Chapter 1
Introduction

Abstract Salinity is a major abiotic stress limiting crop yields in many parts of the world. The FAO (Food and Agriculture Organization) Land and Plant Nutrition Management service estimates that over 400 million hectares (6 %) of the Earth's land is affected by salt. Breeding for salt tolerance is a major goal for cereal researchers for which screens are required to select out tolerant lines. Screening for salt tolerance in the field is difficult as soil salinity is dynamic, the level of salt varies both horizontally and vertically in the soil profile and changes with time. These environmental perturbations can be overcome by testing in hydroponic system where the testing environment is controlled.

1.1 Background

Soil salinity affects more than 800 million hectares worldwide, equivalent to over 6 % of all land on Earth. Of the 1500 million hectares cultivated in dry regions, 2 % are affected by salt. Of the 230 million hectares that are irrigated, 20 % are salt affected (Munns 2005). Irrigation exacerbates the problem as the irrigation waters bring dissolved salts which are deposited in the soil. History tells us of several civilisations collapsed because of salinisation of agricultural land due to irrigation, for example, the ancient Mesopotamian civilisation (now part of Iraq) faded away some 4400–3700 years ago due to crop failures caused by salinity. Crop records of Sumeria indicate a change of crop from wheat (salt sensitive) to barley (salt tolerant) and then a subsequent decline of barley yields as soils became increasingly saline. The Peruvian culture of the Viru Valley, which peaked 1200 years ago, was forced to retreat up into the highlands because of salinisation of fields (Pearce 1987; Jacobsen and Adams 1968).

Irrigation without adequate soil and salt management systems inevitably leads to salinisation of cultivated land. This is due to continual additions of soluble salts of sodium, calcium, magnesium and potassium, usually as chlorides or sulphates, which are concentrated in the soil as water is lost due to evaporation and crop plant transpiration. In addition, excess sodium (sodicity) promotes slaking of soil

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aggregates that degrades the soil structure and impedes water movement and root growth.

Saline environments are generally grouped as being either wet or dry. Wet saline habitats tend to occur in coastal regions and are dominated by salt marshes. Since these areas border the sea, they are subject to periodic inundations, and as a result the level of salinity fluctuates over time. Dry saline habitats are usually located inland, often bordering deserts (Tal 1985; Neumann 1997; Flowers 2004). Other types of saline environments include seashore dunes, where salt spray is a salinising factor, and dry salt lakes. Common features of saline environments are the salinity of the soil and/or of their associated water resources and specialised flora and fauna. The most abundant salts in saline soils are sodium chloride (NaCl) and sodium sulphate (Na_2SO_4), which may be associated with magnesium (Mg) salts.

Sustainable irrigation systems incorporate one or more forms of leaching and drainage of brackish water (slightly saline water). Leaching may be achieved by natural rainfall and run-off or by irrigation with fresh water and artificial drainage systems. In both systems, drainage needs to be provided. These may be small scale for subsistence farming communities or may involve massive civil engineering projects such as the West Bank Outfall drain of the river Indus in Pakistan (Khan et al. 2013). Cropping systems also need to be devised that maximise the benefit of seasonal conditions, e.g. exploitation of monsoon rains to leach out salts and early maturing crops that avoid high saline periods.

With increasing human populations, there is an increasing demand for food. Throughout the world, the best agricultural land is already fully utilised, and hence marginal land, including saline land, is being brought into agriculture. Unfortunately, most crop plants are sensitive to salt (glycophytes). Salinity is therefore a major environmental constraint to crop production throughout the world.

1.2 Biology of Salt Tolerance

Salt-tolerant plants have evolved in many taxa of the plant kingdom. Aronson (1989) noted over 100 plant families which contain salt-tolerant species. Most plant families contain a few salt-tolerant species (halophytes), but the Chenopodiaceae is an exception in containing over 350. It has been suggested that salt tolerance evolved in many higher plants as a consequence of becoming established in estuaries (O'Leary and Glenn 1994) and then spreading to inland environments. More than 30 % of extant plant families have halophytic members (*circa* 2500 species) which are mainly found in salt marshes or desert flats (Glenn 1997).

Ungar (1991) defined salt-loving plant, halophytes, as those that tolerate relatively high soil salinity and are capable of accumulating relatively high quantities of sodium and chloride; glycophytes on the other hand are defined as species that show little tolerance to elevated saline levels in the root zone and do not accumulate high concentrations of salts in growing tissues and organs. Extreme halophytes such as

Salicornia europaea and *Suaeda maritima* can tolerate saline water above that of sea water, whereas glycophytes are intolerant of salinities above 10 % of sea water.

In general, three physiological mechanisms are deployed by plants growing in saline conditions: (1) osmotic adjustment, (2) ion exclusion and (3) tissue tolerance to accumulated ions. The effects of salinity are first observed by a reduction in plant growth (Munns 1993), which has two response phases: (1) a rapid response to the increase in external osmotic pressure (the osmotic phase), which starts as soon as the salt concentration increases around the roots to a threshold level (approximately 40 mM NaCl for most plant), and (2) a slower response in which harmful ions accumulate in leaves (the ionic phase). When the death rate of older leaves is greater than the production of new leaves, the photosynthetic capacity will no longer be optimum and growth rate retards (Munns and Tester 2008).

Genetic variation exists for these major mechanisms of salt stress (osmotic stress, ion exclusion and tissue tolerance) and their component parts (ion compartmentalisation, ion transport, toxicity, etc.). Genetic variation can be found within as well as between species. The former is good news for plant breeders as it allows salt tolerance traits to be transferred through normal cross-breeding, whereas interspecific crosses may provide a means of transferring genes from one species (a donor) to another (a recipient).

1.3 Screening Methods

Plant growth responses to salinity vary with plant life cycle; critical stages sensitive to salinity are germination, seedling establishment and flowering (Ashraf and Waheed 1990; Flowers 2004). Criteria for evaluating and screening salinity tolerance in crop plants vary depending on the level and duration of salt stress and the plant developmental stage (Shannon 1985; Neumann 1997). In general, tolerance to salt stress is assessed in terms of biomass production or yield compared to non-stress conditions. In conditions of low to moderate salinity, the production capacity of the genotype is often the most pertinent measure, whereas survival ability is often used at relatively high salinity levels (Epstein et al. 1980). The physiological mechanisms that play a major role in maintaining the production capacity of a genotype are not the same as those that contribute to tolerance at extremely high salt concentrations.

Genotypes are generally evaluated using phenotypic observations.

Phenotypic selection parameters include:

(a) Germination

Germination tests are easy to perform and may be important where the crops are required to germinate and establish in saline conditions. However, germination in saline conditions is not often associated with salinity tolerance in subsequent growth stages (Dewy 1962; Shannon 1985; Flowers 2004).

(b) Plant survival

Selection on the basis of plant survival at high salt concentrations has been proposed as a selection criterion for tomato, barley and wheat (Rush and Epstein 1976; Espstein and Norlyn 1977). The ability of a genotype to survive and complete its life cycle at very high salinities, irrespective of yield potential under moderate salinity levels, is considered as being tolerant in the absolute sense.

(c) Leaf damage

Since most crops are glycophytes, they are unable to restrict toxic salt ions being translocated from roots into shoots and leaves. Consequently, salinity damage may be readily observed by leaf symptoms of bleaching and necrosis. Screening for salt tolerance by leaf damage is therefore common (Richards et al. 1987; Gregorio et al. 1997).

(d) Biomass and yield

For plant breeders, yield and biomass are obvious parameters in assessing salt tolerance (Richards et al. 1987). These parameters, however, do not provide information on the underlying physiological mechanisms. In the past, plant breeders have not been interested in physiological mechanism; that a genotype was tolerant was sufficient, the physiological mechanisms were regarded as academic. However, with the emergence of gene function studies, this view is changing.

(e) Physiological mechanisms

Physiological mechanisms that confer tolerance to salt may be harnessed for screening. These may include measurements of tissue sodium content, ion discrimination and osmotic adjustment. Surrogates such as carbon isotope discrimination ($\delta^{13}C$) which give a general indication of plant stress may also be used (Flowers and Yeo 1981; Pakniyat et al. 1997).

1.4 Breeding for Salt Tolerance

1.4.1 Traditional Breeding

Subbarao and Johansen (1994) suggested the following pragmatic considerations in initiating a programme for genetic improvement of crop plants:

1. Define the target environment.
2. Define the level of improvement necessary.
3. Define the growth stage response.
4. Choose the screening method.
5. Choose the selection criteria.
6. Assess the genotypic variation for the various traits under consideration that may have a functional role in improving salinity tolerance.
7. Identify genetic resources for the various components (traits) of salinity tolerance.

8. Determine the genetic basis for traits under consideration, and estimate their heritability.
9. Initiate breeding programmes that combine various traits from different sources into a locally adapted germplasm for ultimate development of a salt-tolerant cultivar.
10. Test selected genotypes in target locations, in a range of saline soils within a production environment, to assess their potential adaptability as new cultivars.

As a comparison, Flowers and Yeo (1995) suggested five strategies in developing salt-tolerant crops:

1. Develop naturally tolerant species (halophytes) as alternative crops.
2. Use interspecific hybridisation to raise the tolerance of current crops.
3. Exploit genetic variation already present in crop gene pools.
4. Generate variation within existing crops by using recurrent selection, mutation induction and/or tissue culture.
5. Breed for yield rather than tolerance.

The use of conventional cross-breeding for salt tolerance has met with little success. This is largely because the required salt tolerance is not present in the primary gene pool of breeding materials. For many crop species, salt tolerance traits are not present in the secondary gene pool (within the species), and for some crops breeders have to resort to interspecific and intergeneric crosses involving wild species to tap into genes that may be transferred by sexual reproduction and recombination. As a consequence, novel genetic variation needs to be produced.

1.4.2 Induced Mutation in Breeding for Salt Tolerance

Mutation induction is one means of increasing biodiversity in crop plants. Mutation induction can be achieved within minutes by gamma-ray or X-ray irradiation of plant materials (usually seed). Mutation may also be produced easily through the use of chemical agents. The detection of mutants carrying the desired variation is more time-consuming and usually involves the screening of thousands of individuals either phenotypically (response to salinity) or genotypically (searching for changes in target genes). Screening for desired mutants is often a major bottleneck in crop improvement.

Once desired mutants are found, these may be entered directly into breeding programmes. However, it is more common that some pre-breeding is performed to ‘clean up’ the genetic background of the mutant lines before entry into breeding programmes. Various genetic marker techniques may be deployed in marker-assisted selection to increase breeding efficiency.

1.5 Need for Reliable Screening Techniques for Pre-field Selection

Salt-affected soils can be classified into three types: (1) saline, (2) sodic and (3) saline–sodic soils. Soil salinity can decrease water availability in the soil and produce toxic effects on particular plant processes. Measuring soil salinity is difficult as it varies with space and time. As a result, soil must be sampled at various times in various places to analyse the effects of salinity on plant growth. A large number of samples are needed to characterise a specific field fully, and sampling should follow all changes in conditions; thus, in many cases, soil sampling requires considerable time and effort in the field.

Abiotic stress tolerance, especially salinity stress, is complex because of variation in sensitivity at various stages in the life cycle. Rice is comparatively tolerant to salt stress during germination, active tillering (vegetative growth) and the later stages of maturity. It is most sensitive during seedling establishment and reproductive stages. Screening at an early growth stage (2–4 weeks) is more convenient than at flowering. This is due to the fact that it is (1) quick, (2) seedlings take up less space, (3) tolerant seedlings may be recovered for seed production and (4) seedling tests are more efficient in terms of time and costs. Seedling screening offers the possibility of preselection of putative individual mutants, mutant populations, breeding lines and progeny and cultivars before large-scale field evaluation.

The rice seedling test described in this booklet is an adaptation of that originally devised in collaboration with the International Rice Research Institute (IRRI). The current system however does not use a floating support and is designed to be robust, reusable and multiple functional; it can be adapted to evaluate individual genotypes or large mutant populations. The hydroponics set-up uses plastic tanks with tight-fitting polyvinyl chloride (PVC) support plates (platforms). A prototype system used bulky styrofoam supports, but these are difficult to maintain and become brittle and contaminated with algae and other microbes with time. The PVC supports are more robust, easily cleaned and can be used repeatedly with minimal maintenance. The PVC platforms are also strong enough to support several hundred seedlings. The test is rapid taking 4–6 weeks. A simplified non-aerated system is used for rice, but forced air aeration and higher salt concentrations are used in screening wheat and barley seedlings.

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