the salt-tolerant crops, the switch to drainage water would usually occur after seedling establishment (pre-plant irrigations and initial irrigations being made with river water).

The feasibility of this strategy is supported by the observations that maximum soil salinity in the root zone resulting from continuous use of drainage water does not occur when such water is only used for short time; substantial alleviation of salt build-up resulting from irrigation of salt-tolerant crops with drainage water occurs during the time salt-sensitive crops are irrigated with river water; and proper pre-plant irrigation and careful irrigation management during germination and seedling establishment leaches salts out of the seed area and from shallow soil depths. The qualities of all these crops were never inferior, and were often superior, when they were grown using the drainage water for irrigation (Rhoades (1990 a).

There are also uncertainties associated with the long-term effects of the reuse strategy, which include reduction of the soil infiltration capacity, soil salinization, and accumulation of certain elements (selenium, molybdenum, heavy metals...) in soils and plants that are toxic to the consumers of the crops (humans and animals) Rhoades (1990b) describes the management considerations of the reuse strategy, including the criteria for crop selection. Drainage water can also be used for tree plantation and agro-forestry (Schofield, 1990). Wood lots can reduce the volume of drainage water that needs to be evaporated or ultimately disposed of by 75 per cent. Same uncertainties, as explained above, with this agroforestry-evaporation pond approach have also been identified.

The Serial Biological Concentration of Salts (SBCS) concept later proposed by Heuperman (1995) is almost similar and is drummed up in Australia that it may provide an alternative salt management option in badly salt-affected areas, especially where groundwater pumping or reuse of the groundwater in the farm irrigation system is not possible. The SBCS system involves the reuse of drainage water on progressively increasing salt tolerant crops. Each crop is underlain by tile drain for the collection of water to be used to irrigate the next stage. Along the crop sequence, the volume of drainage water collected is reduced due to plant water use and the salinity of the drainage water increases since there is little or no salt uptake by plants. The final effluent is contained in relatively small evaporation ponds making it feasible to consider the use of floor lining to eliminate leakage. These ponds could also be used for fish farming. The highly saline water may be collected in a series of ponds where through evaporation the salts may be harvested, and may have commercial value.

## **8. SALINE AGRICULTURE TECHNOLOGY**

Efforts have been made towards economic utilization of salt-affected soils and brackish ground water with Saline Agriculture Technology. Saline Agriculture is the profitable and integrated use of genetic resources (plants, animals, fish, insects and microorganisms) and improved agricultural practices to obtain better use from saline land and saline irrigation water on a sustained basis. It is not one simple system as one might consider. In fact it is a rich collection of possible systems for the use of saline land, involving combinations of salt tolerant trees, shrubs, crops and livestock. The main component of this approach is growing salt-tolerant plants which can successfully be grown under existing saline conditions and are capable of showing minimum depression in yield.

Saline Agriculture has many dimensions such as; 1) selection/breeding of productive and salt-tolerant crop species/ genotypes or halophytes of agricultural significance; 2) domestication of salt-tolerant wild plant species for economic exploitation of salt-affected lands; 3) introduction into the cultivated crops/other plant species genes of salt tolerance from their wild relatives through conventional breeding or genetic engineering; 4) special agronomic practices including methods of land preparation to improve their productivity, planting, irrigation and drainage management strategies and fertilizers application; 5) physiological studies with the objectives to: a) determine critical plant factors controlling yield under saline conditions; b) study physiological differences between salt tolerant and salt sensitive genotypes with a view to develop selection criteria for salt tolerance and assist breeding programs; and c) improve yield through special treatments at critical stages during the ontogeny of plants.

### **8.1 Salt tolerance in plants**

Plant salt tolerance: the salt tolerance of a plant can be defined as the plant's capacity to endure the effects of excess salt in the medium of root growth (Mass, 1990).

#### **8.1.1 Screening techniques**

Selection of appropriate screening techniques for salt tolerance is one of the most crucial steps in the selection and/or development of salt-tolerant varieties. These techniques can be used in green house or in the field. These techniques have recently been described by Aslam (2008).

Salt tolerance of a plant species/variety can be studied at germination and at later growth stages at a range of salt concentrations. At germination, salt tolerance of a plant is studied by placing seed in a Petri dish lined with a filter paper moistened with desirable salt concentration. Salt tolerance in plants at vegetative and maturity growth stages, can be studied in soil, sand, gravel or aerated solutions, employing pots or small plots.

A large number of grasses, shrubs, trees and crop plants have been studied so far. The overall picture emerged from salt-tolerance studies on different plants is that the maximum amount of salts that can be tolerated by salt-tolerant plants varies among species and even varieties of the species. The salt-tolerance of some of these plants may enable them to produce yields under saline conditions that are comparable to those obtained from salt-sensitive crops grown under non-saline conditions.

For studying salt tolerance in plants, we routinely use a gravel culture method employing cemented tanks. In gravel/sand culture facilities at NIAB, the seeds are first grown in river sand for their germination and then seedlings of various genotypes at 2-4 leaf stage are transferred to plastic buckets or cemented tanks having Hoagland solution. Using a mixture of salts, salinity is then increased stepwise and maintained at different levels EC (in dS/m) levels of 0, 5, 10, 15, 20, 25 etc, in respective buckets/tanks. Seedlings are then allowed to grow for a few weeks and then dry shoot weights are taken. The relative reduction in dry weight of various cultivars compared with respective controls is taken as the criterion for salt-tolerance. The data so obtained should be correlated with the salt tolerance data obtained in the soil culture and in the field.

In soil culture technique the varieties/cultivars are grown in the artificially salinized soil (pots or small plots) or natural saline field. The plants are allowed to grow for a due course of time. The dry matter and/or grain yields are taken as criterion of salt tolerance compared to control.

### **8.1.2 Diversity for salt tolerance in plants**

Many plants have developed special methods to avoid or tolerate salinity by salt exclusion or avoidance, immobilization within the plant, excretion and adjustment in osmotic pressure and thus grow in the hostile environment. Therefore, there is an approximate 10 fold range in salt tolerance of agricultural crops; for example, production of wheat may decrease up to 25% with a salinity level of  $8 \text{ dSm}^{-1}$ , while production of barley is not affected at this salinity level (Ayers and Westcot, 1989).

### **8.1.3 Improvement of crop growth on slightly and moderately salt-affected soil**

Tolerance of variable crops to salinity levels has been under investigation in numerous research centers. The pioneering work was done in USA. Crop tolerance tables for different field, forage, vegetables and trees crops were assembled and published lists of crops and the salinity levels that might cause 50% decrement in their yield. Later, Mass, (1980) and Maas and Hoffman (1985) summarized salt tolerance data on crops collected from various sources.

All plants can tolerate salinity up to a certain level without a measurable loss in yield (threshold salinity). As a general rule, the more the crop is salt tolerant, the higher the threshold level. Crop yields reduce linearly as salinity increases above threshold level, as shown in the equation.  $Y_r = 100 - s (EC_e - t)$ , where  $Y_r$  is crop yield relative to the same conditions without salinity,  $t$  is the

threshold salinity,  $s$  is the % linear rate of yield loss with 1 ECe (dS/m) increase above the threshold value. ECe is the electrical conductivity of the soil saturation extract, which represents the average root zone salinity.

Expected yield of a crop on a specific level of salinity (ECe) can be calculated. For example the ECe of a farmer field is 7 dS/m and the farmer is willing to use this piece of land for a potato crop, the potato yield relative to a non saline conditions would be  $Y_r = 100 - 12(7 - 1.7)$ , therefore, the expected yield would be 36.4%. The farmer should think very carefully in selecting the crop for various levels of soil salinity at his farm. Salinity mapping at the farm level and an appropriate use of salt-tolerance data is the correct solution/ guide for farmers to predict yield losses. Please see Mass, (1980) for salt-tolerance data on a range of crops.

#### **8.1.3.1 Factors affecting salt tolerance in plants**

The salt tolerance of a plant depends on many factors, conditions and limits including environmental factors (soil fertility, physical condition of soil, salt distribution in the profile, irrigation method and climate) and biological factors (stage of growth, varieties and rootstocks) and actual plant response to salinity varies with growing conditions (Balba, 1995). Thus, salt tolerance data cannot indicate accurate quantitative crop yield losses from salinity and predict crop yield under saline condition. Rhoades (1982) concluded that salt-tolerance data should be taken as a relative rating of the tolerance of crops to salinity. However, data do tell that some plants are more tolerant to high salinity conditions than others. Generally most fruit trees are more sensitive to salts than are vegetables, while forages are the most tolerant.

#### **8.1.3.2 Facilitating seed germination and seedling establishment in saline soil**

It is emphasized that crops tolerance published by the do not represent salt tolerance for germination and early seedling growth (Rhoades, 1982). Crop species which are salt tolerant during the late stages of growth may be quite sensitive to salinity during germination (U. S. Salinity Laboratory, 1954), and particular attention should be given to salt tolerance of crops during seedling development because low yield frequently result from failure to obtain strong stand. Hamdy (1990) suggested increasing plant density to overcome the salt adverse effect on seed germination. Bernstein et al (1974) recommended that seeds should not be placed in the upper center of the furrow where salts are concentrated. They recommended placing the seeds in double row beds; each row is located at the furrow shoulder away from the area of greatest salt accumulation. Ayers and Westcot (1985) recommended a heavy pre-planting irrigation. Establishment of crops can also be facilitated by germinating them in non-saline soil in small bags, hardening them at seedling stage and then placing them in saline environments. But practically it is only possible with some row crops like cotton. It would be a great service to Saline Agriculture Technology, if some techniques are developed to facilitate seed germination and early establishment under saline conditions.

A selection of appropriate crops is essential to choose a correct plant type that can produce satisfactorily under saline condition. In addition to the choice of suitable salt-tolerant species, the choice of cropping pattern, seed placement, and method of raising plants, water application, and frequency of irrigation, use of mulches and proper uses of plant nutrients should also be taken into consideration while managing the saline soils. It must be emphasized that salt-tolerance in the crop

plants is only appropriate for slightly or moderately salt-affected soils and is too low to cover highly salt-affected soils. Research work in breeding and genetic engineering enhances hopes to have cultivars of economical crops that can tolerate high salinity.

#### **8.1.4 Revegetation of highly saline soils**

Saline soils which cannot be reclaimed due to variable circumstances may be left for grazing pastures. Halophytes are one choice for such soils. They can grow under very saline conditions. For example, *Leptochloa fusca* (kallar grass), a halophyte from Pakistan, native to salt marshes in Pakistan, has also been found a suitable plant for forage, green manure/compost, and pulp for paper production. According to some reports, its distribution area includes Egypt, India, Sri Lanka and Australia. Kallar grass is a deep-rooted, perennial tall grass which grows to a height of 1.5 m. It is a highly salt-tolerant grass and possesses an excellent salt excretory mechanism through its leaves. Therefore, it does not retain most of the salts taken up; hence it remains reasonably palatable for farm animals. Kallar grass can survive at a very high salinity ( $EC_e$ ) of 40 dS<sup>-m</sup>, but remains economical up to a salinity of 20 dS<sup>-m</sup>. It also tolerates sodic soils and waterlogged conditions.

Kallar grass, harbors nitrogen-fixing bacteria and obtains 60 to 80 per cent of its nitrogen requirements from the air. Therefore it grows very well without fertilizer. It produces about 50 tones of biomass per hectare, even when irrigated with brackish water. Although kallar grass can be propagated through seeds, the best field establishment is obtained by the sowing of stem cuttings or root stubbles. Kallar grass can be sown at any time of the year in the plains of Pakistan. However, the best sowing time is in March (see Malik et al., 1986 and Qureshi et al., 1982 for further information concerning kallar grass and its plantation for amelioration of salt-affected soils).

The Nuclear Institute for Agriculture and Biology (NIAB) has successfully demonstrated the potential of agricultural production systems using salt-tolerant plants, in particular the potential of kallar grass (*Leptochloa fusca*) for the saline, sodic and waterlogged soils of Pakistan (Malik et al., 1986). Kallar grass is most commonly used as a forage plant and is readily incorporated into the local farming systems. It is now an established and a profitable technology. Due to the usefulness of the grass as a forage species, farmers have been encouraged to plant their salt-affected soils with this plant. As a result its use as forage is generally increasing. The economic returns to private farmers of raising buffaloes with this grass are particularly encouraging. Kallar grass has been grown on a large scale as a primary colonizer of highly salt-affected soils in certain areas in by the Saline Agriculture Development Project, and elsewhere by progressive farmers.

#### **8.1.5 Some other highly salt tolerant plant species**

Kallar grass is a summer plant and grows under wet conditions. Therefore, with its sole cultivation, the supply of forage during winter and utilization of dry salt-affected areas remains unattended. Such issues have also been tackled by selection of plant species which remain productive during winter and/or capable of growth under water deficit conditions. Work is in progress on salt-tolerant winter forage species like *Agropyron*, *Salicornia* and *Atriplex* as possible sources of fodder from dry salt-affected areas. Research indicates successful results growing



salt-tolerant plants like *Acacia ampliceps*, *A. nilotica*, *Prosopis juliflora*, *Eucalyptus carnaldulensis* and *Tamarix aphylla*.

There are many other halophytes which can be grown for economically utilizing salt-affected soils. For example, *Juncus rigidus* and *J. acutus* can grow in saline marshes or under irrigation with brackish water or even sea water. The culms provide fibers for high quality paper production and the seeds have the medicinal value. Mangroves are other options; they provide fuel and fodder in coastal areas in many countries. *Atriplex* species are well suited to forage production on saline soils, and have been planted on a large scale in Australia for grazing (Malcom et al, 1980). In-fact now there is increasing interest in several countries for cultivating, breeding and managing selected *Atriplex* species for intensive forage production on highly salt-affected soils, with highly saline water. Malcolm (1992) gives criteria, field test methods and guidelines for the selection of shrub species suitable for forage production on saline land.

The National Research Council (1990) examines some of the plants that may be suitable for economic production in saline environments in developing countries. The four sections of this publication highlight the salt-tolerant plants that may serve as food, fuel, fodder and other products such as essential oils, pharmaceuticals and fiber. In each of these sections, plants are described that have potential for productive use. Each section also contains an extensive list of recent papers and other publications that contain additional information on these plants.

#### **8.1.6 Revegetation with tree species**

Different tree species have also a lot of potential for growing under salt-affected and waterlogged soil conditions and are capable of producing very attractive economic returns. Australia has a native flora rich in halophytes including trees. In Western Australia more than 100 species of Australian woody plants have been *screened* for salt and waterlogging tolerance under glasshouse conditions (van der Moezel and Bell, 1990). Highly salt-tolerant tree species (e.g. *Eucalyptus occidentalis*, *Melaleucit halmaturorum* and *Casuarina glauca*) have great potential in saline drainage water reuse schemes and with salt and/or water logging tolerant tree-based land management strategies, productive use of salt-affected land and control of salinity is reasonably achievable (Marcar 1990 and 1992). Certain native tree species, such as *E. camaldulensis*, have also been extensively examined, and salt-tolerant clones developed. Significant scope exists for intraspecific selection within species of proven commercial value, for example, *E. glauca*.

##### **8.1.6.1 Choice of tree species**

Substantial progress has been made toward answering such basic questions as which species to plant, how to plant, where to plant and what density to plant. In deciding which tree species to plant, three important criteria (adaptation, water use and multiple uses) should be addressed (Schofield, 1992):

Adaptation is the primary criterion to be satisfied and includes adaptation to such factors as climate, soil, pests, waterlogging and salinity.

Knowledge of the potential water use of species could allow trees to be selected to minimize the area required for tree planting. This is particularly important where there are strong reasons for maintaining land under agriculture.

#### **8.1.6.2 Tree establishment on saline soil**

Successful establishment of suitable salt-tolerant trees and shrubs on saline sites requires the use of appropriate pre-and post-planting strategies for reducing site environmental stress (Marcar, 1992). Any strategy should aim to minimize the shock to transplanted seedlings or direct seeded plants by ensuring as low soil salinity and waterlogging regime as possible. This will mean that the time of planting will need to coincide with the period of maximum leaching. Several strategies have been shown to be effective, which include: construction of mounds, particularly double-ridge mounds; application of mulch, particularly straw, newspaper or plastics; and pre-conditioning of seedlings to salt and waterlogging.

#### **8.1.6.3 Agroforestry**

Tree planting, in combination with other vegetation (agroforestry), is also regarded as a leading solution to dry land salinity and has a potential role in controlling irrigation salinity (Schofield, 1992). Planting trees can significantly lower water tables, and thereby reverse the causal process of salinization and allow under storey plants to grow successfully. The multiple benefits deriving from innovative configurations of trees combined with agriculture, such as wide timber belts, are now being realized. Multiple uses refer to the range of beneficial uses of trees other than salinity control which may influence selection. These include commercial tree planting for timber, pulp, firewood, fodder and other products, shelter and shade, wind and water erosion control, waterlogging control and aesthetics. Although salinity control may be the prime objective, the attractiveness and feasibility of tree planting may depend strongly on some of these other beneficial uses.

Such systems can improve the productivity of crops, pasture and livestock, often by providing shelter, can provide products from the trees and can also control salinity.

### **9. FARMER PARTICIPATORY SALINE AGRICULTURE**

#### **9.1 Salinity mapping at farm level and participatory planning**

Many plants either fail to grow in saline soils or their growth is retarded significantly; few plants grow well on saline soils. Therefore, salinization often restricts options for cropping in a given land area. The assessment of salinity at surface and subsoil layers at the farm level would identify different salinity zones in the farms and will help to select crops/cultivars on respective salinity level in the farms. It can help growers to improve crop productivity and get more value from each piece of farm land. Salinity maps also help farmers to understand subtle difference in soil properties across their fields, allowing them to develop more precise management zones and, ultimately, potentially higher yields. For salinity assessment and mapping at farm and landscape level, a description of destructive and non-destructive techniques is given elsewhere in this manual. These techniques can be employed in consultation and if possible, in participation of farming communities. Salinity mapping on whole farm scale is the best practice for crop selection. Farmers should realize the significance of salinity mapping for the sustainable use of their farms. Advisory program is developed to help farmer's plan and use salinity control practices on their farms.

## 9.2 Farming Community education

Controlling salinization of land and water resources cannot be achieved by individual landholders working in isolation taking local measures. Also, government action in the form of isolated management programs run by different agencies is not the most effective way of tackling the problem. Instead, governments should develop a coordinated plan of action with the participation of their agencies and the communities. Government support for community education and their participation in development, implementation, monitoring and cost-sharing of action plans is crucial for effectiveness and success of the management plans.

Education to farming community is vital in increasing people's awareness and understanding of salinity so that the above strategy is widely supported and acted upon.

The Government of Pakistan has taken a leading role in helping farming community by starting an integrated program on farmer participatory management and reclamation of saline soils. It is a partnership between production and conservation. The program is about whole community caring for their land, government agencies, farming community, awareness in schools and interested individuals. These aspects include formation of the working groups, participatory planning processes, the role of departmental officers, and community education. Salinity exhibitions for community education are arranged in government institutes, demonstration days at the farmer's field are also useful. Introductory brochures for salinity control and management at the farm level are being prepared and their distribution to the farming community is being arranged to enhance their understanding to tackle salinity in a sustainable way. This program has resulted in a semi-large scale rehabilitation of salt-affected soils and a substantial enhancement in the income of project farmers.

## REFERENCES

- Abrol, I.P., Yadav, J.S.P. and Massoud, F.I. 1988. Salt-affected Soils and Their Management. Rome: FAO (Soils Bulletin 39). 131 pp.
- Adams, W. M., and F. M. R. Hughes. 1990. Irrigation development in desert environments. In: Techniques for desert reclamation. A. S. Goudie (ed). John Wiley, New York. Pp. 135-160.
- Alben, W., Bamm, A. and Gonsowski, P. 1987. Employment of tensiometers for irrigation control. In. Thirteenth International Congress on Irrigation and Drainage, Rabat, Morocco, September 1987. New Delhi: International Commission on Irrigation and Drainage. Transactions, v.1B: 1061-1080.
- Al-Gabri, I. M. 1997. Salt-affected soils in Oman. Proc. Regional Workshop on Management of Salt-Affected Soils in the Arab Gulf States. Abu Dhabi, UAE 29 Oct. - 2 Nov. 1995. Published by the FAO of the United Nations Regional Office for the Near East Region Cairo, p. 133.
- Al-Ghanem, G.A. 1997. Salt-affected soils in Qatar. Proc. Regional Workshop on Management of Salt-Affected Soils in the Arab Gulf States. Abu Dhabi, UAE 29 Oct. - 2 Nov. 1995. Published by the FAO of the United Nations Regional Office for the Near East Region Cairo, p. 1-5.
- Al-Ghawas, S. 1997. Salt-affected soils in Kuwait. Proc. Regional Workshop on Management of Salt-Affected Soils in the Arab Gulf States. Abu Dhabi, UAE 29 Oct. - 2 Nov. 1995. Published by the FAO of the United Nations Regional Office for the Near East Region Cairo, p. 132.

- Al-Mehrizi, R. 1997. The use of salt-affected soils and saline water in agriculture in the UAE. Proc. Regional Workshop on Management of Salt-Affected Soils in the Arab Gulf States. Abu Dhabi, UAE 29 Oct. - 2 Nov. 1995. Published by the FAO of the United Nations Regional Office for the Near East Region Cairo, p. 129.
- Amer, M.H. 1990. Design of drainage system with special reference to Egypt. In Symposium on Land Drainage for Salinity Control in Arid and Semi-Arid Regions. February 25<sup>th</sup> to March 2<sup>nd</sup> 1990, Cairo, Egypt. Delta Barrage, Cairo: Drainage Research Institute. 377 pp.
- Aslam, Z. 1985. Growth, ion relations and carbohydrate levels of *Atriplex amnicola* at high salinities. Ph. D. Thesis. School of Agriculture, The University of Western Australia, Australia
- Aslam, Z. 2008. Salt tolerance and screening techniques for salt tolerance in plants. In Proceeding: International Conference on sustainable crop production on salt-affected land. saline Agriculture research Centre, UAF, Faisalabad, Pakistan. 4-6 December, 2006.
- Ayers, R., D.W. Westcot. 1985. Water quality for Irrigation. FAO Irrigation and Drainage Paper, No.29 Rev. 1p.173.
- Ayers, R.S. and Westcot, D.W. 1989. Water Quality for Agriculture. Rome: Food and Agriculture Organization of the United Nations. 174 pp.
- Balba, A.M. 1995. Management of Problem soils in arid ecosystems. Lewis Publishers, Newyork-London, pp. 250.
- Bernstein, L., L. E. Francis, and L. A. Clark. 1974. Interactive effects of salinity and fertility on yields of grains and vegetables. *Agron. J.*, 66:412.
- Boyden, S. 1987. Western Civilization in Biological Perspective: Patterns in Biohistory. Oxford: Oxford University Press. 370 pp.
- Boyle Engineering Corporation. 1986. Evaluation of On-Farm Agricultural Management Alternatives. (Prepared for the San Joaquin Valley Drainage Program). Fresno, California: Boyle Engineering Corporation. (Various pagings, about 300 pp).
- Brown, P.L., Halvorson, A.D., Siddoway, F.H., Maylan, H.F. and Miller, M.R. 1983. Saline seep Diagnosis, Control and Reclamation. Washington DC: US Department of Agriculture, Agricultural Research Service. (Conservation Research Report no. 30). 22pp.
- Campbell, G.S. and Campbell, M.D. 1982. Irrigation scheduling using soil moisture measurements: Theory and practice. In. Hillel, D. ed. *Advances in Irrigation*. New York: Academic Press. v.1: 25-42.
- Casey, H.E. 1972. Salinity Problems in Arid Lands Irrigation: A Literature Review and Selected Bibliography. Tucson, Arizona: University of Arizona, Office of Arid Land Studies. 300 pp.
- Castle, D.A., McCunnall, J. and Tring, I.M. 1984. Field Drainage: Principles and Practices. London: Batsford Academic and Educational. 250 pp.
- Driscoll, F.G. 1989. Groundwater and Wells. Second edition. St. Paul, Minnesota: Johnson Filtration Systems Inc. 1089 pp.
- Euroconsult, 1989. Agricultural Compendium for Rural Development in the Tropics and Subtropics. Third edition. Amsterdam: Elsevier. 740 pp.
- Evans, R.S. 1989. Saline water disposal options in the Murray Basin. *BMR Journal of Australian Geology & Geophysics*. 11(213): 167-185.

- Evans, R.S. and Nolan,. 1989. A groundwater management strategy for salinity mitigation in Victorian riverine plain, Australia. In. Groundwater Management: Quantity and Quality. Proceedings of the Benidorm Symposium, October 1989. (IAHS Publication no. 188).487-499.
- Food and Agriculture Organization of the United Nations (FAO). 1980. Drainage Design Factors: 28 Questions and Answers. Rome: FAO. 52 pp.
- Francois, L.E. 1981. Alfalfa management under saline conditions. *Agronomy Journal*, 73:1042-1046.
- George, P.R. 1985. Sub-surface drainage methods for salinity control. *Journal of Agriculture-Western Australia*. 26(4): 112-114.
- Ghassemi, F., A. J. Jakeman and H. A. Nix. 1995. Salinization of land and water resources: Human causes, extent management and case studies. Center for Resources and Environmental Studies. The Australian National University, Pub. CAB International, Wallingford, Oxon.
- Greenway and Munns, 1980. Mechanism of salt tolerance in non-halophytes. *Annual review of Plant Physiology*. 31:149-90.
- Gutteridge Haskins & Davey Pty Ltd, Aill Australia Pty Ltd, Australian Groundwater Consultants Pty Ltd and Melbourne University School of Agriculture and Forestry.1983. The Application of Salinity Control Techniques in Victoria. Melbourne:Gutteridge Haskins & Davey. 131 pp.
- Hamdy, A. 1990. Crop management under saline water irrigation, page 108 in *Water, Soil and Crop Management Relating to the Use of Saline Water*.FAO-AGL/MISC/16/90, FAO,Rome.
- Hawkins, G.P. 1978. Modern trends in mechanisation of construction of irrigation and drainage projects. In. Framji, K.K. ed. *State-of-the-Art I"igation Drainage and Flood Control*. New Delhi: International Commission on Irrigation and Drainage. no. 1. 107-254.
- Heuperman, A.F. 1995. Salt and water dynamics beneath a tree plantation growing on a shallow watertable.Report of the Dept. of Agriculture, Energy and Minerals Victoria, Institute for Sustainable Irrigated Agriculture, Tatura Center, Australia, pp. 61.
- Hillel, D. 1990. Ecological aspects of land drainage for salinity control in arid and semiarid regions. In. *Symposium on Land Drainage for Salinity Control in Arid and Semi-Arid Regions*. February 25th to March 2nd 1990, Cairo, Egypt. Delta Barrage, Cairo: Drainage Research Institute. v.1 (Keynote): 125-135.
- Jackson, R.D. 1982. Canopy temperature and crop water stress. In. Hillel, D. ed. *Advances in Irrigation*. New York: Academic Press. v.1. 43-85.
- Maas, E. V. and G. J. Hoffman. 1985. Crop salt tolerance current assessment.U. S. Salinity Laboratory Publication. 1976. FAO Irrigation and Drainage Paper Nov. 29, Rev. p.36.
- Maas, E.V. 1990. Crop Salt Tolerance. In *Agricultural Salinity Assessment and Management Manual*. K. K. Tanji (ed). ASCE Manuals and Reports on Engineering No. 71, New York, pp. 262-304.
- Maas, E.V. 1990. Crop salt tolerance. In. Tanji, KK ed. *Agricultural Salinity Assessment and Management*. New York: American Society of Civil Engineers. 262-304.
- Mako\m, C:V. 1992. Selecting forage shrubs for productive use of saline land. In. *National Workshop on Productive Use of Saline Land*. Waite Agricultural Research Institute, 22-24 September 1992. Adelaide: Department. Department of Agriculture.52-58.
- Malcom, C.V., T.S.Swan, and H.I.Ridings, 1980. Niche seeding for broad scale forage shrub establishment on saline soils. *Intl. Symp. Salt-Affected Soils*. India, 539.

- Malik, K.A., Aslam, Z. and Naqvi, M. 1986. Kallar Grass: A Plant for Saline Land. Faizalabad, Pakistan: Nuclear Institute for Agriculture and Biology. 93 pp.
- Marcar, N.E. 1990. Tree option for utilisation of salt-affected land. In. Myers B.A. and West, D.W. eds. Revegetation of Saline Land. Proceedings of a workshop held at the Institute for Irrigation and Salinity Research, 29-31 May 1990. Tatura, Victoria: Institute for Irrigation and Salinity Research. 53-59.
- Marcar, N.E. 1992. Trees for salt-affected land. In. National Workshop on Productive Use of Saline Land. Waite Agricultural Research Institute, 22-24 September 1992. Adelaide: Department of Agriculture. 12-23.
- Mashali, A. M. 1995. Network on integrated soil management for sustainable use of salt-affected soil. Proc. Intl. Symp. On Salt-Affected Lagoon Ecosystems ISSALE-95, Valencia (Spain) 18-25 September, 1995. P.267-283.
- McWhorter, D.B. 1977. Drain spacing based on dynamic equilibrium. Journal of the Irrigation and Drainage Division. v.103, IR2:259-271.
- National Research Council (1990). Saline Agriculture, Salt-Tolerant Plants for developing countries. Washington DC:National Academy Press.143pp.
- Oldeman, L.R., R.T.A.Hakkeling and W.G.Sombroek. 1991. Second revised edition. World map of the status of human-induced soil degradation. An explanatory note. Wageningen. International Soil Reference and Information Center (ISRIC). 35pp.
- Oster, J. D. 1984. Plant water uptake; or, how do plants integrate. Soil Water 52:11-13.
- Postel, S. 1989. "Water for agriculture: facing the limits". Worldwatch Paper 93. Worldwatch Institute, Washington, DC. Price, F. and D.A.Jenkins. 1980. Removal of resin from standard soil thin sections by low temperature ashing as a means of following transmitted optical by scanning electron microscopy. Clay Minerals, 15:309-315.
- Postel, S. 1990. Saving water for agriculture. In. state of the World 1989. A worldwatch institute report on progress toward a sustainable society. Washington DC: World Watch Institute. 39-58.
- Qureshi R.H., Salim, M., Abdullah, M. and Pitman, M.G. 1982. *Diplachne fusca*: An Australian salt-tolerant grass used in Pakistan agriculture. The Journal of the Australian Institute of Agricultural Science. 48(4): 195-199.
- Rhoades, J. D. 1980. Determining leaching fraction and field measurements of soil electrical conductivity. Agricultural Water Management, 2:205-215.
- Rhoades, J.D. 1982. Reclamation and management of salt-affected soils. Proc. of the First Annual Western Provinces Conf., pp.123.
- Rhoades, J.D. 1984. Reusing saline drainage waters for irrigation: A strategy to reduce salt loading of rivers. In. French, R.B. ed. Salinity in Watercourses and Reservoirs: Proceedings of the 1983 International Symposium on State-of-the-Art Control of Salinity. Salt Lake City, Utah, 13-15 July 1983. Boston: Butterworth Publishers. 455-464.
- Rhoades, J.D. 1990a. Assessing suitability of water quality for irrigation. In. Kandiah, A.ed. Water, Soil and Crop Management Relating to the Use of Saline Water. Rome: Food and Agriculture Organization of the United Nations. 52-70.

- Rhoades, J.D. 1990b. Strategies to facilitate the use of saline water for irrigation. In. Kandiah, A. ed. Water, Soil and Crop Management Relating to the Use of Saline Water. Rome: Food and Agriculture Organization of the United Nations. 125-136.
- Schofield, N.J. 1990. Salinity problems and remedies (with particular reference to agroforestry) in the San Joaquin Valley, California. Land and Water Research News. (Western Australian Steering Committee for Research on Land Use and Water Supply).6: 14-17.
- Schofield, N.J. 1992. Tree planting for dryland salinity control in Australia. Agroforestry Systems. 20(1/2): 1-23.
- Schofield, N.J., Loh, I.e., Scott, P.R., Bartle, J.R., Ritson, P., Bell, R.W., Borg, H., Anson, B. and Moore, R. 1989. Vegetation Strategies to Reduce Stream Salinities of Water Resources Catchments in South- West Western Australia. Leederville:Water Authority of Western
- Shahid, S. A. and D. A. Jenkins. 1992. Utilization of a simulation system for quick screening of soils against salinity and sodicity. In Jan Feyen, Emmanuel Mwendera and Moussa Badji (Editors). Advances in Planning, Design and Management of Irrigation Systems as Related to Sustainable landuse, Vol.2:615-626.
- Szabolcs, I. 1989. Salt-Affected Soils. Boca Raton, Florida: CRC Press. 274 pp.
- U.S.Salinity Lab. Staff (Richards, L.A., ed). 1954. Diagnosis and Improvement of Saline and Alkali Soils.USDA. Hand Book No. 60, p.160.
- U.S.Salinity Lab. Staff.1981. Minimizing salts in return flow through irrigation management. Final Report, interagency project EPA-1ag-D6-0370. Roberts S. Kerr, Environ. Res Lab, USEPA, AdA, OK. University of Calif. 1985. Committee of Consultants. van der Moezel, P.G. and Bell, D.T.1990. Saltland reclamation: Selection of superior Australian genotypes for discharge sites. Proceedings of the Ecological Society of Australia. 16: 545-549.
- Zijlstra, G. and van Someren, c.L. 1980. Development in subsurface drainage techniques. In. Wiersma-Roche, M.F.L. ed. Land Reclamation and Water Management: Development, Problems and Challenges. Wageningen: International Institute for Land Reclamation and Improvement (ILRI). 171-180.

# PART II MONOGRAPHIC REVIEWS

## CHAPTER 2

### EFFECTS OF SALINITY ON SOILS

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#### 1. INTRODUCTION

Soil and water salinity and sodicity are major abiotic constraints affecting agriculture in arid areas of the World. The purpose of this Chapter is to describe the causes and extent of the problem, and their effects upon soils, as well as to describe the methods of assessment of both soil and water salinity. To do this it is first necessary to give an assessment of the current state of the World's land and water resources and population.

#### 2. WORLD LAND AND WATER RESOURCES

##### 2.1 Land resources

Table 1 shows the total area and the arable cultivated and irrigated areas in each continent. Table 2 shows the world's total land resources and those under different types of crop. Clearly, the potential arable land is unevenly distributed around the world, and of course much of it also has marginal soils or climate. Just below 50% of the potential arable land is cultivated, so over 1,700 M ha remain to be developed – just more than is currently used. However, the best land is already in use, so any increase in food production will have to come from marginal or degraded lands.

**Table 1** Total land area, potential arable land, cultivated and irrigated land (M ha) (derived from Ghassemi et al, 1995)

	Land area	Potential arable	Cultivated	Irrigated
Africa	2,964	734	185	11
Asia	2,679	627	451	142
Oceania	843	153	49	2
Europe	473	174	140	17
N America	2,138	465	274	26
S America	1,753	681	142	9
Ex-USSR	2,227	356	233	20
Total	13,077	3,190	1,474	227



**Table 2** World land resources (000 ha) (FAO, 2007a)

Category	Year	Area	% of total
Arable	1980	1,345,989	10.35
	1990	1,395,973	10.73
	2000	1,397,656	10.75
Permanent crops	1980	102,020	0.78
	1990	119,883	0.92
	2000	135,821	1.04
Pasture	1980	3,244,404	24.95
	1990	3,368,403	25.90
	2000	3,442,078	26.47
Irrigated	1980	209,657	1.61
	1990	244,196	1.88
	2000	275,090	2.12
Total land area		13,004,202	

Arable land world wide, increased by only 4.8% between 1970 and 1990, and of this only 0.3% was in developed countries, with a 9% increase in developing countries (FAO, 2007a). *Per capita*, it fell from 0.38 ha to 0.28 (in developing countries this was a fall from 0.28 to 0.20 ha per person). At the current level of use, without new land being cultivated and with none being lost, *per capita* land availability will fall to 0.14 ha in a few years.

## 2.2 Water resources

Freshwater is a very scarce resource in the World – it accounts for around 2.5% of the total water supply, and of it only around 30% (or less than 1% of the total water in the world) is readily available for agriculture (Table 3). Average annual precipitation is about 800 mm, but this is not distributed evenly and many areas receive considerably less than that.

**Table 3** World water resources (Derived from Table 2 in Ghassemi et al, 1995)

	Vol (km <sup>3</sup> x 10 <sup>3</sup> )	% world reserves	
		Of total	Of FW
Oceans	1,338,000	96.5	
Fresh GW	10,530	0.76	30.1
Soil water	17	0.001	0.05
Glaciers & snow	24,064	1.74	68.7
Permafrost	300	0.022	0.86
FW lakes	91	0.007	0.26
SW lakes	85	0.006	
Marsh & rivers	14	0.001	0.04
Biological water	1	0.0001	0.003
Atmosphere	13	0.001	0.04
Total water	1,385,985	100	
Freshwater	35,029	2.53	100

### 2.3 Global water use

By 2000, we were using more than half our available water each year, and over the course of the 20th century, global water use increased tenfold. In 1900, agriculture used about 90.5% of the available global water, and by 2000 this was down to 70%, mainly as a result of increasing competition from industry and domestic consumers (WRI, 2008).

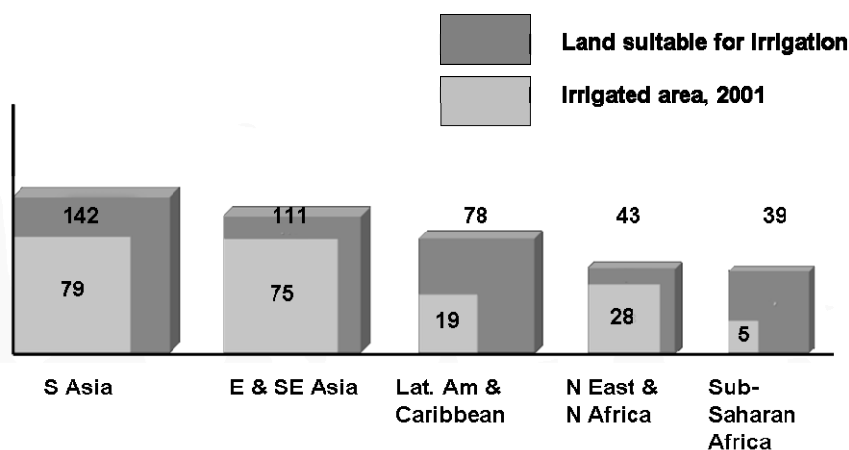
**Figure 1.** Irrigated area and land suitable for irrigation, 2001 (M ha) (FAO, 2007b)

Figure 1 clearly shows that there is little potential for further irrigation in Asia, the Near East or North Africa, so future increases in food production will need to come from already-irrigated land. In Latin America and, particularly, Sub-Saharan Africa, there is still great potential to expand irrigation, and it is likely that this will lead to increasing problems due to salinity in these regions.

## 2.4 Population

The world's population doubled between 1950 and 1990, and is expected to almost double again by 2030 (WRI, 2008). In 1990, 77% of people lived in the developing world (WRI, 2008), and this is expected to be over 80% by 2030. Population growth increases demand for food, goods and services, and increases competition for water. Population growth peaked at 2.0% between 1965 – 1970 and is currently at about 1.2%, although expected to fall to about 0.75% by 2030 (WRI, 2008).

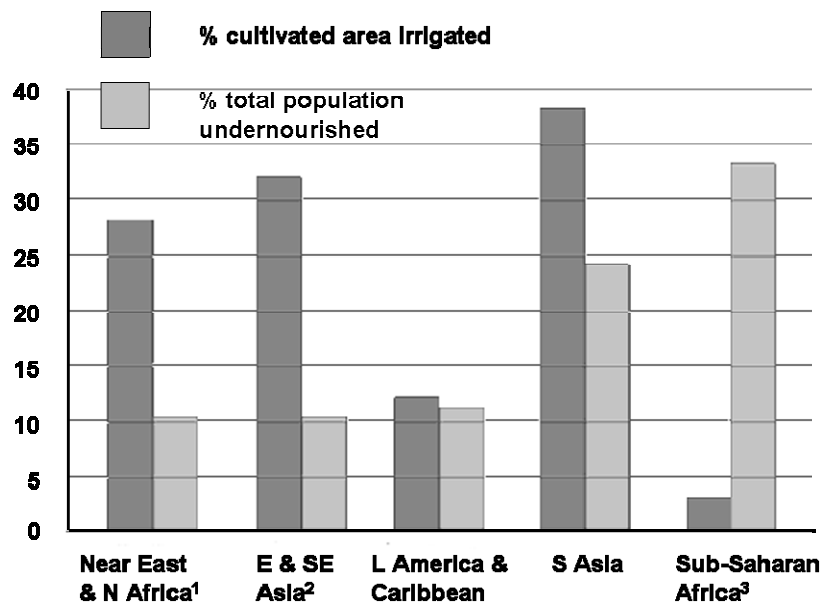
A comparison of growth in irrigated area with population, 1980 – 2003 (Table 4) shows that most of the production to feed the increasing populations of the developing world has come from irrigated lands (FAO, 2007a). It is highly likely that this will become even more the case in the future. This has two major implications. The first is that, with the increasing competition for water, will there be enough water available for irrigation to feed future populations? The second is that, as salinity and irrigation are inextricably linked, will irrigated lands be able to continue feeding the world in the future?

**Table 4** Comparison of growth in world population with irrigated areas (FAO, 2007a)

Country	Irrigated area (M ha)			Population (M)		
	1980	2003	% incr.	1980	2003	% inc r.
Bangladesh	1.5	4.7	212.5	85	147	72.6
China	45.3	54.6	20.5	1,004	1,312	30.6
India	38.4	55.8	45.2	689	1,065	54.6
Iran	5.2	7.7	47.7	39	69	74.9
Mexico	5.0	6.3	26.9	68	103	53.1
Pakistan	14.8	18.2	23.6	81	154	90.0
Russian Fed	*	4.6	*	*	143	*
Thailand	3.0	4.9	65.8	46	63	35.6
Turkey	2.7	5.2	92.3	46	71	54.6
USA	20.6	22.4	8.8	231	294	27.0

The importance of irrigated agriculture is shown in Figure 2, which shows the relationship between the area irrigated and the proportion of the population undernourished. The large-scale adoption of irrigated agriculture in South Asia has played a major role in reducing malnutrition, particularly when compared with Sub-Saharan Africa.

Overall the prognosis is not good – increasing world populations will require feeding from a diminishing pool of land suitable for irrigation and with increasing competition for water from domestic and industrial users. Add in to this the possible effects of climate change, and it is likely that salinity, already a major constraint to production, will become even more of a problem in the future.



<sup>1</sup>excluding Israel, <sup>2</sup>excluding Japan, <sup>3</sup>excluding South Africa

**Figure 2.** The relationship between irrigation and malnutrition, 1998-2000 (FAO, 2007b)

### 3. HISTORICAL ASPECTS

#### 3.1 Mesopotamia

Soil salinity has been known for thousands of years – the first record of what were noticeably salinity effects was in Mesopotamia (now Iraq) 6000 years ago (Tanji, 1990). This civilisation was built upon a highly productive wheat-based irrigated agriculture in the valleys of the Tigris and Euphrates rivers, but in common with many since, no provision was made by the engineers for any form of drainage. This led to raised watertables, and as sodium chloride built up in the soil the wheat had to be replaced by barley, more tolerant but less nutritious. After about 1000 years, similar amounts of barley and wheat were being grown, and by 2100 BC wheat made up less than 2% of the grain production. The result of this was that the civilisation declined and eventually disappeared. Similar problems have been recorded from the early civilisations in the Americas (Tanji, 1990).

#### 3.2 India

In India, salinity was first noted during the Vedic era around 1400 BC (Singh, 1998). At this time, lands began to be distinguished as salt-affected (*usara*). The term *kalar* also came into use about then for salt-affected land, and the terms have been in use for salt-affected lands throughout India and Pakistan since then. Salt was clearly a problem in Punjab and Sindh in particular well before the large-scale irrigation that began in British India in the 19<sup>th</sup> century. However, it was only with the development of these very large irrigation schemes, without attention to drainage, that salinity became of concern to the government. In 1855 a grower near what is now the Central Soil Salinity Research Institute (CSSRI) at Karnal, India, complained about secondary salinisation following the remodelling of the Western Yamuna Canal, and the fear of the government that salinity would lead to a rejection of large-scale irrigation led them to commission a number of

reports and analyses. A Committee of 1879, the *Reh* (another term for salt-affected land) Committee made a number of recommendations towards solving the problem, including mapping, and experiments to test the effects of drainage and different cultivation techniques. Work on the problem has since continued in India and, since partition in 1948, in Pakistan and later in Bangladesh.

#### 4. CAUSES OF SOIL SALINITY

Salinity and sodicity affect a large proportion of irrigated lands throughout the world, and salt-affected soils are natural parts of arid landscapes in which evapotranspiration exceeds rainfall for a large part of the year. They are a major cause of desertification, reduced yields and increased poverty. Salinity results from the presence of excess ions in the soil solution. The most important anions (ions with a positive charge) are  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$ , while major cations (ions with a negative charge) are  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{NO}_3^-$  and, at high pH,  $\text{CO}_3^{2-}$ . Salinity results from excess  $\text{Na}^+$  and, to some extent, other anions together with associated cations, in particular  $\text{Cl}^-$  and, to a lesser extent,  $\text{SO}_4^{2-}$ . Sodicity results from excess  $\text{Na}^+$  and an excess of  $\text{HCO}_3^-$  or  $\text{CO}_3^{2-}$  over  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ .

Salinity can be defined as either **Primary salinity**, which is mainly due to natural causes, or as **Secondary salinity**, which is largely a result of poorly-managed irrigation or other human activity. Primary salinity is the result over the long term of natural processes leading to a salt accumulation in a particular area. This could include the long-term weathering of rocks, or be the result of previous submergence in sea water. Examples are:

- High saline groundwater tables, due to weathering of rocks, topography etc
- Saline seeps
- Pollution
- Seawater intrusion
- Blowing of salt-laden sand from coastal areas

Secondary salinity principally results from irrigation. It is the result of the salt in the soil profile being mobilised by extra water, which either raises watertables or increases the pressure of confined aquifers, forcing saline water upwards. Once close to the soil surface, the water evaporates and leaves the salts behind. Typical causes are:

- Seepage from irrigation canals
- High salinity in irrigation water
- Insufficient leaching of salts
- Types of salts in the irrigation water

In both cases, the salt exists as dissolved solids – in rainwater, within the soil profile or in the irrigation water. Rainwater can contain up to  $50 \text{ mg l}^{-1}$  of salt, and even a salt content as low as  $10 \text{ mg l}^{-1}$  can deposit  $10 \text{ kg ha}^{-1}$  of salt to soil over a year for each 100 mm of rainfall (Ghassemi et al., 1995). Over geological time, this is a huge amount. The amount of salt in the soil varies with soil type – it is generally less in sandy soils, and is less in areas of high rainfall. The salt stored in aquifers can be enormous. An aquifer 40 m thick can, for example, store from up to 400 t of salt per hectare. Finally, irrigation water can also add substantial quantities of salt to the soil. Even reasonable quality water, containing 500 mg salt per litre, will add 0.5 t of salt per  $1000 \text{ m}^3$  of

water, and irrigating with 10,000 m<sup>3</sup> per year will add 5 t salt to every hectare (Ghassemi et al., 1995).

Waterlogging is often but not always associated with salinity. Where it occurs it tends to be highly seasonal, particularly in areas with monsoonal climate. Waterlogging itself is detrimental to plant growth, but when combined with salinity the effect is greatly multiplied (Barrett-Lennard, 2003). For this reason, drainage should be recorded when sites assessed for salinity. The effects of salinity and waterlogging together on plant growth will be covered in detail in the next chapter.

#### **4.1 Effect of land clearing**

Land clearing for agriculture can alter the hydrological balance and is a major cause of rising water tables and salinity problems. What tends to happen is that there is less evapotranspiration from crops and pastures than from deep-rooted, native vegetation, so more water percolates through the soil profile and recharges the aquifers. If the aquifers are confined or semi-confined then their pressure increases, forcing water upwards towards the soil surface and leading to the salinisation not just of the soil but also of watercourses. This has been a particular problem in Australia, where large scale clearance of native vegetation in the 19<sup>th</sup> and 20<sup>th</sup> centuries has led to severe salinity problems, including the pollution of domestic, industrial and irrigation water supplies (Ghassemi et al., 1995).

#### **4.2 Dryland salinity**

Salinity is also a major problem in many rainfed areas, for example in the southern half of Australia, and in the Great Plains in Canada and the USA. In Australia, such areas are known as seepage salinity or scalds – the latter resulting from wind or water erosion removing topsoil and exposing a saline or sodic subsoil. Saline seeps result from discharge from saline groundwater, often accelerated by farming which allows water to move through saline strata below the rootzone. Saline seeps typically occur where native vegetation has been replaced by agriculture with a lower potential evapotranspiration.

### **5. SOIL AND WATER SALINITY AND SODICITY**

Salt-affected soils can be broadly categorised into 3 types, based on their salinity and sodicity, and a similar classification is also broadly followed for irrigation waters. These are

- Saline soils
- Sodic soils
- Saline-sodic soils

#### **5.1 Saline soils**

In saline soils, the salt concentration is sufficient to adversely affect crop growth, i.e. there is an excess of soluble salts, usually chlorides, sulphates and bicarbonates of sodium, calcium and magnesium. In general, the electrical conductivity of the saturation extract of a saline soil (EC<sub>e</sub>) is above 4 dS m<sup>-1</sup>, with pH between 7.5 and 8.5 and a sodium adsorption ratio (SAR) greater than 13. Very often, the soil surface has a white crust, but there may be little effect on the soil structure –

the soil remains permeable, and is well drained, and adverse effects on plant growth are generally due to the osmotic effects of the excess salts.

## 5.2 Sodic soils

Sodic, or black alkali soils, have high concentrations of exchangeable Na. The term sodic is generally preferred to alkali these days, as not all have a high pH. The high Na concentration causes soil organic matter to dissolve – this gives the soil a dark brown or black colour.

In these soils, the structure is deteriorated, with permeability decreased, and root growth restricted. Typically, they have an  $EC_e$  less than  $4 \text{ dS m}^{-1}$ , pH above 8.5, and SAR above 13. Such soils affect plant growth due to the excessive sodium on the exchange complex of the soil, which is associated with the altered physical properties. The high ESP causes soil colloids to disperse, which leads to the blocking of the soil pores with consequent impeding of soil and water movement. Rainfall or irrigation water will stagnate on or near the soil surface, creating unfavourable conditions for plant growth. Such soils are very difficult to reclaim.

## 5.3 Saline-sodic soils

Saline sodic soils have the characteristics of both saline and sodic soils, and are particularly common in Pakistan. In general, they have an  $EC_e$  above  $4 \text{ dS m}^{-1}$ , the pH is usually less than 8.5, and the SAR is usually above 13. Such soils initially have good permeability, but if attempts are made at reclamation by leaching without the use of gypsum, then the structure deteriorates rapidly, and hydraulic conductivity is reduced. The properties of the three soil types are summarised in Table 5 below.

**Table 5** USDA classification of soil salinity (based upon Richards, 1954)

Soil	$EC_e$ ( $\text{dS m}^{-1}$ )	ESP	pH	Description
Saline	> 4	< 15	Usually < 8.5	Non-sodic soil with sufficient soluble salts to interfere with the growth of most crops
Saline-sodic	> 4	> 15	Usually < 8.5	Soils with sufficient exchangeable sodium to interfere with the growth of most plants, and containing appreciable quantities of soluble salts
Sodic	< 4	> 15	Usually > 8.5	Soils with sufficient exchangeable sodium to interfere with the growth of most plants, but without appreciable quantities of soluble salts

### 5.3.1 Classification of saline and sodic soils

This is based mainly upon pH, EC and ESP/SAR, as noted above. In addition, the topography and drainage status of the soils affect both the degree of salinisation, and the ease of reclamation, while the clay mineralogy of the soil controls the effect of Na on soil structure and permeability. Under the FAO system, saline soils are classified as *solonchaks*, while sodic soils are *solonetz* (Dudal and Purnell, 1986; Table 6), and other soil groups can have saline or sodic phases. In the USDA system, salt-affected soils are placed in great groups such as the salorthids. For further detailed classification of salt-affected soils using different systems see Szabolcs (1989).

**Table 6** FAO classification of salt-affected soils (Dudal and Purnell, 1986)

Grouping	Characterisation
Solonchak	$EC_e > 15 \text{ dS m}^{-1}$ in the upper 0.75 – 1.25 m
Saline phase	$EC_e 4 - 15 \text{ dS m}^{-1}$ in the upper 1.0 m
Solonetz	$ESP > 15$ in the upper 0.4 m
Sodic phase	$ESP 6 - 15$ in the upper 1.0 m

Heavy soils such as vertisols and those with marked textural changes such as planosols are more susceptible to salinisation, and very often these have greater problems of reclamation and drainage. Table 7 shows the USDA classification of saline soils, and the effect of different levels of salinity on crop growth. It is important to bear in mind that these are generalised classifications, and local conditions may be different in some respects. For Pakistan, Qureshi (1993), and Rafiq (1975) have developed similar systems.

**Table 7** Explanation of  $EC_e$  values (Adapted from Table 8.1 in Landon (1991))

USDA soil class	Designation	$EC_e$	Total salt content (%)	Crop reaction
0	Salt free	0 – 2	< 0.15	Salinity effects are negligible except in very sensitive plants
1	Slightly saline	4 – 8	0.15 – 0.35	Yields in many crops are restricted
2	Moderately saline	8 – 15	0.35 – 0.65	Only tolerant crops yield well
3	Strongly saline	> 15	> 0.65	Only very tolerant crops yield well

**Table 8** Farmers' classification of salt-affected soils in the Pakistan Punjab (Kielen, 1996)

Soil type	Local name
White surface, with either a good structure below the crust or hard underneath	<i>Chitta kalar</i> (white salinity)
Some patches of white crust, or the crust is very thin	Chitta kalar
Black appearance and with a hard upper layer	<i>Kala kalar</i> (black salinity)
Look good but have a hard layer deeper in the profile, sometimes with "stones" at about 30 cm	<i>Kalrathi</i> (hard layer) and <i>roor</i> (stones)
A lot of white salts on the surface, appear dry but are muddy underneath	Kalar shoor
Waterlogged	Sam

In addition to formal classification systems, indigenous knowledge and classification systems have developed in many of those communities affected by the problem. A typical one is that used by farmers in Pakistan's Punjab (Kielen, 1996), which includes indicators related to the appearance of the soil and those based upon crop performance. The farmers recognise six types of salt-affected soil (Table 8), based upon soil colour and texture.



**Table 9** Physical indicators of salinity and farmers' explanations for them in the Pakistan Punjab (Kielen, 1996)

Indicator	Explanation
Standing water visible 3 – 4 days post-irrigation	If good quality water has been used the soil has a salinity problem
Soil cracks after irrigation	If the soil has a good structure, then poor quality water has been used and the soil will eventually harden
Walking through the field sounds different before and after irrigation	Poor quality water has been used, and a flour-like layer developed on the soil surface with a hard layer below it
Walking leaves oily footprints	The soil has a salinity problem
White soil	The first sign of "white salinity." May occur after irrigation with poor quality water, or during an extended period without irrigation
White patches	"White salinity" in some areas, either due to poor quality water or to salts coming from the soil
White soil surface	"White salinity" as above
Black soil	Severe salinity problems with major consequences for crop production
Muddy soils but with a dry-looking surface due to a white, flour-like layer	Waterlogged and very saline. Often also have "black salinity" and are very difficult for growing crops

**Table 10** Crop indicators of salinity and farmers' explanations for them in the Pakistan Punjab (Kielen, 1996)

Indicator	Explanation
Poor germination	Salinity. Used for many different levels of salinity by all farmers, includes both "white" and "black" salinity
Irregular growth	Salinity
Stunted growth	Salts deeper in the soil profile. The crop grows post-germination but the roots eventually meet the salts and in the worst cases the crop dies off
Yellow leaf burn	Too many salts in the soil burn the crop

Tables 9 and 10 show how farmers assess the physical effects of salinity upon the soil, and present a number of crop indicators. Farmers also have similar classifications for water quality. These indicators correlate well with existing schemes (Kielen, 1996).

## 6. EFFECTS OF SALINITY

Salinity has two main effects on plants – the decreased water availability in the soil which leads to reduced germination, growth and yields, and the toxicity effect of specific ions on particular plant processes. These are discussed in detail in Chapter 4. It is important to remember that the typical effect of salinity is very patchy, and varies over the season, particularly after irrigation and rainfall.

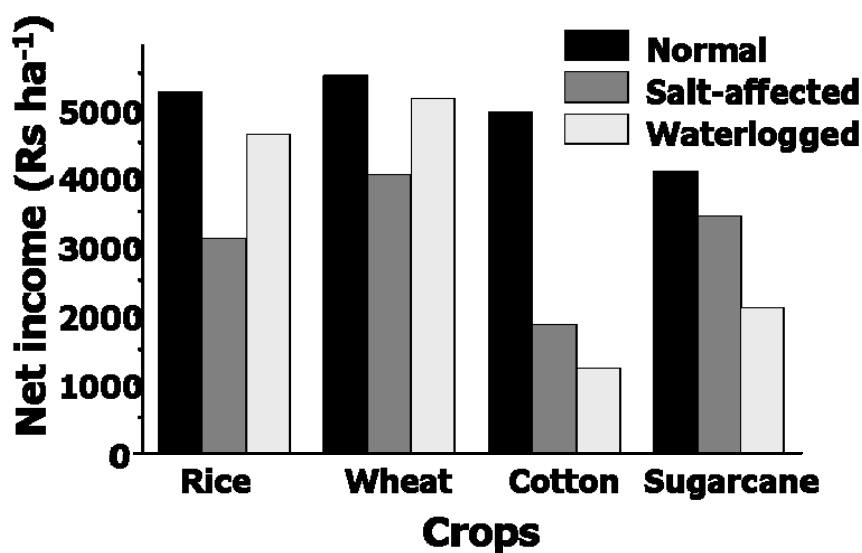
Table 11 shows the economic damage due to secondary salinity in a number of countries, estimated at 1995 currency levels. It can be inferred that, globally, the income loss due to salinity in irrigated areas was about 11.4 billion US dollars in 1995, and about 1.2 billion in non-irrigated lands. When the costs to industry of damage from using salty water are added, the total is likely to be around USD 15 billion.

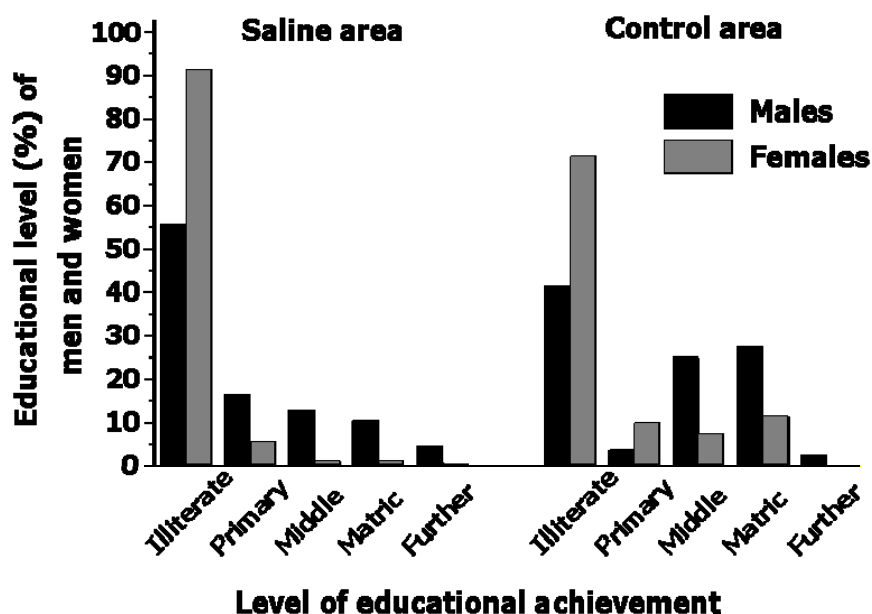
**Table 11** Estimates of economic damage due to secondary salinity (Ghassemi et al., 1995)

Country	Region	Estimated damage (M \$ y <sup>-1</sup> , 1995 equivalent)	Reference
Pakistan	Punjab and NWFP	300 <sup>a</sup>	WAPDA (1988)
Australia	Murray-Darling Basin	208 <sup>a</sup>	Murray-Darling Basin Ministerial Council (1989)
	Murray-Darling Basin	52 <sup>b</sup>	Simmons et al. (1991)
	SW Western Australia	50 <sup>a</sup>	WA Legislative Assembly (1991)
	SW Western Australia	72 <sup>c</sup>	WA Legislative Assembly (1991)
	SW Western Australia	32 <sup>d</sup>	Water Authority of WA (Personal Communication)
USA	Colorado River Basin	750 <sup>e</sup>	Colorado River Basin Salinity Control Forum (1993)
	San Joaquin Valley, California	31 <sup>a</sup>	El-Ashry et al. (1985)
S Africa	Pretoria, Witwatersrand, Vereeniging and Sasolburg complex	29 <sup>e</sup>	Heynike (1981)

<sup>a</sup>Agricultural loss; <sup>b</sup>Water supplies; <sup>c</sup>Waterlogging; <sup>d</sup>Stream salinity; <sup>e</sup>Total damage

Social costs are not easy to quantify, but some work has been done in India and in Pakistan. In NW India, Joshi et al., (1996) calculated the income loss from different crops as a result of salinity (Figure 3). These showed that farm incomes were affected by both salinity and waterlogging, and that losses were particularly severe in cotton, and due to salinity in rice.

**Figure 3.** Effect of soil type on net income from various crops (Joshi et al., 1996)

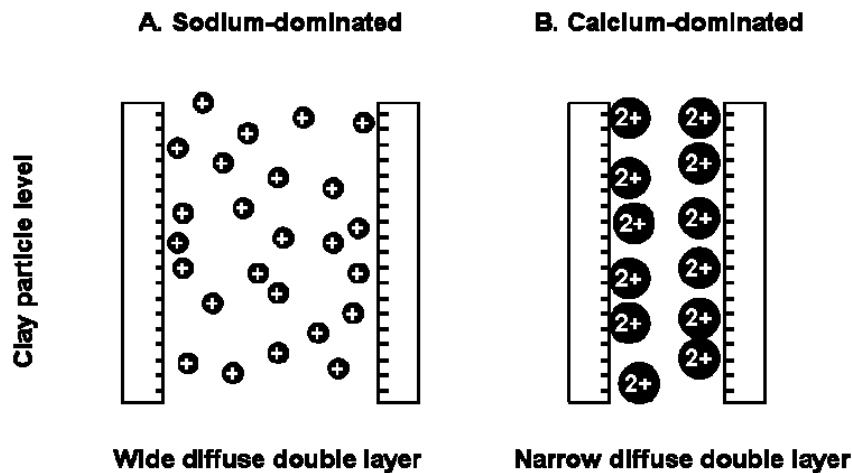


**Figure 4.** Educational achievement by gender, saline and non-saline areas, Pakistan Punjab. (Ijaz and Davidson, 1997)

These losses have effects on the entire development of communities, as is shown by Figure 4 from work in Pakistan (Ijaz and Davidson, 1997). Education levels in villages in salt-affected areas were much lower, particularly for women, and the pattern was repeated in a wide range of socio-economic indicators. Other effects may result in population movements and the abandonment of land, and may be felt on a regional level.

## 7. SOIL SODICITY

Soils become sodic when the exchange surfaces of the minute clay particles become dominated by  $\text{Na}^+$  rather than by  $\text{Ca}^{2+}$  ions. The colloidal clay particles are negatively charged, and are associated with positively-charged exchangeable cation “envelopes”. As a result, interactions occur, as they do between the same ions and their counterparts in the soil solution. Two antagonistic processes occur. First, the envelope of exchangeable cations is attracted to the negatively-charged clay surface, in proportion to the size of the charge. Secondly, they tend to diffuse from the surface of the clay, where their concentration is higher, and into the soil solution where the concentration is lower. The attractive force decreases exponentially with distance from the clay particle, and is twice as strong in divalent cations like  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$ , compared to monovalent  $\text{Na}^+$ . As a result, in soils with high proportions of  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  relative to  $\text{Na}^+$ , or with high concentrations of total dissolved ions (for example saline soils), then the exchangeable cation envelope is compressed towards the soil particle surface (Figure5). This leads a decrease in the repulsion between like-charged envelopes, and so to an aggregation of the particles. As a result, pores in the soil are larger, and permeability and tilth improved compared to non-aggregated soils.



**Figure 5.** Effects of sodicity and Ca on clay particles and aggregates (Redrawn from Qureshi and Barrett-Lennard, 1998)

Clay particles are subject to electrostatic forces. These cause them to move apart (disperse) or together (aggregate). In non-sodic soils, aggregative forces operate, so the clay remains stable when wet. The soil pores remain open, and water can infiltrate into the plant. In sodic soils, dispersive forces arise due to excess  $\text{Na}^+$ . As a result, the soil structure collapses, there is reduced pore size and deflocculation leading to impermeability.

The dispersive forces allow more soil solution to be imbibed between the clay particles, a phenomenon known as swelling. This reduces the size of the pores and reduces permeability, and is particularly important in soils with expandable phyllosilicate minerals such as smectites, and when the ESP > 15. Dispersion, the release of individual clay particles from the aggregates, and slaking, the breakdown of the aggregates, can both occur below an ESP of 15 if electrolyte concentrations are low. The result can be blocked soil pores, leading to impermeable conditions. In addition, porosity decreases, leading to crusting, reduced permeability and a poor tilth for sowing.

If water is to enter the soil it must infiltrate through the soil surface, so the rate of entry is affected by the properties of both the water and the soil. It is therefore important to assess not just the salinity of the water and of the soil, but also the exchangeable sodium percentage (ESP) or equivalent sodium adsorption ratio (SAR) in the surface soil.

### 7.1 Use of gypsum

Gypsum is widely used to improve sodic soils. As we have seen, when the clay particle surfaces become dominated by  $\text{Na}^+$ , they have relatively thick diffuse double layers. The electrostatic forces cause the particles to swell and become suspended in the water, blocking the soil pores and forming surface crusts. However, if calcium in the form of gypsum is added, the particles become surrounded by  $\text{Ca}^{2+}$  ions, so the diffuse double layers narrow and the soil re-aggregates. Gypsum can either be applied to the soil, or added to the irrigation water.

## 7.2 Effects of soil sodicity

The deterioration of soil physical conditions that results from sodicity leads to a number of problems. Less air and water enters the soil, and in particular the rootzone, so creating a poor environment for crop growth. Waterlogging develops due to poor drainage, causing seeds to rot and roots to die. Surface crusting as a result of the slaking of the aggregates leads to reduced seedling emergence, and the compaction that results from the breakdown in soil structure creates problems for root penetration and for cultivation.

## 8. EXTENT OF SALT AFFECTED SOILS

Estimates of the extent of soil salinisation vary widely due to lack of consistency in the methodologies and criteria used. However, they are distributed all over the world, and are a particular problem in Asia and Australia. There are also severe problems in Africa, some parts of the USA and Canada, and some European countries, for example Spain and Hungary. Globally, over around 955 M ha are affected by primary salinity (Szabolcs, 1989) and about 76 M ha by secondary salinity (Oldeman et al., 1991). Oldeman et al., (1991) catalogued the land degraded by different causes. Light degradation was slightly reduced productivity, manageable by local farming systems. Medium has greatly reduced productivity, with major improvements needed, generally which have to be provided from outside. Strong degradation implies soils that are no longer workable, and need major engineering work or international assistance for restoration, while extremely degraded soils are beyond reclamation.

Table 12 shows these categories as they apply to salinity, while Table 13 estimates the amount of irrigated land that is affected by secondary salinisation. In another classification, Szabolcs (1989) classified the total land affected by salinity or sodicity (Table 14).

**Table 12** Global extent of human-induced salinisation (Mha). (Oldeman et al., 1991 cited in Ghassemi et al., 1995)

Continent	Light	Moderate	Strong	Extreme	Total
Africa	4.7	7.7	2.4	-	14.8
Asia	26.8	8.5	17.0	0.4	52.7
S America	1.8	0.3	-	-	2.1
N and Central America	0.3	1.5	0.5	-	2.3
Europe	1.0	2.3	0.5	-	3.8
Australasia	-	0.5	-	0.4	0.9
World total	34.6	20.8	20.4	0.8	76.6

**Table 13** Irrigated land in selected countries affected by secondary salinity (<sup>1</sup>FAO, 2007c; <sup>2</sup>Ghassemi et al., 1995)

Country	Cultivated area (Mha) <sup>1</sup>	Irrigated area (Mha) <sup>1</sup>	Percentage of irrigated to cropped land <sup>1</sup>	Salt-affected irrigated land (Mha) <sup>2</sup>	Percentage of irrigated land salt-affected <sup>2</sup>
China	156.3	53.82	34.8	6.70	15.0
India	169.6	57.28	33.8	7.00	16.6
Kazakhstan	22.5	3.56	13.3	3.70	18.1
USA	177.2	21.40	12.0	4.16	23.0
Pakistan	22.1	17.82	80.0	4.22	26.2
Iran	18.1	6.91	38.8	1.72	30.0
Thailand	17.8	5.00	25.5	0.40	10.0
Egypt	3.5	3.42	99.9	0.88	33.0
Australia	49.7	2.54	5.1	0.16	8.7
Argentina	29.5	1.55	5.4	0.58	33.7
S Africa	15.7	1.49	9.5	0.10	8.9
Subtotal	842.8	158.70	18.8	29.62	20.0
World	1473.7	227.11	15.4	45.40	20.0

**Table 14** Global distribution of salt-affected soils (Szabolcs, 1989)

Continent	Area affected (M ha)		
	Saline	Sodic	Total
N America	6.2	9.6	15.8
Central America	2.0	-	2.0
S America	69.4	59.6	129.0
Africa	53.5	27.0	80.5
S Asia	83.3	1.8	85.1
N and Central Asia	91.6	120.1	211.7
SE Asia	20.0	-	20.0
Europe	7.8	22.9	30.7
Australasia	7.4	340.0	357.4
Total	351.4	581.0	932.2

### 8.1 Extent of salt-affected soils in China

China is suffering from increasing salinity problems as new irrigation schemes are established and older ones are rehabilitated.

It has about 20 M ha of salt-affected soils, widely distributed over the country, and with around 20% of them being alkaline. This accounts for about 21% of the arid area of China (Dregne and Chou, 1992). Saline soils are widespread in coastal areas. The country has been divided into eight salinisation regions, and 27 subregions, according to the natural characteristics of salt formation, migration and accumulation in the soils, and the soil geochemistry.

The eight regions are:

- Coastal humid and semi-humid regions with seawater submergence
- Northeastern semi-humid and semi-arid region
- Huang-Huai-Hai Plain
- Mongolian Plateau, arid and semi-desert
- Middle and upper Huanghe River, semi-arid and semi-desert
- Gansu-Xinjiang desert
- Qinghai-Xinjiang extremely arid desert
- Xizang (Tibet) Plateau alpine frozen desert

Sodic soils are widely distributed from Inner Mongolia in the North to the Huang-Huai-Hai Plain, and from the East Coast to Xinjiang in the West, including the Tibet Plateau. Sodic and saline soils often coexist. Naturally problem areas for salinity are the gravel soils in Xinjiang, and the Tibetan Plateau (Dregne et al., 1996).

There is no whole-China data for secondary salinisation, although in 1965 about 6.7 M ha were reported to be affected (Vermeer, 1977). More recently, it is reported that just under 5 M ha have been reclaimed (James, 1989). Major problem areas in irrigated lands are the Ningxia and Hetao irrigated plains along the Yellow River, and there are many areas subject to secondary salinisation in the Northeast, the North China Plain, the Hexi Corridor in Gansu Province, and the oases in the Xinjiang Desert.

## **9. MEASUREMENT AND ASSESSMENT OF SALINITY**

The problem with measuring salinity in the field is that it changes, both with space and with time. This is a result of changes and interactions between a large number of edaphic (soil), climate and management factors, all of which are constantly changing. The edaphic factors include the permeability, the water table depth, the salinity of the perched groundwater, the topography, the soil parent material, and the geohydrology. Management factors include such things as irrigation, drainage, tillage, and cropping, while climatic factors include the rainfall amount and distribution, temperature, relative humidity, and windspeed and direction. As a result, a large number of samples are needed to characterise a particular field, and the measurements must be constantly updated as conditions change, or to see if they are changing! Salinity assessment is therefore extremely slow and expensive using traditional methods such as the laboratory analysis of soil samples, as well as being very labour intensive.

Due to the variability, soils must be sampled when analysis allows useful predictions to be made about likely effects of salinity on growth. If we are conducting an experiment, for example a screening trial for salt tolerance, then intensive sampling is needed over the whole season, particularly after any irrigation or rainfall, and in a large number of the experimental plots, in order to identify the environmental (E) component of the G x E interactions. However, in an agricultural situation, then sampling mid-way through the growing season, when the soil is moist but not

freshly leached, is a good compromise. In very variable fields, samples should be taken from strongly, moderately, and slightly affected areas. Depending upon the crop, sampling depth increments should be chosen as appropriate to ensure that the whole of the rootzone is sampled.

In many cases, the sampling will require considerable time and effort in the field, although very often a number of samples will be composited together for analysis. In addition, the chemical analysis of soil samples for  $EC_e$ , pH and so on is laborious, time consuming and expensive.

However, other methods using electromagnetic induction and advanced software are now available, including various electromagnetic (EM) techniques, and time-domain reflectometry (TDR). Although these are far more efficient, and cheaper once the initial outlay is overcome, they still require that the instruments are calibrated with actual samples from the field, and as well the analysis of the physical characteristics of the soil is still important, for example texture, structure, mottling and gleying, root distribution etc.

The new methods give a practical, cost-effective, faster, and more informative outcome. For example, they have the potential to identify the causes of salinisation, they allow the salinity survey to be integrated automatically with GIS, and they can also identify any needs for mitigation.

### **9.1 Determination of salinity from conductivity**

Salinity refers to presence of one or more of a number of major dissolved inorganic ions ( $Na^+$ ,  $Mg^{2+}$ ,  $Ca^{2+}$ ,  $K^+$ ,  $SO_4^{2-}$ ,  $HCO_3^-$ ,  $NO_3^-$  and  $CO_3^{2-}$ ) in the soil solution. In soils, it refers to both the soluble and the readily dissolvable salts either in the soil or in an aqueous extract of a soil sample. We can quantify salinity either in terms of the total concentration of these salts, or in terms of electrical conductivity, which is generally an easier option. These two are very closely related to each other, even though the conductivity is also affected by the temperature, and by the particular ions involved.

The best measure of soil salinity is the conductivity of the soil water. However, it is not yet practical to measure this, so we use an aqueous extract of a soil sample, typically a saturated soil paste. Both the water content (saturation percentage, or SP) and the water:soil ratio vary with soil texture and are related to the soil water content in field. As a result of this, and to obtain accurate and repeatable results that stand comparison with others, we use the saturated soil paste extract, or saturation extract ( $EC_e$ ). Conductivity can also be measured as the conductivity of the bulk soil ( $EC_a$ ). Along the same lines, the salinity of irrigation water is measured as its conductivity  $EC_w$ .

Conductivity is easy to measure, and provides a good index of the total concentration of ionised solutes in the soil solution. Using the SI system, electrical conductivity is reported in siemens per metre ( $S\ m^{-1}$  or  $S/m$ ), or for smaller amounts as decisiemens per metre ( $dS/m$  or  $dS\ m^{-1}$ ). Decisiemens per metre are the same as the older units of millimhos per centimetre ( $mmho\ cm^{-1}$ ). It is important to be aware that salinity work is bedevilled by a plethora of units, some related and some not. We recommend that you stick to using  $dS\ m^{-1}$  for any field-based work, although in the laboratory and for some plant physiology, it is permissible to use units of concentration.

Conductivity is the reciprocal of the resistance, so to measure it we need to measure the resistance of the soil or of the soil solution. Resistance is inversely proportional to the area, and directly



proportional to the length, so the resistance depends to a large extent upon the size of the sample we are measuring, and the distance between the electrodes we are using to do the measurement. The procedure to determine  $EC_e$  using the saturated paste extract is straightforward.

To prepare the saturated soil paste:

- Add distilled water slowly to 200 – 400 g air-dried soil, stirring all the time. The soil will reach a glistening, flowing texture that fills-in quickly after a spatula is passed through it – at this stage it has become saturated.
- Allow to stand for several hours, and overnight if possible. This allows the soil to fully imbibe water and the salts to dissolve.
- Use a funnel and filter-paper, or a vacuum extractor, to obtain the extract.
- Measure EC and temperature using standard equipment.
- Correct for temperature and convert to  $EC_e$

The adjustment for temperature is made by standardising the measurement to 25°C. This needs to be done as conductivity increases with temperature. For practical purposes, this is done by using a temperature coefficient.

$$f_t = 1 - 0.20346 (T) + 0.03822 (T^2) - 0.00555 (T^3)$$

where  $T = (\text{temperature in degrees Celsius} - 25) / 10$

From this, the conductivity at 25°C ( $EC_{25}$ ) is calculated as:

$$EC_{25} = f_t * EC_t$$

where  $EC_t$  = the EC at the measured temperature  $T$

The  $EC_{25}$  thus calculated is the  $EC_e$

As well as the  $EC_e$ , conductivity can be measured on a range of paste extracts such as a 1:5 soil – water ( $EC_{1:5}$ ) or 1:1. Values obtained using these ratios can be converted to  $EC_e$ , but they are less reliable and are subject to error. Example formulae which can be used for rough estimates are:

$$EC_e = 2.2 \times EC_{1:1} \text{ (a general value)}$$

$$EC_e = 6.4 \times EC_{1:5} \text{ (Loveday, 1972)}$$

They are also less well related to meaningful soil properties, and the formulae developed tend to be location-specific. If you do want a quicker method, then the EC of the saturated soil paste ( $EC_p$ ) described in Rhoades et al., (1999) is the best method.

Once the  $EC_e$  of the soil has been determined, then other analyses should be carried out, for example for the individual ions. From these, such parameters as ESP and SAR can be calculated.

Some other rule-of-thumb conversions, all at 25°C, are given below:

- Total ion (cation OR anion) concentration ( $\text{mmol l}^{-1}$ ) is approx.  $10 \times EC_{25}$  ( $\text{dS m}^{-1}$ )
- Total dissolved solids (TDS) ( $\text{mg l}^{-1}$ ) is approx  $640 \times EC_{25}$
- Osmotic potential (MPa) is approx  $0.04 \times EC_{25}$

## **9.2 Determination of field salinity from bulk soil conductivity**

As an alternative to the saturated extract, which is laborious and impractical on a large scale, salinity can be measured either through direct measurements of the conductivity of a saturated soil paste ( $EC_p$ ), without the need to collect extracts, or of the conductivity of the bulk soil ( $EC_a$ ). The first is a laboratory measurement, or obtained using simple field equipment, while  $EC_a$  is measured either by electrodes in contact with soil, or electromagnetically. From these measurements of  $EC_a$  and  $EC_p$ , then the  $EC_e$  can be obtained easily. The equations for this are given in Rhoades et al., (1999) and are not repeated here – corrections for soil physical conditions and organic matter content are needed.

The ideal situation is to know the “real time” concentrations of all the solutes in the soil water over range of field water content. This is not yet possible, but the relatively new electromagnetic techniques enable total solutes to be easily determined, and the determination of particular ions can be done in the laboratory if needed as an adjunct to the calibration process. The sampling strategies and sampling positions needed for these analyses can now be easily determined using computer software.

### **9.2.1 Measurements on the bulk soil**

The techniques involve mobile instruments that can rapidly measure bulk  $EC_a$  directly as a function of the landscape position, together with procedures and software to infer salinity ( $EC_e$ ) directly from the  $EC_a$  after the instruments have been calibrated. The software can also be used for computer-assisted mapping of the field salinity, and can use spatial statistics to infer rootzone salinity distribution and detect changes with space and time. It can link with GIS and remote sensing, and the information obtained can be used to help determine the underlying causes of salinity problems, to develop solute transport models, and to predict the consequences of alternative management options.

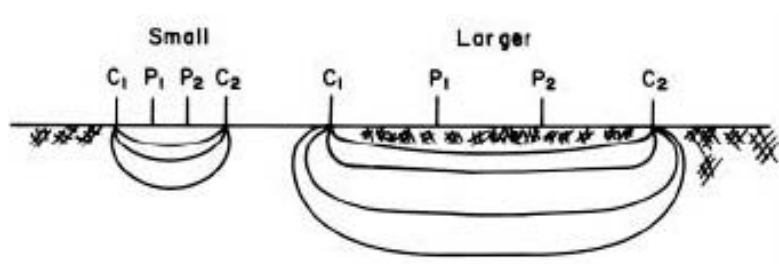
Three methods are available, all of which have been proved in the field, and are portable. These are the four-electrode probes, electromagnetic induction sensors, and time-domain reflectometry, or TDR. The latter however has not yet been shown to be sufficiently accurate, simple, robust or fast enough for everyday use, and is still very expensive.

#### **9.2.2 4-electrode sensors**

These are covered in detail in Rhoades et al., (1999) and, as the name would suggest, make use of four separate electrodes inserted in the soil. The instrument also involves a current-generator and resistance meter, and a connecting wire. The depth of sampling and the volume of measurement

can be altered by changing the spacing of the electrodes. Basically, when the distance between the outer pair of electrodes (the “current” electrodes) is small, then the flow of current is shallower than when they are further apart – the depth of measurement being about one third of the distance between them (Figure 6). To calculate the  $EC_a$ , we need to know the spacing between the “current” electrodes and the inner, or “potential” electrodes. A typical instrument is the one produced by Martek – this was specifically designed for salinity use and reads directly in conductivity, corrected to 25°C, rather than in resistance as some other models do.

Four-electrode probes can be tractor-mounted and connected to data-loggers, and can also be connected to a GPS to record positions accurately. They can also be mounted together on a board, so that all the electrodes can be inserted in the soil at once, to save time and make the instrument more portable.

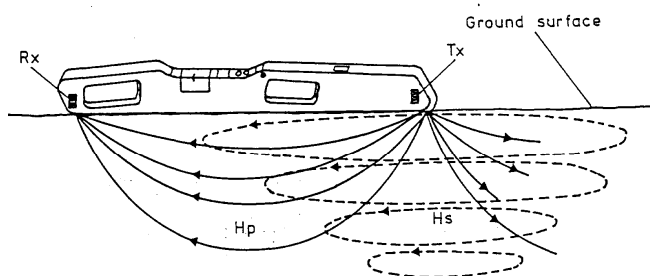


**Figure 6.** Effect of electrode spacing on depth and volume sampled (Fig 32 from Rhoades et al., 1999)

### 9.2.3 Electromagnetic induction sensors

Conductivity can be measured remotely by electromagnetic induction (EM). A transmission coil at one end of the instrument induces circular, eddy-current loops in the soil which are proportional to the conductivity. In turn, these loops generate secondary magnetic fields proportional to the loop current which are part intercepted by a receiver coil at the opposite end of the instrument. The quantity that the instrument records is known as the “depth-weighted” electrical conductivity, or  $EC_a^*$ .

The most common instrument in use is the Geonics EM38. It works through a transmitter coil at the rear, which generates a primary magnetic field (Figure 7). This induces small eddy currents in the ground, the secondary magnetic field. A receiving coil at the front of the instrument measures both the strong primary field and the much weaker secondary field arising from the eddy currents, which is proportional to the soil electrical conductivity.



where: Tx is the transmitter coil, Rx is the receiver coil,  $H_p$  is the primary magnetic field, and  $H_s$  is the secondary magnetic field

**Figure 7.** Simplified diagram of the nature of the electromagnetic fields created by the EM38 (Fig. 3 in Norman, 1990, © State of Victoria, Department of Primary Industries)

The EM38 can also be used to estimate the shape of the salinity profile within the rootzone, as it has different depth responses when placed upright on the soil surface (the vertical dipole) and when it is lying on its side (the horizontal dipole) (Figure 8). If the vertical reading is greater than the horizontal, then soil conductivity is greater at depth than in the shallow rootzone, and the profile is said to be leached. Conversely, if the horizontal reading is greater than the vertical, then the profile is said to be inverted. These profile descriptions may change shape over the year as a result of alternating leaching and capillarity cycles. The vertical configuration allows readings to 2 m depth, while the horizontal configuration allows readings to 1 m depth. Figure 9 shows the instrument in the field in Pakistan in its horizontal position.



**Figure 8.** EM38 in horizontal (A, EMh) and vertical (B, EMv) orientation (Rhoades et al., 1999)

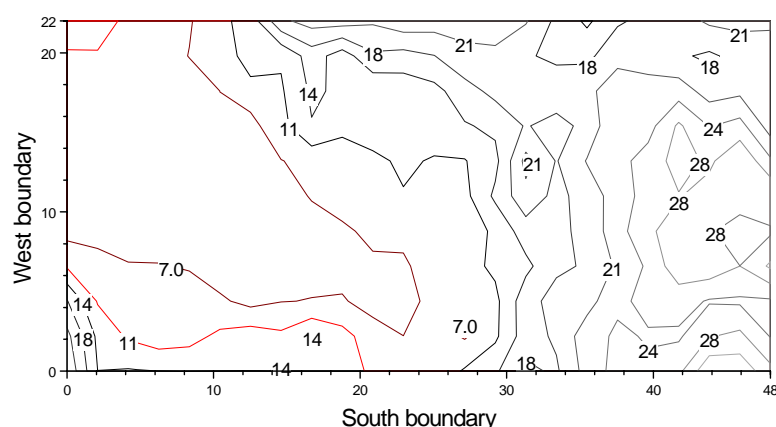
Other EM units are available for deeper readings, and as with the 4-electrode probe, the system can be mobilised, combined with GPS or other sensors, and automated. There has also been some work to assess the possibilities of using it from an aircraft or helicopter, and this shows promise for large-scale surveys (George and Green, 2000).



**Figure 9.** An EM 38 in the field in Pakistan. Note that the instrument was not actually in use at this moment, and so the demonstrator's (Dr Ramon Aragüés) watch had not been removed!

To carry out a survey using the EM38 requires calibration of the instrument to transform the depth-weighted  $EC_a$  readings to either  $EC_e$  or  $EC_{1.5}$ , using the vertical ( $EM_v$ ) and horizontal ( $EM_h$ ) readings of the EM38. It is vital to ensure that the calibrations remain accurate over time. The number of samples required for calibration depends upon the degree of variation within the field measurements, and ideally should result in a coefficient of variation for the regression of less than about 25%. The sampling pattern and number of samples required can easily be obtained using the ESAP software available from the US Salinity Laboratory (available at [www.ars.usda.gov/Services/docs.htm?docid=8918](http://www.ars.usda.gov/Services/docs.htm?docid=8918)). As noted earlier, depending upon the crop, sampling depth increments should be chosen as appropriate. When carrying out a survey using the instrument, it is also vital that soil temperature is recorded using an insertion probe, and the readings then converted to give the  $EC_a$  at 25° C using the relationship given above.

Once the readings have been obtained, they can be plotted by a range of methods – we recommend the use of ESAP to do this as it integrates everything together into one package.



**Figure 10.** Example of the type of plot produced of salinity levels in the field (Gundheri, NWFP Pakistan, 13 November 1997). These are  $EC_e$ , produced from the  $EC_a$  after calibration of the EM38.

## 9.2.4 Important considerations

In most field situations, the topsoil is too dry to use for sampling and assessment – electrical currents need moisture to flow in soil, and dry soil is an insulator – so no useful information about salinity or other soil properties can be inferred from  $EC_a$  measurements on the surface layers of the soil. This is a particular problem in dryland situations, but also possibly on irrigated land, where very often the surface soil is highly salinised due to evaporation.

The instrument is provided with a comprehensive set of instructions, and it is important that these are followed carefully, in particular to zeroing the instrument and calibration. It is also important to note that, if the reading for  $EM_h$  is less than half that for  $EM_v$ , then either there is an error with the instrument, or there is some form of electrical or magnetic interference occurring.

It may be useful to use a block of wood, grooved to hold the instrument, to support the EM38 10 cm above ground level. Some researchers have found that this gives more accurate readings.

Operators should develop a routine to carry out accurate sampling:

NEVER include the surface mulch in the soil sample for calibration!

ALWAYS scrape away dry surface soil before taking an EM38 reading

ALWAYS record the soil temperature at regular intervals to allow the readings to be standardised

ALWAYS check you have no metal objects on your person – any metal (watches, rings, some coins) will affect the working of the instrument and lead to incorrect readings.

ALWAYS sample away from metal poles, pylons and overhead lines, also buried electric cables and drains if possible. Water conducts electricity!

### 9.3 Measuring soil sodicity

It is important when assessing soil sodicity that it is the exchangeable Na rather than the soluble Na in the soil that is measured. To do this, the quantity of soluble Na, determined from the saturation extract, must be subtracted from the total Na in the leaching agent.

Exchangeable Na = Extracted Na – Soluble Na

The Exchangeable Sodium Percentage (ESP), which is the percentage of the total exchangeable charge neutralised by  $\text{Na}^+$ , is commonly used to assess the sodicity of the soil. It is calculated by dividing the soil exchangeable Na by the cation exchange capacity (CEC), taking care to calculate the CEC accurately.

$\text{ESP} = (\text{exchangeable Na} / \text{CEC}) \times 100$

An ESP of 15 has been proposed as the arbitrary boundary between sodic and non-sodic soils, although some workers have proposed that the level should be at an ESP of 13, 10 or even 6. In general, however, it is safe to regard any soil with an exchangeable Na content greater than 1 me / 1100 g as potentially sodic. The effect of exchangeable Na on crops is covered in Chapter 4.

Another important quantity is the Sodium Adsorption Ratio (SAR) of the soil solution or irrigation water, which reflects the balance between  $\text{Na}^+$  and the divalent cations ( $\text{Ca}^{2+}$  plus  $\text{Mg}^{2+}$ ). It is used to estimate water quality (see Section 10) and also to calculate the equilibrium values for ESP in irrigated soils. SAR is defined as

$$\text{SAR} = \frac{(\text{Na}^+)}{\sqrt{\frac{(\text{Ca}^{2+}) + (\text{Mg}^{2+})}{2}}}$$

Where ( $\text{Na}^+$ ), ( $\text{Ca}^{2+}$ ) and ( $\text{Mg}^{2+}$ ) are the ion concentrations in meq  $\text{l}^{-1}$  of solution. The units are important, as if others are used then SAR assumes a different value.

For soils in equilibrium with applied irrigation water, the equation

$\text{ESP} = (100 * (0.01475 \text{ SAR} - 0.0126)) / (0.01475 \text{ SAR} + 0.9874)$

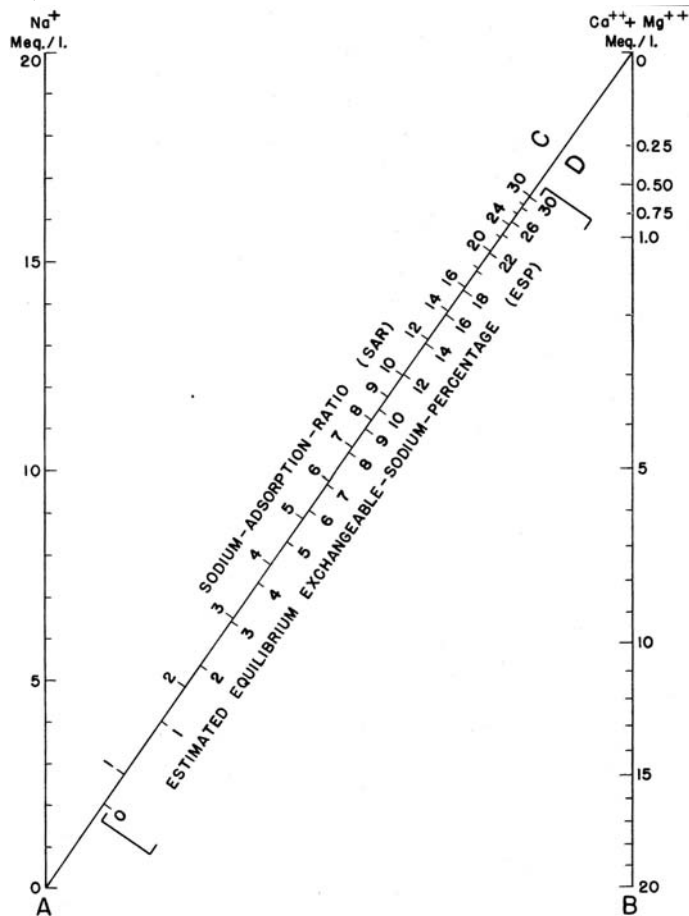
gives a good correlation with measured ESP (Richards, 1954) for total cation concentrations between 39 and 110 meq l<sup>-1</sup>, except where the waters contain high carbonate contents, when other equations should be used. This can be shown diagrammatically, as shown in Figure 11.

Although this equation is now not recommended for all situations, it is still acceptable for most of the irrigation water used (Ayers and Westcot, 1985). An adjusted SAR (adj SAR) was developed by Ayers and Westcot (1979) to take account of this when high carbonate waters were used, but this is not longer recommended as it is thought to overstate the sodium hazard.

The recommended procedure is now the adjusted RNa (adj RNa) procedure of Suarez (1981). This takes account of the effects of CO<sub>2</sub>, HCO<sub>3</sub> and salinity on the calcium in the irrigation water, and assumes a soil source of calcium in addition. The calculated adj RNa can be substituted for SAR in any equations where that term appears. The equation to calculate adj RNa is given by

$$adjRNa = \frac{Na}{\sqrt{\frac{Ca_x + Mg}{2}}}$$

Where Na is the sodium in the irrigation water (meq l<sup>-1</sup>), Ca<sub>x</sub> is a modified calcium value obtained from Table 11 in Ayers and Westcot (1985), and Mg is the magnesium in the irrigation water (meq l<sup>-1</sup>).



**Figure 11.** Nomogram to determine SAR of irrigation water and to estimate corresponding soil ESP (Richards, 1954)

## 10. IRRIGATION WATER QUALITY

Water quality for crop production is an enormous but important topic and will only be outlined here. For more detailed information, refer to Ayers and Westcot (1985) and to Rhoades et al. (1992). In general, salts are present in irrigation water only in small amounts, but they can have a major effect on the soils and crops they are applied to, which depends not only on the concentration of the salts but also what they are and their relative proportions. On the whole, a long-term view should be taken of the potential problems when determining water quality.

Problems fall in four main areas:

Salinity (Table 15), where the salts in the water or deposited in the soil reduce the water availability to the crop so that yield is affected

Water infiltration rate is reduced by high  $\text{Na}^+$  or low  $\text{Ca}^{2+}$  in the soil or the water to the extent that the amount of water infiltrating into the soil is not sufficient to supply the needs of the crop. The main factors influencing quality here are the salinity of the water, and the ratio of sodium to the sum of magnesium and calcium – high salinity water increases infiltration, but water with either low salinity, or with a high ratio of sodium to  $\text{Ca}^{2+} + \text{Mg}^{2+}$ , will decrease it. If this problem persists, then crusting of seedbeds may occur, together with waterlogging, increase in disease-carrying insect vectors, and weed infestations.

Specific ion toxicity may occur due to the accumulation of particular ions ( $\text{Na}^+$ ,  $\text{Cl}^-$  or borate  $[\text{BO}_3^{3-}]$ ). This can be a particular problem when using overhead sprinkler irrigation, and tree crops are particularly sensitive to this type of damage.

Other problems may result from the accumulation of excessive nutrients, the formation of unsightly deposits, and from corrosion of the equipment used for irrigating. A particular problem is the deterioration of pumps and wells, but in some cases the concrete lining irrigation channels can be seriously damaged as well.

A sodium hazard (Table 16) in the irrigation water can have a negative effect on crop production not by impairing the uptake of water by the crop, but by reducing the infiltration rate of water into the soil and so making it unavailable to the crop. The sodicity hazard potential of water is usually assessed in terms of its SAR and its salinity (see section 9.3). At the same SAR, the potential of the water to damage soil structure through dispersing the soil aggregates is greater at low salinity than at higher salinity. However, there are a great many interactions with other soil properties, and it is always best to directly test the effect of your water on the soil of interest.

Care also needs to be taken when analysing groundwater samples. These tend to be lower in pH than surface waters due to their equilibration with much higher partial pressures of  $\text{CO}_2$  ( $\text{PCO}_2$ ). When exposed to atmospheric  $\text{CO}_2$ , the pH rises and precipitation of calcium compounds might occur. In such cases, the adjusted SAR needs to be used.



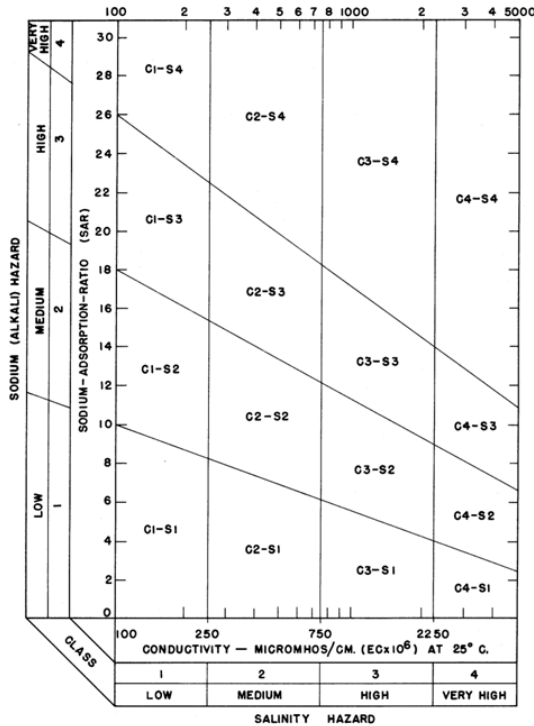
**Table 15** USDA classification of irrigation water salinity (adapted from Richards, 1954)

Salinity class and description		EC range (dS m <sup>-1</sup> )	Equivalent salt concentration (Approx)		
			TDS (g l <sup>-1</sup> )	TDS (ppm)	Cl (ppm)
C1	Low salinity water. Can be used for irrigation with most crops on most soils, with little risk of developing a salinity problem. Some leaching is needed, but except in very impermeable soils this will occur with normal irrigation	< 2.5	< 0.2	< 200	< 60
C2	Medium salinity water. Can be used if moderate leaching is carried out. Plants of moderate salt tolerance can usually be grown without the need for special salinity control measures	2.5-7.5	0.2-0.5	200-500	60-200
C3	High salinity water. Cannot be used if drainage is restricted. Even if drainage is adequate, special management may be needed to control salinity, and plants with good salt-tolerance are needed	7.5-22.5	0.5-1.5	500-1500	200-600
C4	Very high salinity water. Unsuitable for irrigation, except under very special circumstances. Soils must be permeable, drainage adequate, and excess irrigation is needed to provide considerable leaching. Only very salt-tolerant crops should be grown	> 22.5	1.5-3.0	> 1500	> 600

**Table 16** USDA classification of irrigation water sodicity (adapted from Richards, 1954)

Sodium class and description		SAR
S1	Low sodium water. Usable for irrigation on almost all soils with little danger of developing harmful levels of exchangeable sodium. Sodium-sensitive crops, for example stone fruit, may accumulate injurious concentrations of sodium.	0-10
S2	Medium sodium water. Has an appreciable sodium hazard in fine-textured soils with high CEC, especially under low-leaching conditions, unless gypsum is added. May be used in coarse or organic soils with good permeability.	10 - 18
S3	High sodium water. May produce harmful levels of exchangeable sodium in most soils and will require good soil management, good drainage, high leaching and the addition of organic matter. Gypsiferous soils may not develop harmful levels of exchangeable sodium from such waters. Chemical amendments may be needed to replace exchangeable sodium, but may not be feasible if the water also has high salinity.	18 – 26
S4	Very high sodium water. Generally unsatisfactory for irrigation except under low or possibly medium salinity conditions, where the solution of calcium from the soil or the use of gypsum or other amendments may make it feasible.	> 26

Water of high pH (> 8.5) has an excess of alkalinity over Ca and usually poses a substantial sodicity hazard. If pH is below 8.5 it could also have high alkalinity depending on the PCO<sub>2</sub>. Very low salinity (EC around 0.1 dS m<sup>-1</sup>) also makes waters more hazardous in terms of sodicity. Very high pH (> 9.0) adversely affects infiltration, and limits Ca concentrations and high SAR. Water sodicity hazard also depends on its management, and there are greater infiltration problems associated with sprinkler irrigation than with other methods.



**Figure 12.** Diagram for the classification of irrigation waters (Richards et al, 1954)

Figure 12 summarises the various criteria for the evaluation of water quality, using the salinity and sodicity classes from Tables 3.14 and 3.15. It should be noted that, conventionally, the conductivity of irrigation water is described in  $\mu\text{S cm}^{-1}$ . It is important to note that enough calcium may sometimes be dissolved from calcareous soils to decrease the Na hazard significantly, and this needs to be taken into account when using these waters, particularly those of class C1-S3 and C1-S4. For calcareous soils of high pH, or for non-calcareous soils, sodium status may be improved by adding gypsum to the water for waters in classes C1-S3, C1-S4 and C2-S4. For waters of C2-S3 and C3-S2, gypsum added to the soil is beneficial.

Soils become sodic if they are irrigated with water of high “sodium hazard.” In irrigation water, beneficial  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  ions can be precipitated out as insoluble compounds if the water has high  $\text{CO}_3^{2-}$  or  $\text{HCO}_3^-$  concentrations. The residual sodium carbonate (RSC) is calculated to determine irrigation water quality in terms of whether it has an excess of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , or of  $\text{CO}_3^{2-}$  or  $\text{HCO}_3^-$ . It is determined as:

$$\text{RSC} = (\text{CO}_3^{2-} + \text{HCO}_3^-) - (\text{Ca}^{2+} + \text{Mg}^{2+})$$

where the items in parentheses are ion concentrations in  $\text{me l}^{-1}$ .

An  $\text{RSC} < 1.25$  means that the water is probably safe for irrigation. If it is between 1.25 and 2.5, then the water is marginal for irrigation, and if greater than 2.5 then the water is unsuitable for irrigation.

The computer program WATSUIT can be used to predict the salinity, sodicity and toxic-solute concentration of the soil-water resulting from the use of a particular irrigation water of given

composition and at a specified leaching fraction. It can be used to predict the effect of a particular salinity level on crop yield, and of a given sodicity level on soil permeability. Like the other models, the program is available for download at

<http://ars.usda.gov/Services/docs.htm?docid=8968>.

## ACKNOWLEDGEMENTS

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## REFERENCES

- Ayers, R. S., and Westcot, D. W., 1979, Water quality for agriculture. FAO Irrigation and Drainage Paper 29, 1<sup>st</sup> edition, Food and Agriculture Organisation of the United Nations, Rome, Italy
- Ayers, R. S., and Westcot D. W., 1985, Water quality for agriculture. FAO Irrigation and Drainage Paper 29, revision 1, Food and Agriculture Organisation of the United Nations, Rome, Italy, ISBN 92-5-102263-1. Available at <http://www.fao.org/docrep/003/T0234E/T0234E00.htm> (April 7, 2008).
- Barrett-Lennard, E. G., 2003, The interaction between waterlogging and salinity in higher plants: causes, consequences and implications, *Plant Soil* 253:35-54.
- Colorado River Basin Salinity Control Forum, 1993, 1993 review: water quality standards for salinity, Colorado River System. Bountiful, Utah: Colorado River Basin Salinity Control Forum, 115 pp.
- Dregne H. E. and Chou, Nant-Ting, 1992, Global desertification dimensions and costs, in: *Degradation and Restoration of Arid Lands*, H. E. Dregne, ed., Texas Tech. Univ. Press, Lubbock, Texas, USA, pp. 249 – 282.
- Dregne, H. E., Zhixun Xiong and Siyu Xiong, 1996. Soil salinity in China. *Desertification Control Bulletin* 28, pp. 28-33.
- Dudal, R., and Purnell, M. F., 1986, Land resources: salt-affected soils, *Rec. Reveg. Res.* 5:1-9.
- El-Ashry, M. T., Schilfsgaarde, J.V., and Schiffman, S., 1985, Salinity pollution from irrigated agriculture. *J. Soil Wat. Cons.* Jan – Feb, 48 – 52.
- FAO, 2007a, The State of Food and Agriculture 2007. Food and Agriculture Organisation of the United Nations, Rome, Italy, available at <ftp://ftp.fao.org/docrep/fao/010/a1200e/a1200e00.pdf>, (April 29, 2008).
- FAO, 2007b, Water at a Glance. Food and Agriculture Organisation of the United Nations, Rome, Italy, available at <http://www.fao.org/nr/water/docs/waterataglace.pdf>, (April 29, 2008).
- FAO, 2007c, Aquastat Database. Food and Agriculture Organisation of the United Nations, Rome, Italy, available at <http://www.fao.org/nr/water/aquastat/dbase/index.stm>, (April 30, 2008).
- George, R. J., and Green, A., 2000, Position paper on airborne geophysics for salinity and land management. Airborne Geophysics Advisory Group, Sustainable Land and Water Resources Management Committee, Agriculture Western Australia. 32 pp.
- Ghassemi, F., Jakeman, AJ and Nix, HA 1995. *Salinisation of Land and Water Resources: Human Causes, Extent, Management and Case Studies*, CAB International, Wallingford, UK, ISBN 0 86840 198 6. 526 + xviii pp.
- Heynike, J. J. C., 1981, The Economic Effects of the Mineral Content Present in the Vaal River Barrage on the Community of the PWVS Complex (A Desk Study). Water Research Commission, Pretoria, South Africa, 131 pp.
- Ijaz, K., and Davidson, A. P., 1997, Baseline socio-economic survey – Joint Satiana Pilot Project. Lahore, Pakistan, International Waterlogging and Salinity Research Institute. 77 pp + appendices.

- James, C. V., ed., 1989, *Information China: the Comprehensive and Authoritative Reference Source of New China* (organised by the Chinese Academy of Social Sciences), Pergamon Press, Oxford, UK, Vi 1 – 440, v2 441 – 929, v3 930 – 1621.
- Joshi, P. K., Datta, K. K., Gajja, B. L., and Singh, J., 1996, Saline and waterlogged soils: Impact on agricultural economy and feasibility of reclamation, in: *Reclamation and management of waterlogged saline soils*, K. V. G. K. Rao, M. C. Agarwal, O. P. Singh, and R. J. Oosterbaan, eds., National Seminar Proceedings, 5-8 April 1994. CSSRI, Karnal, India, pp 384-398.
- Kielen, N. C., 1996, Farmers' ability to cope with salinity and sodicity: farmers' perceptions, their strategies and practices for dealing with salinity and sodicity in their farming systems. Research Report R6, International Irrigation Management Institute, Lahore, Pakistan. 78 pp.
- Loveday, J., 1972, Moisture content of soils for making saturation extracts and effect of grinding. Division of Soils Technical Paper No. 12a, Commonwealth Scientific and Industrial Research Organization, Canberra City, A.C.T. 2601, Australia.
- Murray-Darling Basin Ministerial Council, 1989, Draft Murray-Darling Basin Natural Resources Management Strategy, Murray-Darling Basin Ministerial Council, Canberra, Australia, 19 pp.
- Norman, C., 1990, Training Manual on the use of the EM38 for Soil Salinity Appraisal, Tech. Rept 181, Victorian Department of Primary Industries, Australia.
- Oldeman, L. R., van Engelen, V. W. P., and Pulles, J. H. M., 1991, The extent of human-induced soil degradation, in: *World map of the status of human-induced soil degradation: as explanatory note*, L. R. Oldeman, R. T. A. Hakkeling, and W. G. Sombroek, eds., International Soil Reference and Information Centre, Wageningen, Netherlands, pp. 27 – 33.
- Qureshi, R. H., and Barrett-Lennard, E. G., 1998, *Saline Agriculture for Irrigated Land in Pakistan: a Handbook*, ACIAR Monograph No 50, Australian Centre for International Agricultural Research, Canberra, Australia, vi + 142 pp.
- Rhoades, J. D., Chanduvi, F. and Lesch, S., 1999, Soil salinity assessment. Methods and interpretation of electrical conductivity measurements. FAO Irrig. Drain. Paper 57, Food and Agriculture Organisation of the United Nations, Rome, Italy, 160 pp. ISSN 0254-5284. Available at <ftp://ftp.fao.org/agl/aglw/docs/idp57.pdf> (April 7, 2008).
- Rhoades, J. D., Kandiah, A., and Mashali, A. M., 1992, The use of saline waters for crop production, FAO Irrig. Drain. Paper 48, Food and Agriculture Organisation of the United Nations, Rome, Italy, 94 + ix pp. ISBN 92-5-103237-8. Available at <ftp://ftp.fao.org/agl/aglw/docs/idp60.pdf> (April 7, 2008).
- Richards, L. A., ed., 1954, *Diagnosis and improvement of saline and alkali soils*. USDA Handbook 60, US Salinity Laboratory, Riverside, California, USA, 160 pp.
- Simmonds, P., Poukter, D., and Hall, N. H., 1991, Management of irrigated water in the Murray-Darling Basin, Discussion paper 91.6, Australian Bureau of Agricultural and Resource Economics, Canberra, Australia, 42 pp.
- Singh, N. T., 1998, Historic perspective. Chapter 2 in *Agricultural Salinity Management in India*, N. T. Tyagi, and P. S. Minhas, eds., Central Soil Salinity Research Institute, Karnal, India, pp 9-19.
- Suarez D. L., 1981, Relation between pHc and Sodium Adsorption Ratio (SAR) and an alternate method of estimating SAR of soil or drainage waters. *Soil Sci. Soc. Amer. J.* 45:469–475.
- Szabolcs, I., 1989, *Salt-Affected Soils*, CRC Press, Boca Raton, Florida, USA, 274 pp.
- Tanji, K. K., 1990, Nature and extent of agricultural salinity. Chapter 1 in *Agricultural Salinity Assessment and Management*, K. K. Tanji, ed., ACSE Manuals and reports on engineering practice No. 71, ASCE, New York, USA, ISBN 0-87262-762-4, pp.1-17.
- Vermeer, E. B., 1977, *Water Conservancy and Irrigation in China: Social, Economic and Agrotechnical Aspects*, Leiden University Press, The Hague, Netherlands, 350 pp.
- WAPDA 1988 Water and Power Development Authority, Lahore, Pakistan. Personal Communication cited in Ghassemi et al., 1995.
- Western Australia Legislative Assembly 1991 Select Committee into Land Conservation. Final report. Perth, WA: Legislative Assembly. 171 pp.
- WRI, 2008, World Resources Institute, online databases at <http://earthtrends.wri.org>, (April 29, 2008).

# CHAPTER 3

## EFFECTS OF SALINITY ON PLANTS

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### 1. INTRODUCTION

Salinity, either of the soil or of the irrigation water, is one of the major abiotic stresses affecting crop production world wide. Chapter Three dealt in detail with the extent of the problem, its causes, and its effects upon soils. This chapter looks in detail at the ways in which salt affects plant growth and survival, and the mechanisms plants adopt to cope, focusing in particular upon crop plants. There is a fundamental division into two classes of plant, the halophytes, which adapted to growth in saline areas, and the glycophytes, which are not, and most crop plants fall into the glycophyte category.

#### 1.1 Halophytes

Halophytes (from the Greek for “salt plants”) have evolved in salt-affected regions of the world. They generally show increased growth at low salt concentrations compared with no salt at all, but growth is decreased at very high concentrations. Despite this, many grow at very high salinities indeed. The halophytes are widely distributed, and make up about one third of angiosperm (flowering plant) families. Both monocotyledonous and dicotyledonous examples are found, and halophytes are particularly common in the Chenopodiaceae. Typical examples of halophytes include the river saltbush (*Atriplex amnicola*), which has a 10% growth increase at 5 dS m<sup>-1</sup>, suffers a 50% decrease at 40 dS m<sup>-1</sup>, but is still alive at 75 dS m<sup>-1</sup> (Qureshi and Barrett-Lennard, 1998). Others include quailbrush (*A. lentiformis*), *Sueda fruticosa*, and *Salicornia bigelovii*. Another typical example is *Kochia indica*, an indicator species which, if it is found growing, shows that the land is almost certainly saline. Few, if any, agricultural crops can be regarded as halophytes.

#### 1.2 Glycophytes

The term glycophyte is again from the Greek, this time for “sweet plants.” Glycophytes are more common than halophytes, and supply the great majority of agriculturally important plants, although most cope poorly with salinity or sodicity. There are two groups. Salt-tolerant glycophytes can maintain growth at low salinity, but show decreased growth at higher levels. A typical examples is cotton (*Gossypium hirsutum*), in which the salinity level at which growth is reduced by 50% (the EC<sub>50</sub>) is 17 dS m<sup>-1</sup>. Others include barley (*Hordeum vulgare*, EC<sub>50</sub> = 18 dS m<sup>-1</sup>); sugarbeet (*Beta vulgaris*, 15 dS m<sup>-1</sup>); and date palm (*Phoenix dactylifera*, 18 dS m<sup>-1</sup>). Salt-sensitive glycophytes are those in which growth is sensitive even to low salt concentrations.

They include beans (*Phaseolus vulgaris*) and many other legumes, with a typical  $EC_{50}$  of around  $3.5 \text{ dS m}^{-1}$ . Other examples of salt-sensitive glycophytes include rice,  $EC_{50} = 7.2 \text{ dS m}^{-1}$ ; carrot (*Daucus carota* subsp. *Sativus*,  $EC_{50} = 4.6 \text{ dS m}^{-1}$ ) grapefruit (*Citrus*  $\times$  *paradisi*,  $EC_{50} = 4.9 \text{ dS m}^{-1}$ ); and peach (*Prunus persica*,  $EC_{50} = 4.1 \text{ dS m}^{-1}$ )

## 2. EFFECTS OF SALTS ON PLANTS

### 2.1 Plant response to salinity

Salinity adversely affects plants in two ways. A depression of the external water potential, a physical effect, occurs first, and specific chemical effects as a result of excess concentrations of certain ions follow. Although the effects of salinity are usually damaging, they may occasionally be beneficial. For example, salinity may increase yields of cotton, or improve tomato (*Solanum lycopersicum*) quality, and it has been shown to increase citrus tolerance to freezing. These benefits may, to some limited extent, compensate for the more serious adverse effects.

#### 2.1.1 The time sequence

Responses to salinity were neatly summarised by Munns (2002), which has been drawn upon extensively for this section. She used plants grown either in sand or in solution culture to elucidate the effects. Salinity initially reduces root water potential, leading to reduced flow of water from the soil into the plant. The first consequence is a rapid but temporary drop in growth rate, due to short-term changes in water relations, followed by gradual recovery to a new but reduced growth rate. Effects after this tend to be more permanent, and involve alterations to metabolism and the specific effects of particular ions.

The depression of external water potential by salinity, which occurs instantaneously with the increase in salinity, narrows the gap between the external (soil) and internal (plant) water potentials, and as a result the leaves show an almost immediate reduction in growth rate. The rate of growth reduction is so fast that it can only be due to changes in water relations (Passioura and Munns, 2000), and as similar effects can be induced using other osmotica, for example KCl or mannitol (Yeo et al., 1991), “salt” in the broad sense cannot be involved. Similar effects have been induced in root growth, again using either NaCl or other osmotica, (Frensch and Hsiao, 1994, 1995), so in the roots as well the changes are not due to “salt” but to altered water relations. However, some indications of a “salt”-induced effect on root growth have been noted, as salt-induced  $Ca^{2+}$  deficiency is greater for genotypes with inherent low uptake of  $Ca^{2+}$  (Cramer et al., 1988; Colmer et al., 1996). After several minutes, growth recovers gradually to a new steady-state, although this is lower than before the application of stress, particularly in the case of the roots. The concentration of the salt solution affects both the level of the new steady-state, and the time taken for this recovery.

After a few days, leaf and root growth have usually stabilised at their new, reduced, steady rate. This is usually lower for leaf growth than for roots, especially in dry soil (Hsiao and Xu, 2000; Munns and Sharp, 1993), again probably due to water stress rather than salts. The evidence for this is that  $Na^+$  and  $Cl^-$  concentrations in the growing cells are always below toxic concentrations, both in the leaves (Hu and Schmidhalter, 1998) and in the roots (Jeschke, 1984; Jeschke et al., 1986). It is likely that the rapid expansion of the growing cells helps to maintain  $Na^+$  and  $Cl^-$  at low levels.

After several days at high salinity, salt-specific effects may begin to occur in particularly salt-sensitive species. For example, some plants are unable to exclude NaCl when it is at high levels in the growing medium, and as a result show severe injury to the older leaves at this time. This is due to the accumulation of excessive levels of  $\text{Na}^+$  and  $\text{Cl}^-$  in the transpiring leaves. When this exceeds the capacity of the cells to compartmentalise them in the vacuole, the ions rapidly build up, either in the cell walls where they dehydrate the cell, or in the cytoplasm where they inhibit enzyme activity.

If the ion concentration in the cell wall rises, the cell shrinks and the internal ion concentration rises. The efflux of some of these ions adds to those in the transpiration stream coming from the roots, so that the ion concentration in the cell wall rises even faster and the cell dehydrates rapidly. If the site of increased concentration of  $\text{Na}^+$  and  $\text{Cl}^-$  ions, once the vacuole is full, is not the cell wall, then they build up in the cytoplasm, which has equally severe effects. The rate of concentration increase in the cytoplasm is much more (typically ten times faster) than it is in the vacuole, due to the cytoplasm's much smaller volume. As an example, Munns (2002) cited barley. If  $\text{Na}^+$  was accumulating at  $5 \text{ mol m}^{-3}$  per day on a leaf water basis, the increase in the cytoplasm would be at the rate of  $50 \text{ mol m}^{-3}$  per day, and so in just two days would exceed  $100 \text{ mol m}^{-3}$  and be potentially toxic.

Wherever the ion accumulation occurs, the cells will die, either of salt toxicity or of dehydration, within a day or two of the vacuole ceasing to take up salt. Ion toxicity occurs in older rather than younger leaves as they have been transpiring longest.  $\text{Na}^+$  and  $\text{Cl}^-$  increase with time and will eventually reach toxic levels in any transpiring leaves. How long this takes (days or weeks) will depend upon the salinity level and other environmental factors, as well as on genetic differences between species and genotypes in the ability of the roots to keep salt out of the transpiration stream. Toxicity to low or moderate concentrations is quite common in woody plants, and several fruit trees are unable to exclude  $\text{Na}^+$  or  $\text{Cl}^-$  from the leaves, and so suffer toxicity at low concentrations.

After a period of weeks, the effects of salinity become more pronounced in all species, particularly in more sensitive ones which either take up more salt, or which cannot compartmentalise it into the vacuoles, where the injury may be visible as leaf yellowing or death in the older leaves. In more tolerant species, the plant will continue to show responses similar to those of plants under water stress.

As time continues, in the more sensitive species in which salt is not excluded from the transpiration stream it will reach toxic levels in the longest transpiring leaves, so that there will be a progressive loss of older leaves with time. The leaf death rate determines survival: if new leaves are produced faster than the old ones die, then enough photosynthetic area will remain to allow the plants to begin reproductive growth. If they are not, then the proportion of injured leaf increases and the number of green leaves falls as leaves die faster than they can be replaced. The relative rates of these two processes determine whether the plant flowers and produces seeds while enough green leaf remains to supply photosynthate.

After several months, as would be expected, there are substantial differences between annuals and perennials. In perennial species, leaf death continues, and whether the plant lives or dies depends on its ability to prevent salts reaching toxic levels in the older leaves (determined by ion exclusion

from the transpiration stream, the plant's ability to compartmentalise, and the rate of new leaf growth as determined by soil water potential. In annuals, the formation and viability of the reproductive organs is affected. In cereals, the number of florets per ear is reduced, and flowering and maturity times are altered, as happens with drought. This is thought unlikely to be a salt-specific effect, as the concentrations of both  $\text{Na}^+$  and  $\text{Cl}^-$  in the reproductive primordia remain too low to affect the metabolism. Most apical cells are unvacuolated, and so any salt accumulates in the cytoplasm. However, it is likely that both  $\text{Na}^+$  and  $\text{Cl}^-$  transport in the phloem to the apices is well-enough controlled to prevent concentrations reaching toxic levels (Munns, 2002).

### **2.1.2 Osmotic effects**

Recently there has been increased interest in the osmotic effects of salinity, in particular in the case of durum wheat. This has been linked to interest in whether there is genotypic variation in this trait which could be exploited for breeding, in combination with two recently-discovered major genes for  $\text{Na}^+$  exclusion in this species (Lindsay et al., 2004; James et al., 2006). Photosynthesis in salt-affected plants is limited by salinity, and it is known that stomatal conductance is more sensitive to salinity than are the non-stomatal components of photosynthesis (James et al., 2002). Stomatal conductance reduces immediately upon exposure to salt, which indicates that it is a response to the osmotic changes in the root zone, whereas non-stomatal factors occurred over several weeks. James et al. (2008) tested durum wheat genotypes, and found two or three-fold differences in the size of the stomatal conductivity response to salinity stress, with a positive correlation between high conductance and relative growth rate in saline conditions. The decline in stomatal conductance began almost as soon as salinity was applied, and continued for at least one week. Similar conclusions regarding the importance of stomatal conductance have been made for a number of other crops, for example barley (Chen et al., 2005; Jiang et al., 2006) and bread wheat (El Hendawy et al., 2005). The mechanism by which the osmotic stress reduces stomatal conductance is not yet known, but is thought likely to be through signals from the roots (James et al., 2008). As turgor is maintained (James et al., 2002), it is clearly not hydraulic.

### **2.1.3 Specific ion effects**

There may also be specific ion effects in some cases. High concentrations of a given ion may cause nutritional disorders, for example high  $\text{Na}^+$  may cause deficiencies of other elements such as  $\text{K}^+$  or  $\text{Ca}^{2+}$ . Even plants that have a high ability to select  $\text{K}^+$  over  $\text{Na}^+$ , for example mangrove (*Avicennia marina*), may show evidence of salt-induced  $\text{K}^+$  deficiency under some conditions (Ball et al., 1987). During the initial water deficit stage, slow water flow limits nutrient uptake to the plant, but when uptake resumes,  $\text{Na}^+$  and  $\text{Cl}^-$  ions can inhibit the uptake of others, leading to excess shoot  $\text{Na}^+/\text{K}^+$  ratios as a result of  $\text{Na}^+$  entry and  $\text{K}^+$  exclusion. Deficiencies of other ions, in particular of  $\text{Ca}^{2+}$  (e.g. LaHaye and Epstein, 1969; Läuchli and Epstein, 1970; Cramer et al., 1988) and  $\text{NO}_3^-$  (Aslam et al., 1984), can also occur.

Certain ions, such as  $\text{Cl}^-$ , may have specific toxic effects which are not always distinguishable from deficiencies. These are particularly common in woody plants, when they occur at even moderate concentrations (Bernstein, 1965). Many disorders result from the disruption by  $\text{Na}^+$  of  $\text{Ca}^{2+}$  transport and membrane function, which can occur very rapidly in some species, and may sometimes be ameliorated by the addition of  $\text{Ca}^{2+}$  to the growing medium.



#### 2.1.4 Trace elements

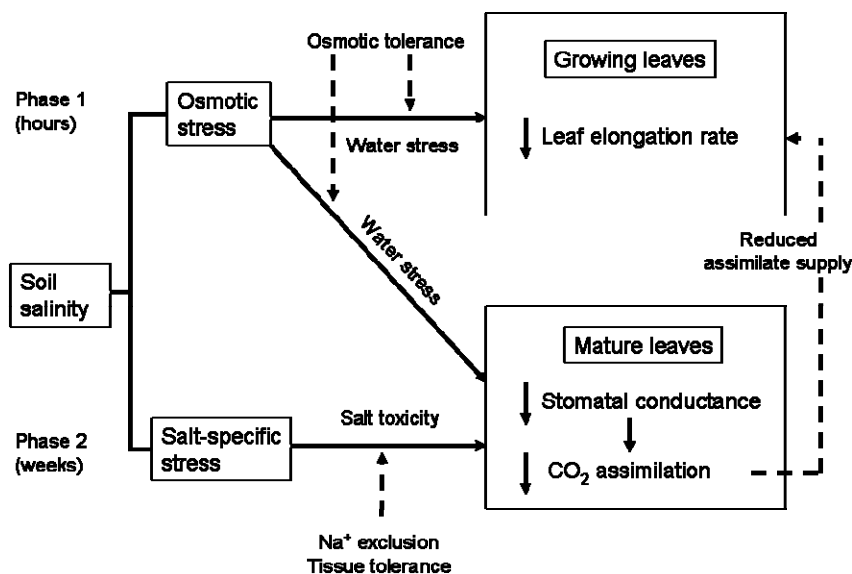
In salt-affected soil, deficiencies of a number of trace elements are likely. These include copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn). Deficiencies of boron (B) are rarely a problem in saline soils, although B toxicity may be. Some trace elements may also accumulate in the tissues of plants growing in saline soils to levels that may be toxic either to animals or to humans. Trace element levels in irrigation water also need to be monitored carefully to prevent adverse effects.

#### 2.1.5 Summary of responses to salinity

Plant response to salinity occurs at different time scales, and the effects of salinity on tolerant plants are basically those due to soil water deficits, as shown in the Table 1 below (Munns, 2002). It is only the more sensitive plants that suffer salt-specific effects. Munns (1993) proposed a 2-phase salinity response model, which has been validated in wheat (Munns et al., 1995) and in maize (*Zea mays*) (Cramer et al., 1994; Fortmeier and Schubert, 1995). Similar effects were also noted in rice (Yeo et al., 1990). However, recent advances in the understanding of the osmotic effects of salinity have led to the model being refined, and a new version (Figure 1) was proposed by James et al. (2008). The initial response to salinity is osmotic stress due to salt concentration in the rootzone (Phase 1), followed by a salt-specific stress resulting from the accumulation of salts in the older leaves (Phase 2).

**Table 1** Response of plants to salinity with time (after Munns, 2002)

Time	Water-stress (osmotic) effects	Salt-specific effects
	Effect on salt-tolerant plants	Effect on salt-sensitive plants
Minutes	Immediate and rapid reduction in leaf and root growth, followed by a partial and rapid recovery	
Hours	Steady but reduced rate of leaf and root growth	
Days	Greater reduction in leaf growth than in root growth. Reduction in rate of leaf emergence	Visible signs of injury in oldest leaves
Weeks	Smaller final leaf size. Possibly also reduced number of lateral shoots	Death of oldest leaves
Months	Effects on flowering time. Reduced seed production	Younger leaves dead, possible death of whole plant before maturity



**Figure 1.** Revised 2-phase model for plant growth under salinity (James et al., 2008), developed from the initial model of Munns (1993). Reproduced by permission of CSIRO Publishing.

The osmotic stress produces hormone signals that result in reduced leaf elongation rates (Termaat et al., 1985; Passioura and Munns, 2000), and also probably reduce the stomatal conductance. This results in reduced photosynthesis, which reduces the supply of carbohydrate to the growing leaves and so further reduces their growth. Salt accumulation in the older leaves eventually reaches toxic levels and so further inhibits photosynthesis, with the result that carbohydrate supply is the main factor limiting leaf growth.

The various changes can be summarised as follows:

- Seconds or minutes after exposure to salt the cells lose water and shrink.
- Within hours, they regain their original volume but with reduced elongation rates, and there are lower rates of leaf and root growth.
- In days, there are changes in cell elongation and division leading to lower leaf appearance rate and smaller leaf size. Leaf growth is usually more affected than root growth, and where there is high salt uptake, the oldest leaf may begin to show injury.
- In weeks, the lateral shoots are clearly inhibited, and in plants with high salt uptake many leaves may be dead, although rates of leaf *production* may not differ between genotypes.
- In months, there are clear and obvious differences between plants with high and low salt uptakes, and there may be high leaf injury and complete death in some cases at high salinity levels.

## 2.2 Effect of sodicity

Sodicity, the result of high concentrations of Na<sup>+</sup> ions relative to Ca<sup>2+</sup> and Mg<sup>2+</sup>, affects the mineral nutrition of the plant, and may lead to symptoms of Na<sup>+</sup> toxicity. Any disruption to the

water balance of the plant as a result of sodicity will be due to its effects on the soil permeability. Crop tolerance to different levels of sodicity is shown in Table 2.

**Table 2** Crop tolerance to ESP (Bower, 1959)

ESP	Type of crop affected	Growth responses under field conditions	Crop examples
2 - 10	Extremely sensitive	Sodium toxicity symptoms even at low ESP values	Deciduous fruit, nuts, avocado, cassava, citrus
10 - 20	Sensitive	Stunted growth at low values even though the physical condition of the soil may be good	Beans, sugarcane
20 - 40	Moderately tolerant	Stunted growth due to both nutritional factors and adverse soil conditions	Clover, oats, tall fescue, rice, dallis grass
40 - 60	Tolerant	Stunted growth usually due to adverse physical condition of the soil	Wheat, cotton, lucerne, barley, tomatoes, beets
> 60	Most tolerant	Stunted growth usually due to adverse soil physical condition	Crested fairway and tall wheat grasses, Rhodes grass

### 2.3 Salinity tolerance mechanisms

Salt tolerance is a complex, multigenic trait (Shannon, 1997; Flowers, 2004). Most crops are adversely affected by salinity, although in general, cereals are more tolerant than legumes (Reynolds et al., 2005), and many wild relatives of crop plants show greater tolerance than their domesticated descendents. Of the major cereals, bread wheat (*Triticum aestivum*) is generally regarded as moderately tolerant, durum wheat (*T. durum*) as somewhat less so, while rice (*Oryza sativa*) is regarded as susceptible (Francois and Maas, 1994). Salt-tolerant and salt-sensitive plants are distinguished from one another by the inability of the latter to stop salts reaching toxic levels in transpiring leaves. The enzymes in salt-tolerant halophytes are no more tolerant to salt than are those in glycophytes (Greenway and Osmond, 1972; Flowers et al., 1977), and are inhibited by both  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations above about  $100 \text{ mol m}^{-3}$  (Munns, 2002). Tolerance mechanisms must therefore either minimise salt entry to the plant, or minimise salt concentration in the cytoplasm. Halophytes have both mechanisms – they are efficient salt excluders, and also compartmentalise into the vacuoles any salt that does enter. Some glycophytes are also good salt excluders, although unable to compartmentalise salt taken up as well as do the halophytes, but most are quite poor excluders, with the result that salt reaches toxic levels in the transpiring leaves.

In wheat, the key traits for salt tolerance were discussed by Colmer et al. (2005): one of the most important is a combination of low rates of  $\text{Na}^+$  (high  $\text{K}^+/\text{Na}^+$  discrimination) and  $\text{Cl}^-$  uptake and transport to the leaves (salt exclusion) (Munns et al., 2002). However, in rice, transpirational flow is highly correlated with  $\text{Na}^+$  uptake and is an order of magnitude greater than in wheat (Garcia et al., 1997), reducing the importance of  $\text{K}^+/\text{Na}^+$  discrimination in that crop.

#### 2.3.1 Salt exclusion – low transport to leaves

Salt exclusion reduces the accumulation of salt in transpiring organs. Plants transpire between 30 and 70 times more water than is used for cell expansion, so any solutes that are not excluded by the roots would be between 30 and 70 times more concentrated in the plant than in the soil solution. An efficient filtering mechanism at the roots reduces this substantially. Rana Munns and her group in Australia originally demonstrated this in the early 1980s (Munns et al., 1983).

Chloride concentrations in the xylem are between 0.2 and 5% of those in the external medium, which means that the roots filter out 95 – 99.8% of the salt in the soil. If a plant transpires 50 times more water than it retains, but filters out 98% of the salt at the roots, then a shoot ion concentration greater than that of the soil will never be reached, and the plant could grow indefinitely in saline soil (Munns, 2002). Bread wheat is a good example of a plant that shows efficient  $\text{Na}^+$  exclusion, and maintains a high ratio of  $\text{K}^+/\text{Na}^+$  in the shoots (Gorham et al., 1990). However, individual leaves cannot be protected indefinitely, and as the plant transpires, dissolved salts are deposited in the leaves as water passes out of the plant. These concentrations gradually increase, and eventually there will be much higher salt concentrations in older (i.e. those that have been transpiring for longer) than in younger leaves, which will eventually be high enough to kill the cells.

There are three main mechanisms to exclude salts from the leaves. The first is ion uptake selectivity at the roots. The cells that control this are not yet known, but it is believed that the initial  $\text{Na}^+$  and  $\text{Cl}^-$  uptake could occur either at the epidermis, the exodermis or at the endodermis. The second mechanism is xylem loading, and Gorham et al. (1990) provided evidence for genetically-controlled loading of  $\text{K}^+$  rather than  $\text{Na}^+$  in the stele cells. The third mechanism is salt removal from the xylem in the upper roots, or the stem, petiole or leaf sheaths. In many species,  $\text{Na}^+$  is retained in these areas, suggesting that  $\text{K}^+$  is exchanged for  $\text{Na}^+$  in the cells lining the transpiration stream.

In halophytes, salt exclusion is the most important regulator of the internal salt load, even in those species with salt glands or bladders. It is particularly important in perennial species with long-lived leaves, as these clearly need to regulate salt uptake for longer than annuals, whose leaves may be alive for only a few months. Some glycophytes can also control salt transport to the leaves fairly well, but only at low or moderate salinity levels. For example, barley excludes 95% of both  $\text{Na}^+$  and  $\text{Cl}^-$  at the xylem level when grown in  $100 \text{ mol m}^{-3} \text{ NaCl}$  (Munns, 1985).

There is now considerable evidence for the importance of these exclusion mechanisms (for example see Greenway and Munns (1980), and specifically Laüchli (1984) for legumes, Storey and Walker (1999) for citrus species, and Jeschke (1984) for  $\text{K}^+/\text{Na}^+$  selectivity). We also have a good understanding of the electrophysiology of the channels and transporters that regulate salt movement across the membranes, and know which genes are involved in the process (e.g. Amtmann and Sanders, 1999; Hasegawa et al., 2000; Byrt et al., 2007).

A number of other features also help maintain low rates of salt accumulation in the leaves. For example, high shoot:root ratios and high intrinsic growth rates reduce the rate at which salt enters the transpiration stream and accumulates in the shoot (Pitman, 1984). The extent of the apoplastic pathway in the roots also influences salt movement across the root and into the xylem (Garcia et al., 1997). A third trait is the export of salts from the leaves via the phloem, although this is relatively minor compared with salt import via the transpiration stream. The evidence for this is that salt remains in leaves for a long time after salts around the roots are removed (Munns, 2002). It appears that salt-tolerant species are better at excluding  $\text{Na}^+$  and  $\text{Cl}^-$  from the phloem than are less tolerant ones, so ensuring that those ions do not reach the growing tissues (Munns et al., 1988). Some halophytes possess salt glands or bladders which can help to export salts from the leaves (Flowers et al., 1977; 1986) and so maintain salt balance over longer periods (Ball, 1988).

As a plant's capacity for salt exclusion is limited, above a particular threshold the exclusion mechanism breaks down, leading to high rates of transport of  $\text{Na}^+$  and  $\text{Cl}^-$  to the shoots. Even with exclusion, leaf water deficits will develop, so as a result, salt exclusion may not be entirely useful as a mechanism of salt tolerance. An example can be taken from Laüchli and Epstein (1990), who compared the uptake of  $\text{Na}^+$  and  $\text{Cl}^-$  in sensitive and less sensitive genotypes of avocado (*Persea americana*) (Downton, 1978), grapevine (*Vitis* spp.) (Bernstein et al., 1969), maize (Schubert and Laüchli, 1986) and soybean (*Glycine max*) (Laüchli and Wieneke, 1979). In avocado, the less-sensitive cultivar excluded  $\text{Na}^+$  from the shoot better than the sensitive one, although both took up similar amounts of  $\text{Cl}^-$ . In soybean, the less-sensitive cultivar excluded  $\text{Na}^+$  and  $\text{Cl}^-$  better than the sensitive one. The tolerant grapevine was able to exclude  $\text{Cl}^-$  much more effectively than the sensitive one (measurements of  $\text{Na}^+$  were not made), while in contrast the more tolerant maize cultivar showed poor exclusion of  $\text{Na}^+$ .

### 2.3.2 Intracellular ion compartmentation

Sequestration of  $\text{Na}^+$  and  $\text{Cl}^-$  ions in the vacuole occurs in most species, as is shown by the concentrations of these ions ( $> 200 \text{ mol m}^{-3}$ ), which would severely inhibit enzyme activity if the enzymes were exposed to them, that are found in still-functioning leaves. When sequestration occurs,  $\text{K}^+$  ions and organic solutes accumulate in the cytoplasm to balance the osmotic pressure (OP) of the vacuole. The most frequent organic solutes involved are proline and glycinebetaine, although in some species others can reach high concentrations (Hasegawa et al., 2000). As well as accumulating in response to salinity, these compounds accumulate under water stress, and are also found at high concentrations in plants adapted to dry or saline soils. Their role is not entirely clear, as we know that they can accumulate to such high levels that they are not confined to the cytoplasmic compartments, and that their accumulation relates more to osmotic stress than to specific salt effects (Wyn Jones and Storey 1978).

Barley is a good example of a crop that uses intracellular ion compartmentation as its main tolerance mechanism. Both  $\text{Na}^+$  exclusion and  $\text{K}^+/\text{Na}^+$  selectivity are lower than in bread wheat, but the  $\text{Na}^+$  is effectively compartmentalised into the vacuole, and so its toxicity is reduced (Greenway and Munns, 1980). Barley has an additional mechanism in that  $\text{K}^+$  is compartmentalised into the mesophyll rather than the epidermis. This results in higher  $\text{K}^+/\text{Na}^+$  ratios in the cytoplasm of the mesophyll, which helps to maintain photosynthesis.

Recently it has been realised that tissue tolerance to high  $\text{Na}^+$  may be of much greater importance for breeding tolerant varieties of wheat than had previously been thought (Genc et al., 2007). The work showed that, in a diverse collection of genotypes, there was no relationship between salt tolerance and  $\text{Na}^+$  exclusion (as measured by  $\text{K}^+/\text{Na}^+$  ratio), either from individual leaf blades or from the shoot as a whole, and  $\text{Na}^+$  exclusion and tissue tolerance were found to vary independently. The suggestion was that future efforts in selecting for salt tolerance in wheat should concentrate both on sodium exclusion and on tissue tolerance to high levels of  $\text{Na}^+$ .

### 2.3.3 The cost to the plant of salinity tolerance

Tolerance costs energy, and this is particularly important in the case of plants growing under saline waterlogged conditions. The three processes involved – excluding the salt, the intracellular compartmentation, and excretion through salt glands in certain species, all have metabolic costs,

although these are much smaller (around a whole order of magnitude) than those incurred by synthesising organic solutes for osmotic adjustment (Raven, 1985). This suggests that plants might grow faster in saline than in dry soils of the same water potential, although there is little evidence that this is in fact the case. Energy costs assume particular importance when considering combined salinity and waterlogging stress (Section 3).

## 2.4 Genetic control of salt tolerance

Over the last few years a much clearer picture has emerged of the genes responsible for controlling salt tolerance. We now know that the HKT (**H**igh-affinity **K**<sup>+</sup> **T**ransporter) genes control sodium transport in higher plants (Munns and Tester, 2008), and these have been identified in wheat (Huang et al., 2006; Byrt et al., 2007), rice (Ren et al., 2005), barley (Haro et al., 2005) and *Arabidopsis* (Berthomieu et al., 2003). Huang et al. (2008) provide a detailed discussion of the role of these genes in salt tolerance and Na<sup>+</sup> transport in rice, wheat and barley.

Bread wheat has a low rate of Na<sup>+</sup> transport to the shoot and maintains a high K<sup>+</sup>/Na<sup>+</sup> ratio in the leaves (Gorham et al., 1990). A trait for enhanced K<sup>+</sup>/Na<sup>+</sup> discrimination at low salinity (Gorham et al., 1987), is conferred by the locus *Kna1* (Dvořák and Gorham, 1992), which is situated on the distal part of chromosome 4DL (Dubcovsky et al., 1996). It is likely that *Kna1* controls net xylem loading rather than net Na<sup>+</sup> uptake, as it does not produce differences in root Na<sup>+</sup> concentrations (Gorham et al., 1990). QTLs for salt tolerance in bread wheat have been located on chromosome 5A (Semikhodskii et al., 1997), homoeologous to two yield QTL clusters on 5B and 5D in a different population grown under saline conditions (Quarrie et al., 2005).

In durum wheat, which in general has a poorer ability to exclude Na<sup>+</sup> (Gorham et al., 1990), a line with low leaf Na<sup>+</sup> concentrations and high K<sup>+</sup>/Na<sup>+</sup> ratios has been identified (Munns et al., 2000). Munns et al. (2003) identified two genes, *Nax1* and *Nax2* as being responsible for this trait. *Nax1* reduces the transport of Na<sup>+</sup> from the roots to the shoots, and causes retention of Na<sup>+</sup> in the leaf blades (James et al., 2006). It has been mapped to chromosome 2AL (Lindsay et al., 2004), and a candidate gene identified as a sodium transporter *HKT7-A2* mapped to the same region (Huang et al., 2006). This is consistent with *Nax1*'s physiological role in reducing Na<sup>+</sup> in the leaf blades by retaining the ions in the leaf sheaths, and was postulated as controlling Na<sup>+</sup> unloading from the xylem in the roots and sheaths.

*Nax2* also confers a lower rate of Na<sup>+</sup> transport from the roots to the shoots, but has a higher rate of K<sup>+</sup> transport than *Nax1*, leading to enhanced leaf K<sup>+</sup>/Na<sup>+</sup> discrimination (James et al., 2006). It was suggested that the gene only functions in the roots, as lines containing *Nax2* did not retain Na<sup>+</sup> in the bases of the leaves (James et al., 2006). It has been mapped to the distal region of chromosome 5AL (Byrt et al., 2007), which coincides with the locus for a putative Na<sup>+</sup> transporter *HKT1;5-A* (*HKT8*). The *Nax2* region on 5AL is homoeologous to the region on 4DL that carries *Kna1*, and it is therefore highly probable that both *Nax2* and *Kna1* are strongly associated with *HKT1;5* genes. In consequence, Byrt et al. (2007) proposed *HKT1;5-D* as the candidate gene for this locus. Davenport et al. (2005) suggested that the two genes *Nax1* and *Nax2* interacted via net Na<sup>+</sup> xylem loading and net leaf sheath sequestration to control Na<sup>+</sup> exclusion.

In *Thinopyrum* species, related to wheat, tolerance is associated with shoot Na<sup>+</sup> and Cl<sup>-</sup> exclusion, particularly at higher NaCl concentrations (Gorham, 1994). In *Th. elongatum*, Dvořák et al. (1988)

and Omielan et al. (1991) reported major effects of chromosomes 3, 4 and 7 on ion exclusion. Koebner et al. (1996) proved the involvement of chromosomes of homoeologous group 5 in salt tolerance in wheat. These effects are closely linked to genes controlling vernalisation (group 5) and photoperiod (group 2) response (Taeb et al., 1992; Martin et al., 1993). In *Th. bessarabicum*, group 2 chromosomes carry genes conferring susceptibility to salt, and chromosome 5E<sup>b</sup> has a major dominant gene or genes for tolerance (Forster et al., 1988, Mahmood and Quarrie, 1993). Zhong and Dvořák (1995a, b) suggested that Triticeae species generally shared common mechanisms of tolerance to sudden salinity stress.

In rice, Flowers et al. (2000) identified AFLP markers for ion transport and selectivity. In the tolerant variety Pokkali, a major gene, possibly *SalT* (Causse et al., 1994), has been mapped (IRRI 1998), as were QTLs governing high K<sup>+</sup> and low Na<sup>+</sup> absorption, and high K<sup>+</sup>/Na<sup>+</sup> ratio (Gregorio et al., 2002). A common major QTL was found on chromosome 1 for three traits associated with salt tolerance (IRRI, 1998). One QTL for salt tolerance on rice chromosome 1 has been fine mapped and the gene, *SKC1*, identified as a sodium transporter (Ren et al., 2005). It is not yet clear whether it is responsible for other QTLs on chromosome 1, or if there is a cluster of relevant genes in this region. Other markers in rice have been identified for traits associated with productivity in saline environments, although many are associated with tolerance to submergence, and micronutrient deficiency or toxicity (Gregorio et al., 2002). Koyama et al. (2001), Lin et al. (2004) and Takehisa et al. (2004) have also mapped other QTLs for salt tolerance.

However, overall, relatively few QTLs have been identified for salt tolerance. This could either be because the traits are actually determined by a limited number of loci, or that the genes associated with the traits were clustered on particular chromosomes (Flowers, 2004).

## 2.5 Response during development

The sensitivity of plants to salinity changes according to environmental conditions, and over the course of their development (Shannon, 1997), with the critical stages being germination, vegetative growth and reproductive growth. The vegetative growth of some halophytes is stimulated by salinity, but they are not tolerant at germination. While vegetative growth in glycophytes is generally sensitive to salinity, some crops are more tolerant during reproductive development, although the picture is complex.

Some salt-sensitive species, for example maize (Maas et al., 1983) and tomato (Kurth et al., 1986, Foolad and Lin, 1993), may germinate well at high salinity. In triticale ( $\times$  *Triticosecale*), tolerance varies between germination and emergence according to the genotype, and may be improved by calcium (Norlyn and Epstein, 1984). Both cotton and sugar beet are more salt-tolerant than triticale, but are more sensitive during germination than later in their development (Kent and Lauchli, 1985; Beatty and Ehlig, 1993), although cotton yield may be only slightly reduced by salt, despite large decreases in vegetative growth (Rains et al., 1987), possibly as a result of shifts in the hormonal balance. Grain yield in rice is more depressed by salt than is vegetative growth, and germination is relatively resistant to salt (Khatun and Flowers, 1995). The developmental shifts vary with genotype. For example, in barley a cultivar very sensitive early on, Briggs, was more resistant than another, which showing early tolerance, at a later stage (Lynch et al., 1982).

Several cereals, for example wheat, sorghum and oats, develop and mature more rapidly under saline conditions, but others, for example barley and rye, are unaffected (Shannon et al., 1994). Grieve et al. (1994) found that phyllochron in spring wheat increased with salinity. Salinity decreased the number of tillers of spring and durum wheat (Maas and Grieve, 1990), and this was the main cause of yield reductions in both greenhouse-grown plants and in the field (Francois et al., 1994).

### **3. EFFECT OF COMBINED SALINITY AND WATERLOGGING ON PLANTS**

#### **3.1 Extent of the problem**

Waterlogging is commonly associated with secondary salinity, and has been a major problem in some areas of NW India and Pakistan, for example (CSSRI, 1997). It is due either to the presence of shallow water tables as a result of seepage from canals, monsoonal flooding or over-irrigation, or is the result of poor infiltration due to soil sodicity (Ghassemi et al., 1995). The problem is exacerbated in rice-wheat systems such as those of NW India and Pakistan due to the subsoil compaction that is done to produce a favourable environment for rice (Samad et al., 2001). The combination of waterlogging and salinity is far worse than the presence of either stress on its own. Barrett-Lennard (1986) suggested that a substantial proportion of salt-affected land worldwide was subject to occasional, intermittent or prolonged waterlogging.

#### **3.2 Effects on plant ion uptakes**

When a soil is waterlogged, oxygen availability is greatly reduced – the diffusion rate of oxygen is about 10,000 times lower through water than it is through air, its solubility in water is very low, and bacteria and roots use up much of the oxygen that is there – and the soil is said to have become anaerobic or hypoxic. In addition to this, carbon dioxide and various other products of root and bacterial anaerobic metabolism accumulate, leading to the development of reducing conditions in the soil. As a result, energy production from the breakdown of carbohydrate (respiration) is very much lower than is possible under aerobic (oxygenated) conditions.

As noted in section 2.3.1, plants filter out salts at the root surface to a greater or lesser extent. However, this filtering is very expensive in terms of energy, and anything that decreases its efficiency has a severe effect on plant growth and survival. Barrett-Lennard (1986) calculated that  $\text{Na}^+$  exclusion from the roots required about 2.5% of the total energy available to a root in drained soil, and it is likely that  $\text{Cl}^-$  exclusion needs about the same. In aerobic soils, glucose in the roots oxidises to produce up to 6 mol each of  $\text{H}_2\text{O}$  and  $\text{CO}_2$ , and up to 38 mol of ATP – adenosine triphosphate, the molecule that powers cell metabolism. However, in anaerobic waterlogged conditions, the process produces 2 mol each of ethanol and  $\text{CO}_2$ , and only 2 mol of ATP, a reduction of about 95% in the ATP and hence in the fuel available for the filtration mechanism.

As a result, a major impact of waterlogging under saline conditions is to prevent the roots from screening out salts, so that there are large increases in salt uptake and shoot salt concentrations in the plant. Barrett-Lennard et al. (2003) comprehensively reviewed this, and noted:



- Waterlogging interacted with salinity to increase shoot concentrations of both  $\text{Na}^+$  and  $\text{Cl}^-$ . This was initially due to increases in the net transport of these ions to the shoots, and later due to reduced shoot growth and impairment of root functions.
- This interaction led to increased  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations in the shoots, which had severe effects on plant growth and survival.
- The interaction also had profound implications for the management of salt-affected areas and for breeding crops or plant for revegetation of these lands.

### **3.3 Effect on plant growth**

Previous exposure to waterlogging can improve a plant's ability to cope with saline waterlogging. This may be due to stimulation of aerenchyma (large pores) formation in the roots (Kriedemann and Sands, 1984). These allow oxygen to diffuse through the roots, and so the plant is able to maintain energy levels.

Barrett-Lennard et al. (1999) concluded from laboratory experiments that the effects of combined salinity and hypoxia in wheat were in the main a result of adverse ion relations in the oldest leaves. These results were in agreement with those of a number of other authors (e.g. John et al., 1977; Barrett-Lennard, 1986; Marcar, 1993). The sequence of events after the onset of saline hypoxia was as follows (assuming a solution with an NaCl concentration of  $30 \text{ mol m}^{-3}$  or greater).

After one week, roots ceased to grow, and increased uptake of  $\text{Na}^+$  and  $\text{Cl}^-$ , combined with decreased  $\text{K}^+$  uptake, led to increased  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations in the oldest leaves and decreased  $\text{K}^+$  concentrations in the youngest leaves. In a further week's time, the older leaves of the main tiller began to senesce, and after another week this was at the same rate, or faster, than new leaves were being produced. In one month, or seven weeks after the onset of the stress, the plants would be more or less dead, and in any case unable to produce grain.

### **3.4 A possible screening technique for tolerance to salinity and waterlogging**

The severe effects of combined salinity and waterlogging may be a reason for the lack of success in breeding successful crop varieties for saline lands – breeding in the past has simply been for salinity and has not taken account of waterlogging as well. Barrett-Lennard et al. (1999) proposed a novel screening technique to overcome this by simultaneously testing for salinity and waterlogging tolerance. This would be done through a simple comparison of the rates of production and senescence of main culm leaves.

We tested the method in Faisalabad, Pakistan (Hollington et al., 1999) using 25 genotypes. Rates of leaf emergence and senescence were recorded over a thirty-day period. The emergence and senescence rates for each genotype were plotted, and the point at which the two slopes crossed over noted. The hypothesis was that genotypes with a long time to crossover, i.e. those in which the rate of emergence was much greater than that of senescence under saline hypoxia, would be tolerant to the combined effect of the two stresses, and that the technique will provide an easy and rapid way to identify these genotypes. Unfortunately, the relationship between rates of leaf emergence and senescence was not a good indicator of tolerance to salinity and waterlogging in

terms of shoot weight, although there was some correlation with root weight. We believe the method has potential, but that it needs much more testing and further refinement before it can be recommended.

## 4. EFFECTS OF SPRINKLER IRRIGATION

Everything so far in this Chapter has referred to the effects of soil salinity, or salinity in irrigation water where the water is applied to the soil surface and is taken up through the roots. This type of flood irrigation is still the most common in many parts of the world. However, for high-value crops, and in more modern irrigated systems, some form of sprinkler application is often used. This has major implications in areas where the waters contain salts, as tolerance to saline water applied through sprinklers is often very different to that to saline water applied through flood irrigation. The problem is that a variety that takes up or excludes  $\text{Na}^+$  or  $\text{Cl}^-$  efficiently through the roots may not show the same efficiency through the leaves. This has long been known to be the case for fruit trees and other woody plants, as shown in Table 3, but was not thought to be of concern in arable crops. However, Aragüés et al. (1994) noted that barley took up  $\text{Cl}^-$  more readily through the roots than through the leaves, and that maize in particular and barley also took up more  $\text{Na}^+$  through the leaves than through the roots (Benes et al., 1996).

**Table 3** Relative susceptibility of crops to foliar injury from sprinkler irrigation with saline water. (Maas, 1990 cited in Rhoades et al., 1992)

Na or Cl concentration causing foliar injury ( $\text{mol m}^{-3}$ )			
<5	5 – 10	10 – 20	> 20
Almond	Grape	Lucerne	Cauliflower
Apricot	Pepper	Barley	Cotton
Citrus	Potato	Maize	Sugar beet
Plum	Tomato	Cucumber	Sunflower
		Safflower	
		Sesame	
		Sorghum	

## 5. ASSESSMENT OF CROP SALINITY TOLERANCE

There are three ways in which to assess the salt tolerance of a crop. These are in terms of its survival, its absolute yield in saline conditions, or its relative yield compared with non-saline controls. Each of these has its use, but the most common assessment so far as agricultural crops is concerned is the relative yield.

### 5.1 Survival

The survival of a crop is the best method ecologically, but is pretty much useless from an agricultural point of view as it gives no measure of the agronomic usefulness of the plant, and survival is often at the expense of growth and yield.

## 5.2 Absolute yield in saline conditions

The absolute yield in saline conditions, i.e. the yield in terms of tonnes or kilograms per hectare, is the best when tolerance is looked at from economic point of view. However, a major drawback is that it does not allow comparisons to be made between different crops which may have very different growth habits. A simple, if extreme, example will make this clear.

Both sugar cane (*Saccharum officinarum*) and wheat can be grown on saline land. In normal conditions, a crop of sugar cane should yield between 70 and 100 t ha<sup>-1</sup> under rainfed conditions, and 110 – 150 t ha<sup>-1</sup> under irrigation. Similarly, bread wheat could produce around 1.3 – 2.0 t ha<sup>-1</sup> under rainfed, and 3.0 – 5.0 t ha<sup>-1</sup> under irrigated conditions. There is thus an enormous difference between the yield potential of the two crops. However, at an EC<sub>e</sub> of 10 dS m<sup>-1</sup>, irrigated sugarcane might yield 50 – 60 t ha<sup>-1</sup>, and wheat 2.5 – 3.75 t. Even though the yield of sugarcane is higher than wheat, we cannot say it is more tolerant, as its yield has been reduced much more than has that of wheat. In economic terms, it might be a more worthwhile crop, but it is NOT more tolerant.

## 5.3 Relative yield compared with non-saline controls

The advantage of using relative yield is that crops can be compared where their yields are different. Relative yield can simply be defined as the yield under saline conditions as a proportion (or percentage) of the yield under non-saline conditions. It makes no difference whether we take the yield under non-saline conditions as 100% or as 1, so we can say the relative yield is, for example, either 60% or 0.6. The use of relative comparisons is not confined to the grain yield – we can use it for any part of the plant in which we are interested, for example number of grains, height, total biomass, or oil yield. We are also not confined to using it for salinity – the same idea can be used under any other stress condition, for example to assess drought tolerance or response to heat stress.

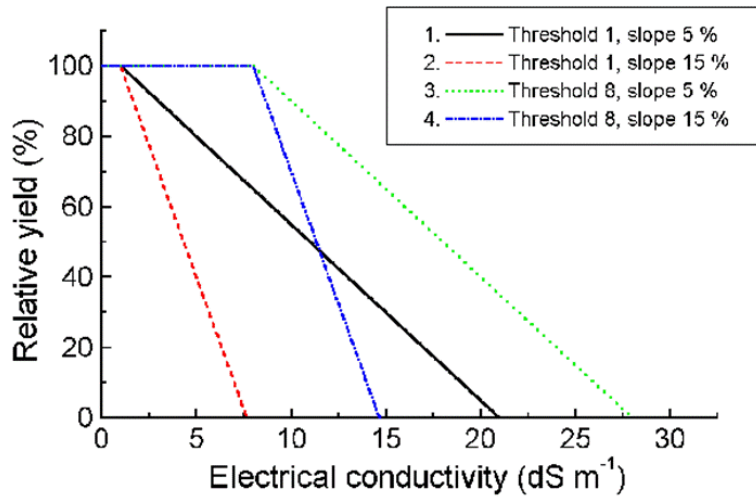
### 5.3.1 Response models

Earlier work on relative yields used the “bent stick” model of Maas and Hoffmann (1977). This described response in terms of the threshold (the salinity level below which there was no effect on growth or yield) and the slope (the reduction per unit increase salinity in the yield or other trait of interest). Using this method, workers at the US Salinity laboratory produced numerous tables of crop salt tolerance, quantifying crops as tolerant, medium tolerant, medium susceptible or susceptible. Examples include Maas (1984; 1990), and Maas and Grattan (1999), which have been used by workers all over the world. These tables also have a problem as many of the varieties and genotypes used are old, are often not specified, and are not those adapted to areas such as those in India and Pakistan where salt-tolerant crops are required. This needs to be borne in mind by anyone using them.

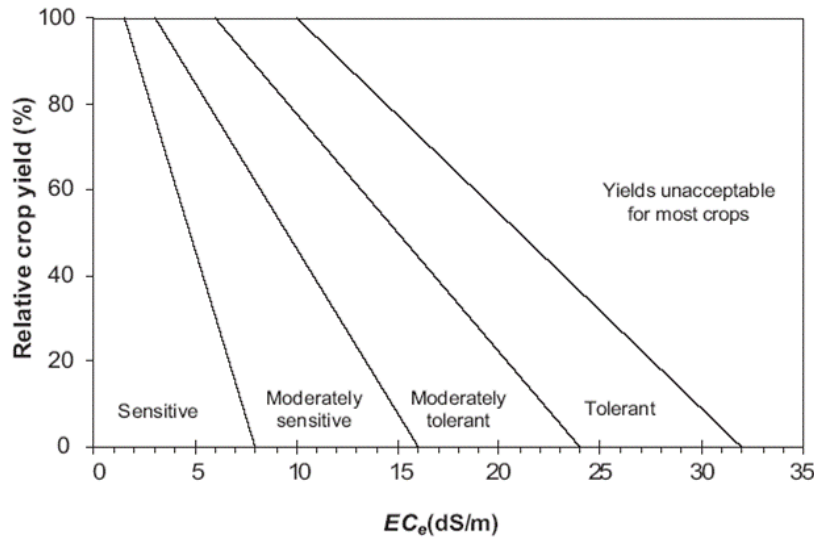
The response can be described in terms of a simple equation:

$$Y_r = 100 - b (EC_e - a)$$

where  $a$  = the salinity threshold in  $\text{dS m}^{-1}$ ,  $b$  = the slope expressed in percent per  $\text{dS m}^{-1}$ , and  $EC_e$  = the mean conductivity of the saturated paste from the rootzone. An example of the model is shown in Figure 2 below, while Figure 3 shows how this model can be used to classify crop salinity tolerance, based upon their calculated threshold and slope values.



**Figure 2.** Example of the “bent stick” model of Maas and Hoffman (1977)



**Figure 3.** Division for classifying crop tolerance to salinity (Tanji and Kielen, 2002)

Figure 2 shows the response of four hypothetical genotypes to salinity. Genotype 1 has a threshold salinity level of  $1 \text{ dS m}^{-1}$ , and a slope of 5%, i.e. for every  $\text{dS m}^{-1}$  increment in conductivity, the relative yield declines by 5%. It is somewhat more tolerant than genotype 2, which has the same threshold of  $1 \text{ dS m}^{-1}$  but a slope of 15% and is clearly the least tolerant of the four genotypes. Similarly, genotype 3, with a threshold of  $8 \text{ dS m}^{-1}$  and a slope of 5% is more tolerant than

genotype 4, which has a threshold of 8 dS m<sup>-1</sup> and a slope of 15%. Genotype 3 is the most tolerant of all. It is debatable which of genotypes 1 and 4 is the most tolerant – it would depend upon the situation in the field. Under low salinity, then genotype 4 would be preferred, but at higher levels then genotype 1 would do better.

The Maas and Hoffman (1977) model is of value to give general tolerance data and to enable managers and farmers to make decisions. For example, it is important to know roughly the salinity level at which the yield of a certain crop or variety will start to decrease, and at what rate this will occur. However, the model has a problem if there is little data to the left of the threshold value, and predictions become unreliable (Royo et al., 1991).

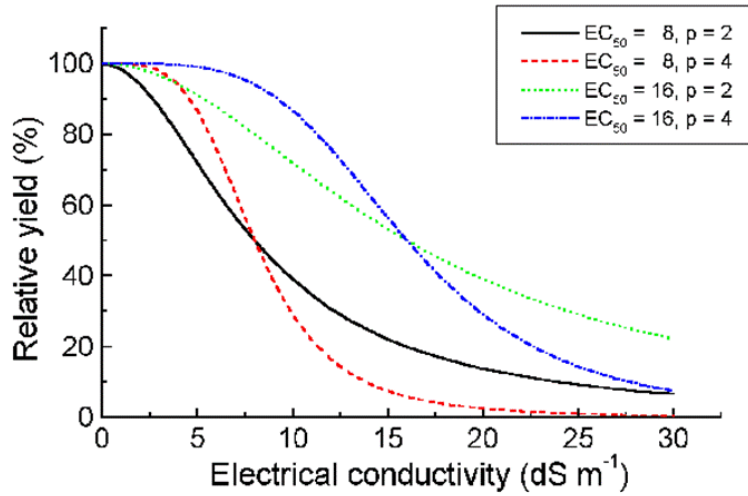
Where the Maas and Hoffman (1977) model is inappropriate, or for more precise modelling of crop response, then the non-linear model of van Genuchten and Hoffman (1984) may be preferred. This produces a continuous sigmoidal response to changes in salinity. The computer programmes to carry out these calculations, as well as those for the other models, were described by van Genuchten (1983) are available from the US salinity laboratory at <http://ars.usda.gov/Services/docs.htm?docid=8957>. The most usual model is of the form:

$$Y_r = Y_m / (1 + (EC_e / EC_{50})^P)$$

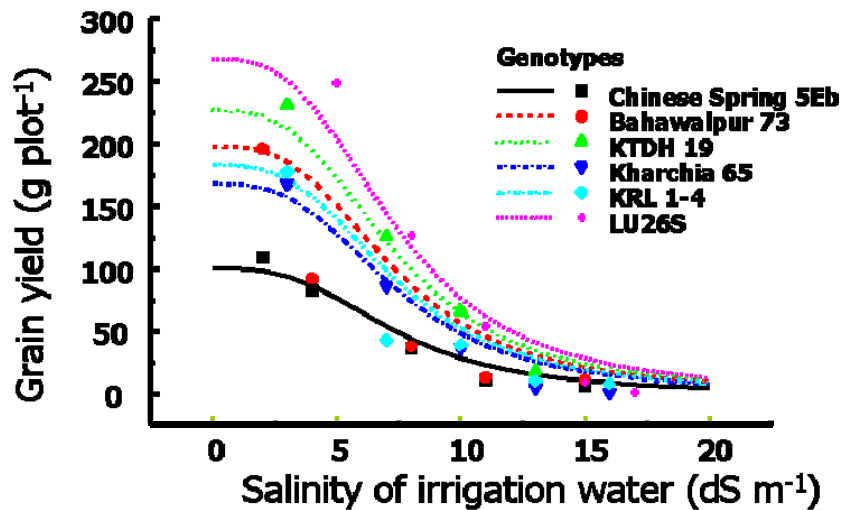
where  $Y_r$  is the relative yield for a given  $EC_e$ ,  $Y_m$  is the maximum yield that can be obtained under non-saline conditions,  $EC_{50}$  is the  $EC_e$  that reduces yield by 50% and  $P$  is a constant describing the shape of the curve. The values of  $EC_{50}$  and  $P$  are calculated by the model, and can also be calculated using non-linear regression in statistical software such as Genstat or SAS. Figure 4 shows an example of the van Genuchten model.

In Figure 4 we again have four hypothetical genotypes. This time, genotypes 1 and 2 have  $EC_{50} = 8$  dS m<sup>-1</sup>, and for genotypes 3 and 4  $EC_{50} = 16$  dS m<sup>-1</sup>, i.e. the salinity at which yield is reduced by 50% is twice as high for genotypes 3 and 4 as for genotypes 1 and 2. The values of  $P$ , the coefficient that describes the shape of the curve, are  $P = 2$  for genotypes 1 and 3, and  $P = 4$  for genotypes 2 and 4. This time, genotypes 1 and 2 are clearly less tolerant than genotypes 3 and 4, but the distinction within the tolerance groups is less clear.

Figure 5 shows the data from an actual experimental situation in a field trial in Spain (P A Hollington, unpublished data). The data is from the triple-line-sprinkler system described in Section 6, and was part of a trial to test the tolerance of wheat genotypes, including a novel addition line (Forster et al., 1988) incorporating an extra chromosome from *Thinopyrum bessarabicum* in Chinese Spring wheat (Chinese Spring 5E<sup>b</sup>). The other genotypes were tolerant Indian varieties KRL 1-4 and Kharchia 65, developed at the Central Soil Salinity Institute, Karnal, Bahawalpur, a non-tolerant Pakistani variety, LU26S, a salt-tolerant selection made by Qureshi et al. in Faisalabad from the non-tolerant Pakistani variety LU26, and KTDH 19. This was a doubled haploid line developed by Quarrie and Mahmood (1993) at the John Innes Centre, UK from a cross between the tolerant Kharchia 65 and the highly-efficient Na<sup>+</sup>-excluding line TW 161. Figure 5 also shows that the model can be used with absolute data, rather than just with relative yields.



**Figure 4.** Example of the non-linear model of van Genuchten and Hoffman (1984)



**Figure 5.** Response of wheat genotypes and hybrids to irrigation water salinity under controlled conditions in Spain (P A Hollington, unpublished data).

Figure 5 shows that, unexpectedly, on this occasion there was no yield advantage due to the additional chromosome in Chinese Spring. LU26S had the highest yield potential, but yield differences between genotypes became smaller as salinity increased.

## 6. EXPERIMENTATION FOR SALINE AREAS

Field assessment of salinity tolerance is extremely difficult due to the high variability of salinity, both spatially and temporally. However, controlled-environment trials (for example those conducted in hydroponics, floodbenches, sand culture, etc) are not a realistic prediction of field behaviour. This is because of the many differences in the stresses existing in the two situations. Even so, controlled environment trials should not be ruled out, as they are useful for physiological studies, attempts to understand the mechanisms of tolerance, and so on. Most of the early experiments carried out at the US Salinity Lab to determine salt tolerance classes for different crops were done in such conditions, and they have proved useful, in general terms, for decades.

Whatever system is used, it is imperative to ensure that it is testing tolerance to salinity, rather than to osmotic shock. Many published papers have applied the salinity all at once, rather than gradually increasing it, and do not include the addition of  $\text{Ca}^{2+}$  to the growth medium. As a result, what they test is the ability of the plant to withstand osmotic shock rather than salinity. Great scepticism should be used on data that show the results of trials in which salinity increases at a greater rate than  $50 \text{ mol m}^{-3} \text{ d}^{-1}$ , for example. Other common failings in experimental techniques include a failure to carry out experiments under conditions where plants are transpiring – this is a common failing of work on cell culture and transgenics (Flowers, 2004). Assessments need to be made of growth and vigour, and ideally, for agricultural crops, reported in terms of the yield in the field, as in the end this is the only thing that matters.

### 6.1 Field experiments

The idea is to minimise the experimental variation as much as possible in order to be able to distinguish differences between treatments (i.e. minimise the ratio of experimental to genotypic or treatment error). A method was developed for testing under natural field conditions in Spain (Ramon Aragüés, personal communication). This involves the following stages:

- Locate a plot with visual symptoms of salinity (for example white patches in some areas, differences in surface texture) and, preferably, with a regular gradient in crop yield decrements.
- Construct a detailed salinity map of the plot as described in Chapter 3. The only way to do this is through  $\text{EC}_a$  (apparent soil electrical conductivity) readings of the electromagnetic sensor on a regular (ideally 2 m x 2 m) grid. Delineate the  $\text{EC}_a$  isolines, preferably using geostatistical techniques (e.g. using computer programs such as Surfer or ESAP).
- Sow the crop or cultivars along the direction of the maximum salinity gradient. Sow in bands of 4 or 5 rows, depending upon between-row spacing, in rows of at least 25 m.
- Apply conventional cultural and agronomic practices. A regular practice of farmers is to give pre-sowing or post-sowing irrigations to leach the salts from the seedbed.
- Take regular  $\text{EC}_a$  readings (for example every 15 days and/or after each irrigation) on the previously-used grid to ascertain the temporal variation of soil salinity.

- Carry out several (e.g. 3)  $EC_a$  -  $EC_e$  calibrations ( $EC_e = a + b.EC_a$ ) during the growing season. Ensure the number of data points each time is greater than 10. Measure the  $EC_e$  of the soil in the crop rootzone. From the average calibration, convert the  $EC_a$  maps to  $EC_e$  maps.
- Harvest grain yield (or other target crop variable) in selected small areas by hand, preferably where the crop appears to be uniform (area around 0.5 - 1.0 m<sup>2</sup>). In an experiment in Spain, an area of 4 m<sup>2</sup> was harvested by machine with poor results. Harvest or sample over the maximum number of possible points (ideally 20 per genotype or other treatment) from areas of low to high yields.
- Plot the yield -  $EC_e$  observations. These  $EC_e$  values are the time-weighted average values obtained from the  $EC_e$  maps delineated in point 6 above. Decide the salt response model that best fits these observations. If necessary, carefully delete “extraneous” points. Estimate the salinity tolerance parameters (e.g. the threshold  $EC_e$  and the slope of the line, or the  $EC_{50}$  and values of  $p$  and  $Y_{max}$ ).

The system we have used for our field work in India and Pakistan modifies this to some extent. A field with visible variability in salinity levels is selected and an EM38 survey and calibrations carried out as before. If ESAP is not available, then sample positions can be selected using other methods, ensuring they are representative of the range of salinity in the field, and the plots can be made using any graphing package.

From the “contour” map of field salinity, areas of homogeneous salinity should be identified, and between eight and nine blocks selected in these. Relatively long but narrow plots should then be sown, with five or six rows and length 4 or 5 m. After every irrigation, additional sampling with the EM38 should be carried out at two or three points within each of these. At harvest, the yield and yield components should be assessed on two 1 m row lengths in the central three rows, again taking a salinity reading from the sampling point. We have found the use of eight or nine blocks to be the minimum necessary to ensure a reasonable ratio of error to experimental variation. If necessary, precision can be increased further by adjusting the data statistically, using nearest neighbour analysis or covariance on adjacent plots.

## 6.2 Controlled field trials

Controlled field trials are a useful half-way house between the variability of the natural field, and the artificial environment of a greenhouse experiment. Examples include triple-line sprinkler systems and controlled drip irrigation systems, which were developed to allow a more accurate control of field salinity without the drawbacks of the greenhouse.

The triple line source sprinkle (TLS) was developed and tested in Zaragoza, Spain (Aragüés et al., 1992; Royo and Aragüés, 1993). It consists of two outer laterals supplying fresh water ( $EC < 2$  dS m<sup>-1</sup>), and a third lateral midway between them to supply saline water  $EC$  20 dS m<sup>-1</sup>). The lateral spacing is equal to the sprinkler’s wetted radius, so the layout duplicates a salinity gradient each side of the centre line, and maintains a uniform supply of fresh water. The system provides 10 salinity treatments perpendicular to the laterals. The TLS was used for over 10 years, but over time a number of problems became apparent. The first was excess ion absorption through the leaves. Some crop varieties show different patterns of uptake by the roots compared to the leaves (e.g. Gorham et al., 1994 for barley), and ion absorption through the leaves could distort the assessment



of tolerance. As a result, the TLS is not a realistic simulation of the situation in flood irrigated areas. In addition, the TLS could only be used in very light wind conditions (wind speeds  $< 2 \text{ m s}^{-1}$ ), and was comparatively labour intensive to operate.

Despite altering the operating protocols to include pre- and post-irrigation washings of the leaves with fresh water, it was decided to develop a better system, and this led to the first controlled drip irrigation system (Isla et al., 1997). This consisted of three 3000 l PVC tanks, a pumping system, three 75 mm-diameter polyethylene lines (one for each saline treatment), and 25-mm diameter laterals with emitters located 0.2 m apart. Plot size was 1.45 x 1.25 m, allowing for six rows of plants. Within each plot there were three irrigation laterals located 0.42 m apart, and 23 evenly-spaced emitters delivering a flow of  $12 \text{ l h}^{-1}$  to provide uniform and complete wetting of the soil. The system was used for testing in the 1990s, but the frequent irrigations led to shallow rooting and a new system was developed.

This was a drip-injection irrigation (DIS) (Aragüés et al., 1999; Royo et al., 2000). This system used a parallel pump system with a centrifugal pump for fresh water and an injection pump for saline water, combined with a conventional drip irrigation system. The injection pump had a fixed discharge, while the discharge of the centrifugal pump was controlled by varying the number of emitters in each sector. The number of emitters installed in that sector therefore controlled the salinity developed in each sector. The DIS was validated, and found to be a low cost (at 1998 values about \$5000 automated and \$3000 manual) and effective system for evaluating salt tolerance under field conditions.

### 6.3 Implications of the response models for screening for salt tolerance

The models described above provide a number of parameters which have been proposed for use in screening genotypes for salt tolerance. The value of these will depend upon the particular situation being screened for. Ramon Aragüés and his group in Zaragoza, Spain, working mainly on barley under both controlled and natural field conditions, have carried out a great deal of work on this. Under controlled conditions using the TLS, both the linear and the non-linear models proved adequate for describing the response of a large number of barley genotypes to salinity (Royo and Aragüés, 1999). There was no correlation between  $EC_{50}$  and  $Y_m$ , suggesting that the most productive barley genotypes were not necessarily the least tolerant to salinity, in contrast to data from Shannon (1997) and Pasternak and De Malach (1994). In fact, the most productive genotypes under both moderate ( $6 \text{ dS m}^{-1}$ ) and high ( $12 \text{ dS m}^{-1}$ ) salinity were those with the highest yield potential,  $Y_m$ . It was also concluded that the threshold,  $EC_t$ , was not efficient for screening.  $EC_t$  was not correlated with yields at  $6 \text{ dS m}^{-1}$ , and was negatively correlated with the yield at  $12 \text{ dS m}^{-1}$ , so selecting for high  $EC_t$  would select genotypes with low yields under high salinity conditions. Under natural field conditions (Isla et al., 2003) they concluded that breeding barley for moderately saline soils (average  $EC_e$  5 – 7  $\text{dS m}^{-1}$ ) should use  $Y_m$  as the selection criterion, but on soils of high salinity ( $EC_e$  around  $15 \text{ dS m}^{-1}$ ) then selection should be based upon a combination index  $EC_{50} \times (Y_m \times 10^{-3})$ .

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## REFERENCES

- Amtmann, A., and Sanders, D., 1999, Mechanisms of Na<sup>+</sup> uptake by plant cells, *Adv. Bot. Res.* 29:75-1122.
- Aragüés, R., Playán, E., Ortiz, R., and Royo, A., 1999, A new drip-irrigation system (DIS) for crop salt tolerance evaluation, *Soil Soc. Sci. Am. J.* 63:1397-1404.
- Aragüés, R., Royo, A., and Faci, J., 1992, Evaluation of a triple line source sprinkler system for salinity crop production studies, *Soil Sci. Soc. Am. J.* 56:377-383.
- Aragüés, R., Royo, A., and Grattan, S., 1994, Foliar uptake of sodium and chloride in barley sprinkler-irrigated with saline water: effect of pre-irrigation with fresh water, *Eur. J. Agron.* 3:9-16.
- Aslam, M., Huffaker, R. C., and Rains, D. W., 1984, Early effects of salinity on nitrate assimilation in barley seedlings, *Plant Physiol.* 76:321-325.
- Ball, M. C., 1988, Salinity tolerance in the mangroves *Aegiceras corniculatum* and *Avicennia marina*. I. Water use in relation to growth, carbon partitioning and salt balance, *Aust. J. Plant Physiol.* 15:447-464.
- Ball, M. C., Chow, W. S., and Anderson, J. M., 1987, Salinity-induced potassium deficiency causes loss of functional photosystem II in leaves of the grey mangrove, *Avicennia marina*, through depletion of the atrazine-binding polypeptide, *Aust. J. Plant Physiol.* 14:351-361.
- Barrett-Lennard, E. G., 1986, Effects of waterlogging on growth and NaCl uptake by vascular plants under saline conditions, *Rec. Reveg. Res.* 5:245-261.
- Barrett-Lennard, E. G., 2003, The interaction between waterlogging and salinity in higher plants: causes, consequences and implications, *Plant Soil* 253:35-54.
- Barrett-Lennard, E. G., van Ratingen, P., and Mathie, M. H., 1999, The developing pattern of damage in wheat (*Triticum aestivum* L.) due to the combined stresses of salinity and hypoxia: experiments under controlled conditions suggest a methodology for plant selection, *Aust. J. Agric. Res.* 50:129-136.
- Beatty, K. D., and Ehlig, C. F., 1973, A technique for testing and selecting for salt tolerance in sugar beet, *J. Am. Soc. Sugar Beet Tech.* 17:295-299.
- Bernstein, L., 1965, Salt Tolerance of Fruit Crops, USDA Inf. Bull. 292, US Govt. Printing Office, Washington DC, USA, 8pp.
- Bernstein, L., Ehlig, C. F., and Clark, R. A., 1969, Effect of grape rootstocks on chloride accumulation in leaves, *J. Am. Soc. Hort. Sci.* 94:584-590.
- Berthomieu, P., Conéjéro, G., Nublat, A., Brackenbury, W. J., Lambert, C., Savio, C., Uozumi, N., Oiki, S., Yamada, K., Cellier, F., Gosti, F., Simonneau, T., Essah, P. A., Tester, M., Véry, A. A., Sentenac, H., and Casse, F., 2003, Functional analysis of AtHKT1 in *Arabidopsis* shows that Na<sup>+</sup> recirculation by the phloem is crucial for salt tolerance, *EMBO J.* 22:2004-2014.

- Bower, C. A., 1959, The Chemical Amendments for Improving Sodium Soils, Agric. Info. Bull. 195, USDA, Washington, D.C., USA.
- Byrt, C. S., Platten, J. D., Spielmeyer, W., James, R. A., Lagudah, E. S., Dennis, E. S., Tester, M., and Munns, R., 2007, KLT1;5-like transporters linked to Na<sup>+</sup> exclusion loci in wheat, *Nax2* and *Kna1*, *Plant Physiol.* 143:1918-1928.
- Causse, M. A., Fulton, T. M., Cho, Y. G., Ahn, S. N., Chunwongse, J., Wu, K., Xiao, J., Yu, Z., Ronald, P. C., Harington, S. E., Second, G., McCouch, S. R., and Tanksley, S. D., 1994, Saturated molecular map of rice genome based on an interspecific backcross population, *Genet.* 138:1251-1274.
- Chen, Z., Newman, I., Zhou, M., Mendham, N., Zhang, G., and Shabala, S., 2005, Screening plants for salt tolerance by measuring K<sup>+</sup> flux: a case study for barley, *Plant Cell Environ.* 28:230-246.
- Colmer, T. D., Fan, T. W.-M., Higashi, R. M., and Läuchli, A., 1996, Interactive effects of Ca<sup>2+</sup> and NaCl salinity on the ionic relations and proline accumulation in the primary root tip of *Sorghum bicolor*, *Physiol. Plant.* 97:421-424.
- Colmer, T. D., Munns, R., and Flowers, T. J., 2005, Improving salt tolerance of wheat and barley: future prospects, *Aust. J. Exp. Agric.* 45:1425-1443.
- Cramer, G. R., Alberico, G. J., and Schidt, C., 1994, Salt tolerance is not associated with the sodium accumulation of two maize hybrids, *Aust. J. Plant. Physiol.* 21:675-692.
- Cramer, G. R., Epstein, E., and Läuchli, A., 1988, Kinetics of root elongation of maize in response to short-term exposure to NaCl and elevated Ca concentration, *J. Exp. Bot.* 39:1513-1522
- CSSRI ,1997, Vision 2020. CSSRI Perspective Plan, Central Soil Salinity Research Institute, Karnal, Haryana, India, 95 pp.
- Davenport, R., James, R. A., Zakrisson-Plogander, A., Tester, M., and Munns, R., 2005, Control of sodium transport in durum wheat, *Plant Physiol.* 137:807-818.
- Downton, W. J. S., 1978, Growth and flowering in salt-stressed avocado trees, *Aust. J. Agric. Res.* 29:523-534.
- Dubcovsky, J., Santa Maria, G., Epstein, E., Luo, M.-C., and Dvorak, J., 1996, Mapping of the K<sup>+</sup>/Na<sup>+</sup> discrimination locus *Kna1* in wheat, *Theor. Appl. Gen.* 92:448-454.
- Dvořák, J., and Gorham, J., 1992, Methodology of gene transfer by homoeologous recombination into *Triticum turgidum*: transfer of K<sup>+</sup>/Na<sup>+</sup> discrimination from *T. aestivum*, *Genome* 30:639-646.
- Dvořák, J., Edge, M., and Ross, K., 1988, On the evolution and the adaptation of *Lophopyrum elongatum* to growth in saline environments, *Proc. Nat. Acad. Sci, USA* 85:3805-3809.
- El Hendawy, S. E., Hu, Y., and Schmidhalter, U., 2005, Growth, ion content, gas exchange, and water relations of wheat genotypes differing in salt tolerances, *Aust. J. Agric. Res.* 56:123-134.
- Flowers, T. J., 2004, Improving crop salt tolerance, *J. Exp. Bot.* 55:307-319.
- Flowers, T. J., Hajibagheri, M. A., and Clipson, N. J. W., 1986, Halophytes, *Q. Rev. Biol.* 61:313-337.
- Flowers, T. J., Koyama, M. L., Flowers, S. A., Sudhakar, C., Singh, K. P., and Yeo, A. R., 2000, QTL: their place in engineering tolerance of rice to salinity, *J. Exp. Bot.* 51:99-106.
- Flowers, T. J., Troke, P. F., and Yeo, A. R., 1977, The mechanism of salt tolerance in halophytes, *Ann. Rev. Plant Physiol.* 28:183-121.

- Foolad, M. R., and Lin, G. Y., 1997, Absence of a genetic relationship between salt tolerance during seed germination and vegetative growth in tomato, *Plant Breed.* 116:363-367.
- Forster, B. P., Miller, T. E., and Law, C. N., 1988, Salt tolerance of two wheat - *Agropyron junceum* disomic addition lines, *Genome* 30:559-564.
- Fortmeier, R., and Schubert, S., 1995, Salt tolerance of maize (*Zea mays* L): the role of sodium exclusion, *Plant Cell Environ.* 18:1041-1047.
- Francois, L. E., and Maas, E. V., 1994, Crop response and management on salt-affected soils, in *Handbook of plant and crop stress*, M. Pessarakli, ed., Marcel Dekker, New York., pp. 149-181.
- Frensch, J., and Hsaio, T. C., 1994, Transient responses of cell turgor and growth of maize roots as affected by changes in water potential, *Plant Physiol.* 104:247-254.
- Frensch, J., and Hsaio, T. C., 1995, Rapid response of the yield threshold and turgor regulation during adjustment of root growth to water stress in *Zea mays*, *Plant Physiol.* 108:303-312.
- Garcia, A., Senadhira, D., Flowers, T. J., and Yeo, A. R., 1997, The effects of selection for sodium transport and of selection for agronomic characteristics upon salt resistance in rice (*Oryza sativa* L.), *Theor. Appl. Gen.* 90:1106-1111.
- Genc, Y., McDonald, G. K., and Tester, M., 2007, Reassessment of tissue  $\text{Na}^+$  concentration as a criterion for salinity tolerance in bread wheat, *Plant Cell Environ.* 30:1486-1498.
- Ghassemi, F., Jakeman, A. J., and Nix, H. A., (eds.) 1995, *Salinisation of Land and Water Resources: Human Causes, Extent, Management and Case Studies*, CAB International, Wallingford, UK.
- Gorham, J., 1994, Salt tolerance in the Triticeae: K/Na discrimination in some perennial wheatgrasses and their amphiploids with wheat, *J. Exp. Bot* 45:441-447.
- Gorham, J., Hardy, C., Wyn Jones, R. G., Joppa, L. R., and Law, C. N., 1987, Chromosomal location of a K/Na discrimination character in the D genome of wheat, *Theor. Appl. Gen.* 74:584-588.
- Gorham, J., Papa, R., and Aloy-Lleonart, M., 1994, Varietal differences in sodium uptake in barley cultivars exposed to soil differences or salt spray, *J. Exp. Bot.* 45:895-901.
- Gorham, J., Wyn Jones, R. G., and Bristol, M., 1990, Partial characterisation of the trait for enhanced  $\text{K}^+$ - $\text{Na}^+$  discrimination in the D genome of wheat, *Planta* 180:590-597.
- Greenway, H., and Munns, R., 1980, Mechanisms of salt tolerance in non-halophytes, *Ann. Rev. Plant Physiol.* 31:149-190.
- Greenway, H., and Osmond, C. B., 1972, Salt responses of enzymes from species differing in salt tolerance, *Plant Physiol.* 49:256-259.
- Gregorio, G. B., Senadhira, D., Mendoza, R. D., Manigbas, N. L., Roxas, J. P., and Guerta, C. Q., 2002, Progress in breeding for salinity tolerance and associated abiotic stresses in rice, *Field Crops Res.* 76:91-101.
- Grieve, C. M., Francois, L. E., and Maas, E. V., 1994, Salinity affects the timing of phasic development in spring wheat, *Crop Sci.* 34:1544-1549.
- Haro, R., Banuelos, M. A., Senn, M. E., Berrero-Gil, J., and Rodríguez-Navarro, A., 2005, HKT1 mediates sodium uniport in roots: pitfalls in the expression of HKT1 in yeast, *Plant Physiol.* 139:1495-1506

- Hasegawa, P. M., Brennan, R. A., Zhu, J.-K., and Bohnert, H. J., 2000, Plant cellular and molecular responses to high salinity, *Ann. Rev. Plant Physiol. Mol. Biol.* 51:463-499
- Hollington, P. A., Akhtar, J., Aragüés, R., Gill, K. S., Hussain, Z., Quarrie, S. A., and Rashid, A., 1999, First Annual Report, EU INCO-DC Project ERBIC 18CT 980305 "Assessment and development of salinity, sodicity and waterlogging tolerant wheat genotypes for India and Pakistan," Centre for Arid Zone Studies, University of Wales, Bangor, 126 pp.
- Hsaio, T. C., and Xu, L.-K., 2000, Sensitivity of growth of roots versus leaves to water stress: biophysical analysis and relation to water transport, *J. Exp. Bot.* 51:1595-1616.
- Hu, Y., and Schmidhalter, U., 1998, Spatial distributions and net deposition rates of mineral elements in the elongating wheat (*Triticum aestivum* L.) leaf under saline soil conditions, *Planta* 204:212-219.
- Huang, S., Spielmeier, W., Lagudah, E. S., and Munns, R., 2008, Comparative mapping of HKT genes in wheat, barley, and rice, key determinants of Na<sup>+</sup> transport, and salt tolerance, *J. Exp. Bot.* 59:927 – 937.
- Huang, S., Spielmeier, W., Lagudah, E. S., James, R. A., Platten, J. D., Dennis, E. S., and Munns, R., 2006, A sodium transporter (HKT7) is a candidate for Nax1, a gene for salt tolerance in durum wheat, *Plant Physiol.* 142:1718-1727.
- IRRI, 1998, Programme Report for 1997, International Rice Research Institute, Los Baños, Philippines.
- Isla, R., Aragüés, R., and Royo, A., 2003, Spatial variability of salt-affected soils in the middle Ebro valley (Spain) and implications in plant breeding for increased productivity, *Euphytica* 134:325-334.
- Isla, R., Royo, A. and Aragüés, R. (1997) Field screening of barley cultivars to soil salinity using a sprinkler and a drip irrigation system, *Plant Soil* 197:105-117.
- James, R. A., Davenport, R., and Munns, R., 2006, Physiological characterization of two genes for Na<sup>+</sup> exclusion in wheat: Nax1 and Nax2, *Plant Physiol.* 142:1537-1547.
- James, R. A., Rivelli, A. R., Munns, R., and van Caemmerer, S., 2002, Factors affecting CO<sub>2</sub> assimilation, leaf injury and growth in salt-stressed durum wheat, *Func. Plant Biol.* 29:1393-1403.
- James, R. A., von Caemmerer, S., Condon, A. G., Zwarf, A. B., and Munns, R., 2008, Genetic variation in tolerance to the osmotic stress component of salinity stress in durum wheat, *Func. Plant Biol.* 35:111-123.
- Jeschke, W. D., 1984, K<sup>+</sup>-Na<sup>+</sup> exchange at cellular membranes, intracellular compartmentation of cations, and salt tolerance, in: *Salinity Tolerance in Plants: Strategies for Crop Improvement*, R. C. Staples, and G. H. Toenniessen, eds., John Wiley and Co., New York, USA, pp 37-66.
- Jeschke, W. D., Aslam, Z., and Greenway, H., 1986, Effects of NaCl on ion relations and carbohydrate status of roots and on osmotic regulation of roots and shoots of *Atriplex amnicola*, *Plant Cell Environ.* 9:559-569.
- Jiang, Q., Roche, D., Monaco, T. A., and Hole, D., 2006, Stomatal conductance is a key parameter to assess limitations to photosynthesis and growth potential in barley genotypes, *Plant Biol.* 8:515-521.
- John, C. D., Limpinuntana, V., and Greenway, H., 1977, Interaction of salinity and anaerobiosis in barley and rice, *J. Exp. Bot.* 28:133-141.
- Kent, L. M., and Lauchli, A., 1985, Germination and seedling growth of cotton: salinity-calcium interactions, *Plant Cell Environ.* 8:155-159
- Khatun, S., and Flowers, T. J., 1995, Effects of salinity on seed set in rice, *Plant Cell Environ.* 18:61-67.

- Koebner, R. M. D., Martin, P. K., Orford, S. M., and Miller, T.E., 1996, Responses to salt stress controlled by the homoeologous group 5 chromosomes of hexaploid wheat, *Plant Breed.* 115:81-84.
- Koyama, M. L., Levesley, A., Koebner, R. M. D., Flowers, T. J., and Yeo, A. R., 2001, Quantitative trait loci for component physiological traits determining salt tolerance in rice, *Plant Physiol.* 125:406-422.
- Kriedemann, P. E., and Sands, R., 1984, Salt resistance and adaptation to root-zone hypoxia in sunflower, *Aust. J. Plant Physiol.* 11:287-301.
- Kurth, E., Jensen, A., and Epstein, E., 1986, Resistance of fully imbibed tomato seeds to very high salinities, *Plant Cell Environ.* 9: 667-676.
- LaHaye, P. A., and Epstein, E., 1969, Salt tolerance by plants: enhancement with calcium, *Science* 166:395-396.
- Landon, J. R., ed, 1991, *Booker Tropical Soil Manual: a handbook for soil survey and agricultural land evaluation in the tropics and subtropics.* Booker Agriculture International Ltd., London, UK, ISBN 0-582-00557-4. 475 pp.
- Laüchli, A., 1984, Salt exclusion: an adaptation of legumes for crops and pastures under saline conditions, in: *Salinity Tolerance in Plants: Strategies for Crop Improvement*, R. C. Staples, and G. H. Toenniessen, eds., John Wiley and Co., New York, USA, pp 171-187.
- Laüchli, A., and Epstein, E., 1970, Transport of potassium and rubidium in plant roots. The significance of calcium, *Plant Physiol* 45:639-641.
- Laüchli, A., and Epstein, E., 1990, Plant responses to saline and sodic conditions. Chapter 6 in: K. K. Tanji, ed., *Agricultural Salinity Assessment and Management.* ACSE Manuals and reports on engineering practice No. 71, ASCE, New York, USA, ISBN 0-87262-762-4, pp. 113-137.
- Laüchli, A., and Wieneke, J., 1979, Studies on growth and distribution of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  in soybean varieties differing in salt tolerance, *Z. Pflanzennähr. Bodenkde.* 142:3-13.
- Lin, H. X., Zhu, M. Z., Yano, M., Gao, J. P., Liang, Z. W., Su, W. A., Hu, X. H., Ren, Z. H., and Chao, D. Y., 2004. QTLs for  $\text{Na}^+$  and  $\text{K}^+$  uptake of the shoots and roots controlling rice salt tolerance, *Theor. Appl. Genet* 108:253-260.
- Lindsay, M. P., Lagudah, E. S., Hare, R. A., and Munns, R., 2004, A locus for sodium exclusion (Nax1), a trait for salt tolerance, mapped in durum wheat, *Funct. Plant Biol.* 31:1105-1114
- Lynch, J., Epstein, E., and Laüchli, A., 1982,  $\text{Na}^+$  -  $\text{K}^+$  relationships in salt-stressed barley, in: A. Scaife, (ed), *Plant Nutrition 1982. Proc. 9<sup>th</sup> Int. Plant Nutrition Colloq.*, CAB, Slough, UK, pp 347-352.
- Maas, E. V., 1984, Salt tolerance of plants, in: *Handbook of Plant Science in Agriculture*, B. R. Christie, ed., CRC Press, Boca Raton, Florida, USA.
- Maas, E. V., 1990, Crop salt tolerance, chapter 13 in: *Agricultural Salinity Assessment and Management*, K. K. Tanji, ed., ACSE Manuals and reports on engineering practice No. 71, ASCE, New York, USA, ISBN 0-87262-762-4, pp. 262-304.
- Maas, E. V., and Grattan, S. R., 1999 Crop yields as affected by salinity, in *Agricultural Drainage*, R. W. Skaggs, and J. van Schilfgaarde, eds., US Salinity Lab., Riverside, California, USA. pp. 55-108
- Maas, E. V., and Grieve, C. M., Spike and leaf development in salt-stressed wheat, *Crop Sci.* 30:1309-1313.

- Maas, E. V., and Hoffman, G. J., 1977, Crop salt tolerance - current assessment, J. Irrig. Drain. Div., Am. Soc. Civ. Eng. 103 (IR2):115-134.
- Maas, E. V., Hoffman, G. J., Chaba, G. D., Poss, J. A., and Shannon, M. C., 1983, Salt sensitivity of corn at various growth stages, Irrig. Sci. 4:45-57.
- Mahmood, A., and Quarrie, S. A., 1993, Effects of salinity on growth, ionic relations and physiological traits of wheat, disomic addition lines from *Thinopyrum bessarabicum*, and two amphiploids, Plant Breed. 110:265-276.
- Martin, P. K., Taeb, M., and Koebner, R. M. D., 1993, The effect of photoperiod insensitivity on the salt tolerance of amphiploids between bread wheat (*Triticum aestivum*) and sand couch grass (*Thinopyrum bessarabicum*), Plant Breed. 111: 283-289.
- Munns, R., 1985,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$  in xylem sap flowing to shoots of NaCl-treated barley, J. Exp. Bot. 36:1032-1042.
- Munns, R., 1993, Physiological processes limiting plant growth in saline soil: some dogmas and hypotheses, Plant Cell Environ. 16:15-24.
- Munns, R., 2002, Comparative physiology of salt and water stress, Plant Cell Environ. 25:239-250.
- Munns, R., and Sharp, R. E., 1993, Involvement of abscisic acid in controlling plant growth in soils of low water potential, Aust. J. Plant Physiol. 20:425-437.
- Munns, R., and Tester, M., 2008, Mechanisms of salinity tolerance, Ann. Rev. Plant. Biol. 59:651-681.
- Munns, R., Greenway, H., and Kirst, G. O., 1983, Halotolerant eukaryotes, in Physiological Plant Ecology III. Responses to the Chemical and Biological Environment, O. Lange, P. S. Nobel, C. B. Osmond, and H. H. Zeigler, eds., Encyclopaedia of Plant Physiology, New Series, Vol 12C, Springer Verlag, Berlin, Germany, pp 59-135.
- Munns, R., Hare, R. A., James, R. A., and Rebetzke, G. J., 2000, Genetic variation for improving the salt tolerance of durum wheat, Aust. J. Agric. Res. 51:69-74.
- Munns, R., Rebetzke, G. J., Husain, S., James, R. A., and Hare, R. A., 2003, Genetic control of sodium exclusion in durum wheat, Aust. J. Agric. Res. 54:627-635
- Munns, R., Schachtman, D. P., and Condon, A. G., 1995, The significance of a two-phase growth response to salinity in wheat and barley, Aust. J. Plant Physiol. 22:561-569.
- Norlyn, J., and Epstein, E., 1984, Variability in salt tolerance of four triticale lines at germination and emergence, Crop Sci. 24:1090-1092.
- Omielan, J. A., Epstein, E., and Dvořák, J., 1991, Salt tolerance and ionic relations of wheat as affected by individual chromosomes of salt-tolerant *Lophopyrum elongatum*, Genome 34:961-974.
- Passioura, J. B., and Munns, R., 2000, Rapid environmental changes that affect leaf water status induce transient surges or pauses in leaf expansion rate, Aust. J. Plant Physiol. 27:941-948.
- Pasternak, D., and De Malach, Y., 1994, Crop irrigation with saline water, in Handbook of Plant and Crop Stress, M. Pessarakli, ed., Marcel Dekker, New York, USA.
- Pitman, M. G., 1984, Transport across the root and shoot/root interactions, in Salinity Tolerance in Plants: Strategies for Crop Improvement, R. C. Staples, and G. H. Toenniessen, eds., John Wiley and Co., New York, USA, pp 92-123

- Quarrie, S. A., and Mahmood, A., 1993, Improving salt tolerance in hexaploid wheat, Annual Report 1992, AFRC Institute of Plant Science Research Cambridge Laboratory John Innes Institute Nitrogen Fixation Laboratory and Sainsbury Laboratory, p 4.
- Quarrie, S. A., Steed, A., Calestani, C., Semikhodskii, A., Lebreton, C., Chinoy, C., Steele, N., Pljevljakusic, D., Waterman, E., Weyen, J., Schondelmaier, J., Habash, D. Z., Farmer, P., Saker, L., Clarkson, D. T., Abugalieva, A., Yessimbekova, M., Turuspekov, Y., Abugalieva, S., Tuberosa, R., Sanguineti, M.-C., Hollington, P. A., Aragüés, R., Royo, A., and Dodig, D., 2005 A high density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring x SQ1 and its use to compare QTLs for grain yield across a range of environments, *Theor. Appl. Genet.* 110:865-880.
- Qureshi, R. H., and Barrett-Lennard, E. G., 1998, Saline Agriculture for Irrigated Land in Pakistan: a Handbook, ACIAR Monograph 50, ACIAR, Canberra, Australia, ISBN 1 86320 220 X vi + 142 pp.
- Rains, D. W., Goyal, S., Weyrauch, R., and Laüchli, A., 1987, Saline drainage water reuse in a cotton rotation system, *Cal.Agric.* 41 (9-10):24-26.
- Raven, J. A., 1985, Regulation of pH and generation os osmolarity in vascular plants: a cost-benefit analysis in relation to efficiency of use of energy, nitrogen and water, *New Phytol.* 101:25-77.
- Ren Z. H., Gao J. P., Li L. G., Cai X. L., Huang W., Chao D. Y., Zhu M. Z., Wang Z. Y., Luan S., and Lin H. X., 2005, A rice quantitative trait locus for salt tolerance encodes a sodium transporter, *Nat. Genet.* 37:1029-30.
- Reynolds, M. P., Mujeeb-Kazi, A., and Sawkins, M., 2005, Prospects for utilising plant-adaptive mechanisms to improve wheat and other crops in drought- and salinity-prone environments, *Ann. Appl. Biol.* 146:239-259.
- Royo, A., and Aragüés, R., 1993, Validation of salinity crop production functions obtained with the triple line source sprinkler system, *Agron. J.* 85:795-800.
- Royo, A., and Aragüés, R., 1999, Salinity-yield response functions of barley genotypes assessed with a triple line source sprinkler, *Plant Soil* 209:9-20.
- Royo, A., Aragüés, R., and Quilez, D., 1991, Descripcion y evaluacion de cuatro modelos de respuesta de cultivares de cebada a la salinidad, *Invest. Agr: Prod. Veg.* 6:319-330.
- Royo, A., Aragüés, R., Playan, E., and Ortiz, R., 2000, Salinity-grain yield response functions of barley cultivars assessed with a drip-injection sprinkler system, *Soil Sci. Soc. Am. J.* 64:359-365.
- Samad, A., Meisner, C. A., Saifuzzaman, M., and van Ginkel, M., 2001, Waterlogging tolerance, in: *Application of Physiology in Wheat Breeding*, M. P. Reynolds, J. I. Ortiz-Monasterio, and A. McNab, eds., CIMMYT, Mexico City, Mexico.
- Schubert, S., and Laüchli, A., 1986, Na<sup>+</sup> exclusion, H<sup>+</sup> release, and growth of two different maize cultivars under NaCl salinity, *J. Plant Physiol.* 126:145-154.
- Semikhodskii, A. G., Quarrie, S. A., and Snape, J. W., 1997, Mapping quantitative trait loci for salinity responses in wheat, in: *Drought and Plant Production Vol.2*, S. Jevtic and S. Pekic, eds., Proceedings of International Symposium, Donji Milanovac, Serbia, Sept 1996. Agricultural Research Institute, Belgrade, Serbia, pp 83-92.
- Shannon, M. C., 1997, Adaptation of plants to salinity, *Adv. Agron.* 60:75-120.
- Storey, R., and Walker, R. R., 1999, Citrus and salinity, *Sci. Hort.* 78:39-81.



- Taeb, M., Koebner, R. M. D., Forster, B. P., and Law, C. N., 1992, Association between genes controlling flowering time and shoot sodium accumulation in the Triticeae, *Plant Soil* 146:117-121.
- Takehisa, H., Shimodate, T., Fukuta, Y., Ueda, T., Yano, M., Yamaya, T., Kameya, T., and Sato, T., 2004 Identification of quantitative trait loci for plant growth of rice in paddy field flooded with salt water, *Fld. Crops Res.* 89:85-95.
- Tanji, K. K., and Kielen, N., 2002, *Agricultural Drainage Water Management in Arid and Semi-arid Areas*, FAO Irrig. Drain. Paper 61, FAO, Rome, Italy, 188 pp.
- Termaat, A., Passioura, J. B., and Munns, R., 1985, Shoot turgor does not limit shoot growth of NaCl-affected wheat and barley, *Plant Physiol.* 77:869-872.
- van Genuchten, M. T., 1983, *Analyzing Crop Salt Tolerance Data: Model Description And Users' Manual*, USDA-ARS US Salinity Lab. Res. Rep. No. 120.
- van Genuchten, M. T., and Hoffman, G. J., 1984, Analysis of crop salt tolerance data, in: *Soil Salinity under Irrigation – Process and Management*, I. Shainberg, and J. Shalhevet, eds., *Ecological Studies* 51, Springer Verlag, Berlin, Germany, pp 258-271.
- Wyn Jones R. G., and Storey, R., 1978, Salt stress and comparative physiology in the Gramineae. II. Glycinebetaine and proline accumulation in two salt- and water-stressed barley cultivars, *Aust. J. Plant Physiol.* 5:817-829.
- Yeo, A. R., Lee, K.-S., Izard, P., Boursier, P. J., and Flowers, T. J., 1991, Short and long-term effects of salinity on leaf growth in rice (*Oryza sativa* L), *J. Exp. Bot.* 42:881-889.
- Yeo, A. R., Yeo, M. E., Flowers, S. A., and Flowers, T. J., 1990, Screening of rice (*Oryza sativa* L) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance, *Theor. Appl. Genet.* 79:377-384.
- Zhong, G.Y., and Dvořák, J., 1995a, Evidence for common genetic mechanisms controlling the tolerance of sudden salt stress in the tribe Triticeae, *Plant Breed.* 114:297-302.
- Zhong, G.Y., and Dvořák, J., 1995b, Chromosomal control of the tolerance of gradually and suddenly imposed salt stress in the *Lophopyrum elongatum* and wheat, *Triticum aestivum* L, genomes, *Theor. Appl. Genet.* 90:229-236.

## **CHAPTER 4**

# **ADVANCES IN BREEDING WHEAT FOR SALT TOLERANCE**

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## **1. INTRODUCTION**

Salinization and drought are main abiotic stresses that seriously affect the growth and development of cultivated plants and agricultural production. Over 7% of the world's land is affected by either salinity or sodicity. The total area of saline-alkali soil in China is about 33.5 million ha, among which 6.7 million ha have been brought under cultivation. The rest 20 million more ha of saline-alkali land are waiting to be opened up and utilized. In addition, more than 6.7 million ha of fertile farmland have become secondary salinized soil due to the inappropriate irrigation methods, making up about 10% of the total area under cultivation all over China.

Wheat is one of the most important world food crops, and its productivity directly affects human survival and quality of life. Wheat is now the second important crop with the annual cultivation area of more than 26 million ha in China. The food production and the living standard of the people in arid and saline-alkali areas are increasingly restricted. The genetic improvement of salt tolerance and stress resistance in wheat has aroused great concern and is paid more and more attention by the breeders. Salt tolerance and drought tolerance are quantitative characters. There is a shortage in available germplasm that can be utilized. Salt tolerant variety development by conventional method has been generally limited. The development of the nuclear technology and related biotechnology and the great achievements obtained provide a possibility to tackle this difficult breeding problem effectively. This paper will review the present status and further perspectives of the improvement of salt-tolerant varieties by use of mutation techniques and biotechnology in saline-alkali areas.

## **2. SOME BASIC PHYSIOLOGICAL PARAMETERS OF PLANT SALT TOLERANCE**

Plant salt tolerance is generally defined as the inherent ability of the plant to withstand the effects of high salts in the root or the plant's leaves without significant adverse effects on plant productivity. Salt tolerance is a complex trait involving responses to cellular ionic stresses and

osmotic stresses and their consequent stresses such as oxidative stress and whole plant coordination. These developments have been summarized in excellent reviews (Flowers, 1977; Greenway and Munns, 1980; Zhu, 2000; Hasegawa et al; 2000; Munns and Tester, 2008; Pang and Wang, 2008).

Despite intensive scientific efforts during the last decade, the physiological processes and mechanisms involved in plant sensitivity and tolerance to salinity are not well understood. For lack of a better option, identification of traits and genes for genetic manipulation towards increased tolerance relies on unproved dogmas for tolerance mechanisms.

By far, the problems in designing proper selection criteria for salinity tolerance are a major setback in making progress in breeding for resistance. However, some criteria have been available for salt tolerance screening in the breeding programme or for mechanism studies. Zhao and Dou (1998) reviewed more than ten kinds of criteria to be used in salt tolerance screening and identification in wheat. Under salt stress, seedling survival ratio, root length, shoot height and seedling weight, tiller number in spring and grain yield are the useful morphological and growth indicators. At the same time,  $K^+/Na^+$  ratio in leaves, cell membrane permeability, ATP and SOD enzyme activities, glycinebetaine and polyamine accumulation, cell wall hydroxyproline and cell surface glycoprotein content, and salinity stress-induced tissue specific protein could also be used as the physiological and biochemical criteria under salt stress conditions. Munns et al (2002) recommended rates of  $Na^+$  or  $Cl^-$  accumulation in leaves, degree of leaf injury, seedling root length, and germination percentage as the key traits for salt tolerance screening in wheat.

Seed germination in saline media is often used as a singular criterion or in combination with other criteria. Seed germination in saline media is a legitimate criterion for improving seed germination in saline media (Blum, 1988). Plants may be more tolerant or more susceptible to salinity at germination than at subsequent growth stages (Noble, 1983). It has therefore been proposed that the use of germination in saline media is an inappropriate criterion for species in which germination is more resistant to salinity because the problem lies in subsequent susceptible stages. On the other hand, Noble (1983) suggested that selection at germination is important in species that are relatively susceptible at this stage.

Selection during vegetative growth may involve several criteria. Seedling or plant dry matter as an expression of total growth is an important criterion. Wheat plants grown at 100 mM NaCl produced less dry matter and chlorophyll content than those without NaCl, and supplementary Si at both 0.25 and 0.5 mM ameliorated the negative effects of salinity on plant dry matter and chlorophyll content (Tuna, et al, 2008). It integrates the various possible effects of response to salinity into one measure of resistance. There are, however, several problems in growth measurement. First, this is a destructive method which is unacceptable for selection at early generations. Secondly, genotype may differ in their potential growth, and their growth differences under salinity stress may represent their potential capacity rather than their specific tolerance. As the case for several other environmental stresses, growth under stress can be used as a criterion of tolerance only if it is compared with growth under non-stress conditions in all genotypes. The measurement of the rate of growth reduction under stress as compared with non-stress conditions entails double the work load and facilities.

Other criteria could be used as indirect estimates of the effect of salinity during vegetative growth. Leaf desiccation and death caused by salinity stress has been estimated either in terms of leaf numbers affected or by a visual estimate of the affected leaf area in proportion to total leaf area (Noble and Rogers, 1992). In extreme cases the situation is simple enough, where the population clearly truncates into surviving and death plants (Blum, 1988). In species where salinity tolerance is conditioned largely by salt exclusion, leaf analysis for the specific ion content would seem to be an important criterion (Yeo and Flowers, 1983). Salt tolerance in the Triticeae is associated with sodium exclusion, which limits the entry of sodium into the plant and its transport to leaves. Sodium exclusion from the transpiration stream reaching the leaves is controlled at three stages: (1) selectivity of the root cells taking up cations from the soil solution, (2) selectivity in the loading of cations into the xylem vessels in the roots, and (3) removal of sodium from the xylem in the upper part of the roots and the lower part of the shoot (Munns et al., 2002).

In wheat, salt tolerance is associated with low rates of transport of  $\text{Na}^+$  to shoots with high selectivity for  $\text{K}^+$  over  $\text{Na}^+$  (Ding et al, 2006). The most successful relate to rates of  $\text{Na}^+$  or  $\text{Cl}^-$  accumulation in leaves, measured as the increase in salt in a given leaf over a fixed period of time. Bread wheat (hexaploid) cultivars are able to exclude  $\text{Na}^+$  from the leaves, however, durum wheat (tetraploid) cultivars lack this trait (Dubcovsky et al., 1996). Recently, a novel source of  $\text{Na}^+$  exclusion was identified in a durum landrace (Munns et al., 2000). The landrace had very low rates of  $\text{Na}^+$  accumulation in the leaf blade, as low as bread wheat cultivars, and maintained a high rate of  $\text{K}^+$  accumulation, with consequent high  $\text{K}^+/\text{Na}^+$  discrimination. The low  $\text{Na}^+$  trait was shown to confer a significant yield advantage at moderate soil salinity (Husain et al., 2003), indicating that this novel germplasm provides the opportunity to improve the salt tolerance of cultivated durum wheat. The low rates of  $\text{Na}^+$  uptake and accumulation in a given leaf as a non-destructive and accurate quantitative trait has been used in durum wheat (Munns et al, 2002). But the  $\text{Na}^+$  and  $\text{Cl}^-$  exclusion did not always reflect the salt tolerance, whereas  $\text{K}^+$  in the leaves and  $\text{Ca}^{2+}$  in the leaves and stems were closely associated with genotypic differences in salt tolerance (Salah et al, 2005). Significantly positive correlations among  $\text{K}^+/\text{Na}^+$ , reproductive growth period and total growth period were noted in salt-sensitive varieties, however, none significant relations appeared among those parameters in salt-tolerant varieties, indicating higher salinity tolerance varieties of winter wheat could relieve senescence at the reproductive stage (Zheng et al, 2008).

Criteria for the capacity for osmoregulation as a component for tolerance were suggested (Blum, 1988; Liang et al, 2006)). Membrane stability has been widely used to differentiate stress-tolerant and -susceptible cultivars (Blum and Ebercon 1981) and usually higher membrane stability is correlated with abiotic stress tolerance (Premachandra et al. 1991). Cell membrane stability technique was proved to be suitable for screening wheat tolerance to high salinity and for detecting differences that may arise due to cumulative effects of salinity and reduced water contents. Membrane stability as a salt tolerant criterion could be used more effectively than grain yield for screening large quantities of germplasm at seedling stage ( Farooq and Farooq, 2006). The detail introduction of this technique is also seen in this volume.

As root growth often express well the relative tolerance of a plant to mineral toxicity, it was considered also as a possible criterion of tolerance to salinity (Blum, 1988). Due to the genetic basis and significant correlation with grain yield, root length could be used as a selection criterion to identify salt tolerant wheat genotypes (Zulfiqar, 2004).

Physiological studies, geared toward identification of specific molecular cellular processes of sensitivity or tolerance, might potentially shorten the duration required for development of tolerant crop species. Most crop species are sensitive to salt stress at all stages of plant development, including seed germination, vegetative growth and reproduction. As a result, their growth and economic yield are substantially reduced under salt stress. In addition, stress tolerance appears to be a developmentally regulated, stage-specific phenomenon; tolerance at one stage of plant development is often not correlated with tolerance at other developmental stages. Thus, specific ontogenic stages, including seed germination and emergence, seedling survival and growth, and vegetative growth and reproduction, should be evaluated separately for assessment of tolerance and the identification, characterization and utilization of useful genetic components. Each developmental stage (which may be considered as a separate trait) may require a different screening procedure, and simultaneous or sequential screening may be impractical or impossible. Partitioning of stress tolerance into component traits related to ontogenic stages may facilitate a better understanding of the genetic basis of stress tolerance and development of stress tolerant plants (IAEA, 2002).

### **3. GENETIC DIVERSITY FOR SALT TOLERANCE**

To breed crop plants tolerant to salinity, there must be available significant amount of variation in salinity tolerance in the gene pool. Norlyn (1908) and Shannon (1984) discussed the subject of genetic variability among plants in responses to salinity. Genetic variation for salt tolerance has been found in world collections of bread wheat (Quershi et al., 1980; Kingsbury and Epstein, 1984; Sayed, 1985). Singh and Chatrath (1992) screened and found variation for salt tolerance in tissue-culture-derived wheat lines developed at CIMMYT. The chromosomal locations of genes controlling tolerance are located on all seven homoeologous chromosome groups of the Triticeae. However, group 4 and group 5 chromosomes are predominant for most stresses (Forster, 1994).

Guo et al (2001) screened 161 Tibetan wheat accessions for salt tolerance under three different salt concentrations and found 9.5% of the accessions possessed high salt tolerance and three among them were salt-insensitive, revealing the rich variations in salt tolerance in the Tibetan wheat germplasm. Zhu et al (1996) identified sixty-one with better salt tolerance and agronomical characters screened from 900 collections of wheat varieties or advanced lines. Ma and Wang (2005) screened 28 spring wheat varieties introduced from America for salt-tolerance at the stage of both germination and seedling. There were 13 of them showed high salt tolerance in respect of grain yield and its components, in which two varieties SW10 and SW12 performed higher salt tolerance and grain yield under the heavy salinity affected soil conditions.

Zulfiqar (2004) reported the variations found in the seedling responses of 98 wheat accessions to increasing NaCl concentrations in rooting medium and indicated the potential variability within this species. The genetic variation for salt tolerance was shown to be influenced predominantly by the genes with additive effects based on root length. The heritability estimated under low and high salinity was appreciable and seems to be promising for genetic improvement of salt tolerance in wheat.

Trethowan and Mujeeb-Kazi (2008) reviewed three sources of novel genetic variability, namely synthetic wheat, landrace cultivars, and alien introgressions and their applicability to applied wheat breeding. Synthetic hexaploid wheat, derived by crossing tetraploid wheat with *Aegilops tauschii*, provides new genetic variability for adaptation to drought, high temperature, salinity, waterlogging. Synthetic-derived materials have performed well in many stressed environments globally. There is significant unexploited variation among landraces and modern wheat cultivars to improve the stress adaptation of cultivated wheat. They concluded that there is sufficient genetic variation in the wheat gene pool to ensure the continued improvement of wheat adaptation to abiotic stress.

#### **4. CONVENTIONAL BREEDING FOR SALT TOLERANCE**

The conventional breeding methods have been used to improve wheat salt tolerance in different countries such as Indian, Pakistan and China, etc. In India, efforts to improve salt tolerance of crops by scientists at Central Soil Salinity Research Institute, Karnal over the last two decades, have led to the development and release of varieties of wheat, rice, chickpea, and Indian mustard suitable for cultivation in alkali and saline soils (Sharma and Goyal, 2003). The first salt tolerant wheat variety has been the Indian KRL1-4, derived from a cross of the highly salt tolerant Indian landrace Kharchia 65 with a popular high yielding wheat variety WL 711, was released in 1990. Another salt tolerant wheat variety, KRL 19, was released in 1999. It has better tolerance to rusts and other diseases and a safe replacement of KRL1-4. In India, almost all salt tolerant wheat germplasm is derived from Kharchia 65 (Hollington, 2000). In Pakistan, LU26S and a series of salt tolerant wheat varieties named after Saline Agriculture Research Cell (SARC-1,2,3, and 4) were developed and released.

In China, a high salt tolerant variety Jimai32, developed by the State Sino-Czechic Farm in Hebei province through a cross of local high yield selection Nongda311 and a Kefan68 which was from a wide hybridizing combination, was released in 1992. It is still currently cultivated in the saline areas in Hebei province for its high tolerance to salt and drought as well as good yield performance (Zhao et al, 2000). Using Jimai32 as male parent to make cross with a high yield variety Lifan6145, the separating progenies were advanced along with the parallel alternative selection in two environments of salt stress and non-salt stress at the same time. This led to another new variety Cang6001 with good tolerance to salt and drought stress was released in 1998 and planted as one of the best varieties up to now in the saline areas of Hebei Province (Zhao et al, 2000). In the past decade, the recurrent selection via a *Taigu* genic male-sterile wheat line to pyramid different sources of salt tolerant genes has been employed in the breeding programme for wheat salt tolerance, and some promising advanced lines such as Cang026, Cang030 and Cang036 have been put into multi-location regional for release in Cangzhou Academy of Agricultural Sciences in Hebei Province (Yu Liang et al, personal com).

Dezhou Academy of Agricultural Sciences in Shandong Province is another specialized institute on crop salt tolerance improvement in China. Some elite salt tolerant wheat varieties such as Dexuan1, Lumai10, Lude1 and Dehang961 have been successfully developed by using re-selection or cross breeding method and put into production since the sixties of the 20th century.

There are also several salt tolerant wheat varieties such as Changfangbai, 166, Shidong5, Shidong7 and Xindong26, etc. have been developed and officially released in Xinjiang province since 1980s. Both single cross and multi-cross between or among the salt tolerant selections and local high yielding varieties were usually made to upgrade the breeding efficiency for salt tolerance improvement (Luo and Ren, 2001; Luo et al, 2005; Luo et al, 2006).

## 5. WIDE HYBRIDIZATION IN WHEAT

Some recent extensive reviews of the use of wild relatives to improve the salt tolerance of wheat are available (Colmer et al. 2006; Munns and Richards, 2007). *Thinopyrum bessarabicum* is a perennial species within the graminaceous tribe Triticeae and is more salt resistant than the annual *Triticum aestivum* (bread wheat). The amphidiploid produced (Forster and Miller, 1985) by hybridizing the bread wheat Chinese Spring and *Th. bessarabicum* was found to be more resistant in terms of survival and ability to produce grain at moderate salinity (250 mol ma), than Chinese Spring or even Kharchia. The greater resistance of the amphidiploid was attributed to its inheritance of more efficient exclusion of Na<sup>+</sup> and Cl<sup>-</sup> from younger leaves and reproductive tissue (Gorham et al., 1986).

It is also known that bread wheat (AABBDD) expresses more K<sup>+</sup>/Na<sup>+</sup> selectivity than tetraploid (AABB) wheats, as K<sup>+</sup>/Na<sup>+</sup> was found to be associated with chromosome 4 of the D genome (Gorham et al., 1987). Although there appears to be little allelic variation for this character in hexaploid wheat, variation may exist in *Aegilops squarrosa*, donor of the D genome, and other relatives of wheat carrying this genome. Variation in the salt tolerance of the D genome was shown to influence the salt tolerance of synthetic hexaploids. Schachtman *et al* (1992) produced synthetic hexaploids from five *Ae. squarrosa* accessions varying in salt tolerance and two salt-sensitive *T. turgidum*. The relative grain yield of the hexaploids in 150 mol m<sup>-3</sup> NaCl was greater than that of the tetraploid parents, primarily due to the maintenance of grain weight under salinity. *Aegilops tauschii* (DD) has been hybridized with durum wheat (AABB) to produce synthetic hexaploid wheat (Schachtman et al., 1992; Mujeeb-Kazi and Diaz de Leon, 2002).

An interesting result from the Chinese scientists showed that *Ae. crassa* 6x cytoplasm were able to produce significant genetic effects on salt tolerance of common wheats, the character and range of effect value were related to the nuclear genotype of alloplasmic common wheat, the salt tolerance of alloplasmic common wheat could be significantly increased in specific NC-combination. The salt-tolerant results from experiments of callus inducing and development in salt culture-medium, seed germination in salt solution and seedling development in salt Hoagland -solution indicated that alloplasmic lines *Ae. crassa* 6x-Jian 26 and *Ae. crassa* 6x-SMH1694 were more salt-tolerant than their nuclear parents. The results at recovering stage and mature stage demonstrated that some alloplasmic common wheats with improved nuclear genotypes were more salt-tolerant than or equal to salt-tolerant control variety Keyi 26 (Hou et al, 2004).

Studies using wheat tetrasomic lines (2x=44) and wheat/*Agropyron junceum* disomic lines (2x=44) have shown that chromosomes 2A, 2B, and 2D of wheat and 2J of *A. junceum* carry genes that confer salt susceptibility. However, chromosome 2J also appears to carry genes for salt tolerance (Forster et al., 1988).

These findings suggest the existence of genes with major effects that might be exploited to increase salt tolerance.

A successful programme to introduce tolerance from *Ae. cylindrica*(CCDD) to hexaploid wheat was undertaken in Pakistan (Farooq *et al* 1992a;1992b). They obtained the backcrossed lines produced from hybrids between *Ae. cylindrica* and the Pakistani cultivars LU26 and Pak81. These were tested in both saline and non-saline fields and shown to be both salt and drought-tolerant (Farooq *et al.*, 1995; Farooq, 2004). Wheat lines WL1076 and WL41 out yielded LU26, the salt-tolerant parent, and require less irrigation water and fertilizer than other genotypes. They performed higher yield than the current wheat variety Inqlab (Farooq, 2004; Farooq and Azam, 2005). The amphidiploid wheat plants were also produced through crossing two moderately salt-tolerant wheats, namely *Triticum turgidum durum* (AABB )and *Aegilops speltoidessubsp. speltoides*(SS),. They showed greater salinity tolerance than either parent assessed with the criterion of root growth in different NaCl concentrations (Ahmad and Noori, 2005).

Tall wheatgrasses (E or J genomes) are very salt tolerant. At low to moderate salinity, they have a similar decline in biomass as does barley and the more tolerant bread wheat varieties, but continue to grow at high salinity even up to seawater concentrations and beyond (Munns and Richards, 2007).

King *et al* (1996) assessed the salt tolerance of the addition lines and 5Eb(5A) and 5Eb(5D) substitution lines. The 5Eb addition line survived better than Chinese Spring, while the substitution lines not only survived better than Chinese Spring, but also better than the addition line, showing that the gene or genes had a greater effect when substituted for a homoeologous wheat group 5 chromosome than when present as an additional chromosome. King *et al* (1997) developed a new cereal (*Tritipyrum*) as was triticale. The tetraploid wheats were hybridized with *Th. bess*, and wheat-like amphiploids produced. They tested some of these materials with the octaploid amphiploid Chinese Spring x *Th. bess*, and the wheat parents, at 4 salinity levels. Survival was far better in the Tritypyrum at 150 mol m<sup>-3</sup> NaCl, where 90% of the wheat died, but was much less at 200 and 250 mol m<sup>-3</sup> although little or no wheat survived at these levels.

*Thinopyrum ponticum* (decaploid, E genome) is the “tall wheatgrass” commonly used as a forage crop in saline land, and is very salt tolerant. Somatic hybridization techniques were used to transfer *Th. ponticum* chromosomes into bread wheat, and introgression lines derived from somatic hybrids between *Th. ponticum* and *Triticum aestivum* L. cv. Jinan 177 were screened for salt-tolerance in hydroponic experiments. Their growth rates, salt-tolerance index and the content of free proline, Na<sup>+</sup>, K<sup>+</sup> and Na<sup>+</sup>/K<sup>+</sup> in the leaf were compared with those of wheat cv. Jinan 177. The F4 and F5 generation lines were tested under natural saline conditions in two locations. One line expressed higher salt-tolerance than its parental wheat and a check salt-tolerant cultivar. Karyotype analysis showed that the two lines possessed 42 chromosomes of wheat introgressed with small chromosome segments from *Th. ponticum*. Thus, salt tolerance of *Th. ponticum* appears to have been introgressed into bread wheat, with the *Th. ponticum* chromatin stably inherited (Chen *et al.*, 2004). This new line named Shanrong 3 has been officially released in Shandong province in 2004. Salt tolerant germplasm, developed from a translocated segment of *Thinopyrum junceum* has been recently registered as W4909 and W4910 (Wang *et al.*, 2003).



## **6. INDUCED MUTATIONS FOR SALT TOLERANCE IMPROVEMENT IN WHEAT**

Induced mutations have been used mainly to improve particular characters in well-adapted local varieties or to generate variation difficult to be found in germplasm collections. The high efficiency of classical mutagenesis to generate mutations valuable for breeders has been widely proven and documented through the official release of 2,570 mutant varieties including, as indicated in the FAO/IAEA Mutant Varieties Database (<http://www.infocris.iaea.org/MVD/>). New varieties with increased resistance to disease, salinity, high and low temperatures and lodging have been developed by induced mutation with physical and chemical mutagens (Jain, 2001). Making a general survey of the progress in the past years research work, the induced mutations for improvement of salt tolerance of wheat and other crops can be summarized as the following approaches: induction and selection by radiation of seed; in vitro selection through tissue culture-derived variation; indirect utilization or cross of mutants for salt-tolerant improvement (Liu et al, 2003).

### **6.1 Mutation induction from seed treatment**

Seeds are the most commonly used materials for induced mutation. Mutagenic treatments fall into two main classes: physical ( $\gamma$  rays, x rays, laser, UV, etc.) and chemical (EMS and  $\text{NaN}_3$  etc.). Once seed of a parental line has been treated with a mutagen it is referred to as  $M_0$  seed and produces  $M_1$  plants. Because of the possible chimeras and physiological disorders, selection is not normally practiced until later generations. Generally, individual mutation could be screened and selected in the  $M_2$  or  $M_3$  population. For the targeted trait of salt tolerance, mutation screening and selection of mutants should be conducted under the certain salt stress pressure. This method has been employed in Chinese wheat improvement since nineteen seventies. Some wheat mutant varieties with improved salt tolerance have been developed, officially released, and put into production, such as Jiaxuan No.1 examined and approved in 1974, Changwei No.19 in 1978 and Yuandong No. 3 in 1986 (Liu et al, 2003).

Guo and his colleagues (1997) obtained saline-tolerant wheat lines by using 8.6Gy X-ray irradiation. In their experiment, germinating wheat seeds soaked in 1mg/ml caffeine for 3h before irradiation or treated with heat (43°C, 20min) after irradiation were cultured in 1.9%NaCl solution for saline-tolerant mutant induction. After continuous selection of survival wheat seedlings in 1.9% and 2.0%NaCl solution, twelve stable saline-tolerant wheat selections have been put into field test, in which two lines, i.e., Zhishen No.7 and Zhishen No.2 yielded twice as that of control cv. Lumai No. 10 when planted in 0.6% saline soil.

Another successful example is the very famous barley variety Golden Promise, which is a gamma rays-induced high-yielding and short-height mutant from the original variety Maythorpe. This mutant variety has made a major impact on the brewing industry in Europe and has also been used as parent of more than 150 leading barley varieties worldwide. It was showed that Golden Promise was able to limit the amount of sodium taken up during salt stress and showed more salt tolerance

than Maythorpe in terms of yield and shoot  $\text{Na}^+$ . The most beneficial effect may result from the reduction in the number of sterile spikelets in Golden Promise compared to Maythorpe (Forster et al, 1994; 2001).

## **6.2 Tissue culture-derived salt tolerance**

Of all environmental stresses, the possibilities of selection in vitro were explored most for salinity tolerance. This due to the importance of the issue, the difficulties in achieving final results in plant selection, and the ease of applying salinity stress to any in vitro system (Blum, 1988). In vitro selection for salt tolerance is commonly occurs as a result of a temporary adaptation; cells are able to compartment the excessive salts into vacuoles, and survive by adjusting the osmotic pressure. The first stage after the establishment of a cell culture from any suitable source material is the induction and isolation of salt-tolerant cells and cell lines in the culture. Salinity stress is commonly applied by the addition of NaCl to the culture. In order to eliminate variants that have only some transient adaptation to the saline culture, salinity stress has to be high enough to kill more than 95% of the cells (Blum, 1988). But Barakat and Abdel-latif (1996) also reported that the stepwise method of increasing NaCl in the medium was more effective for plant regeneration than other methods. The second stage involves the regenerating plants from resistance cell lines. Irrespective of salinity tolerance, plant regeneration from cell lines is a well established routine in wheat. However, because of the genotype dependence to some extent, it is probably highly advised to establish varieties with high regeneration capacity before work in cell culture is initiated. A high efficient regeneration system for in vitro culture of young spike in wheat has well established to decrease or overcome the genotype dependence (Liu et al, 2001).

Regenerated plantlets may or may not carry salt tolerance, thus the salinity tolerance of the regenerated plants must be retained through plant reproduction. Dracup (1991) thought that the selection of cultured cells may be more productive if focused on specific cell-based physiological traits (such as  $\text{Na}^+$  accumulation, turgor regulation or tolerance to high  $\text{Na}^+ : \text{Ca}^{2+}$ ) rather than on tolerance to high NaCl only. While the successful production of salt-tolerant regenerated plants revealed that, although there is a large difference between the conditions where the cultured cells are selected and conditions where the salt tolerance of the regenerated plants is evaluated, at least some of the cellular mechanisms of salt tolerance operating in cultured cells operate also in the regenerated plants. The correlation between the mechanisms operating in cultured cells and in the whole plant have been found from the comparison of salt tolerance in the whole plant and in cells isolated from it (Tal, 1994).

Cell or tissue culture generates a wide range of genetic variation in plant species which can be incorporated in plant breeding programmes. It could further be enhanced by applying various physical and chemical mutagen treatments. Combined with in vitro selection, mutants with useful agronomic traits, e.g. salt or drought tolerance or disease resistance, can be isolated in a short duration. This approach has been successfully employed in various crop species to obtain salt-tolerant cell lines (Jain, 2001; Hossain et al, 2006; Liu et al, 2003, 2007).

Use of doubled haploid (DH) breeding through anther culture and microspore cultures saves many generations normally needed to produce pure breeding lines. It also enhances the effectiveness of

selection of desired recombinants. The advantages of DH technologies have long been recognized by breeders and resulted in more than 300 varieties produced with the use of various DH methods in several crops (Szarejko and Forster, 2007). In China, pure breeding diploid lines have been developed through anther culture and microspore culture *in vitro* to produce DH lines; nearly 100 new varieties officially released have been developed in crop plants such as wheat, rice, rapeseed and barley (Liu, unpublished).

Mutagenesis in combination with DH-systems has been successful in developing valuable mutations for other characters such as salt and drought tolerant crop lines. Increasing certain physical/chemical mutagens and salt stress on the culture has become a very successful way to select salt-tolerant mutants of wheat.

A technical system of salt-tolerant wheat selection through DH technique combined with nuclear irradiation has been well established in the Institute of Crop Science of Chinese Academy of Agricultural Sciences (Liu et al, 2006). Salt tolerant variants could be obtained via anther culture in salt stressed media. Wheat anthers were irradiated with 1.5Gy gamma rays and then cultured on medium containing 0.5% NaCl for callus induction. After recurrent selection on NaCl-enriched medium, stably resistant cultures were isolated and regenerated into plantlets for progeny test. A serious good line with prominent salt-tolerant and drought-tolerant characters has been developed, in which H6756 was officially released in Shandong province in 2004. The average yield of H6756 in the regional multi-location test was 12.5% higher than the local control variety DK961. Dozens of demonstration spots in Beijing, Shandong and Hebei province were investigated and its yield potential was 6.75-7.50 t per ha in the saline land areas with about 0.3-0.4% NaCl content (Liu et al, 2007).

The process of selecting salt-tolerant varieties can be sped up effectively by using the salt-tolerant mutants as cross parents and combining with DH technology. The anthers of F<sub>1</sub> generation of Yong445 × Yuandong 3 were induced on the medium containing 0.4% NaCl for the first selection and then callus differentiation was conducted on the medium containing the same concentration of NaCl for the second selection. Regenerated pollen plants grew for propagation in the soil without salt-stress for one generation to remove the possible existing physiological adaptability or postnatal genetic salt-tolerant phenotype. After three generations in succession of field identification and selection for the salt tolerance, the salt-tolerant line H89 was obtained. The field identification of salt tolerance of H89 for many years all reached the first grade. H89 can grow in soil containing NaCl as high as 0.73%. In the salinized soil containing 0.17-0.73% NaCl, the yield of H89 was obviously higher than that of the local control variety Laizhou 953 (Zheng et al, 1996).

A series of salt-tolerant mutants have been also developed by using calli of anther or matured embryo after mutagenizing with EMS, Pingyangmycin(PYM) or Zhengdingmycin(ZDM). Salt-tolerant calli were induced in inducing medium containing certain amount of mutagenic agent, and the salt-tolerant plants could be easily regenerated after such calli were transferred to the differential medium with same amount of salt as the selective medium. Genetic analysis of the high generation materials of salt-tolerant mutants showed that the wheat salt-tolerant trait is not only controlled by nuclear gene, but also affected by cytoplasmic factor (Shen et al, 1993; 1997; see also this volume Zhao et al). The stable salt-tolerant variants of wheat from anther culture *in vitro* selection could be also found in the literatures (Xiao et al, 1989; Zhao et al, 1994; 1995).

## 7. IDENTIFICATION OF CANDIDATE GENES FOR SALINITY TOLERANCE

A comprehensive discussion of candidate genes for salt tolerance is given by Tester and Davenport (2003), particularly in relation to roots, and by Munns (2005) particularly in relation to leaves and an expected phenotype. In salt resistance, specific changes in the profile of proteins, whose biological functions are related to such environmental stress tolerance, have been observed in many plants (Gulick and Dvorak 1987, Caruso, et al, 2008), and several proteins have been characterized to play prominent roles in response to salt stress. It was found that some proteins could be newly synthesized or increased under salt stress conditions. By using SDS-PAGE analysis, Zheng et al (1994) and Zhu et al (1996) observed the significant increased change for the proteins of 26 kDa and 52 kDa in the salt tolerant variety, while the change rate was very lower in the salt sensitive genotypes, implying these two kinds of proteins may be related to wheat salt tolerance.

Huo et al (2004) analyzed the proteome of the salt tolerant mutant of wheat RH8706249 and the salt sensitive mutant of wheat H8706234 which had been treated by 1 % NaCl for 72 hours. The qualitative and quantitative differences were identified between the two materials for five candidate proteins: H<sup>+</sup>-transporting two sector ATPase , glutamine synthetase 2 precursor , putative 33 kD oxygen evolving protein of photosystem II and ribulose-1 , 5-bisphosphosphate carboxylase/ oxygenase small subunit. The se five proteins are all belong to chloroplast proteins. They are likely to play a crucial role in keeping the function of the chloroplast and the whole cells when the plant was under salt stress. Two-dimensional electrophoresis and matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) was used to identify proteins affected by salinity in *Triticum durum*. A set of control plants was grown without NaCl addition under the same conditions as the salt-treated plants. 38 proteins whose levels were altered in response to salt stress were identified. In particular, ten proteins were downregulated and 28 were upregulated (Caruso, et al, 2008). The abundance of proteins, involved in carbohydrate and energy metabolism, decreased after NaCl treatment, whereas the abundance of some proteins involved in ROS scavenging increased, indicating that the identified proteins in wheat leaf are implicated in diverse physiological and defence processes, and while some are probably part of a general stress response to help plants survive in suboptimal conditions, others may contribute to the reduction of the negative physiological effects of the salt treatment (Caruso, et al, 2008).

Zhao et al (2007) analyzed the expression of all genes in root of wheat salt-tolerant mutant RH8706-49 by using gene chip technology. The differential expression profile of 61215 genes was gained. The clustering analysis of hybridization data showed that gene expression changed greatly in root under salt stress. Both salt-induced genes and salt-inhibited genes were found, revealing the mechanism of salt-tolerance in wheat is very complicated and it is the result of a lot of genes expression in line.

Expression of the genes encoding group 3 LEA proteins is thought to related to stress tolerance in young seedling. Yu et al (2004) cloned a novel gene in the group 3 *Lea* genes, named as *TaLEA 3*, from wheat and the results showed that *TaLEA3* was mainly located in cytoplasm and was induced

by high salt, low temperature and exogenous ABA treatments. Han et al (2006) cloned the sequence of *glutamine synthase 2* (*GS2*) from the salt tolerant wheat mutant RH8706-49. It was showed that *GS2* expressed in both salt tolerant wheat RH8706-49 and salt sensitive wheat H8706-34 which was the offspring propagated by one-seed mutant, but the expression was significant higher in the former than in the later, indicating that *GS2* is a kind of salt inhibitory gene and may be regulated at the transcription level. Ge et al (2007) cloned the full-length cDNA sequence of *TaSTK* gene from the salt tolerant wheat mutant RH8706-49 by RACE method. Northern blotting results showed *TaSTK* was a salt induced gene in wheat. Under salt stress, the expression of *TaSTK* was strongly promoted in the salt tolerant line BH870Fr49 than in the salt sensitive line H8706-34. But the hybridization signals were all very weak, indicating the *TaSTK* belongs to the low expression gene in the wheat seedling tissue. Mi et al (2006) obtained a full-length of cDNA of a vacuolar ATPase C subunit (*TaVHA-C*) from a wheat line 98-160, which has a excellent salt tolerance controlled by major genes, via cDNA-AFLP. Northern blot analysis showed both salt and drought stress had a similar effect on the expression of *TaVHA-C*. This gene could be useful to improve plant stress tolerance.

## **8. MARKER-ASSISTED SELECTION (MAS) FOR SALINITY TOLERANCE**

The direct selection of salt-tolerant genotypes under field conditions is hindered by the significant influence that environmental factors have on the response of plants to salinity. There is also evidence supporting the notion that salt tolerance is a complex trait involving the function of many genes. The development of molecular biology techniques has enabled the development of DNA markers that can be used to identify QTLs. The use of QTLs has improved the efficiency of selection, in particular for those traits that are controlled by several genes and are highly influenced by environmental factors. QTLs and marker-assisted selection provide several advantages over direct phenotypic screening, particularly because the PCR-based methodologies used to detect the markers reduce the time needed to screen individuals and reduce the impact of environmental effects on the trait under study. There is considerable evidence to support the view that salt tolerance and its sub-traits are determined by multiple QTLs and that both additive and dominance effects are important in the inheritance of many of the traits associated with salt tolerance (Foolad, 2004; Flowers, 2004).

Salt related QTLs have been mapped in tomato (Foolad 2001; Foolad and Jones 1993), barley (Yoshiro and Kazuyoshi 1997), soybean (Lee et al. 2004), Arabidopsis (Quesada et al. 2002) and rice (Gong et al. 1998; Gu et al. 2000; Lin et al. 1998, 2004; Zhang et al. 1995). Hollington (1998) reported productivity under saline conditions was associated largely with genes affecting flowering time and ion accumulation, and many QTL effects were around the vernalization response gene *Vrn1* on chromosome 5A. Traits regulating productivity under stress conditions can be identified by comparative QTL analysis and measures of productivity such as biomass production or yield. The coincidence of major QTLs for 2 traits such as leaf ion content and grain yield would indicate their likely association, and the identification of alleles of markers associated

with major QTLs for improved salt-tolerance would allow the techniques to be used to improve the efficiency of selecting for better salt-tolerance.

In comparisons between hexaploid, tetraploid and diploid types, it was suggested that bread wheat cultivars have a low rate of Na<sup>+</sup> accumulation and enhanced K<sup>+</sup>/Na<sup>+</sup> discrimination, a character located on the long arm of chromosome 4D (Gorham et al., 1987). This character is controlled by a single locus (*Kna1*) and has been linked to molecular markers on the distal third of chromosome 4DL (Dubcovsky et al., 1996), is likely an *HKT1;5* gene (Byrt et al., 2007)). *Kna1* is associated with a higher leaf K<sup>+</sup>/Na<sup>+</sup> ratio and was attributed with providing bread wheat with its superior salinity tolerance over tetraploid wheats (Gorham et al., 1987).

The gene or genes associated with this locus have not been identified. A number of attempts were made to map the QTL controlling Na exclusion, but only one QTL was successfully located to chromosome 2AL (Munns et al. 2002; Lindsay et al. 2004). In a study of yield under salt stress, Quarrie et al. (2005) identified two candidate QTL, mapping to probable homoeologous regions in the proximal parts of chromosomes 5B and 5D. The identification of salt tolerance at both the germination and seedling stages is particularly important (Yoshiro and Kazuyoshi 1997), Ma et al (2007) used a population of 114 recombinant inbred lines derived from the cross Opata85 · W7984, to find the QTL related to the response of wheat to salt stress. 47 QTL mapping to all wheat chromosomes except 1B, 1D, 4B, 5D and 7D were identified. Of these QTL, 10 were effective during the germination stage, and 37 at the seedling stage. The QTL in the intervals Xglk683–Xcdo460 (chromosome 3AS) and Xfbb168–Xbcd147 (chromosome 3BL) were effective at both the germination and seedling stages.

Two dominant genes with major effect for exceptionally low rates of Na<sup>+</sup> accumulation in leaves, which have been named *Nax1* and *Nax2* (for Na<sup>+</sup> exclusion) were identified (Munns et al., 2003). Both genes appear to derive from a *T. monococcum* accession, and to be absent in modern tetraploid and hexaploid wheat (James et al., 2006). *Nax1* was mapped to the long arm of chromosome 2A (Lindsay et al. 2004) and one very tightly linked marker, *gwm312*, is being used routinely to select low Na<sup>+</sup> progeny in the durum breeding program. *Nax2* has recently been mapped (Byrt et al., 2007) and a tightly linked marker is being used for selection of lines containing *Nax2*. The *Nax* genes have also been transferred into hexaploid wheat cultivars by inter-specific crosses, and progeny selected using the markers (Munns and Richards, 2007). Wu et al (2006) found that the green fluorescence of fused protein TaGSK-GFP distributed in cytoplasm of the transgenic plant cells, and mainly in root meristem and stelar sheath, where new roots would develop. *TaGSK1* localized in cytoplasm and could improve cell proliferation, development, anti-osmotic and anti-stress ability of transgenic plants. Li et al (2006) used Chinese Spring null-tetrasomic lines as materials and located the gene *TaGSK1* on the short arm of the homoeologous chromosome group 1 in *Triticum aestivum*. These results could be helpful to the research on heredity and mechanism in wheat salt-tolerance.

Wu et al (2007) identified 6 QTLs associated with salt-tolerance in F2 and F2:3 populations from the cross between the high yield variety Taikong 6 and salt tolerant variety Dekang 961. Among which one QTL for germination rate was detected on chromosome 5D, two QTLs for seedling height on chromosome 5D and 5A, two QTLs for root length on chromosome 5B, and 1 QTL for fresh weight on chromosome 5D. These results implied that the chromosomal locations of genes

controlling wheat salt tolerance at germination stage may be predominantly located on the homoeologous chromosome group 5. Weng and Chen (2002) identified a major gene controlling the salt tolerant character from the salt tolerant variety Chadianhong and cloned a 591bp marker OPZ09-591, which laid a foundation for the further studies on the molecular marker assistant selection and fine cloning and mapping of salt-tolerant gene for salt-tolerant breeding of wheat. Suo et al (2001) used 280 primers for RAPD analysis of Jimai 24 and its salt-tolerant progeny 8901-17 and selected one RAPD marker pronQ4 closely linked with the salt-tolerant mutation.

The facultatively halophytic *Lophopyrum elongatum*, closely related wheat and their amphiploid tolerate salt stress better if they are gradually exposed to it than if they are suddenly stressed. *Lophopyrum elongatum* has greater tolerance of both forms of salt stress than wheat, and its genome partially confers this tolerance on their amphiploid. Chromosomal control of the tolerance of both sudden and gradual salt stress in the disomic and ditelosomic addition lines and disomic substitution lines of *L. elongatum* chromosomes in wheat were studied by Zhong et al (1995). Wheat chromosomes in homoeologous groups 1, 3, and 7 and chromosomes in homoeologous groups 1, 4, and 6 were shown to enhance the tolerance of suddenly and gradually imposed stress, respectively. Liu et al (2001) identified a wheat-*Leymus* hybrid line 98-160 with remarkable tolerance to salt stress and found SSR markers WMS67 and WMS213 mapped on 5BL were linking to the salt tolerance which might be controlled by major gene(s).

Wang et al (2004) used a F2 wheat population derived from the hybrids of salt tolerant mutant RH8706-49 and salt sensitive mutant H8706-34 to map the salt tolerant genes by 246 pairs of micro-satellite primers, and Xgwm299 located on the 3BL was found to link to the relative salt tolerance. Shan et al (2006) used a F2 population of hybridized combination between a salt-tolerant wheat asymmetric somatic hybrid variety Shantung No.3 and salt-sensitive variety Jinan 17. The results showed the salt tolerance of Shantung No.3 was likely controlled by a major gene and a wheat specific SSR marker Xgwm304 located chromosome 5AS was decided to link with its salt-tolerant locus. But just afterwards, Chen (2007) revised their results as that the major gene was located on the 5AS of wheat chromosome, between the markers Xgwm304 and Xgwm666.

## **9. IMPROVEMENT OF SALINITY TOLERANCE BY TRANSFORMATION**

Recently, the knowledge of gene composition, expression and regulation and signal transformation enabled people to understand the stress-tolerant mechanisms of plants at molecular level. The method of transferring the exogenous target genes related to salt tolerance into wheat via gene engineering so as to improve the salt tolerance of wheat intendedly are paid more and more attention by the vast numbers of breeders.

The total DNA from *Agropyron elongatum* ( $2n=70$ ) was introduced into winter wheat through pollen tube channels. Variations in plant height, plant type, spike type, disease resistance and stress tolerance have been observed. A new variety, Jinan18, which has a higher saline and drought stress tolerance than the control cv. Lumai 10, has been developed and released (Huang et

al, 2000). Xue et al (2004) successfully transformed the vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter gene AtNHX1 into wheat. Grain yield of the best T3 transgenic wheat line grown in moderately saline (ECe 10.6 dS/m) field plots was 50% of that under non-saline conditions, whereas in the non-transformed control the yield was only 34% of that under non-saline conditions. At a higher salinity (ECe 13.7 dS/m), yield of the best transgenic line was 18% of its non-saline control, while yield of the non-transformed control was still lower at 11%. These gains in salt tolerance appear modest and convincing (Xue et al. 2004). Munns and Richards(2007) reviewed that the ornithine-daminotransferase (OAT) gene has been transformed into two Australian commercial wheats by Grain Biotech Australia. Glasshouse studies showed two lines that had 2–5 fold higher yields and 2-fold higher levels of free proline than the control varieties when the plants were salt stressed. The transgenic lines were assessed for yield on a salt gradient under field conditions. They had significantly higher yields at the high salinity levels than the commercial parents, although of course not as great as when grown in non-saline soil.

Liu et al (2006) introduced the antisense phospholipase D $\gamma$  (PLD $\gamma$ ) gene into wheat variety Fuxiwumai mediated by ear stem injecting and 10 transformed individual plants were obtained from T1 generation with PCR and Southern test. Among which, 6 plants have got increased tolerance to cold injury, and two plants with improved salt tolerance in MS containing NaCl. Ji et al (2002) successfully introduced HVA1 gene, one of the members of LEA (Late Embryogenesis Abundant) protein family, played an important role in plant survival under stress such as salt and drought, into wheat by the method deflating pollen tube, in order to produce salt tolerant wheat lines. A trehalose-6-phosphate synthase(TPS) gene from *Saccharomyces cerevisiae* related to drought and salt tolerance of plant was transferred into common wheat variety CB9945 by biolistic bombardment. 15 transgenic lines with improved stress tolerance to some extent were obtained under the simulated drought and salt stress conditions. Some of the lines have been put into the controlled field test (Du et al (2007). The *Tag1*(Triticum aestivum L. glycogen synthase kinase 1)gene cloned from the genome of wheat salt-tolerance mutant RH8706-49 was introduced into the callus induced from mature embryos of salt-sensitive wheat H8706-34 and cv. Chinese Spring by particle bombardment. The transformed callus stressed by Kanamycin and 0.5%NaCl showed higher ability of salt tolerance and could differentiate roots and shoots on the medium containing 0.5% NaCl (Xu et al, 2006).

Guo et al (2000) reported that they transferred betaine aldehyde dehydrogenase (BADH) cDNA cloned from *Atriplex hortensis* L. into wheat. Transgenic plants were obtained with BADH activity 100-300% higher than the control under salt-stressed condition. Further identification under the simulated salt / drought stress conditions showed that the transgenic wheat lines have many obvious advantages over their donor plants in germinating ability, seedlings growth and root development under drought stress condition, as well as the improved plasma membrane protection of excised leaf and the lower transpiration rate under field condition(Zhang et al, 2003).

It was found that under stress conditions such as drought, high-salinity and low-temperature, the transcription factor of DREB (dehydration responsive element binding proteins)improved efficiently stress resistance by regulating the super expressions of a series of genes related to stressful environment such as high salt and drought under normal or stressed conditions in plants. Cloning and transforming some main transcription factors into plant is becoming an effective



approach to enhancing crop stress resistance. Based on the wheat *in vitro* culture, an new efficient regeneration system for wheat genetic transformation without genotype dependence has been explored and set up (Liu et al, 2001). By use of this system, DREB cloned from *Arabidopsis* were transferred into wheat and transgenic plants were obtained (Liu et al, 2003). GmDREB gene encoding a stress-inducible transcription factor was also cloned and transformed into wheat variety Lumai 22 by bombardment. By investigating some main agronomical traits and the identification of stress tolerance, some transgenic lines containing GmDREB gene with good drought or salt tolerance, as well as similar agronomical characters to Lumai22, were obtained. The drought and salt tolerances of T1 transgenic lines with were demonstrated to be improved as compared to wild type (Gao et al, 2005).

## REFERENCES

- Ahmad Seyed and Noori Sadat, 2005, Assessment for salinity tolerance through intergeneric hybridisation: *Triticum durum* × *Aegilops speltoides*, *Euphytica*, 146: 149-155.
- Barakat M. N and Abdel-Latif T. H. 1996, In vitro selection of wheat callus tolerant to high levels of salt and plant regeneration, *Euphytica*, 91:127-140.
- Blum A, Ebercon A, 1981, Cell membrane stability as measure of drought and heat tolerance in wheat. *Crop Science* 21, 43-47.
- Blum, A, 1988, *Plant Breeding for Stress Environments*, CRC Press.
- Byrt, C., Platten, J.D., Spielmeier, W., James, R.A., Lagudah, E.S., Dennis, E.S., Tester, M. and Munns, R., 2007, HKT1; 5-like cation transporters linked to Na<sup>+</sup> exclusion loci in wheat, *Nax2* and *Kna1*, *Plant Physiol.*, 143: 1918-1928.
- Caruso Giuseppe, Chiara Cavaliere, Chiara Guarino, Riccardo Gubbiotti, Patrizia Foglia and Aldo Lagan, 2008, Identification of changes in *Triticum durum* L. leaf proteome in response to salt stress by two-dimensional electrophoresis and MALDI-TOF mass spectrometry, *Anal Bioanal Chem*, 391:381-390.
- Chen Fang, 2007, The major Salt-relative gene Located by SSR markers in salt-tolerance introgression F2 population of Shanrong No. 3 with Jinan I7. Shandong University Master's Thesis.
- Chen, S. Y., Xia, G. M., Quan, T. Y., Xiang, F. N., Yin, J., Chen, H. M., 2004, Introgression of salt-tolerance from somatic hybrids between common wheat and *Thinopyrum ponticum*, *Plant Sci.*, 167:773-779.
- Colmer, T. D., Flowers, T. J., Munns, R., 2006, Use of wide crosses and wild relatives to improve salt tolerance of wheat, *J. Exp. Bot.*, 57:1059–1078.
- Ding Tonglou, Duan Pei, Wang Baoshan, 2006, Na<sup>+</sup>/K<sup>+</sup> selectivity of leaf sheath in wheat cultivars differing in salt tolerance, *Journal of Plant Physiology and Molecular Biology*, 32 (1): 123-126.
- Dracup M, 1991, Increasing salt tolerance of plants through cell culture requires greater understanding of tolerance mechanisms, *Australian Journal of Plant Physiology* 18(1) 1 -15.
- Du Li-pu, Xu Hui-jun, Ye Xing-guo, Lin Zhong-ping, 2007, Transgenic wheat plants with trehalose-6-phosphate synthase (tps) gene and identification of their function, *Journal of Triticeae Crops*, 27(3):369-373.

- Dubcovsky J, Santa G, Epstein E, Luo MC, Dvorak J. 1996. Mapping of the K/Na discrimination locus *Knal* in wheat. *Theor Appl Genet* 92:448-454.
- Farooq Shafqat and Farooqe Azam, 2006, The use of cell membrane stability (CMS) technique to screen for salt tolerant wheat varieties, *Journal of Plant Physiology*, 163(6):629-637.
- Farooq, S, Iqbal, N, Asghar, M and Shah, TM, 1992a, Intergeneric hybridization for wheat improvement .IV. Expression of salt tolerance gene(s) of *Aegilops cylindrica* in hybrids with hexaploid wheat. *Cer Res Comm* 20: 111- 118.
- Farooq, S, Iqbal, N, Asghar, M and Shah, TM , 1992b, Intergeneric hybridization for wheat improvement. VI. Production of salt tolerant germplasm through crossing *wheat (Triticum aestivum* L.) with *Aegilops cylindrical* and its significance in practical agriculture. *J Gen Breed* 46: 125-132.
- Farooq, S. 2004, Salt tolerance in *Aegilops* species: A success story from research and production to large-scale utilization of salt tolerant wheat. In: Taha, F. K., Ismail, S. and Jaradat, A., eds, *Prospects of Saline Agriculture in the Arabian Peninsula*, Massachusetts, Amherst Scientific Publishers, 121-134.
- Farooq, S. and Azam, F., 2005, Salinity tolerance in Triticeae, *Czech J. Genet. Plant Breed.*, 41:252-262.
- Farooq, S., Asghar, M., Iqbal, N., Askari, E., Arif, M., and Shah, T. M., 1995, Production of salt-tolerant wheat germplasm through crossing cultivated wheat with *Aegilops cylindrica* .2. Field evaluation of salt-tolerant germplasm, *Cer. Res. Commun.*, 23:275-282.
- Flowers T.J, 2004, Improving crop salt tolerance, *J. Exp. Bot.* 55: 307-319.
- Flowers TJ, Troke PF, Yeo AR. 1977. The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.* 28: 89-121.
- Foolad M.R, 2004, Recent advances in genetics of salt tolerance in tomato, *Plant Cell Tissue Organ Cult.* 76:101-119.
- Foolad M.R, Jones RA. 1993. Mapping salt tolerance genes in tomato (*Lycopersicon esculentum*) using trait-based marker analysis. *Theor Appl Genet* 87:184-192.
- Foolad M.R.2001. Identification and validation of QTLs for salt tolerance during vegetative growth in tomato by selective genotyping. *Genome* 44:444-454.
- Forster, B.P. 1994. Cytogenetic manipulations in the Triticeae. In: *Monographs on Theoretical and Applied Genetics*. Vol. 21. A.R. Yeo and T.J. Flowers (eds.). Springer-Verlag, Berlin Heidelberg.
- Forster, B.P., 2001. Mutation genetics of salt tolerance in barley: an assessment of Golden Promise and other semi-dwarf mutants. *Euphytica* 120: 317-328.
- Forster, B.P., and Miller, T.E. 1985. 5b deficient hybrid between *Triticum aestivum* and *Agropyron junceum*. *Cereal Res. Comm.* 13:93-95.
- Forster, B.P., Gorham, J., and Taeb, M. 1988. The use of genetic stocks in understanding and improving the salt tolerance in wheat. In: *Cereals Breeding Related to Integrated Cereal Production*. M.L. Joma and L.A.J. Sloodmaker (eds.). Wageningen: PUDOC.
- Forster, B.P., H. Pakniyat, M. Macaulay, W. Matheson, M.S. Phillips, W.T.B. Thomas & W. Powell, 1994. Variation in the leaf sodium content of *Hordeum vulgare* (barley) cultivar Maythorpe and its derived mutant cv. Golden Promise. *Heredity* 73: 249-253.

- Gao Shiqing , Xu Huijun , Cheng Xianguo , Chen Ming , Xu Zhaoshi , Li Liancheng , Ye Xingguo , Du Lipu , Hao Xiaoyan , Ma Youzhi. 2005. Improvement of wheat drought and salt tolerance by expression of a stress-inducible transcription factor GmDREB of soybean (*Glycine max*), *Chinese Science Bulletin* .50(23): 2714-2723.
- GE R C, Zhao B C, Chen G P, Mi C L, Shen Y Z, Huang Z J, 2007, Cloning of salt tolerance related gene *tastk* in *Triticum aestivum*, *Acta Agronomica Sinica*, 33( 5):857-860.
- Gong JM, He P, Qian Q, Chen LS, Zhu LH, Chen SY . 1998. Mapping QTLs related salt tolerance in rice. *Sci Bull* 43:1847-1850.
- Gorham J, Bridges J, Dubcovsky J, Dvorak J, Hollington PA, Luo MC, Khan JA. 1997. Genetic analysis and physiology of a trait for enhanced  $K^+/Na^+$  discrimination in wheat. *New Phytologist* 137, 109-116.
- Gorham J, Forster BP, Budrewicz E, Wyn Jones RG, Miller TE, Law CN. 1986. Salt tolerance in the Triticeae: solute accumulation and distribution in an amphidiploid derived from *Triticum aestivum* cv. Chinese Spring and *Thinopyrum bessarabicum*. *Journal of Experimental Botany* 37, 1435-1449.
- Gorham, J., Hardy, C., Wyn-Jones, R.G., Joppa, L., and Law, C.N. 1987. Chromosomal location of a K/Na discrimination character in the D genome of wheat. *Theor. Appl. Genet.* 74:484-488.
- Greenway H, Munns R. 1980. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant Physiol.* 31: 149-190.
- Gu XY, Mei MT, Yan XL. 2000. Preliminary detection of quantitative trait loci for salt tolerance in rice. *Chinese J Rice Sci* 14:65-70.
- Gulick, P and Dvorak, J ,1987, Gene induction and repression by salt treatment in roots of the salinity-sensitive Chinese Spring wheat and the salinity-tolerant Chinese Spring X *Elytrigia elongatum* amphiploid. *Proc Nat Acad Sci USA* 84: 99-103.
- Guo B.H, Zhang Y M, Li H J., et al, 2000, Transformation of wheat with a gene encoding for betaine aldehyde dehydrogenase(BADH), *Acta Botanica Sinica*, 42(3): 279-283.
- Guo B.S., Yang,K., Song, J.Z., Huang, H.L. and Weng Y.J. 2001. Identification and analysis of salt tolerance in Tibetan wheat. *J Plant Genetic Resources* 2(2):36-39.
- Guo F. Q. Li Q, Gu R Q.1977, Mutation, selection and comparison of several saline-tolerant wheat strains, *Acta Agriculturae Nucleatae Sinica*, 11(1): 1-8
- Han N, Ge R C, Zhao B C, Shen Y Z, Huang Z J, 2006, Cloning and analysis of glutamine synthase II precursor in *Triticum aestivum* L. *Acta Agronomica Sinica*, 32(11):1756-1758.
- Hasegawa PM, Bressan RA, Zhu J-K, Bohnert HJ. 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51: 463-499.
- Hollington, P. A., 2000, Technological breakthroughs in screening/breeding wheat varieties for salt tolerance, in: *Proceedings of the National Conference Salinity management in agriculture* (Eds.Gupta, S. K. Sharma, S. K., and Tyagi, N. K.), December 1998, Karnal, India: Central Soil Salinity Research Institute, 273-289.
- Hossain Zahed, Abul Kalam Azad Mandal, Subodh Kumar Datta and Amal K. Biswas, 2006, Isolation of a NaCl-tolerant mutant of *Chrysanthemum morifolium* by gamma radiation: in vitro mutagenesis and selection by salt stress, *Functional Plant Biology* 33(1):91-101.

- Hou Ning, Liu Chun-guang, Liu Gen-qi, Wu Yu-wen, Zhang Cui-lan and Zhang Yan. 2004. Genetic effects of *Aegilops crassa* 6x cytoplasm on salt tolerance of common wheat. *Journal of Triticeae Crops*, 24(2):5-10.
- Huo Chen-Min, Zhao Bao-Cun, Ge Rong-Chao, Shen Yin-Zhu, Huang Zhan-Jing, 2004, Proteomic analysis of the salt tolerance mutant of wheat under salt stress, *Acta Genetica Sinica*, 31 (12) : 1408-1414.
- IAEA, 2002, Working material-Novel approaches for improving crop tolerance to salinity and drought, Report of a group consultant meeting, Vienna, Austria, 12-16 November 2001 under TC project INT5144.
- Jain S. M, 2001, Tissue culture-derived variation in crop improvement, *Euphytica*, 118: 153-166.
- James, R. A., Davenport, R., and Munns, R., 2006, Physiological characterisation of two genes exclusion in wheat: *Nax1* and *Nax2*, *Plant Physiol.*, 142:1537-1547.
- Ji Junli, Sheng Changzhong, Shi Ming, An Chunju, Wu Xuefeng, Li Desen, Du Rongqian. 2002, A study on the transformation of wheat with salt-tolerant gene *HVA1* by method deflating pollen tube, *Acta Triticeae Crops*, 22(2):10-13.
- King, IP, Law. CN, Cant, KA, Orford, SE, Reader, SM and Miller, TE (1997) *Triticum*, a potential new salt-tolerant cereal. *PI Breed* 116: 127-132
- King, IP, Orford, SE, Cant, KA, Reader, SM and Miller, TE (1996) An assessment of the salt tolerance of wheat/*Th.bessarabicum* 5Eb addition and substitution lines. *PI Breed* 115: 77-78.
- Kingsbury, R.W., and Epstein, E. 1984. Selection for salt-resistant spring wheat. *Crop Sci.* 24:310-315.
- Kingsbury, R.W., and Epstein, E. 1984. Selection for salt-resistant spring wheat. *Crop Sci.* 24:310-315.
- Lee GJ, Boerma HR, Villagarcia MR, Zhou X, Carter TE, Li Z, Gibbs MO. 2004. A major QTL conditioning salt tolerance in S-100 soybean and descent cultivars. *Theor Appl Genet* 109:1610-1619.
- Li Ya-qing, Mao Xin-guo, Zhao Bao-cun, Ge Rong-chao, Shen Yin-zhu, Huang Zhan-jing, 2006, The Chromosomal Location of *Triticum aestivum* Glycogen, *Acta Agriculturae Boreali-Sinica*, 21(5):39-41.
- Liang Chao, Wang Chao, Yang Xiufeng, Zhang Xiutian, Wang wei, 2006, Salt tolerant physiological characters of wheat variety Dekang 961, *Acta Bot. Boreali-Occident. Sin.*, 26(10):2075-2082.
- Lin HX, Zhu MZ, Yano M. 2004. QTL for Na and K uptake of the shoot and roots controlling rice salt tolerance. *Theor Appl Genet* 108:253-260.
- Lin HX, Yanagihara S, Zhuang JY, Senboku T, Zheng K, Yashima S. 1998. Identification of QTL for salt tolerance in rice via molecular markers. *Chinese J Rice Sci* 12:72-78.
- Lindsay MP, Lagudah ES, Hare RA, Munns R. 2004. A locus for sodium exclusion (*Nax1*), a trait for salt tolerance, mapped in durum wheat. *Funct Plant Biol* 31:1105-1114
- Liu Fang, Lv Weitaoh, Cui Decaib, Zhao Tanfang, 2006, Transformation of wheat with anti-phospholipase D  $\gamma$  gene, *Letters in Biotechnology*, 17(2):189-191.
- Liu Luxiang, Zhao Linshu, Liang Xinxin, Zheng Qicheng, Liu Qiang, Wang Jing, Guo Huijun, Zhao Shirong, Chen Wenhua, 2003, Study on production of transgenic wheat with a stress-inducible transcription factor gene *DREB1A* by microprojectile bombardment. *China Biotechnology*, 23(11): 53-56.
- Liu LX, Zheng QC, Zhao LS, Zhang YF, Wang J, Zhao SR and Chen WH, 2001, Study on receptor system for wheat genetic transformation without genotype dependence. *Acta Agriculturae Nucleatae Sinica* 15(5): 308-310.

- Liu LX, Zheng QC, Zhao LS, Zhang YF, Wang J, Zhao SR and Chen WH, 2001, Study on receptor system for wheat genetic transformation without genotype dependence. *Acta Agriculturae Nucleatae Sinica* 15(5): 308-310.
- Liu X, Shi J, Zhang X Y, Ma Y S, Jia J Z, 2001, Screening salt tolerance germplasm and tagging the tolerance genes using microsatellite(SSR)Markers in wheat, *Acta Botanica Sinica*, 43(9):948-954.
- Liu, L.X. , L.S. Zhao, H.J. Guo, S.R. Zhao, J. Wang, W.H. Chen and Q.C. Zheng. 2007, A salt tolerant mutant wheat cultivar 'H6756'. *Plant Mutation Reports*, 1(3):50-51.
- Liu, L.X., L.S. Zhao, Q.C. Zheng, H.J. Guo, S.R. Zhao and J. Wang, 2006, Salt tolerance improvement through doubled haploid and nuclear radiation technique in wheat. In: Abstracts of 11th IAPTC&B Congress Biotechnology and Sustainable Agriculture 2006 and Beyond, Beijing, China, 13-18 August 2006, p191.
- Liu, L.X., Q.C. Zheng, L.S. Zhao, H.J. Guo, X.X. Liang, J. Wang and S.R. Zhao, 2003, Salt tolerance improvement by using mutation techniques and biotechnology in wheat, In: Proceedings on Developing Salt-tolerant Crops for Sustainable Food and Feed Production in Saline Lands, Bangkok, Thailand, 10-14 November 2003, pp.10-19.
- Luo T B and Ren W, 2001, Breeding and characters of the new line of salt-tolerant and high-yielding wheat "101" in Xinjiang, *Agricultural Research in the Arid Areas*, 19(1):131-132.
- Luo T B, Ren W, Li Y, Wang B J, 2005, Salt tolerance of early combinations in wheat diallel crossings, *Agricultural Research in the Arid Areas*, 23(5):80-84.
- Luo T B, Ren W, Li Y, Wang B J, 2006, Primary agronomic properties and breeding process of new lines of salt tolerant wheat in Xinjiang, *Agricultural Research in the Arid Areas*, 24(2):18-21.
- Ma, Y.Q. and Weng, Y.J. 2005. Evaluation for salt tolerance in spring wheat cultivars introduced from abroad. *Acta Agronomica Sinica*. 31(1):58-64.
- Mi C L, Zhang X Y, Wen X J, Liu X, 2006, Isolation of TaVHA-C, a gene in wheat related to salt tolerance via cDNA-AFLP, *Scientia Agricultura Sinica*, 39(9):1736-1742.
- Mujeeb-Kazi, A., Diaz de Leon, J. L., 2002, Conventional and alien genetic diversity for salt tolerant wheats: focus on current status and new germplasm development. In: Ahmad, R. and Malik, K. A., eds, *Prospects for Saline Agriculture*, vol. 37, Dordrecht, Kluwer Academic Publishers, 69-82.
- Munns R, Husain S, Rivelli AR, James RA, Condon AG, Lindsay MP, Lagudah ES, Schachtman DP, Hare RA. 2002. Avenues for increasing salt tolerance of crop and the role of physiologically based selection traits. *Plant Soil* 247:93-105
- Munns Rana and Mark Tester, 2008, Mechanisms of salinity tolerance, *Annu. Rev. Plant Biol.* 59: 651-68.
- Munns, R. 2005, Genes and salt tolerance: bringing them together, *New Phytol.*, 167: 645-663.
- Munns, R. and James, R. A., 2003, Screening methods for salinity tolerance: a case study with tetraploid wheat, *Plant Soil*, 253: 201-218.
- Noble, C L, 1983, The potential for breeding salt tolerant plants, *Proc. R. Soc. Victoria*, 95, 133.
- Pang Cai-Hong and Wang Bao-Shan, 2008, Oxidative stress and salt tolerance in plants, U. Lüttge et al. (eds.), *Progress in Botany* 69, 231-245.
- Premachandra GS, Soneoka H, Kanaya M, Ogata S, 1991, Cell membrane stability and leaf surface wax content as affected by increasing water deficits in maize. *Journal of Experimental Botany* 42, 167-171.

- Quarrie SA, Steed A, Calestani C, Semikhodskii A, Lebreton C, Chinoy C, Steele N, Pljevljakusic D, Waterman E, Weyen J, Schondelmaier J, Habash DZ, Farmer P, Saker L, Clarkson DT, Abugalieva A, Yessinbekova M, Turuspekov Y, Abugalieva S, Tuberosa R, Sanguineti M-C, Hollington PA, Aragues R, Royo A, Dodig D. 2005. A high-density genetic map of hexaploid wheat (*Triticum aestivum* L.) from the cross Chinese Spring X SQ1 and its use to compare QTLs for grain yield across a range of environments. *Theor Appl Genet* 110:965-990.
- Quershi, R.H., Ahmed, R., Ilyas, M., and Aslam, Z. 1980. Screening wheat (*Triticum aestivum* L.) for salt tolerance. *Pak. J. Agric. Sci.* 17:19-25.
- Quesada V, Garcia-Martinez S, Piqueras P. 2002. Genetic architecture of NaCl tolerance in *Arabidopsis*. *Plant Physiol* 130:951-963.
- Rahman MH, Krishnaraj S, Thorpe TA, 1995, Selection for salt tolerance in vitro using microspore-derived embryos of *Brassica napus* cv Topas, and the characterization of putative tolerant plants. *In vitro Cell Dev Biol Plant* 31:116-121.
- Salah E. El-Hendawy,, Yuncai Hua and Urs Schmidhalter, 2005, Growth, ion content, gas exchange, and water relations of wheat genotypes differing in salt tolerances, *Australian Journal of Agricultural Research* 56(2) :123-134.
- Sayed, J. 1985. Diversity of salt tolerance in a germplasm collection of wheat (*Triticum aestivum*). *Theor. Appl. Genet.* 69:651-657.
- Schachtman, D. P., Lagudah, E. S., and Munns, R., 1992, The expression of salt tolerance from *Triticum tauschii* in hexaploid wheat, *Theor. Appl. Genet.*, 84:714-719.
- Shan L, Zhao S Y, Chen F, Xia G M, 2006, Screening and localization of SSR markers related to salt tolerance of somatic hybrid wheat Shanrong No.3, *Scientia Agriculture Sinica*, 39(2):225-230.
- Sharma, S K, Goyal S S, 2003, Progress in plant salinity resistance research :need for an integrative paradigm, *J. of Crop Production*, 7(1/2):387-407.
- Shen Yinzhu, Liu Zhiyi, Zhang Zhaoduo, Si Zhihai, Huang Zhanjing, Shi Lanbo. 1993, A study of the salt-resistant variation of inducing matured embryo's callus and regenerated plants in wheat. *Acta Genetica Sinica*. 20(3): 283-291.
- Shen Yinzhu. Liu Zhiyi, He Congfen, Huang Zhanjing, 1997, A Study of Salt-resistant Variations Induced in Anther Calli and Regenerated Plants in Wheat. *Hereditas*. 19(6): 7-11.
- Singh, K.N., and Chatrath, R. 1992. Genetic variability in grain yield and its component characters and their associations under salt stress conditions in tissue culture lines of bread wheat (*Triticum aestivum* L. em Thell.) *Wheat Information Service* 75:46-53.
- Suo G L, Huang Z J, He C F, Shen Y Z, Wang J, 2001, Identification of the molecule- markers linked to the salt-resistance locus in the wheat using RAPD-BSA technique. *Acta Bot Sinica*, 43(6):598- 602
- Szarejko I and B. P. Forster, 2007, Doubled haploidy and induced mutation, *Euphytica*, 158:359-370.
- Tal M, 1994, In vitro selection for salt tolerance in crop plants: Theoretical and practical considerations, *In Vitro Cellular & Developmental Biology - Plant*, 30:175-180.
- Tester, M., and Davenport, R., 2003, Na<sup>+</sup> tolerance and Na<sup>+</sup> transport in higher plants, *Ann. Botany*, 91, 503-527.

- Trethowan R.M. and A. Mujeeb-Kazi, 2008, Novel germplasm resources for improving environmental stress tolerance of hexaploid wheat, *Crop Sci* 48:1255-1265.
- Tuna, A L, Cengiz Kaya, David Higgs, Bernardo Murillo-Amador, Salih Aydemir and Ali R. Girgin, 2008, Silicon improves salinity tolerance in wheat plants, *Environmental and Experimental Botany*, 62(1):10-16.
- Wang H Y, Zhang C, Huang Z J, Zhu Z G, Guo G Y, Shen Y Z, 2004, Mapping of relative salt tolerance gene in wheat salt tolerant mutant by using microsatellite marker, *Acta Agronomica Sinica*, 30(7):697-699.
- Wang, R.R.C., S.R. Larson, W.H. Horton, and N.J. Chatterton. 2003. Registration of W4909 and W4910 bread wheat germplasm lines with high salinity tolerance. *Crop Sci*. 43:746.
- Weng YueJin and Chen Daoming, 2002, Molecular markers and its clone for salt tolerance gene, *Acta Genetica Sinica*, 29(4):343-349.
- Wu Li-zhu, Zhao Bao-cun, Qi Zhi-guang, Ge Rong-chao, Ma Wen-shi, Shen Yin-zhu, Huang Zhan-jing, 2006, Localization and function analysis of wheat glycogen synthetase kinase (TaGSK 1), *Scientia Agricultura Sinica*, 39(4):842-847.
- Xiao Hailin Zhao Shixu ,1989, The selection of NaCl-tolerant mutants by tissue culture in wheat, *Acta Agriculturae Nucleatae Sinica*, 3(2):85-90.
- Xu Tao, Zhao Bao-Cun, Ge Rong-Chao, Shen Yin-Zhu and Huang Zhan-Jing. 2006, Introduce Tag1 into salt-sensitive callus to improve the capacity of salt-tolerance by microparticle bombardment, *Chinese Journal of Biotechnology*, 22(2):211-214.
- Xue, Z. Y., Zhi, D. Y., Xue, G. P., Zhang, H., Zhao, Y. X., and Xia, G. M., 2004, Enhanced salt tolerance of transgenic wheat (*Triticum aestivum* L.) expressing a vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter gene with improved grain yields in saline soils in the field and a reduced level of leaf Na<sup>+</sup>, *Plant Sci.*, 167:849–859.
- Yeo A R and Flowers T J, 1986, Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Aust. J. Plant Physiol.* 13, 161-173.
- Yoshiro M, Kazuyoshi T. 1997. Mapping quantitative trait loci for salt tolerance at germination stage and the seedling stage in barley (*Hordeum vulgare* L.). *Euphytica* 94:263–272.
- Yu J N, Zhang L S, Zhang J S, Shan L, Chen S Y, 2004, Cloning of a novel stress tolerant gene- TaLEA3 from wheat and the functional analysis in yeast, *Chinese J. of Biotechnology*, 20(6):832-838.
- Zhang GY, Guo Y, Cheng SL, Chen SY. 1995. RFLP tagging of a salt tolerance gene in rice. *Plant Sci* 110:227–334.
- Zhang Yamin, Guo Beihai, Jiang Chunzhi, Wen Zhiyu, Ding Zhansheng, Li Hui, Li Hongjie, He Sijie, Chen Shouyi, Zhu Zhiqing, 2003, Salt and drought stress tolerance in transgenic wheat expressing betaine aldehyde dehydrogenase gene, *Acta Agriculturae Boreo-orientalis—sinica*, 18(1):29-32.
- Zhao Baocun, Zhao Qian, Ge Rongchao, Shen Yinzhong, Huang Zhanjing, 2007, Study on the expression profile of salt-tolerance mutant under salt-stress in wheat using gene microarray, *Scientia Agriculture Science*, 40(10):2355-2360.
- Zhao Ruitang Gao Shugou Qiao Yake Zhu Huimei Bi Yanjuan, 1995, Studies on the Application of Anther Culture in Salt-tolerance Breeding in Wheat (*Triticum aestivum* L.), *Acta Agronomica Sinica*, 21(2):230-234.

- Zhao Ruitang, Gao Shuguo, Qiao Yake, Zhu Huimei, Bi Yanjuan, 1994, A new approach of screening salt-tolerance variants by anther culture to cultivate salt-tolerance wheat variety, *Acta Agriculturae Boreali-Sinica*, 9(1):34-38.
- Zhao S L and Dou Y L, 1998, A review of identification indicators for wheat salt tolerance, *Acta Univ. Borealioccidentalis*, 26(6): 80-85.
- Zhao S S, Wang F Z, Lu L, Zhang H Y, Zhang X Y, 2000, Breeding and selection of drought resistant and salt tolerant wheat variety Cang6001, *Acta Agriculturae Boreali-Sinica*, 15(supplement):113-117.
- Zheng Q C, Zhu Y L, Chen W H, Tang X M, 1994, A study on salt stress induced proteins in the salt tolerant mutant in wheat, *J. Nucl. Agric. Sci.*, 15(3):101-104.
- Zheng Q.C. Chen W H, Zhao S R, Fu X R, Zhang G X. 1996, Screening of salt-tolerant wheat via an unicell and haploid system, *Journal of Nuclear-Agricultural Sciences*, 17(6): 253-255.
- Zheng Yanhai, Zhenlin Wangb, Xuezhen Sun, Aijun Jia, Gaoming Jiang, Zengjia Li, 2008, Higher salinity tolerance cultivars of winter wheat relieved senescence at reproductive stage, *Environmental and Experimental Botany*, 62:129-138.
- Zhong, G.Y. & J. Dvorak, 1995. Chromosomal control of the tolerance of gradually and suddenly imposed salt stress in *Lophopyrum elongatum* and wheat, *Triticum aestivum* L., genomes. *Theoretical and Applied Genetics* 90: 229-236.
- Zhu J K. 2002. Salt and drought signal transduction in plants. *Annu. Rev. Plant Biol.* 53: 247-273.
- Zhu Z H, Chang X P, Song J Z, Gao J Y, 1996, Identification and studies of salt tolerance in wheat, in: *Advances in Wheat breeding Research in China* (Eds, Zhang Q S and Du Z H), China Agriculture Press, 287-294.
- Zulfiqar Ali, 2004, Genetic basis of salt tolerance in wheat. PhD thesis, University of Agriculture, Faisalabad.



# CHAPTER 5

## PROGRESS OF PHYSIOLOGY AND GENETIC RESEARCH ON SALINE-ALKALINE TOLERANCE IN RICE (ORYZA SATIVA L.)

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### 1. INTRODUCTION

Saline-alkaline harm is a serious problem affecting world-wide crop production. It is a threat to normal crop growth and crop yields. Some statistics show that the degree of reduction of crop output because of the harm from drought stress and saline-alkaline stress was above 40%. The amount of saline-alkaline-affected soil is about  $9 \times 10^8$  ha globally, which accounts for 20% of total soil area (Munns, 2002); the saline-alkaline area in China is about  $2 \times 10^7$  ha (Wang, 2000). With the increase of Chinese population and the rapid development of towns, the available soil continuously decreases, while drought and inadvisable irrigative methods extends saline-alkaline areas. Furthermore, freshwater resources have become depleted in recent years. As a result, the crop production in arid, semiarid and coastal land faces seriously threat. Therefore, how to develop and use these saline-alkaline soils and enhance crop production and quality has been an important problem urgently requiring solutions for some saline-alkaline agriculture fields and saline-irrigated soil. Generally speaking, the definition for rice saline-alkaline tolerance is a capability of endurance and resistance under saline-alkaline stress conditions (Han, 2006). Rice is moderately sensitive to salinity and alkaline stress. However, saline and alkaline soils cause serious effects and lead to reduced rice production. In-depth study of rice salt-alkaline tolerance is critical to improve the potential of rice yield in saline and alkaline soils, to enlarge the rice planting area, and ensure the food safety in rice growing areas. It is also significant to improve peoples' living standard and the environment. This paper described the recent progress of the genetic improvement for saline-alkaline tolerance in rice, rice growth and development and agricultural traits affected by saline-alkaline stress, physiological mechanisms, transporters, genetics, QTL analysis based on

molecular markers, molecular signal conduction, gene cloning and transformation for saline-alkaline tolerance in rice.

## **2. GENETIC DIFFERENCE IN SALINE-ALKALINE TOLERANCE IN RICE**

### **2.1 The capacity of saline-alkaline tolerance in rice**

Rice is a crop, which originates from wetlands and is moderately sensitive to salt-alkaline (Wang et al., 1986). Chen et al. (1994) reported that root length, seedling length and root number in rice were obviously restrained under 200 Mmol/L NaCl concentration at rice budding period, and rice seedlings at 4-leaf-stage died quickly under 283 Mmol/L Na<sub>2</sub>CO<sub>3</sub> concentration (Cheng et al., 1994 ). Under saline-alkaline stress, tillering capability per plant in rice decreases obviously (Liang et al., 2004). Cheng et al. (1995) indicated pH 8.6 as the screening concentration for alkaline tolerance in the middle of tillering and boot stage.. Salt tolerance and alkaline tolerance are not correlated within a rice variety (Cheng et al., 1996).

### **2.2 The difference of saline-alkaline tolerance among rice varieties**

The haloduric limit is different among different japonica rice varieties (Zhang et al., 2006). Ying (1993) identified 13029 rice germplasm resources, finding that salt tolerance was obviously different among varieties and salt tolerance was abnormal in distribution for these materials. The difference of salt tolerance among rice germplasm resources could be closely correlative with their historic origin, evolution and genetic background.

### **2.3 The difference of saline-alkaline tolerance among different developmental stages**

Guo et al. (2004) indicated saline-alkaline tolerance for the same rice variety between germination and the seedling stage was different. Akbar et al.(1985) pointed out the capacity for salt tolerance in rice was stronger at the growth beginning, and rice became more sensitive to salt-alkaline stress at flowering and seeding starting. The difference of salt-alkaline tolerance among different growth stages could be closely correlated with the genetic characteristics and physiological/chemical response.

### **2.4 The difference of saline-alkaline tolerance among different parts in rice plant**

Plant height and length of internodes at rice maturity stage is not sensitive to salt stress, but some developmental tissue such as young leaf, radicle and inflorescences are more sensitive to salt stress, and furthermore, the above-ground part of young seedlings in rice is more sensitive to salt stress than the underground part (Han et al., 1998). Plant height and length of internodes at rice maturity can not be used as indicators of level of salt tolerance, but tillering capability could be used as the criterion of salt-alkali tolerance of rice varieties (Liang et al., 2004). Salinity tolerance rating at the seedling stage, relative plant height and relative dry weight of roots among different rice varieties are observably different at the same temperature and salt stress level.

### **2.5 Effect of salt-alkaline stress on development and agricultural traits in rice**

Excessive Na<sup>+</sup> accumulation in paddies antagonizes the absorption of nutrient elements and microelements, and the antagonizing induces crop physiological disorder and interrupted metabolism (Song et al., 2001).

## **2.6 Germination and emergence**

Salinity stress can delay germination and seedling grow of rice (Girdhar, 1992). Salt-alkaline stress at rice seed germination stage will lead to germination variability, reduced germination vigour and decreased germination rate (Dong et al., 2006). Conversely, up to now, mechanisms of salt-alkaline stress at rice seed-set is still poorly understood. Qin et al. (1989) considered salt stress mainly constrained developing rice seed physiological water absorption. Yan et al. (1995) indicated salinity stress destroyed membranes during imbibition of rice seeds and then physiological processes were affected.

## **2.7 Seedling growth**

Salt-alkaline stress leads to leaf curling or death, restrained leaf expansion and reduced new leaf and new root growth, and also root length and seedling length decrease at the seedling stage (Khan et al., 1997; Shan et al., 2006)

## **2.8 Development and maturity**

Salt-alkaline stress is one of main unfavorable factors affecting plant photosynthesis (Zhao, 2006). Liang et al. (2004) indicated that heading stage was obviously delayed or tillering was impaired, and the heading stage was prolonged, with the heading stage of salt-alkaline sensitive early rice varieties being later than the moderately salt-alkaline tolerant late rice varieties. Under salt-alkaline stress, rice plant height reduced, and the number of tillers and green leaves decreased (Cheng, et al., 1996). Rice leaves at tillering stage under salt-alkaline stress are most affected; the production index including the length of internodes and flowers seriously declines, the effective heading number, 1000-grain-weight and the number of tillers diminishes (Lee et al., 2002). Rice seedling sensitive to salt stress are obviously harmed by salt ions. These ions affect photosynthesis by disturbing trans-stomatal movement and reducing CO<sub>2</sub> uptake (Wang, et al., 2002). Brugnoli et al. (1991) considered the effect of salinity on assimilation rate was mostly due to the reduction of stomatal conductance. Stomatal closedown for salt-sensitive rice varieties under salt stress was the main reason for this (Li et al., 2006). Rice panicle forming is seriously hampered and seed setting rate is strongly reduced by salinity (Khan et al., 2003). Salinity generally reduced vigour, stunted growth, reduced straw and grain weight, and induced panicle sterility with large variations between lines (Aisha, et al., 2002; Zhang et al., 2006).

# **3. PHYSIOLOGICAL MECHANISM FOR SALT-ALKALINE TOLERANCE IN RICE**

Salt-alkaline soil engenders two kinds of stress, namely osmotic stress and ion stress (Shu et al., 2007). If they can overcome these stresses, plants can grow normally in salt-alkaline affected soil (Zhao, 2002). Salt-alkaline tolerance of higher plants includes mechanisms both of ion uptake and

ion exclusion. Restriction to ions entering into above-ground parts of plants is an important indicator of the capability of salt-alkaline tolerance (Greenway, et al., 1980).

### 3.1 Uptake and transport adjustment for mineral ions

Under NaCl stress, the content of  $\text{Na}^+$  in the stele parenchyma is relatively higher than in the other parts of the roots for the salt-tolerant genotypes, while the  $\text{Na}^+$  content is evenly distributed in the roots for the salt-sensitive genotypes, implying that the stele parenchyma might be the site controlling  $\text{Na}^+$  translocation from the root to the shoot (Zheng et al., 1996). Short-term (up to 24hr) NaCl stress was investigated to evaluate the ion transport and nutritional and water status in the ion composition of the xylem sap of rice. The results showed that the NaCl stress markedly affected the ion composition of the xylem sap of rice, and a rapid and remarkable increase in the concentrations of  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mg}^{2+}$ . In rice, the  $\text{Na}^+$  transport is restricted and the  $\text{K}^+$  transport rapidly increases, which results in a high ability of osmotic adjustment and an adequate nutritional status (Baba et al., 2003). The concentration and uptake of ions at the rice seedling were studied under stress of different salinity levels. The results indicate that the root dry matter was significantly reduced; the  $\text{K}^+$  concentration decreased significantly in shoots at all the levels and indicated that selective ion absorption may be the principal salt tolerance mechanism of rice (Hussan et al., 2003). Some experiments show the damage of rice leaves is attributed to accumulation of  $\text{Na}^+$  in the shoot by transport of  $\text{Na}^+$  from the root to the shoot under high external concentration (Lin et al., 2004).

### 3.2 Osmotic adjustment

Osmotic stress is one of the main restricting factors for crop production. The capability of osmotic adjustment is one of the basic characteristics of saline-alkaline tolerance of crops (Yu et al., 1998). At the cell level, the capability of osmotic adjustment of cells dictates the capacity for crop salt tolerance (Abdellatif, et al., 2003). Salinity induces accumulation of soluble sugars to reduce cell osmosis (Dubey et al., 1999). Mass proline accumulates in the rice leaves under salt stress (Zhang et al., 1997). Some nitrogen compounds accumulate in response to salt stress, and the quantity of accumulation in rice roots is highly correlated with  $\text{Na}^+$  concentration (Nguyen et al., 2003). Proline content of rice young seedling under salt-alkaline stress is an important index for degree of stress (Xie et al., 2005).

Endogenous free ABA is accumulated rapidly in the seedlings of the rice varieties after salt stress treatments. Higher levels of ABA are accumulated and maintained longer in tolerant varieties than in susceptible varieties (Liu et al., 2003). The quantity of proline accumulation in rice roots is a physiological index for osmotic stress of rice varieties (Do et al., 2003).

### 3.3 Transporters and trans-membrane movement

Molecular responses and signal transduction are induced under salt-alkaline stress. Salt-alkaline stress induces gene expression of some important functional proteins and regulatory proteins to protect plant cells from injury. A wild rice (the *O. latifolia*) thylakoid membrane shows high salt tolerance and still maintains photosynthetic activity even under high NaCl conditions; the wild rice also has specific proteins which may be partially responsible for salt tolerance (Nakamura et al., 2004). The quantity of vacuolar  $\text{Na}^+/\text{H}^+$  “antiporter” induced from rice under salt stress is one of most important indexes in deciding salt tolerance capability (Fukuda et al., 2004). LEA proteins

accumulated during the salinity-triggered growth arrest of young seedlings and are mobilized during the recovery of seedlings from salinity stress (Karuna et al., 2003).  $\text{Na}^+$  and  $\text{K}^+$  uptake in rice are mediated by different transporters, TaHKT1 transporting  $\text{K}^+$  and  $\text{Na}^+$ , and OsHKT1 only  $\text{Na}^+$ . OsHKT transporters are involved in  $\text{Na}^+$  movements in rice, and that OsHKT1 specifically mediates  $\text{Na}^+$  uptake in rice roots when the plants are  $\text{K}^+$  deficient (Garcia-deblás et al., 2003)

#### **4. GENETICS OF SALT-ALKALINE TOLERANCE IN RICE**

Genetic research shows that salt-alkaline tolerance in rice is a complicated phenomenon of various physiological reactions and a multigenic trait, which is controlled by many genes distributed on different chromosomes (Flowers 2004). Inheritance variance rates of leaf death and seedling dry matter under salt stress shows additive effects and interactive effects (Moeljo et al., 1981). Salt tolerance at the rice seedling stage is possibly controlled by only a few genes and shows mainly additive and dominance effects and has no epistatic interaction (Jones et al., 1985). Akbar et al. (1985) studied genetic variation of rice seedling characters under salt stress. The results showed seedling height,  $\text{Na}^+$  and  $\text{Cl}^-$  concentration of above ground parts, dry matter content of shoot, leaf and roots were stronger additive effects and showed high heritability; while  $\text{Na}^+$  and  $\text{Cl}^-$  concentration in root, and root length had stronger dominant effects and lower heritability.  $\text{Na}^+$  and  $\text{Cl}^-$  concentration in rice root, and root length were controlled by at least three genes, and root, shoot and leaf length, and dry matter of shoot were controlled by two genes. A  $\text{BC}_1$  [Peta/ Pokkali (salt tolerant) Peta ( salt sensitive)] population was employed to detect the quantitative trait loci (QTLs) for salt tolerance at different stages of rice growth. There are 4 putative QTLs for salt tolerance at the seedling stage and all of the positive alleles came from Pokkali. The favorite alleles of the QTLs for the salt tolerance at the mature stage came from both parents. Two QTLs in the vicinity of RG678 and RZ400B-RZ792 exhibited salt tolerance over the whole growth period. The saline tolerance of seedling stage and mature stage in rice had common genetic bases (Gu et al., 2000). Salt tolerant mutant lines were obtained. Their salt tolerant traits have been stably inherited for thirteen generations. The effect of a major gene may be present in these mutants (Guo et al., 1981). The seedling stage was analyzed in most of above studies. The responses of most indexes manifest as a quantitative trait to salt stress even though different rice varieties and indexes of salt tolerance were used by the researchers. We can conclude that the genetic variation of rice to salt tolerance is mainly controlled by additive effects and dominant effects, which are the important genetic basis of salt tolerance at rice seedling stage. With regard to heredity of the hybrid rice to salt tolerance, some scholars think that traits for salt tolerance in the hybrid rice are not obvious. The reason to this is still unclear.

#### **5. MOLECULAR MECHANISM OF SALT-ALKALINE IN RICE**

##### **5.1 Molecular mapping**

The salt-alkaline tolerance in rice are quantitative traits controlled by many genes (Akbar and Yabuno, 1977; Gong et al., 1999; Flower, 2004). Lin et al. (2004) identified three QTLs for survival rate of seedlings under salt stress on chromosomes 1, 6, and 7. Among them, SNC-7 and SKC-1 were main genes, which explained 48.5% and 40.1% of the observed phenotypic variances, respectively. Gu et al. (2000) found four QTLs associated with salt tolerance at the seedling stage

and all of their alleles came from tolerant varieties. Lin et al. (1998) detected one QTL for survival period of seedlings on chromosome 5, accounting for 11.6% of observed phenotypic variance. Seven QTLs, which were associated with salt tolerance at the seedling stage, were detected on chromosome 5, 6, 7 and 10 (Prasad et al., 2000). Koyama et al. (2001) identified eleven QTLs, which were associated with Na<sup>+</sup> concentration, K<sup>+</sup> concentration, Na<sup>+</sup> uptake, K<sup>+</sup> uptake, and Na<sup>+</sup>/K<sup>+</sup> ratio. Twenty-three QTLs for the six traits on the ten chromosomes except chromosomes 5 and 10 were identified, including five for SST, six for SDS, four for SKC, four for SNC, one for RKC, and three for RNC (Sun et al., 2007).

## 5.2 Signal transduction

Salt-alkaline tolerance in plants is the process of stress-induced and molecular signal transduction. The whole process includes plant response to external stress, initiation of signal transduction, signal identification and transduction. The gene expression relative to salt-alkaline tolerance is adjusted by stress induced signals. Hashimota et al (2004) researched the roots of two week old seedling in rice under salt stress and considered a novel rice PR10 protein specifically being induced in root by salt stress possibly via the jasmonic acid signalling pathway. The signal for salt stress induction was expression of genes encoding antioxidant enzymes in seedlings of rice and initiated a precise system of antioxidant adjustment. The adjustment system keeps the environmental balance of oxidation-reduction in the cell and keeps organelles protected from ion toxicity (Menezes et al., 2004). Two rice transduction initiation factors OseIF5A-1 and OseIF5A-2 are induced and regulated by salt stress (Chou et al., 2004). Some research results show rice flavonoid pathway genes OsDfr and OsAns, are induced by high salt stress responsive promoter elements that interact with the transcription activator, Osc-MYB. The flavonoid signal transduction pathway is possibly one of important stress signal transduction pathways (Nagabhushana et al., 2004).

## 6. GENE CLONE AND TRANSFORM OF SALT-ALKALINE TOLERANCE IN RICE

Great progress has been made in recent years in the identification of genetic resources and the isolation of useful genes in rice. Fifty-seven induced genes in rice were detected using cDNA microarrays and RNA imprinting technology (Rabbani et al., 2003). Some QTL associated with salt tolerance traits have been located and cloned (Huang 2003; Mohanty, 2002; Suprasanna, et al., 2003; Hayaashi, et al., 1997; Qian, et al., 2003). The aconitifolia-pyrroline-5- carboxylate synthetase gene was successfully transformed into indica rice CV IR-50, and the transgenic plant shows tolerance to high salt (Anoop, et al., 2003). Over-expression of a barley aquaporin gene in rice using transformation increases the shoot/root ratio and raises salt sensitivity in transgenic rice plants (Katsuhara, et al., 2003). The *OPBP1* gene was transformed into japonica rice Xiushui 11 and shows improve resistance to salt stress. Under the same salt stress conditions, transgenic plants grows faster, has significantly higher biomass and chlorophyll content compared to non-transgenic plants (Li, et al., 2006). Some important salt tolerance genes from a wild rice (*Porteresia coarctata* T.) were transformed into cultivated rice using tissue culture, wild-hybridization, and biological

technology approaches (Brar, et al., 2002). Experiments show that the growth of shoot and root for  $F_3$  transgenic rice has stronger tolerance under high salt stress (Su, et al., 2006).

**Table 1** Status of QTL analysis for salt tolerance in rice

Salt tolerance trait	Mapping material	Population and marker	Number of QTL (Chromosome)	Variance explained (%)	Reference
SDS	Nona Bokra×Koshihikari	$F_{2,3}$ , RFLP	3(1, 6, 7)	13.9-18.0	Lin et al., Koyama et al.
NC			2(4, 6)	6.4-19.6	
KC			2(1, 4)	8.8-10.6	
NU			1(1)	8.9	
KU			3(4, 6, 9)	6.8-19.6	
NKR			2(1, 4)	9.1-9.6	
DM			1(6)	9.7	
SG	IR4630×IR15324	RIL ( $F_6$ ), AFLP	2(6, 7)	16.3-19.5	Prasad et al.,
SSL			1(6)	18.9	
SDM			3(5, 6, 10)	13.5-17.9	
SV			1(6)	16.3	
RSI			2(5, 7)	—	
NC			1(9)	—	
SFW/DMW			1(6)	—	
SW	IR64×Azucena	DH ( $F_1$ ), RFLP	3(1, 7, 10)	—	Gu et al.,
SSR			1(3)	—	
MPL			1(5)	—	
PH			1(9)	—	
NSMP			1(6)	—	
SDS			1(5)	11.6	
ASDS			1(5)	11.6	
ASDS	ZYQ8×JX17	DH ( $F_1$ ), RFLP	8(1, 2, 2, 3, 7, 8, 8, 12)	10.2-38.4	Gong et al.,
ISSVF	M-20×77-170	$F_{2,3}$ , RFLP	1(7)	—	Zhang et al.,
SST	IR64×Tarom Mola	$BC_2 F_8$ , SSR	5(2, 3, 4, 7)	8.77-14.47	Sun et al.,2007
SDS			6(2, 3, 4, 7, 9)	8.87-13.03	
SKC			4(1, 2, 3, 8)	9.59-18.9	
SNC			4(2, 3, 11, 12)	9.91-11.5	
RNK			1(6)	9.55	
RNC			4(6, 7, 9)	9.89-14.75	

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SDS: Survival days of seedling; NC: Na<sup>+</sup> concentration; KC: K<sup>+</sup> concentration; NU: Na<sup>+</sup> uptake; KU: K<sup>+</sup> uptake; NKR: Na<sup>+</sup>: K<sup>+</sup> ratio; DM: Dry mass; SG: Seed germination; SSL: Seedling shoot length; SDM: Seedling dry matter; SV: Seedling vigour; RSI: Ranking of salt injury at seedling stage; SFW/DMW: The ratio of shoot fresh weight/dry matter weight of the seedling; SW: Straw weight at mature stage; SSR: Seed setting rate; MPL: Main panicle length; PH: Plant height; NSMP: Number of spikelets in main panicle; ASDS: Average survival days of seedling; ISSVF: The individual of the sum of scores for vigour and fertility

## **7. THE BREEDING OF SALINE TOLERANCE IN RICE**

We present below a succinct overview of achievements made in addressing salinity in rice *in vitro* techniques (doubled haploidy), genetic engineering and mutagenesis.

### **7.1 In vitro techniques for enhancing saline tolerance in rice**

Seventy-nine di-haploid lines were produced in rice through anther culture of the cross of two indica breeding lines. In 1996, some high-yielding salt-tolerant AC-derived lines (IR51500-AC11-1, IR51500-AC17, IR51485-AC6534-4, IR72132-AC6-1, IR69997-AC1, IR69997-AC2, IR69997-AC3 and IR69997-AC4) had been generated in just 3 years (Senadhira et al., 2002; Gregorio et al., 2002). IR51500-AC11-1 was released as a salt-tolerant cultivar in the Philippines with the name PSBRc50 or “Bicol” (Senadhira et al., 2002). This is the first F<sub>1</sub> AC-derived line from an indica/indica cross to be released as a cultivar for cultivation in saline-prone areas. In India, IR51500-AC17 and IR51485-AC6534-4 were named as commercial cultivars CSR21 and CSR28, respectively, for cultivation on saline-alkaline soils (IRRI, 1997). Pokkali and other salt tolerant donors underwent cell culture to induce somaclonal variation. Somaclonal variants of Pokkali with improved agronomic traits were identified. The variant (TCCP 266-2-49-B-B-3) had desirable levels of all tested characteristics and retained salinity tolerance equal to Pokkali. It is superior to Pokkali as a donor of salt tolerance in hybridization programs (Gregorio et al., 2002). Afza reported the development of semi dwarf mutants derived from the salt tolerant landrace Pokkali via anther culture because some rice landraces exhibited greater tolerance to salt, but were agronomically unacceptable because of their tall stature (Afza et al., 2006).

### **7.2 Genetic transformation for salinity tolerance in rice**

Transgenic rice expressing the codA gene had enhanced salt tolerance and could recover to normal growth at a faster rate than the wild type after an initial growth inhibition under salt stress (Sakamoto et al., 1998). Overproduction of proline in transgenic rice (Zhu et al., 1998) increased biomass production and enhanced flower development under salinity stress. Hoshida et al. (2000) examined the potential role of photorespiration in protection against salt stress with transgenic rice. Roy and Wu (2001) reported that the introduction of oat arginine decarboxylase (ADC) into rice, led to salt stress-induced upregulation of ADC activity and polyamine accumulation in transgenic rice plants, and showed all increased in biomass under salinity stress conditions. Flowers (2004) also reviewed the work of Garg et al. (2002) involving the transformation of rice to overexpress genes that led to the synthesis of trehalose. SNAC1-overexpressing transgenic rice showed enhanced salt tolerance (Hu et al., 2006).



### **7.3 Mutation induction in rice for salinity tolerance**

Six mutants with higher salt tolerance than the parent variety Bicol and two salt tolerant varieties from the salt susceptible variety IR29 were obtained in the year 2001. The development of useful salt-tolerant mutants from IR29 that retains superior plant vigour and will be a major contribution to addressing the scourge of salinity in rice agriculture in SE Asia as this variety is well-adapted and widely cultivated by farmers in this region. One mutant variety Shua-92 and two mutants of rice, derived through mutation breeding from the two standard varieties IR8 and Pokkali, were evaluated for two years for their yield performance in salt affected soils with pH 7.63 to 7.68 and EC 7.11 to 8.0 dSm<sup>-1</sup>. The mutant variety Shua-92 produced 40 and 49% higher paddy yield on salt affected soils than the famous salt tolerant varieties Nona Bokra and Pokkali, respectively (Baloch, et al., 2003).

## **8. FUTURE PERSPECTIVES**

### **8.1 Study on exploitation and utilization of rice germplasm for salt-alkaline tolerance**

Rice germplasm resources are the basic material and gene pool for rice breeding and cultivar improving. The capability of salt-alkaline tolerance in rice is mainly dictated by its genetic characteristics. Nowadays, narrower genetic background of salt-alkaline tolerant parents is an important limiting factor on salt-alkaline tolerant breeding in rice, which is still not resolved. It is important to exploit rice material of strong salt-alkaline tolerance for breeding work, taken from present rice germplasm subjected to laboratory and field screening.

### **8.2 Research on physiological mechanisms for saline-alkaline tolerance in rice**

Nowadays, osmoregulation and ion uptake/alteration affects the capability of salt-alkaline tolerance in rice. The pathways for regulation are still poorly understood. There are different opinions regarding the action of Na<sup>+</sup> ions and proline in the salt-alkaline stress process (Flowers, 1981; Zhang, et al., 1997). The function of trans-membrane channel proteins in the process of stress response and ion transporters in membranes is unresolved. In addition, salt tolerance is different between the tissue level and the whole plant level in rice. Therefore, these problems will not be solved until physiological mechanism for saline-alkaline tolerance in rice can be deeply and comprehensively researched.

### **8.3 Study on salt-alkaline tolerance using molecular marker mapping**

Some QTLs associated with salt tolerance were detected in rice chromosomes in recent years (Gong et al., 1999; Prasad et al., 2000; Lin et al., 2004). Since different scholars selected different mapping populations, experimental conditions and statistical analysis methods, the number of QTL and the position of QTL are different. Consequently, some QTL with stable inheritance, repeatedly detected, and consistently observed phenotypic variance should studied consistently using the salt-alkaline tolerance examination over different years and places; these QTL can be further used in rice breeding for salt-alkaline tolerance by means of marker-aided selection.

#### 8.4 Study of signal transduction for salt-alkaline tolerance in rice

Under salt-alkaline stress, there is a need to study the whole process and multipathway of molecular response and signal transduction, especially factors of gene expression and promoters, and to build a genetic network to reveal the essentials of salt-alkaline tolerance from signal transduction.

#### 8.5 Study on core germplasm for salt-alkaline tolerance in rice

We should use conventional methods and current molecular biology means to characterize and evaluate thoroughly rice elite germplasm to decipher salt-alkaline tolerance genetics, using the approaches of conventional crossing, tissue culture, interspecific hybridization, marker-aided selection, transgene technology and so on. Strongly salt-alkaline tolerant rice germplasm characterization will be utilized quickly in rice breeding for salt-alkaline and improved field production.

### REFERENCES

- Abdellatif Bahaji, Aniento F, Cornejo M J, 2003. Uptake of an endocytic marker by rice cells: variations related to osmotic and saline stress. *Plant and Cell Physiol*, 44(10):1100–1111
- Ahloowalia, BS; Maluszynski, M; Nichterlein, K. 2004. Global impact of mutation-derived varieties. *Euphytica* 135: 187–204
- Aisha Shereen, Ansari R Flowers, et al, 2002. Rice cultivation in saline soil. Dordrecht, the Netherland: Kluwer Academic Publishers ,189–192
- Akbar M, Khush G S, et al, 1985. Genetics of salt tolerance in rice. *Rice Genetics Proceeding of International Rice Genetics Symposium, IRRI*, 5:399–409
- Anoop N, Gupta A K. 2003, Transgenic indica rice CV IR-50 over-expressing vigna aconitifolia –pyrroline-5- carboxylate synthetase cDNA shows tolerance to high salt. *J Plant Biochem and Biotechnol*, 12(2):109–116
- Baba T, Fujiya Ma H, 2003. Short-term response of rice and tomato to NaCl stress in relation to ion transport. *Soil Sci. Plant Nutr* , 49(4):513–519
- Binh, DQ; Heszszy, LE. 1990. Restoration of the regeneration potential of long term culture in rice (*Oryza sativa* L) by salt pretreatment. *J. Plant Physiol*. 136:336–340
- Binh, DQ; Heszszy, LE; Gyulai, G; Csillag, A. 1992; Plant regeneration of NaCl-pretreated cells from long-term suspension culture of rice (*Oryza sativa* L.) in high saline conditions. *Plant Cell, Tissue and Organ Culture* 29:75–82
- Brar D S, Buu B C, Khush G S, 2002. Transferring agronomically important genes from wild species into rice: application of tissue culture and molecular approaches. In: Abstract of International Conference on Wild Rice. Katmandu, Nepal, 17–18
- Brugnoli E, Lauteri M , 1991. Effects of salinity on stomatal conductance, photosynthetic capacity, and carbon isotope discrimination of salt-tolerant and salt-sensitive C<sub>3</sub> non-halophytes. *Plant Physiol*, 95:628–635
- Chou Wan Chi, Huang Ya Wen, Tsay Wen Su, et al, 2004. Expression of genes encoding the rice transaction

- initiation factor EIF5A is involved in development and environmental responses. *Physiol Plant*, 121(1):50–57
- Do Thu Hien, Jacobs M, Angenon G, et al, 2003. Proline accumulation and  $\Delta$ -pyrroline-5-carboxylate synthetase gene properties in three rice cultivars differing in salinity and drought tolerance. *Plant Sci*, 165(5):1059–1068
- Dubey R S, Singh A K, 1999. Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolising enzymes in rice plants. *Biol Plant*, 42(2):233–239
- Flower T J, 2004. Improving crop salt tolerance. *J Experimental Botany*, 55(396):307–319
- Flowers T J, Yeo A R, 1981. Variability in the resistance of sodium chloride salinity within rice varieties. *New Phytol*, 88: 363
- Fukuda A, Nakamura A, Tagiri, et al, 2004. Function, intracellular localization and the importance in salt tolerance of a vacuolar  $\text{Na}^+/\text{H}^+$  antiporter from rice. *Plant and Cell Physiol*, 45(2):146–159
- Garciadeblás B, Senn M E, Bañuelos M A , et al, 2003. Sodium transport and HKT transporters: the rice model. *Plant J*, 34(6):788–801
- Girdhar I K, 1992. Effect of sodic water irrigating on the growth development product and chemistry system of rice on the salt soil. *Salt-alkaline Soil Use*, 1:45–50
- Greenway H, Munns R., 1980 Mechanisms of salt tolerance in nonhalophytes. *Ann Rev Plant Physiol*, 31:149–190
- Gregorio, GB; Senadhira, D; Mendoza, RD; Manigbas, NL; Roxas, JP; Guerta, CQ. 2002. Progress in breeding for salinity tolerance and associated abiotic stresses in rice. *Field Crops Research* 76: 91–101.
- Gu X Y, Mei M T, Yan X L, 2000. Preliminary detection of quantitative trait loci for tolerance in rice. *Chinese J Rice Sci*, 14(2):65-70 (in Chinese with English abstract)
- Guo Y, Chen S L, Zhang G Y, et al, 1997. Salt-tolerance rice mutant lines controlled by a major effect gene were obtained by cell engineering technique. *Acta Genetica Sinica*, 24(2):1221–1226 (in Chinese with English abstract)
- Hashimota M, Kisseleva L, Sawa S, et al, 2004. A novel rice PR10 PROTEIN, RsOsPR10, specifically induced in roots by biotic and abiotic stresses, possibly via the jasmonic acid signaling pathway. *Plant and Cell Physiol*, 45(5):5501–559
- Hayaashi Hale Mustardy L, Murata N, 1997. Transformation of *Arabidopsis thaliana* with the coda gene for choline oxidase accumulation of glycine betaine and enhanced tolerance to salt and cold stress. *Plant J*, 12:1331–142
- Hu, H; Dai, M; Yao, J; Xiao, B; Li, X; Zhang, Q; Xiong, L. 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *PNAS* 103(35):12987–12992
- Huang J, Zhang H S, Wang J F, et al. 2003, Molecular cloning and characterization of rice 6-phosphogluconate dehydrogenase gene that is up-regulated by salt stress. *Molecular Biology Reports*, 30(4):2231–227
- Hussan N, Ali A, Sarwar G, et al. 2003, Mechanism of salt tolerance in rice. *Pedosphere*, 13(3): 2331–238
- Jones M P. 1985, Genetic analysis of salt tolerance in mangrove swamp rice. *Rice Genetics Proceeding of*

International Rice Genetics Symposium, IRRI, 5:411–122

Karuna Chourey, Saradha Ramani, Apte S, et al. 2003, Accumulation of LEA proteins in salt (NaCl) stressed young seedling of rice (*Oryza sativa* L.) cultivar Bura Rata and their degradation during recovery from salinity stress. *J Plant Physiol*, 160(100):11651–1174

Katsuhara M, Koshuo K, Shiba M, et al. 2003, Over-expression of a barley aquaporin increased the shoot/root ratio and raised salt sensitivity in transgenic rice plants. *Plant and Cell Physiol*, 44(12):13781–1383

Khan M A, Abdullah Z. 2003, Salinity-Sodicity induced changes in reproductive physiology of rice under dense soil conditions. *Environmental and Experimental Botany*, 49(2):451–147

Khan M S A, Hamid A. Karim M A. 1997, Effect of sodium chloride on germination and seedling characters of different types of rice. *Agron & Crop Sci*, 179:1631–169

Koyama M L, Levesley A, Koebner R M D, et al. 2001, Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiol*, 125:4061–422

Lee Chung Kuen, Yoon Young Hwan, Shin Jincheol, et al. 2002, Growth and yield of rice as affected by saline water treatment at different growth stages. *Korean J Crop Sci*, 47(6): 4021–408

Lee, SY; Lee, JH; Kwon, TO. 2003. Selection of salt-tolerant doubled haploids in rice anther culture, *Plant Cell. Tiss. Org. Cult.* 74(2): 143–149

Li H B, Chen W F, Li Q Y. 2006, Responses of rice leaf photosynthetic parameters to light intensity under NaCl stress. *Chinese Journal of Applied Ecology*, 17(9):1588–1592

Li N Y, Guo Z J. 2006, Overexpression of two different transcription factors, OPB P1 and OsiWRKY enhances resistance against pathogen attack and salt stress in rice. *Chinese J Rice Sci*, 20(1): 13–18

Li, SN; Heszszy, LE. 1986. Testing of salt tolerance and regeneration in callus (n, 2n) of rice. In: Horn, W, Jensen, JC. Odenbach, W & Schieder, JO (eds): *Genetic manipulation in Plant Breeding*. Pp 617–619 Walter de Gruyter and Co, Berlin-New York

Lin H X, Yanagihara S, Zhuang J Y. 1998, Identification of QTL for salt tolerance in rice via molecular markers. *Chinese J Rice Sci*, 12(2):72–78 (in English)

LIN H X, Zhu M Z, Yano M, et al. 2004, QTLs for Na<sup>+</sup> and K<sup>+</sup> uptake of the shoots and roots controlling rice salt tolerance . *Theor Appl Genet*, 108:253–260

Liu C L, Chen H P, Liu E E, et al. 2003, Multiple tolerance of rice to abiotic stresses and its relationship with ABA accumulation. *Acta Agron Sin*, 29(5):725–729 (in Chinese with English abstract)

Maluszynski, M; Nichterlein, K; van Zanten, L; Ahloowalia, BS. 2000. Officially released mutant varieties –the FAO/IAEA database Mutation Breeding Reviews. The Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria. Pp 88.

Menezes-Benaavente L, Teixeira F K, Kamei C L A, et al. 2004, Salt stress induces altered expression of genes encoding antioxidant enzymes in seedlings of a Brazilian indica rice (*Oryza sativa* L.). *Plant Sci*, 166(2):323–331

Moeljo Pawira, Ikehashi S H. 1981, Inheritance of Salt tolerance in rice. *Euphytica*, 30:291–230

Mohanty A, Kathuria H, Ferjani A, et al. 2002, Transgenics of an elite indica rice variety pusa basmatil harbouring the codA gene are highly tolerant to salt stress . *Theor Appl Genet*, 106(1):51– 57

- Munns R. 2002, Comparative physiology of salt and water stress. *Plant, Cell and Environment*, 25:239–250
- Nagabhushana Ithal, Reddy A R. 2004. Rice flavonoid pathway genes OsDfr and OsAns, are induced by dehydration, high salt and ABA and contain stress responsive promoter elements that interact with the transcription activator, Osc-MYB. *Plant Sci*, 166(6):1505–1513
- Nakamura I, Agarie S, Tobita S, et al. 2004, Salt tolerance of the chloroplast thylakoid membrane in wild *Oryza species latifolia* Desv. *Japanese J Crop Sci*, 73(1):84–92
- Nguyen Thi Thu Hoai, IE Sung Shim, Kobayashi K, et al. 2003, Accumulation of some nitrogen compounds in response to salt stress and their relationships with salt tolerance in rice (*Oryza sativa* L.) seedling. *Plant Growth Regulation*, 41(2):159–164
- Prasad S R, Bagali P G, Hittalmani S, et al. 2000, Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.). *Curr Sci*, 78:162–164
- Qian Q, Yanagihara S, Teng Sheng, et al. 2003, Isolation expression characteristics and chromosomal locations of three cDNA fragments under salt stress in rice. *Acta Botanica Sinica*, 45(9):1090–1095
- Rabbani M A, M aruyama K, Abe H, et al. 2003, Monitoring expression profiles of rice genes under cold drought and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gelblot analyses. *Plant Physiol*, 133(4):1755–1767
- Reddy, PJ; Vaidyanath, K. 1986. *In vitro* characterization of salt stress effects and the selection of salt tolerant plants in rice (*Oryza sativa* L.) *Theor. Appl. Genet.* 71. 757–760
- Rogbell J E, Subbaraman N, Karthikeyan C. 1998, Heterosis in rice (*Oryza sativa* L.) under saline stress condition. *Crop Res Hisar*, 15(1): 68–72
- Senadhira, D; Zapata-Arias, FJ; Gregorio, GB; Alejar, MS; De la Cruz, HC; Padolina, TF; Galvez, AM. 2002. Development of the first salt-tolerant rice cultivar through indica/indica anther culture. *Field Crops Research* 76:103–110
- Sivritepe H O, Eris A, Sivritepe N. 1999, The effect of NaCl Priming on salt tolerance in melon seedlings. *Acta Horticul*, 492:77–84
- Su J, Wu R. 2004, Stress-inducible synthesis of proline in transgenic rice confers faster growth stress conditions than that with constitutive synthesis. *Plant Sci*, 66(4):941–948
- Suprasanna Penna. 2003, Building stress tolerance through over-producing trehalose in transgenic plant. *Trends in Plant Sci*, 8(8):355–357
- Wang J F, Chen H Y, Yang Q L, et al. 2004, Effects of salt concentration and temperature on the screening of salt tolerance in rice. *Chinese J Rice Sci*, 18(5):449–454(in Chinese with English abstract)
- Zhang G Y, Guo Y, Chen S Y, et al. 1995, RFLP tagging of a salt tolerance gene in rice. *Plant Sci*, 110:227–234
- Zhang H,Zhou J M,Guo Y, et al. 1997, A physiological study on the salt-tolerant mutant of rice. *Acta Phytophysiological Sinica*, 23(2):181–186 (in Chinese with English abstract)
- Zhang R Z, Shao X W, Tong S Y, et al, 2006. Effect of saline-alkali stress on source-sink and yield of rice. *Chinese J Rice Sci*, 20(1):116–118
- Zhao K F 2002. The plant adaptation to salt stress. *Biology Bull*,37(6):7–10 (in Chinese with English abstract)

# CHAPTER 6

## ADVANCES IN BREEDING BARLEY FOR SALT TOLERANCE

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### 1. INTRODUCTION

Almost three quarters of the surface of our world is covered by salt water and so it is not surprising that salt is dramatically dominant on the earth's land surface. Moreover, salinization of soil in arable land is ever-increasing due to poor irrigation and drainage practices, and to the expansion of irrigated agriculture into arid zones with high evapotranspiration rates (Flowers, 1977). Salinity is one of the main problems that negatively affect soil fertility and physical properties, thus severely affecting the growth and economic yield of many important crops (Maas and Hoffman, 1977). Salinity can be alleviated through either soil reclamation or growing tolerant crops. However, soil reclamation is a very expensive process, and hence the cultivation of tolerant species and varieties is the most practical solution when the salinity is low. It is well known that there are significant genotypic differences with respect to salt tolerance between and within plant species (Rana, 1986; Suhayda et al., 1992; Zhong et al., 1995). Compared with other cereal crops, including wheat, rice, rye and oat, barley is highly tolerant to salinity, thus offering a means for efficient utilization of saline soil and improvement of productivity in these environments. However, barley still suffers from salt toxicity in many areas of the world. Similarly, distinct genetic difference can be found among and within the barley species for salinity tolerance, thus laying the fundamental basis for improvement of salt tolerance. This chapter summarizes progress on physiology, screening methods, molecular genetics, genetic improvement through conventional and biotechnological approaches for barley salinity tolerance.

### 2. SALINITY TOXICITY AND PHYSIOLOGICAL MECHANISMS FOR TOLERANCE IN BARLEY

#### 2.1 Salinity toxicity

The presence of high concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{Mg}^{2+}$ , and  $\text{SO}_4^{2-}$  ions in saline soils inhibits growth of many plants. The problems associated with high salinity are threefold:

1) A high salinity is associated with a low soil water potential, giving rise to symptoms similar to those of water stress

2) Specific ions, especially  $\text{Na}^+$ ,  $\text{Cl}^-$ , may be toxic.

3) High levels of NaCl may give rise to an ion imbalance (mainly calcium), and lead to deficiency symptoms.

Toxicity effects of salinity may include inhibition of nitrate uptake by chloride, probably because both ions are transported across the plasma membrane by the same carrier. High  $\text{Na}^+$  may replace  $\text{Ca}^{2+}$  on root cell membranes, which may give rise to leakage of  $\text{H}^+$  from the root cells. It may also reduce the influx and enhance the efflux of calcium. The decreased influx probably results from competition for binding sites in the cell wall, which decreases the concentration at the protein in the plasma membrane responsible for calcium influx. The toxicity of specific ions may subsequently lead to an ion imbalance and ion deficiency, especially calcium deficiency (Rengel, 1992).

Ion imbalance will happen when plants are exposed to salinity stress, thus resulting in disorder of nutrition, excessive accumulation of some nutrient elements or deficiency of some other elements. In most cases, salinity stress reduces uptake and accumulation of  $\text{K}^+$ , leading to imbalance of  $\text{K}^+/\text{Na}^+$  ratio, which has been considered as the major reason of salinity toxicity to plants. Leonova *et al* (2005) found that soil salinization increased the sodium content in barley seedlings as compared to the control plants. In general, salt-susceptible cultivars accumulated more  $\text{Na}^+$  in their shoots than salt-tolerant cultivars; the reciprocal pattern was found in the roots. On the other hand, soil salinization decreased  $\text{K}^+$  content in the shoots of the salt-susceptible cultivar as compared to the control, whereas in the most tolerant cultivars, the potassium content increased. Gorham *et al.* (1990) compared the difference of  $\text{K}^+$  and  $\text{Na}^+$  concentrations in the plant tissues of *Triticeae* species. *Triticum aestivum*, *Secale cereale*, and *Ae. squarrosa* had the low leaf Na and high leaf K concentrations. *T. durum* and the *Hordeum* species did not have this character, and the better growth of *H. vulgare* than of *T. durum* with similar salt concentrations in the youngest fully-expanded leaves may be attributed to better compartmentation of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{K}^+$  ions among the plant tissues or between different compartments within cells.

## 2.2 Physiological mechanisms for salinity tolerance

Salinity tolerance is a complex trait involving different mechanisms, and is defined as the ability of the plant to survive under salinity and complete the growth cycle with an acceptable growth and yield. Plants employ various strategies in response to salinity including (1) minimising initial  $\text{Na}^+$  entry and  $\text{Na}^+$  loading to the xylem; (2) maximising  $\text{Na}^+$  efflux from root, its recirculation out of the shoot, intracellular compartmentation or allocation to old leaves (3) maintaining a relatively high cytosolic  $\text{K}^+/\text{Na}^+$  ratio; (4) accumulating/synthesising optimal amounts of compatible solutes; (5) increasing enzymatic and non-enzymatic antioxidant defence systems (Bohnert *et al.*, 1995; Hasegawa *et al.*, 2000; Tester and Davenport, 2003).

For most plants,  $\text{Na}^+$  is not an essential nutrient, although low  $\text{Na}^+$  concentrations often stimulating plant growth in many species. To a large extent this is attributed to the role of  $\text{Na}^+$  as an osmoticum in the vacuole, reducing the need for  $\text{K}^+$  (Marschner, 1995). Sodium has been shown to be essential for maximal growth in certain halophytic  $\text{C}_4$  plant species, such as bladder saltbush (*Atriplex vesicaria*), fire bush (*Kochia childsii*), proso millet (*Panicum miliaceum* L.), and saltgrass (*Distichlis spicata* L.) (Brownell and Crossland, 1972; Flowers et al., 1977; Subbarao et al., 2003). In  $\text{C}_4$  species,  $\text{Na}^+$  is considered to be a beneficial element and, to some extent, can replace certain  $\text{K}^+$  functions such as an internal osmoticum, in stomatal function, photosynthesis, as a counter-ion in long-distance transport, and in enzyme activation (Marschner, 1995; Cramer, 1997; Subbarao et al., 2003). However, in glycophytes, elevated  $\text{Na}^+$  levels are detrimental, causing specific ion toxicity and negatively affecting root nutrient uptake, especially ions such as  $\text{K}^+$  and  $\text{Ca}^{2+}$  (Verslues et al., 2006).

Land plants do not appear to have specific transport systems for  $\text{Na}^+$ . Under high external  $\text{Na}^+$  concentration,  $\text{Na}^+$  enters cells passively via several routes (Cheeseman, 1982; Xiong and Zhu, 2002). The initial high unidirectional influx of  $\text{Na}^+$  into roots is found in species like barley (Kronzucker et al., 2006), wheat (Davenport, 1998), maize (Jacoby and Hanson, 1985; Zidan et al., 1991) and *Arabidopsis* (Elphick et al., 2001; Essah et al., 2003). This influx is mostly mediated by non-selective cation channels (NSCCs) (Demidchik et al., 2002), whose usual function is in the uptake of other cations such as  $\text{K}^+$  (Uozumi et al., 2000), and  $\text{Ca}^{2+}$  (White and Davenport, 2002). The unidirectional  $\text{Na}^+$  influx rates can exceed net  $\text{Na}^+$  uptake rates by an order of magnitude, implying the involvement of high  $\text{Na}^+$  efflux (Davenport et al., 1997; Essah et al., 2003).

$\text{Na}^+$  competes with  $\text{K}^+$  for binding sites essential for cellular function. With over 50 enzymes activated by  $\text{K}^+$ , this disrupts numerous enzymatic processes in the cytoplasm (Bhandal and Malik, 1988; Marschner, 1995). For example, protein synthesis requires high concentrations of  $\text{K}^+$  for the binding of tRNA to ribosomes, but this is inhibited by high  $\text{Na}^+$  *in vitro* (Hall and Flowers, 1973; Wyn Jones et al., 1979). Enzymes including malate dehydrogenase, aspartate transaminase, glucose 6-P dehydrogenase, and isocitrate dehydrogenase isolated from salt-sensitive broad bean (*Phaseolus vulgaris* L.) and salt-tolerant pop saltbush (*Atriplex spongiosa* F. Muell.) and glasswort (*Salicornia australis* Sol. ex F. Muell.) are equally sensitive to  $\text{Na}^+$  up to 500 mM *in vitro* (Greenway and Osmond, 1972). Thus, it is essential for plants to employ all sorts of strategies to reduce salt toxicity. Among these,  $\text{Na}^+$  exclusion and vacuolar compartmentation are crucial for plant salt adaptation.

Exclusion of  $\text{Na}^+$  from the cytosol has been suggested to be a crucial mechanism for salt tolerance in plants (Schubert and Läuchli, 1990; Tester and Davenport, 2003). Physiological mechanisms of exclusion that operate at the cellular and whole-plant level have been extensively reviewed (Greenway and Munns, 1980; Schachtman and Liu, 1999; Munns, 2002; Véry and Sentenac, 2002; Shabala, 2003). Ion exclusion mechanisms could provide a degree of tolerance to relatively low concentrations of NaCl but not at high salinity (Yamaguchi and Blumwald, 2005). Salt-tolerant wild *Hordeum* species had better  $\text{Na}^+$  excluding ability than cultivated barley (Garthwaite et al., 2005). When grown in 50 mM NaCl, bread wheat excluded around 98%  $\text{Na}^+$ , but barley, durum wheat, and rice excluded about 94% (Munns, 2005). There is a strong correlation between salt exclusion and salt tolerance in cereals such as barley, rice and wheat (Flowers and Yeo, 1986; Chhipa and Lal, 1995; Ashraf and Khanum, 1997; Munns and James, 2003). In contrast to the  $\text{Na}^+$



excluding ability of roots of rice, barley has the ability to prevent root to shoot  $\text{Na}^+$  translocation at high external NaCl (Nakamura et al., 1996). However, a highly salt-tolerant wild relative of tomato (*Lycopersicon esculentum* Mill.) accumulates higher concentrations of  $\text{Na}^+$  than the salt-sensitive domesticated tomato (Santa-Cruz et al., 1999). The sensitivity of wild-type *Arabidopsis* and some mutants does not appear to be closely related to shoot levels of  $\text{Na}^+$  (Zhu et al., 1998; Nublat et al., 2001; Essah et al., 2003). Therefore, the correlation between plant salt tolerance and its ability to exclude  $\text{Na}^+$  from uptake is not straightforward as initially believed.

Compartmentation of  $\text{Na}^+$  into the vacuole is also vital for the growth and survival of halophytes and of many non-halophytes, such as barley. Barley is more tolerant to salt than wheat partially due to its greater ability to sequester  $\text{Na}^+$  in the vacuole (James et al., 2006). This mechanism would avoid toxic effects of salt on photosynthesis and other key cytosolic metabolic processes (Maathuis et al., 1992; Fricke et al., 1996; Blumwald and Gelli, 1997; James et al., 2006). However, such  $\text{Na}^+$  compartmentation is not sufficient unless the plant also possesses the ability to efficiently retain  $\text{K}^+$  in the cytosol.

Excessive accumulation of salt ion should be toxic to plant cells, thus they must be separated from the metabolic machinery of the cells if the toxic effect is alleviated or avoided. In general, this is achieved by ion compartmentation, storing salt ion in vacuoles, where there is very weak metabolic activity (Flowers and Yeo, 1986). A possible survival strategy of plants under saline conditions is to sequester absorbed  $\text{Na}^+$  in the vacuole, thus maintaining a higher  $\text{K}^+/\text{Na}^+$  ratio in the cytoplasm (Greenway and Munns, 1980). The  $\text{K}^+/\text{Na}^+$  antiport in vacuolar membranes transports  $\text{Na}^+$  from the cytoplasm to vacuoles using a pH gradient generated by  $\text{H}^+$ -ATPase and  $\text{H}^+$ -PPase, which was considered to be related to salt tolerance of plants (Atsunori et al., 1998). It has been demonstrated that the proton pump and  $\text{K}^+/\text{Na}^+$  antiport in vacuolar membranes were important in ion selective absorption and compartmentation of  $\text{Na}^+$  in barley seedlings (Garbarino and Dupont, 1988). Takuji *et al* (1996) investigated the mechanisms of salt tolerance in terms of the ATPase activity in roots, the Na excluding ability of roots, and the regulation of Na translocation from roots to shoot by comparing barley and rice grown at high NaCl concentrations. It was found that high salt tolerance of barley was ascribed to the inhibition of Na translocation from roots to shoot, and less reduction of ATPase activity in roots. ATPase activities in the plasma membrane and tonoplast of barley roots were higher than those of rice when roots were exposed to NaCl stress. Recently, Ershov *et al.* (2005) confirmed that the changes in the activity of ion transporters under salt stress conditions correlated with the barley cultivar-specific tolerance to elevated NaCl concentrations.

In order to maintain water uptake in salt environments, plants will reduce water potential, which is realized by accumulating solutes and synthesizing some new chemicals. Within the cytoplasm, osmotic adjustment is influenced by compatible solutes, such as glycinebetaine, mannitol and proline. When a plant is exposed to salt stress, it responds initially to the changed water conditions caused by the lowering of the external water potential. These initial effects of salinity are probably the same for the cultivars differing in salt tolerance. However, when ions accumulation lasts more times, difference in salt tolerance among genotypes appear (Munns, 1993), with sensitive cultivars accumulating ions more quickly than tolerant ones.

Ions enter plant cells through a membrane-across protein, and the process is driven by energy-consuming ion pumps, which use the energy stored in ATP to move protons by generating a difference of hydrogen ion concentration (pH) and electric potential ( $\Delta E$ ). It is assumed that  $\text{Na}^+$  is 'mistaken' for potassium by a  $\text{K}^+$  carrier or channels, but it is also possible that  $\text{Na}^+$  enter cells through non-selective cation channels (Maser et al., 2002). It has been reported that a specific phospholipid environment is required for optimal ATPase activity, and changes in phospholipids and free sterols of the cell membranes may contribute to salt tolerance (Norberg and Liljenberg, 1991; Mansour et al., 1994). Yamaguchi and Kasamo (2001) found that exogenously added tonoplast phospholipids would stimulate the activity of purified tonoplast  $\text{H}^+$ -ATPase. Meanwhile, fatty acids are considered to be important in salt tolerance of plants and micro-organisms (Somerville, 1995; Malkit et al., 2002). By using genetic mutants, Allakhverdiev *et al.* (1999) has proved that unsaturated fatty acids in membrane lipids could protect the photosynthetic machinery against salt stress-induced damage. Zhao and Qin (2005) found that addition of linoleic acid (LA) at 1 mM into culture solution possessed protective effects on root tonoplast function against salt stress in the barley seedlings; and the effect was accompanied with a significant suppression of phospholipids and PAs degradation in tonoplast vesicles. Moreover, these salt-ameliorating effects of linoleic acid on tonoplast function were also indicated by the increase in  $\text{H}^+$ -ATPase and  $\text{H}^+$ -PPase activities. An application of LA under saline condition increased the activity of a vacuolar  $\text{K}^+/\text{Na}^+$  antiport.

Polyamines (PAs) have been found to be related to salt tolerance in plants. The poly-cationic nature of PAs at physiological pH endows their ability of mediating biological activity. They are able to bind negatively charged molecules, such as DNA (Basu et al., 1990), membrane phospholipids and proteins (Tassoni et al., 1996), and pectic polysaccharide (D'Oraci and Bagni, 1987). In addition, PAs can also be covalently bound to some specific proteins catalysed by a class of enzymes known as trans-glutaminases (TGase) to form bound PAs (Serafini-Fracassini et al., 1995). Both free and bound PAs associated to the tonoplast vesicles from barley seedlings were detected, and their contents were found to be closely related to salt tolerance of the plants (Zhao et al., 2000).

The normal calcium metabolism in plants is quite important to develop salt tolerance. Lynch and Läuchli (1985) investigated the influence of salt stress on calcium nutrition in barley. Soil salinity decreased the Ca contents of shoots of two cultivars of barley grown under field conditions, particularly in younger leaves. Reduction in Ca content was more pronounced in the salt-sensitive cultivar than in the salt-tolerant cultivar. In the seedlings grown in solution culture, relatively low concentrations of NaCl inhibited the transport of Ca to the shoot and the inhibition was not due to effects of NaCl on Ca influx into the root or on transpiration. It was found that NaCl inhibited Ca transport from root to shoot by interfering with the release of Ca into the root xylem, possibly via an effect on the active loading of Ca into xylem vessels. Similarly, Cramer *et al.* (1989) reported that salt-stressed plants often show symptom of Ca deficiency. The transport and tissue concentrations of Na were significantly affected by supplemental Ca. In contrast, calcium transport and tissue concentrations were markedly inhibited by salinity. There were significant Na-Ca interactions in ion transport and accumulation, and as well as growth. Lynch *et al.* (1988) proposed that leaf growth in salt-stressed barley plants was reduced by sub-optimal Ca availability in the leaf meristem. One cause of reduced Ca availability is that Na replaces Ca in the leaf apoplast (Zid

and Grignon, 1985). Salinity stress has been shown to stimulate a release of Ca from intracellular compartments (Lynch and Lauchli, 1988). Calcium transport to the shoot is reduced in NaCl-stressed plants (Lynch and Lauchli, 1985; Wolf et al., 1990), and indeed, the ability to transport Ca to the shoot during salt stress has been proposed as an index of salt tolerance (Lahaye and Epstein, 1971). According to Tobe *et al.* (2003), Ca<sup>2+</sup> present in saline soils would alleviate the toxic effects of other salt components on seed germination of barley.

In addition, ultra-structural alternations happen in root cells of some species including barley when the plants are exposed to salt stress. Kramer (1984) suggested that the appearance of various alternations under salt stress might have a function in the adaptation of plant to salinity. Huang *et al.* (1990) observed the structural changes occurring in meristematic cells of barley in response to moderate salinity stress. In the apical region of the root, salinity stress caused an increase in vacuolation, which may provide a means for accumulation of excess ions. Salt treatment also induced the formation of many plastids in the cortical cells, often appearing to enclose part of the cytoplasm, which was less dense than the surrounding cytoplasm. It was suggested that plastid morphology might allow or alternatively result from adaptive change in protein synthesis or cytoplasmic composition.

### **3. PHENOTYPIC SCREENING METHODS FOR SALINITY TOLERANCE**

A efficient breeding program for salinity tolerance requires new genetic resources, but breeding efforts are constrained by a shortage of field and laboratory screening tests (Zhu, 2000) and unfortunately, few screening procedures have proven successful for identifying salinity tolerance (Shannon, 1997) for a number of reasons. Firstly, screening a large number of genotypes for salinity tolerance is difficult due to the complexity and polygenic nature of salinity tolerance, which involves responses to cellular osmotic, ionic and oxidative stresses (Shannon, 1997; Zhu, 2000). Secondly, evaluating field performance under saline conditions is difficult because of the variability of salinity within fields (Richards, 1983; Daniells et al., 2001) and due to interactions with other environmental factors (Shannon and Noble, 1990; Flowers, 2004). Thus, physiological traits measured under controlled conditions are employed for rapid and cost-effective selection techniques (Shannon and Noble, 1990; Munns et al., 2002). Thirdly, evaluation of the degree of salinity tolerance between and within species is likely to vary according to the criteria used for evaluation (Shannon, 1997), so developing an effective screening procedure may in itself be difficult (Flowers and Yeo, 1995). Last but not least, evaluating salinity tolerance is made more complicated by the variation in salinity sensitivity at different growth stages: the tolerance of the plants at one growth stage is not always correlated with tolerance at other stages (Greenway and Munns, 1980; Flowers, 2004; Foolad, 2004). Barley has been found to be tolerant to salinity at germination, sensitive at the seedling and early vegetative growth stages, then again tolerant at maturity (Epstein et al., 1980; Munns, 2002).

Over the past few decades, various screening methods based on physiological traits have been practically and intensively employed in many plant breeding programs (Noble and Rogers, 1992; Shannon, 1997). Those include germination percentage, degree of leaf injury, root length and plant

height, shoot and root dry weight, shoot number, maintenance of flowering, seed and fruit set, canopy volume and quality, plant survival under salinity, tissue and specific accumulation of ions in different cell compartments (e.g.  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$ ), or their ratio (e.g.  $\text{K}^+/\text{Na}^+$ ,  $\text{Ca}^{2+}/\text{Na}^+$ ), and the production of certain metabolites or enzymes (e.g. proline, glycine betaine, sucrose, antioxidative enzymes) (Shannon, 1997; Munns et al., 2002; Munns and James, 2003; Foolad, 2004; Colmer et al., 2005). The use of physiological traits as screening methods has also been investigated for rice (Garcia et al., 1995) where it has proved successful in generating salt-resistant lines (Gregorio et al., 2002). Thus it may be also applicable to other crops (Cuartero et al., 1992; Ellis et al., 1997; Foolad, 1997; Munns et al., 2002). Despite this, there is still controversy about the use of a simple screening method for salt tolerance and its complex nature. Isla *et al.* (1997, 1998) reported that some physiological traits, including leaf ion concentrations, canopy temperature, stomatal conductance and grain ash content, would not be useful as screening tools for salinity tolerance in barley, and that grain yield under salinity remains the only reliable measure of identifying salt-tolerant barley. This section will review the most frequently used screening methods for salt tolerance and the recent development on the screening methods.

### 3.1 Germination and emergence

High salt concentrations in the seed-planting layer of soil frequently lead to germination failure (Fowler, 1991), although as one of the most salt-tolerant crop species, some barley genotypes are reported to be able to germinate in seawater (i.e.  $47 \text{ dS m}^{-1}$ ) (Mano and Takeda, 1995, 1997). Salinity affects germination by multiple mechanisms including prevention of water uptake and imposition of ionic stress (Bewely and Black, 1982; Poljakoff-Mayber et al., 1994), reduction in hydrolysis and enzyme activities (Filho and Sodek, 1988; Guerrier, 1988), disturbance to nitrogen metabolism (Yapsanis et al., 1994; Dell'Aquila and Spada, 1993), and imbalance of plant growth regulators (Khan and Rizvi, 1994).

Al-Karaki (2001) and Tajbakhsh *et al.* (2006) showed that salt-tolerant barley maintained a much higher germination rate and shorter germination time than salt-sensitive barley grown under high salinity in Petri dishes. Germination percentage has a strong positive correlation with barley seed and straw yield (Thalji and Shalaladeh, 2007). Germination is a convenient test for a large number of genotypes, but little or no correlation has been found between genotypic differences in germination and later growth in salinity for many species (Kingsbury and Epstein, 1984; Ashraf and McNeilly, 1988; Mano and Takeda, 1997; Almansouri et al., 2001; Shannon, 1997; Munns and James, 2003). For example, Donovan and Day (1969) found that a third out of 39 barley cultivars exceeded the germination of the best-known salt-tolerant California Mariout under high salinity. Emergence rate has also been reported as one of the criteria for salinity tolerance (Tajbakhsh et al., 2006) and been proposed to be a more practical screening criterion than germination rate (Shannon, 1997; Murillo-Amador et al., 2001).

### 3.2 Plant growth in response to salinity

Salinity in the soil reduces plant water uptake, leading to slower growth (Munns et al., 2006) along with a clear plant stunting as salt concentration increases (Wang and Nil, 2000). The components of this effect on plant growth are the reduction in plant height, fresh and dry weight of leaves, stems, and roots, yield and deterioration of the quality of the product (Kumar, 1995; AliDinar et al., 1999; Chartzoulakis and Klapaki, 2000). One of the major causes for this growth reduction is

inadequate photosynthesis owing to stomatal closure, inhibition of photosynthetic enzymes and limited CO<sub>2</sub> uptake (Zhu, 2001; Munns, 2002).

Plant water status changes rapidly and relative water content (RWC) is a convenient method of assessing plant water status. Jensen (1982) reported that the decrease in leaf osmotic potential was due partly to dehydration (58%) and partly to an increase in leaf solute content (42%). The turgor pressure was unaffected, but the RWC was significantly decreased by salinity in some wheat genotypes that accumulated high levels of leaf Na<sup>+</sup>. At the same time, the low leaf Na<sup>+</sup> accumulating genotypes significantly reduced both turgor pressure and RWC after 5 d of salt treatment (James et al., 2002; Rivelli et al., 2002), suggesting a rather complex relationship between plant water status and ionic relations under saline conditions.

Plant growth components such as plant height, leaf and root elongation rate, and biomass production at saline relative to non-saline conditions are frequently employed as simple and effective screening criteria (Kingsbury and Epstein, 1984; Munns and James, 2003). Plant height was used as an indicator of salinity tolerance in many species (Joshi and Nimbalkar, 1983; Forster, 2001; Houshmand et al., 2005). Seedlings of the salt-tolerant barley cultivar California Mariout (from which CM72 and Numar originated) showed no growth retardation at 400 mM NaCl, while other genotypes were severely affected at lower salt concentrations (Epstein et al., 1980).

In wheat and maize, it is reported that there are varietal differences in the elongating rate of leaves and roots in early growth responses to salinity (Kingsbury et al., 1984; Mladenova, 1990). Aslam *et al.* (1993) and Moons *et al.* (1995) showed early varietal difference in rice and subsequent reductions in vegetative and reproductive yields in response to salinity. Moreover, early varietal differences in leaf growth responses to salinity were detected after only 3 d in two *Brassica* varieties whose leaf Na<sup>+</sup> and Cl<sup>-</sup> levels were identical (He and Cramer, 1993).

Genotypic differences in the effect of salinity on the rate of leaf growth in barley and wheat took a few weeks time to appear (Munns and James, 2003). However, within two weeks dead leaves became visible on the more sensitive genotype (Munns et al., 1995; Munns et al., 2002). Similarly, two maize cultivars with a two-fold difference in Na<sup>+</sup> accumulation in leaves, showed the same growth reduction. It was not until after eight weeks that a growth difference was clearly seen (Cramer et al., 1994; Fortmeier and Schubert, 1995). Thus, long-term experiments (several weeks to months) are necessary to detect genotypic differences in the effects of salinity on growth in cereal crops such as maize, wheat, barley, and rice (Kingsbury and Epstein, 1984; Aslam et al., 1993; Fortmeier and Schubert, 1995; Munns et al., 1995; Zhu et al., 2001).

### **3.3 Leaf element accumulation**

#### **3.3.1 Tissue Na<sup>+</sup> content**

Leaf Na<sup>+</sup> content analysis has the advantage of being directly related to the rate of Na<sup>+</sup> transport to shoot thus is specific to Na<sup>+</sup> toxicity in the mesophyll. Na<sup>+</sup> exclusion is frequently used as a screening technique, as genetic differences in exclusion are highly correlated with differences in salinity tolerance between durum and bread wheat (Francois et al., 1986; Gorham et al., 1987). When it comes to barley, Forster *et al.* (1994) showed that a salt-tolerant mutant was able to limit the amount of Na<sup>+</sup> uptake during salt treatment compared to its isogenic parent. Salt-tolerant barley varieties showed significantly lower Na<sup>+</sup> concentrations than that of susceptible varieties

under saline conditions (Chen et al., 2005; Tajbakhsh et al., 2006). Extensive screening for salt tolerance in barley, based on  $\text{Na}^+$  content, has been conducted in thousands of accessions from the world barley collections (Kingsbury and Epstein, 1984). Salt tolerance in barley varieties such as CM67 has been correlated with their ability to exclude  $\text{Na}^+$  from the shoot (Wyn Jones and Storey, 1978; Royo and Aragues, 1993; 1999). In addition, leaf  $\text{Na}^+$  accumulation has been shown to relate to salt sensitivity in genotypes of rice, sorghum, and wheat (Yeo and Flowers, 1986; Munns, 2002; Munns et al., 2006; Krishnamurthy et al., 2007; Thalji and Shalaldeh, 2007). It is no exaggeration to say that this trait is the most popular among plant breeders working on improving salt tolerance in plants. There is one hurdle, however, which limits its applicability and reduces the prognostic value of such approach. Tissue  $\text{Na}^+$  analysis fails to take into account a plant's ability for  $\text{Na}^+$  sequestration, both at the cellular and the tissue level. Therefore, some salt-tolerant genotypes with efficient vacuolar compartmentation (enhanced  $\text{Na}^+/\text{H}^+$  activity for example) may be simply missed when selection is made based on shoot  $\text{Na}^+$  content analysis.

### 3.3.2 Tissue $\text{K}^+$ content

Potassium is the most abundant cation in higher plants and comprises up to 10% of the total plant dry weight (Marschner, 1995). In plants,  $\text{K}^+$  plays central roles including osmoregulation, maintenance of turgor pressure, leaf and stomatal movement, enzyme activation, cell elongation, phloem solute transport, cation:anion balancing, control of membrane polarisation, cytoplasmic pH regulation, protein and starch synthesis, and energy conservation across membranes (Wyn Jones et al., 1979; Clarkson and Hanson, 1980; Kochian and Lucas, 1988; Maathuis et al., 1997; Leigh, 2001; Palmgren, 2001; Mäser et al., 2002; Subbarao et al., 2003). The overall contribution of  $\text{K}^+$  to the total solute potential, studied in over 200 plant species, varies from 66% to 90% (Wagner, 1982; Hsiao and Lauchli, 1986). The formation of chloroplast structure, the translocation of assimilates and storage in the sink tissue all critically depend on adequate tissue  $\text{K}^+$  concentrations (Flowers and Lauchli, 1983). Finally, many metabolic processes and enzymatic reactions in the cytoplasm have a specific requirement for  $\text{K}^+$  (Wyn Jones et al., 1979; Marschner, 1995).

$\text{K}^+$  levels in soil solution range from 1 to 10 mM, but intracellular  $\text{K}^+$  levels in plants are maintained at 100–200 mM (Kochian and Lucas, 1988). Cytoplasmic  $\text{K}^+$  levels are well buffered against change by the large vacuolar pool of  $\text{K}^+$ , but high salt causes a decrease in cellular  $\text{K}^+$  content and in the  $\text{K}^+/\text{Na}^+$  ratio (Storey and Wyn Jones, 1987). Compared with barley, salt-tolerant wild *Hordeum* species maintain higher leaf  $\text{K}^+$  under severe salt treatment (Garthwaite et al., 2005). Salt toxicity on seeds is reflected by decreases in seed  $\text{K}^+$  concentration, and leakage of  $\text{K}^+$  has been used as a measure of membrane damage from salinity (Nassery, 1979; Petruzzelli et al., 1992). Soil salinization (170 mM NaCl) significantly decreases  $\text{K}^+$  content in the shoots of salt-susceptible barley cultivars, but the  $\text{K}^+$  content in the most tolerant one increased (Leonova et al., 2005). Salt treatment also reduces potassium activity in the barley epidermal leaf cell vacuoles from 224 to 47 mM and in the cytosol from 68 to 15 mM, while the corresponding changes in the mesophyll were from 235 to 150 mM (vacuole) and 79 to 64 mM (cytosol) (Cuin et al., 2003).

Plants that are more tolerant to salt have a greater ability of maintaining high levels of  $\text{K}^+$ . This has been found in crops screened and bred for salinity tolerance, as well as in wild relatives of certain crop species (Colmer et al., 1995; Dubcovsky et al., 1996; Flowers and Hajibagheri, 2001; Zhu et

al., 2001). For example, Thalji and Shalaldehy (2007) reported that, for barley and wheat,  $K^+$  content at the three-leaf stage showed a strong positive correlations with seed yield: the ultimate criterion of salinity resistance. Chen *et al.* (2007a) also found that  $K^+$  flux from the root in response to NaCl treatment (the ability to maintain  $K^+$  under salt stress) was highly inversely correlated with relative grain yield, shoot biomass, plant height, net CO<sub>2</sub> assimilation, survival rate and thousand-seed weight measured in glasshouse experiments after 4–5 months of salinity treatment.

### 3.3.3 $K^+/Na^+$ and $Ca^{2+}/Na^+$ ratios

The capacity of plant to maintain a high cytosolic  $K^+/Na^+$  ratio is one of the key determinants of plant salt tolerance (Serrano *et al.*, 1999; Amtmann *et al.*, 2004). Under typical physiological conditions, plants contain about 100 mM  $K^+$  and maintain a high  $K^+/Na^+$  ratio in their cytosol (Binzel *et al.*, 1988), rarely tolerating cytosolic  $Na^+$  levels above 20 mM (Blumwald *et al.*, 2000). *In vitro* activities of enzymes extracted from the halophytes pop saltbush or Seablite (*Suaeda maritima* L.), and even enzymes from the pink salt-lake alga (*Dunaliella parva*) that tolerates 10-fold-seawater salinity, were just as sensitive to NaCl as were those of beans or peas (Greenway and Osmond, 1972; Flowers *et al.*, 1977). In contrast to durum wheat, barley is more efficient on  $Na^+$  and  $K^+$  partitioning in cellular and subcellular compartments, leading to the preservation of a higher cytoplasmic  $K^+/Na^+$  ratio at high leaf  $Na^+$  concentrations (Munns *et al.*, 2006). This has been confirmed by electrophysiological analysis of root cation channels: all major  $K^+$  influx channels exhibit higher  $K^+/Na^+$  selectivity in salt cress (*Thellungiella halophila*) than in *Arabidopsis* (Volkov *et al.*, 2004). Rivelli *et al.* (2002) showed that in low- $Na^+$  wheat genotypes, osmotic adjustment was enabled by a higher  $K^+$  content,  $Na^+$  exclusion being associated with maintenance of higher  $K^+$  levels. Cytoplasmic  $Na^+$  concentration in salt-sensitive barley was almost 1.4 times greater than that of the salt-resistant one, the latter also having a higher  $K^+$  concentration. It is evident that a salt-sensitive cultivar has a higher  $Na^+$  concentration in its cytoplasm than a salt-resistant variety (Flowers and Hajibagheri, 2001).

The higher  $Na^+$  uptake and lower  $K^+$  content exhibited by salt-sensitive barley has been contrasted with lower  $Na^+$  and higher  $K^+$  in salt-tolerant California Mariout and its derivative CM67 (Wyn Jones and Storey, 1978; Gorham *et al.*, 1994). Similar results have also been reported by Al-Karaki (2001). Tissue  $K^+/Na^+$  ratio has been used successfully for the selection for salinity tolerance in many crops (Janardhan *et al.*, 1979; Chhipa and Lal, 1985; Dvořák *et al.*, 1994; Asch *et al.*, 2000; Tajbakhsh *et al.*, 2006; Thalji and Shalaldehy, 2007). Thalji and Shalaldehy (2007) suggested that the barley and wheat  $K^+/Na^+$  ratio can be used as a selection criterion for salt tolerance because it is highly correlated with biomass, seed and straw yields. The  $Ca^{2+}/Na^+$  ratio also appears a more reliable indicator of salt stress than  $Na^+$  content alone (Ben-Hayyim *et al.*, 1987; Krishnamurthy *et al.*, 2007). Indeed, the maintenance of higher  $K^+/Na^+$  and  $Ca^{2+}/Na^+$  ratios in young growing tissues does appear to be an important mechanism contributing to improved barley salt tolerance (Wei *et al.*, 2003).

Controversially, bulk leaf ion concentrations of  $Na^+$ ,  $K^+$ ,  $Ca^{2+}$ , and the  $K^+/Na^+$  and  $Ca^{2+}/Na^+$  ratios were not judged to be the cause of the differences in grain yield that were observed in wheat and barley cultivars. Thus it was suggested that these ratios could not be used in screening for salt tolerance (Rawson *et al.*, 1988; Isla *et al.*, 1997). Moreover, Fricke *et al.* (1996) found that studies

using the bulk leaf ion concentrations may be misleading because they do not detect potential ion exclusion mechanisms by the cytoplasm. On the other hand, they showed  $\text{Na}^+$  and  $\text{Cl}^-$  in different leaf compartments could be relevant to barley salt tolerance. However, bulk leaf ion concentrations of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and the ratio of  $\text{K}^+/\text{Na}^+$  and  $\text{Ca}^{2+}/\text{Na}^+$  are reliable indicators of salt tolerance as shown that they are some of the most frequently used physiological screening methods (Munns and James, 2003) and phenotyping indices in molecular marker studies (Dubcovsky et al., 1996; Koyama et al., 2001; Lin et al., 2004). In an experiment with 69 randomly selected barley varieties, Chen *et al.* (2007a) found that 62 out of 69 cultivars followed an inverse relationship between  $\text{K}^+$  efflux (release of  $\text{K}^+$  under salt stress) and salt tolerance. In a few cultivars, however, high salt tolerance (measured as grain yield at harvest) was observed for plants showing only modest ability to retain  $\text{K}^+$  in the root cells. Tissue elemental analysis showed that these plants had a much better ability to prevent  $\text{Na}^+$  accumulation in plant leaves and, thus, to maintain a higher  $\text{K}^+/\text{Na}^+$  ratio. They concluded that a plant's ability to maintain high  $\text{K}^+/\text{Na}^+$  ratio (either retention of  $\text{K}^+$  or preventing  $\text{Na}^+$  from accumulating in leaves) is a key feature for salt tolerance in barley.

### 3.4 Photosynthetic parameters

#### 3.4.1 Photosynthesis

Under mild salt treatment (150 mM NaCl), photosynthetic rate ( $P_n$ ) of barley plants was only slightly affected by NaCl treatments (Fricke et al., 1996). However, exposure of a number of barley genotypes to high salinity significantly decreased  $P_n$ ,  $g_s$ , and  $C_i$  (Jiang et al., 2006). Furthermore, these responses differed greatly between salt-tolerant and -sensitive genotypes (Huang et al., 2006). Tajbakhsh *et al.* (2006) reported that photosynthetic rate of barley decreased with the addition of salt and cultivars showed significant differences in the rate of decline under greater salinity stress. The impact of salinity on  $\text{CO}_2$  assimilation also differed substantially among barley cultivars, with tolerant genotypes maintaining net  $\text{CO}_2$  assimilation 3- to 4-fold higher than the sensitive ones (Chen et al., 2007a). Munns and James (2003) and James *et al.* (2006) reported that the maintenance of photosynthetic capacity parameters of barley compared to durum wheat at higher leaf  $\text{Na}^+$  levels was associated with the maintenance of higher  $\text{K}^+$ , lower  $\text{Na}^+$  and a resultant higher  $\text{K}^+/\text{Na}^+$  in the cytoplasm of mesophyll cells in barley. If the major limitation to photosynthesis is stomatal conductance, this parameter may be an effective way of selecting wheat genotypes that will continue to grow in saline soils (Rivelli et al., 2002; Munns and James, 2003). However, screening methods based on photosynthesis are not feasible, except stomatal conductance measured by viscous flow porometry, to handle large numbers (Rebetzke et al., 2000; James et al., 2002).

#### 3.4.2 Chlorophyll fluorescence

Chlorophyll fluorescence was employed for measuring salinity-induced inhibition of PSII (Abadia et al., 1999; Fedina et al., 2002) and thus for laboratory screening of barley genotypes for salinity tolerance (Belkhdja et al., 1994; 1999). Two-week exposure to 20 dS  $\text{m}^{-1}$  saline conditions significantly reduced a number of chlorophyll fluorescence parameters such as the  $\Phi_{\text{PSII}}$  and  $qP$  in barley (Jiang et al., 2006). In rice (Yamamoto et al., 2004) and wheat (Muranaka et al., 2002) seedlings,  $\Phi_{\text{PSII}}$  markedly decreased for both salt-tolerant and -sensitive genotypes in 100 mM NaCl, but photosynthetic activity was maintained in salt-tolerant lines (Muranaka et al., 2002). Under high salinity, both slowing of electron transport at the PSII in sunflower (Rivelli et al.,



2002) and reduced photochemical efficiency of olive (Loreto et al., 2003) have been reported, although neither was associated with photosynthetic reduction.  $F_v/F_m$  values have been widely used as a non-destructive and non-invasive tool to determine effects of environmental stresses on the photosynthetic apparatus (Maxwell and Johnson, 2000; Shabala, 2002; Sayed, 2003). However, salt stress showed no apparent effect of salt stress on maximal quantum efficiency of PSII ( $F_v/F_m$ ). Regardless of severity of the salt stress, the  $F_v/F_m$  values remained above 0.8, indicating optimal functioning of PSII (Chen et al., 2005), indicating that even under severe stress conditions leaf photochemistry in barley is well protected. In maize,  $F_v/F_m$  was also not significantly affected by NaCl treatment (Shabala et al., 1998), and  $F_v/F_m$ ,  $\Phi_{PSII}$ ,  $qP$ , and nonphotochemical quenching (NPQ) showed little difference between wheat genotypes in response to 150 mM NaCl, indicating that the efficiency of PSII photochemistry was not affected by salinity (Rivelli et al., 2002). Thus, it is unlikely that chlorophyll fluorescence measurements can be used as a reliable tool in breeding programmes aimed to improve salinity tolerance in plants. Although measuring gas exchange and chlorophyll fluorescence is not suitable for screening large number of genotypes for salinity tolerance, it is still valuable to investigate effects of salinity on photosynthetic mechanisms in a small number of germplasms.

### 3.5 Yield

The grain yield is the ultimate criterion and the main aim of the entire breeding process. It is therefore obvious that grain yield of salt-tolerant barley cultivars is less affected by NaCl than that of the salt-susceptible cultivars (Chauhan et al., 1980; Isla et al., 1998; Flowers, 2004; Leonova et al., 2005). Salinity treatment significantly reduced grain yield, shoot biomass and thousand seed weight of barley plants. The impact of salinity differed substantially between barley cultivars, with sensitive genotypes giving zero grain yield under saline conditions, while tolerant genotypes yielded 25–30% of their control values (Chen et al., 2007a). This reduction in grain yield and shoot biomass was most likely a consequence of reduced  $CO_2$  assimilation under saline conditions (Chen et al., 2007a). Forage yield of Omani Batini barley at tillering stage at 10 and 20 dS  $m^{-1}$  can be predicted with high and moderate accuracy by forage yield under 0.85 dS  $m^{-1}$  (Jaradat et al., 2004). Importantly, by testing the grain yield of 124 barley genotypes in ten salinity treatments over five consecutive years, Royo and Aragües (1999) found that the most productive genotypes were not necessarily the least salt-tolerant, so it might be useful to select for the most productive barley genotypes under medium and high saline conditions. Also, controlled environment chambers or glasshouses cannot provide adequate space, light, and pot size required to predict maximum field yield, hence, field evaluation of yield potential under salinity is critical in breeding for salt-tolerant crops. However, field experiments for yield may be more appropriate at the final stages of breeding programmes, rather than at the initial stages when screening for salt-tolerant germplasms is best done under controlled environments (Shannon, 1997; Zeng et al., 2002).

### 3.6 Survival

Survival under high salinity is also a convenient test of salinity tolerance (Kingsbury and Epstein, 1984; Sayed, 1985; Tal, 1985). The rate (percentage) of survival in saline conditions was used to evaluate salt-tolerance in 24 barley genotypes and eight wild *Hordeum* in glasshouse and controlled environment cabinet (Flowers and Hajibagheri, 2001; Garthwaite et al., 2005). In an experiment with 69 barley varieties, survival rate was greatly limited by severe salinity. The

survival rate was correlated strongly with plant salt tolerance (estimated as grain yield) (Chen et al., 2007a). Furthermore, survival under salinity was employed as a physiological indicator and its QTLs were detected in rice (Lin et al., 2004). The physiological and genetic factors that contribute to growth of crops at very high salt concentrations were found to be proportionally related to survival more than to high yields, despite survival not being of interest to farmers (Shannon and Noble, 1990). Despite showing considerable genetic diversity among 5000 hexaploid and tetraploid wheat lines, marginal correlation was found between survival of high salinity and performance in the field (Sayed, 1985). Survival at high NaCl as a selection criterion is rapid and simple, but it does not necessarily imply healthy growth and carries the risk of selecting against productivity.

### **3.7 Accumulation of compatible solutes**

Accumulation of compatible solutes is a typical plant response to salinity exposure (Yancey et al., 1982; Hare et al., 1998). Compatible solute accumulation has long been emphasised as a selection criterion in traditional crop breeding programs (Morgan, 1984; Ludlow and Muchow, 1990). The recent progress in molecular biology has made this approach central to molecular breeding programs, largely due to the fact that osmolyte accumulation is often controlled by only a single gene (Serraj and Sinclair, 2002). At 300 mM NaCl, glycine betaine and proline together contribute almost 15% to osmotic potential in leaves of halophytic sea barleygrass, compared with only 8% in barley (Garthwaite et al., 2005). Glycine betaine and proline concentrations in the flag leaf of plants exposed to 200 mM NaCl were much higher in salt-tolerant sea barley-grass than those in salt-sensitive bread wheat, and salinity tolerance was found expressed in their amphiploid (Islam et al., 2007). In barley, the impact of H<sub>2</sub>O<sub>2</sub> (one of the components of salt stress) on K<sup>+</sup> flux (a measure of stress 'severity') and the mitigating effects of glycine betaine and proline on NaCl-induced K<sup>+</sup> efflux were found to be significantly higher in salt-sensitive barley genotypes (Chen et al., 2007a) with a 2-fold higher accumulation of leaf and root proline and leaf glycine betaine in salt-sensitive cultivars. The total amino acid content was less affected by salinity in salt tolerant cultivars. In salt-sensitive genotypes, glycine betaine and proline contributed substantially to cell osmolality, compensating for reduced cytosolic K<sup>+</sup>. They concluded that hyperaccumulation of known major compatible solutes in barley does not appear to play a major role in salt-tolerance, but rather, may be a symptom of salt-susceptibility (Chen et al., 2007a).

Due to the controversy over whether high accumulation of compatible solutes is actually beneficial for salinity tolerance in glycophytes, the use of salt-induced compatible solute accumulation is not validated as potential screening tool for salinity tolerance. It also varies among different plant species.

### **3.8 The MIFE technique**

The proposal to use ion concentrations or electrochemical potentials measured outside plant tissues to calculate tissue flux of the ion came from B. Lucas (Lucas and Kochian, 1986). Over the past twenty years, the MIFE technique has been employed in a broad range of research areas in plant sciences (Newman, 2001; Shabala, 2006), including the response of plants to salinity (Shabala et al., 1998; 2003; 2005a; 2005b), waterlogging (Pang et al., 2006a; 2006b), Al<sup>3+</sup> toxicity (Wherrett et al., 2005), Ca<sup>2+</sup> and Mg<sup>2+</sup> deficiency (Shabala et al., 2003; Shabala and Hariadi, 2005), high and low temperature (Shabala, 1996; 1997), rhythmic patterns of nutrient acquisition (Shabala and

Knowles, 2002; Shabala et al., 2006b), plant response to blue light (Babourina et al., 2002), fluctuations of light intensity (Živanović et al., 2005), and plant ion transporter studies combined with patch clamp and other techniques for detailed ion transporter studies (Tyerman et al., 2001; Shabala and Lew, 2002; Demidchik et al., 2003; Shabala et al., 2006a).

The use of the MIFE technique has advanced research into plant salinity tolerance (Shabala, 2006; Chen et al., 2005; 2007a; 2007b). For example, bioelectric response measured by MIFE was reported to be a sensitive indicator of NaCl stress in maize leaves (Shabala et al., 1998). Indeed, it has been shown that NaCl stress results in a significant net  $K^+$  efflux (prevented by 10 mM  $Ca^{2+}$ ) from leaf mesophyll of broad bean (*Vicia faba* L.). In contrast, plants showed a net  $K^+$  uptake in response to isotonic mannitol application. These differences reflect the involvement of both the ionic and osmotic components of salinity stress (Shabala et al., 2000). In suspension cells from wheat, Fusicoccin prevents NaCl-induced  $K^+$  loss from the cell by direct activation of  $H^+$ -ATPases and other metabolic changes crucial for the plant's adaptation to high salinity (Babourina et al., 2000). It was found that there was no effects of NaCl on the net  $Ca^{2+}$  flux in protoplasts from broad bean mesophyll, indicating that the large transient NaCl-induced  $Ca^{2+}$  efflux from tissue originates from cell wall ion exchange (Shabala and Newman, 2000). Shabala *et al.* (2003) reported that NaCl causes rapid and prolonged efflux of  $H^+$ ,  $K^+$ , and  $NH_4^+$  from the root epidermis with a more positive plasma membrane potential ( $E_m$ ). The relative efficiency on stimulating barley  $Na^+$  efflux and  $K^+$  uptake was shown to be  $Ba^{2+} > Zn^{2+} = Ca^{2+} > Mg^{2+}$  (Shabala et al., 2005a). In addition to their ability to block NSCCs, divalent cations also control the activity of  $K^+$  transporters to maintain the high  $K^+/Na^+$  ratio required for optimal leaf photosynthesis. It has been proposed that compatible solutes prevent NaCl-induced  $K^+$  efflux from barley roots, through enhancing the activity of  $H^+$ -ATPase, thereby controlling DAPCs and creating the electrochemical gradient necessary for secondary ion transport processes (Cuin and Shabala, 2005). In addition, Cuin and Shabala (2007) found that a large number of amino acids cause a significant mitigation of the NaCl-induced  $K^+$  efflux from barley root epidermis, thus suggesting that free amino acids might also contribute to plant adaptation to salinity by regulating  $K^+$  transport across the PM. A very strong negative correlation was found between the magnitude of  $K^+$  efflux from the root and salt tolerance of a particular cultivar (Chen et al., 2005).  $K^+$  efflux from the mature root zone of intact 3-day-old seedlings following 40 min pretreatment with 80 mM NaCl was found to be a reliable screening indicator for salinity tolerance in barley (Chen et al., 2005). Further experiments showed that the MIFE technique can distinguish contrasting barley varieties differing substantially in salt tolerance (Chen et al., 2007a).

In summary, various physiological, electrophysiological, genetic, and biochemical methods can be employed to reveal the mechanisms underlying the difference in response to salinity between salt-tolerant and -sensitive barley genotypes. Also, a newly developed screening method based on NaCl-induced ion flux measurement (MIFE technique) can also facilitate rapid screening of plant species and cultivars for their salinity tolerance.

#### **4. BARLEY GERMPLASM FOR SALINITY TOLERANCE**

The gene pool of wild relatives may represent a valuable source of new loci for salt tolerance. Mano and Takeda (1998) evaluated salt tolerance of 340 accessions of *Hordeum*, consisting of 41

brittle-rachis forms of *Hordeum vulgare* L. subsp. *vulgare* (*H. agriocrithon*) accessions, 154 *H. vulgare* L. subsp. *spontaneum* (*H. spontaneum*) accessions, and 145 accessions of ten other species or subspecies of wild *Hordeum*. They found the levels of salt tolerance for seed germination in wild *Hordeum* species were generally lower than those in cultivated barley and the NaCl tolerance level of the different species were as follows: *H. agriocrithon* > *H. spontaneum* > other wild *Hordeum* species. The higher tolerant level of *H. agriocrithon* probably originated from natural hybridization between *H. spontaneum* and cultivated barley (Briggs, 1978). In addition, when leaf injury index was used to assess tolerance at the seedling stage, the levels of salt tolerance in wild *Hordeum* species were generally higher than those found in cultivated barley. Most wild *Hordeum* species showed high NaCl tolerance at the seedling stage and were considered good sources of germplasm for salt tolerance breeding.

The natural populations of *H. spontaneum* showed rich genetic variation in tolerance to physiological stresses such as salinity. This was highlighted by the tremendous variation both between and within populations observed in the extreme desert station at Sede Boqer (Nevo et al., 1984). Subsequently, populations and genotypes of wild barley *H. spontaneum* and wild emmer wheat *T. dicoccoides* were tested for salt tolerance under controlled conditions, using 150, 250 and 350 mM NaCl (equivalent to 25, 40 and 60% sea water) with non-saline controls (Nevo, 1992). They found wide difference in high salt tolerance within and between populations of *H. Spontaneum* and *T. dtkoccoides*, and identified wild barley genotypes with high tolerance from coastal Mediterranean populations (e.g. Caesarea), the Jordan Valley (Mehola) and the northern Negev desert (Makhtesh Gadol). The tolerant lines could spike normally under 350 mM NaCl. Natural populations of wild barley extend southwards to the northern Negev, yet they only grow there in dry watercourses, thereby effectively utilizing the extra run-off water. However, under a water regime of 130 mm and highly saline soil conditions, some populations and superior genotypes have proved relatively outstanding in both growth and yield, despite these extremely harsh ecological conditions. Thus, the genetic resources of the progenitor display remarkable morphological and physiological performances, which are of potential economic importance for barley improvement.

There is variation among barley varieties for salinity tolerance. Almost 5000 barley lines were screened in Syria for germination, seedling vigor and adult plant vigor (Srivatava and Jana, 1984). Promising lines were identified, but the results were not resulted in release of new variety with salinity tolerance. Ramagopal (1988) found obvious difference between salt-tolerant barley genotypes, CM72 (California Mariout 72) and sensitive Prato in protein synthesis during seed germination. Salinity stress induced both quantitative and qualitative changes in the expression of some proteins *in vivo*. Around 8% of the nearly 400 resolved proteins in a tissue were affected this way. Some of the proteins in this category were specific to each genotype. About 1% of the total showed qualitative changes; these proteins were expressed only during salinity stress. In roots, two proteins were detected in CM72 and five in Prato. In shoots, four proteins were found only in Prato and these were similar to those induced in roots. The four new proteins in germinating embryos were apparently induced only in CM72. It is indicated that ontogeny plays an important role in the expression of tissue-specific proteins during salinity stress in the salt tolerant and sensitive barley genotypes.

## 5. DEVELOPMENT OF SALINITY TOLERANT GERMPLASM THROUGH IN VITRO CULTURE

Ye *et al.* (1987) demonstrated that *in vitro* culture with salt in media was an effective approach to develop salinity tolerant barley lines. Anthers of two six-rowed barley cultivars Diamond (salinity sensitive for germination) and Men Yuan Liang Lan (salinity tolerance), and their F<sub>1</sub> from reciprocal crosses were cultured in liquid media containing 0, 0.4, 0.6, and 0.8% Na<sub>2</sub>SO<sub>4</sub>. It was found that among 37 progenies from F<sub>1</sub> pollen in Na<sub>2</sub>SO<sub>4</sub> free medium, 11 were as sensitive as “Diamond”, 12 were intermediate to the two parents, 7 were equal to the salt tolerant parent and 7 were more tolerant to Na<sub>2</sub>SO<sub>4</sub> than ‘Men Yuan Liang Lan’. Whereas, no progeny from F<sub>1</sub> pollen in high salt media was as susceptible as the susceptible parent; 2 were intermediate, 2 were equal to the salt tolerant parent and 2 were more tolerant than the salt tolerant parent. The results demonstrated that culturing anthers in Na<sub>2</sub>SO<sub>4</sub> media effectively eliminated salt susceptible progenies. All 16 microspore-derived lines of Diamond were as susceptible as ‘Diamond’ to Na<sub>2</sub>SO<sub>4</sub>. The 5 lines from ‘Men Yuan Liang Lan’ microspores were as resistant to Na<sub>2</sub>SO<sub>4</sub> as ‘Men Yuan Liang Lan’. The results indicate that the lines exhibiting elevated levels of tolerance to salt probably resulted from recombination of genes rather than from spontaneous mutation.

## 6. DEVELOPMENT OF SALINITY TOLERANT GERMPLASM THROUGH MUTATION

In combination with irradiation, EMS induction and salt-induced tissue culture, 4 salinity tolerant mutants were identified from *Hordeum brevisubulatum* (Lu *et al.*, 2002). The most successful example for barley salinity tolerance using mutation is Golden Promise, a gamma-ray induced semi-dwarf mutant of the cultivar ‘Maythorpe’, although salinity tolerance was not the initial purpose. Forster (2001) reviewed the research made at the Scottish Crop Research Institute (SCRI) on the effects of semi-dwarfing genes on salt tolerance. The work was initiated in 1993 with the fortuitous and unexpected result that the cultivar Golden Promise showed considerable tolerance to salt. The parent and mutant showed significant differences in their responses to salt stress. The positive and pleiotropic effects of the mutant gene *Gpert* were found to be effective in a number of genetic backgrounds. The *Gpert* mutation was allelic to the *ari-e* mutants in barley. The *ari-e* mutants were salt tested and found to show the same positive responses to salt stress as Golden Promise, supporting the allelism tests, and consequently the *Gpert* symbol was changed to *ari-e.GP*. The semi-dwarf mutant *sdw1* and the *erectoides* semidwarf mutant *ert-k32* were also tested for their effects on salt tolerance, but did not show any positive effects. Salt tolerance was therefore not a general phenomenon of semi-dwarf stature but specific to mutations at the *ari-e* locus in these lines. The barley varieties Dash and Hindmarsh with the *ari-e.GP* gene showed excellent yield potential and adaptation in Australian environment. As there are closely linked molecular markers available for the *ari-e.GP*. It should be a good target for MAS to improve barley salinity tolerance. Wei *et al.* (2001) found a gene encoding the barley vacuole ATPase subunit B (BSVAP), which was differentially expressed between near isogenic barley cultivars, Golden Promise and Maythorpe. The gene was inducible under long-term salinity stress in the salt

sensitive cultivar Maythorpe, but less so in the relatively salt tolerant Golden Promise and was highly expressed under control conditions in Maythorpe. It was concluded that the short-term down-regulation of BSVAP under high salinity was an important mechanism contributing to Golden Promise. This gene could be the target for genetic modification or marker-assisted selection for improvement of salinity tolerance. Dizetz *et al.* (2001) suggested the ability to respond to salinity stress with changes in gene expression of the vacuolar ATPase might be a prerequisite and a characteristic of salt tolerance in plants.

## **7. MARKER-ASSISTED SELECTION (MAS) FOR SALINITY TOLERANCE**

### **7.1 Barley molecular marker techniques**

Successful implementation of marker-assisted selection in a breeding program consists of nine key steps (Barr *et al.*, 2001), which include; characterizing genetics of traits, choosing parents, constructing genetic population, multiplying seeds, constructing map, phenotyping mapping population, identifying genes/QTL for target traits, validating putative markers and implementing molecular markers in breeding programs. Map construction is the most expensive and time-consuming step and it usually costs more than 40% of the total cost for MAS (Barr *et al.*, 2000). Developments of the marker technologies in the last few years have significantly reduced the cost for map construction. Since development of the first generation of RFLP barley molecular maps (Graner *et al.*, 1991; Kleinhofs *et al.*, 1993), PCR-based molecular markers have become the dominant marker type for map construction and MAS (Varshney *et al.*, 2004). Among different types of molecular markers available for barley, microsatellite or simple sequence repeats (SSRs) have become the mostly commonly-used markers for genetic diversity study, map construction and MAS in breeding programs, as SSR markers are abundant in genomes, multi-allelic, high informative, co-dominantly inherited. In addition, SSR markers are easy detection and amenable to high throughput analysis. Several thousands of barley SSR markers have been developed from different research groups including SCRI (Bmac, Bmag, EBmac, EBmag, HVGeneName, scsssr), IPK (GBM, GBMS), WUR (GBM), Virginia Polytechnic Institute (HVM), and MPI for Plant Breeding (HVGeneName). These SSR markers have been mapped in various mapping populations.

Diversity arrays technology (DArT) is a new high-through molecular marker system, which belongs to a hybridization-based molecular marker method deployed on a microarray platform. The Technology was developed and commercialized by Triticarte P/L (<http://www.diversityarrays.com>). DArT is genome sequence-independent and allows high throughput screening of hundreds of molecular markers simultaneously. It is especially suitable for generating genome-wide markers for genetic linkage mapping and identifying markers closely linked to genes or QTLs for MAS applications. Barley was the first species for which DArT markers were made available. Approximately 2.3 million data points for 4,000 lines have been generated for barley breeders and researchers (Wenzl *et al.*, 2006). More than 2000 DArT markers have been mapped in various barley populations and integrated into a consensus map with RFLP, SSR and STS markers (Wenzl *et al.*, 2006). The consensus map comprised 2,935 loci (2,085 DArT, 850 other loci) and spanned 1,161 cM. It contained a total of 1,629 'bins' (unique loci), with an

average inter-bin distance of  $0.7 \pm 1.0$  cM (median = 0.3 cM). More than 98% of the map could be covered with a single DArT assay.

Single Nucleotide Polymorphisms (SNPs) are the most abundant form of DNA polymorphism and now predominate applications in modern genetic analysis. Over 17 000 candidate SNPs have been identified from mining 350 000 public barley expressed sequences in barley (Appleby et al., 2008). However, it should be cautious about this number as the greatest challenge of *in silico* SNP discovery is the differentiation between true polymorphisms and sequence error. In another study, more than 2,000 genome-wide barley single nucleotide polymorphisms (SNPs) were developed by resequencing unigene fragments from eight diverse accessions (Rostoks et al., 2005). The average genome-wide SNP frequency observed in 877 unigenes was 1 SNP per 200 bp DNA sequence. However, SNP frequency was highly variable with the least number of SNP and SNP haplotypes observed within European cultivated germplasm reflecting effects of breeding history on genetic diversity. More than 300 SNP loci were mapped genetically in three experimental mapping populations which allowed the construction of an integrated SNP map incorporating a large number of RFLP, AFLP and SSR markers (1,237 loci in total). Recently, a total of 12,615 SNPs were identified in 3509 EST-derived contigs using 36 pairwise comparisons between nine barley genotypes (Bhat et al., 2007). A linear relationship was observed between the number of SNPs and the product of the number of ESTs for each pair of genotypes. An additional ~800 SNPs from genomic amplicon sequences were also used. An Illumina oligonucleotide pool assay (OPA) was designed for high throughput SNP genotyping, representing 1524 different barley unigenes with one SNP each. SSR, DArT and SNP provide efficient molecular tools for map construction, QTL identification and MAS for salinity tolerance in barley.

## 7.2 Mapping the genes/QTLs for salinity tolerance

A number of QTLs affecting salinity tolerance were detected on all chromosomes in *H. spontaneum* and *H. vulgare* and the crosses of the two species. In cultivated barley, Mano and Takeda (1997) identified QTLs controlling salt tolerance at germination and the seedling stage in barley by interval mapping analysis using marker information from two doubled haploid (DH) populations derived from the crosses Steptoe x Morex and Harrington x TR306. The results revealed that the QTLs for salt tolerance at germination in the DH lines of Steptoe x Morex were located on chromosomes 4H, 6H, and 5H, and in the DH lines of Harrington/TR306 on chromosomes 1H and 5H. In both DH populations, the most effective QTLs were found at different loci on chromosome 5H. Genetic linkage between salt tolerance at germination and ABA response was found from QTL mapping. The QTLs for the most effective ABA response at germination were located very close to those for salt tolerance on chromosome 5H in both crosses. The QTLs for salt tolerance at the seedling stage were located on chromosomes 2H, 1H, 6H, and 5H in the DH lines of Steptoe x Morex, and on chromosome 5H in the DH lines of Harrington x TR 306. Their positions were different from those of QTLs controlling salt tolerance at germination, indicating that salt tolerance at germination and at the seedling stage were controlled by different loci. QTLs were also identified for traits hypothesised to associate with salinity tolerance (Ellis et al., 1997). However, it is not found to be a useful indicator for screening barley for grain yield under saline conditions (Isla et al., 1998). In a cross of Derkado  $\times$  B83-12/21/5, the largest individual effects to salt tolerance were associated with the chromosomal regions around the two dwarfing genes *sdw1* (3H) and *ari-e.GP* (5H). The *sdw1* gene resulted in an overall

yield increase, but was only detected as a secondary QTL (Ellis et al., 2002). In a winter barley mapping population Angora  $\times$  W704/137, a QTL related to sodium uptake was found on chromosome 1H in the field trial and another QTL responding to salt stress on chromosome 3H was found in both development stages and was related to yield and yield reduction in the later developmental stages, probably indicating a gene location related to translocation of carbohydrates (Weidner et al., 2007). Recently, we identified QLTs controlling salt tolerance at later growth stage in barley by interval mapping analysis using marker information from a doubled haploid (DH) populations derived from the cross, CM72 and Gairdner, examining tillers per plant, plant height, spikes per line, spikes per plant, shoot dry weight, grains per plant, grain yield, shoot Na<sup>+</sup>, K<sup>+</sup> content and Na<sup>+</sup>/K<sup>+</sup> ratio under the conditions of salinity and no stresses. Totally 31 QTL were detected, 16 under the control condition, and 15 under salt stress condition. All these QTL are located on the 7 chromosomes. Moreover, QTL affecting the different characters or same character under different treatment were clustered on the chromosomes. Interestingly, 4 QTL were detected in the region of bPb-1278~bPb-9504 on chromosome 4, and these QTL is associated with tillers per plant, spikes per line, spikes per plant and shoot dry weight, respectively, and characterized by pleiotropic effect. Moreover, the additive effect is mainly attributed to salt-tolerant parent CM72.

However, it has been reported that such QTL were dependent on the conditions under which the plants were grown (Foolad et al., 1999; Monforte et al., 1997). In addition, it was suggested that QTL associated with salt tolerance vary with the developmental stage. A further limitation to the use of QTL in plant breeding is the fact that QTLs may be specific to particular crosses. Therefore it may be argued that whilst markers will be of value in using elite lines from the mapping population in backcrossing, the result cautions against any expectation of a general applicability of markers for physiological traits. Although there is no successful report for using MAS to improve salinity tolerance, the identified loci/QTLs may provide basis to integrate MAS with conventional approaches for improvement of salinity tolerance in barley.

## **8. IDENTIFICATION OF CANDIDATE GENES FOR SALINITY TOLERANCE**

Plants respond to high salinity conditions by adjusting their physiological and metabolic processes (Rhode and Hanson, 1993). There are many genes for maintaining ion homeostasis and metabolism including synthesis of compatible solutes (osmoprotectants), stress proteins for cell rescue and defense, proteins for signal transduction, components of protein synthesis and others affecting morphology (Bohnert and Jensen, 1996; Kasuga et al., 1999; Kawasaki et al., 2001).

Candidate gene approaches have been used to study salinity tolerance. Munns (2005) and Colmer *et al.* (2005) reviewed the potential genes involved in salt tolerance. There are four categories of genes which control traits that might be expected to increase salinity tolerance. (1) proteins that control the transport of ions across membranes; (2) enzymes that synthesise organic solutes that contribute to control of plant water relations or functions; (3) proteins involved in protecting against oxidative damage and other consequences osmotic stress and (4) genes encoding components of signal transduction pathway pathways.



Development of microarray technology provides an opportunity to identify a large number of genes involved salinity tolerance. Barley cultivar Morex was used for transcriptional profiling during salinity stress using a microarray containing 22,750 probe sets (Walia et al., 2006). The experiment was designed to target the early responses of genes to a salinity stress at seedling stage. A number of probe sets were found up-regulated and down-regulated in response to salinity. A prominent feature of the response to salinity was the induction of genes involved in jasmonic acid biosynthesis and genes known to respond to jasmonic acid treatment. A large number of abiotic stresses (heat, drought, and low temperature) related genes were also found to be responsive to salinity stress. The results indicated osmoprotection to be an early response of barley under salinity stress. Unfortunately, the results of this study with two other reports characterizing gene expression of barley under salinity stress found very few genes in common (Ozturk et al., 2002; Ueda et al., 2004). The gene-encoding P5CS is the only gene found to be commonly up-regulated in all three studies.

A differential display using randomly amplified polymorphic DNA (RAPD) primers isolated a salt-inducible gene encoding a putative methionine synthase in barley leaves (Muramoto, 1999; Shi et al., 2001). The gene was named as *HvMS* (*Hordeum vulgare* methionine synthase), and it was found that the expression of this gene is induced in barley leaves within 1 h by salt stress. This is one of the early responsive genes in barley leaves. It was assumed that the salt-inducible *HvMS* could play an important role as a member of this cycle for salt tolerance in barley plants (Shi et al., 2001). Eckermann *et al.* (2000) reported that the expression of methionine synthase (*MS*) is induced under salt stress. A *MS* gene from potato was cloned and characterized (Zeh, 2002). RNA transcription from this *MS* gene was regulated by a day/night rhythm, but protein levels did not alter. Therefore, *MS* was not considered as one of the important components in salt-stress tolerance of plants. However, Narita *et al.* (2004) reported *HvMS* protein levels were increased under salt stress and suggested that *MS* may indeed be important in salt tolerance in higher plants. Furthermore, they found that this gene complemented a yeast mutant lacking the ability to synthesize methionine under both non-stress and high-salinity conditions.

A late-embryogenesis-abundant (LEA) gene family was induced by osmotic condition, dehydration, salt and ABA treatment. The gene family was up regulated by drought and salinity. Several genes from this gene family were regulated or induced by drought and salinity. These genes include *HVA1* (Hong et al., 1992), *ABA2* and *ABA3* (Gulli et al., 1995), *Pa93* (Grossi et al., 1995), dehydrins (Close, 1996) and a *B19* gene family (Hollung et al., 1994). Cattivelli *et al.* (2002) have summarized this gene family isolated from barley.

Accumulation of glycinebetaine is one mechanism for barley response to drought or salt stress. Ishitani *et al.* (1995) reported a gene up-regulated by drought stress and encoding an enzyme of known function - betaine aldehyde dehydrogenase (BADH). This enzyme is the last step in the betaine synthesis pathway. The mRNA level of BADH increased significantly when barley was subjected to drought or saline conditions. Sorbitol has a role in osmoregulation. The enzyme aldose reductase involved in the accumulation of sorbitol was regulated by ABA (Bartels et al., 1991).

However, application of the knowledge about the candidate genes for improvement of salinity tolerance is very limited. One reason may be that the biochemical function of the gene is unknown

and the second is that many experiments are not designed to expose genes that confer salinity tolerance under natural conditions (Munns, 2005).

## **9. IMPROVEMENT OF SALINITY TOLERANCE BY TRANSGENETIC APPROACH**

There has been substantial progress in identifying genes for tolerance to various abiotic stresses. An alanine aminotransferase isolated from barley roots could increase drought tolerance of transformed tobacco (Muench and Good, 1994). Xu *et al.* (1996) has used a transgenic approach to investigate the function of the *HVA1* protein in stress protection of rice. *HVA1* is a group 3 LEA protein that is expressed in barley aleurone and embryo during late seed development correlating with the seed desiccation stage (Hong *et al.*, 1988). The transgenic rice plants exhibited a high constitutive expression of *HVA1* protein in leaves and roots. The progeny of three transgenic plants was used for evaluation of the growth performance under water deficit and salt stress treatment. The appearance and development of the major damage symptoms such as wilting, dying of old leaves and necrosis of young leaves caused by the stress conditions were delayed in the transgenic plants. The better performance of the transgenic lines under stress conditions was correlated with higher level of *HVA1* protein accumulated in the plants.

Although there are some successful reports for improvement of salinity tolerance through transgenic approach, in almost of all cases so far there is no clear supporting data (Flowers, 2004). As salinity tolerance is determined by the coordinated action of many genes, it might be not expected to significantly improve the tolerance by modifying a single gene. The second wave of transformation attempts to transform plants stress-induced regulatory genes (Bhantnagar-mathur *et al.*, 2008). Many genes involved in stress response can be simultaneously regulated by a single gene encoding stress inducible transcription factor (Kasuga *et al.*, 1999). Transcription factors activate cascades of genes that act together in enhancing tolerance towards multiple stresses. One example is the genes involved in the ABA signalling pathway. Salt hypersensitivity was observed in ABF3- and ABF4-overexpressing plants at germination and seedling stages, which indicated that the two genes may participate in salinity response. Pardo *et al.* (1998) achieved salinity tolerant transgenic plants by over expressing calcineurin, a protein phosphatase involved in salt stress signal transduction. Similarly a truncated tobacco mitogen-activated protein kinase (NPK) activated an oxidative signal cascade resulting salinity tolerance.

## **10. BREEDING FOR SALT-TOLERANT BARLEY**

As physiology and the genetics of salt tolerance are so complicated, is it going to be possible to breed for salt-tolerant crops? To date, there have been only limited successes. However, a variety of approaches have been advocated, including conventional breeding, wide crossing, the use of physiological traits and, more recently, marker-assisted selection and the use of transgenic plants. None of these approaches could be said to offer a universal solution. Conventional breeding programs have rarely delivered enhanced salt tolerance (Flowers and Yeo, 1995), while wide crossing generally reduces yield to unacceptably low levels (Yeo and Flowers, 1989). There has been success using physiological criteria as the basis of selection in rice (Dedolph and Hettel,

1997) and such an approach has recently been advocated for wheat (Munns et al., 2002). A recent analysis has shown that whilst it is possible to produce a wide range of transgenic plants where some aspect of a trait relating to salt tolerance was altered, none has been tested in the field and few claims for success meet even minimal criteria required to demonstrate enhanced tolerance (Flowers, 2004). With the development of molecular marker research, marker-assisted selection provides a more powerful tool in barley salt-tolerant breeding.

Combining the DNA technology and advanced statistical methods (Kearsey, 1998), chromosomal regions that contain the genes that determine quantitative traits can be identified. By crossing parents that differ in one or more aspects of salt tolerance (their phenotype), and then analyzing the phenotype and the genotype of their offspring, it has been possible to locate QTL for salt tolerance. For a plant breeder, such QTLs are particularly attractive, as they can, in principle, be developed to produce markers to aid selection. Such markers can be used in the selection of lines following a crossing program and without the need to determine their phenotype or to take all the lines to seed (Asins, 2002).

In selection of barley with salt tolerance, Koval *et al.* (2000) investigated the contribution of the gametophyte in the inheritance of salt tolerance by crossing F<sub>3</sub> and BC<sub>1</sub> hybrids of the tolerant cultivars Rannii 1 and Pirkka with the sensitive cultivar K-30356, and found that the progenies of heterozygous plants grown in saline conditions showed elevated salinity tolerance. A comparison of the BC<sub>1</sub> hybrid progenies showed that the male and female gametophytes contributed to the inheritance of salt tolerance. Gametic selection is maximally efficient during the formation of the female gametophyte and the germination of pollen grains on the stigma.

There is one successful report for development of salinity tolerance barley variety through conventional crossing and selection strategy (Zhao and Liu, 2002). The new barley variety Shanglong50245 was derived from a salinity tolerance variety CM72 crossing with a local breeding line 26-2. Shanglong50245 showed similar salinity tolerance as CM72 at germination, seedling and adult plant stages. Under soil salt concentrations of 0.35-0.56‰, the new breeding variety increased grain yield by 18.3%.

## REFERENCES

- Abadia, A., Belkhodja, R., Morales, F., and Abadia, J., 1999. Effects of salinity on the photosynthetic pigment composition of barley (*Hordeum vulgare* L.) grown under a triple-line-source sprinkler system in the field, *J. Plant Physiol.* 154:392–400.
- AliDinar, H.M., Ebert, G., and Ludders, P., 1999. Growth, chlorophyll content, photosynthesis and water relations in guava (*Psidium guajava* L.) under salinity and different nitrogen supply. *Gartenbauwissenschaft* 64: 54–59.
- Al-Karaki, G.N., 2001. Germination, sodium, and potassium concentrations of barley seeds as influenced by salinity. *J. Plant Nutr.* 24: 511–522.
- Allakhverdiev, S. I., Nishiyama, Y., Suzuki, I., Tasaka, Y., Murata, N., 1999. Genetic engineering of the non-saturation of fatty acids in membrane lipids alters the tolerance of *Synechocystis* to salt stress. *Proc. Natl Acad Sci USA* 96: 5862–5867.

- Almansouri, M., Kinet, J.M., and Lutts, S., 2001. Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant Soil* 231: 243–254.
- Amtmann, A., Armengaud, P., and Volkov, V., 2004. Potassium nutrition and salt stress. In: *Membrane Transport in Plants*, MR Blatt, (ed.), Blackwell Publishing, Oxford.
- Appleby, N., Duran, C., Imelfort, M., Wood, D.L.A., Edwards, D., Batley J., 2008. Discovery and annotation of barley SNP and SSR markers with autosnpdb. *Plant & Animal Genomes XVI Conference*, San Diego, CA, January 12-16, 2008
- Asch, F., Dingkuhn, M., Dörffling, K., and Miezian, K., 2000. Leaf  $K^+/Na^+$  ratio predicts salinity induced yield loss in irrigated rice. *Euphytica* 113: 109–118.
- Ashraf, M., and Khanum, A., 1997. Relationship between ion accumulation and growth in two spring wheat lines differing in salt tolerance at different growth stages. *J Agron Crop Sci.* 178: 39–51.
- Ashraf, M., and McNeilly, T., 1988. Variability in salt tolerance of nine spring wheat cultivars, *J. Agron. Crop Sci.* 160: 14–21.
- Aslam, M., Qureshi, R.H., and Ahmed, N., 1993. A rapid screening technique for salt tolerance in rice (*Oryza sativa* L.). *Plant Soil* 150: 99–107.
- Asins M.J., 2002. Present and future of quantitative trait locus analysis in plant breeding. *Plant Breed.* 121: 281–291
- Atsunori, F., Yoshiaki, Y., Ishikawa, T., Setsuo, K., Yoshiyuki, T. 1998.  $Na^+/K^+$  antiporter in tonoplast vesicles from rice roots. *Plant Cell Physiol* 39: 196–201
- Babourina, O., Leonova, T., Shabala, S., and Newman, I., 2000. Effect of sudden salt stress on ion fluxes in intact wheat suspension cells, *Ann. Bot.* 85: 759–767.
- Babourina, O., Newman, I., and Shabala, S., 2002. Blue light-induced kinetics of  $H^+$  and  $Ca^{2+}$  fluxes in etiolated wild-type and phototropin-mutant *Arabidopsis* seedlings. *Proc. Natl. Acad. Sci. USA* 99: 2433–2438.
- Barr, A.R., Jefferies, S.P., Warner, P., Moody, D.B., Chalmers, K.J., Langridge, P., 2001. Marker assisted selection in theory and practice. 8<sup>th</sup> International Barley Genetics Symposium, pp 167–178
- Basu, H.S., Schwietert, H.C.A., Feuerstein, B.G., Marton, L.J., 1990. Effect of variation in the structure of spermine on the association with DNA and the induction of DNA conformational changes. *Biochem J* 269: 329–334
- Bartels, D., Engelhardt, K., Roncarati, R., Schneider, K., Rotter, M., Salamini, F., 1991. An ABA and GA modulated gene expressed in the barley embryo encodes an aldose reductase related protein. *EMBO J* 10:1037–43
- Belkhodja, R., Morales, F., Abadia, A., Gómez-Aparisi, J., and Abadia, J., 1994, Chlorophyll fluorescence as a possible tool for salinity tolerance screening in barley (*Hordeum vulgare* L.). *Plant Physiol.* 104: 667–673.
- Belkhodja, R., Morales, F., Abadia, A., Medrano, H., and Abadía, J., 1999. Effects of salinity on chlorophyll fluorescence and photosynthesis of barley (*Hordeum vulgare* L.) grown under a triple-line-source sprinkler system in the field. *Photosynthetica* 36: 375–387.
- Ben-Hayyim, G., Kafkafi, U., and Ganmore-Neumann, R., 1987. Role of internal potassium in maintaining growth of cultured Citrus callus on increasing NaCl and  $CaCl_2$  concentrations. *Plant Physiol.* 85: 434–439.

- Bewely, J.D., and Black, M., 1982. Physiology and biochemistry of seeds in relation to germination. Vol. 2. Springer-Verlag, Berlin, Germany.
- Bhandal, I.S., and Malik, C.P., 1988. Potassium estimation, uptake, and its role in the physiology and metabolism of flowering plants. *Internat. Rev. Cytol.* 110: 205–254.
- Bhatnagar-Mathur, P., Vadez V., Sharma K.K., 2008. Transgenic approaches for abiotic stress tolerance in plants: retrospect and prospects. *Plant Cell Reports* 20:411-424
- Bhat, P.R., Ramsay, L., Rostoks, N., Stein, N., Wanamaker, S., Svensson, J.T., Mandal, J., Fenton, R.D., Madishetty, K., Condamine, P., Varshney, R., Graner, A., Marshall, D., Waugh, R., Roose, M., Close, T.J., 2007. High-throughput SNP genotyping and mapping in barley provide ample markers for plant breeders. *Plant & Animal Genomes XV Conference*, San Diego, CA, 2007.
- Binzel, M.L., Hess, F.D., Bressan, R.A., Hasegawa, P.M., 1988. Intracellular compartmentation of ions in salt adapted tobacco cells, *Plant Physiol.* 86: 607–614.
- Blumwald, E., Ahraon, G.S., Apse, M.P., 2000. Sodium transport in plant cells. *Biochim. Biophys. Acta.* 1465: 140–151.
- Blumwald, E., Gelli, A., 1997. Secondary inorganic ion transport at the tonoplast. *Adv. Bot. Res.* 25: 401–417.
- Bohnert, H.J., Nelson, D.E., Jensen, R.G., 1995. Adaptation to environment stresses, *Plant Cell* 7: 1099–1111.
- Bohnert H. J., Jensen R. G., 1996. Strategies for engineering water-stress tolerance in plants. *Trends Biotechnol* 14: 89-97
- Briggs, D.E., 1978. The origin and classification of barleys. In: 'Barley', pp. 76-88, Chapman & Hall, London.
- Brownell, P.F., and Crossland, C.J., 1972. The requirements of sodium as a micronutrient by species having the C<sub>4</sub> dicarboxylic photosynthetic pathway. *Plant Physiol.* 49: 794–797.
- Cattivelli L., Baldi P., Crosatti C., Grossi M., Vale G., Stanca A.M. 2002. Genetic bases of barley physiological response to stressful conditions. In: GA Slafer, Molina-Cano JL, Savin R, Aruas JL, Romagosa I (eds) *Barley science Recent advances from molecular biology to agronomy of yield and quality*. Food Products Press, New York, pp. 387-411
- Chartzoulakis, K., and Klapaki, G., 2000. Response of two greenhouse pepper hybrids to NaCl salinity during different growth stages. *Sci. Hort.* 86: 247–260.
- Chauhan, R.P.S., Chauhan, C.P.S., Kumar, D., 1980. Free proline accumulation in cereals in relation to salt tolerance. *Plant Soil* 57: 167–175.
- Cheeseman, J.M., 1982. Pump-leak sodium fluxes in low salt corn roots. *J. Membr. Biol.* 70: 157–164.
- Chen, Z., Newman, I., Zhou, M.X., Mendham, N., Zhang, G., and Shabala, S., 2005. Screening plants for salt tolerance by measuring K<sup>+</sup> flux: a case study for barley. *Plant Cell Environ.* 28: 1230–1246.
- Chen, Z., Zhou, M.X., Newman, I., Mendham, N., Zhang, G., and Shabala, S., 2007a. Potassium and sodium relations in salinised barley tissues as a basis of differential salt tolerance. *Funct. Plant Biol.* 34: 150–162.

- Chen, Z.H., Pottosin, I.I., Cuin, T.A., Fuglsang, A.T., ester, M., Jha, D., Zepeda-Jazo, I., Zhou, M.X., Palmgren, M.G., Newman, I.A., and Shabala, S., 2007b. Root plasma membrane transporters controlling  $K^+/Na^+$  homeostasis in salt-stressed barley. *Plant Physiol.* 145:1714-1725.
- Chhipa, B.R., and Lal, P., 1985. Effect of soil salinity on yield, yield attributes and nutrient uptake by different varieties of wheat. *Anal. Edaf. Agrobiol.* 11: 1681–1691.
- Chhipa, B.R., and Lal, P., 1995.  $Na^+/K^+$  ratios as the basis of salt tolerance in wheat. *Aust. J. Agric. Res.* 46: 533–539.
- Clarkson, D.T., and Hanson, J.B., 1980. The mineral nutrition of higher plants. *Annu. Rev. Plant Physiol.* 31: 239–298.
- Close T.J., 1996. Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Physiol Plant* 97:795–803
- Colmer, T.D., Epstein, E., and Dvorak, J., 1995. Differential solute regulation in leaf blades of various ages in salt-sensitive wheat and a salt-tolerant wheat  $\times$  *Lophopyrum elongatum* (Host) A. Love amphiploid, *Plant Physiol.* 108: 1715–1724.
- Colmer, T.D., Munns, R., and Flowers, T.J., 2005. Improving salt tolerance of wheat and barley: future prospects. *Aust. J. Exp. Agric.* 45: 1425–1443.
- Cramer, G.R., Alberico, G.J., and Schmidt, C., 1994. Salt tolerance is not associated with the sodium accumulation of two maize hybrids. *Aust. J. Plant. Physiol.* 21: 675–692.
- Cramer, G., Epstein, E., Lauchli, A., 1989. Na-Ca interactions in barley seedlings: relationship to ion transport and growth. *Plant Cell and Environment* 12: 551-558
- Cuartero, J., Yeo, A.R., and Flowers, T.J., 1992. Selection of donors for salt-tolerance in tomato using physiological traits. *New Phytol.* 121: 63–69.
- Cuin, T.A., Miller, A.J., Laurie, S.A., and Leigh, R., 2003. Potassium activities in cell compartments of salt-grown barley leaves. *J. Exp. Bot.* 54: 657–661
- Cuin, T.A., and Shabala, S., 2005. Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley roots. *Plant Cell Physiol.* 46: 1924–1933.
- Cuin, T.A., and Shabala, S., 2007. Amino acids regulate salinity-induced potassium efflux in barley root epidermis. *Planta* 225: 753–761.
- Daniells, I.G., Holland, J.F., Young, R.R., Alston, C.L., and Bernardi, A.L., 2001. Relationship between yield of grain sorghum (*Sorghum bicolor*) and soil salinity under field conditions. *Aust. J. Expl. Agric.* 41: 211–217.
- Davenport, R.J., 1998. Mechanisms of toxic sodium influx in wheat. PhD Thesis, University of Cambridge, UK.
- Davenport, R.J., Reid, R.J., and Smith, F.A., 1997. Sodium-calcium interactions in two wheat species differing in salinity tolerance. *Physiol. Plant* 99: 323–327.
- Day A.D., Ludeke K.L., Ottman M.J. 1985. Registration of Arizona 8501 Barley germplasm for disturbed land reclamation. *Crop Sci* 26: 387
- Dedolph C., Hettel G. (Eds.) 1997. Rice varieties boost yield and improve saline soils. *Partners Making a Difference*. IRRI, Manila, p. 37

- Dell'Aquila, A., and Spada, P., 1993. The effect of salinity stress upon protein synthesis of germinating wheat embryos. *Ann. Bot.* 72: 97–101.
- Demidchik, V., Davenport, R.J., and Tester, M., 2002. Non-selective cation channels in plants. *Annu Rev Plant Physiol Plant Mol Biol* 53: 67–107.
- Demidchik, V., Shabala, S.N., Coutts, K.B., Tester, M., and Davies, J.M., 2003. Free oxygen radicals regulate plasma membrane  $\text{Ca}^{2+}$ - and  $\text{K}^{+}$ -permeable channels in plant root cells. *J. Cell. Sci.* 116: 81–88.
- D'Oraci D., Bagni N. 1987. In vitro interactions between polyamines and pectic substances. *Biochem. Biophys. Res. Commun* 148: 1159-1163
- Dietz K.J., Tavakoli N., Kluge C., Mimura T., Sharma S.S., Harris G.C., Chardonnens A.N., Golldack D. 2001. Significance of the V-type ATPase for the adaptation to stressful growth conditions and its regulation on the molecular and biochemical level. *J Exp Bot* 52: 1969-1980
- Donovan, T.J., and Day, A.D., 1969. Some effects of high salinity on germination and emergence of barley (*Hordeum vulgare* L. emend Lam.). *Agron. J.* 61: 236–238.
- Dubcovsky, J., Santa Maria, G., Epstein, E., Luo, M.C., and Dvorak, J., 1996. Mapping of the  $\text{K}^{+}/\text{Na}^{+}$  discrimination locus *Kna1* in wheat. *Theor. Appl. Genet.* 92: 448–454.
- Dvořák, J., Noaman, M.M., Goyal, S., and Gorham, J., 1994. Enhancement of the salt tolerance of *Triticum turgidum* L. by the *Kna1* locus transferred from the *Triticum aestivum* L. chromosome 4D by homoeologous recombination. *Theor. Appl. Genet.* 87: 872–877
- Eckermann C., Eichel J., Schröder J. 2000. Plant methionine synthase: new insights into properties and expression. *Biol Chem* 381: 695-703
- Ellis, R.P., Forster, B.P., Waugh, R., Bonar, N., Handley, L.L., Robinson, D., Gordon, D.C., and Powell, W., 1997. Mapping physiological traits in barley. *New Phytol.* 137: 149–157.
- Ellis, R.P., Forster B.P., Gordon, D.C., Handley, L.L., 2002. Phenotype/genotype associations for yield and salt tolerance in a barley mapping population  
*J Experimental Botany* 53:1163-1176
- Elphick, C.H., Sanders, D., and Maathuis, F.J.M., 2001. Critical role of divalent cations and  $\text{Na}^{+}$  efflux in *Arabidopsis thaliana* salt tolerance. *Plant Cell Environ.* 24: 733–40.
- Epstein, E., Norlyn, J.D., Rush, D.W., Kingsbury, R.W., Kelly, D.B., Cunningham, G.A., and Wrona, A.F., 1980. Saline culture of crops: a genetic approach. *Science* 210: 399–404.
- Ershov P., Reshetova O., Trofimova M., Babakov A.V. 2005. Activity of ion transporters and salt tolerance in barley. *Russian J Plant Physiol.* 52: 765-773
- Essah, P.A., Davenport, R., and Tester, M., 2003. Sodium influx and accumulation in *Arabidopsis*. *Plant Physiol.* 133: 307–318.
- Fedina, I.S., Georgieva, K., and Grigorova, I., 2002. Light-dark changes in proline content of barley leaves under salt stress, *Biol. Plant* 45: 59–63.
- Filho, E.G., and Sodek, L., 1988. Effect of salinity on ribonuclease activity of *Vigna unguiculata* cotyledons during germination. *Plant Physiol.* 132: 307–311.
- Flowers, T. J., Troke, P. F., Yeo, A. R., 1977. The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.* 28: 89-121

- Flowers, T.J., 2004. Improving crop salt tolerance. *J. Exp. Bot.* 55: 307–319.
- Flowers, T.J., and Hajibagheri, M.A., 2001. Salinity tolerance in *Hordeum vulgare*: ion concentrations in root cells of cultivars differing in salt tolerance. *Plant Soil* 231: 1–9.
- Flowers, T.J., and Lauchli, A., 1983. Sodium versus potassium: Substitution and compartmentation. In: *Encyclopedia of Plant Physiology*, new series, Pirson A, Zimmermann MH (eds.), vol. 15B, Springer-Verlag, Berlin. pp. 651–681.
- Flowers, T.J., Troke, P.F., and Yeo, A.R., 1977. The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.* 28: 89–121.
- Flowers, T.J., and Yeo, A.R., 1986. Ion relations of plants under drought and salinity, *Aust. J. Plant Physiol.* 13: 75–91.
- Flowers, T.J., and Yeo, A.R., 1995. Breeding for salinity resistance in crop plants: where next? *Aust. J. Plant Physiol.* 22: 875–884.
- Foolad, M.R., 1997. Genetic basis of physiological traits related to salt tolerance in tomato, *Lycopersicon esculentum* Mill, *Plant Breed.* 116: 53–58.
- Foolad, M.R., 1999. Comparison of salt tolerance during seed germination and vegetative growth in tomato by QTL mapping. *Genome* 42: 727–734
- Foolad, M.R., 2004. Recent advances in genetics of salt tolerance in tomato. *Plant Cell Tiss. Org.* 76: 101–119.
- Forster, B.P., H. Pakniyat, M. Macaulay, W. Matheson, M.S. Phillips, W.T.B. Thomas & W. Powell, 1994. Variation in the leaf sodium content of *Hordeum vulgare* (barley) cultivar Maythorpe and its derived mutant cv. Golden Promise. *Heredity* 73: 249–253.
- Forster, B.P., 2001. Mutation genetics of salt tolerance in barley: An assessment of Golden Promise and other semi-dwarf mutants. *Euphytica* 120: 317–328.
- Fortmeier, R., and Schubert, S., 1995. Salt tolerance of maize (*Zea mays* L.): the role of sodium exclusion. *Plant Cell Environ.* 18: 1041–1047.
- Fowler, J.L., 1991. Interaction of salinity and temperature on the germination of crambe. *Agron. J.* 83: 169–172.
- Francois, L.E., Maas, E.V., Donovan, T.J., and Youngs, V.L., 1986. Effect of salinity on grain yield and quality, vegetative growth, and germination of semi-dwarf and durum wheat. *Agron. J.* 78: 1053–1058.
- Fricke, W., Leigh, R.A., and Tomos, A.D., 1996. The intercellular distribution of vacuolar solutes in the epidermis and mesophyll of barley leaves changes in response to NaCl. *J. Exp. Bot.* 47: 1413–1426.
- Garbarino, J., Dupont, F.M., 1988. NaCl induces a Na<sup>+</sup>/H<sup>+</sup> antiport in tonoplast vesicles from barley roots. *Plant Physiol* 86: 231–236
- García, A., Senadhira, D., Flowers, T.J., and Yeo, A.R., 1995. The effects of selection for sodium transport and of selection for agronomic characteristics upon salt resistance in rice. *Theor. Appl. Genet.* 90: 1106–1111.
- Garthwaite, A.J., von Bothmer, R., and Colmer, T.D., 2005. Salt tolerance in wild *Hordeum* species is associated with restricted entry of Na<sup>+</sup> and Cl<sup>-</sup> into the shoots. *J. Exp. Bot.* 56: 2365–2378



- Gorham, J., Hardy, C., Wyn Jones, R.G., Joppa, L.R., and Law, C.N., 1987. Chromosomal location of a  $K^+/Na^+$  discrimination character in the D genome of wheat. *Theor. Appl. Genet.* 74, 584–588.
- Gorham, J., Bristol, A., Young, E.M., Wyn Jones R.G., Kashour, G., 1990. Salt tolerance in the Triticeae: K/Na discrimination in barley. *Journal of Experimental Botany* 41:1095–1101
- Gorham, J., Papa, R., and Aloy-Lleonart, M., 1994. Varietal differences in sodium uptake in barley cultivars exposed to soil salinity or salt spray. *J. Exp. Bot.* 45: 895–901.
- Graner, A., Jahoor, A., Schondelmaier, J., Siedler, H., Pillen, K., Fischbeck, G., Wenzel, G., Herrmann, R.G., 1991. Construction of an RFLP map of barley. *Theor Appl Genet* 83:250–256.
- Greenway, H., and Munns, R., 1980. Mechanisms of salt tolerance in nonhalophytes. *Annu. Rev. Plant. Physiol. Mol. Biol.* 31: 149–190.
- Greenway, H., and Osmond, C.B., 1972. Salt responses of enzymes from species differing in salt tolerance. *Plant Physiol.* 49: 256–259.
- Gregorio, G.B., Senadhira, D., Mendoza, R.D., Manigbas, N.L., Roxas, J.P., and Guerta, C.Q., 2002. Progress in breeding for salinity tolerance and associated abiotic stresses in rice. *Field Crop Res.* 76: 91–101.
- Grossi, M., Gulli, M., Stanca, A.M., Cattivelli, L., 1995. Characterization of two barley genes that respond rapidly to dehydration stress. *Plant Science* 105: 71-80
- Guerrier, G., 1988. Comparative phosphatase activity in four species during germination in NaCl media. *J. Plant Nutr.* 11: 535–546.
- Hall, J.L., and Flowers, T.J., 1973. The effect of salt on protein synthesis in the halophyte *Suaeda maritima*. *Planta* 110: 361–368.
- Hare, P.D., Cress, W.A., and Van Staden, J., 1998. Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ.* 21: 535–553.
- Hasegawa, P.M., Bressan, R.A., Zhu, J.K., and Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Biol.* 51: 463– 499.
- He, T., Cramer, G.R., 1993. Salt tolerance of rapid-cycling Brassica species in relation to potassium-sodium ratio and selectivity at the whole plant and callus levels. *J Plant Nutr* 16: 1263-1277
- Hong, B., Uknes, S., Ho, T.H.D. 1988. Cloning and characterization of a cDNA encoding a mRNA rapidly induced by ABA in barley aleurone layers. *Plant Molecular Biology* 11: 495-506
- Hong, B., Barg, R., Ho, T.D. 1992. Developmental and organ-specific expression of an ABA- and stress induced protein in barley. *Plant Molecular Biology* 18: 663-674
- Hollung, K., Espelund, M., Jakobsen, K.J., 1994. Another Lea B19 gene (Group 1 Lea) from barley containing a single amino acid hydrophilic motif. *Plant Molecular Biology* 25: 559-564
- Houshmand, S., Arzani, A., Maibody, S.A.M., and Feizi, M., 2005. Evaluation of salt-tolerant genotypes of durum wheat derived from in vitro and field experiments. *Field Crop Res.* 91: 345–354.
- Hsiao, T.C., and Lauchli, A., 1986. Role of potassium in plant-water relations. *Adv. Plant Nutr.* 2: 281–312.
- Huang C.X., Van Steveninck R.F.M., 1990. Salinity induced structural changes in meristematic cells of barley roots. *New Phytol* 115: 17-22

- Huang, Y., Zhang, G., Wu, F., Chen, J., and Zhou, M., 2006. Differences in physiological traits among salt-stressed barley genotypes. *Commun. Soil Sci. Plant Anal.* 37: 557–570.
- Ishitani M., Nakamura T., Han S.Y., Takabe T. 1995. Expression of the betaine aldehyde dehydrogenase gene in barley in response to osmotic stress and abscisic acid. *Plant Molecular Biology* 27: 307-315
- Isla, R., Aragüés, R., and Royo, A., 1998. Validity of various physiological traits as screening criteria for salt tolerance in barley. *Field Crop Res.* 58: 97–107.
- Isla, R., Royo, A., and Aragüés, R., 1997. Field screening of barley cultivars to soil salinity using a sprinkler and a drip irrigation system. *Plant Soil* 197: 105–117.
- Islam, S., Malik, A.I., Islam, A.K.M.R., and Colmer, T.D., 2007. Salt tolerance in a *Hordeum marinum*-*Triticum aestivum* amphiploid, and its parents. *J. Exp. Bot.* 58: 1219–1229.
- Jacoby, B., and Hanson, J.B., 1985. Controls on  $^{22}\text{Na}^+$  influx in corn roots. *Plant Physiol.* 77: 930–934.
- James, R.A., Munns, R., von Caemmerer, S., Trejo, C., Miller, C., and Condon, T. 2006, Photosynthetic capacity is related to the cellular and subcellular partitioning of  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  in salt-affected barley and durum wheat, *Plant Cell Environ.* 29: 2185–2197.
- James, R.A., Rivelli, A.R., Munns, R., and von Caemmerer, S., 2002. Factors affecting  $\text{CO}_2$  assimilation, leaf injury and growth in salt-stressed durum wheat. *Funct. Plant Biol.* 29: 1393–1403.
- Janardhan, K.V., Panchaksharaiah, S., Balkishna, K.R., and Patil, B.N., 1979, Effect of various K/Na ratios in saline irrigation water on grain yield and ionic composition of wheat, *Curr. Sci.* 48: 739–771.
- Jaradat, A.A., Shahid, M., and Al-Maskri, A., 2004. Genetic diversity in the Batini barley landrace from Oman: II. Response to salinity stress. *Crop Sci.* 44: 997–1007.
- Jensen, C.R., 1982. Effect of soil water osmotic potential on growth and water relationships in barley during soil water depletion, *Irrig. Sci.* 3: 111–121.
- Jiang, Q., Roche, D., Monaco, T.A., and Durham, S., 2006. Gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination of 14 barley genetic lines in response to salinity. *Field Crop Res.* 96: 269–278.
- Joshi, S., and Nimbalkar, J.D., 1983. Effect of salt stress on growth and yield in *Cajanus cajan* L. *Plant Soil* 74: 291–294.
- Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki, K., 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol* 17: 287-291
- Kawasaki, S., Borchert, C., Deyholos, M., Wang, H., Brazille, S., Kawai, K., Galbraith, D., Bohnert, H.J., 2001. Gene expression profiles during the initial phase of salt stress in rice. *Plant Cell* 13: 889-905
- Kearsey, M.J., 1998. The principles of QTL analysis (a minimal mathematics approach). *J Exp Bot* 49: 1619-1623
- Khan, M.A., and Rizvi, Y., 1994. Effect of salinity, temperature and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. Stocksii, *Can. J. Bot.* 72: 475–479.
- Kingsbury, R.W., and Epstein, E., 1984, Selection for salt-resistant spring wheat, *Crop Sci.* 24: 310–315.
- Kingsbury, R.W., Epstein, E., and Percy, R.W., 1984. Physiological responses to salinity in selected lines of wheat. *Plant Physiol.* 74: 417–423

- Kleinhofs, A., Kilian, A., Saghai Maroof, M.A., Biyashev, R.M., Hayes, P., Chen, F.Q., Lapitan, N., Fenwick, A., Blake, T.K., Kanazin, V., Ananiev, E., Dahleen, L., Kudrna, D., Bollinger, J., Knapp, S.J., Liu, B., Sorrells, M., Heun, M., Franckowiak, J.D., Hoffman, D., Skadsen, R., Steffenson, B.J., 1993. A molecular, isozyme and morphological map of barley (*Hordeum vulgare*) genome. *Theor Appl Genet* 86:705–712.
- Kochian, L.V., and Lucas, W.J., 1988. Potassium transport in roots, *Adv. Bot. Res.* 15: 93–178.
- Koval V. S., 2000. Male and female gametophyte selection of barley for salt tolerance. *Hereditas* 132: 1-5
- Koyama, M.L., Levesley, A., Koebner, R.M.D., Flowers, T.J., and Yeo, A.R., 2001, Quantitative trait loci for competent physiological traits determining salt tolerance in rice. *Plant Physiol.* 125: 406–422.
- Kramer, D., 1984. Cytological aspects of salt tolerance in higher plants. In: *Salinity Tolerance in Plants* (Ed. by Staples R.C. and Toenniessen G.H.). John Wiley and Sons, New York. pp. 3-5
- Krishnamurthy, L., Serraj, R., Hash, C.T., Dakheel, A.J., and Reddy, B.V.S., 2007. Screening sorghum genotypes for salinity tolerant biomass production. *Euphytica* 156: 15–24.
- Kronzucker, H.J., Szczerba, M.W., Moazami-Goudarzi, M., and Britto, D.T., 2006. The cytosolic  $\text{Na}^+:\text{K}^+$  ratio does not explain salinity-induced growth impairment in barley: a dual-tracer study using  $^{42}\text{K}^+$  and  $^{24}\text{Na}^+$ . *Plant Cell Environ.* 29: 2228–2237.
- Kueh, J.S.H., Bright, S.W.J., 1982. Biochemical and genetic analysis of three proline accumulating barley mutants. *Plant Sci Lett* 27: 233-241.
- Kumar, D., 1995. Salt tolerance in oilseed brassicas—present status and future prospects. *Plant Breed. Abst.* 65: 1438–1447.
- Lahaye, P.A., Epstein, E., 1971. Calcium and salt toleration by bean plants. *Physiologia Plantarum* 25: 223-218
- Leigh, R.A., 2001. Potassium homeostasis and membrane transport. *J. Plant Nutri. Soil Sci.* 164: 193–198.
- Leonova, T.G., Goncharova, E.A., Khodorenko, A.V., and Babakov, A.V., 2005. Characteristics of salt-tolerant and salt-susceptible cultivars of barley. *Russ. J. Plant Physiol.* 52: 774–778.
- Loreto, F., Centritto, M., Chartzoulakis, K., 2003. Photosynthetic limitations in olive cultivars with different sensitivity to salt stress. *Plant, Cell & Environment* 26: 595-601
- Lin, H.X., Zhu, M.Z., Yano, M., Gao, J.P., Liang, Z.W., Su, W.A., Hu, X.H., Ren, Z.H., and Chao, D.Y., 2004. QTLs for  $\text{Na}^+$  and  $\text{K}^+$  uptake of the shoots and roots controlling rice salt tolerance. *Theor. Appl. Genet.* 108: 253–260.
- Lu, Y.M., Li, Y.F., Cao, M.F., Li, X.W., Zhang, Y.L., Yang M.F., Yang, S.T., Cheng X.R., 2002. Physiological-biochemistrical and Molecular Biological Analysis of Salt-tolerant New Lines of *Hordeum brevisubulatum*. *Sci Agriculhura Sinica* 35:282-286
- Lucas, W.J., and Kochian, L.V., 1986. Ion transport processes in corn roots: an approach utilizing microelectrode techniques. In: *Advanced Agricultural Instrumentation: Design and Use*, Gensler WG, (eds.), Martinus Nijhoff, Dordrecht, The Netherlands, pp. 402–425.
- Ludlow, M.M., Muchow, R.C., 1990. A critical evaluation of traits for improving crop yields in water-limited environments. *Adv. Agron.* 25: 107–153.
- Lynch, J., Läuchli, A. 1985. Salt stress disturbs the calcium nutrition of barley (*Hordeum vulgare* L.). *The New Phytologist* 99: 345-354

- Lynch, J., Lauchli, A., 1988. Salinity affects intracellular calcium in corn root protoplasts. *Plant Physiology* 87: 351-356
- Lynch, J., Thiel, G., Lauchli, A., 1988. Effects of salinity on the extensibility and Ca availability in the expanding region of growing barley leaves. *Botanica Acta* 101: 355-361
- Ma, S.S., Gong, Q.Q., and Bohnert, H.J., 2006. Dissecting salt stress pathways, *J. Exp. Bot.* 57: 1097–1107.
- Maas, E.V., Hoffman, G. J. 1977. Crop salt tolerance--current assessment. *Journal of the Irrigation and Drainage Division* 103: 115-134
- Maathuis, F.J.M., Flowers, T.J., and Yeo, A.R., 1992. Sodium chloride compartmentation in leaf vacuoles of the halophyte *Suaeda maritima* (L.) Dum. and its relation to tonoplast permeability. *J. Exp. Bot.* 43: 1219-1223.
- Maathuis, F.J.M., Ichida, A.M., Sanders, D., and Schroeder, J.I., 1997. Roles of higher plant K<sup>+</sup> channels. *Plant Physiol.* 114: 1141–1149.
- Malkit, A., Sadka, A., Fisher, M., Goldschlag, P., Gokhman, I., Zamir, A., 2002. Salt induction of fatty acid elongase and membrane lipid modifications in the extreme halotolerant Alga *Dunaliella salina*. *Plant Physiol* 129: 1320-1329
- Mano, Y., and Takeda, K., 1995. Varietal variation and effects of some major genes on salt tolerance in barley seedlings. *Bull. Res. Inst. Bioresour. Okayama Univ.* 3: 71–81.
- Mano, Y., Takeda, K., 1996. Genetical studies on salt tolerance at germination in recombinant, inbred, iso-genic and doubled haploid lines of barley (*Hordeum vulgare* L.). *Bull Res. Ins Okayama Univ* 4: 79-88
- Mano, Y., Takeda, K., 1997. Mapping quantitative trait loci for salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.). *Euphytica* 94: 263-272
- Mano, Y., and Takeda, K., 1997, Diallel analysis of salt tolerance at germination and the seedling stage in barley (*Hordeum vulgare* L.), *Breed. Sci.* 47: 203–209.
- Mano, Y., Takeda, K., 1998. Genetic resources of salt tolerance in wild *Hordeum* species. *Euphytica* 103: 137-141
- Marschner, H., 1995. Mineral nutrition of higher plants. 2<sup>nd</sup> edn. London: Academic Press.
- Mansour M.M.F., van Hasselt P.R., Kuiper P.J.C. 1994. Plasma membrane lipid alterations induced by NaCl in winter wheat roots. *Physiol. Plant* 92: 473-478
- Maser, P., Eckelman, B., Vaidyanathan, R., Horie, T., Fairbairn, D. J., Kubo, M., Yamagami, M., Yamaguchi, K., Nishimura, M., Uozumi, N., Robertson, W., Sussman, M. R., Schroeder, J. I., 2002. Altered shoot/root Na<sup>+</sup> distribution and bifurcating salt sensitivity in *Arabidopsis* by genetic disruption of the Na<sup>+</sup> transporter AtHKT1. *FEBS Lett* 531: 157-161
- Mäser, P., Gierth, M., and Schroeder, J., 2002. Molecular mechanisms of potassium and sodium uptake in plants. *Plant Soil* 247: 43–54.
- Maxwell, K., and Johnson, G.N., 2000. Chlorophyll fluorescence: a practical guide. *J. Exp. Bot.* 51: 659–668.
- Mladenova, Y.I., 1990. Influence of salt stress on primary metabolism of *Zea mays* L. seedlings of model genotypes. *Plant Soil* 123: 217-222.

- Monforte, A.J., Asins M.J., Carbonell E.A. 1997. Salt tolerance in *Lycopersicon* species 6. Genotype-by-salinity interaction in quantitative trait loci detection: constitutive and response QTLs. *Theor Appl Genet* 95: 706-713
- Moons, A., Prinsen, E., Bauw, G., Van Montagu, M., 1997. Antagonistic effects of abscisic acid and jasmonates on salt stress-inducible transcripts in rice roots. *Plant Cell* 9:2243– 2259
- Morgan, J.M., 1984. Osmoregulation and water stress in higher plants. *Annu. Rev. Plant Physiol.* 25: 299–319.
- Muench, D.G., Good, A.G., 1994. Hypoxically inducible barley alanine aminotransferase: cDNA cloning and expression analysis. *Plant Mol Biol* 24: 417-427
- Munns, R., 1993. Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses. *Plant Cell and Environ* 16: 15-24
- Munns, R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.* 25: 239–250.
- Munns, R., 2005. Genes and salt tolerance: bringing them together. *New Phytol.* 167: 645–663.
- Munns, R., Hare, R.A., James, R.A., and Rebetzke, G.J., 2000. Genetic variation for improving the salt tolerance of durum wheat. *Aust. J. Agric. Res.* 51: 69–74.
- Munns, R., Hussain, S., Rivelli, A.R., James, R.A., Condon, A.J., Lindsay, M.P., Lagudah, E.S., Schachtman, D.P., and Hare, R.A., 2002. Avenues for increasing salt tolerance of crops, and the role of physiologically based selection traits. *Plant Soil* 247: 93–105.
- Munns, R., and James, R.A., 2003. Screening methods for salinity tolerance: a case study with tetraploid wheat. *Plant Soil* 253: 201–218.
- Munns, R., James, R.A., and Läuchli, A., 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* 57: 1025–1043.
- Munns, R., Schachtman, D.P., and Condon, A.G., 1995. The significance of a two-phase growth response to salinity in wheat and barley. *Aust. J. Plant Physiol.* 22: 561–569.
- Muramoto, Y., Watanabe, A., Nakamura, T., Takabe, T., 1999. Enhanced expression of a nuclease gene in leaves of barley plants under salt stress. *Gene* 234: 315-321
- Muranaka, S., Shimizu, K., and Kato, M., 2002. A salt-tolerant cultivar of wheat maintains photosynthetic activity by suppressing sodium uptake. *Photosynthetica* 40: 509–515.
- Murillo-Amador, B., Troyo-Diéguez, E., López-Cortés, A., Jones, H.G., Ayala-Chairez, F., and Tinoco-Ojanguren, C.L., 2001. Salt tolerance of cowpea genotypes in the emergence stage. *Aust. J. Exp. Agric.* 41: 81–88.
- Nakamura, T., Osaki, M., Ando, M., and Tadano, T., 1996. Differences in mechanisms of salt tolerance between rice and barley plants *Soil Sci. Plant Nutr.* 42: 303–314.
- Narita, Y., Taguchi, H., Nakamura, T., Ueda, A., Shi, W., Takabe, T. 2004. Characterization of the salt-inducible methionine synthase from barley leaves. *Plant Science* 167: 1009-1016
- Nassery, H., 1979. Salt-induced loss of potassium from plant roots. *New Phytol* 83: 27–32
- Nevo, E., Beiles, A., Gutterman, Y., Stroch N., Kaplan, D., 1984. Genetic resources of wild cereals in Israel and the vicinity: II. Phenotypic variation within and between populations of wild barley, *Hordeum spontaneum*. *Euphytica* 33: 737–756

- Nevo E., 1992. Origin, evolution, population genetics and resources for breeding of wild barley, *Hordeum spontaneum* in the fertile crescent. In P.R. Shewry (ed.) *Barley genetics, biochemistry, molecular biology and biotechnology*. CAB International, Wallingford, UK. pp.19–43.
- Newman, I.A., 2001. Ion transport in roots: measurement of fluxes using ion-selective microelectrodes to characterise transporter function., *Plant Cell. Environ.* 24: 1–14.
- Noble, C.L., and Rogers, M.E., 1992. Arguments for the use of physiological criteria for improving the salt tolerance in crops. *Plant Soil* 146: 99–107.
- Norberg, P., Liljenberg, C., 1991. Lipids of plasma membranes prepared from oat root cells: effect of induced water deficit tolerance. *Plant Physiol* 96: 1136–1141
- Nublat, A., Desplands, J., Casse, F., and Berthomieu, P., 2001. *sas1*, an Arabidopsis mutant overaccumulating sodium in the shoot, shows deficiency in the control of the root radial transport of sodium. *Plant Cell* 13: 125–137.
- Ozturk, Z.N., Talame, V., Deyholos, M., Michalowski, C.B., Galbraith, D.W., Gozukirmizi, N., Tuberosa, R., Bohnert, H.J. 2002. Monitoring large-scale changes in transcript abundance in drought- and salt-stressed barley. *Plant Molecular Biology* 48: 551–573
- Palmgren, M.G., 2001. Plant plasma membrane H<sup>+</sup>-ATPases: powerhouses for nutrient uptake. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 52: 817–845.
- Pang, J.Y., Newman, I., Mendham, N., Zhou, M.X., and Shabala, S., 2006a, Microelectrode ion and O<sub>2</sub> flux measurements reveal differential sensitivity of barley root tissues to hypoxia, *Plant Cell Environ.* 29: 1107–1121.
- Pang, J.Y., Cuin, T., Shabala, L., Zhou, M.X., Mendham, N., and Shabala, S., 2006b, Effect of secondary metabolites associated with anaerobic soil conditions on ion fluxes and electrophysiology on barley roots, *Plant Physiol.* 145:266–276.
- Pardo, J.M., Reddy, M.P., Yang, S., Maggio, A., Huh, G.H., Matsumoto, T., Coca, M.A., Paino-D'Urzo, M., Koiwa, H., Yun D.J., Watad, A.A., Bressan, R.A., Hasegawa P.M., 1998. Stress signaling through Ca<sup>2+</sup>/calmodulin-dependent protein phosphatase calcineurin mediates salt adaptation in plants. *Proc Natl Acad Sci USA* 95: 9681–9686
- Petrizzelli, L., Melillo, M.T., Zacheo, T.B., and Taranto, G., 1992. Physiological and ultrastructural changes in isolated wheat embryos during salt and osmotic shock. *Ann. Bot.* 69: 25–31.
- Poljakoff-Mayber, A., Somers, G.F., Werker, E., and Gallagher, I.L., 1994, Seeds of *Kosteletzkya virginica* (Malvaceae): their structure, germination and salt tolerance. *Am. J. Bot.* 81: 54–59.
- Ramagopal, S., 1988. Regulation of protein synthesis in root, shoot and embryonic tissues of germinating barley during salinity stress. *Plant, Cell and Environment* 11: 501–515
- Rana, R.S., 1986. Genetic diversity for salt-stress resistance of wheat in India. *Rachis* 5: 32–37.
- Rathore, A.K., Sharma, R.K., Lal, P., 1977. Relative salt tolerance of different varieties of barley (*Hordeum vulgare* L.) at germination and seedling stage. *Ann Arid Zone* 16: 53–60
- Rawson, H.M., Richards, R.A., and Munns, R., 1988. An examination of selection criteria for salt tolerance in wheat, barley and triticale genotype. *Aust. J. Agric. Res.* 39: 759–772.
- Rebetzke, G.J., Read, J.J., Barbour, M.M., Condon, A.G., and Rawson, H.M., 2000. A hand-held porometer for rapid assessment of leaf conductance in wheat. *Crop Sci.* 40: 277–280.

- Rengel, Z., 1992. The role of calcium in salt toxicity. *Plant Cell Environ.* 15:625-632
- Rhode, D., Hanson, A.D., 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Ann Rev Plant Physiol Plant Mol Biol* 44: 357-384
- Richards, R.A., 1983. Should selection for yield in saline regions be made on saline or in non saline soils? *Euphytica* 32: 431-438.
- Richards, R.A., 1996. Defining selection criteria to improve yield under drought. *Plant Growth Regul.* 20: 157-166.
- Rivelli, A.R., James, R.A., Munns, R., and Condon, A.G., 2002. Effect of salinity on water relations and growth of wheat genotypes with contrasting sodium uptake. *Funct. Plant Biol.* 29: 1065-1074.
- Rostoks, N., Mudie, S., Cardle, L., Russell, J., Ramsay, L., Booth, A., Svensson, J.T., Wanamaker, S.I., Walia, H., Rodriguez, E.M., Hedley, P.E., Liu, H., Morris, J., Close, T.J., Marshall, D.F., Waugh, R., 2005. Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. *Mol Genet Genomics* 1-13.
- Royo, A., and Aragüés, R., 1993. Validation of salinity crop production functions obtained with the triple line source sprinkler system. *Agron. J.* 85: 795-800.
- Royo, A., and Aragüés, R., 1999. Salinity-yield response functions of barley genotypes assessed with a triple line source sprinkler system. *Plant Soil* 209: 9-20.
- Santa-Cruz, A., Acosta, M., Rus, A., and Bolarin, M.C., 1999. Short-term salt tolerance mechanisms in differentially salt tolerant tomato species. *Plant Physiol. Biochem.* 37: 65-71.
- Sayed, H.I., 1985. Diversity of salt tolerance in a germplasm collection of wheat (*Triticum* spp.). *Theor. Appl. Genet.* 69: 651-657.
- Sayed, O.H., 2003. Chlorophyll fluorescence as a tool in cereal crop research. *Photosynthetica* 41: 321-330,
- Schachtman, D.P., and Liu, W., 1999. Molecular pieces to the puzzle of the interaction between potassium and sodium uptake in plants. *Trends Plant Sci.* 4: 281-287.
- Schubert, S., and Lauchli, A., 1990. Sodium exclusion mechanisms at the root surface of two maize cultivars. *Plant Soil* 123: 205-209.
- Serafini-Fracassini D., Del Duca S., Beninati S. 1995. Plant transglutaminases. *Phytochemistry* 40: 355-365
- Serraj, R., and Sinclair, T.R., 2002. Osmolyte accumulation: can it really help increase crop yield under drought conditions? *Plant Cell Environ.* 25: 333-341.
- Serrano, R., Mulet, J.M., Rios, G., de Marquez, J.A., Larrinoa, I., Leube, M.P., Mendizabal, I., Pascual-Ahuir, A., Proft, M., Ros, R., and Montesinos, C., 1999. A glimpse of the mechanisms of ion homeostasis during salt stress. *J. Exp. Bot.* 50: 1023-1036.
- Shabala, L., Cuin, T.A., Newman, I., and Shabala, S., 2005a. Salinity-induced ion flux patterns from the excised roots of *Arabidopsis* sos mutants. *Planta* 222: 1041-1050.
- Shabala, S., 1996. Leaf temperature kinetics measure plant adaptation to extreme high temperatures. *Aust. J. Plant. Physiol.* 23: 445-452.
- Shabala, S., 1997.  $H^+$  flux kinetics around plant roots after short-term exposure to low temperature: Identifying critical temperatures for plant chilling tolerance. *Plant Cell Environ.* 20: 1401-1410.

- Shabala, S., 2000. Ionic and osmotic components of salt stress specifically modulate net ion fluxes from bean leaf mesophyll. *Plant Cell Environ.* 23: 825–837.
- Shabala, S., 2002. Screening plant for environmental fitness: chlorophyll fluorescence as a “Holy Grail” for plant breeders. In: *Advance in plant physiology*, Hemantaranjan, A. (ed.), Vol. 5 Scientific Publishers: Jodhpur, India pp. 287–340.
- Shabala, S., 2003. Regulation of potassium transport in leaves: from molecular to tissue level. *Ann. Bot.* 92: 627–634.
- Shabala, S., 2006. Non-invasive microelectrode ion flux measurements in plant stress physiology. In: *Plant electrophysiology - theory and methods*, Volkov, A., ed. Berlin, Germany: Springer-Verlag, 35–71.
- Shabala, S., Babourina, O., and Newman, I.A., 2000. Ion-specific mechanism of osmoregulation in bean mesophyll cells. *J. Exp. Bot.* 51: 1243–1253.
- Shabala, S., Demidchik, V., Shabala, L., Cuin, T.A., Smith, S.J., Miller, A.J., Davies, J.M., and Newman, I.A., 2006. Extracellular  $\text{Ca}^{2+}$  ameliorates NaCl-induced  $\text{K}^{+}$  loss from *Arabidopsis* root and leaf cells by controlling plasma membrane  $\text{K}^{+}$ -permeable channels. *Plant Physiol.* 141: 1653–1665.
- Shabala, S., and Hariadi, Y., 2005. Effects of magnesium availability on the activity of plasma membrane ion transporters and light-induced responses from broad bean leaf mesophyll. *Planta* 221: 56–65.
- Shabala, S., and Knowles, A., 2002. Rhythmic patterns of nutrient acquisition by wheat roots. *Funct. Plant Biol.* 29: 595–605.
- Shabala, S., and Lew, R.R., 2002. Turgor regulation in osmotically stressed *Arabidopsis thaliana* epidermal root cells: direct support for the role of inorganic ion uptake as revealed by concurrent flux and cell turgor measurements. *Plant Physiol.* 129: 290–299.
- Shabala, S., and Newman, I.A., 2000. Salinity effects on the activity of plasma membrane  $\text{H}^{+}$  and  $\text{Ca}^{2+}$  transporters in bean leaf mesophyll: masking role of the cell wall. *Ann. Bot.* 85: 681–686.
- Shabala, S., Shabala, L., Gradmann, D., Chen, Z., Newman, I., and Mancuso, S., 2006b Oscillations in plant membrane transport: Model predictions, experimental validation, and physiological implications. *J. Exp. Bot.* 57: 171–184.
- Shabala, S., Shabala, L., Van Volkenburgh, E., and Newman, I., 2005b. Effect of divalent cations on ion fluxes and leaf photochemistry in salinised barley leaves. *J. Exp. Bot.* 56: 1369–1378.
- Shabala, S., Shabala, L., and Volkenburgh, E., 2003. Effect of calcium on root development and root ion fluxes in salinised barley seedlings. *Funct. Plant Biol.* 30: 507–514.
- Shabala, S., Shabala, S.I., Martynenko, A.I., Babourina, O., and Newman, I.A., 1998. Salinity effect on bioelectric activity, growth,  $\text{Na}^{+}$  accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. *Aust. J. Plant. Physiol.* 25: 609–616.
- Shannon, M.C., 1997. Adaptation of plants to salinity. *Adv. Agron.* 60: 75–120.
- Shannon, M.C., and Noble, C.L., 1990. Genetic approaches for developing economic salt tolerant crops. In: *Agricultural salinity assessment and management*. ACSE Manuals and reports on engineering practice No. 71, Tanji, K.K. (ed.) ASCE, New York. pp. 161–185.
- Shi W.M., Muramoto Y., Ueda A., Takabe T. 2001. Cloning of peroxisomal ascorbate peroxidase gene from barley and enhanced thermotolerance by overexpressing in *Arabidopsis thaliana*. *Gene* 273: 23–27



- Somerville, C., 1995. Direct tests of the role of membrane lipid composition in low-temperature-induced photoinhibition and chilling sensitivity in plants and cyanobacteria. *Proc Natl Acad Sci USA* 92: 6215–6218
- Srivastava, J.P., Jana, S., 1984. Screening wheat and barley germplasm for salt tolerance. In: *Salinity tolerance in plants: strategies for crop improvement*, Staples, R.C., Toenniessen, G.H. (eds.).- New York (USA): Wiley, p. 273-283
- Storey, R., and Wyn Jones, R.G., 1978. Salt stress and comparative physiology in the Gramineae. I. Ion relations of two salt- and water-stressed barley cultivars, California Mariout and Arimar. *Aust. J. Plant Physiol.* 5: 801–816.
- Subbarao, G.V., Ito, O., Berry, W.L., and Wheeler, R.M., 2003. Sodium: a functional plant nutrient. *Crit. Rev. Plant Sci.* 22: 391–416.
- Suhayda, G.G., Redmann, R.E., Harvey, B.L., Cipywnyk, A.L., 1992. Comparative response of cultivated and wild barley species to salinity stress and calcium supply. *Crop Science* 32: 154-163.
- Takuji, N., Mitsuru, O., Michiko, A., Toshiaki, T., 1996. Differences in mechanisms of salt tolerance between rice and barley plants. *Soil sci plant nutri.* 42: 303-314
- Tajbakhsh, M., Zhou, M.X., Chen, Z.H., and Mendham, N.J., 2006. Physiological and cytological response of salt-tolerant and non-tolerant barley to salinity during germination and early growth. *Aust. J. Exp. Agric.* 46: 555–562.
- Tal, M., 1985. Genetics of salt tolerance in higher plants: theoretical and practical considerations. *Plant Soil* 89: 199–226.
- Tassoni A., Antognoni F., Bagni N. 1996. Polyamine binding to plasma membrane vesicles from zucchini hypocotyls. *Plant Physiol* 110: 817-824
- Tester, M., and Davenport, R., 2003. Na<sup>+</sup> tolerance and Na<sup>+</sup> transport in higher plants. *Ann. Bot.* 91: 503–527.
- Thalji, T., and Shalaldehy, G., 2007, Screening wheat and barley genotypes for salinity resistance. *J. Agron.* 6: 75–80.
- Tobe, K., Zhang, L. P., Omasa, K., 2003. Alleviatory effects of calcium on the toxicity of sodium, potassium and magnesium chlorides to seed germination in three non-halophytes. *Seed Sci Res.* 13:47-54
- Tyerman, S.D., Beilby, M., Whittington, J., Juswono, U., Newman, I., and Shabala, S., 2001. Oscillations in proton transport revealed from simultaneous measurements of net current and net proton fluxes from isolated root protoplasts: MIFE meets patch-clamp. *Aust. J. Plant Physiol.* 28: 591–604.
- Ueda, A., Kathiresan, A., Inada, M., Narita Y., Nakamura, T., Shi, W., Takabe, T., and Bennett, J., 2004. Osmotic stress in barley regulates expression of a different set of genes than salt stress does. *Journal of Experimental Botany* 55:2213-2218
- Uozumi, N., Kim, E.J., Rubio, F., Yamaguchi, T., Muto, S., Tsuboi, A., Bakker, E.P., Nakamura, T., and Schroeder, J.I., 2000. The Arabidopsis HKT1 gene homolog mediates inward Na<sup>+</sup> currents in *Xenopus laevis* oocytes and Na<sup>+</sup> uptake in *Saccharomyces cerevisiae*. *Plant Physiol.* 122: 1249–1260.
- Varshney, R.K., Prasad, M., Graner, A., 2004. Molecular marker maps of barley: a resource for intra- and interspecific genomics. In: Lorz H, Wenzel G (eds) *Biotechnology in agriculture and forestry. Molecular markers systems*. Springer, Heidelberg, 55: 229–243.

- Verslues, P.E., Agarwal, M., Katiyar-Agarwal, S., Zhu, J., and Zhu, J.K., 2006. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. *Plant J.* 45: 523–539.
- Véry, A.A. and Sentenac, H., 2002. Cation channels in the Arabidopsis plasma membrane. *Trends Plant Sci.* 7: 168–175.
- Volkov, V., Wang, B., Dominy, P.J., Fricke, W., and Amtmann, A., 2004. *Thellungiella halophila*, a salt-tolerant relative of *Arabidopsis thaliana*, possesses effective mechanisms to discriminate between potassium and sodium. *Plant Cell Environ.* 27: 1–14.
- Wagner, G.J., 1982. Compartmentation in plant cells: The role of the vacuole. In: *Cellular and Subcellular Localisation in Plant Metabolism*, Creasy, L.L., and Hrazdina, G. (eds.), Plenum Press, New York. pp. 1–45.
- Walia, H., Wilson, C., Wahid, A., Condamine, P., Cui, X., Close, T.J., 2006. Expression analysis of barley (*Hordeum vulgare* L.) during salinity stress. *Funct Integr Genomics* 6: 143–156
- Wang, Y., and Nil, N., 2000. Changes in chlorophyll, ribulose biphosphate carboxylase–oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. *J. Hortic. Sci. Biotechnol.* 75: 623–627.
- Wei, W.X., Bilsborrow, P., Hooley, P., Fincham, D., Foster, B., 2001. Variation between two near isogenic barley (*Hordeum vulgare*) cultivars in expression of the B subunit of the vacuolar ATPase in response to salinity. *Hereditas* 135: 227–231
- Wei, W., Bilsborrow, P.E., Hooley, P., Fincham, D.A., Lombi, E., and Forster, B.P., 2003. Salinity induced differences in growth, ion distribution and partitioning in barley between the cultivar Maythorpe and its derived mutant Golden Promise. *Plant Soil* 250: 183–191.
- Weidner, A., Asch, F., Buck-Sorlin G. H., Börner, A., 2007. QTLs for Salt Resistance Vary with Development Stage in Field-Grown Barley. In: *Utilisation of diversity in land use systems: Sustainable and organic approaches to meet human needs. Tropentag 2007*, October 9 - 11, Witzenhausen, Germany
- Wenzl, P., Li H., Carling, J., Zhou, M., Raman, H., Paul, E., Hearnden, P., Maier, C., Xia, L., Caig, V., Ovesna, J., Cakir, M., Poulsen, D., Wang, J., Raman, R., Smith, K.P., Muehlbauer, G.J., Chalmers, K.J., Kleinhofs, A., Huttner, E., Kilian, A., 2006. A high-density consensus map of barley linking DArT markers to SSR, RFLP and STS loci and phenotypic traits. *BMC Genomics* 7: 206.
- Wherrett, T., Ryan, P.R., Delhaize, E., and Shabala, S., 2005. Effect of aluminium on membrane potential and ion fluxes at the apices of wheat root. *Funct. Plant Biol.* 32: 199–208.
- White, P.J., and Davenport, R.J., 2002. The voltage independent cation channel in the plasma membrane of wheat roots is permeable to divalent cations and may be involved in cytosolic Ca<sup>2+</sup> homeostasis. *Plant Physiol.* 130: 1386–1395.
- Wolf O., Munns R., Tonnet M.L., Jeschke W.D. 1990. Concentrations and transport of solutes in xylem and phloem along the leaf axis of NaCl-treated *Hordeum vulgare*. *Journal of Experimental Botany* 41: 1133–1141
- Wyn Jones, R.G., Brady, C.J., and Speirs, J., 1979. Ionic and osmotic relations in plant cells. In: *Recent Advances in the Biochemistry of Cereals*, Laidman, D.L., Wyn Jones, R.G., (eds.), Academic Press, London. pp. 63–103.

- Wyn Jones, R.G., Storey, R., 1978. Salt stress and comparative physiology in the Gramineae. II. Glycine betaine and proline accumulation in two salt- and water-stressed barley cultivars. *Aust. J. Plant. Physiol.* 5: 817–829.
- Xiong, L., Zhu, J.K., 2002. Molecular and genetic aspects of plant responses to osmotic stress. *Plant Cell Environ.* 25: 131–139.
- Xu, D., Duan, X., Wang, B., Hong, B., Ho, T.-H.D., Wu, R., 1996. Expression of a late embryogenesis abundant protein gene, HVAJ, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiol* 110:249–257
- Yamaguchi, M., Kasamo, K., 2001. Modulation in the activity of purified tonoplast H<sup>+</sup>-ATPase by tonoplast glycolipids prepared from cultured rice (*Oryza sativa* L. var. Boro) cells. *Plant Cell Physiol* 42: 516–523.
- Yamamoto, A., Shim, I.S., Fujihara, S., Yoneyama, T., and Usui, K., 2004. Effect of difference in nitrogen media on salt-stress response and contents of nitrogen compounds in rice seedlings. *Soil Sci. Plant Nutr.* 50: 85–93.
- Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D., and Somero, G.N., 1982. Living with water stress: evolution of osmolyte systems. *Science* 217: 1214–1222.
- Yapsanis, T., Moustakas, M., and Domiandou, K., 1994, Protein phosphorylation dephosphorylation in alfalfa seeds germinating under salt stress, *J. Plant Physiol.* 143: 234–240
- Ye, J. M., Kao K. N., Harvey, B. L., Rossnagel, B. G., 1987. Screening salt-tolerant barley genotypes via F<sub>1</sub> anther culture in salt stress media. *Theoretical and Applied Genetics* 74: 426–429
- Yeo, A.R., and Flowers, T.J., 1986. Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Aust. J. Plant Physiol.* 13: 161–173.
- Yeo A.R., Flowers T.J.. 1989. Selection for physiological characters – examples from breeding for salt tolerance. In: Jones, H.G., Flowers, T.J., Jones, M.B. (Eds.), *Plants under Stress Biochemistry, Physiology and Ecology and their Application to Plant Improvement*. Cambridge University Press, Cambridge, pp. 217–234.
- Zeh, M., Leggewie, G., Hoefgen, R., Hesse, H., 2002. Cloning and characterization of a cDNA encoding a cobalamin-independent methionine synthase from potato (*Solanum tuberosum* L.). *Plant Mol Biol* 48: 255–265.
- Zeng, L., Shannon, M.C., and Grieve, C.M., 2002. Evaluation of salt tolerance in rice genotypes by multiple agronomic parameters. *Euphytica* 127: 235–245.
- Zhao F.G., Qin P. 2005. Protective effects of exogenous fatty acids on root tonoplast function against salt stress in barley seedlings. *Environmental and Experimental Botany* 53: 215–223
- Zhao F.G., Sun C., Liu Y.L., Liu Z.P., 2000. Effects of salinity stress on the levels of covalently and noncovalently bound polyamines in plasma membrane and tonoplast isolated from leaves and roots of barley seedlings. *Acta Bot Sin* 42: 920–926.
- Zhong, G.Y., J. Dvorak, J., and Zhong, G.Y., 1995. Evidence for common genetic mechanisms controlling the tolerance of sudden salt stress in the tribe Triticeae. *Plant Breeding* 114: 297–302.
- Zhu, J.K., 2000. Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiol* 124: 941–948
- Zhu, G.Y., Kinet, J.M., Lutts, S., 2001. Characterisation of rice (*Oryza sativa* L.) F<sub>3</sub> populations selected for salt resistance. I. Physiological behaviour during vegetative growth. *Euphytica* 121: 25–263.

- Zhu, J.K., 2000. Genetic analysis of plant salt tolerance using Arabidopsis. *Plant Physiol.* 124: 941–948.
- Zhu, J.K., 2001. Plant salt tolerance. *Trends Plant Sci.* 6: 66–71.
- Zhu, J.K., Liu, J., and Xiong, L., 1998. Genetic analysis of salt tolerance in Arabidopsis: evidence for a critical role of potassium nutrition. *Plant Cell* 10: 1181–1191.
- Zidan, I., Jacoby, B., Ravina, I., and Neumann, P.M., 1991. Sodium does not compete with calcium in saturating plasma membrane sites regulating  $^{22}\text{Na}$  influx into salinised maize roots. *Plant Physiol.* 96: 331–334.
- Zid, E., Grignon, C., 1985. Sodium-calcium interactions in leaves of *Citrus aurantium* grown in the presence of NaCl. *Physiologie Vegetale* 23: 895-203.
- Živanović, B.D., Pang, J., and Shabala, S., 2005. Light-induced transient ion flux responses from maize leaves and their association with leaf growth and photosynthesis, *Plant Cell Environ.* 28: 340–352.

# CHAPTER 7

## GENETICS AND BREEDING FOR SOYBEAN SALT TOLERANCE

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### 1. INTRODUCTION

Soybean, *Glycine* is one of the most important oil-seed crops in the world. The demand for soybean is continuing to increase due to the increasing world population. However, enormous areas of formerly arable land are being lost from crop production due to increasing soil salinity (Epstein et al. 1980). It is estimated that one billion hectares of the earth's surface are covered by saline soils. In China, 8 million hectares of agricultural land are becoming increasingly salinized due to improper irrigation and fertilization (Gao and Lin 2005). Soil with electric conductivity greater than 40mM NaCl (about 4ds/m) is considered saline (Stoddard et al., 2006). Blumwald and Grover (2006) predicted about 50% of the arable land will be affected by salt stress by 2050.

### 2. SALINITY EFFECTS ON SOYBEAN

#### 2.1 Yield

Soybean is classified as a salt-sensitive glycophyte (Launchli 1984). In soybean, salinity stress inhibits seed germination and seeding growth. The threshold of salt electric conductivity (EC) is 5.0 dS m<sup>-1</sup>. Salinity stress induced a significant increase of mortality rate and leaf necrosis when EC increased to 10.2 dS m<sup>-1</sup> (Able and MacKenize, 1964). Soybean yield (versus 100% at 0.8 dS m<sup>-1</sup>) is dramatically decreased from 80% at 4.0 dS m<sup>-1</sup>, and 44% at 6.7 dS m<sup>-1</sup> v under salt stress. The effect of soil salinity on the relative biological nitrogen contribution of the soil was 77% at 4.0 dS m<sup>-1</sup>, and 28% at 6.7 dS m<sup>-1</sup> verse 100% at 0.8 dS m<sup>-1</sup> (Katerji et al., 2003).

#### 2.2 Seed quality

Lower salt stress (14-15 dS m<sup>-1</sup>) increased oil content and decreased seed protein, linoleic acid, linolenic acid content and oleic acid significantly. But protein content increased and oil content decreased under higher salt stress (18-20 dS m<sup>-1</sup>). The increase of linolenic acid and protein content may increase the salt tolerance of soybean (Chang et al., 1994).

#### 2.3 Nodulation

Soybean nodulation is also adversely affected by salt stress. Studies have shown salinity significantly decreases nodule number and dry-weight (Bernstein and Ogata, 1966). Availability of oxygen to nodules is reduced and fermentative pathways are stimulated (Serraj et al., 1994). The ability of tissues to supply water to root cells under salt stress is reduced (Joly, 1989). In other studies nuclear deformation of the meristematic root cells occurred and was followed by degradation of nuclei in the apical region of the root tip (Liu et al., 2000).

#### 2.4 Others

Salinity stress induced a significant increase in soybean leaf sodium and chloride and reduced the accumulation of potassium, calcium, and magnesium (Abel, 1969; Essa, 2002).

### 3. MECHANISM OF SALT TOLERANCE

The mechanism of salt tolerance is complicated, which is thought to consist of two principle components (Greenway and Munns, 1980). One component is an 'osmotic effect' which limits water absorption due to salinity in the rhizosphere. The other component is an 'ionic effect' which is able to overcome intercellular toxicity from excess ions. Soybean salt tolerance is thought to be primarily from the ionic component in which tolerant plants limit the accumulation of excess ions to reduce injury (Lauchli and Wieneke, 1979; Umezawa et al., 2000; Umezawa et al., 2002). Salt tolerant soybean genotypes 1) prevent salt ions from moving from the roots to other plant parts, 2) do not accumulate as much salt in leaves and stems, and 3) have better osmotic adjustment in plant cells.

Abel (1969) reported that the chloride concentration of tolerant soybeans in soybean leaves was 10 times less than salt susceptible soybeans. Transport of chloride ions in salt tolerant cultivars from the root to stems and leaves was exceedingly low (Able and MacKenize, 1964). Plants of the tolerant cultivar Lee were taller, maintained lower  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations, a higher  $\text{K}^+$  concentration and a higher  $\text{K}^+/\text{Na}^+$  ratio at higher salinity levels than salt sensitive cultivars Colquitt and Clark 63 (Essa, 2002). Dare another tolerant cultivar showed a higher relative shoot and root growth, water extraction ability, root pressure, better root osmotic adjustment and less sodium accumulation in plant tissue than salt sensitive cultivars. The salt tolerance of Dare was associated with high water uptake, and  $\text{Na}^+$  and  $\text{Cl}^-$  exclusion from being transported from roots to upper portions of plants. Tolerance in soybean was also shown to be related to maintenance of stable water content in shoots and the accumulation of soluble saccharides, soluble proteins, the amino acid, proline, and  $\text{K}^+$  and  $\text{Ca}^+$  for osmotic adjustment (Abd El-Samad and Shaddad, 1997).

Differences in salt tolerance among *Glycine* species were reported (Pantalone et al., 1997; Luo et al., 2005; Kao et al., 2006). Greater variation in sodium chloride tolerance was shown among the perennial *Glycine* accessions than among the *G. max* cultivars. The sodium chloride tolerance thresholds ranged from 3.0 to 17.5 g/L NaCl for the perennial accessions, but only ranged from 5.2 to 8.0 g/L NaCl in the *G. max* cultivars based on the Weibull model for measuring leaf chlorosis (Pantalone et al., 1997). Luo et al. (2005) reported that the salt tolerance mechanism of cultivated soybean (*G. max*) was different from wild soybean (*G. soja*). Comparing differential sensitivity of *G. max* and *G. soja* to chloride and sodium ions, *G. max* genotypes were more heavily damaged in the  $\text{Cl}^-$  solution than  $\text{Na}^+$  solution. Salt tolerance in *G. max* was mainly due to prevention of  $\text{Cl}^-$  ion transport from the roots to the upper portion of the plant preventing toxic accumulation in

stems and leaves. In contrast leaves of salt tolerant wild soybeans, *G. soja* strains were not as susceptible as *G. max* to  $\text{Cl}^-$  toxicity as that of  $\text{Na}^+$ . Salt tolerance in *G. soja* was primarily from exclusion of sodium ions from the roots preventing accumulation at toxic concentrations in stems and leaves. The descendants of a cross between tolerant wild and cultivated soybean strains were more tolerant to salt stress of NaCl and  $\text{Cl}^-$  salts than those of cross between tolerant *G. max* cultivars (Luo et al., 2005). This indicates that inter specific crosses between *G. max* and *G. soja* offer the possibility of improving salt tolerance in soybean cultivars.

## 4. IDENTIFICATION OF SALT TOLERANT GERMPLASM

### 4.1 Identification and classification method

Two screening techniques have been used for selection of salt tolerant soybeans, 1) soil with high salt content, 2) hydroponics with high salt added to a nutrient solution. However the evaluation of genotypes in fields with high salt content can be difficult because of variability of salt levels across experiment conditions. Some studies developed the classification methods such as direct grading method, and indexing method (Shao et al., 1987; Ma et al., 1994; Lee et al., 2004). In these experiments, salt tolerance can be identified according the phenotype or the toxic index (Salt toxic index =  $\frac{\sum(\text{individual number with the same grade} \times \text{grade number}) \times 100}{\text{surveyed plant number before treatment} \times 5}$  ).

### 4.2 Screening germplasm for salt tolerance

Shao et al. (1995) evaluated 1716 Chinese soybean and 415 accessions for salt tolerance with three replications in Shandong province. The soil was a sandy loam with moderate saline content. They screened soybean lines at the V2 or V3 seedling stage using 15-17  $\text{ds m}^{-1}$  saline water made from a mixture of fresh water and underground salt water. A total of 85 salt tolerant Chinese soybean germplasm at seeding stage were identified and only 10 germplasm from the United States were salt tolerant. Parker et al. (1986) planted 15 soybean genotypes in two fields with a history of leaf scorch symptoms from  $\text{Cl}^-$  toxicity from KCl fertilizer. Soil types were both Leefield sand (arenic Plinthaquic Paleudults) and Alapaha sand (arenic Plinthic Paleaquults). They found 10 of 15 soybean cultivars were tolerant to high chloride by rating for leaf scorch and leaf  $\text{Cl}^-$  concentration. Yang and Blanchard (1993) used Mexico silt loam soil with and without added  $\text{Cl}^-$ . A total of 673  $\text{Kg Cl}^- \text{ ha}^{-1}$  was added as a  $\text{CaCl}_2$  solution for the high  $\text{Cl}^-$  plot. Nineteen of 60 soybeans were salt tolerant based on leaf scorch ratings and leaf  $\text{Cl}^-$  concentration. These studies show that different soil types can be used for screening for phenotyping soybean genotypes for salt tolerance. Hydroponics modified by Johnson et al. (1957) and Hoagland and Arnon (1953) with added NaCl to the nutrient solution is widely used to screen soybeans for tolerance. Many genotypes can be screened in limited space like the greenhouse and the salt rate can be easily controlled.

**Table 1** Screening method for salt tolerant soybean

Screening techniques	Classifying method	Reference
1) Germinating soybean in sand and placing 5 seedlings per replication in nutrient solution 14–21 days after emergence 2) adding NaCl to the solution for 14–31 days	0 = healthy (no apparent symptoms of scorch) 1 = slight scorch (25% of the leaf area showed scorch symptoms) 2 = moderate scorch (50% of the leaf area showed scorch symptoms) 3 = severe (75% of the leaf area showed scorch symptoms) 4 = dead (plants were brown and withered)	Pantalone et al., 1997
1) Plants were grown in 27 cm diameter pots filled with builder's washed sand 2) 14 days after emergence, 100mM NaCl was added to the nutrient solution, and this modified solution was applied daily 3) visual ratings of leaf scorching and chlorosis were taken 45 days after emergence	0 = all plants dead; 3 = plants with light green or chlorotic appearance; 5 = plants with normal green leaves	Lee et al., 2004
1) Soybean was planted in sandy loam soil with moderate saline content 2) at germination and the V2 or V3 seedling stage using 15-17 ds m <sup>-1</sup> saline water made from a mixture of fresh water and underground salt water 3) plants were screened 3~5 days after treatment	1 = normal; 2 = Less than 30% plants showed scorch symptoms; 3 = most of the plants showed scorch symptoms, less than half of the plants dead; 4 = plant growth was decreased, less than 80% of the plants dead; 5 = more than 80% plants dead. Grades and toxic indexes were corresponded as: 1 = 0~20%; 2 = 20.1~35%; 3 = 35.1~65% 4 = 65.1~90%; 5 = 90.1~100%	Shao, 1987
1) Soybean was planted in alkaline chernozem soil with 0.2% saline content 2) at the V3 seedling stage using 10 hos cm <sup>-1</sup> saline water made from a mixture of fresh water, refined salt and alkali 3) plants were screened 5 days after treatment	0 = normal 1 = basically normal, less than 10% leaf area showed scorch symptoms or more than 4 green leaves per plant 2 = basically normal, More than 10% leaf area showed scorch symptoms or more than 2 green leaves 3 = plant growth was decreased, Half of the leaf area showed scorch symptoms or 2 green leaves per plant 4 = plant growth was decreased badly, 75% of leaf area showed scorch symptoms or only one green leaf per plant 5 = nearly dead, more than 75% of leaf area showed scorch symptoms or only one central leaf was alive per plant. Grades and toxic indexes were corresponded as: 0 = 0; 1 = 0.1~20%; 2 = 20.1~35%; 3 = 35.1~65%; 4 = 65.1~90%; 5 = 90.1~100%	Ma et al., 1994

The results of the identification depend on genotype, salt concentration, and other environmental factors such as temperature and light. Salt concentration is the most critical factor for phenotyping genotypes for tolerance. Threshold values to detect salt tolerance in soybean have varied among



different studies. Chinnusamy et al. (2005) determined in their study that the threshold salinity to detect tolerance was  $3.2 \text{ dS m}^{-1}$ . The cultivar ‘Lee’ (tolerant) produced more than twice the relative shoot fresh weight and was significantly lower in chlorosis score than other genotypes at a  $6.0 \text{ dS m}^{-1}$  salt level. Soybean genotypes were able to differentiate for tolerance or sensitivity at  $7.5 \text{ dS m}^{-1}$ , but all genotypes were sensitive at  $10.9 \text{ dS m}^{-1}$  and had similar leaf chloride levels. The most significant phenotypic differences for salt tolerance between ‘Dare’ (tolerant) and ‘Tachiyutaka’ (sensitive) were obtained at  $40 \text{ mM NaCl}$  (An et al., 2002). Seventeen soybean cultivars were evaluated for salt tolerance at five locations in two years. Three cultivars with higher yield at saline field were selected, which will increase soybean planting area by using selected cultivars in the saline field of Hebei province in China (Pan et al., 2007).

**Table 2** Salt tolerant germplasm identified by different researches

Serial number	No. Germplasm	Tolerant germplasm		Stage	Reference
		Number	Ratio (%)		
1	1716	242	14.1	Germination	Shao et al., (1986)
2	1716	85	5.0	Seeding	Shao et al., (1986)
3	1716	82	4.8	Mature	Shao et al., (1986)
4	1514	201	13.3	Seeding	Shao, (1988)
5	17	3	17.6	Full-life	Pan, (2007)
6	15	10	66.7	Seeding	Parker et al., (1986)
7	60	19	31.7	Seeding	Yang and Blanchar, (1993)

## 5. INHERITANCE OF SALT TOLERANCE AND MAPPING THE SOYBEAN SALT TOLERANCE GENE

### 5.1 Inheritance

To characterize the inheritance of salt tolerance, genotypes were screened for chloride inclusion (sensitive) and exclusion (tolerant). Salt solution was added to screen F<sub>2</sub> populations from parents differing in chloride accumulation, populations segregated in ratios of 3 non-necrotic plants very low in chloride to 1 necrotic plant very high in chloride with a 1:1 segregation ratio from a test cross. It was concluded that the factor to exclude or include chlorides in soybean leaves and stems is controlled by a single dominant gene. Gene symbols Ncl and ncl were proposed as the dominant chloride excluder allele for tolerance and the recessive chloride includer for sensitivity, respectively (Able, 1969). Shao et al. (1994) studied the inheritance of salt tolerance on soybean based on progenies segregation performance under salt stress by means of cross salt tolerance and sensitive cultivars. F<sub>1</sub> plants expressed salt tolerance, and F<sub>2</sub> populations segregated in ratio of 3 tolerance to 1 sensitive. The result indicated that the salt tolerance was based on a single dominant gene, and it was suggested that this gene was the same as that reported by Abel (1969).

### 5.2 Tagging salt tolerance gene/QTL

Once sources of tolerance are identified and their genetics are determined, it is important to identify closely linked genetic markers for use in marker assisted selection. Zhong et al. (1997) suggested that three DNA amplified fingerprinting (DAF) markers present only in cultivars Morgan and Wenfeng 7 might be linked to salt tolerance. Six RAPD markers were identified only in salt tolerant soybeans indicating a possible relationship between the molecular markers and salt-tolerance (Zhang et al. 1999). Guo et al. (2000) developed a co-dominant RAPD marker (600bp band in the susceptible parent and a 700bp band in tolerant parent) closely linked to a salt tolerance gene, but the location of the marker was not determined. Now this RAPD marker has been converted to SCAR marker and mapped to soybean linkage group N near SSR marker Satt255 (unpublished data). Lee et al. (2004) mapped a major QTL for salt tolerance in cultivar S-100 near marker Sat\_091 on linkage group N. This gene accounted for 79% of the genetic variation in combined environments. The association between salt tolerance and the same marker allele was consistent in related salt tolerant cultivars. They predicted that the major QTL on LG-N is likely to be the *Ncl* locus initially named by Abel (1969). It thus appears that the same gene is relatively common although tests of allelism are yet to be conducted.

## 6. CLONING OF CANDIDATE GENES FOR SALT TOLERANCE

Molecular analysis has led to the identification of a number of genes induced by NaCl in soybean (Table 1). Most of the genes in the functional groups have been identified as salt inducible under stress conditions. Some of the genes could be induced by other abiotic stress, such as cold and draught (Luo et al., 2005; Sun et al., 2006). By using cDNA-AFLP, Umezawa et al. (2002) found several Glycine max Stress Responsive genes (GSR) genes, GSR-8, 98, 110, and 112, induced by the NaCl treatment, and these genes showed tissue-specific expression. They suggested that salt tolerance of soybean is achieved from the response to both ionic effects and osmotic effects. The gene expression was abundant in soybean under salt stress. Transcripts which could be determined from ionic (NaCl-specific) and osmotic effects (common from NaCl with PEG) were 44 and 40 %, respectively. The gene expression dependent ionic effect was more abundant in roots indicating a greater response to ionic stress in roots than shoots. On the other hand, GSR gene expression from osmotic effects was more in shoots than roots. To evaluate the response of soybean to salt stress, Aghaei et al. (2008) evaluated the changes in protein expression using the proteomic approach. Proteins from the hypocotyl and root treated with 100 mM NaCl were extracted and separated by two dimensional polyacrylamide gel electrophoresis; 321 protein spots were detected. In response to salt stress, seven proteins were reproducibly found to be up- or down-regulated by two to sevenfold: late embryogenesis-abundant protein,  $\beta$ -conglycinin, elicitor peptide three precursor, and basic/helix-loop-helix protein were up-regulated, while protease inhibitor, lectin, and stem 31-kDa glycoprotein precursor were down-regulated. These results indicate that salinity can change the expression level of some special proteins in the hypocotyl and root of soybean that may in turn play a role in the adaptation to saline conditions.

**Table 3** Genes/protein induced by salt stress in soybean

Salt responsive genes/proteins	Characteristic feature	Reference
<i>GmCAX1</i>	Expressed in all tissue of the plants, over expression in <i>Arabidopsis</i> accumulated less Na <sup>+</sup> , K <sup>+</sup> and Li <sup>+</sup> , may function as an antiporter for Na <sup>+</sup> , K <sup>+</sup> and Li <sup>+</sup>	Luo et al., (2005)
<i>GmDREBa</i> <i>GmDREBb</i> <i>GmDREBc</i> <i>GmDREB2</i>	<i>GmDRBa</i> and <i>GmDRBb</i> in soybean leaves were induced by salt, drought, and cold stress, expression of <i>GmDRBc</i> was induced in roots by salt, drought, and abscisic acid treatments Classified into A-5 subgroup in DREB subfamily in AP2/EREBP family, over expression in <i>Arabidopsis</i> , resulting in enhanced tolerance to drought and high-salt stresses	Li et al., (2005) Chen et al., (2007)
<i>GmTDFs</i>	A novel cytosolic leucine-zipper-like protein, induced in the stem and lower-expanded leaf	Aoki et al., (2005)
<i>GmPAP3</i>	Plays a role in the adaptation of soybean to NaCl stress	Liao et al., (2003)
<i>GmNHX1</i>	Expression was increased by ABA treatment, NaCl, KCl, LiCl and dehydration stress, over expression in <i>Lotus corniculatus</i> L. got lower Na <sup>+</sup> and K <sup>+</sup> content, and higher K <sup>+</sup> / Na <sup>+</sup> than that in the control line	Sun et al., (2006)
STL	Shared 63.6% identity with <i>Arabidopsis thaliana</i> salt tolerance protein STO	Li et al., (2006)
GmOSBP	May be involved in some physiological reactions for stress-response and cotyledon senescence in the soybean	Li et al., (2008)
GmbZIP132	Induced by drought and high salt treatments, over expression in <i>Arabidopsis</i> could increase the salt tolerance during germination and seedling stage.	Liao et al., (2008)
GmOLPa	The mature GmOLPa protein without the signal peptide has a calculated molecular mass of 21.5 kDa and a pI value of 4.4	Onishi et al., (2006)

## 7. PERSPECTIVES OF BREEDING FOR SALT TOLERANCE SOYBEAN

### 7.1 Fine mapping of tolerance gene

To date conventional breeding programs for generation of salt-tolerant genotype have made progress. In China, few varieties had been released, such as Tiefeng 8, Zhonghuang 13 etc. This lack of success is resulted by poorly understanding genetic and physiological mechanisms of the salt tolerance. More concentrated efforts are needed to screen germplasm, determine new genes, develop useful molecular markers and gene tagging methodologies, and to combine genes for higher levels of tolerance to these stresses in soybean. Despite significant efforts have been made to understand molecular and physiological aspects of drought and salt tolerance. However, further efforts are needed to better understanding of the mode of action for ion exclusion under salt stress will facilitate the ability to develop tolerant cultivars.. A significant number of QTLs/genes have been identified for different traits in soybean, but only a few associated with salt tolerance. More studies are needed to elucidate and determine novel genes and their mode of action for high tolerance. Although there are numerous cultivated and wild soybean accessions in the soybean germplasm collections of the world, little of this germplasm with salt tolerance were further

identified. Combining genes from both wild and cultivated species shows promise to obtain genotypes with higher levels of tolerance (Luo et al., 2005). Mapping for new QTL/gene and determination of gene action under salt stress will provide key resources to improve tolerance to drought and salt stress in soybean.

## **7.2 Pyramiding genes by marker assisted selection in soybean breeding**

Marker assisted selection (MAS) will be important for pyramiding genes at two or more loci to elevate salt tolerance in soybean. Limitations of molecular markers have been surpassed with the discovery and use of gene-based abundant SNP markers. SNP and other markers have helped to develop a high density soybean genetic map for the identification and characterization of QTLs/genes conditioning salt tolerance related traits facilitating MAS programs. The United States Department of Energy and Joint Genome Institute (DOE-JGI) have released preliminary whole genome shotgun at the beginning of 2008 (<http://www.phytozome.net/soybean.php>). With the availability of soybean genome sequence information, integrated soybean genetic and physical map and traits specific SNP markers will make a significant and successful contribution in molecular breeding for abiotic stress. Genetic engineering technology is an attractive approach to improve soybean for drought and salt stress tolerance. Genes have been identified and some are being used successfully to develop genetically engineered drought and salt tolerant rice and canola. Introduction of drought and salt tolerance genes from soybean and other crops into elite soybeans that have high yield, enhanced resistance to pathogens and improved seed quality is highly desirable. Molecular techniques like QTL mapping, gene cloning, gene transformation and DNA microarray and gene expression analysis related to specific QTL regions are rapidly advancing and will play a vital role in the development of stress tolerant soybeans. Effective use of available genetic resources, understanding of tolerance mechanisms, construction of a fine map of the genome, development of marker assistant selection techniques and well-designed breeding strategies will advance the development of soybean varieties with significantly greater salt tolerance. By using the salt tolerant germplasm Wenfeng 7 as a parent, Shao developed a salt tolerant cultivar Zhonghuang 10, which were planted 26666.7 ha in salinity field. The evaluation and utilization of salt tolerance germplasm has been awarded the Beijing Sci-Tech Progress Award (II) in 2002. Three populations of salt-tolerant cultivars, which including Wenfeng 7, and susceptible cultivars were used to map the salt tolerance gene, and a co-dominant RAPD marker was identified via analysis of the segregation of F<sub>2</sub> plants (Guo et al., 2000). The markers were closely linked with salt-tolerant/susceptible alleles, and are now being utilized to select salt-tolerant, high yielding lines. The identification and utilization of this marker has got the Chinese patent in 2004. Zhang et al. (2005) evaluated Chinese soybean germplasm by SSR marker linked to salt tolerance QTL (Lee et al., 2004). The 212bp at SSR locus Satt339 in salt tolerance germplasm accounted for 74%, which indicated the potential usefulness of this marker in salt tolerance soybean breeding. By convert the RAPD marker the Guo et al. (2000) reported into SCAR marker, we developed a co-dominant SCAR marker recently. The identity of genotype and phenotype was 85.1% when the marker was used to analyze 55 salt tolerant and 90 salt sensitive germplasm (unpublished data). This result indicated that this marker will be useful for marker assisted selection of salt tolerance gene in different soybean genetic backgrounds.

## REFERENCES

- Abd El-Samad, H. M., and Shaddad, M. A. K., 1997, Salt tolerance of soybean cultivars, *Biologia Plantarum*. 39(2):263–269.
- Abel, G. H., 1969, Inheritance of the capacity for chloride inclusion and chloride exclusion by soybeans, *Crop Sci.* 9:697–698.
- Able, G. H., and MacKenzie, A. J., 1964, Salt tolerance of soybean varieties (*Glycine max* L. Merrill) during germination and later growth, *Corp Sci.* 4:157–161.
- Aghaei, K., Ehsanpour, A. A., Shah, A. H., and Komatsu, S., 2008, Proteome analysis of soybean hypocotyl and root under salt stress, *Amino Acids*. DOI 10.1007/s00726–008–0036–7.
- An, P., Inanaga, S., Cohen, Y., Kafkafi, U., and Sugimoto, Y., 2002, Salt tolerance in two soybean cultivars, *Journal of Plant Nutrition*. 25(3):407–423.
- Aoki, A., Kanegami, A., Mihara, M., Kojima, T., Shiraiwa, M., and Takahara, H., 2005, Molecular cloning and characterization of a novel soybean gene encoding a leucine–zipper–like protein induced to salt stress, *Gene*. 356:135–145.
- Bernstein, L., and Ogata, G., 1966, Effects of salinity on nodulation, nitrogen fixation, and growth of soybeans and alfalfa, *Agron. J.* 58:201–203.
- Blumwald E., Grover, A., 2006, Salt tolerance, in: *Plant Biotechnology: Current and future uses of genetically modified crops*, Nigel G. Halford, eds., John Wiley and Sons Ltd, UK, pp. 206–224.
- Chang, R., Chen, Y., Shao, G., Wan, C., 1994, Effect of salt stress on agronomic characters and chemical quality of seeds in soybean, *Soybean Science*. 13(2):101–105.
- Chen, M., Wang, Q., Cheng, X., Xu, Z., Li, L., Ye, X., Xia, L., and Ma, Y., 2007, GmDREB2, a soybean DRE-binding transcription factor, conferred drought and high-salt tolerance in transgenic plants. *Biochem Biophys Res Commun*. 353(2):299–305.
- Chang, R., Qiu, L., Sun, J., Chen, Y., Li, X., and Xu, Z., 1999, Collection and conservation of soybean germplasm in China, in: *Proceedings at the World Soybean Research Conference VI*, H. E. Kauffman, ed., Chicago, Illinois. Superior Printing, Champagne, Illinois. pp. 172–176.
- Chinnusamy, V., Jagendorf, A., and Zhu, J. K., 2005, Understanding and improving salt tolerance in plants, *Crop Sci.* 45:437–448.
- Epstein, E., Norlyn, J. D., Rush, D. W., Kings, R. W., and Kelly, D. B., 1980, Saline culture of crops: A general approach. *Science*. 210:399–404.
- Essa, T. A., 2002, Effect of salinity stress on growth and nutrient composition of three soybean (*Glycinemax* L. Merrill) cultivars, *J. Agron. Crop Sci.* 188(2):86–93.
- Gao, J., Lin, H., 2005, Review of salt tolerant mechanism on rice—a research on salt tolerant quantitative trait locus (QTL) SKC1. *Chinese Bull Life Sci.* 17:563–565.
- Guo, P., Qiu, L., Shao, G., Chang, R., Liu, L., Xu, Z., Li, X., and Sun, J., 2000, Tagging a salt tolerant gene using PCR markers in soybean. *Sci Agric Sin.* 33(1):10–16.
- Guo P., Shao, G., Qiu, L., and Xu, Z., 2002, Marker assisted-identification of the salt tolerant accessions in soybean. *Soybean Sci.* 21(1):56–61.

- Greenway, H., and Munns, R., 1980, Mechanisms of salt tolerance in nonhalophytes, *Ann. Rev. Plant Physiol.* 31:149–190.
- Hoagland, D. R., and Arnon, D. I., 1938, The water–culture method for growing plants without soil, *Calif. Agric. Expt. Circ.* 347:1–39.
- He, C., Zhang, J., and Chen, S., 2002, A soybean gene encoding a proline–rich protein is regulated by salicylic acid, an endogenous circadian rhythm and by various stresses. *Theor Appl Genet.* 104(6-7):1125–1131.
- Hu, H., Dai, M., Yao, J., Xiao, B., Li, X., Zhang, Q., and Xiong, L., 2006, Over expressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice, *Proc. Natl. Acad. Sci.* 103(35):12987–12992.
- Johnson, C. M., Stout, P. R., Broyer, T. C., and Carlton, A. B., 1957, Comparative chlorine requirements of different plant species, *Plant Soil.* 8:337–353.
- Joly, R. J., 1989, Effect of sodium chloride on the hydraulic conductivity of soybean root systems, *PlantPhysiol.* 91(4):1262–1265.
- Kao, W. Y., Tsai, T. T., Tsai, H. C., and Shih, C. N., 2006, Response of three Glycine species to salt stress, *Environ. Expt. Bot.* 56(1):120–125.
- Katerji, N. Hoorn, J. W., Hamdy, A., and Mastrorilli, M., 2003, Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods, *Agri. Water Mangt.* 62(1):37–66.
- Lauchli, A., 1984, Salt exclusion: an adaptation of legumes for crops and pastures under saline conditions: an adaptation of legumes for crops and pastures under saline conditions. In :Staples RC, Toenniessen GH (eds) *Salinity tolerance in plants. Strategies for crop improvement.* Wiley, New York, pp. 171–187.
- Lauchli, A., and Wieneke, J., 1979, Studies on growth and distribution of Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>–</sup> in soybean varieties differing in salt tolerance, *Zeitschrift Fur Pflanzenernahrung und Bodenkunde.* 142:3–13.
- Lee, G., Boerma, H., Villagarcia, M., Zhou, X., Carter, T., Li, Z., and Gibbs, M., 2004, A major QTL conditioning salt tolerance in S–100 soybean and descendent cultivars. *Theor Appl Genet.* 109(8):1610–1619.
- Li, D., Inoue, H., Takahashi, M., Kojima, T., Shiraiwa, M., and Takahara, H., 2008, Molecular characterization of a novel salt–inducible gene for an OSBP (oxysterol–binding protein)–homologue from soybean, *Gene.* 407(1-2), 12-20.
- Li, F., Zhang, L., Wang, G., Cao, Y., Wang, J., and Tang, K., 2006, Cloning and Characterization of a Salt–tolerance Related Gene from Glycine max, *Molecular Plant Breeding*, 4(4):464–468.
- Li, X. P., Tian, A. G., Luo, G. Z., Gong, Z. Z., Zhang, J. S., and Chen, S. Y., 2005, Soybean DRE–binding transcription factors that are responsive to abiotic stresses, *Theor Appl. Genet.* 110(8):1355–1362.
- Liao, H., Wong, F. L., Phang, T. H., Cheung, M. Y., Francisca Li, W. Y., Shao, G., Yan, X., and Lam, H. M., 2003, GmPAP3, a novel purple acid phosphatase–like gene in soybean induced by NaCl stress but not phosphorus deficiency, *Gene.* 318:103–111.
- Liao, Y., Zhang, J., Chen, S., and Zhang, W., 2008, Role of soybean GmbZIP132 under abscisic acid and salt stresses, *Journal of integrative plant biology.* 50(2):221–230.

- Liu, T., Staden J. V., and Cress W.A., 2000, Salinity induced nuclear and DNA degradation in meristematic cells of soybean (*Glycine max* (L.)) roots, *Plant Growth Regulation*. 30(1): 49–54.
- Luo, G., Wang, H., Huang, J., Tian, A., Wang, Y., Zhang, J., and Chen, S., 2005, A putative plasma membrane cation/proton antiporter from soybean confers salt tolerance in *Arabidopsis*, *Plant Mol. Biol.* 59(5):809–820.
- Luo, Q., Yu, B., Liu, Y., 2005, Differential sensitivity to chloride and sodium ions in seedlings of *Glycine max* and *G. soja* under NaCl stress, *J. Plant Physiol.* 162(9):1003–1012.
- Ma, S., Wang W., 1994, Research of salt tolerance of soybean germplasm, *J. Jilin Agricultural Sciences*. (4): 69~71
- Onishi M., Tachi, H., Kojima, T., Shiraiwa, M., and Takahara, H., 2006, Molecular cloning and characterization of a novel salt-inducible gene encoding an acidic isoform of PR-5 protein in soybean(*Glycine max* [L.] Merr.), *Plant Physiology and Biochemistry*. 44(10):574–580.
- Parker, M. B., Gaines, T. P., and Gascho, G. J., 1986, Sensitivity of soybean cultivars to soil chloride, *Research Bulletins* 347, The Georgia Agricultural Experiment Stations, University of Georgia, pp. 1–14.
- Pan, R., 2007, Identification of salt tolerant soybean at sea shore in Hebei province. *Anhui Agri. Sei. Bull.* 13(7):158–159.
- Pantalone, V. R., Kenworthy, W. J., Slaughter, L. H., and James, B. R., 1997, Chloride tolerance in soybean and perennial *Glycine* accessions, *Euphytica*. 97(2):235–239.
- Serraj, R., Roy, G., and Dreven, J. J., 1994, Salt stress induces a decrease in the oxygen uptake of soybean nodules and in their permeability to oxygen diffusion. *Physiologia Plantarum*. 91(2), 161–168.
- Shao, G., Chang, R., Chen, Y., and Yan, S., 1994, Study on inheritance of salt tolerance in soybean, *Acta Agron Sin.* 20(6):721–726.
- Shao, G., Chang, R., and Chen, Y., 1995, Screening for salt tolerance to soybean cultivars of the United States, *Soybean Genet. Newsl.* 22:32–42.
- Stoddard, F. L., Balko, C., Erskine, W., Khan, H. R., Link, W., and Sarker, A., 2006, Screening techniques and sources of resistance to abiotic stresses in cool-season food legumes, *Euphytica*. 147(1-2):167–186.
- Sun, Y., Wang, D., Bai, Y., Wang, N., and Wang, Y., 2006, Studies on the over expression of the soybean GmNHX1 in *Lous corniculatus*: The reduced Na<sup>+</sup> level is the basis of the increased salt tolerance, *Chinese Sci. Bull.* 51(11):1306–1315.
- Umezawa, T., Mizuno, K., and Fujimura, T., 2002, Discrimination of genes expressed in response to the ionic or osmotic effect of salt stress in soybean with cDNA-AFLP, *Plant Cell Environ.* 25(12): 1617–1625.
- Umezawa, T., Shimizu, K., Kato, M., and Ueda, T., 2000, Enhancement of salt tolerance in soybean with NaCl pretreatment, *Physiologia Plantarum*. 110(11):59–63.
- Yang, J., and Blanchar, R. W., 1993, Differentiation chloride susceptibility in soybean cultivars, *Agron. J.* 85:880–885.
- Zhang, Q., Wang, H., and Hu, Z., 1999, RAPD markers associated with salt tolerance in wild soybean populations. *Soybean Gene Newst.* <http://www.soygenetics.org/articles/sgn1999-010.html>

Zhang, H., Guan, R., Li, Y., Wang, L., Luan, W., Chang, R., Liu, Z., and Qiu, L., 2005, Genetic diversity analysis and marker assisted identification of salt tolerant soybean by using SSR marker. *Journal of Plant Genetic Resources*. 6(3):251–255.

Zhong, M., Hu, Z., and Gresshoff, P. M., 1997, Search for molecular markers of salt tolerance of soybean by DNA amplification fingerprinting, *Soybean Genet. Newsl.* 24:81–82.



# PART III PROTOCOLS

## CHAPTER 8

### ASSESSMENT/MEASUREMENT OF SALINITY PROBLEM IN SOIL AND WATER SYSTEM

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#### 1. INTRODUCTION

Land degradation is a principal constraint in meeting the needs of food for burgeoning world populations. A major factor contributing to land degradation in arid zones is soil and water salinization due to accumulation of soluble salts in soil and water systems. Accumulation of salts may be the result of a gradual weathering of parent materials/ bed rocks or submergence of soil under sea water.

Natural and human-induced salinities are also called primary and secondary salinity respectively. The salinity problem particularly secondary salinity occurs both in irrigated and dry land agriculture. Secondary salinity is reflected as saline seeps in dry land agriculture and secondarily-salinized soils in irrigated areas. The global extent of primary salt-affected soils is 955 Mha (Szablocs, 1989) and of secondary salt-affected soils is 76.6 Mha (Oldman *et al* 1991.b).

Salt-affected soils are classified into two broad groups: saline and/or sodic soils. The dominant soluble salts in saline soils mostly comprise chlorides, sulphates and bicarbonates of sodium, calcium and magnesium. The physical properties of saline soils are normally good and they usually have no permeability problems.

Associated with soil salinization may be the process of sodication, whereby the clay fraction of the soil becomes saturated with exchangeable  $\text{Na}^+$ . The effect of  $\text{Na}^+$  is to disperse the fine clay particles and cause the desirable crumb structure to collapse. As a result, the soil tends to swell and its aggregates to slake down and clog the soil pores creating a less permeable condition that restricts water penetration and aeration (Hillel, 1990). Consequently air and water movement is impeded. Therefore, the adverse effect of exchangeable sodium on plant growth is mainly associated with changes in the physical properties of the soil.

Assessment and measurement of salinity and sodicity in land and water systems is of paramount importance for devising site-specific management practices and preventive measures for preserving and sustaining productivity in saline environments. Modern electromagnetic induction

equipment EM38 (Rhoades, 1992) help collect salinity information for appropriate farming operations by selecting crops commensurate with salinity level. The EM38 measures salinity by transmitting an electric current through the soil, the resulting electromagnetic field is measured by a sensor in the device. This paper briefly describes various destructive and non-destructive techniques for the measurement and estimation of concentration of salts in soil and water systems.

## **2. TECHNIQUES FOR ASSESSMENT /MEASUREMENT OF SALT-AFFECTED SOILS**

The effect of salts on plant growth depends on their concentration in the soil solution and the extent of adsorption on the soil exchange complex. There are several techniques, destructive and non-destructive, which could be employed for the assessment/measurement of salts in soil and/or water systems. Soil conductivity can be measured rapidly using sensitivity or electromagnetic techniques (Rhoades 1979, Rhoades and Corwin, 1981), these methods may reduce number of measurements required. Choosing particular method may depend upon the precision required, effort and expenditure involved and time available to complete the task. A brief mention of merits and scope of each method is given below.

### **2.1 Destructive methods**

#### **2.1.1 Measurement of soil salinity**

Where a high level of precision is required in measurement of total salts in soil and water system, it is always advisable to collect the sample from the field in the clean containers. Two methods can be employed.

##### **2.1.1.1 Gravimetric method**

Total salts in a soil sample can be measured by dissolving them in water and evaporating the water by heat and estimating them by their weight. (This applies to water samples also). However this method is outmoded now.

##### **2.1.1.2 Electrical conductivity of saturated soil paste**

Total salt concentration in water can be estimated approximately by the measurement of the electrical conductivity (Shahid, 2008). The standard procedure to assess soil salinity (and solution chemistry also) is the analysis of soil saturation extract collected from a saturated soil paste under vacuum.

The measurement of electrical conductivity in the saturation extract obtained from the saturated soil paste is a routine very widely used standard procedure in assessing soil salinity. It is important that the saturated soil paste must match the required standards that: 1) the surface of the paste glistens in the light; 2) if a ditch is made in the paste, no water fills in it and; 3) on tilting the spatula it must slipped down. When the paste meets the criteria, the soil saturation extract is then obtained under the vacuum, and the EC measured on an electrical conductivity meter in deci Siemen per meter (dS/m).

The EC of solution extracted from a saturated soil paste (which has water content about double than at field capacity) has been correlated with the response of various crops. This measure, known as electrical conductivity of the soil saturation extract (EC<sub>e</sub>), is now the generally accepted measure of soil salinity. It should be noted that EC measurement on extracts or suspensions of fixed soil: water ratio (commonly 1:1, 1:2.5 or 1:5) do not give a reliable correlation. Such extracts or wider ratio are more convenient where the soil sample is limited. This is because the amount of water held at a given tension varies from soil to soil, depending on texture, the type of clay mineral and other factors. Various steps involved in this method are:

#### **2.1.1.2.1 Saturated soil paste preparation**

- Place about 300 grams of sieved < 2mm air-dried soil into plastic beaker of about 500 ml capacities.
- Add deionized water gradually until all the soil is moist and then mix with a spatula until a smooth paste is obtained, adding more water or soil as necessary. The paste should glisten and just flow when the container is tilted and have no free water on the surface but be in a condition whereby it slides cleanly off the spatula.
- Keep the saturated soil paste overnight with lid.
- Check the following morning by first remixing the paste and adding water or soil as is needed to bring the paste to the saturation point, as described above.

#### **2.1.1.2.2 Collection of soil saturation extract for salinity assessment**

- Put a round Whatman No.42 filter paper in the Buchner funnel attached to the filtration rack and vacuum suction. Then moisten the filter paper with deionized water and make sure that it is tightly attached to the bottom of the funnel and that all holes of the Buchner funnel are covered.
- Start the vacuum pump. Open the suction, and add saturated soil paste to Buchner funnel.
- Continue filtration until the paste on the Buchner funnel starts cracking.
- If the filtrate is not clear, the procedure must be repeated. Transfer the clear filtrate into a 50-ml bottle.
- Switch on the conductivity meter and immerse the electrode in the saturation extract and record the reading.
- Remove the conductivity cell from the filtrate, rinse thoroughly with deionized water, and carefully dry excess water with a tissue.
- If accurate comparisons of EC<sub>e</sub> are to be made, measure the temperature of the extract and apply a correction factor to 25°C. Some instrument automatically present reading at 25°C. The correction factor is given in table below.

**Table** Temperature correction factor

Temp °C	Factor
22	1.06
23	1.04
24	1.02
25	1.00
26	0.98
27	0.96
28	0.94
29	0.93
30	0.91
31	0.89
32	0.87
33	0.86
34	0.84
35	0.83

(The procedure to measure electrical conductivity in soil extract and a water sample is the same)

#### **2.1.1.2.3 Notes to be remembered**

The EC readings are recorded in milli-mhos per centimeter (mmhos/cm), deci-Siemens per meter (dS/m) or milli Siemens per cm (mS/cm).

The use of the dS/m or mS/cm is preferred over the unit mmhos/cm. All units are equal, that is, 1 dS/m = 1mmho/cm = 1 mS/cm.

- Readings are usually taken and reported at a standard temperature of 25°C.
- Check accuracy of the EC meter using a 0.01 NKCl solution, which should give a reading of 1.413 dS/m at 25°C.

#### **2.1.1.2.4 Electrical conductivity and soil salinity:**

Salinity is an important laboratory measurement since it reflects the extent to which the soil is suitable for growing crops. Electrical conductivity is an indirect measurement of salinity of soil solution or soil saturation extract. Soil is usually considered saline when the electrical conductivity of an extract from the saturated soil ( $EC_e$ ) exceeds 4 dS/m. This value is generally used worldwide, although the Soil Science Society of America has recommended that this limit be reduced to 2 dS/m because many crops can be damaged in the range of 2-4 dS/m (Abrol et al., 1988). On the basis of a saturation extract, values of 0 to 2 dS/m are safe for all crops: yields of very sensitive crops are affected between 2 to 4 dS/m; many crops are affected between 4 and 8 dS/m; while only tolerant crops grow well above that level. The ESP value of these soils is less than 15, which is equivalent to an SAR value of 13. Saline soils have a pH value of 8.5 or less.

#### **2.1.2 Measurement of soil sodicity**

In the laboratory soil sodicity can be measured more accurately by determining the proportion of exchangeable sodium (ES) present in the cation-exchange-capacity (CEC).

$$ES (\%) = (ES/CEC) \times 100$$

where ES and CEC (exchangeable  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) are in meq/100g soil.

This provides an ESP (exchangeable sodium percentage) value, which determines the sodicity of the soil. If the ESP is more than 15 the soil is sodic (Richards, 1954). Sodic soils have the tendency to swell on wetting. The ESP is also calculated through a standard formula using the Sodium Adsorption Ratio values (SAR) obtained from the ionic ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) concentrations in the soil saturation extract. The SAR can be calculated by the following formula:

$$\text{SAR} = \frac{\text{Na}^+}{[(\text{Ca}^{2+} + \text{Mg}^{2+})/2]^{1/2}} \quad (\text{mmoles/l})^{1/2}$$

Where the concentration of  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  are in meq/l.

SAR is good measure of sodicity. From the value of SAR, ESP can be easily calculated if needed. Sodic soils have an electrical conductivity of their saturated soil extract of less than 4 dS/m, ESP of 15 or more, SAR of 13 or more and pH value of 8.5 to 10.

## **2.2 Non-destructive methods of estimating salinity**

Soil salinization is a dynamic process due to the influence of a wide range of parameters including soil permeability, climatic and hydro geological conditions and management practices. Soil salinity information is quickly out of date as management, water table depth and climatic conditions change. Monitoring of soil salinity and preparation of soil salinity maps are essential tools for the management of salt-affected lands. However, the expenditure of time and effort to characterize the salinity conditions of a large area with conventional sampling and laboratory analysis procedures becomes prohibitive. It is thus obvious that a practical means of measuring soil salinity directly in the field is advantageous, if not essential, to obtain timely information required for making appropriate management decisions (Rhoades, 1990c).

### **2.2.1 Feeling salinity in the field**

The presence of salinity in the field can be felt by these hints:

- presence of white salt efflorescence (crust)
- reduced plant vigor or stunting in crops
- salt stains visible on dry soil surface
- small to large bare areas can be seen
- affected area may be worsen after high seasonal rainfall
- some species show marked changes in leaf color and shape due to salt stress
- only salt-tolerant species are present
- trees are dead or drying
- high water table, usually less than 2 meter
- collapse of green belts, buildings and roads due to corrosion effects of salinity

### **2.2.2 Feeling sodicity in the field**

The qualitative tests of soil sodicity can be made in the field (dry and simulated states)

#### **2.2.2.1 Dry state**

A small dry soil aggregate is placed in a Petri-dish containing distilled water. If the soil is sodic (i.e., dispersive), a cloud of clay-sized particles will usually form around the aggregates. A further

visual assessment of the amount of dispersion taking place is made at a 2 hour and a 24 interval in the laboratory.

#### **2.2.2.2 Simulated cultivated state**

Soil is remolded (worked between thumb and forefinger to assess dispersion) in a moist state or at field capacity and then placed in distilled water to assess dispersion. The remolded procedure simulates the effect of cultivation on a moist soil. If the dispersion is more in the simulated than dry state, care should be taken when cultivating in a moist state.

### **2.3 Geophysical methods for soil salinity mapping**

Technological advances in the last two decades have revolutionized soil salinity assessment, however, in the field a number of instruments have been developed and applied for the purpose of providing reasonable estimates of salinity *in situ* (Corwin and Rhoades, 1982) as well as describing the spatial distribution of soil salinity in the field (Cameron et al., 1981). These methods have been developed on the basis of the geophysical techniques used by geophysicists and geologists for mineral exploration and hydro geological investigations (see Milson, 1989). They estimate the soil salinity via the measurement of bulk electrical conductivity of the soil, which depends on the salinity of soil solution, porosity and the type and amount of clay in the soil. Global positioning systems have become available for accurate and rapid mapping. These techniques, linked with salinity monitoring in a geographic information system, can be used for salinity mapping purposes. The following sections briefly describe ground and airborne geophysical methods that allow rapid field-wide measuring of soil salinity.

#### **2.3.1 Ground resistivity method**

A number of devices are available for soil salinity measurements using the resistivity method (Rhoades, 1990c).

##### **2.3.1.1 Four electrode sensor**

Four electrode sensors have an array of four electrodes, a few centimeters apart. The four electrodes may be equally spaced or have a variable spacing. The two external electrodes are connected to the source of electric current and the voltage difference is measured between the two internal electrodes. Current electrodes are made of stainless steel, copper, brass or any other corrosion-resistant metal. Potential electrodes are made of stainless steel. Electrodes have a diameter of 1 to 1.25 cm and a length of 45 cm. The probe provides the measurement of soil conductivity at the measuring point at a depth which depends on the distance between the electrodes. For survey or traverse work the array electrodes may be mounted in a hoard with a handle so that the soil conductivity measurements can be made quickly.

##### **2.3.1.2 Electromagnetic method**

Instruments for assessing bulk soil conductivity and soil salinity and its distribution in the soil profile by means of electric current and electromagnetic induction (EM) have advanced since 1971 (Rhoades, 1990). The modern salinity measurement equipment EM38 is designed to be particularly useful for agricultural salinity surveys and help collect salinity information for appropriate farming operations by selecting crops commensurate with salinity level. The EM38 measures salinity by

transmitting an electric current through the soil, the resulting electromagnetic field is measured by a sensor in the device. The EM38 is very lightweight and only one meter long; the EM38 provides rapid surveys with excellent lateral resolution. Measurement is normally made by placing this instrument on the ground and recording the meter reading. It covers large area quickly without ground electrodes. The EM38 provides depth of exploration of 1.5 meters and 0.75 meters in the vertical and horizontal dipole modes respectively. It sounds complicated, but EC mapping is one of the simplest, least expensive salinity measurement tools. A resulting computer generating salinity maps can add value to farms by helping farmers interpret yield variation.

This technique offers the outstanding advantage of permitting measurement of ground conductivity without ground contact, that is, without the use of any probes. All problems of attempting to obtain good electrical connection with the ground are avoided and, since there are no or fewer cables to manipulate, the speed at which a conductivity survey can be carried out is now substantially increased. Furthermore, since these instruments automatically average the measurement over a lateral area which is approximately equal to the depth of exploration, they give an accurate value for the bulk conductivity of the soil and are able to detect very small variations in this quantity. For these reasons, electromagnetic ground conductivity meters are replacing conventional resistivity techniques for many survey applications. Multifrequency EM surveys can also provide a rapid means for delineating the major recharge and discharge areas (Williams and Arunin ,1990). Although the initial expense is higher, the savings resulting from faster, more accurate surveys usually quickly offset this factor (McNeill, 1986).

### **2.3.2 Remote sensing**

In its broadest sense, remote sensing is the measurement or acquisition of information of some property of an object or phenomenon by a recording device that is not at physical or intimate contact with the object or phenomenon under study (Johannsen and Sanders, 1982). Remote sensing has a wide range of applications in investigation and management of natural resources including water, land and forest resources. Robbins and Wiegand (1990) describe the following remote sensing methods for salt-affected soils.

#### **2.3.2.1 Airborne electromagnetic method**

The airborne electromagnetic method can be used for soil salinity investigations (Street, 1992 and Street and Duncan, 1992).The aircraft normally operates at an altitude of about 120 m. Measurements made of the electromagnetic fields at rapid sampling rates are subject to a large signature from the aircraft, which is itself a good conductor. A stable technique for the separation of aircraft and ground response has been designed and implemented for work in highly saline areas. Electromagnetic interference caused by atmospheric discharge has also been reduced. Of the different types of film used for remote sensing, color infrared film has produced the most useful data on how the salinity-induced plant stress varies. With this film, dark-green vegetation produces a bright red image; light-green foliage produces a pink image; barren saline soil produces a white image; non-saline soil a grey, bluish-grey or greenish-grey image; salt-stressed crops a reddish-brown to reddish-black image; clear water a very dark-blue to black image, and sediment-laden water a blue image. Thus, plant responses to salinity, soil and water can be readily identified. Modern image analysis systems can digitize infrared photographs, cluster the scene into classes of salinity severity, and produce

fractional area estimates that fall into each class. The response of crops to salinity severity usually varies from season to season and from crop to crop and depends mainly on patterns of precipitation and crop tolerance to salinity. Infrared photography is also very useful for recording year-to-year and crop-to-crop responses to salinity and changes in management practices.

#### **2.3.2.2 Videography**

Video cameras, because of their recording ease, immediate playback, real time display and declining prices, are replacing traditional photographic cameras. The video signals are digitized and an image-processing system has been used to distinguish between saline and non-saline areas and to estimate the area of land affected by salinity. Although resolution is lower than with photographic cameras, videography adequately surveys and documents salinity patterns.

#### **2.3.2.3 Infrared thermometry**

Temperatures of the crop canopy can be measured by infrared thermometers and correlated with the crop water stress index to monitor the water stress caused by soil salinity. Infrared thermometry can also be used to monitor saline seeps, since the temperatures of wet and dry soil differ. Infrared thermometers can be hand-held or flown across fields in transects.

#### **2.3.2.4 Satellite imagery**

Remotely sensed data can be used to identify soil salinity at the broadest level on a large area at various scales which could be small 1:1,000,000 or large 1:5,000 etc.. This can be further supplemented with ground truthing and map unit boundaries may be drawn. The sites of observation may be accurately located with global positioning system (GPS).

Efforts are also being made to map sodic soils with remote sensing. However, the technique for sodicity mapping is fraught with difficulties. In addition to increase in reflectance with salt content, high-salt content may mask detection of other ions. One more important factor about saline soils is the fact that in modern agriculture, farmers are adding gypsum to sodic soils for soil reclamation (Singh, 1994). The artificial increase of the gypsum content in such soils may alter the soil reflectance spectra significantly and hence, require attention. Moreover, Metternicht, and. Zinck (1996) Showed that by using six reflective combined Landsat bands, it is possible to discriminate saline and sodic soils with varying confident limits.

## **REFERENCES**

- Abrol, I.P., Yadav, J.S.P. and Massoud, F.I. 1988. Salt-affected Soils and Their Management. Rome: FAO (Soils Bulletin 39). 131 pp.
- Cameron, D. R., E. De Jong, D. W. L. Read and M. Oosterveld. 1981. Mapping salinity using resistivity and electromagnetic inductive techniques. Canadian Journal of Soil Science, 61:67-78.
- Corwin, D. L. and J. D. Rhoades. 1982. An improved technique for determining soil electrical conductivity-depth relations from above ground electromagnetic measurements. Soil Science Society of America Journal, 46:517-520.



- Hillel, D. 1990. Ecological aspects of land drainage for salinity control in arid and semiarid regions. In: Symposium on Land Drainage for Salinity Control in Arid and Semi-Arid Regions. February 25th to March 2nd 1990, Cairo, Egypt. Delta Barrage, Cairo: Drainage Research Institute. v.1 (Keynote): 125-135.
- Johannsen, C.J. and Sanders, J.L. eds. 1982. Remote Sensing for Resource Management. Ankeny, Iowa: Soil Conservation Society of America. 665 pp.
- McNeill, J.D. 1986. Rapid, Accurate Mapping of Soil Salinity Using Electromagnetic Ground Conductivity Meters. Mississauga, Ontario: Geonics Limited. (Technical Note TN-18). 15 pp.
- Milson, J. 1989. Field Geophysics. Milton Keynes, England: Open University Press. 182 pp.
- Oldeman, L.R., van Engelen, V.W.P. and Pulles, J.H.M. 1991b. The extent of human-induced soil degradation. In: Oldeman, L.R., Hakkeling, R.T.A. and Sombroek, W.G. World Map of the Status of Human-Induced Soil Degradation: An Explanatory Note. Wageningen: International Soil Reference and Information Centre (ISRIC). 27-33.
- Parasnis, D. S. 1986. Principles of applied geophysics. Fourth Edition. New York: Chapman and Hill. 402pp.
- Rhoades, J. D. 1979. Monitoring soil salinity: A review of methods.
- In: Everett LG, Schmidt KD (eds) Establishment of water quality monitoring program. Am. Water Resour Assoc, St Anthony Falls Hydraul Lab, Minneapolis, pp. 150-165.
- Rhoades, J.D. 1990. Measuring and monitoring soil salinity. In: Kandiah, A. ed. Water, Soil and Crop Management Relating to the Use of Saline Water. Rome: Food and Agriculture Organization of the United Nations. 71-88.
- Rhoades, J.D. 1992. Recent advances in the methodology for measuring and mapping soil salinity. Proc. Intl. Symp. On Strategies for Utilizing Salt-Affected lands, Bangkok, Thailand, Feb. 17-25, pp. 39-58.
- Rhoades, J. D. and D. L. Corwin. 1981. Determining soil electrical conductivity-depth relations using an inductive electromagnetic soil conductivity meter.
- Soil Science Society of America Journal, 45:255-260.
- Richards, L.A. (ed). 1954. Diagnosis and improvement of saline and alkali soils. U. S. Dept. Agric. Handb. 60, U. S. Gov. Printing Office, Washington DC.
- Robbins, C.W. and Wiegand, C.L. 1990. Field and laboratory measurements. In: Tanji, KK ed. Agricultural Salinity Assessment and Management. New York: American Society of Civil Engineers. 201-219.
- Shahid, A.S. 2006. Salinization in Irrigated Lands and Reclamation. International Center for Biosaline Agriculture, Dubai UAE. 1-24pp
- Singh, A.N (1994) Monitoring Changes in the extent of salt-affected soils in northern India. International J. of Remote Sensing 16: 3173-3182
- Street, G. 1992. Airborne geophysics - a tool to identify strategic areas for revegetation. In: Catchments of Green: A National Conference on Vegetation and Water Management. Adelaide, 23-26 March 1992. Canberra: Greening Australia Ltd. Conference J Proceedings. v.B. 43-53.
- Street, G. and Duncan, A.C. 1992. The application of airborne geophysical surveys for land management. In: Proceedings, 7th International Soil Conservation Organisation. Sydney, 27-30 September 1992. v.2. 762-770.
- Szabolcs, I. 1989. Salt-Affected Soils. Boca Raton, Florida: CRC Press. 274 pp.
- Williams and Arunin, 1990. Inferring recharge/discharge area from multifrequency electromagnetic induction measurements. Canberra: CSIRO Division of Water Resources (Technical Memorandum 90/11). 17pp.
- Metternicht, G.L, and J. A. Zinck (1996). Modeling salinity-alkalinity classes for mapping salt-affected top soils in the semiarid valleys of Cochabamba (Bolivia) ITCJ. 44, 1282-1285

# CHAPTER 9

## A PROTOCOL FOR MEASUREMENT OF SOIL SALINITY

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### 1. INTRODUCTION

The measurement of soil salt content is very important for plant salt tolerance study. The most common and simple method used to measure soil salt content is the field test. The characteristics of saline soil areas include microtopography, complicated soil types and significant differences in local soil conditions. In order to reduce test error caused by differences in local soil conditions, the number of repeats and the density of the sample in the field should be increased accordingly. The soil samples should be collected from different soil layers to different depths based on the plant species. For deep rooted plants, sample soil layers from 0 - 5 cm, 5 - 10 cm, 10 - 20 cm, 20 - 40 cm, 40 - 60 cm and so on to at least 1 m deep. The samples from different layers should be mixed uniformly. For plants with shallow roots, sample soil layers to 60 cm deep. The salt content in saline soil has great instability over time and inhomogeneity from location to location. It changes with the year and the month, and even with the morning, afternoon and evening during one day. Considering seasonal climate change, the sampling times should include spring when salt accumulates, summer when salt leaches and fall when salt accumulates again, so soil salt in different seasons and under different conditions can be analyzed.

Saline soil has excessive water-soluble salts. The measurement of the content of water-soluble salts in soil has two main steps. First prepare the sample solution according to a certain water/soil ratio. Then analyze the soil salt concentration and ionic components in the soil. In general studies on dynamic changes of water and salt contents in the soil, the water/soil ratio of 5:1 is usually used, whereas the water/soil ratio of 1:1 is suitable for the analysis of alkaline soil. The method of saturated soil extract is rarely used because the execution of this method is tedious and it is difficult to determine the right saturation point. The sample solution in the following tests refers to 5:1 water/soil extract if it is not noted.

### 2. PREPARATION OF SAMPLE SOLUTION OF WATER-SOLUBLE SALTS IN SOIL

#### 2.1 Instruments and reagents

Instruments: reciprocating shaker, 1/100 balance, Buchner funnel, vacuum pump, centrifuge (4000r/min), gas extraction bottle.

Reagent: 0.1%  $\text{NaPO}_3$ .

#### 2.2 Methods

##### 2.2.1 Preparation of 5:1 water/soil extract

Weigh 100 g air-dried soil sample that passes through a 1 mm sieve. Put the sample soil in an Erlenmeyer flask. Add 500 ml CO<sub>2</sub> free distilled water based on a water/soil ratio of 5:1. Seal the flask mouth with a rubber stopper and put the Erlenmeyer flask in a reciprocating shaker to shake for 3 min. Right after shaking, perform the air pump filtration with a Buchner funnel. Collect the clear liquid in a 500 ml Erlenmeyer flask. Add 1 drop of 0.1% NaPO<sub>3</sub> for every 25 ml. Reserve for the test.

### **2.2.2 Preparation of 1:1 water/soil extract**

Weigh an air-dried soil sample that passes through a 1 mm sieve. Put the soil sample in an Erlenmeyer flask. Add CO<sub>2</sub> free distilled water based on a water/soil ratio of 1:1. The rest of the operations are the same as above.

## **3. POINTS FOR ATTENTION**

(1) When extracting with the 5:1 water/soil ratio, hygroscopic water of the air-dried soil can be neglected due to the high percentage of water. When extracting with the 1:1 water/soil ratio, hygroscopic water of the air-dried soil must be corrected to avoid test error.

(2) In the process of the extraction of the water-soluble salts in the soil, 3 min of shaking is enough for the water-soluble chlorides, carbonates and sulfates to dissolve in the water. With the extension of shaking time or standing time, the neutral salts and water-insoluble salts will also enter the extract causing greater error.

(3) Both the partial pressure of CO<sub>2</sub> in the air and the dissolved CO<sub>2</sub> in the distilled water will affect the solubility of some salts including CaCO<sub>3</sub>, CaSO<sub>4</sub> and MgSO<sub>4</sub>. As a result, the salt content in the extract will be affected. Therefore, CO<sub>2</sub> free distilled water must be used in the extraction.

(4) The standing time of the sample solution should not exceed 1 day.

(5) Adding a small amount of NaPO<sub>3</sub> solution in the soil extract can prevent the formation of CaCO<sub>3</sub> precipitate when standing. Although NaPO<sub>3</sub> will slightly increase the Na<sup>+</sup> concentration in the extract, the error caused by NaPO<sub>3</sub> is much smaller than the error caused by CaCO<sub>3</sub> precipitate.

## **REFERENCES (OMITTED)**

# CHAPTER 10

## A PROTOCOL FOR MEASUREMENT OF TOTAL WATER-SOLUBLE SALT IN SOIL

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The main methods of measuring the total water-soluble salt in the soil are weight method, conductivity method and salinometer method. The result of the weight method is reliable but the operation is tedious and time consuming. The conductivity method is simple. The salinometer method is even more simple and rapid and can be used in the field directly.

### 1. WEIGHT METHOD

Draw a certain amount of soil extract, evaporate to dryness in a water bath, and then dry at 105 -110 °C to a constant weight. This total dried residue contains both water-soluble salts and water-soluble organic matter. Use  $\text{H}_2\text{O}_2$  to remove the organic matter in the residue. What remains is the total water-soluble salt in the soil.

#### 1.1 Instruments and reagents

Instruments: evaporating dish, water bath, dryer, electrothermal drying oven, analytical balance.

Reagents: 15%  $\text{H}_2\text{O}_2$ , 2%  $\text{Na}_2\text{CO}_3$ .

#### 1.2 Method

Draw 50.0 ml of sample solution and put it in an evaporating dish weighing  $w_0$ . Evaporate to dryness in a water bath, and then dry in an electrothermal drying oven at 105 -110 °C for 4 hours. Take out and put in a dryer for 30 min. Use an analytical balance to weigh. Put back in the electrothermal drying oven for 2 more hours, cool down. Repeat the steps till at a constant weight ( $w_1$ ), meaning the weight difference between two times is not more than 1 mg. Calculate the weight of total dried residue.

Add 15%  $\text{H}_2\text{O}_2$  in drops to wet the residue. Evaporate to dryness in the water bath. Treat repeatedly until the entire residue turns white. Dry the remained residue to constant weight ( $w_2$ ) according to the method described above. Calculate the content of total water-soluble salt in the soil.

### 1.3 Calculation

$$\text{Total dried residue} = \frac{w_1 - w_0}{w} \times 100\%$$

where  $w$ : the weight of the sample soil (g) that the drawn extract is equivalent to.

### 1.4 Points for attention

- ① The volume of soil extract to draw is determined by the soil salt content. When the soil salt content is higher than 0.5%, draw 25 ml; when the soil salt content is lower than 0.5%, draw 50 ml or 100 ml. Make sure the measured total salt content is 0.02 - 0.2 g.
- ② If the residue has high contents of  $\text{CaSO}_4 \cdot 2 \text{H}_2\text{O}$  and  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ , drying at 105 -110 °C can not completely remove the crystal water in these hydrates. As a result the constant weight is hard to get. In this case, the drying temperature should be increased to 180 °C. If there are high contents of  $\text{CaCl}_2 \cdot 6 \text{H}_2\text{O}$  and  $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$  in the saline soil, it is hard to get a satisfactory result even if raising the drying temperature to 180 °C because these compounds very easily absorb moisture and hydrolyze. In this case, first add 10 ml of 2%  $\text{Na}_2\text{CO}_3$ . When evaporating to dryness in the water bath  $\text{NaCl}$ ,  $\text{Na}_2\text{SO}_4$  and  $\text{MgCO}_3$  will generate. Then dry at 150 -180 °C for 2 hours, the weight will be constant. The amount of  $\text{Na}_2\text{CO}_3$  added should be deducted from the result of the total salt.
- ③ Because the salt easily absorbs water in the air, the conditions for cooling and weighing should be the same.
- ④ When using  $\text{H}_2\text{O}_2$  to remove the organic matter, the residue only needs to be wet. Too much  $\text{H}_2\text{O}_2$  will generate excessive foam when  $\text{H}_2\text{O}_2$  is decomposing organic matter, causing splashing and the loss of salt. Repeated treatment with a small amount of  $\text{H}_2\text{O}_2$  each time should be used.

## 2. CONDUCTIVITY METHOD

Water-soluble salts in the soil are strong electrolytes. So the soil solution has conductivity. The electrical conductivity reflects the conductive capacity of the soil solution. Within a certain range of concentration, the salt content in the soil solution is positively related to the electrical conductivity. So the electrical conductivity of the soil extract can reflect the soil salt content. But it can not reflect the components of the mixed salt. If the ratios of different salts in the soil solution are relatively constant, the salt concentration determined by the electrical conductivity is very accurate. The conductivity method is a rapid and accurate method to measure the soil salt content. The present tendency is to use the electrical conductivity to represent the total salt content in the soil directly. The units for the electrical conductivity are S/m or mS/cm.

### 2.1 Instruments

Conductivity meter, thermometer ranging 1 – 60 °C.

### 2.2 Method

Draw 20 – 30 ml of sample solution and put it in a small beaker. Adjust the conductivity meter according to the user's manual. Read the value of the electrical conductivity (mS) after the pointer is stable. Measure the temperature of the sample solution every 10 min.

## 2.3 Calculation

The electrical conductivity of the soil extract at 25°C (EC<sub>25</sub>) is used to reflect the soil salt content. It is calculated as follows:

$$EC_{25} = EC_t \times f_t$$

where EC<sub>25</sub>: electrical conductivity of the soil extract at 25°C, EC<sub>t</sub>: measured electrical conductivity of the soil extract at t °C, f<sub>t</sub>: the corrected value of electrical conductivity at t °C (see Table).

**Table** The corrected values of electrical conductivity rate under different temperatures

Temperature (°C)	Correct -ed value	Temperature (°C)	Correct -ed value	Temperature (°C)	Correct -ed value	Temperature (°C)	Correct -ed value
3.0	1.709	20.0	1.112	25.0	1.000	30.0	0.907
4.0	1.660	20.2	1.107	25.2	0.996	30.2	0.904
5.0	1.613	20.4	1.102	25.4	0.992	30.4	0.901
6.0	1.569	20.6	1.097	25.6	0.988	30.6	0.897
7.0	1.528	20.8	1.092	25.8	0.983	30.8	0.894
8.0	1.488	21.0	1.087	26.0	0.979	31.0	0.890
9.0	1.448	21.2	1.082	26.2	0.975	31.2	0.887
10.0	1.411	21.4	1.078	26.4	0.971	31.4	0.884
11.0	1.375	21.6	1.073	26.6	0.967	31.6	0.880
12.0	1.341	21.8	1.068	26.8	0.964	31.8	0.877
13.0	1.309	22.0	1.064	27.0	0.960	32.0	0.873
14.0	1.277	22.2	1.060	27.2	0.956	32.2	0.870
15.0	1.247	22.4	1.055	27.4	0.953	32.4	0.867
16.0	1.218	22.6	1.051	27.6	0.950	32.6	0.864
17.0	1.189	22.8	1.047	27.8	0.947	32.8	0.861
18.0	1.163	23.0	1.043	28.0	0.943	33.0	0.858
18.2	1.157	23.2	1.038	28.2	0.940	34.0	0.843
18.4	1.152	23.4	1.034	28.4	0.936	35.0	0.829
18.6	1.147	23.6	1.029	28.6	0.932	36.0	0.815
18.8	1.142	23.8	1.025	28.8	0.929	37.0	0.801
19.0	1.136	24.0	1.020	29.0	0.925	38.0	0.788
19.2	1.131	24.2	1.016	29.2	0.921	39.0	0.775
19.4	1.127	24.4	1.012	29.4	0.918	40.0	0.763
19.6	1.122	24.6	1.008	29.6	0.914	41.0	0.750
19.8	1.117	24.8	1.004	29.8	0.911		

In addition, when the temperature of the soil extract is 17 – 35 °C, the electrical conductivity of the soil extract increases or decreases about 2% for every 1 °C in the difference of the soil extract temperature and the standard temperature (25 °C). So the electrical conductivity of the soil extract at 25°C can also be calculated according to the following formula when the soil extract temperature is 17 – 35 °C.

$$EC_{25} = EC_t \times [1 - (t - 25) \times 2\%]$$

where:  $EC_{25}$ : electrical conductivity of the soil extract at 25°C,  $EC_t$ : measured electrical conductivity of the soil extract at t °C, t: the temperature of the soil extract (°C).

#### **2.4 Points for attention**

- ① It is best that the measuring time for each sample is relatively consistent after the electrodes are inserted into the solution.
- ② The solution used for conductivity measurement should be clear. Do not use suspension liquid. Otherwise the platinum back layer on the platinum electrode will be damaged, therefore causing test error.
- ③ High conductivity solution should be diluted before the measurement. Or else the sensitivity of the instrument will decrease and the electrode will be polarized.

#### **REFERENCES (OMITTED)**

# CHAPTER 11

## A PROTOCOL FOR SCREENING SALT TOLERANT WHEAT GENOTYPES USING CELL MEMBRANE STABILITY TECHNIQUE

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### 1. INTRODUCTION

Salinity of arable land is a global problem that has restricted productivity on 955million hectares (Mha). Nevertheless, the inherent plasticity in some of the crop plants identified through extensive screening enables them grow on such lands as reported for different wheat varieties, wild relatives of wheat, and other crops. These screening techniques include hydroponics, gravel culture, and natural or artificially saline fields. However, since major impact of salinity on sensitive plants is modification of cell membrane which can result in increased permeability and leakage of ions to be readily measured by the efflux of electrolytes. The method was developed and modified by Sullivan (1972) for assessing heat tolerance in sorghum and maize and established that lesser leakage is always associated with more stability of the cell membrane. Further studies provided information on how permeability of membrane increased and/or decreased in different crops such as wheat (Blum and Ebrecon, 1981; Sairam et al., 2002; Farooq and Azam , 2002a), Maize (Premachandra et al., 1989; 1991a; 1991b), *Populus deltoids* (Michael et al., 1994) and rice (Tripathy et al., 2000). In all these studies, cell membrane dysfunction has been reported as one of the parameters affected in sensitive plants when they were growing under different stresses and not as a technique for screening against stress tolerance. To do this, it is imperative to understand the cell membrane and its functions which are of two types. One is as barrier to keep all elements of the cell in and unwanted substances out of the cell and second is to be gate keeper allowing transport into the cell of essential substances and movement from the cells of waste products. If a cell is placed in a hypertonic solution of NaCl, it will increase the salt and decrease water concentration in outer environment of the cell thereby forcing the protoplast to shrink and the water molecules to come out of the membrane. Since membrane is semi permeable, it would allow only water molecules to pass through the membrane while larger molecules such as sugars, Na or K would not be able to cross the barrier. However, if the temperature is raised, it will disrupts ordered structure of the cell creating holes in it and the cell will automatically lose its contents. Similarly, if the cell freezes, the water inside will expand and disrupt the membrane. Upon rising the temperature, the frozen water inside the cell will melt and ruptured membrane will lose its contents. Under drought conditions also, the low water potential outside the cell will force the cell to plasmolyse. All these conditions thus, clearly described that under stress conditions water play significant role in disruption of cell membrane hence it is quite



possible that varieties which can tolerate water deficiency can have more stable membrane than the plants that tolerates salinity. To ascertain this, we used cell membrane stability (CMS) as criteria for screening wheat varieties known for its tolerance to i) salinity, ii) salinity and water deficiency and iii) water deficiency/drought (Table 1). The objective was to see how this technique is different from hydroponics, gravel culture and/or field salinity screening techniques. And whether CMS can provide extra information that has not been obtained during screening through other procedures?

**Table 1** Material used to test CMS technique

#*	Variety/ Line	Pedigree	Description	Status	Reference
V1	LU-26	Khushal / Blue Silver	Medium tall, early maturing, rust susceptible	Salt tolerant variety, high $K^+$ - $Na^+$ ratio	Qureshi et al., 1985.
V2	WL-1073	LU-26/ <i>Ae.cylindrica</i> D// LU-26	Medium tall, early maturing,	Salt tolerant line with strong ability of ion discrimination/selectivity	Farooq et al., 1992.
V3	WL-1076	LU-26/ <i>Ae. cylindrica</i> D// Pak-81	Normal height and maturity	Salt and water deficiency tolerant line	Farooq and Azam, 2002b.
V4	WL-41	LU-26/ <i>Ae. cylindrica</i> D// Pak-81	High tillering, high yielding	Water deficiency tolerant	Farooq et al., 1992.
V5	Inqbal-91	A selection from Int. CIMMYT nurseries	Medium tall, high yielding	Salt sensitive cultivar	Anonymous 1992.

\*Wheat material is mentioned according to decreasing order of salinity tolerance (recorded with respect to reduction in grain yield) i.e. V1 most salt tolerant and V5 least salt tolerant (or salt sensitive)

## 2. THE PROTOCOL

### 2.1. Genotypes to be tested

Any wheat genotypes can be tested but it is important that the test material should be of different genetic background.

### 2.2 Things to be required

- Uniform sized seeds of the wheat not less than 150 or about 4-5 grams,
- Commercial sodium chloride (NaCl),
- Hoagland nutrient solution,
- Six volumetric flasks (1000 ml),
- Test tubes of 1 ½ inch diameter
- Filter papers (commercial),
- Petri plates (5 of 8 inch diameter )
- Forceps

- A pair of scissors,
- Controlled temperature incubator,
- Electrical conductivity measuring (EC) meter
- Flame photometer
- A Net house with natural sunlight,
- Oven,
- Refrigerator
- Balance
- Incubator, and
- Plastic pots (12 & 15 inches diameter, 75 each)

### **2.3 Preparation of saline solution**

- Weigh about 5.8 gram of NaCl and dissolved it in one liter of Hoagland nutrient solution Hoagland and Arnon, (1950) to make 100 mM of saline solution,
- Check the electrical conductivity (EC) with EC meter: the meter should read approximately  $10\text{dS m}^{-1}$ ,
- Weigh 8.7, 11.6 and 14.6 grams of NaCl and dissolved each of the lots it in one liter of Hoagland nutrient solution to make 150, 200 and 250 mM of saline solutions, respectively
- Check EC of every solution. It should be approximately 15, 20 and 25  $\text{dS m}^{-1}$ .

### **2.4 Germination of seeds**

- Take 5 grams of uniform seeds (100 grain weight ranging between 3-3.5 grams) of all the varieties/lines to be tested,
- Cut the filter paper to the size of bottom of the Petri plate,
- Take about 10 ml of Hoagland nutrient solution and pour it on to clean and dry Petri plate,
- Hold the filter paper with the forceps and insert it in the solution lying on one end of slightly tilted Petri plate. Try to place filter paper on the bottom of the plate as soon as it starts absorbing water. Care should be taken that no air bubble entered beneath the soaked filter paper. This Petri plate with its two other replication will act as non-treated control,
- Similarly, made another four sets of Petri plates lined with filter papers soaked using all the four (100, 150, 200 and 250 mM) saline solutions. These five Petri-plates are enough only for one genotypes, and one replication. For three replications, (minimum requirement) 15 Petri-plates are required. If the genotypes to be tested are more than one, the Petri-plates should be increased accordingly to accommodate each genotype and three replications for each genotype,
- After preparation of plates, take 30 seeds out of the each seed lot (5 lots in our experiments),
- Dust them with any fungicide (We used Vitavax) and placed them on the soaked filter paper at appropriate distance from each other with germination point facing downward,
- Repeat this step with each genotypes and each saline solution.

- Cover the Petri plates and put them in incubator pre-set at 25/15°C day/night temperature and 60-70% relative humidity,
- Record germination data from the day when radical and plumule first appear.

#### 2.4.1 Recording data on seed germination

Genotype	Seed germination under un-treated control						Total germinated	% germinated
	Day-1	Day-2	Day-3	Day-4	Day-5	Day-6		
V1								
V2								
V3								
V4								
V5								

Data would be recorded the same way for germination under various saline treatments

#### 2.4.2 Analyzing data on seed germination

Genotypes	Mean seed germination (%) + SD under NaCl concentrations of:				
	0(Control)	100mM	250mM	200mM	250mM
V1					
V2					
V3					
V4					
V5					
One way Analysis of Variance	F= df= P<0.05				

ANOVA would be followed by Duncan's Multiple Range Test (DMRT)

#### 2.4.3 Presenting the seed germination data

The seed germination data can be presented either in a tabulated form. The values shown in the table would be marked by ANOVA to describe their significance. Data can also be presented in the form of bar charts with standard deviation plotted on the bars. When we conducted this experiment on wheat we presented the data as bar charts.

*It is worth mentioning here that since CMS is based on water depletion, screening for salt tolerance through this technique requires pre-selection of salt concentration that can reduced water availability to a level capable of inducing water stress and subsequently the injury to the cell membrane. Seed germination under saline conditions is one of such criteria used for this purpose. Since wheat can germinate under considerably high level of salt hence a level that could*

*reduce > 20% of seed germination was used. For example a salt sensitive cultivar V5 exhibited 50% reduction in seed germination and 39.5 % reduction in RWC when grown under 250mM NaCl. RWC up to 70% (a level below which plant cannot survive even if it is re-watered) usually appeared in plants when subjected to sever drought conditions. That is how we selected salt concentrations amenable to induced water stress. Such standardization should be made for every genotype required to be tested through CMS.*

## **2.5 Transplanting the seedlings into pots**

- Take plastic post of approximately 12 inches diameter with one inch diameter hole at the bottom,
- Fill the pot with gravel, flush it with Hoagland nutrient solution (make three such pots for three replications)
- Transplant nine, one week old seedlings in one pot and again make three replications (total 27 seedlings are to be planted per treatment and genotype)
- Take another 15 inches diameter plastic post and place 6 inches diameter plastic ring with one inch height at the bottom of the pot,
- Insert the pot with plants inside this pot so that it is placed on the ring. This is done to allow the extra water pass out of the pot which will keep accumulating in the pot and disturb the required salinity level,
- Similarly, make another set of 12 pots (3 pots genotype<sup>-1</sup> for four genotypes: total 15 pots will thus be used for one genotypes and one treatment) and transplant all the seedling in pots @ nine plants pot<sup>-1</sup> and three pots genotypes<sup>-1</sup> and insert them into the bigger pot. (This set is prepared for non-treated control),
- In the same way, make 4 more sets of 15 pots: each flushed with 100, 150, 200 and 250 mM saline solution for all the remaining genotypes and transplant the seedlings @ nine seedlings pot<sup>-1</sup> and three pots genotypes<sup>-1</sup>,
- Put all the 75 pots inside the net house. If the natural day/night temperature is other than 10-20/5-10°C, respectively, then make sure that the pots be placed in incubators preset on these temperatures with 55% relative humidity and approximately 10 hours of light not less than 35,000 lux,
- Irrigate the plants twice a day with their respective solutions (control plants with Hoagland nutrient solution and rest of the plants with various saline solutions),
- Flush the entire pot (as was done initially before transplanting the seedlings) twice a week with their respective solutions so that salt may not accumulate on the gravel pieces which may disturb the salinity level in particular pot,
- Let the plants grow there of which three plants replicate<sup>-1</sup>, genotype<sup>-1</sup> and treatment<sup>-1</sup> will be harvested at maturity for recording yield data and remaining six plants will be used for measuring Relative Water Contents (RWC), cellular injury and chemical analysis (two plant each).

## **2.6 Measuring Relative Water Contents**

- Take fresh leaves from two of the six plants of each replicate, treatment and genotype that are earmarked for analysis, clean the surfaces with moist tissue paper and weigh immediately to record fresh weight (FW)

- Dip half of the leaf portion in distilled water filled in a test tube and keep it there for 12 hours,
- Pulled the leaf out of water, wipe its surface immediately with blotting paper and weigh it again to get the fully turgid weight (TW),
- Put the leaves immediately in glass Petri-plates and place them for 24 hours in oven pre-heated to 70°C,

Genotype	Replicate	Parameters to be measured:			
		FW	TW	DW	RWC
V1	R1				
	R2				
	R3				
	Mean+SD				

- After drying, weigh the leaves again to record dry weight (DW).
- The RWC will be calculated as  $RWC = \frac{FW - DW}{TW - DW} \times 100$

### 2.6.1 Recording data for RWC

SD= Standard Deviation

### 2.6.2 Analyzing data on RWC

Genotypes	Mean RWC recorded for:				
	Control	100mM	150mM	200mM	250mM
V1					
V2					
V3					
V4					
V5					
One way analysis of Variance	F= df= P<0.05				

ANOVA would be followed by Duncan's Multiple Range Test (DMRT)

### 2.6.3 Presenting the data on RWC

The data on RWC can be presented either in line graphs or as bar charts with standard deviation plotted on the bars to show significance. When we conducted the experiment, we presented the data as bars charts.

*Measuring RWC is essential for CMS technique because it will describe the water status of plants at the time of analysis. Screening for salt tolerance through CMS will not be meaningful until and unless the plants show symptoms of being grown under water stress.*

### 2.7 Measuring cell membrane stability

- Take fully expanded (preferably 5<sup>th</sup> leaf) leaves from the remaining two plants earmarked for analysis in each genotype, replicate and treatment.

- Remove dirt and dust from the surface of the leaves with moist tissue paper and cut them into small pieces not smaller than one cm<sup>2</sup>.
- Take four glass test tubes marked as Tu-1, Tu-2, Tu-3 and Tu-4 each with 1 ½ inches diameter.
- Divide the leaf discs into four lots and put them into four tubes containing 20-30 ml of distilled water enough to submerged the discs completely in water.
- Keep the tubes at 10°C for 24 hours.
- Warm the tubes at 25°C and measure its electrical conductivity (EC). This measurement would be called C1.
- Put all the four tubes in autoclaving bucket and autoclave them along with water and leaf discs for 15 minutes and measure the electrical conductivity again. This measurement would be called C2. Both C1 and C2 would be from the leaves taken from plants growing under Hoagland nutrient solution.
- Similarly, repeat all the seven steps mentioned above for leaves taken from various saline treatments. In this case electrical conductivity measured before and after autoclaving would be termed as T1 and T2 (instead of C1 and C2),
- The cell membrane stability would be calculated as  $[1-(T1/T2)] / [1-(C1/C2)] \times 100$ . Here T and C refer to as treatment and control, respectively and 1 & 2 refer to as conductivity 1 & 2.
- If cellular injury is to be calculated the formula would be as follow:

Cellular injury=  $[1-(1-T1/T2)/(1-C1/C2)] \times 100$ . However 100-CMS would give % cellular injury anyway

### 2.7.1 Recording data on CMS

V1	Replicate-1				Replicate-2				Replicate-3				Mean of means +SD
	Tu-1	Tu-2	Tu-3	Tu-4	Tu-1	Tu-2	Tu-3	Tu-4	Tu-1	Tu-2	Tu-3	Tu-4	
C1													
Mean													
C2													
Mean													
T1													
Mean													
T2													
Mean													

Tu=Tube; C=Control; T=100mM.

Data should be recorded similarly for C+150mM, C+200mM and C+250mM

### 2.7.2 Analyzing the data on CMS

Geno- types	Mean values															
	100mM				150mM				200mM				250mM			
	C <sub>1</sub>	C <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>	C <sub>1</sub>	C <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>	C <sub>1</sub>	C <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>	C <sub>1</sub>	C <sub>2</sub>	T <sub>1</sub>	T <sub>2</sub>
V <sub>1</sub>																
CMS																
V <sub>2</sub>																
CMS																
V <sub>3</sub>																
CMS																
V <sub>4</sub>																
CMS																
V <sub>5</sub>																
CMS																

CMS= Cell Membrane Stability; V<sub>1</sub>-V<sub>5</sub>= various wheat genotypes

### 2.7.3 Presenting the data on CMS

CMS data can be presented as CMS or as cellular injury both as line graph or bar charts with standard deviation plotted above the bars. We however, presented our data as cellular injury which is inversely related with CMS.

## 3. EFFICIENCY AND APPLICATION OF CMS TECHNIQUE FOR SCREENING AGAINST SALINITY

Screening for salt tolerance through hydroponics, gravel culture, and natural or artificially saline fields have provided clear indications on sensitivity or resistance of plants based on simple observation of reduced germination, survival, and yield or otherwise in the test material. The reason(s) why these parameter are affected by salinity were mostly physiological and based on ion selectivity, ion exclusion, ion accumulation, ion discrimination, compatible solute production, osmo-regulation and membrane stability index. Plants that accumulate toxic ions like Na<sup>+</sup> and Cl<sup>-</sup> into cytoplasm were termed as salt sensitive and those that limit uptake of such ions were termed as salt tolerant. However, none of these three techniques, and the mechanisms of salt tolerance studied therein explains, why some of the tolerant plants limit uptake, discriminate, or try to exclude toxic ions reaching its cytoplasm? Whether all these tolerance mechanism work separately or cumulatively in one genotype? Answer to these questions is necessary if we have to use these parameters for improvement and/or development of new crops for salinity tolerance especially through transferring gene(s) from other plants. For example, *T. aestivum* (hexaploid wheat) and *Aegilops tauschii*: a highly salt tolerant species (Farooq et al. 1989) and D genome donor species to *T. aestivum* discriminate between K<sup>+</sup> and Na<sup>+</sup> (ion discrimination) and maintain high K<sup>+</sup>/Na<sup>+</sup> ratio (Ion regulation) during ion uptake. None of the *Aegilops* species (or their derivatives) has so far been reported to possess ion selectivity enabling them simultaneously to ion

regulation and ion discrimination. Thus it is difficult to ascertain whether these two different salt tolerance mechanisms exist simultaneously in one genotype and if they interact with each other? Also as we know, salt tolerance of plants is not simply a tolerance against toxicity of  $\text{Na}^+$  but also an adaptation to its secondary effects like water deficiency/depletion. None of the techniques and/or mechanisms clearly established that “salt tolerance of V3 and V4 mentioned in table 2 is because of its tolerance to water deficiency. It is only when we used CMS technique to discriminate between salt and drought tolerance *Aegilops* species we learnt that “salinity and drought co-exist” (Farooq and Azam 2002a) and it is salinity induced drought that aggravates the effect of salts in certain plants. We then used the technique to screen the known salt tolerant genotypes to know exactly the exact difference between them. The results indicated that it is only the screening through CMS that one can find whether or not salt tolerance exhibited by a genotype of wheat is due to its ability to i) discriminate between toxic and nutrient ions as in V1, ii) restrict uptake of toxic ions as in V2, iii) take less water (and hence passive uptake of salt) because of its tolerance to water deficiency or iv) possess simultaneously two mechanisms that is ion discrimination ( $\text{K}^+$ - $\text{Na}^+$  discrimination) and restricted ion uptake due to less water requirement: a unique mechanism that was found operative in V3 and V4 and was investigated for the first time only through using CMS technique. For more detailed information on these lines and the CMS, please read the paper by Farooq and Azam appeared in Plant and Cell Physiology 163: 629-637, 2006.

## REFERENCES

- Anonymous. Annual wheat report, 1992, Ayub Agriculture Research Institute, Faisalabad, Pakistan.
- Blum, A., and Ebrecon, A., 1981, Cell membrane stability as measure of drought and heat tolerance in wheat. *Crop Sci.* 21: 43-47.
- Farooq, S., and Azam, F., 2002a, Production of low input and stress tolerance wheat germplasm through the use of biodiversity residing in the wild relatives, *Hereditas.* 135: 211-215.
- Farooq, S., and Azam, F., 2002b, Co-existence of salt and drought tolerance in *Triticeae*. *Hereditas.* 135: 205-210.
- Farooq, S., and Azam, F., 2006, Cell membrane stability technique for screening salt tolerant wheat genotypes, *J. Plant Physiol.* 163:629-637.
- Farooq, S., Iqbal, N., Asghar, M., and Shah, T.M., 1992, Intergeneric hybridization for wheat improvement-V1. Production of salt tolerant germplasm through crossing wheat with *Aegilops cylindrica* and its significance in practical agriculture, *J Genet Breed.* 46: 125-132.
- Farooq, S., Niazi, M.L.K., Iqbal, N., and Shah, T.M., 1989, Salt tolerance potential of wild resources of the tribe *triticeae*-11. Screening of species of the genus *Aegilops*, *Plant and Soil.* 119: 255-260.
- Hoagland, D. R., and Arnon, D.I., 1950, The water culture method for growing plants without soil. *Uni Calif Berkeley college Agric Exp Stn. Cric.* No. 347.
- Michael, G. G., Michael, R.K., and James, R. B., 1994, Organic solute accumulation and dehydration tolerance in three water stressed *Populus deltoids* clones, *Tree Physiol.* 14: 575-587.
- Premachandra, G. S., Saneoka, H., and Ogata, S., 1989, Nutrio-physiological evaluation of the polyethylene glycol test of cell membrane stability in maize, *Crop Sci.* 29: 1292-1297.
- Premachandra, G. S., Saneoka, H., Kanaya, M., and Ogata, S., 1991b, Cell membrane stability and leaf surface wax content as affected by increasing water deficit. *J. Exp. Bot.* 42: 176-171.
- Premachandra, G.S., Saneoka, H., and Ogata, S., 1991a, Cell membrane stability and leaf water relations as affected by potassium nutrition of water stressed maize, *J. Exp. Bot.* 42: 739-745.



Qureshi, R. H., 1985, Selection of crop varieties and plant species suitable for salt affected areas of Pakistan. In: Proc. Nat Workshop on Biosaline Research in Pakistan, Qureshi, R.H, ed., University of Agriculture, Faisalabad, pp. 28-42.

Sairam, R. K., Rao, K.V., and Srivastava, G.C., 2002, Differential response of wheat genotypes to longterm salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration, Plant Sci. 163: 1037-1046.

Sullivan, C. Y., 1972, Mechanism of heat and drought resistance in grain sorghum and methods of measurement. In: Sorghum in the seventies, Rao, N. G. P., and House, L. R, ed., Oxford & IBH publishing Co, New Delhi, pp. 247-264.

# CHAPTER 12

## A PROTOCOL FOR BREEDING SALT-TOLERANT WHEAT VIA IN VITRO MUTAGENESIS

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### 1. INTRODUCTION

An excess accumulation of salt in the soil can cause rhizosphere salinization. Soil salinization has become a worldwide problem restricting agricultural production. Normal growth of crops will usually be affected when soil salt content reaches 0.2-0.5%. More seriously, high soil salt content causes wilting and even death of crops, resulting in a severe reduction in crop yield (Dai *et al.*, 2003; Vinocur *et al.*, 2005). With the reduction day by day of available cultivated land, people are starting to pay attention to the utilization of saline-alkali land. Practical experience has demonstrated that the problem of utilizing saline-alkali land can be solved by breeding salt-tolerant varieties based on the difference and heredity of salt tolerance of plants and by making selections of new varieties that adapt to the saline-alkali soil. By breeding salt-tolerant varieties of crops we can make use of the saline-alkali land and increase crop yield on that land. This is of great significance to the sustainable development of agriculture.

Plant cells have totipotency. This means that from a single cell, a whole plant can be regenerated. In plant tissue culture somaclonal variations may occur after calli are induced. The somaclonal variations can provide a large number of usable resources for germplasm enhancement of crops. Mutation breeding is a modern breeding technology that has been increasingly used in the last 50 years. Its rapid development produced a great impact on in vitro selection of salt-tolerant mutants. Research has shown that the combination of in vitro culture and mutation induction can significantly enrich the types of mutations, increase mutation frequency and breeding efficiency (Li *et al.*, 1994). In vitro mutation technique combines in vitro culture and mutation technique to induce and select resistant mutants in the process of plant tissue and cell culture. In 1972 Melchers discussed the advantages of plant tissue culture in the selection of a salt-tolerant mutant for the first time (Melchers *et al.*, 1972). In 1974 Zenk obtained a salt-tolerant cell line using this technique (Zenk *et al.*, 1974). Thereafter in vitro mutation technique has been widely used and played a very important role in plant resistance breeding.

Wheat (*Triticum aestivum* L.) is a very important crop. It is a glycophyte and has poor tolerance to salt. Therefore, breeding salt-tolerant wheat is especially important for agricultural production. Since Shimada first induced calli from young embryos of wheat and then successfully obtained regenerated plants in 1969, there have been more and more studies on wheat tissue culture. Wheat anther culture was first a success in China in the early 1970s (Zhu *et al.*, 1973; Ouyang *et al.*, 1973). With further studies on its theory and application, wheat anther culture has been applied in

the practice of wheat breeding and a batch of new wheat varieties has been developed (Hu *et al.*, 1983; Buyser *et al.*, 1987), showing that the somaclonal variation technique has been well applied in wheat breeding and agricultural production. This article introduces the experimental scheme, technical effect and the application status of the in vitro mutation technique in breeding salt-tolerant wheat.

## 2. PROTOCOL

The breeding process of salt-tolerant wheat by tissue and cell culture includes material determination, callus and mutation induction, mutant selection and identification. Our research group used both wheat mature embryos and anthers as explants to conduct in vitro culture under appropriate conditions. Calli were used to induce mutation and select salt-tolerant mutants. Salt-tolerant wheat lines were obtained from both explants of mature embryos and anthers. The concrete experimental protocol is as follows:

### 2.1 Donor plants and explants

The wheat varieties that donated explants were carefully chosen local commercial wheat varieties with good agronomic characters. The tissues and organs used for explants were mature embryos and anthers. The mature embryos were from the seeds of the above mentioned wheat varieties while the anthers were from F<sub>1</sub> generation of different cross combinations among these wheat varieties.

### 2.2 Callus induction and salt-tolerant mutant selection in mature embryo culture

#### 2.2.1 Establishment of mature embryo culture

Wheat seeds were first disinfested in 0.1% HgCl<sub>2</sub> for 10 minutes, rinsed 3 times in sterile water and then disinfested in 75% ethanol for 5 minutes, rinsed 3 times in sterile water again. Treated seeds were soaked in sterile water and put in the temperature box at 25°C overnight. The mature embryos with scutella were excised in a laminar airflow transfer hood. After being dried on the sterile filter paper, the mature embryos were placed on the surface of the induction media with the scutella facing upwards to initiate the culture. The Murashige-Skoog (MS) basal medium supplemented with different concentrations of mutagens and hormones was used for callus and mutation induction. 0.1µg/ml, 0.5µg/ml and 1.0µg/ml of two mutagens, Ping Yang mycin (PYM) and daunomycin (ZDM), were added in the induction media to determine the optimum concentration for mutation induction. When putting the mature embryos on the media, the scutella must face upwards so as to prevent the germination of the mature embryos (Fig.1).

#### 2.2.2 Callus induction

The mature embryos were cultured in the room at the temperature of 25-28°C. Between 10-12 hours of daily light and 1500 lux light irradiance were provided for callus induction. Under these conditions, the calli formed after 3-4 days of culture. The callus induction frequencies were investigated according to different treatments and were calculated as follows (Fig.2):

$$\text{Frequency of callus induction} = \frac{\text{No. of mature embryos that produced calli}}{\text{No. of mature embryos placed on induction medium}} \times 100\%$$

### **2.2.3 Basic experiment for salt tolerance**

Disinfested seeds were put on the medium MS + NaCl 0.3%, MS + NaCl 0.5% and MS + NaCl 1.0%, respectively. Seed germination on different media was observed. The concentration of NaCl at which the seed germination percentage was 50% was used for the selection of salt-tolerant calli.

### **2.2.4 Selection of salt tolerance**

The calli were transferred to MS based subculture media supplemented with 0.5% and 1.2% NaCl respectively at the same time for subculture and salt tolerance selection. The salt concentration was not raised gradually in salt tolerance selection, thus effectively avoiding a false positive result if the calli had gained the salt-tolerance because of physiological adaptation. After 40-45 days of subculture, the number of surviving calli was counted. All those calli that were able to keep growing or produce fresh calli even if they were browning were counted as live ones. The survival rates of calli were calculated as follows:

$$\text{Survival rate of calli} = \frac{\text{No. of live calli}}{\text{No. of calli transferred on subculture and selection medium}} \times 100\%$$

### **2.2.5 Differentiation of regenerated plantlets**

The surviving calli were transferred to 1/2 MS based differentiation media, supplemented with 0.5%, 0.8% and 1.2% of NaCl respectively to promote the selected salt-tolerant calli to differentiate and generate new plantlets (Fig.3).

### **2.2.6 Transplanting**

The differentiation status was investigated after 25-30 days of differentiation culture. The salt-tolerant plantlets were transplanted to normal soil (salt content lower than 0.3%) (Fig.4). The variations in agronomic characters such as plant height, spike length, seed setting rate and grain plumpness were observed. The variant plants were classified.

### **2.2.7 Identification of salt tolerance**

The stable individual mutant plants or mutant lines obtained in M2 generation were identified for their salt tolerance. Salt tolerance identification included the following:

#### **2.2.7.1 Salt pond trial**

The obtained stable lines, together with the control variety (original wheat variety), were sown, grown and developed in the artificial simulated salt pond of a 0.45% salt concentration until seeds were ripe (Fig.5). The mature plants were harvested and their agronomic traits were inspected separately. The salt tolerance of the new lines was evaluated based on plant characters. The salt tolerance efficiency and salt tolerance index were analyzed, thus determining the salt tolerance grade of the plant. The salt tolerance efficiency and salt tolerance index were calculated as follows:

$$1) \text{ Salt tolerance efficiency} = \frac{\text{No. of survival material in salt treatment}}{\text{No. of survival material in control treatment}}$$

$$2) \text{ Salt tolerance index} = \frac{\text{salt tolerant efficiency of the new bred variety}}{\text{salt tolerant efficiency of the control variety}}$$

3) The corresponding relation between the grade of salt tolerance and salt tolerance index were: Grade 1  $\geq 1.3$ , Grade 2  $\geq 1.1$ , Grade 3  $\geq 0.8$ , Grade 4  $\geq 0.5$ , and Grade 5  $< 0.5$ .

4) To determine the salt tolerance index, 3 repeats of each material and 50 plants of every repeat were investigated.

### **2.2.7.2 Physiological, biochemical and molecular detection**

The stable lines were used to further explore the molecular mechanism of salt tolerance (Shen *et al.*, 1993; Bi *et al.*, 1999; Suo *et al.*, 2001; Wang *et al.*, 2004; Wang *et al.*, 2004).

## **2.3 Callus induction and salt-tolerant mutant selection in anther culture**

### **2.3.1 Sampling**

The developmental stage of the microspores in the F<sub>1</sub> generation of different cross combinations was detected. After staining and slice production, pollen grains of the F<sub>1</sub> generation were observed under the microscope. Anthers with the pollen grains at the middle-uninuclear stage were ready for in vitro culture. When sampling in the field, plants with mid-uninucleate pollens were chosen, the young spikes together with the flag leaves were picked up and put in polyvinyl ethylene (PVC) bags to prevent moisture loss. Samples were then pretreated in the 4°C refrigerator for 72 hours in order to increase the callus induction frequency.

### **2.3.2 Disinfestation of young spikes**

First the flag leaf and the base of the young spike were cut off. Then the leaf sheath was wiped with 75% ethanol. In the laminar airflow transfer hood, young spikes were shelled and put in 0.1% HgCl<sub>2</sub> to be disinfested for 10 minutes, then rinsed 3 times in sterile water and dried on sterile filter paper. The anthers in the middle spikelets were taken off and cultured on N6 medium. The number of anthers placed on the medium depends on the size of the container. Usually it is appropriate to spread the anthers covering the whole surface of the medium.

### **2.3.3 Culture conditions**

The anthers were cultured in an incubator at 27°C to induce calli. A 12/12 (light/dark) hr. photoperiod was provided. Weak scattered light at the intensity of 15  $\mu\text{E}/\text{m}^2/\text{s}$  was proper for callus induction. After 30-35 days, the callus induction frequency was investigated.

$$\text{Frequency of callus induction} = \frac{\text{No. of anthers that produced calli}}{\text{No. of anthers placed on induction medium}} \times 100\%$$

In addition, although dark culture is beneficial to callus formation, it is easier to produce regenerated albino plantlets later.

#### **2.3.4 Mutation induction treatment**

Under aseptic conditions, the obtained calli were cut into small pieces about 2mm<sup>3</sup> each, immersed in 0.4% EMS mutagen solution for 2 hours, rinsed 3 times with sterile water, and then transferred to N6 subculture medium. After 40-45 days, the surviving calli were counted and the survival rate was calculated. The criterion for live calli and the calculation formula for survival rate of calli were the same as mentioned above.

#### **2.3.5 Selection of salt tolerance**

After being cut into small pieces, the surviving calli were transferred to N6 medium supplemented with 0.5% NaCl for subculture and salt tolerance selection. The number of surviving calli was counted after 37 days of subculture. The survival rate of calli was calculated.

#### **2.3.6 Differentiation of regenerated plantlets**

The selected calli were transferred to 1/2 MS medium containing NaCl. The differentiation status was investigated after 20-30 days.

#### **2.3.7 Transplanting**

Regenerated salt-tolerant plantlets were transplanted to normal soil (salt content lower than 0.3%) after adaptation at room temperature.

#### **2.3.8 Haploid detection**

We determined the ploidy levels of the regenerated plants by measuring the guard cell length of stomata on the leaves. First the chlorophyll was extracted completely with 70% ethanol. Then sections were prepared. The size of stomata was measured using an eyepiece micrometer. The stomatal length of haploids ranged from 36µm to 54µm, which were 40-50% shorter than that of diploids. The stomatal width of haploids ranged from 18µm to 24µm. Based on the detection result, the regenerated plants were divided into haploids and naturally doubled diploids.

#### **2.3.9 Doubling treatment on haploid seedlings**

The most critical step in haploid breeding is chromosome doubling. We used the following method and obtained over 70% chromosome doubled plants.

- a. Colchicine was dissolved in dimethyl sulfoxide (DMSO) to prepare 0.4% colchicine solution.
- b. At active tillering stage, the roots of haploid seedlings were washed clean and the tillering nodes were immersed into 0.4% colchicine solution. The treatment was kept at 18°C for 8 hours to double the chromosomes. If the haploid seedlings had too many tillers, they were separated and put in tap water overnight before being treated in the colchicine solution so as to be immersed more thoroughly.
- c. The colchicine was rinsed out of the treated haploid seedling roots. Then the haploid seedlings were planted in common soil. The culture conditions were: night temperature 15°C, day temperature 18°C, photoperiod 16/8 (light/dark) hr., and humidity 70%. Winter wheat needed

vernalization treatment (cultured at 4°C for 28 days) and then moved to normal temperature until seed was set.

#### **2.3.10 Identification of salt tolerance**

The successfully doubled plants were grown for 3 continuous generations. Their agronomic characters were investigated. The 4<sup>th</sup> generation was sown in the artificial simulated salt pond of a 0.45% salt concentration to identify salt tolerance. Those plants having stable salt tolerance were propagated. Physiological and biochemical detection methods were used to explore the mechanism of salt tolerance.

### **3. TECHNICAL EFFECT AND APPLICATION STATUS**

In vitro mutation technique is an economic and practical technique that combines in vitro culture and mutation induction. Using the in vitro mutation technique in breeding can double and redouble the variation frequency, accelerate the purification speed, shorten the breeding period and reduce the population of the selection generation, thus improving the breeding efficiency. This technique has wide application value and development prospects in crop breeding (Gao, 1992). Various mutation induction methods have been applied in this technique. For example, wheat ‘Hezu 8’ was bred through the combination of nuclear mutation technology and in vitro culture. This variety has such advantages as high yield, early maturity, disease resistance, wet soil endurance and good quality (Gao, 1992). While wheat ‘RH 8706—49’ was obtained by chemical mutation combined with in vitro culture. It is highly tolerant of salt (salt tolerance index  $\geq 1.3$ ) and has many other characters of stress resistance (Shen *et al.*, 1997). All this research showed that it is feasible to breed new salt-tolerant wheat varieties (or lines) via in vitro mutation technique.

In our experiment, we simplified the experimental procedure by adding a certain amount of mutagen in induction medium to make the callus induction and mutation occur at the same time. We chose the salt concentration higher than half lethal dose (LD50) to be the salt stress concentration so that the obtained salt-tolerant calli were a genetically stable cell line, thus avoiding the result of physiological adaptation. We also found in our research that the selection effect was better if we used NaCl stress at the callus differentiation stage rather than at the callus formation stage. Practice proves that further identification is needed to tell whether the salt-tolerant mutants obtained via tissue and cell culture are real genetic mutations. Synthesizing the judging criteria of Widholm, Flick and Maliga, one mutant can be identified as a true genetic mutant if: (1) This mutant occurs at low frequency. (2) The variation characters are stable when away from the selection pressure. (3) The callus induced from the regenerated plants with stable mutations should express the phenotype it was selected to be. (4) The mutant can be sexually transmitted. (5) There are changed gene products in variant cells (Gao *et al.*, 2004). We acquired the salt-tolerant variant plant from the differentiation medium containing 0.5% NaCl. After continuous planting in common soil for two years, its progeny still had higher level of proline as compared with the control. Especially under salt stress, the mutagenic progeny showed much better tolerance to salt, indicating that the mutant we got had a certain stability of salt tolerance.

Ever since the advantages of artificial mutation combined with in vitro culture in salt-tolerant mutant selection were found, in vitro mutation technique has made good progress in breeding

salt-tolerant mutants. Mutant plants were obtained on many wheat crops such as Barley (Zhang *et al.*, 1998), wheat (Shen *et al.*, 1993), and Triticale (Li *et al.*, 1992). The mutagens involved are <sup>60</sup>Co-rays, X rays, fast neutrons, Ping Yang mycin (PYM), daunomycin (ZDM), EMS and NaN<sub>3</sub>. In view of different explants reacting differently to different mutagens, explants can help determine what mutagens to use. For example, we used EMS in anther calli mutation induction and use Ping Yang mycin and daunomycin to induce mutations in calli from mature embryos. The research also showed that it was easier to obtain salt-tolerant variations when using the genotypes that are more sensitive to the mutagens (Shen *et al.*, 1997). Compound treatments can significantly increase the salt-tolerance of the mutant.

In the process of selecting salt-tolerant mutants, in vitro-cultured organs and tissues are used to induce mutations. The occurrence of serious chimera makes it difficult to select and identify the mutants. The low mutation frequency greatly influences the selection efficiency, too. Given the characteristics of suspension cells and the advantages and successful examples of suspension culture in selecting salt-tolerant mutants, it is hoped that suspension culture will be used as the main technology to select salt-tolerant mutants in the future. In addition, since the objective of breeding salt-tolerant wheat is to utilize the saline-alkali land and increase the wheat yield, in the future salt tolerance should not be the only character to be studied, and other agronomic characters should also be considered comprehensively in the selection of mutants. Therefore the salt-tolerant mutant will have a high comprehensive score in resistance, fertility, yield and quality. Along with the continuous development of modern science and technology, the combination of the somatic mutant breeding of salt tolerance with conventional breeding, chromosome engineering and genetic engineering can put together various fine traits to develop new varieties with salt tolerance, high yield and good quality. It certainly will be an effective tool for the development and utilization of saline soil and will bring great economic benefits to the practice of agriculture and forestry (Gao *et al.*, 2004).

## REFERENCES

- Bai Shouxin, Liu Cuiyun, Zhang Zhengang, Zhou Shuangjiao. 1979, A study of chromosome doubling of haploid plants of wheat (*triticum vulgare*). *Acta Genetica Sinica*. 6(2): 230-232.
- BI Cai-li, SHEN Yin-zhu, HUANG Zhan-jing et al. 1999, Molecular Biological Identification of Wheat Salt Tolerant Lines. *Hereditas*. 21 (6) : 32-36.
- Buyser J De et al. 1987, 'Florin': A doubled haploid wheat variety developed by the anther culture method. *Plant Breeding*, 98:53-56.
- Dai Gaoxing ; Peng Keqin ; Pi Canhui, 2003, The Effects of Calcium on Salt-tolerance in Plant *Chinese Agricultural Science Bulletin*. 19 (3) :97-101.
- Gao Mingwei. Hezu 8, a New Wheat Variety Developed with in Vitro Mutation Technique. *Nuclear Physics Review*. 1992, 9(4): 45~46.
- GAO Yu-hong, LI Yun. Application of selection of salt-tolerant mutant from plant in vitro. *Acta Agriculturae Nucleatae Sinica*. 2004, 18(6): 448~452.
- Hu Daofan, Tang Yunlian, Yuan Zhendong, Wang Jingin. THE INDUCTION OF POLLEN SPOROPHYTE OF WINTER WHEAT AND THE DEVELOPMENT OF THE NEW VARIETY "JINGHUA NO. 1". *Scientia Agricultura Sinica*. 1983 (1): 29~35.
- Li Suoping, Hu Yuxin, Zhang Dawei. In vitro Culture, Callus Induction and Plant Regeneration of Hybrid Embryo Crossed between *Aegilops tauschii* and Hexaploid Triticale, *Hereditas*. 1992, (6): 137~144.



- Melchers G. Haploid. Higher plants for plant breeding. Z. pflanzenzüchtg, 1972, 67:19~32.
- Ouyang Junwen, Hu Han, Zhuang Jiajun et al. The induction of pollen regeneration plant and observing of the offspring in wheat. Science in China. 1973, (1): 72~78.
- Shen Yinzhū, Liu Zhiyi, Zhang Zhaoduo, Si Zhihai, Huang Zhanjing, Shi Lanbo. 1993, A study of the salt-resistant variation of inducing matured embryo's callus and regenerated plants in wheat. Acta Genetica Sinica. 20(3): 283-291.
- Shen Yinzhū, Liu Zhiyi, He Congfen, Huang Zhanjing, 1997, A Study of Salt-resistant Variations Induced in Anther Calli and Regenerated Plants in Wheat. Hereditas. 19(6): 7-11.
- Shimada T. In vitro culture of wheat, callus formation, organ redifferentiation and single cell culture. Can J Genet Cytol, 1969, 11 (3): 294~304.
- SUO Guang Li, HUANG Zhan Jing, HE Cong Fen, et al. Identification of the Molecular Markers Linked to the Salt-resistance Locus in the Wheat Using RAPD-BSA Technique. Acta Botanica Sinica. 2001, 43 (6) : 598—602.
- Vinocur B, Altman A. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Curr Opin Biotech, 2005, 16: 123~132.
- WANG Cui-Ting, HUANG Zhan-Jing, HE Cong-Fen, BI Cai-Li, SHEN Yin-Zhu. Detection of the Wheat Salt-tolerant-mutant Using PCR-SSCP Combining with Direct Sequenceing. Acta Genetica Sinica, 2001, 22 (1) : 10—13.
- WANG Hong-Ying, ZHANG Cui, HUANG Zhan-Jing, ZHU Zheng-Ge, GUO Guang-Yan, SHEN Yin-Zhu. Mapping of Relative Salt-Tolerance Gene in Wheat Salt-Tolerant Mutant Using Microsatellite Marker. Acta Agronomica Sinica. 2004, 30(7): 679~699.
- Zenk MH. Haploids in physiological and biochemical research. In Haploids In higher plants advances potential. Ed. by Kasha K, J. Guelph Univ. Guelph. 1974, 339~354.
- Zhang Yalan, Li Yanfang, Yang Baiming, Zhang Chengwu, Wu Xiaoming. SELECTION AND CHARACTERIZATION OF SALT-RESISTANT VARIANTS FROM HORDEUM BREVISULATUM. Pratacultural science. 1998, 15(1): 30~32.
- Zhu Zhiqing, Wang Jingju, Sun Jingsan et al. Study on the induction of pollen regeneration plant and morphological forming procedure in wheat. Acta Botanica Sinica, 1973 (15): 1~11.

## **APPENDIX I: FORMULAS OF MEDIA**

### **I. Formulas of media for mature embryo culture**

1. Induction medium: MS + 2,4-D 2.0mg/L + 6-BA 0.5mg/L + NAA 0.5mg/L + LH 500mg/L + sucrose 3%, pH 5.8. Mutagens Ping Yang mycin (PYM) and daunomycin (ZDM?) were dissolved in distilled water and added in the medium as designed.
2. Subculture and selection medium: MS + 2,4-D 1.0mg/L + LH 500mg/L + yeast paste 1000 mg/L + sucrose 3%, pH 5.8. 0.5% and 1.2% NaCl were added in the medium for selection of salt tolerance.
3. Differentiation medium: 1/2MS + 6-BA 1.0mg/L + NAA 0.5mg/L + sucrose 3%, pH 5.8. 0.5%, 0.8% and 1.2% NaCl were added in the medium for further selection of salt tolerance.

### **II. Formulas of media for anther culture**

1. Induction medium: N6 + 2,4-D 2.0mg/L + KT 0.5mg/L + sucrose 9%.
2. Subculture medium: N6 + 2,4-D 1.0mg/L + KT 0.5mg/L + sucrose 9%.
3. Selection medium: N6 + 2,4-D 1.0mg/L + KT 0.5mg/L + NaCl 0.5% + sucrose 9%.
4. Differentiation medium: N6 + KT 2.0 mg/L + IAA 0.3 mg/L + NaCl 0.5% + sucrose 3%.

Note: All the media were sterilized by autoclaving at 1.1kg/cm<sup>2</sup> for 20 minutes.

## Appendix II: Pictures



Fig. 1 Mature embryos of wheat on the induction medium supplemented with mutagens.



Fig. 2 Calli induced from mature embryos of wheat.



Fig. 3 Salt-tolerant calli on differentiation medium containing salt.



Fig. 4 The growth of regenerated salt-tolerant.



Fig 5. Regenerated plants in the artificial simulated salt pond of a 0.45% salt concentration for identification of salt tolerance.

# CHAPTER 13

## A PROTOCOL FOR BREEDING SALT-TOLERANT BARLEY VIA HAPLOID CELL ENGINEERING TECHNOLOGY

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### 1. INTRODUCTION

It is easier to obtain mutants for specific purposes from cell culture (Chaleff, 1983). Anthers contain a large number of haploid microspores, and the embryoids formed from anther culture basically stems from microspore dedifferentiation, so the tolerance to NaCl of barley anthers should be related to the capacity of NaCl tolerance of microspores to a large extent. According to the scientific principles of cell totipotency and biological holography, the survival of anthers (microspores predominantly) after NaCl stress should indicate that they contain the corresponding resistance gene, and the surviving haploid cells can be induced to diploid plants with the pure resistance gene by chromosome doubling. The tolerance to NaCl of barley anthers (microspores) was consistent with donor plants (Yuefang Sun, Jianhua Huang et al., 2006, 2007). It provides a theoretical basis for screening recombinant gametophytes and variants with NaCl-resistance by using haploid cell engineering technology in barley. Selections of barley in haploid cell level can be propagated stably into double haploid plants by chromosome doubling (Jianhua Huang, 2007). This paper describes the protocols for screening barley doubled haploids with salt tolerance developed by barley anther culture employing mutagenesis and NaCl treatment.

### 2. TECHNICAL PROCEDURES

#### 2.1 The technical procedure of anther culture-<sup>60</sup>Co mutagenesis

##### 2.1.1 Donor plant growth

The highest efficiency of barley anther culture is achieved when donor plants are grown in a controlled growth room at the temperature about 18°C during the day and 15°C during the night, 16h photoperiod (light intensity about 10000 Lux), humidity 60-80%. During the whole vegetation period, plants should be properly watered and fertilized to maintain a vigorous growth.

If field grown material was used as donor plants, the seeds should be well spaced, in order to ensure the plants have adequate space for growth and development. A small amount of nitrogen fertilizer in meiosis stage will be beneficial to anther culture.

##### 2.1.2 Selection of donor spike

In barley, the distance between the flag leaf and the penultimate leaf indicates the proper stage of microspore development. Generally speaking, tillers should be collected when the distance

between the flag and the penultimate leaf is 2-3 cm. And the spikes in donor plants with anthers at the mid uninucleate stage should be used for pretreatment.

### **2.1.3 Cold pretreatment**

Donor spikes wrapped with cling film are stored at temperature 3°C for 15-20 days.

### **2.1.4 Spike mutagenesis and anther selection**

After cold pretreatment spikes are treated by  $^{60}\text{Co}$  irradiation at the dose rate of 1.2 Gy / min and dose of 10 Gy

Anthers containing microspores at the mid uninucleate stage of florets in the middle of spikes are selected to cultured *in vitro*.

### **2.1.5 Induction medium**

To N6 medium as a basic stock, add 2,4,5-T 2.0mg/L, KT 0.2mg/L, CH 800mg/L and maltose 60g/L, pH 5.8, solidified with 0.5% agar powder, load approximately 15ml medium in 50ml Erlenmeyer flasks separately, sterilize at 107.87KPa for 15 minutes.

### **2.1.6 Anther culture**

The tillers after cold pretreatment were put in 75% ethanol solution for 3-5 minutes, then rinsed by sterile water 3-5 times. Anthers from the middle florets in each row of the spikes are used for culture *in vitro*. The anthers are removed from florets in sterile conditions. The planting density should be 35-40 anthers per flask in 15ml induction media, then incubated at 20°C in darkness for 25-35 days.

### **2.1.7 The differentiation medium**

To C17 medium as a basic stock, add 6-BA 0.5mg/L, KT 1.5mg/L, NAA 0.1mg/L and maltose 30g/L, pH 5.8, solidified with 0.5% agar powder, load approximately 25ml medium in the 100ml Erlenmeyer flasks separately, sterilize at 107.87KPa for 15 minutes.

### **2.1.8 Differentiation culture of callus**

Calli about 1-2 mm in diameter are transferred into the Erlenmeyer flasks within 25ml differentiation medium, at a density of 8-10 structures per flask. Erlenmeyer flasks with calli are incubated at 25°C and 11h photoperiod.

### **2.1.9 Healthy culture medium**

To 1/2 MS medium as a basic stock, add chlorocholine chloride 5.0mg/L and sucrose 20g/L, pH 6.0, solidified with 0.60 agar powder, load approximately 30ml medium in the 100ml Erlenmeyer flasks separately, sterilize at 107.87KPa for 15 minutes.

### **2.1.10 Healthy culture**

The green seedlings are chosen for transfer onto healthy culture medium, at a density of 3-4 seedlings per bottle, at 20°C and 11h photoperiod for 20-25 days. Then the bottles with seedlings are incubated at 5°C in natural light.

### **2.1.11 Transfer to soil**

When roots have developed, plants are potted in pots containing garden soil, grown at 20/15°C day/night, 11h photoperiod. During prophase 2-3 sprays should be used to keep wet. Ensure adequate nitrogen, phosphorus and potassium fertilizers at tillering and jointing stages.

Because about 80% of anther culture derived barley plants obtained with this protocol are spontaneously doubled and completely fertile, colchicine treatment is not required.

### **2.2 Barley seed mutagenesis and field selection**

Dry barley seeds are treated by  $^{60}\text{Co}$  irradiation at the dose of 200 Gy. The treated seeds are then sown in the field, harvesting one spike per plant at harvest time. The seeds are sown, recording each spike, in the mountainous areas of Yunnan renowned for its cool climate. Choose the plants with excellent variations, harvest the seeds keeping records of each plant, and then sow them in the field separately.

### **2.3 Barley anther culture-NaCl stress screening procedures**

#### **2.3.1 Donor plant**

Use excellent combinations of F1 hybrid plants, and the fine mutations derived by mutagenesis.

#### **2.3.2 Anther culture-NaCl stress medium**

The same as procedure 1, plus 0.3%NaCl.

#### **2.3.3 Differentiation-NaCl stress medium**

The same as procedure 1, plus 0.5%NaCl.

#### **2.3.4 Anther culture and differentiation under NaCl stress**

The same as procedure 1.

### **2.4 Identification methodology of salt tolerance for barley seeds at germination stage**

#### **2.4.1 Seed germination medium**

1.5% NaCl solution, pH 6.0, solidified with 6% agar powder, load approximately 30ml medium in 100ml Erlenmeyer flasks separately.

#### **2.4.2 Seed pretreatment**

Seeds are soaked in 0.5% NaCl solution for 24h.

#### **2.4.3 NaCl stress culture**

Germinated seeds are put onto NaCl stress medium, 5 seeds per bottle, 5 repeats, at 28°C for 5 days.

#### **2.4.4 Trait statistics**

Radicle length, coleoptile length and the number of roots are measured in control and salt stress, separately.

### 2.4.5 Evaluation standard

Radicle growth rate evaluation:  $[(RS-RC)/RC] \times 100\%$

RS: the average length of radicle in salt stress

RC: the average length of radicle in control

Coleoptile growth rate evaluation:  $[(CS-CC)/CC] \times 100\%$

CS: the average length of coleoptile in salt stress

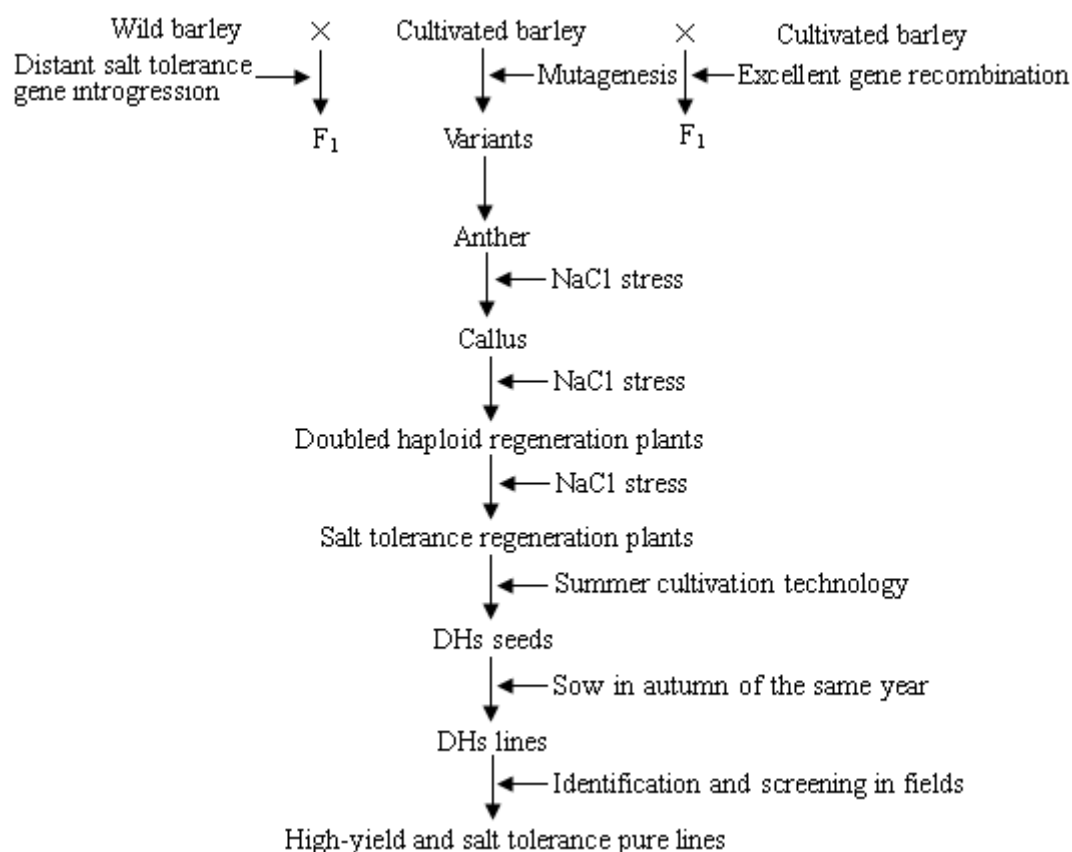
CC: the average length of coleoptile in control

The number of roots growth evaluation:  $[(NS-NC)/NC] \times 100\%$

NS: the average number of roots in salt stress

NC: the average number of roots in control

### 2.5 Heredity improvement methodology of barley double haploids for salt tolerance



**Fig.** Heredity improvement technology procedures of barley double haploids for salt tolerance

## 3. APPLICATION AND EFFICIENCY

Like the population of F<sub>2</sub> generation in the field, the population of F<sub>1</sub> generation derived from microspore culture is also one of the populations with the most variable genotypes. The genotypes

with relevant resistance genes can be screened out under NaCl stress through the *in vitro* anther culture system. The embryoids derived from these microspores are then induced to differentiate to seedlings under stress again. The seedlings with relevant resistance genes are induced to doubled haploids (DH) plants, and immediately become homozygous lines. Excellent plants can be derived from the screening of DHs under salt stress. These plants are cultivated in summer of the same year, and seeds harvested as pure lines in autumn. The fine breeding pure lines can be screened out through field identification in spring of the second year. Variety comparative testing can take place in the following autumn, and then official field test can be arranged in the third year. Homozygous breeding lines with excellent genes originating from four different parents separately can be derived in two years through this breeding system. These fine DHs varieties always possess the characters of high purity. The chromosome configuration of the doubled haploids is shown to be quite stable through karyotype analysis. There are two most important characters of the doubling haploids: 1) we only need to select parents with genes of target traits, while not needing to consider whether the parents are homozygous or not; 2) we can make efficiency selection in DH<sub>1</sub> generation.

Through application of the above procedures, a group of barley germplasm materials with markedly improved salt tolerance has been selected. A number of salt-tolerant high-yielding new lines have been bred through field identification and comprehensive selection for agronomic traits. Hua 22 is one of these new varieties, it has been cultivated in an area of nearly 50 million mu in Shanghai and Jiangsu province, showing high yield, high quality, salt tolerance, large ears and middle-stalked, 85 cm plant height, 7.2 cm of ear length, 24 grains per panicle, about 48g of 1000-grain weight, lenient and green leaves, strong middle-tillers, strong stem, vigorous growth, a high rate of spike and seed-setting, kernels and aristae easily falling at harvest, belongs to two-row long awn hull kernel type spring barley, strong salt tolerance, suitable for cultivation in the coastal areas, strong restoration after freezing damage, moderate resistance to scab and powdery mildew, yellow mosaic disease tolerance, but slight susceptibility to barley stripe disease, strong lodging resistance, lower protein content of about 10.99 percent, and a better quality for beer.

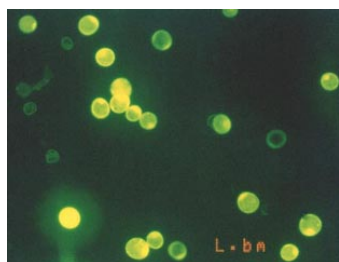
## REFERENCES

- Chaleff R S. 1983. Isolation of agronomically useful mutants from plant cell cultures. *Science*, 219:676-682.
- Sun Yuefang, Lu Ruiju, Wang Yifei, Shan Lili, Huang Jianhu, 2007. Response of *in Vitro* Culture of Anther and Microspore from Different Genotypes of Barley to Cool and NaCl Pretreatments, *Chinese Agricultural Science Bulletin*, 23(4): 46-48.
- Sun Yuefang, Lu Ruiju, Wang Yifei, Zhou Runmei, Huang Jianhua, 2006. Effects of NaCl stress on anther culture for different genotypes in barley. *Journal of Nuclear Agricultural Science*, 20(1): 19-22.
- Huang Jianhu, 2007, Application of microspore and anther culture to directional genetic improvement in barley. *Crop Research*, 3: 167-169.

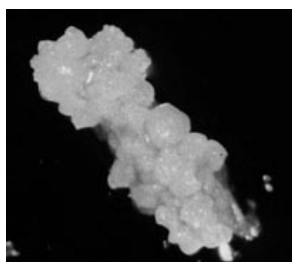


# APPENDIX: THREE COMMON PLANT TISSUE AND CELL CULTURE MEDIUM

Mineral salts	MS		Mineral salts	N6		Mineral salts	C17
<b>Macroelements</b>	mg/L	mM	<b>Macroelements</b>	mg/L	mM	NH <sub>4</sub> NO <sub>3</sub>	200
NH <sub>4</sub> NO <sub>3</sub>	1650	20.6	KNO <sub>3</sub>	2830mg/L	80mg/L	KNO <sub>3</sub>	mg/L
KNO <sub>3</sub>	1900	18.8	NaNO <sub>3</sub>		(1800)	KCl	300
CaCl <sub>2</sub> ·2H <sub>2</sub> O	440	3.0	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	463	(790)	CaCl <sub>2</sub> ·2H <sub>2</sub> O	150
MgSO <sub>4</sub> ·7H <sub>2</sub> O	370	1.5	KCl		65(845)	MgSO <sub>4</sub> ·7H <sub>2</sub> O	250
KH <sub>2</sub> PO <sub>4</sub>	170	1.25	CaCl <sub>2</sub> ·2H <sub>2</sub> O	166		KH <sub>2</sub> PO <sub>4</sub>	325
<b>Microelements</b>	mg/L	μM	Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O		300	NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	150
KI	0.83	5.0	MgSO <sub>4</sub> ·7H <sub>2</sub> O	185	720(1000)	Na-Fe-EDTA	100
H <sub>3</sub> BO <sub>3</sub>	6.2	100	Na <sub>2</sub> C <sub>4</sub>		200	Fe-citrate	17.5
MnSO <sub>4</sub> ·4H <sub>2</sub> O	22.3	100	KHFO <sub>4</sub>	400		MnSO <sub>4</sub> ·4H <sub>2</sub> O	3.0
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	8.6	30	NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O		16.5(300)	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	0.5
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.25	1.0	FeSO <sub>4</sub> ·7H <sub>2</sub> O	27.8		CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.25
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.025	0.1	Na-EDTA	37.3		CuSO <sub>4</sub> ·5H <sub>2</sub> O	0.012
CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.025	0.1	Fe <sub>2</sub> (SO <sub>4</sub> ) <sub>3</sub>		2.5	Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0.012
Na <sub>2</sub> EDTA	37.3	100	MnSO <sub>4</sub> ·4H <sub>2</sub> O	4.4	7	KI	0.012
FeSO <sub>4</sub> ·7H <sub>2</sub> O	27.8	100	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	1.5	3	H <sub>3</sub> BO <sub>3</sub>	0.1
			CuSO <sub>4</sub> ·5H <sub>2</sub> O		(0.001)*		5.0
			MoO <sub>3</sub>		(0.0001)*		
			KI		0.75		
			H <sub>3</sub> BO <sub>3</sub>	1.6	1.5		
Sucrose(g)	30						
pH	5.7						



Microspore vigor test



Calli formed on one anther



Regenerated plants



Variety certificate of Hua-22



Field-growing of Hua-22

## CHAPTER 14

# A PROTOCOL FOR SELECTION OF RADIATION-INDUCED SALT TOLERANT RICE MUTANTS BY IN VITRO MUTAGENESIS

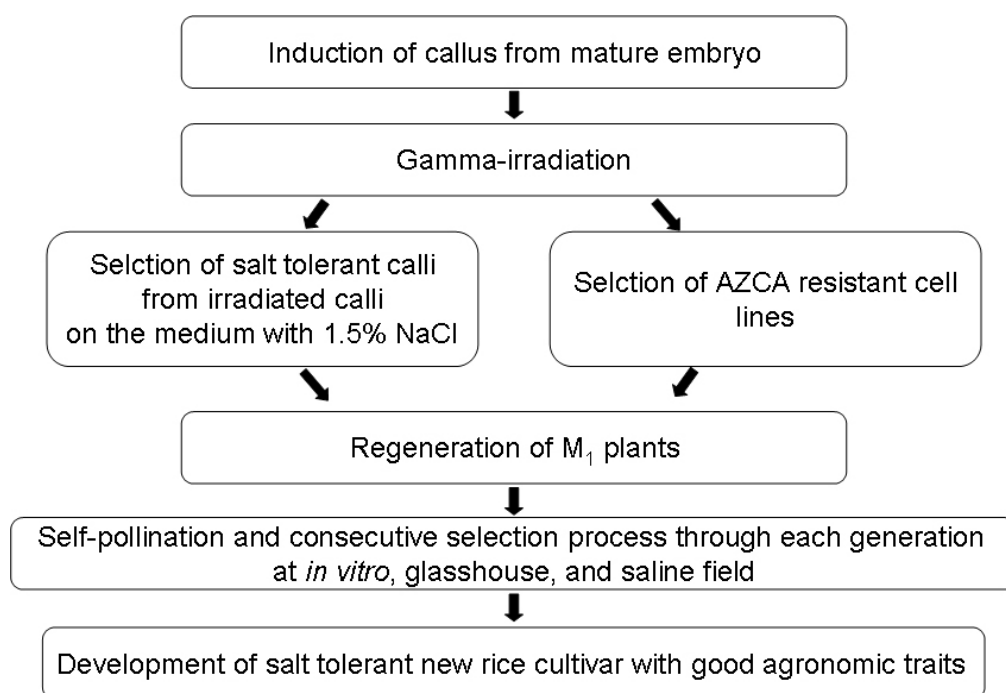
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## 1. INTRODUCTION

Radiation induced mutagenesis has been studied extensively for the identification and isolation of a plant gene and in plant breeding programs. Rapid progress in plant tissue culture methods is attributed to the availability of many mutants that can be generated for specific biochemical events that are difficult or impossible to use with intact plants (Carlson, 1970; Larkin and Scowcroft, 1981; Schaeffer and Sharpe, 1983; Kim *et al.*, 2004). This may be achieved by co-culturing plant tissues with pathogens or their toxins and by manipulating a medium composition, e.g. selection for a resistance to salinity, drought and amino acid analogs. Although, it is possible to select mutants with salt tolerance by a somaclonal variation, mutant frequencies are often lower in plant systems due to inefficient recovery techniques, as well as the presence of multiple gene copies located on different chromosomes, and by cell-to-cell interactions in even the most ideal suspension culture systems (Schaffer and Sharpe, 1983). Therefore, *in vitro* mutation induction techniques provide tools for a rapid creation and increase of the selection frequencies of cell types for which there is no obvious counterpart in field environments (Maluszynski *et al.*, 1995). Such techniques enable a greater use of mutated genes for crop improvement, the cumulative effect of which may result in a more desirable character than the original variety. Rice, an important staple food, is consumed by more than half of the world's population. It is sensitive to a salt stress. Therefore, development of varieties with an increased salt tolerance is required. The possibility of screening for salt stress in a culture makes *in vitro* methods attractive for developing stress tolerant plants (Lee *et al.*, 2003). In the present protocol, we described the selection of salt tolerant cell lines via *in vitro* mutagenesis, a regeneration, a consecutive selection through each generation (M<sub>2</sub>-M<sub>3</sub>), and a screening of salt tolerant lines in a saline field. Figure 1 shows a flow chart of the procedures for the selection of salt tolerant lines.



**Figure 1.** Flow chart of procedures for the selection of salt tolerant lines.

## 2. PROTOCOL

### 2.1 *In vitro* selection of salt tolerant rice mutant cell lines

#### 2.1.1 Embryogenic callus induction

Calli were initiated from embryos of hulled seeds of a japonica rice cultivar ‘Dongjinbyeo’, a high quality and high yielding cultivar with lodging and disease resistance. Seeds were sterilized in 10% sodium hypochlorite with 2–3 drops of Tween 40 for 30 min. The embryos were placed on a callus induction medium that was a N6 basal medium supplemented with 2 mg/L 2,4-D 30 g/L sucrose and 0.4% (W/V) phytigel in sterile petri dishes, and incubated in the dark at 25±1 °C for callus initiation (Table 1). The medium was adjusted to pH 5.8 and autoclaved for 20 min at 1.1 kg/cm<sup>2</sup> pressure at 121 °C. Induced calli were once more subcultured for a proliferation with 4 weeks intervals.

#### 2.1.2 Determining optimum NaCl concentration and irradiation dose for salt tolerant calli

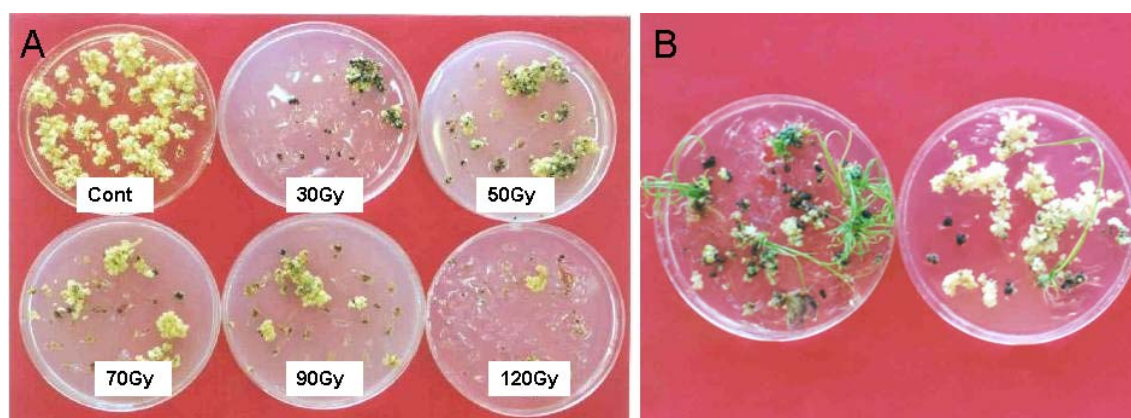
It is very important to decide on the optimal salt concentration for a selection of salt tolerant calli. The calli were divided into small pieces (0.5 ~ 1 mm diameter) and inoculated on a N<sub>6</sub> medium containing various concentration (0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5%) of NaCl, and the survival rate and fresh weight of the surviving calli for 40 days were investigated. The callus pieces were irradiated with 0, 30, 50, 70, 90, and 120 Gy gamma-ray from a <sup>60</sup>Co source, and the fresh weight and survival rate of calli for 40 days were also investigated (Figure 2A). In each experiment, there were three replicates for each treatment.

#### 2.1.3 Selection for NaCl tolerance

The propagated calli were divided into small pieces (0.5~1 mm diameter) and inoculated on a N<sub>6</sub> medium supplemented with 1.5% NaCl and 70 Gy, which were the optimum concentration of NaCl and irradiation dose for the salt tolerant calli, respectively (Table 1). The selected salt-tolerant calli were maintained at the same concentration.

#### 2.1.4 Regeneration

The selected NaCl tolerant calli were transferred and cultured for regeneration on a MS medium supplemented with 0.5 mg/L NAA and 2 mg/L BAP, but without salt for 30 days (Table 1, Figure 2B). Plantlets were cultured at 10 cm on a half-MS medium followed by an acclimation at room temperature. Some of the regenerates were transferred to a medium with 0.75 % NaCl to confirm a transmission of a tolerance from the callus to the regenerated plants. Each of the regenerated plants (M<sub>1</sub>) were assigned numbers, and grown to maturity in a NaCl-free soil in a field. Standard crop management practices were followed, which included an application of 11; 7; 8; kg/10a of N; P; K. Harvested lines were sterilized with a disinfectant for the seed.



**Figure 2.** Comparison of growth calli irradiated with various radiation on the medium with 1.5% NaCl. Control plate containing non-mutagenied callus on the medium without salt (A). Regeneration from salt tolerant calli on the medium (B).

**Table 1** Composition of callus induction and regeneration media for *in vitro* selection of salt tolerant cell lines

Medium type	Components
Callus induction	N <sub>6</sub> basal, 30 g/l sucrose, 2 mg/l 2,4-dichlorophenoxyacetic acid, pH 5.7 - 5.8, 4 g/l phytagel
Callus selection	Same as callus induction medium with 1.0% NaCl
Regeneration	MS basal, 30 g/l sucrose, 0.5 mg/l Naphthaleneacetic, 2 mg/l Benzylaminopurine, pH 5.7 - 5.8, 4 g/l phytagel
Plant selection	MS basal, 30 g/l sucrose, 1.0% NaCl, pH 5.7 - 5.8, 4 g/l phytagel
Root formation	Same as plant selection medium, but no NaCl

## **2.2 Selection of salt tolerant rice using high proline accumulating rice mutants induced by *in vitro* mutagenesis**

When plant cells are exposed to stresses, such as drought, salinity and cold, proline accumulation is a widespread phenomenon. Under stress conditions a proline increase plays a key role for an osmotic adjustment in a large number of plant species (Delauney and Verma, 1993). Mutants resistant to proline analogs are thought to be useful material for studying proline biosynthesis and saline, drought, and cold resistance in higher plants (Mifflin *et al.*, 1983). Azetidine-2-carboxylic acid (AZCA), a proline analog, is a natural product found in some species of *Liliaceae*, which inhibited an irreversible cell growth by competition with proline for an incorporation into protein (Cella *et al.*, 1982; Hyun *et al.*, 2003). This incorporation presumably leads to an altered protein conformation and function, and accounts for the cytotoxic effects of AZCA (Lodato *et al.*, 1984; Song *et al.*, 2007). In many cases, the mechanism of a resistance to amino acid analogs has been shown to be due to the insensitivity to a feedback inhibition (Widholm, 1974). Mutants with an altered feedback mechanism express more resistance to a feedback inhibition resulting in a greatly elevated free proline accumulation. The decrease of uptake inhibitors by a change in cell membrane permeability and an inactivation of inhibitors have also been reported as mechanisms of an amino acid analog resistance.

### **2.2.1 Embryogenic callus induction**

Dehusked seeds cv. ‘Donganbyeon’ were sterilized in 70% ethanol for 30 sec followed by an addition of 5% sodium hypochlorite with 2-3 drops of Tween-20 for 20 min. The embryos of seeds were rinsed 3-4 times with sterile distilled water and placed on a N<sub>6</sub> medium supplemented with 2 mg/L 2,4-D 30 g/L sucrose and 0.4% (W/V) phytagel in sterile petri dishes, and incubated in the dark at 25±1 °C for callus initiation. The medium was adjusted to pH 5.8 and autoclaved for 20 min at a 1.1 Kg/cm<sup>2</sup> pressure at 121 °C. Induced calli were subcultured once more for proliferation.

### **2.2.2 Determining the optimum AZCA concentration**

Induced callus was divided into 30 pieces (about 1- 2 mm) with a sterile razor and transferred to the N<sub>6</sub> basal medium containing 1.5, 2, 3, and 4 mM AZC concentrations. Thirty callus pieces each were plated per concentration with 10 replications. The data on the survival rate and fresh weight were obtained at 40 days after culture. Based on the survival rate and fresh weight, the optimum AZCA concentration was assessed as 3 or 4 mM.

### **2.2.3 Gamma ray treatment and selection of AZCA resistant cell lines**

The effect of gamma rays on growth of the callus was investigated 40 days after an irradiation. Callus was irradiated with 0, 30, 50, 70, 90, and 120 Gy of gamma rays. Selection of AZCA resistant cell lines was conducted on a selection medium containing 3 or 4 mM AZCA.

### **2.2.4 Regeneration of AZCA resistant cell lines**

AZCA resistant callus was transferred to the MS medium containing 0.2 mg/L indoleacetic acid (IAA), 3 mg/L kinetin, and 30 g/L sucrose solidified with 0.5% (W/V) phytagel. The medium was adjusted to pH 5.8 and autoclaved for 20 min at 121 °C. Regenerants were obtained at 30 days after being transferred to the regeneration medium. The plantlets were grown further in a bottle containing a half strength MS medium without hormone. Regenerants from the resistant callus

were grown in a solution containing a half strength MS basal and 2 mM AZCA to determine their resistance. The shoots of the regenerants were removed except for 5 cm in length and the roots were removed thoroughly for the 2-3 leaf stage seedlings for a comparison of their revival ability. AZCA resistant regenerants were selected after 2 weeks.

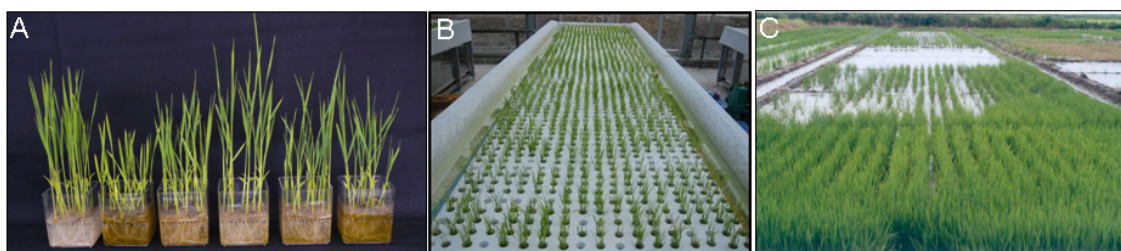
## 2.3 Section 1 & 2 (continued)

### 2.3.1 Selection of the optimum salt concentration at a plant level

It is very important to decide on the concentration resulting in 50 % mortality ( $LD_{50}$ ) for each cloned plant under a salt concentration. Hulled seeds were sterilized with 5% sodium hypochlorite, and cultured on a half strength MS agar medium containing 0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.5 % NaCl concentrations for 40 days. During this period, the highest efficiency of plant culture was achieved when the plants were grown in a controlled growth room at  $27\pm1^{\circ}\text{C}$  under 16 h and 8 h of light and dark conditions, respectively, for the determined optimum salt concentration

### 2.3.2 $M_2$ generation and screening of the $M_3$ seedlings

Three hundred  $M_2$  lines from  $M_1$  plants, except those with a poor plant type (droopy leaves and weak culm), were harvested and numbered from  $M_2$ -1 to 300. The  $M_2$  generation was grown as a plant in rows in a NaCl-free field and harvested as  $M_3$  generation (5,000 lines) showing normal grain fertility (above 80%) on an individual plant basis. Each  $M_3$  line was the progeny of a single  $M_2$  line seed bulk and numbered from  $M_3$ -1-1 to  $M_3$ -1-n and from  $M_3$ -300-1 to  $M_3$ -300-n (n was influenced by lines). Salinity tolerance of the  $M_3$  lines was screened *in vitro* and in a glasshouse with trays for 0.75 % NaCl (Figure 3A & B). In the screening in the glasshouse, each tray had 20 pots ( $60 \times 150 \times 30$  mm), with one from the parent, not cultured *in vitro*. The trays were filled with fine soil, which was commercially made for rice culture, and 80 seeds per line were placed in each pot at a depth of 5 mm. The trays were watered with tap water until the 3 to 4 leaf stages. At that stage, excess water was drained, and the trays with rice seedlings were refilled with solution containing 0.75 % (E.C= 13 mS) salt and 1 g/L fertilizer. The solution was circulated with an underwater rotator to maintain a uniform salt concentration. The E.C measurements were taken daily. After three weeks of salinization with 13 mS, salinity symptoms were scored according to the Standard Evaluation System (1~3: tolerant, 5: moderate and 7~9: sensitive) developed at IRRI. To re-estimate the salt tolerance, thirty seeds each of the tolerant and sensitive lines, were placed in glass bottles ( $5 \times 7$  cm) with a salt-free solution and 0.75% salt treatment and cultured for 30 days and 3 weeks, respectively. Plant height was used to assess the salt tolerance or sensitivity of the rice lines for a quantitative measurement of their salt tolerance.



**Figure 3.** Screening of selected salt tolerant rice mutant lines in *in vitro* (A), glasshouse (B), and saline field (C).

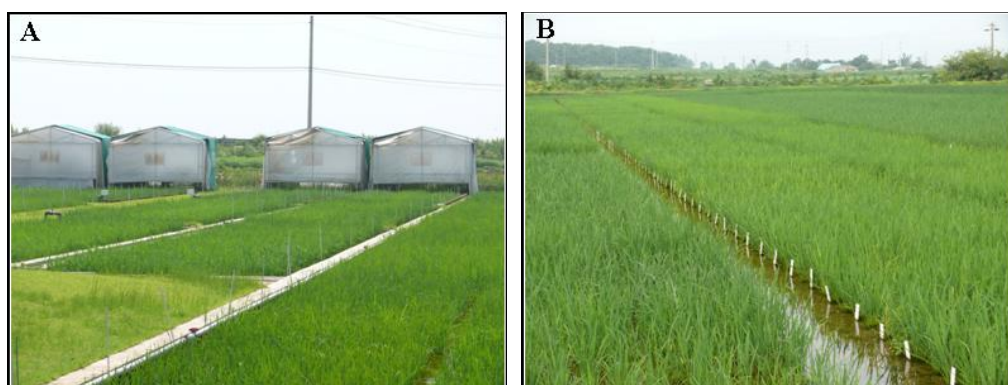


### 2.3.3 Selection of salt tolerant lines in a saline field

M<sub>3</sub> or M<sub>4</sub> lines selected at the seedlings stage were tested in a saline field near the sea coast (Figure 3C). The salinity level of the saline field was cal. 10 – 14 mS. Heterogeneity of the soil salinity in the field was an inherent problem for evaluating their performance. However, this problem was minimized with plot sizes of 2.4 × 4.5 m<sup>2</sup>. From each line, 30 plants were transplanted in two replicates 30 × 15 apart in a saline and normal field. The original variety was used as the control, and transplanted once every 8 lines. Standard crop management practices including an application of 20-8-8 kg/10 a of NPK were applied. Seed was harvested on an individual plant basis.

### 2.3.4 Performance evaluation at maturity

All lines were tested in a field trial in five replications, and evaluated at maturity (Figure 4). Plant height, panicle length, tiller number, survival rate, spikelets per plant and grain weight were recorded. Analysis of the variance and means were compared with the data from the saline and non-saline fields. The t-test value at 5 and 1 % level of significance was used to determine the superiority of the tested lines over the control.



**Figure 4.** Comparison of agronomic characteristics of M<sub>3</sub> lines cultured in the 0.8% saline field at the Gyechwado Substation of the Honam Agricultural Research Institute, the Rural Development Administration (A) and non-saline field (B).

## 3. EFFICIENCY AND APPLICATIONS

Variations in the progenies of the *in vitro* mutagenized plant materials have already been reported and they probably originated from the genetic instability created during a plant tissue culture in combination with induced mutations (Maluszynski et al. 1995). *In vitro* culture in rice in combination with gamma-ray induced mutations is an effective way to improve the salt-tolerance in rice. The mutation and selection frequency were greatly increased, and the selected mutants were stable. Such mutant lines could be utilized as a source of salt-tolerant germplasm. A new rice cultivar ‘Wonhaebyeon’ with salt tolerance was developed by the above mentioned *in vitro* mutagenesis with 70 Gy gamma-rays during 2007 in the KAERI. This rice mutant will be released for cultivation at reclaimed land and used as a control plot for genetic research on salt tolerance.

## REFERENCE

- Carlson, PS. 1970, Induction and isolation of auxotrophic mutants in somatic cell cultures of *Nicotiana tabacum*. Science 168: 487-489.
- Cella R, Parisi B, Nielsen E, 1982. Characterization of a carrot cell line resistant to azetidine-2-carboxylic acid. Plant Sci. Lett. 24:125-135.
- Delauney AJ, Verma DPS, 1993. Proline biosynthesis and osmoregulation in plants. Plant J 4:215-223.
- Hyun DY, Lee IS, Kim DS, Seo YW, Lee YI, 2003. Selection of azetidine-2-carboxylic acid resistant cell lines by in vitro mutagenesis in rice (*Oryza sativa* L.). J Plant Biotech 5(1):43-49.
- Kim DS, Lee IS, Jang CS, Hyun DY, Seo YW, Lee YI, 2004. Selection of radiation induced 5-methyltryptophan resistant rice mutants through embryo culture. Euphytica 135(1):9-19.
- Larkin PJ, Scowcroft WR, 1981. Somaclonal variation - a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet. 60: 197-214.
- Lee IS, Kim DS, Lee SJ, Lim YP, Lee YI, 2003. Selection and characterizations of radiation-induced salinity tolerant lines via rice embryo culture. Breed Sci 53(4) 313-318.
- Lodato RF, Smith RJ, Valle DL, Crane K, 1984. Mutant cell lines resistant to azetidine-2-carboxylic acid: alterations in the synthesis of proline from glutamic acid. J Cell Physiol 119:137-143.
- Maluszynski M, Ahloowalia BS, Sigurbjornsson B, 1995. Application of in vitro mutation techniques for crop improvement. Euphytica 85: 303-315.
- Miflin BJ, Bright SW, Rognes SE, Kueh JHS, 1983. Amino acids, nutrition and stress: the role of biochemical mutants in solving problems of crop quality. In Kosuge T, Meredith CP, Hollaender A (eds), Genetic engineering of plants – an agricultural perspective, pp. 391-414. Plenum Press, London.
- Schaeffer G.W, Sharpe FT, 1983. Mutations and selection: genetic variation for improved protein in rice. Genetic Engineering, Application to Agriculture; Invited papers presented at a symposium, May 16-19, 1982, Beltsville, pp. 237-254.
- Song JY, Kim DS, Lee G-J, Lee IS, Kang KK, Yun SJ, Kang S-Y, 2007. Characterization of salt tolerant rice mutant lines derived from Azetidine-2-carboxylic acid resistant cell lines induced by gamma ray irradiation. J Plant Biotech 34(1):61-68.
- Widholm JM, 1974. Cultured carrot cell mutants: 5-methyltryptophan-resistant trait carried from cell to plant and back. Plant Sci Lett 3:323-330.



# CHAPTER 15

## A POTOCOL FOR FREE PROLINE QUANTIFICATION

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### 1. INTRODUCTION

Free proline accumulation is one of the most frequently reported metabolic modifications induced by different stresses in plants. In spite of this, the precise role of proline in stress physiology, as well as the metabolic adjustments associated with the proline biosynthesis, still remains in controversy. So far there has been no definitive evidence for the adaptive value of proline itself under adverse conditions (Gibon et al., 2000). Nonetheless, many researchers are still being attracted to the study of free proline regarding its significance in the integrated metabolic response to stress in plants.

### 2. A COLORIMETRIC METHOD FOR MEASURING FREE PROLINE

The most often used analytical method for proline quantification is the rapid colorimetric procedure described by Bates et al. (1973). This technique is adequate for studying water stress as described by the authors. Nonetheless, the application of this method to other types of stress, including the biotic stresses like allelopathy (Sanchez-Moreiras, 1996) and resource competition (Pedrol et al., 1999) which are less often studied, has been widely demonstrated.

#### 2.1 Chemical basis

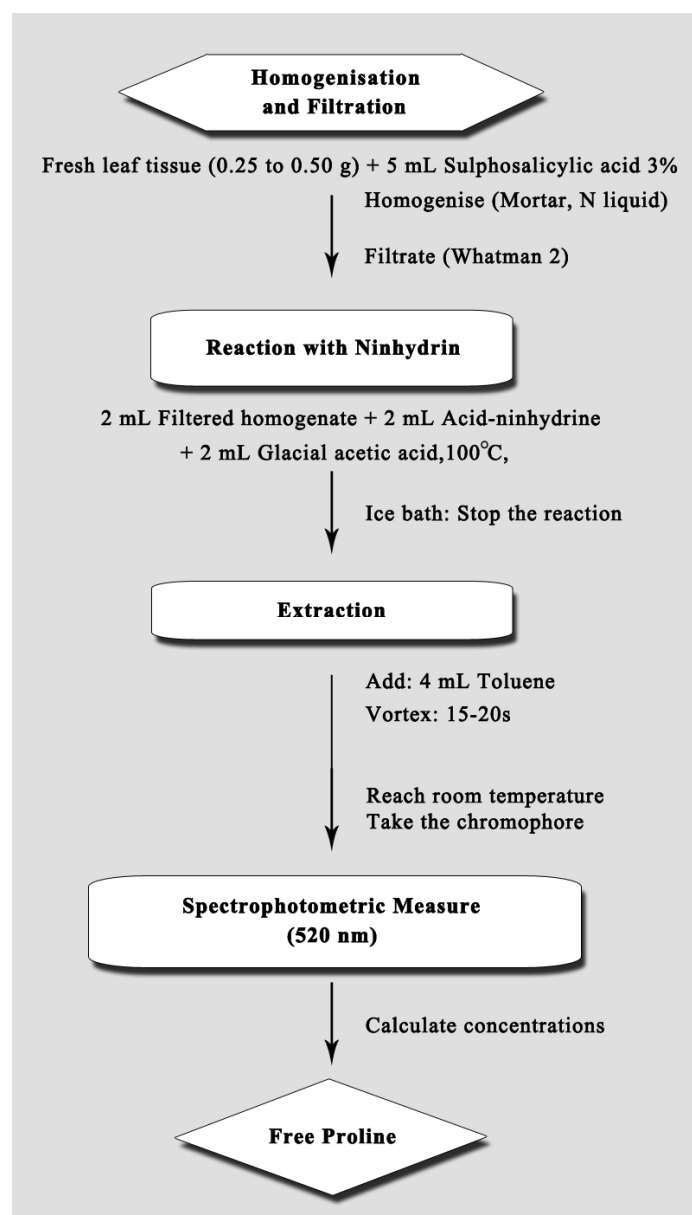
The method proposed by Bates et al. (1973) is based on the reaction that takes place between ninhydrin and amino acids. Ninhydrin is a powerful oxidant that produces the oxidative deamination of the  $\alpha$ -amino group, releasing ammonium,  $\text{CO}_2$ , the corresponding aldehyde and ninhydrin in reduced form. Released ammonium reacts with an additional mol of ninhydrin and with reduced ninhydrin, thus producing a coloured complex. For the amino acids, this purple complex has an absorption maximum at 570 nm where the absorbance is nearly a lineal function of the quantity of amino groups present in the solution. Therefore, this reaction is a very convenient probe for quantitative colorimetric amino acids determination.

Considering that proline is strictly an imino acid (partially substituted amino group), the reaction between proline and ninhydrin is different from the rest of proteic amino acids, forming a coloured complex whose absorption maximum is near 440 nm (Maler and Cordes, 1971).

## 2.2 Accuracy

Although several amino acids can interfere with proline quantification, it is noteworthy to state that the concentrations of other free amino acids normally present in stressed plants are very low if compared to proline. So the absorption interference can be ignored.

The detection range of this method is considered between 0.1 and 36  $\mu\text{mol}$  (free proline) per g of fresh weight (plant tissue) (Bates et al. 1973). On the basis of this colorimetric reaction several authors have made different modifications in function of the stress type, used material (species and tissue) and possible interference of several reactives. The need for pre-treatments when using in vitro callus cultures was also considered (method of Troll and Lindsley, 1955; modified by Magne and Larher, 1992; Lutts et al., 1996; Trotel-Aziz et al., 2000).



**Figure 1.** Main steps of the protocol in the colorimetric determination of free proline (Bates et al., 1973).

### 3. PROTOCOL

#### 3.1 Reagents

- Sulphosalicylic acid 3% (w/v)
- Phosphoric acid 6 M
- Glacial acetic acid
- Acid-ninhydrin, prepared by warming 1.25 g ninhydrin in 30 mL glacial acetic acid, and adding 20 mL phosphoric acid 6 M  
**Warning:** Acid ninhydrin can only keep stable for 24 hours at 4 °C.
- Purified proline
- Toluene

#### 3.2 Methods

##### 3.2.1 Preparation of the standard curve

Sample absorbances can be converted to proline concentration by comparison to a proline standard curve obtained by using known proline concentrations. Every time proline concentration is calculated, a standard curve must be prepared first.

A trial-and-error assay is often necessary to select our adequate standard curve points. A series of standard proline solutions must be prepared with different concentrations that cover the expected range of unknown sample concentrations. Different solutions are prepared by adding distilled water to the 'stock' solution of commercial purified proline.

All tubes in standard curve must follow the same procedure used on the samples, according to ninhydrin reaction (see Step 2).

Take leaf tissue for example, here we describe the method for free proline quantification as the following key steps (see Figure 1).

##### 3.2.2 Homogenisation and filtration

250 to 500 mg of fresh leaf tissue (obtained from the fully expanded young leaves) are powdered in a cold mortar with liquid nitrogen until pulverisation and homogenised with 5 mL of sulphosalicylic acid 3% (to get protein precipitation). Then, the homogenate is filtered through a filter paper. The required amount of fresh sample depends on the tissue's water content. A trial-and-error approach is often necessary.

##### 3.2.3 Reaction with ninhydrin

2 mL of the filtrated sample is mixed with 2 mL of glacial acetic acid and 2 mL of acid-ninhydrin (previously prepared) in test tubes. After agitation, the sample is incubated at 100 °C for 1 hour to produce the coloured complex. After one hour, the reaction is stopped by putting the tubes in an ice bath.

#### **3.2.4. Extraction**

4 mL of toluene is added to each tube. After vortex for 15-20 s, organic and inorganic phases are separated. Obtain the chromophore dissolved in toluene.

#### **3.2.5 Spectrophotometric measurement**

The maximum absorbance of the chromophore when dissolved in toluene is attained at a wavelength of 520 nm. Collect the organic phase of each sample and standard solution containing the chromophore and measure the absorbance at 520 nm spectrophotometrically.

#### **3.2.6 Calculations and data analysis**

Using the data obtained from the standard curve prepared at known concentrations, a lineal regression relationship can be established between the absorbance and proline concentration. The sample concentrations can be calculated according to the regression equation.

Finally, it is suggested that the free proline concentration be expressed as  $\mu\text{mol}$  per g of dry weight of plant material.

### **REFERENCES**

- Schweet RS, 1954. The quantitative determination of proline and pipecolic acid with ninhydrin. *J Biol Chem* 208: 603-613.
- Troll W, Lindsley J, 1955. A photometric method for the determination of proline. *J Biol Chem* 215: 55-660.
- Pilar Ramos Tamayo, Nuria Pedrol Bonjoch, 2003. Handbook of plant ecophysiology techniques. Springer Netherlands. 365-382.

# CHAPTER 16

## TOTAL SOLUBLE SUGAR (TSS) QUANTIFICATION

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Carbohydrates are the most abundant biomolecules on earth and also the main components of plants. In certain plant tissues, the proportion of carbohydrate in dry weight reaches as high as 80%-90%. There are three major size classes of carbohydrates: monosaccharides (such as glucose, fructose), oligosaccharides (such as starch, fructose oligosaccharides, and galactin) and disaccharides (such as sucrose). Under stress conditions, the plant carbohydrate metabolism is influenced so that the content of carbohydrates changes correspondingly.

### 1. EQUIPMENT

Spectrophotometer

Oven

Thermostatic waterbath

### 2. REAGENTS

Anthrone solution: dilute 76 ml of concentrated sulfuric acid with distilled water to 100 ml. Dissolve 150 mg of purified anthrone in 100 ml of dilute sulfuric acid to make anthrone solution.

### 3. METHODS

#### 3.1 Construction of working curve

Prepare a series of standard glucose solutions with different concentrations, such as 0, 5, 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml. Mix 0.1 ml of each standard glucose solution with 0.5 ml of anthrone solution and incubate the mixtures at 90°C for 15 min. Then the optical densities of the mixtures are measured on a spectrophotometer at 620 nm wavelength. The working curve is constructed with the concentrations of the standard glucose solutions as the abscissa and the OD values as the ordinate.

#### 3.2 Extraction of TSS

Fresh leaf sample (0.05 g) is dried to constant weight at 80°C. Extract soluble sugars of dry leaves in 80% ethanol at 80°C for 30 min for three times. After filtration, the combined extract is made up to 10 ml with 80% ethanol.

### **3.3 Measurement of TSS**

The mixture of 0.1 ml of the sample extract and 0.5 ml of anthrone solution is incubated at 90°C for 15 min. Measure the optical density at 620 nm wavelength. The TSS concentration of the sample can be read off from the working curve.

## **REFERENCES**

- Lin Xi Feng, 2004. Study of salt tolerant crops. Science Press, Beijing, China. 255-259.
- Yemm EW, Willis AJ, 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem J* 57: 508-514.

# CHAPTER 17

## PROTEIN SDS-PAGE

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The separation of macromolecules in an electric field is called *electrophoresis*. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), a very common method for separating proteins, uses a discontinuous polyacrylamide gel as a support medium and sodium dodecyl sulfate (SDS) to denature the proteins. This most commonly used system is also called the Laemmli method named after U.K. Laemmli, who was the first to publish a paper employing SDS-PAGE in a scientific study.

SDS (also called lauryl sulfate) is an anionic detergent which means that when dissolved its molecules have net negative charges within a wide pH range. A polypeptide chain binds numbers of SDS molecules whose number is in proportion to the relative molecular mass of the polypeptide. The negative charges on SDS destroy most of the complex structure of the proteins, and are strongly attracted toward an anode (positively-charged electrode) in an electric field.

Polyacrylamide gels restrain larger molecules from migrating as fast as smaller ones. Because the charge-to-mass ratios are nearly the same among SDS-denatured polypeptides, the final separation of proteins is dependent almost entirely on the difference in relative molecular masses of the polypeptides. In a gel of uniform density the relative migration distance of a protein ( $R_f$ ) is negatively proportional to the log of the mass of the protein. If proteins of known masses are run simultaneously with the unknowns, the relationship between  $R_f$  and mass can be plotted and the masses of unknown proteins can be estimated.

Protein separation by SDS-PAGE can be used to estimate the relative molecular mass, determine the relative abundance of major proteins in a sample and to determine the distribution of proteins among fractions.

### 1. EQUIPMENT

Vertical slab electrophoresis chamber

### 2. REAGENTS

#### 2.1 1×Laemmli SDS electrophoresis buffer (running buffer)

Reagent	Amount	Final concentration
Tris base	3.03 g	25 mmol/L
Glycine	14.4 g	192 mmol/L
SDS	1 g	0.1% (w/v)
make up to 1L with distilled water		

## 2.2 Monomer stock solution (30% acrylamide, 0.8% N,N'-methylenebisacrylamide)

Reagent	Amount	Final concentration
acrylamide	300 g	30% (w/v)
N,N'-methylenebisacrylamide	8 g	0.8% (w/v)
make up to 1L with distilled water		

## 2.3 4×resolving separating gel buffer solution

Reagent	Amount	Final concentration
Tris base	18.17 g	1.5 mol/L
Double-distilled water	—	75 ml
HCl	—	Adjust to pH 8.9
make up to 100 ml with distilled water		

## 2.4 8×resolving stacking gel buffer solution

Reagent	Amount	Final concentration
Tris base	5.98 g	0.5 mol/L
Double-distilled water	—	75 ml
HCl	—	Adjust to pH 6.8
make up to 100 ml with distilled water		

## 2.5 10% SDS solution

Reagent	Amount	Final concentration
SDS	5.0 g	10% (w/v)
make up to 50 ml with distilled water		

## 2.6 10% Ammonium persulfate solution

Reagent	Amount	Final concentration
Ammonium persulfate	0.1 g	10% (w/v)
make up to 1 ml with distilled water		

## 2.7 2×Protein resolving solution

Reagent	Amount	Final concentration
Tris-HCl (500 mmol/L, pH 6.8)	25 ml	125 mmol/L
SDS	4 g	4 % (w/v)
Glycerol	20 ml	20% (v/v)
1% Bromophenol blue stock solution	200 µl	0.002% (w/v)
2-Mercaptoethanol	140 µl	0.14% (v/v)
make up to 100 ml with distilled water		



## 2.8 Gel fixing solution

Reagent	Amount	Final concentration
100% ethanol	40 ml	40% (v/v)
100% acetic acid	10 ml	10% (v/v)
make up to 100 ml with distilled water		

## 2.9 Gel staining solution

Reagent	Amount	Final concentration
Coomassie Brilliant Blue R-250	0.25 g	0.25% (w/v)
100% ethanol	40 ml	40% (v/v)
100% acetic acid	10 ml	10% (v/v)
make up to 100 ml with distilled water		

## 2.10 Gel destaining solution

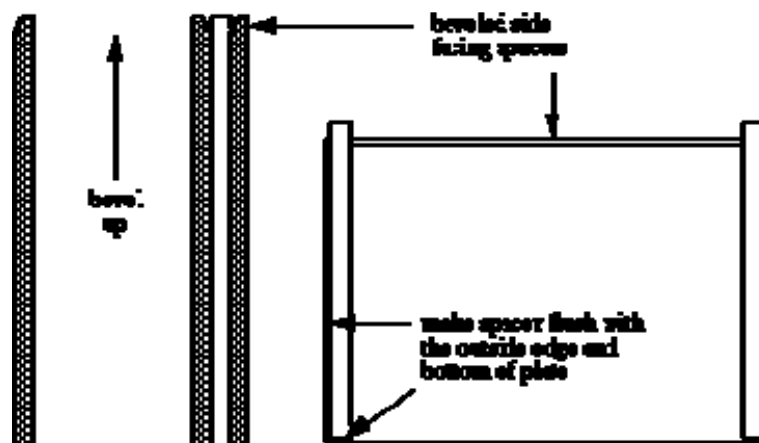
Reagent	Amount	Final concentration
100% ethanol	40 ml	40% (v/v)
100% acetic acid	10 ml	10% (v/v)
make up to 100 ml with distilled water		

# 3. METHODS

## 3.1 Preparing SDS gels

### 3.1.1 Cassette

As shown in Figure 1, two clean plates with two Teflon spacers make a cassette.



**Figure 1.** A gel cassette, properly assembled.

### 3.1.2 Separating gel preparation.

Depending on the range of the proteins that we wish to separate, composition 7% to 20% acrylamide gels are prepared according to Table 1. Acrylamide polymerizes spontaneously in the absence of oxygen, so the polymerization process involves complete removal of oxygen from the

solution. Polymerization is more uniform if the gel mix is de-gassed to remove much of the dissolved oxygen by placing it under a vacuum for 5 minutes or so before polymerization. Polymerization is initiated by adding freshly prepared 10% ammonium persulfate (AP) to the mix followed by TEMED. After swirling to mix, the solution is simply poured into the space occupied by the cassettes.

As shown in Figure 2, immediately after pouring the gel mix, it must be overlaid with water-saturated butanol to an additional height of 0.5 cm or so. Butanol is the top layer in the stock container. The purpose of butanol is to produce a smooth, completely level surface on top of the separating gel so that the bands are straight and uniform.

### 3.1.3 Stacking gel preparation

Stacking gel mix is made according to Table 1. Before adding the last two components which will start polymerization, the butanol should be poured off the separating gels into a sink with the tap water running and the excess butanol and acrylamide should be removed from the surface with a pipet. After adding AP and TEMED, the mix is immediately swirled and poured into the cassettes to the tops of the plates. We insert combs one at a time. Watch and try not to catch bubbles under the teeth. Adjust to make them even if necessary. Scrape the excess stacking mix off later (see Figure 2).

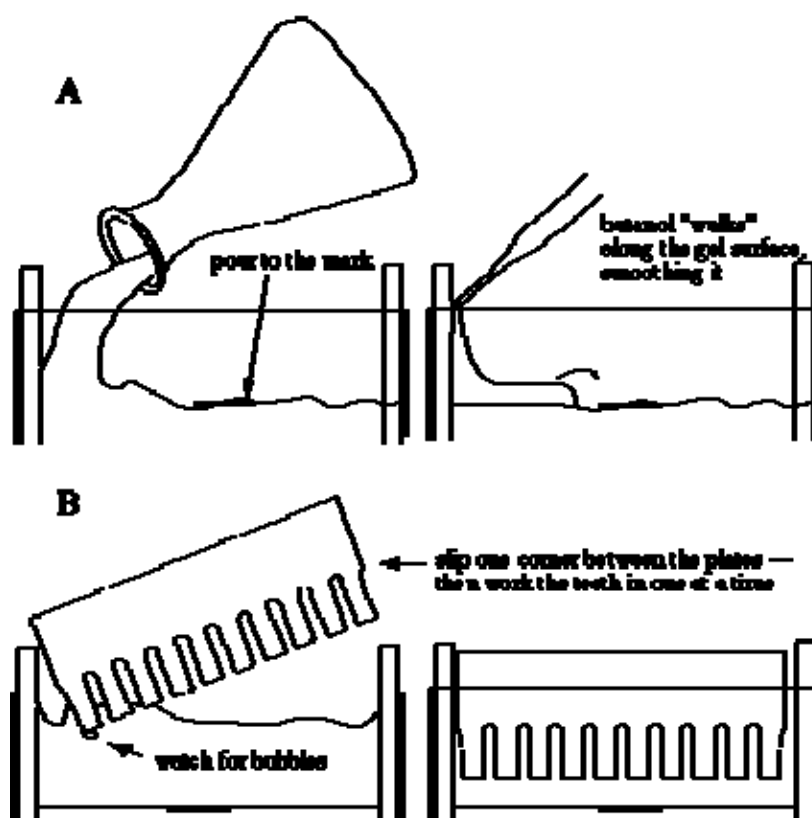


Figure 2. Gel preparation.

**Table 1** The recipe for different concentration gels

Stock solution	30 ml of different concentration separating gels (ml)					10 ml of 3% stacking gel (ml)
	7%	10%	12%	15%	20%	
Monomer stock solution	7	10	12	15	20	1
4×resolving separating gel buffer solution	7.5	7.5	7.5	7.5	7.5	—
8×resolving stacking gel buffer solution	—	—	—	—	—	1.25
10% SDS	0.3	0.3	0.3	0.3	0.3	0.1
Double-distilled water	15	12	10	7	2	6.9
10% Ammonium persulfate	0.15	0.15	0.15	0.15	0.15	0.75
TEMED	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l	5 $\mu$ l

### 3.2. Preparing protein samples for electrophoresis

A polypeptide is a macromolecule consisting of a nonbranching sequence of amino acids, each connected to the next by a single peptide bond. A protein consists of one or more polypeptides and/or additional types of molecules, held together by any number of molecular interactions often including covalent bonds. Such interactions result in several levels of organization which we call primary, secondary, tertiary, and quaternary structures. Intact proteins are notoriously difficult to separate reproducibly. Patterns of bands vary depending on temperature, buffer, variations in pH, quality of a preparation, etc. The purpose of sample preparation is to take proteins apart so that the primary structure is left only.

All of the unknown protein samples are diluted to the same concentration. Then 1 volume prepared sample is mixed to 1 volume 2×Protein resolving solution. Heat the samples to at least 95°C, keep for 5 minutes and cool on ice immediately. Allow SDS to bind in the hydrophobic regions of protein and complete the denaturation.

### 3.3. Assembling, loading, and running gels

Cassettes should be rinsed free of any excess liquid, leaving the combs in place. The assembly of a gel running stand varies with the type of apparatus. The top of the cassette must be continuous with an upper buffer chamber and the bottom must be continuous with a lower chamber so that current will run through the gel itself. Both the upper and lower buffer compartments are filled

with the electrode buffer (running buffer). The comb is removed from the gel before filling the upper buffer compartment.

### **3.3.1 Loading samples**

Microinjector works well for loading samples into the wells. Ideally, the glycerol in a sample makes it sink neatly to the bottom of the well, allowing as much as 20  $\mu\text{l}$  or even more to be loaded. If the combs do not fit well or the plates are not clean, the sample often hangs up and the sample volume is limited to 10  $\mu\text{l}$  or so.

### **3.3.2 Running gels**

The anode (+ electrode) must be connected to the bottom chamber and the cathode to the top chamber. The negatively-charged proteins will move toward the anode, of course. Gels are usually run at a voltage to run the tracking dye to the bottom as quickly as possible without overheating the gels. Overheating can distort the acrylamide or even crack the plates. The voltage to be used is determined empirically.

### **3.4 Disassembly and staining**

When the dye front is nearly at the bottom of the gel it is time to stop the run. For low percent gels with a tight dye front, the dye should be on the verge of running off the gel. Before removing gels the power must be turned off and the cables must be removed. The plates are separated and the gel is dropped into a staining dish containing deionized water. After a quick rinse, the water is poured off and gel fixing solution is added. Fixing requires incubation for 2 hours with agitation at room temperature.

After the gel fixation, the fixing solution is poured off and the gel staining solution is added. Staining is usually done overnight with agitation at room temperature. The agitation circulates the dye, facilitating penetration and helps ensure uniformity of staining.

The dye actually penetrates the entire gel. However it only sticks permanently to the proteins. Excess dye is washed out by gel destaining solution, also with agitation. Properly stained/destained gels should display a pattern of blue protein bands against a clear background. The gels can be dried down or photographed for later analysis and documentation.

### **3.5 Analysis of protein SDS-PAGE gels**

The resources on protein gel analysis focus on "routine" gels that are used to separate polypeptides from samples containing a mix of proteins. Before starting an analysis one's goals should be defined. Here are some observations that one may make using a Coomassie Brilliant Blue stained gel.

- Estimated molecular masses and relative abundance of unknown polypeptides in a complex mixture
- Patterns of bands that suggest presence of isoenzymes or specific complex proteins
- Effectiveness of a separation procedure during cell/tissue fractionation
- Effectiveness of a procedure to purify specific organelles, proteins, or polypeptides

- Condition of a preparation such as extent to which proteins have degraded
- Similarity of one preparation to another
- Changes in gene expression during developmental stages or resulting from experimental intervention

Semi-quantitative analysis of a gel begins with a cursory examination to see if the results make sense. Often an investigator repeatedly runs samples that give reproducible patterns. A change in the pattern may indicate that something is wrong. The following steps describe how one can calibrate and interpret a gel on which a series of aliquots from a fractionation procedure is run.

- Identify top/bottom and left/right
- Identify which lane corresponds to which sample
- Identify the lane or lanes to be analyzed
- Assess the success of the fractionation – do fractions overlap, that is, "share" the same polypeptide band(s)
- Calibrate the gel using standards of known molecular mass (set up a standard curve if necessary)
- Select polypeptide bands in the lane(s) of interest to be analyzed and identify them by some generic label (e.g., a, b, c,... or 1, 2, 3,...)
- Estimate molecular mass or relative molecular mass for each band of interest
- Note differences in intensity of staining that reflect relative abundance of individual polypeptides
- Note unusual patterns that might indicate isoenzymes, incomplete denaturation, degradation, etc.
- Note qualitative differences among bands that suggest presence of hydrophobic regions and/or covalent bonding to non-protein substituents
- Use the available information to characterize the unknowns

This method does have limitations. For example, identification of a band on a protein gel is not considered a positive proof of identity. A great many different polypeptides have very similar molecular masses. One band may mask the presence of more than one polypeptide. Incomplete denaturation, unusual amino acid sequences, and/or presence of non-protein residues can affect mobility, resulting in considerable error when estimating molecular mass. A unique band may be nothing more than a product of degradation of a heavier polypeptide or an aggregate of two or more lighter ones.

## REFERENCE

This protocol mainly referred to the methods from website of Protocol Online ([www.protocol-online.org](http://www.protocol-online.org)).

# CHAPTER 18

## ANALYSIS OF SALT-STRESS PROTEINS

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Plants are constantly subject to adverse environmental conditions including drought, flooding, extreme temperatures, excessive salts, and so on. Because of their immobility, plants have to make necessary metabolic and structural adjustments to cope with the stress conditions. Stress-induced changes in plant metabolism and development can often be attributed to altered patterns of gene expression. In response to stress, some genes are expressed more intensively, whereas others are repressed. The protein products of stress-induced genes often accumulate in response to unfavourable conditions. The functions of these proteins, named stress proteins, and the mechanisms that regulate their expression are currently a central topic of research in stress physiology. The salt-stress proteins are identified as new synthesized or increased proteins in plant as well as callus and suspension cultured plant cells under salt stress conditions. Since Singh (1983) reported that NaCl and PEG could induce synthesis of new polypeptides in cultured tobacco cells, many researchers have been working on salt-stress proteins.

### 1. EQUIPMENT

- Refrigerated centrifuge;
- Vacuum pump;
- Electrophoretic apparatus;
- Vertical slab electrophoresis chamber;
- Homoisothermy shoker;
- Microinjector.

### 2. REAGENTS

- Protein extracting solution: 75 mmol/L phosphate buffer, pH 7.8
- Protein resolving solution
- 1×Laemmli SDS electrophoresis buffer
- Monomer stock solution
- Gel staining solution

- Gel destaining solution
- 1.5 mol/L Tris-HCl, pH 8.9
- 0.5 mol/L Tris-HCl, pH 6.7
- 10% SDS
- 10% Ammonium persulfate

### 3. METHODS

#### 3.1. Extraction of total leaf protein

Leaf samples are harvested from the control and NaCl treated plants after 7, 14, 30 and 45d of treatment. Samples (0.5g) are homogenized with 3ml of protein extracting solution in a chilled pestle and mortar at 4°C. The homogenate is centrifuged in a refrigerated centrifuge (Sigma, 3K15, Germany) at 14,000×g for 10 min. The supernatants are precipitated with 4×V chilled 100% acetone and incubated overnight at -20°C in order to remove the pigments. The pellet is centrifuged at 10,000×g for 10 min, and resolved in protein resolving solution. The resolved samples are heated for 5 min at 95°C, cooled on ice before loading on polyacrylamide slab gels.

#### 3.2. Analysis of protein profile of leaf by SDS-PAGE

Use the vertical slab electrophoresis chamber. The separating gel and the stacking gel are made according to table 1. Samples containing 50 µg of protein are injected in wells. Electrophoresis is accomplished at 10-20 mA for 4 h.

**Table 1** The recipe for making running gels

Reagent	11% separating gel	3% stacking gel
Monomer stock solution	4.95 ml	1.0 ml
1.5 mol/L Tris-HCl, pH 8.9	3.375 ml	—
0.5 mol/L Tris-HCl, pH 6.7	—	2.5 ml
Double-distilled water	4.92 ml	6.3 ml
10% SDS	135 µl	100 µl
TEMED	6.75 µl	15 µl
10% Ammonium persulfate	108 µl	75 µl
Total Volume	13.5 ml	10 ml

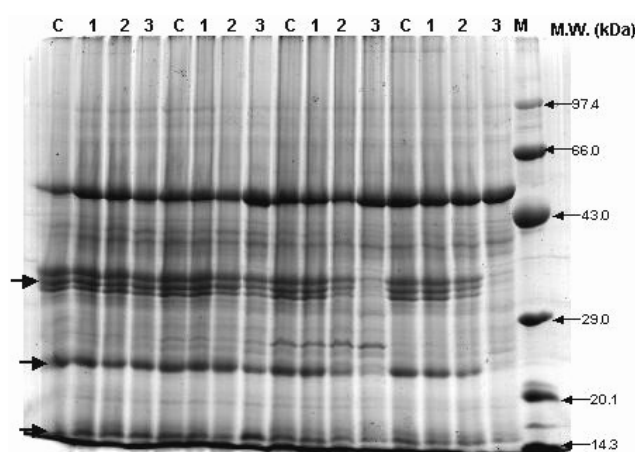
#### 3.3. Gel staining

The gels are stained with gel staining solution for 10 h and destained with gel destaining solution until the background is clear. The gels are photographed.

### 4. DATA ANALYSIS

Total proteins are extracted from leaves of the control and NaCl treated plants after 7, 14, 30 and 45 d of treatment and analysed by SDS-PAGE. As visualized from SDS-PAGE intensity of several protein bands of molecular weight 17, 23, 32, 33 and 34 kDa decreased as a result of NaCl

treatment (Fig. 1). The degrees of decrease of these protein bands seem to be roughly proportional to the external NaCl concentration (0, 100, 200 and 400 mM). The dependence of the dissociation of the protein on the length of exposure of plants to salt is shown in Fig. 1. Plants are transferred to a medium containing 100 to 400 mM NaCl and incubated for 45 d. At the end of 7, 14, 30 and 45 d of incubation period, the protein extracted from leaves is analyzed by SDS-PAGE and Coomassie blue staining (Fig. 1). Analysis of the gel has shown that the decrease in the amount of protein band is dependent on the days of treatment. When plants are grown on 400 mM NaCl decrease of these protein bands is detectable after 2 weeks and more prominent decrease is noticed after 45 d of treatment. After 45 d in 400 mM NaCl, the protein band of molecular weight 23 kDa almost disappear (Fig. 1).



**Fig. 1.** Effects of NaCl (0 to 400 mM) on polypeptide patterns of total protein from leaves as analyzed on SDS-PAGE. Lanes C, 1, 2 and 3 from left to right represent proteins extracted from control, 100, 200 and 400 mM NaCl treated plants after 7, 14, 30 and 45 d of treatment, respectively. Lane M represents the molecular weight marker. The bold arrows on the left indicate the polypeptides, which showed major changes under salt stress.

## REFERENCE

Parida AK, Das AB, Mitra B, Mohanty P, 2004. Salt-stress induced alterations in protein profiles and protease activity in the mangrove *Bruguiera parviflora*. *Z. Naturforsch.* 59c: 408-414



## Appendix 1:

National Standard of  
the People's Republic of China

**Technical Specification for  
Identification and Evaluation of  
Salt Tolerance in Wheat**

(In process of approval)

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*(The main developers of this National Standard of the People's Republic of China were Mr. Yuejin Weng and Mr. Zheng Hu from the Institute of Crop Science, Chinese Academy of Agricultural Sciences. This Standard is in process of approval by General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China & Standardization Administration of the People's Republic of China)*

# Technical Specification for Identification and Evaluation of Salt Tolerance in Wheat

## 1 Scope

This Standard provides the methods, grading judgment and repeat trial circumstances for identification of salt tolerance in wheat (*Triticum aestivum* L.) at germination, seedling stage and throughout the entire growing period.

This Standard is suitable for salt tolerance identification of wheat germplasm resources, genetic materials, new varieties, new lines and gene transgenic products.

## 2 Normative references

The following standards contain provisions that, through reference in this text, constitute provisions of this National Standard of the People's Republic of China. All standards are subject to revision. The amendments (corrigenda not included) and revised editions of the reference standards that are dated are not applicable to this Standard. But parties to agreements based on this National Standard of the People's Republic of China are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. The most recent editions of the reference standards that are undated are applicable to this Standard.

GB/T 3543. 4 Crop Seed Test Agreement      Germination test

GB7871-87 Measurement of Soil Salt content (Conductivity method)

## 3 Terms and definitions

For the purposes of this National Standard of the People's Republic of China, the following terms apply.

### 3.1 Relative salt injury rate

Relative salt injury rate shows the degree of influence of salt stress on germination rate.

$$\text{Relative salt injury rate} = \frac{\text{Decrement of germination percentage caused by salt stress}}{\text{Germination percentage of the control}} \times 100\%$$

### 3.2 Salt injury index

The wheat seedlings are divided into 5 classes according to the injury of seedlings under salt stress.

$$\text{Salt injury index} = \frac{\sum_{i=1}^5 \text{Weight of Class } i \times \text{No. of seedlings in Class } i}{\text{Maximum weight} \times \text{total No. of seedlings}} \times 100\%$$

### 3.3 Salt tolerance index

$$\text{Salt tolerance index} = \frac{\text{Character value under salt stress conditions}}{\text{Character value under control conditions}} \times 100\%$$

### 3.4 Salt tolerance ability

Salt tolerance ability shows the ability of a certain character to bear salt stress.

$$\text{Salt tolerance ability} = \frac{\text{Salt tolerance index of the test variety}}{\text{Salt tolerance index of the control variety}}$$

## 4 Test methods for identification of salt tolerance in wheat

### 4.1 Test at germination

#### 4.1.1 Sample preparation

Mix the sample seeds thoroughly and randomly pick 800 plump and uninjured seeds. Put the seeds in an aluminum box 7 cm in diameter. Keep the aluminum box in an air-dry box at a constant temperature of 35°C for heating and drying for 3 days. Cool to room temperature. Divide the 800 seeds into two halves. 400 seeds are used for salt stress treatment and the other 400 seeds are for control. Sample another 500 seeds, put in an aluminum box, seal and label it, keep it on file.

#### 4.1.2 Culture under salt stress

Place 2 layers of sterile filter paper in a culture dish 11 cm long x 11 cm wide x 3 cm tall. 400 seeds are divided into 4 repeats, 100 seeds per repeat. Place the seeds evenly on the filter paper in the culture dish with the furrows facing down, one culture dish for one repeat, labeled as T1, T2, T3 and T4. Add 10 ml of 350mMol NaCl (C.P.) in each culture dish. Keep the culture dishes in a biochemical incubator at 20°C for 10 days. Keep moist and prevent mildew.

#### 4.1.3 Control

Compare to the culture under salt stress, instead of 350mMol NaCl (C.P.), add 10 ml of sterile water in the culture dishes. The culture dishes for each repeat are labeled as CK1, CK2, CK3 and CK4. Based on the Crop Seed Test Agreement (GB/T 3543. 4), investigate the germination percentage after 7 days.

#### 4.1.4 Observation and result

When identifying salt tolerance at germination, the criteria for seed germination are that the pumule is longer than 1/3 of the seed length and the radical is longer than 1/2 of the seed length. Investigate the germination status of the control after 7 days of culture. Count seed germination number of CK1, CK2, CK3 and CK4. Investigate the germination status of the stress treatment after 10 days of culture. Count seed germination number of T1, T2, T3 and T4. Fill out form D1 (see Annex D). Calculate the relative salt injury rate (  $\alpha$  ) as follows:

$$\alpha (\%) = \frac{X_{CK} - X_T}{X_{CK}} \times 100 \dots \dots \dots (1)$$

where  $\alpha$  : relative salt injury rate,  $X_{CK}$ : average germination percentage of the control,  $X_T$ : average germination percentage of the salt stress treatment. The result of the calculation is accurate to the first position after the decimal point.

## 4.2 Test at the seedling stage

### 4.2.1 Instruments and equipment

Illumination box;

Air pump SP-780 for a fish tank;

Culture slot: 0.46m long x 0.40m wide x 0.3m deep;

Styrofoam plate: 0.45m long x 0.40m wide x 0.01m thick. Drill 100 (10 x 10) holes with a diameter of 0.2cm on the side of 0.45m long x 0.40m wide. Distance between holes on the long side of the rectangle is 5 cm, and distance between holes on the short side of the rectangle is 4 cm.

### 4.2.2 Culture under salt stress

Sample more than 100 plump and uninjured seeds. Based on the Crop Seed Test Agreement (GB/T 3543. 4), seeds are cultured for germination under normal conditions for 10 days. Make sure to get 100 seedlings.

When the seedlings grow to 1-leaf stage, move them individually into the holes on styrofoam plate. Float the styrofoam plate in the culture slot filled with Hoagland solution (see Annex A). The Hoagland solution is 15 cm deep with its surface being 15 cm away from the slot edge. Put the culture slot in an illumination box. Set the temperature in the illumination box at 25°C. Provide 16 hours of daily light and about 1000 Lx of light irradiance. After 3 days, add NaCl in Hoagland solution and measure the conductivity (see Annex C). Keep the stress strength at  $30 \pm 1$  ds/m for 20 days. Pump air in the Hoagland solution twice a day for 4 hours each time.

### 4.2.3 Results

The stressed wheat seedlings are water-cultured in the illumination box at 25°C. After 20 days, investigate the growth of the seedlings (see Annex B). Fill out form D2 (see Annex D). Calculate the salt injury index ( $\beta$ ) as follows:

$$\beta (\%) = \frac{\sum C_i N_i}{5 \times 100} \times 100 \dots \dots \dots (2)$$

where  $\beta$  : salt injury index,  $C_i$ : the class number (i) of the seedlings,  $N_i$ : the number of seedlings in class i, 100: the total number of seedlings, 5: the highest class number of the seedlings. The result of the calculation is accurate to the first position after the decimal point.

### 4.3 Test through the entire period of growth

#### 4.3.1 Salt pond

Build 4 cement ponds with bricks and cement. The size of each pond is 7m long x 1m wide x 1m deep. These 4 ponds serve for the treatment of the control variety under the normal conditions, control variety under the salt stress conditions, test variety under the normal conditions, and test variety under the salt stress conditions, respectively. Fill the ponds with well-mixed loam soil. Provide outfalls underneath the ponds. Build a canopy to keep out the rain with transparent glass at the height of 2 meters above the ground. Provide water storage at the bottom of the salt ponds containing gravel to a depth of 15 cm and fine sand to a depth of 5 cm. Water supply imitates the groundwater recharge by way of infiltration irrigation from the water storage zone.

#### 4.3.2 Regulation of salt in the pond

Add NaCl solution in the two ponds for salt stress treatment to make the salt concentration equivalent to soil salt content of 0.4%. The method is to collect a soil sample at a fixed point in the pond every 10 days. Measure the change of salt content according to the Measurement of Soil Salt content (Conductivity method) (GB7871-87). By spraying brine or freshwater the conductivity of the soil in the pond is adjusted to  $1.6 \pm 0.2$  ds/m which is the equivalent of the standard soil salt content of 0.4%.

#### 4.3.3 Salt stress treatment

Divide 200 seeds of the test variety into 2 groups, 100 seeds per group. Sow one group in the salt-stressed pond and the other group in the non-stressed pond separately. Use variety Kharchia or local salt-tolerant wheat variety as the control variety. Add a special control variety if necessary. For example, it is necessary to add the receptor parent as the control for a transgenic product. The control variety is sown in both the salt-stressed pond and the non-stressed pond. Spaces between rows and between plants are 20 cm and 5 cm, respectively. During the growing period be aware of and control diseases and pests in a timely manner. As much as possible, attempt to simulate the natural conditions and conduct the same water and fertilizer management as in the field.

#### 4.3.4 Results

Harvest the test variety and the control variety when they are ripe. Randomly sample 10 plants in each treatment. Investigate the three evaluating characters - plant height, the number of grains per plant and 1000-grain weight. Fill out form D3 (see Annex D). Calculate the salt tolerance index (  $\gamma$  ) and salt tolerance ability (  $\delta$  ) as follows:

$$\gamma (\%) = \frac{X_i}{Y_i} \times 100 \dots \dots \dots (3)$$

where  $\gamma$  : salt tolerance index of character i,  $X_i$ : average value of character i under salt stress conditions,  $Y_i$ : average value of character i under control conditions. The result of the calculation is accurate to the first position after the decimal point.

$$\delta = \frac{Y_S}{Y_T} \dots\dots\dots (4)$$

where  $\delta$  : salt tolerance ability of a certain character,  $Y_S$ : salt tolerance index of the test variety,  $Y_T$ : salt tolerance index of the control variety (Kharchia or local salt-tolerant variety). The result of the calculation is accurate to the second position after the decimal point.

## 5 Judgment of salt tolerance of wheat

### 5.1 Grading of salt tolerance at germination

The salt tolerance of wheat at germination is divided into 5 grades according to the relative salt injury rate (Table 1).

**Table 1** Grading of salt tolerance at germination of wheat

Salt tolerance	Relative salt injury rate	Grade of salt tolerance
High tolerance (HT)	0.0--20.0%	Grade 1
Tolerance(T)	20.1%--40.0%	Grade 2
Medium tolerance(MT)	40.1%--60.0%	Grade 3
Sensitivity(S)	60.1%--80.0%	Grade 4
High sensitivity (HS)	80.1%--100.0%	Grade 5

### 5.2 Grading of salt tolerance at the seedling stage

The salt tolerance of wheat at the seedling stage is divided into 5 grades according to the salt injury index (Table 2).

**Table 2** Grading of salt tolerance at the seedling stage of wheat

Salt tolerance	Salt injury index	Grade of salt tolerance
High tolerance (HT)	0.0—20.0%	Grade 1
Tolerance(T)	20.1%--40.0%	Grade 2
Medium tolerance(MT)	40.1%--60.0%	Grade 3
Sensitivity(S)	60.1%--80.0%	Grade 4
High sensitivity (HS)	80.1%--100.0%	Grade 5

### 5.3 Grading of salt tolerance throughout the entire growing period

The salt tolerance of wheat throughout the entire growing period is divided into 5 grades according to the salt tolerance ability (Table 3).

**Table 3** Grading of salt tolerance throughout the entire growing period of wheat

<b>Salt tolerance</b>	<b>Salt tolerance ability</b>	<b>Grade of salt tolerance</b>
High tolerance (HT)	>0.80	Grade 1
Tolerance(T)	0.61-0.80	Grade 2
Medium tolerance(MT)	0.41-0.60	Grade 3
Sensitivity(S)	0.21-0.40	Grade 4
High sensitivity (HS)	0.00-0.20	Grade 5

## 6 Repeat trial

A repeat trial needs to be done under the following circumstances.

- Seed germination number of the control is less than 50 when identifying salt tolerance at germination, or the number of seedlings or plants of the control is less than 50 when identifying at the seedling stage or throughout the entire growing period.
- Infection by fungi or bacteria affects the result.
- The trial can not be conducted as planned because of human factors such as power outages.
- Errors between the repeats of the treatment or the control are higher than 10%.

## Annex A: Solution preparation

### A.1 Preparation of 350mMol NaCl solution

Weigh 10.227g of NaCl (C.P.) on a 1/1000 Balance and then put in a 500 ml volumetric flask. Add sterile water, stir, and determine the volume to 500 ml. The sterile water is prepared with distilled water of which conductivity is zero.

### A.2 Preparation of Hoagland solution (C.P.)

Hoagland solution is prepared according to the following formula.

KNO <sub>3</sub>	0.51g/L
Ca(NO <sub>3</sub> ) <sub>2</sub>	0.82g/L
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.49g/L
KH <sub>2</sub> PO <sub>4</sub>	0.136g/L
*FeEDTA	1ml
**A-Z	1ml

Where: \*FeEDTA is prepared by weighing 7.45g of Na<sub>2</sub>EDTA and 5.57g of FeSO<sub>4</sub>.7H<sub>2</sub>O, put in 1 L volumetric flask and then determined to 1 L.

\*\*A-Z is prepared as follows:

H <sub>3</sub> BO <sub>3</sub>	2.80g/L
CuSO <sub>4</sub> .5H <sub>2</sub> O	0.08g/L
ZnSO <sub>4</sub> .7H <sub>2</sub> O	0.22g/L
MnCl <sub>2</sub> .6H <sub>2</sub> O	1.18g/L
MoO <sub>4</sub> .4H <sub>2</sub> O	0.09g/L

### A.3 Preparation of solution with 30 ± 1ds/m conductivity

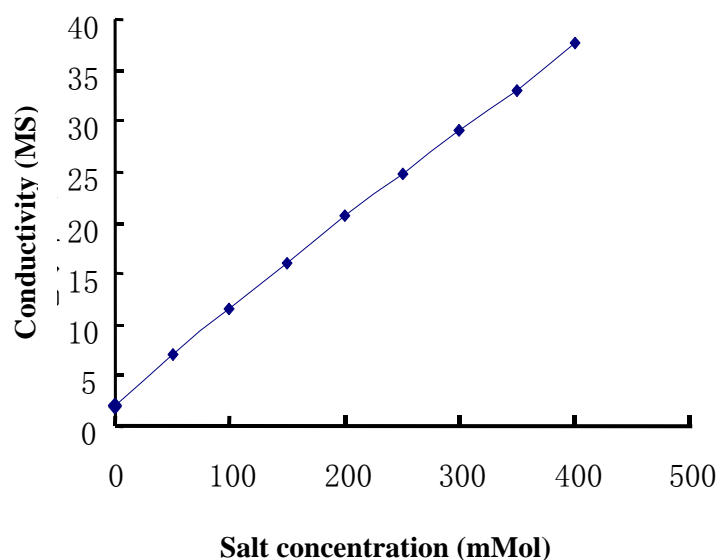
Weigh 72.0g of NaCl (C.P.) on a 1/1000 Balance. Determine the volume to 4 L with Hoagland solution.



## Annex B: Classification of salt stressed wheat seedlings at 4 leaf stage according to seedling condition

Classification of seedlings	Injury of seedling
Class 1	Growth almost normal, having 3-4 green leaves
Class 2	Growth almost normal, having 3-4 green leaves, leaf apexes turning yellow or green wilting
Class 3	Growth inhibited, having 2 green leaves
Class 4	Seriously injured, having only 1 green leaf or only the heart leaf alive
Class 5	The whole seedling dead or dying

## Annex C: Figure of relationship between salt and conductivity



## **Annex D: Tables of Original Record for identification of salt tolerance in wheat**

D1 Table of Original Record for identification of salt tolerance in wheat at germination

D2 Table of Original Record for identification of salt tolerance in wheat at the seedling stage

D3 Table of Original Record for identification of salt tolerance throughout the entire growing period of wheat

## D1 Table of Original Record for identification of salt tolerance in wheat at germination

Total \_\_\_\_\_ pages; page \_\_\_\_\_

Sample name		Sample number		Test date	
Test location		Room temperature (°C)		Basis for Test	
Instrument name and serial number				Test category	

Treatment time		Investigation date on the control		Investigation date on the treatment	
----------------	--	-----------------------------------	--	-------------------------------------	--

Item		Total No. of seeds	Seed germination No.	Seed germination percentage (Ger) %
control	CK1	control		
	CK2			
	CK3			
	CK4			
	Average (X <sub>CK</sub> )			
Stressed treatments	T1			
	T2			
	T3			
	T4			
	Average (X <sub>T</sub> )			
Formula of Relative salt injury rate %		$\alpha = \frac{X_{CK} - X_T}{X_{CK}} \times 100$		
Relative salt injury rate %				

Tested by:

Double Checked by:

Approved by:

Date:

Date:

Date:

## D2 Table of Original Record for identification of salt tolerance in wheat at the seedling stage

Total \_\_\_\_\_ pages; page \_\_\_\_\_

Sample name		Sample number		Test date	
Test location		Room temperature (°C)		Basis for Test	
Instrument name and serial number				Test category	

Treatment time		Investigation date on the treatment	
----------------	--	-------------------------------------	--

	Seedlings in Class 0 (S <sub>0</sub> )	Seedlings in Class 1 (S <sub>1</sub> )	Seedlings in Class 2 (S <sub>2</sub> )	Seedlings in Class 3 (S <sub>3</sub> )	Seedlings in Class 4 (S <sub>4</sub> )	Seedlings in Class 5 (S <sub>5</sub> )
1-10						
11-20						
21-30						
31-40						
41-50						
51-60						
61-70						
71-80						
81-90						
91-100						
Total						
Formula of Salt injury index %	$\beta = \frac{\sum C_i N_i}{5 \times 100} \times 100$					
Salt injury index %						

Tested by:

Double Checked by:

Approved by:

Date:

Date:

Date:

### D3 Table of Original Record for identification of salt tolerance throughout the entire growing period of wheat

Total \_\_\_\_\_ pages; page \_\_\_\_\_

Sample name		Sample Number		Basis for Test	
Test category		Test location		Test date	
Control variety		Sowing date		Investigation date	
Instrument name and serial number					

Plant height (cm)	1	2	3	4	5	6	7	8	9	10	Average
Test variety, salt stress conditions											
Test variety, normal conditions											
Control variety, salt stress conditions											
Control variety, normal conditions											
Salt tolerance index of plant height =						Salt tolerance ability of plant height =					
No. of grains per plant	1	2	3	4	5	6	7	8	9	10	Average
Test variety, salt stress conditions											
Test variety, normal conditions											
Control variety, salt stress conditions											
Control variety, normal conditions											
Salt tolerance index of No. of grains per plant =						Salt tolerance ability of No. of grains per plant =					
1000-grain weight (g)	Test variety, salt stress conditions		Test variety, normal conditions		Control variety, salt stress conditions		Control variety, normal conditions				
Salt tolerance index of 1000-grain weight =						Salt tolerance ability of 1000-grain weight =					
Average salt tolerance ability = (Salt tolerance ability of plant height + Salt tolerance ability of No. of grains per plant + Salt tolerance ability of 1000-grain weight) • 3 <sup>-1</sup>											

Tested by:

Double Checked by:

Approved by:

Date:

Date:

Date:

## Appendix 2:

**GB/T 21127 - 2007**

Approved National Standard of  
the People's Republic of China

### **Technical Specification for Identification and Evaluation of Drought Resistance in Wheat**

Approved and Issued October 16, 2007

**General Administration of Quality Supervision,  
Inspection and Quarantine of the People's Republic of China  
& Standardization Administration of the People's Republic of China**

In practice May 1, 2008

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*(The main developers of this National Standard of the People's Republic of China were Ruilian Jing, Ronghai Hu, Canjun Zhang, Zhihua Zhu, Xiaoping Chang and Juanling Wang from the Institute of Crop Science, Chinese Academy of Agricultural Sciences & Center for Supervision, Inspection and Test of Crop Germplasm Resource, Ministry of Agriculture of the People's Republic of China.)*

## **Technical Specification for Identification and Evaluation of Drought Resistance in Wheat**

### **1 Scope**

This Standard provides methods for the identification of drought resistance in wheat (*Triticum aestivum* L.) and rules for grading wheat's resistance to drought.

This Standard is applicable for the testing of drought resistance in wheat.

### **2 Normative references**

The following standard contains provisions that, through reference in this text, constitute provisions of this National Standard of the People's Republic of China. All standards are subject to revision, and parties to agreements based on this National Standard of the People's Republic of China are encouraged to investigate the possibility of applying the most recent edition of the standard indicated below.

GB/T 3543. 4 Crop Seed Test Agreement (Germination test)

### **3 Terms and definitions**

For the purposes of this National Standard of the People's Republic of China, the following terms and definitions apply.

#### **3.1 Control variety**

Control variety mentioned in this Standard is a wheat variety that is used as the control in the regional trials on dry lands of the same grade.

#### **3.2 Adjusting variety**

Adjusting variety in this Standard refers to a state approved wheat variety that has high resistance to drought. It is the standard variety that is used to adjust the identification results from a different batch of test varieties.

#### **3.3 Survival percentage after a repeated drought stress**

Survival percentage after a repeated drought stress is the percentage of surviving seedlings in the total number of seedlings after a repeated drought stress – rehydration treatment.

#### **3.4 Drought resistance index**

Drought resistance index is a criterion for judging the drought resistance of the test variety. It is based on the grain yield in comparison with the control variety.

## 4 Identification of drought resistance

The time to identify drought resistance can be chosen from germination, seedling stage, water critical period and the entire growing period. It depends on the objectives of the research work.

### 4.1 Identification test at germination

Hypertonic solution method is used.

Use -0.5MPa PEG-6000 solution to conduct water stress on the seeds. Use deionized water cultured seeds as the control. Germination dishes are 10 cm long x 10 cm wide x 5 cm tall plastic boxes. Put one layer of filter paper in the germination dish to be the germinating bed.

#### 4.1.1 Sample preparation

Mix the sample seeds thoroughly and randomly pick 800 seeds.

#### 4.1.2 Preparation of stress solution

Dissolve 192g of PEG-6000 in 1000 ml of deionized water to prepare -0.5MPa PEG-6000 solution.

#### 4.1.3 Culture under stress conditions

400 seeds are divided into 4 repeats, 100 seeds per repeat. Place the seeds evenly on the filter paper in the germination dishes, one dish for one repeat (consult GB/T 3543.4 ). Add 12 ml of -0.5MPa PEG-6000 solution in each germination dish and cover them.

#### 4.1.4 Culture under control conditions

Instead of -0.5MPa PEG-6000 solution, add 12 ml of deionized water in the germination dishes for the culture under the control conditions.

#### 4.1.5 Investigation of germination

Put the germination dishes in a incubator at 20℃. After culture for 8 days (168 hours), investigate the number of germinated seeds.

#### 4.1.6 Germination percentage

Calculate seed germination percentage as follows:

$$Ger_T = X_{Ger.T} \cdot X_{TS.T}^{-1} \cdot 100 \dots\dots\dots (1)$$

$$Ger_{CK} = X_{Ger.CK} \cdot X_{TS.CK}^{-1} \cdot 100 \dots\dots\dots (2)$$

$$R_{Ger} = Ger_T \cdot Ger_{CK}^{-1} \cdot 100 \dots\dots\dots (3)$$

where  $Ger_T$ : germination percentage of the stress culture,  $X_{Ger.T}$ : average number of germinated seeds of 4 repeats of the stress culture at 168 hr,  $X_{TS.T}$ : average total number of seeds of 4 repeats of the stress culture,  $Ger_{CK}$ : germination percentage of the control culture,  $X_{Ger.CK}$ : average number



of germinated seeds of 4 repeats of the control culture at 168 hr,  $X_{TS,CK}$ : average total number of seeds of 4 repeats of the control culture,  $R_{Ger}$ : relative germination percentage.

## **4.2 Identification test at the seedling stage**

The method of repeated drought stress - rehydration is used.

### **4.2.1 Culture temperature**

$20^{\circ}\text{C} \pm 5^{\circ}\text{C}$

### **4.2.2 Trial design**

3 repeats, 50 seedlings per repeat, cultured in the plastic boxes.

### **4.2.3 Sowing**

Load 10 cm of loam soil with middle fertility (unit yield about  $3000\text{kg}/\text{hm}^2$ ) in the plastic boxes of 60 cm long x 40 cm wide x 15 cm tall. Water till  $85\% \pm 5\%$  of field capacity. Sow the seeds and cover 2 cm of soil.

### **4.2.4 First drought stress – rehydration treatment**

Stop watering and start the first drought stress at 3-leaf stage. Rehydrate when the soil water content decreases to 20% - 15% of field capacity. Increase the soil water content to  $80\% \pm 5\%$  of field capacity. After 120 hours of rehydration, investigate the number of surviving seedlings. Those seedlings with leaves turning fresh and green again are counted as live ones.

### **4.2.5 Repeated drought stress - rehydration treatment**

After the first rehydration is done, repeat the drought stress – rehydration treatment once again. Investigate the number of surviving seedlings after the second rehydration for 120 hours.

### **4.2.6 Measured value of survival percentage after a repeated drought stress**

The formula to calculate the measured value of survival percentage after a repeated drought stress is as follows:

$$\begin{aligned} DS &= (DS1 + DS2) \cdot 2^{-1} \\ &= (X_{DS1} \cdot X_{TT}^{-1} \cdot 100 + X_{DS2} \cdot X_{TT}^{-1} \cdot 100) \cdot 2^{-1} \dots\dots\dots(4) \end{aligned}$$

where DS: measured value of survival percentage after a repeated drought stress, DS1: survival percentage after the first drought stress – rehydration treatment, DS2: survival percentage after the second drought stress – rehydration treatment,  $X_{TT}$ : average total number of seedlings of 3 repeats before the first drought stress,  $X_{DS1}$ : average number of surviving seedlings of 3 repeats after the first rehydration,  $X_{DS2}$ : average number of surviving seedlings of 3 repeats after the second rehydration.

### **4.2.7 Corrected value of survival percentage after a repeated drought stress**

First calculate the deviation of the measured value of survival percentage after a repeated drought stress of the adjusting variety according to formula (5). Then calculate the corrected value of survival percentage after a repeated drought stress of a test variety according to formula (6).

$$ADS_E = (ADS - ADS_A) \cdot ADS_A^{-1} \dots\dots\dots(5)$$

$$DS_A = DS - ADS_A \cdot ADS_E \dots\dots\dots(6)$$

where  $ADS_E$ : deviation of the measured value of survival percentage after a repeated drought stress of the adjusting variety,  $ADS$ : measured value of survival percentage after a repeated drought stress of the adjusting variety,  $ADS_A$ : corrected value of survival percentage after a repeated drought stress of the adjusting variety, i.e., the average value of the survival percentage after a repeated drought stress of the adjusting variety in many tests,  $DS_A$ : corrected value of survival percentage after a repeated drought stress of a test variety,  $DS$ : measured value of survival percentage after a repeated drought stress of the test variety.

### 4.3 Identification test during the water critical period

Drought resistance identification test during the water critical period can be done either in a drought-shed or in the field. A field test needs to be done in two locations.

#### 4.3.1 Trial design

Random arrangement and 3 repeats are designed in the trial. The plot size is 2 m<sup>2</sup> in drought-shed test and 6.7 m<sup>2</sup> in the field test. Sow the seeds at a proper time. The basic seedling densities of winter wheat and spring wheat are 2,250,000/ha and 3,750,000/ha, respectively.

#### 4.3.2 Drought stress treatment

Supply enough soil moisture before sowing. Water one time at heading stage and one time at filling stage to make the water content in the soil layer from 0-50 cm reach 80%±5% of field capacity.

#### 4.3.3 Control treatment

Supply enough soil moisture before sowing. Besides heading stage and filling stage, water one more time at jointing-booting stage. Make the water content in the soil layer from 0-50 cm reach 80%±5% of field capacity.

#### 4.3.4 Character to investigate

Grain yield per plot

#### 4.3.5 Drought resistance index

Calculate drought resistance index according to formula (7).

$$DI = GY_{S,T}^2 \cdot GY_{S,W}^{-1} \cdot GY_{CK,W} \cdot (GY_{CK,T}^2)^{-1} \dots\dots\dots(7)$$

where  $DI$ : drought resistance index,  $GY_{S,T}$ : grain yield per plot of test variety under drought stress treatment (kg),  $GY_{S,W}$ : grain yield per plot of test variety under control treatment (kg),  $GY_{CK,W}$ : grain yield per plot of control variety under control treatment (kg),  $GY_{CK,T}$ : grain yield per plot of control variety under drought stress treatment (kg).

### 4.4 Identification test throughout the entire growing period

Drought resistance identification test throughout the entire growing period can be done either in a drought-shed or in the field. A field test needs to be done in two locations. Sow the seeds at a proper time. The basic seedling densities of winter wheat and spring wheat are 2,250,000/ha and 3,750,000/ha, respectively.

#### **4.4.1 Drought-shed test**

##### **4.4.1.1 Trial design**

Random arrangement and 3 repeats are designed in the trial. The plot size is 2 m<sup>2</sup>.

##### **4.4.1.2 Drought stress treatment**

During the time from wheat harvest till next sowing, control the experimental field to accept natural precipitation by moving the drought-shed. Water storage in the soil layer from 0-150 cm is controlled at about 150 mm. If the natural precipitation is insufficient, irrigate to supplement water. Before sowing, make sure there is enough moisture in surface soil for seedling emergence. Proper irrigation is needed if the moisture in surface soil is not sufficient. The experimental field does not accept natural precipitation any more after sowing.

##### **4.4.1.3 Control treatment**

Set up the control treatment near the drought-shed. The conditions of the experimental field of the control including soil nutrient content, soil texture and soil depth should be basically identical with the experimental field in the drought-shed. The management of soil water should guarantee that the water status is suitable for wheat throughout the entire growing period. Before sowing, make sure there is enough moisture in surface soil for seedling emergence. Proper irrigation is needed if the moisture in surface soil is not sufficient. In addition, irrigate at jointing stage, heading stage and filling stage to make the water content in the soil layer from 0-50 cm reach 80%±5% of field capacity.

#### **4.4.2 Field test**

In areas where natural precipitation in normal years is less than 500 mm, or natural precipitation throughout the entire growing period of wheat is less than 150 mm, a field test should be used for identification of drought resistance.

##### **4.4.2.1 Trial design**

Random arrangement and 3 repeats are designed in the trial. The plot size is 6.7 m<sup>2</sup>.

##### **4.4.2.2 Drought stress treatment**

Before sowing, make sure there is enough moisture in surface soil for seedling emergence. Proper irrigation is needed if the moisture in surface soil is not sufficient.

##### **4.4.2.3 Control treatment**

Set up the control treatment near the drought stress treatment. The conditions of the experimental field of the control including soil nutrient content, soil texture and soil depth should be basically identical with that of the drought stress treatment. The management of soil water should guarantee that the water status is suitable for wheat throughout the entire growing period. Before sowing,

make sure there is enough moisture in surface soil for seedling emergence. Proper irrigation is needed if the moisture in surface soil is not sufficient. In addition, irrigate at jointing stage, heading stage and filling stage to make the water content in the soil layer from 0-50 cm reach 80%±5% of field capacity.

#### 4.4.3 Character to investigate

Grain yield per plot

#### 4.4.4 Drought resistance index

Calculate drought resistance index based on grain yield per plot. The formula is the same as formula (7) (see 4.3.5)

#### 4.5 Points for attention

During the period of drought resistance identification, the timely control of disease, pest and bird damage and the prevention of lodging are the points for attention.

### 5 Rules for grading drought resistance

The drought resistance of wheat is divided into 5 grades – highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S), and highly susceptible (HS).

#### 5.1 Grading of drought resistance at germination

See Table 1.

**Table 1** Grading of drought resistance at germination of wheat

Relative germination percentage %	Grade of drought resistance
$\geq 90.0$	Highly resistant (HR)
70.0-89.9	Resistant (R)
50.0-69.9	Moderately resistant (MR)
30.0-49.9	Susceptible (S)
$\leq 29.9$	highly susceptible (HS)

#### 5.2 Grading of drought resistance at the seedling stage

See Table 2.

**Table 2** Grading of drought resistance at the seedling stage of wheat

Corrected value of survival percentage after a repeated drought stress (a) (%)	Grade of drought resistance
$\geq 70.0$	Highly resistant (HR)
60.0 - 69.9	Resistant (R)
50.0 - 59.9	Moderately resistant (MR)
40.0 - 49.9	Susceptible (S)
$\leq 39.9$	Highly susceptible (HS)

### 5.3 Grading of drought resistance during the water critical period

See Table3

**Table 3** Grading of drought resistance during the water critical period of wheat

Drought resistance index	Grade of drought resistance
$\geq 1.30$	Highly resistant (HR)
1.10-1.29	Resistant (R)
0.90-1.09	Moderately resistant (MR)
0.70-0.89	Susceptible (S)
$\leq 0.69$	Highly susceptible (HS)

### 5.4 Grading of drought resistance throughout the entire growing period

The criterion used to judge the drought resistance throughout the entire growing period is the drought resistance index based on the grain yield per plot. See Table 3 for the grading of drought resistance throughout the entire growing period.

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