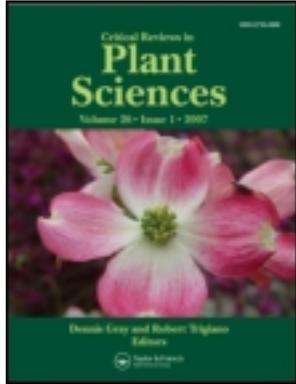


This article was downloaded by: [McGill University Library]

On: 06 October 2012, At: 19:37

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Critical Reviews in Plant Sciences

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/bpts20>

### Breeding for Salinity Tolerance in Plants

M. Ashraf<sup>a</sup> & Dr. Lin Wu<sup>b</sup>

<sup>a</sup> Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan

<sup>b</sup> Department Environmental Horticulture, University of Davis, Davis, CA, 95616, USA

Version of record first published: 30 Mar 2011.

To cite this article: M. Ashraf & Dr. Lin Wu (1994): Breeding for Salinity Tolerance in Plants, Critical Reviews in Plant Sciences, 13:1, 17-42

To link to this article: <http://dx.doi.org/10.1080/07352689409701906>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.tandfonline.com/page/terms-and-conditions>

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae, and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand, or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

# Breeding for Salinity Tolerance in Plants

M. Ashraf

Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan, Pakistan

Referee: Dr. Lin Wu, Department Environmental Horticulture, University of Davis, Davis, CA 95616

**ABSTRACT:** Accumulation of high levels of salts in the soil is characteristic of arid and semi-arid regions. Although different curative and management measures are being used to render salt-affected soils fit for agriculture, they are extremely expensive and do not provide permanent solutions to overcome the salinity problem. In contrast, a biotic approach for overcoming salinity stress has gained considerable recognition within the past few decades in view of the vast experimental evidence from what has happened in nature concerning the evolution of highly salt-tolerant ecotypes of different plant species, and also from the remarkable achievements that have been made in improving different agronomic traits through artificial selection.

Considerable improvements in salt tolerance of important crop species have been achieved in the past 2 decades using barley, rice, pearl millet, maize, sorghum, alfalfa, and many grass species. Such achievements relied solely on assessment of the phenotypic expression of the features involved. Knowledge of the underlying physiological mechanisms producing those salt-tolerant individuals was not clearly known. The present review highlights the relationships between different physiological/biochemical variables being recommended as selection criteria, and salt tolerance of different plant species. This paper also lists different sources of genetic variation for salt tolerance since it is evident that for successful improvement in a character there must be a great amount of genetic variation present in the gene pool of a species.

**KEY WORDS:** artificial selection, breeding, evolution, genetic variation, genetics, salt tolerance, selection intensity, selection pressure.

## I. INTRODUCTION

For plants, stress can be defined as any factor that inhibits plant growth. Stress due to drought, waterlogging, salinity, low mineral nutrients, extremes of temperature and pH, and metals, including heavy metals and others such as aluminum and manganese, are common throughout the world. These stresses have caused many social and economic problems, particularly in developing countries. According to Dudal,<sup>1</sup> only about 10% of the total arable land in the world may be considered as free from stress.

Salt stress and drought stress are the most prevalent in the world. Christiansen<sup>2</sup> estimated that, of the total agricultural land available on earth (14 billion ha), 6.0 billion ha are arid or semi-arid, and about 1.0 billion ha are affected by excess salt. Irrigated land is estimated as occupy-

ing 0.23 billion ha,<sup>3</sup> of which one third is believed to be salt affected.<sup>4</sup>

Massoud<sup>5</sup> examined the distribution of the 343.5 million ha of saline soils throughout the world. Of this total, 53.5 million ha was located in Africa, 17.4 million ha in Australia, 1.96 million ha in Mexico and central America, 6.2 million ha in North America, 69.4 million ha in South America, 91.7 million ha in North and Central Asia, 83.3 million ha in Southern Asia, and 20.0 million ha in Southeast Asia.

Salinity stress is not a new problem for man. After examination of the historical records of the past 6000 years of civilization, it is evident that humans have never been able to continue a progressive civilization in one locality for more than 800 to 2000 years.<sup>6</sup> The major reason for the decline of any civilization seems to have been the destruction of the resource base of the area.

Gelburd<sup>7</sup> found that in Mesopotamia major salinity damage occurred from 2400 B.C. to 1700 B.C., and the slow increase in salinity caused a decline in agricultural productivity. For example, in 2400 B.C., the average barley yield was estimated as 29 bushels per acre. By about 2100 B.C., its yield had declined to 17 bushels per acre and by 1700 B.C. to an average of only 10 bushels per acre, i.e., an approximately 65% decline over a 700-year period.

Although salinity problems have caused a continuing degrading of arable land since ancient times, in some cases land has been devastated by salinity within a short time span. This is certainly true in California, where irrigated agriculture in most areas is less than 100 years old. Nonetheless, of 8.6 million acres of arable land in California, 4.5 million acres are now affected by salinity or sodicity, and the area damaged by salinity is expected to increase from 4.5 to 5.2 million acres by the turn of the century. In the rich San Joaquin Valley, which has 4.5 million acres of land under irrigation, more than 0.4 million acres are now severely affected by salinity. It is expected that by the year 2000 a further one million acres could be lost due to salinity.<sup>8</sup> This means a loss of many millions of dollars in lost crop production. Similarly, in Pakistan, soil salinity is one of the most common stress problems in crop production under irrigation because the climate of Pakistan varies from arid to semi-arid. It has been reported<sup>9</sup> that the total salt-affected land is 14 million acres of the agriculturally available land. Of the saline land, 4.7 million acres are saline, 7.2 million acres saline-sodic, and 0.07 million acres sodic.

Elevated soil salinity arises mainly from two sources, natural and man-made. Soils derived from saline parent rocks contain amounts of salt sufficient to suppress crop production. Soils of coastal regions may receive salt from sea spray, or may be directly affected by seawater as in salt marshes, the mangrove swamps of the subtropics and tropics, salt deserts, and smaller areas around salt springs. Such soils support a limited number of plant species, all of which are, to varying degrees, salt tolerant. It is, however, increasing salinity caused by man that is posing enormous problems worldwide, especially in arid and semi-arid regions where crop production is based on irrigation.

## II. SALINITY AS A NATURAL ENVIRONMENTAL FACTOR

The oceans occupy 71% of the global surface and contain about 97% of the total water supply of the world.<sup>10</sup> In addition to appreciable concentrations of other salts, the major component of seawater is NaCl, with a concentration of about 500 mol m<sup>-3</sup>, a concentration highly toxic to normal plants. Nonetheless, oceans and the shallower seas of the world comprise a major environment colonized by various forms of plant life, the plants at some point in their evolution having developed adaptations to this highly saline environment. However, such plants are not the only ones adapted to highly saline conditions. The seashores, salt marshes, and the salt deserts of the world's arid regions represent habitats having green plants with similar adaptations to high salinity, i.e., the halophytes.

Coastal salt-marsh plant communities range from assemblages of highly salt-tolerant species occurring in lower salt marsh zones subjected to almost daily inundation by saline water, to those on upper marsh areas submerged in saline waters only during high spring and autumn tides. Upper salt marsh communities are more diverse and usually contain salt-tolerant ecotypes of a small number of glycophytes in addition to highly adapted halophytic species, which must have a very long evolutionary history of adaptation in such habitats. By contrast, most crop species upon which we rely for food are not species with any prehistory in saline habitats and hence there will have been no requirement or possibility of evolving any adaptation to them. The vast majority of them cannot tolerate salinity higher than about 10 to 20‰ seawater, and many fail to grow at even lower salt concentrations.

The occurrence of marine plant life and of terrestrial halophytes, both of which have arisen as a consequence of evolutionary change, clearly shows that there is no basic incompatibility between plant life and saline conditions.

Most naturally occurring saline environments provide situations in which high selection pressures exist, which would tend to promote rapid evolution of salinity tolerance in potential colonizing plant species. What is surprising is that relatively few plant species occur on such sites.

Those that do are salt tolerant and can survive because they maintain their growth under high salt concentrations. Naturally saline habitats must have existed for very long periods during which there would have been ample opportunity for natural selection to act on the surrounding plant species to promote the evolution of salt-tolerant individuals and populations. Despite this, the number of salinity tolerant species has remained relatively small.

Saline habitats affected by sea spray also are common in coastal areas, and inputs of saline sea spray can be considerable.<sup>11-13</sup> Coastal spray zone habitats, because of their lower soil salinity,<sup>14</sup> are colonized by a greater diversity of species, including *Agrostis capillaris* L.,<sup>15</sup> *A. stolonifera* L.,<sup>16-18</sup> *Festuca rubra* L.,<sup>19</sup> *Holcus lanatus* L.,<sup>13,20</sup> and *Dactylis glomerata* L.<sup>13</sup> Of these species, however, only *A. stolonifera* and *F. rubra* are known to have evolved ecotypes tolerant of soil salinity.<sup>13,21-23</sup> Recent work,<sup>13</sup> however, has shown that several species not normally associated with highly saline habitats (*H. lanatus*, *D. glomerata*, *A. castellana*, and *Lolium perenne*) possess considerable genetically based variation in tolerance to salt. Yet, salt-tolerant ecotypes of these species have not been recorded. It seems possible that they are excluded from such habitats because they lack heritable variation for other habitat features such as waterlogging, poor soil aeration, soil physical structure, and nutrient imbalance, which have to be overcome by successful colonizers. It is thus clear that for evolution of tolerance to naturally saline habitats to occur, the relevant variability available in a species is not just that affecting salinity tolerance, but must include variability affecting other obligatory adaptational characters. Within a single individual, the lack of the appropriate combination of variability in several adaptive features will lead to a lack of response to selection.

### III. SALINITY AND AGRICULTURE

Accumulation of salts at the soil surface is characteristic of arid and semi-arid regions, particularly where irrigation systems have been used for agricultural production. Salinity occurs mainly as a result of either an accelerated redistribution

of salts in the soil profile due to high water tables in most areas, or the use of insufficient irrigating water to leach salts out of the soil. The major potential sources of irrigation water in most arid areas are canals derived from rivers, tubewells, or seawater. Water from these sources contains variable amounts of salts, and their uncontrolled use has caused considerable salinization worldwide. The extent of deterioration of lands can be illustrated by reference to Pakistan, where an estimated 40,000 ha are being lost annually to agriculture due to salinity. This means a loss of about  $300 \times 10^6$  ha per annum.<sup>150</sup> This threat is not confined to only Asian countries. Several Middle East countries, Australia, Egypt, Iraq, Iran, Israel, Saudi Arabia, and the San Joaquin Valley in California also face similar problems. In these countries, economic utilization of salinity affected lands has been successfully made by the introduction of highly salt-tolerant crops with some appropriate soil amelioration treatments. In addition, soil scientists have devised many reclamation methods and management practices to reduce the problem.<sup>24</sup> However, the applicability of these methods depends on the type of salinity. For reclamation of saline soils, leaching of surface salts has been widely used because of the normal permeability of those soils, and the salts they contain are usually leached below the root zone whenever the amount of water infiltrated exceeds that lost by evapotranspiration. By contrast, in arid and semi-arid regions where rainfall is low and irrigation waters are saline, appropriate provision for adequate leaching is difficult. In addition, leaching is not applicable for saline-sodic and sodic soils that have a high sodium content because their high sodium content makes them almost totally impervious to water. Hence, chemical amendments, such as addition of gypsum ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ) or  $\text{H}_2\text{SO}_4$ , have been recommended for reclaiming alkali soils because they result in the production of soluble salts that are easily removed from the rooting zone with addition of sufficient good quality water. Such chemical methods are, however, very expensive and also are only short-term solutions to overcome salinity.

Salt-affected soils are variable in their chemical and physical properties. Thus, they differ in salt content, type of salt, soil pH, distribution of salts in the profile, nature and content of clay, and

temperature. Salinity also may vary in space across a particular field and throughout the season, generally increasing with time. Under such variable conditions, only a few plant species that are highly tolerant occur naturally. Similarly, very few crop species that have inherent salt tolerance can be grown on such soils. Maas and Hoffman<sup>25</sup> have categorized crops species into four groups: tolerant, moderately tolerant, moderately sensitive, and sensitive on the basis of their tolerance. Barley, cotton, tall wheatgrass, sugar beet, and bermuda grass are tolerant because their threshold salinity level (maximum salinity without yield loss) ranges from 6.9 to 8.0 dS m<sup>-1</sup> (Table 1), whereas sorghum, wheat, perennial ryegrass, soybean, and cowpea are ranked as moderately tolerant, their threshold salinity level ranging from 4.9 to 6.8 dS m<sup>-1</sup>. Moderately sensitive crops include rice, tomato, alfalfa, potato, sugarcane, berseem, etc., whereas most fruit trees and carrot, onion, and pulses are in the sensitive group, where the threshold salinity level is below 2.0 dS m<sup>-1</sup>.

The tolerant crops listed above clearly have inherent genetically based differences in their tolerance to salt. They also have been shown to have genetically based variations in that tolerance. However, attempts to fully exploit this variability for the improvement of salt tolerance have, with a few exceptions (Table 2), not yet been made. Thus, the cotton variety NIAB 78 from Pakistan has recently been shown to be highly salt tolerant, although it was originally bred for high yield and good quality fiber. Similarly, the wheat cultivar LU 26S also is highly salt tolerant compared with most other local/exotic available accessions, and it is as tolerant as the Indian salt-tolerant wheat variety, Kharchia.<sup>151</sup> This variety again was initially bred for high yield and high protein content, not for its salinity tolerance. A sorghum variety Double TX has been developed to be tolerant to high levels of salts.<sup>26</sup> Similarly, rice varieties Pokkali, Johna 349, Nona Bokra, Kalarata, and Damodar were found to be relatively highly salt tolerant.<sup>27</sup> Recently, two highly salt-tolerant hybrids of rice, RSR-1-84 and RSR-3-84, were developed at the Nuclear Institute for Agriculture and Biology Faisalabad, Pakistan.<sup>28</sup>

These are some of the very few successful attempts that have been made in the past few decades to improve salt tolerance within plant

**TABLE 1**  
**Degrees of Salt Tolerance of Some Important Crops**

Crop	Threshold <sup>a</sup> salinity level (EC <sub>e</sub> = dS/m)
<b>Tolerant crops</b>	
Sugarbeet ( <i>Beta vulgaris</i> )	7.0
Cotton ( <i>Gossypium hirsutum</i> )	7.7
Barley ( <i>Hordeum vulgare</i> )	8.0
Bermudagrass ( <i>Cynodon dactylon</i> )	6.9
Tall wheatgrass ( <i>Agropyron longatum</i> )	7.5
<b>Moderately tolerant crops</b>	
Cowpea ( <i>Vigna unguiculata</i> )	4.9
Soybean ( <i>Glycine max</i> )	5.0
Perennial ryegrass ( <i>Lolium perenne</i> )	5.6
Wheat ( <i>Triticum aestivum</i> )	6.0
Durum wheat ( <i>T. turgidum</i> )	5.7
Sorghum ( <i>Sorghum bicolor</i> )	6.8
<b>Moderately sensitive crops</b>	
Berseem clover ( <i>Trifolium alexandrinum</i> )	1.5
White clover ( <i>T. repens</i> )	1.5
Red clover ( <i>T. pratense</i> )	1.5
Alfalfa ( <i>Medicago sativa</i> )	2.0
Corn ( <i>Zea mays</i> )	1.7
Rice ( <i>Oryza sativa</i> )	3.0
Tomato ( <i>Lycopersicon lycopersicum</i> )	2.5
Sugarcane ( <i>Saccharum officinarum</i> )	1.7
Lettuce ( <i>Lactuca sativa</i> )	1.3
<b>Sensitive crops</b>	
Bean ( <i>Phaseolus vulgaris</i> )	1.0
Carrot ( <i>Daucus carota</i> )	1.0
Onion ( <i>Allium cepa</i> )	1.2
Orange ( <i>Citrus sinensis</i> )	1.7
Peach ( <i>Prunus persica</i> )	1.7
Plum ( <i>P. domestica</i> )	1.5
Apricot ( <i>P. armeniaca</i> )	1.6

<sup>a</sup> Maximum salinity level at which a crop has no yield loss.

After References 25 and 147.

species. They are, however, minimal when the problem is viewed in light of the rapid annual increase in salinization throughout the world. From this viewpoint, it is crucial to launch extensive research projects to develop salt-tolerant lines/varieties of each crop that may thrive on salt-affected soils without substantial financial inputs for reclamation or management.

**TABLE 2**  
**Salt Tolerant Varieties/Lines of Different**  
**Crops That Have Been Developed Through**  
**Selection and Breeding**

Crop	Salt tolerant variety/line	Ref.
Alfalfa	AZ-GERM SALT-I	148
	CUF 101-T <sub>2</sub>	103
	Euver S <sub>1</sub>	77
Barley	Selection from Composite Cross XXI	115
Rice	Pokkali, Johna 349	27
	Nona Bokra, Kalarata	
	Damodar	
Sorghum	Double TX	26
Tomato	Selection line from the cross, <i>Lycopersicon esculentum</i> × <i>L. cheesmanii</i>	123
Wheat	Kharchia	149
	LU 26S	119
	Accessions, 178704	114
	178012, 180988	

#### IV. EVOLUTION OF SALINITY TOLERANCE

Natural selection is the major factor determining the relative frequency of alleles in wild populations. Natural selection discriminates between phenotypes of a population with respect to their ability to grow and produce offspring; individual environments selectively allow certain of those offspring to flourish and propagate more efficiently than others, and their genotypes come to predominate in the overall gene pool. Over many generations, significant evolutionary changes may arise.

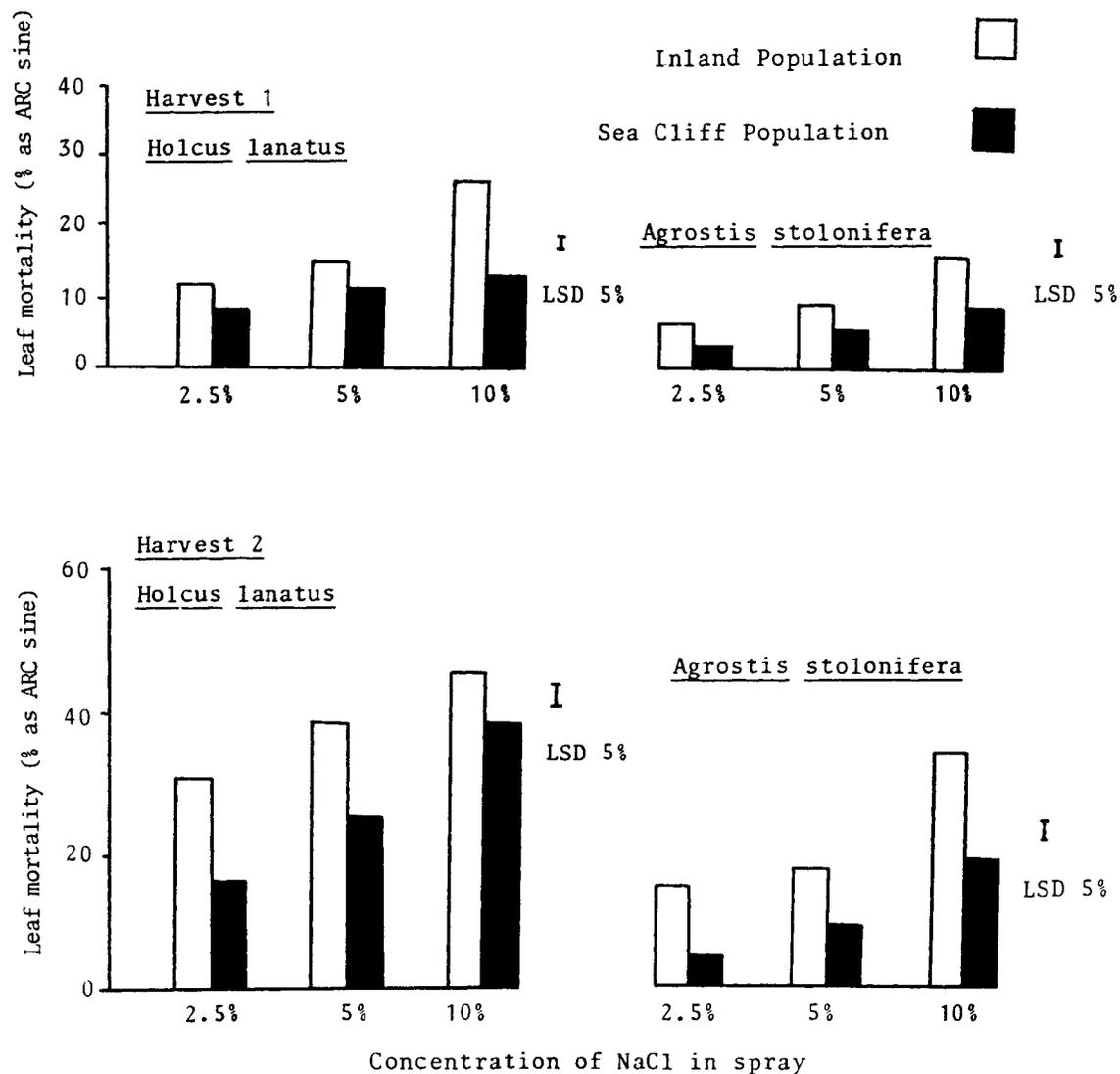
Selection can, however, act only on genetic variation. If the appropriate genetic variation is absent within a given population or species, then evolution clearly is not possible unless the variation arises through mutation or segregation and recombination. Where appropriate variability is available, evolution may be very rapid in those situations in which a population has not already been subjected to selection because, in relation to the particular selection pressure, there can be considerable free and potential unselected variability. In such a situation, evolution of a particular character would not therefore seem to be dif-

ficult. In such situations, we can expect very rapid evolutionary changes.

It also must be realized that evolution is a response to stress conditions of some form or other, whether physical, chemical, or biological in origin. This can be observed by the salt tolerance exhibited by different populations of certain grass species.<sup>13</sup> Thus, a population of *A. stolonifera* L. from an exposed sea cliff showed no tolerance to salt applied to the rooting medium. However, the same population showed considerable resistance to salt spray due to adaptation of leaf epidermis (Figure 1).<sup>29</sup> All this relates to the fact that the sea cliff soil, in spite of being very exposed to salt spray, remains salt free because of frequent freshwater drainage through it. It is clear from this example that evolutionary change in response to any stress can be highly specific.

There is now accumulating evidence that selection has produced constitutive adaptations in some plant species in response to salt, but not in others.<sup>30</sup> A similar situation has long been observed on sites contaminated by heavy metals, where only those species occur that have the appropriate genetic variability. Other species that are not found on metal-contaminated habitats do not possess such variability.<sup>31,32</sup> There also is evidence that not all populations within a particular species contain the variability necessary to respond to selection.<sup>31</sup>

Naturally saline environments provide situations in which high selection pressures favoring salinity tolerance must exist; the salt marsh habitat is one such environment. Yet, surprisingly, salt marshes are colonized by relatively few species.<sup>33</sup> In view of the long period of time that salt marsh habitats have existed, and therefore for selection for tolerance to have operated, it is somewhat surprising that a greater number of species have not evolved to survive on them. There could be two main reasons for the exclusion of species from the salt marsh habitat. First, they may not have the appropriate variability for salt tolerance, and second, they may have sufficiently appropriate variability for salt tolerance to NaCl, but not for other features of saline environments. Confirmation of this second possibility comes from a study of seven grass species:<sup>30</sup> *A. stolonifera* L., *A. capillaris*, *H. lanatus* L., *L. perenne* L., *D. glomerata* L., *F. rubra* L., and *Puccinellia distans*



**FIGURE 1.** Percentage of leaf mortality (arcsine transformed) of one inland and one sea cliff population of *Agrostis stolonifera* and *Holcus lanatus* to three concentrations of NaCl spray, after 3- (Harvest 1) and 6-weeks (Harvest 2) spraying.

(L.) Parl. Of these, only three, *F. rubra*, *A. stolonifera* and *P. distans*, are known to be from highly saline soils. Yet, surprisingly, in laboratory screenings, all seven species varied considerably in their tolerance to NaCl. The failure of some of them to have evolved salt-tolerant populations in the wild seems likely to be due to the lack of appropriate variability for tolerance of other limiting factors in naturally saline environments, such as waterlogging, anaerobic soil conditions, and other salts like  $\text{CaCl}_2$  and  $\text{MgCl}_2$ .

In addition to the necessity for the availability of appropriate genetic variability within a species for evolutionary change to occur, the intensity of

selection pressure related to variation in salinity level within particular saline environments can produce a diversity of tolerance within a particular species. This is well illustrated by the contrasting salt tolerance of upper and lower marsh populations of *F. rubra*,<sup>21</sup> with upper marsh plants being more tolerant than those from the lower marsh. The salt content of lower salt marsh areas are subjected to a regular almost complete inundation by seawater and thus have an almost constant salt content. By contrast, upper salt marsh zones are submerged only at high tides, and at certain times of the year they can have significantly greater salt content due to evaporation of water.

Similar intraspecific variation in salt tolerance has been found in natural populations of *F. rubra*, *A. stolonifera*, *H. lanatus*, and *D. glomerata* from salt marsh, coastal zones, and inland habitats.<sup>13</sup> The differing tolerances of the populations could be related to differences in the selection pressures due to salinity in particular habitats. Two populations of *F. rubra* and *A. stolonifera* from highly saline habitats had insignificant additive genetic variation for salt tolerance. This reflects the fact that populations under strong directional selection tend to show low values for the additive component of genetic variation. In such populations, it can be argued that strong directional selection must, over the long period of time these populations have been growing on these sites, have led to fixation of additive variation. By contrast, the remaining populations of all four species had considerably higher narrow- and broad-sense heritabilities for salt tolerance (Table 3) and these clearly correspond with their overall low salt tolerance. This is taken as evidence for low selection pressures operating to promote tolerance. In view of the low selection pressure in these sites, the populations have not evolved full salt tolerance and free additive genetic variation is found and the narrow-sense heritability values are high. If these populations were subject to selection for salinity tolerance, then evolution of salt tolerance in all of them would be expected.

## V. SPEED OF EVOLUTION

It is quite easy to accept the principle of evolution acting over geological periods of time. It is less easy to appreciate that it can occur in the wild in very short periods of time over very few generations in the life of a species, and in highly localized situations. The presence of salts and heavy metals in the environment has provided us with some good examples of such rapid evolution, which is not only of interest to population geneticists, but also of potential value to those who are involved in assessing the effects of these factors on plants and other living organisms. The inundation of agricultural land with saline waters, generally the sea, shows very effectively the dramatic toxicity of such waters; they are very effective destroyers of vegetation. From an evolutionary viewpoint, it can be seen that saline waters exert powerful selection pressures on any appropriate variation in populations present in the inundated areas, which can lead to the evolution of salt-tolerant individuals/populations. Since this process must have occurred many times in the past, populations of many plant species are found on salt-affected soils.<sup>13,15,17,27,32</sup> This raises the problem of their origin and the specific part played by natural selection in their appearance and survival. Salt-tolerant individuals in normal populations of several grass species also have been reported.<sup>15,30</sup> Of particular interest is a small num-

**TABLE 3**  
**Estimates of Narrow-Sense Heritability**

Site	Species	NaCl test concentrations (mol m <sup>-3</sup> )	Narrow-sense heritability (b = 1/2 h <sub>N</sub> <sup>2</sup> )
Abraham's Bosom	<i>Agrostis stolonifera</i>	200	0.32
	<i>Holcus lanatus</i>	150	0.41
	<i>Dactylis glomerata</i>	150	0.52
Foryd Bay	<i>Festuca rubra</i>	150	—
	<i>D. glomerata</i>	150	0.56
Aberdesach	<i>H. lanatus</i>	150	0.29
Treaddur Bay	<i>A. stolonifera</i>	200	—
Parkgate	<i>F. rubra</i>	150	0.29

*Note:* These estimates are based on female parent-progeny regression for different populations of four grass species from saline habitats.

After Ashraf, M., McNeilly, T., and Bradshaw, A. D., *New Phytol.*, 103, 299, 1986. With permission.

ber that do not normally grow on highly saline soils, e.g., *L. perenne*, *D. glomerata*, *A. capillaris*, and *H. lanatus*. In fact, the selected tolerant lines of these outbreeding grass species were the outcome of a single cycle of selection using large numbers of individuals (10,000 seeds) and high selection pressure. The experiments with grasses provide ample evidence that variation for salt tolerance exists in normal populations of the species examined, and that superior tolerant individuals can be readily selected.

Just as artificial selection in the laboratory can rapidly effect a change in population structure, under natural conditions natural selection can similarly promote the survival of individuals having very rare gene combinations. If natural selection is powerful, the genetic constitution of a population may change markedly and rapidly.<sup>30,34</sup>

## VI. PHYSIOLOGY OF SALINITY TOLERANCE AND ITS RELATIONSHIP TO PLANT BREEDING

Salt tolerance means the ability of plants to grow satisfactorily on saline soils. However, Levitt<sup>35</sup> used the term "salt resistance" in a broader sense, which includes

1. Salt tolerance — when plants respond to salinity stress either by accumulating salts generally in their cells or in specific cells such as salt glands.
2. Salt avoidance — when plants avoid salt stress by maintaining their cell salt concentration unchanged either by water absorption or salt exclusion.

Nonetheless, the term "*salt tolerance*" remains in use to describe the capacity of plants to grow on salty soils, whether they accumulate ions in their cells or exclude the salts.

It is evident that high salt concentrations in the soil cause growth inhibition in most plants, but saline conditions affect plant growth in a variety of ways. Thus, salinity can cause (1) a decrease in water uptake in the plant; (2) the accumulation of ions to toxic levels; and (3) reduced nutrient availability.

When the salt concentration of the soil solution increases and the water potential decreases, the pressure potential of plant cells declines and cells ultimately cease to divide and elongate. Under these situations of water stress conditions, in general, stomata close, which results in the reduction of photosynthesis. Protein breakdown is enhanced and plants ultimately show poor or negative growth and may lose biomass.

Saline conditions present two distinct physiological problems to plants, and evoke different responses.

### A. Adverse Effects of Salinity on Growth due to Osmotic Effect

Under saline conditions, the low osmotic potential of soil salt solutions makes it necessary for plants exposed to these media to maintain a lower intracellular osmotic potential, otherwise they would experience osmotic desiccation because water would move osmotically from the cells into the soil.<sup>36-38</sup> Because the growth of cells is primarily correlated with turgor potential, decreased turgor is the major cause of inhibition of plant growth under saline conditions.

Osmotic adjustment under salt stress can occur due to ion uptake from the soil solution or by internal synthesis of organic solutes. Halophytes usually maintain high turgor potential due to accumulation of ions.<sup>38</sup> By contrast, some glyco-phytes are unable to adapt osmotically under salt stress because of reduced accumulation of ions from the external medium. However, the major responses of halophytes and other salt-tolerant plants to high salinity are osmotic adjustments. Osmotic adaptation can be achieved by the accumulation of high concentrations of either inorganic ions or organic solutes. In higher plants, both invariably play crucial roles, but the contribution of both for osmotic adaptation varies considerably in different plants and even differ in different cell compartments within the same plant.

The role of organic solutes in plants subject to salinity stress has been widely reviewed.<sup>36,39</sup> Osmotic adjustment in almost all types of plants involves synthesis and accumulation of organic solutes, which reduce the cell osmotic potential to a level that provides high turgor potential for

maintaining growth.<sup>36,40</sup> However, it has been argued elsewhere<sup>39,41</sup> that not all organic solutes have a positive adaptive significance. For example, Strogonov<sup>41</sup> noted that in some stressed tissues, synthesis of certain amines actually enhanced salt toxicity.

Low molecular weight organic compounds generally synthesized in response to low water potential of soil solution in the cell cytoplasm are sugars, organic acids, amino acids, and their N-methylated derivatives. Of these, organic acids and sugars are considered as major organic osmotica in many higher glycophytes. The role of the organic acid anion malate in stomatal movement is well established in higher plants.<sup>42</sup> Malate is involved in osmoregulation<sup>43</sup> and it balances excess cation uptake.<sup>44</sup> Similarly, oxalate, an organic acid anion, has been found in several species of *Atriplex* and several other species of the Chenopodiaceae as the major organic osmoticum.<sup>44</sup>

Sugars such as sucrose also act as organic osmotica in some plants under salinity stress.<sup>36,45</sup> Cram<sup>40</sup> suggested that sugars contribute up to 50% of the total osmotic potential in glycophytes growing in natural environments.

The role of proline in osmoregulation has long been questioned. Proline has been found to be accumulated in plants subject to the severe conditions of both drought and salt stress.<sup>37,39,46</sup> According to Rains<sup>47</sup> and Wyn Jones,<sup>39</sup> proline can effectively regulate the accumulation of essential N and it is osmotically very active. It also is compatible with other cytoplasmic components and can be easily converted to glutamate. This conversion is very important because glutamate takes part in the synthesis of other essential amino acids. Thus, proline in a plant under salt stress could act as both a nitrogen reserve and in osmoregulation.<sup>47</sup> Proline accumulation is very common in some monocotyledons, such as *Triglochin*, *Puccinellia*, and barley,<sup>48,50</sup> under salinity stress conditions.

Because proline accumulates in plants in response to salt as well as to drought stress, it may be that the synthesis of proline is a nonspecific response to low water potential. Recently, Ashraf<sup>51</sup> found a negative correlation between proline content and salt tolerance in *Vigna mungo* (L.) Hepper inasmuch as the salt-sensitive cultivar Mash 654 had a greater leaf proline content than the more

tolerant cv. Candhari Mash at different NaCl concentrations. Similarly, Moftah and Michel<sup>52</sup> found that the proline content cannot be used as an indicator of salt tolerance in soybean. Wyn Jones<sup>39</sup> was of the view that proline may not play an adaptive role in plants in response to stresses because it accumulates at the extremes of stress. This may help plants to temporarily override highly damaging stress and thus its use as a selection criterion for salt tolerance does not seem to be plausible.

Glycinebetaine accumulation in response to salt-stress has been reported<sup>53</sup> in many members of the Chenopodiaceae, but it rarely occurs in halophytic species of other families such as the Cyperaceae and Plantaginaceae. In the Chenopodiaceae *Suaeda* and *Salicornia*, glycinebetaine accumulates in leaves and appears to have a positive adaptive significance in relation to salinity. In the Poaceae, glycinebetaine accumulation is characteristic of a few tribes such as the Hordeae and the Chlorideae but not others, e.g., Festuceae and the Maydeae. However, no positive relationship was found between glycinebetaine accumulation and salt tolerance in the two highly salt-tolerant excluders *Agropyron junceum* and *Elymus sabulosus* (both Poaceae), where the glycinebetaine content was unaffected by the salt stress.<sup>54</sup>

On the basis of these findings Wyn Jones<sup>53</sup> has suggested that salt tolerance does not depend on an ability to accumulate glycinebetaine. There is thus considerable variation in organic solute accumulation in different plant species. To depend on one type of organic compound as a measure of salt tolerance would thus not be wise in devising appropriate selection protocols for the development of salt-tolerant varieties/lines of important crops.

## B. Ionic Effects of Salinity on Plant Growth

In addition to osmotic effects, total ion activity and the relative proportion of ions in the external environment have considerable adverse effects on plant growth.

Ions that are often found in excess in saline soils include  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ , and

Mg<sup>2+</sup>, whereas K<sup>+</sup> and NO<sub>3</sub><sup>-</sup> are found less often. Halo- and glycophytic species show specific growth responses to high concentrations of Na ions. For example, the dry biomass of *Atriplex* sp. and *Halogeton glomeratus* was less affected by Cl<sup>-</sup> salinity than by sulfate salts.<sup>55</sup>

Specific ionic effects were assessed in beans, which showed that greater growth inhibition occurred in NaCl than in polyethylene glycol<sup>56</sup> solutions of equivalent osmotic potential. Specific ionic effects can be assessed further from crop varieties differing in salt tolerance. For example, leaf injury only occurs in those soybean varieties that accumulate a high concentration of Cl<sup>-</sup> in their leaves.<sup>57</sup> Genetic analysis of this character showed that this varietal difference is controlled by a single gene pair.<sup>58</sup> In another study with barley, Greenway<sup>59</sup> showed that long-term NaCl treatment of this crop leads to higher accumulation of Cl<sup>-</sup> in leaves of salt-sensitive than salt-tolerant varieties. This greater accumulation of Cl<sup>-</sup> by salt-sensitive varieties was due to their greater rate of Cl<sup>-</sup> uptake. In another study, Rush and Epstein<sup>60</sup> assessed the growth response of the cultivated tomato *Lycopersicon esculentum* Mill. and a wild salt-tolerant relative, *L. cheesmanii* ssp. *minor* (Hook) C. H. Mull. from the Galapagos Islands. The wild tomato grew vigorously up to 250 mol m<sup>-3</sup> NaCl, but the growth of the cultivated tomato was adversely affected at 200 mol m<sup>-3</sup>. By contrast, the wild salt-tolerant tomato was highly sensitive to high K ions compared with the cultivated tomato. Wild salt-tolerant populations of *Agrostis stolonifera* and *F. rubra* also have been compared with their normal populations of the same species (*A. stolonifera*,<sup>17</sup> *F. rubra*<sup>23</sup>). The wild tolerant populations accumulate considerably less Na in their leaves than the normal populations. In a similar study, Greenspan and Kessler<sup>61</sup> showed that the salt sensitivity of *Citrus* was attributable to high rates of Na<sup>+</sup> uptake under saline conditions.

Chloride and sulfate salinities have been shown to have different effects on plant growth,<sup>62</sup> the vegetative growth of grain sorghum being inhibited dramatically by sodium sulfate (43% of control), but much less so by sodium chloride (70% of control).

Recently, Ashraf et al.<sup>63</sup> screened a number of cultivars/lines of mash (*V. mungo* [L.] Hepper) at

the germination and seedling stage for their ability to tolerate NaCl, Na<sub>2</sub>SO<sub>4</sub>, CaCl<sub>2</sub>, and MgSO<sub>4</sub>. Considerable variation among cultivars/lines was found in relation to these salts. However, NaCl proved to be more toxic at the germination stage than the other three salts. In *A. stolonifera* L., *A. capillaris* L., *Holcus lanatus* L., and *Lolium preenne* L., of the three salts NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub>, and seawater,<sup>64</sup> MgCl<sub>2</sub> had a considerably greater inhibitory effect on growth of all four than the other salts.

### 1. Ion Exclusion and Inclusion

Several different mechanisms of tolerance enable higher plants to tolerate high levels of salts. Of these, salt inclusion or salt exclusion has long been recognized in different plants in relation to salinity.<sup>36,65</sup> Salt excluders have the ability to restrict the uptake of salts into the shoot. This may be due to toxic ions such as Na<sup>+</sup> being absorbed in considerable amounts, but it is reabsorbed from the root or the shoot and is either stored or retranslocated to the soil.<sup>66,67</sup> By contrast, salt includers take up large quantities of salt and store it in the shoot. In this case, the high amounts of salts in the cytosol of the plant cells present problems for many physiological/biochemical events taking place there. Many salt includers carry out compartmentation of salts into the vacuole and become succulent. Other salt includer species possess special glands on their leaf surface to excrete high concentrations of salts.<sup>68</sup> The mechanism of salt exclusion in legumes was recently reviewed by Lauchli,<sup>69</sup> and he concluded that most of them respond to salt stress by salt exclusion. Jacoby<sup>70</sup> showed that bean plants exclude Na<sup>+</sup> by retaining it in the basal parts of the plants but at the same time readily translocate Cl<sup>-</sup> to their tops. Chloride also has been found to be excluded in some legumes.<sup>69</sup> In soybean, it was observed that salt tolerance of a tolerant cultivar (cv. Lee) was correlated with Cl<sup>-</sup> exclusion, the variety maintaining a low concentration of Cl<sup>-</sup> in both stems and leaves when grown with varying concentrations of NaCl.

It also has been observed that some plants exclude both Na<sup>+</sup> and Cl<sup>-</sup>. For example *Trifolium alexandrinum* L., an important forage legume of

the Indian subcontinent and Middle East, was found to be more salt resistant than *T. pratense*,<sup>71</sup> *Medicago sativa*, and *T. repens*.<sup>72</sup> Its relatively higher salt tolerance was attributable to efficient exclusion of  $\text{Na}^+$  and  $\text{Cl}^-$  relative to the other species examined. Similarly, Wyn Jones et al.<sup>54</sup> demonstrated that *Agropyron junceum* was more salt resistant than *A. intermedium* because it efficiently excluded both  $\text{Na}^+$  and  $\text{Cl}^-$  from its leaves. The same authors also have shown a positive correlation between salt tolerance and exclusion of  $\text{Na}^+$  and  $\text{Cl}^-$  in wheat, and have suggested that this as an efficient selection criterion for this crop and other glycophytic crop species.<sup>37,39</sup>

The correlation of salt tolerance and salt exclusion also has been observed in naturally occurring populations of grasses. Hannon and Barber<sup>73</sup> found that salt-tolerant ecotypes of *F. rubra* and *Agrostis stolonifera* accumulated less  $\text{Na}^+$  and  $\text{Cl}^-$  in their shoots, and maintained a higher shoot K/Na ratio than populations from nonsaline areas.

A question arises as to whether salt exclusion is a universal mechanism in glycophytes to resist salt stress. There can be found in the literature many cases in which tolerant species or cultivars accumulate considerable amounts of salts in their leaves. For instance, Van Steveninck et al.<sup>74</sup> demonstrated that *Lupinus luteus* was more salt resistant than *L. angustifolius*, but the former accumulated more  $\text{Na}^+$  and  $\text{Cl}^-$  than the latter.

Such differences can be found not only between species, but also within them. In rice, for example, it was noted that one tolerant cultivar accumulated less  $\text{Na}^+$  in its leaves but another salt-tolerant variety had a high Na content.<sup>37</sup> In maize, it was found that salt-tolerant and salt-sensitive varieties had similar ion concentrations in their leaves.<sup>75</sup> Croughan et al.<sup>76</sup> showed that a selected salt-tolerant line of *M. sativa* from tissue culture accumulated higher  $\text{Cl}^-$  than the corresponding unselected line. Similar results were found in a selected salt-tolerant line of the same species produced by conversional selection and breeding.<sup>77</sup> Again, a selected salt-tolerant line of *Lolium perenne* contained higher  $\text{Na}^+$  and  $\text{Cl}^-$  contents in the shoot than the unselected line.<sup>78</sup>

From this evidence, it is clear that glycophytes may use both ion exclusion and ion inclusion mechanisms to resist salt stress. These two mechanisms also are dependent on many other physio-

logical processes such as ion redistribution from one leaf to another or ion compartmentation within the cell, i.e., the ions are not contained in compartments, such as vacuoles, separated from cellular sensitive sites.

## 2. K/Na Selectivity

High salt concentrations in the rooting medium will interfere with the nutritional status of the plant. It has been noted that uptake and translocation of major nutrients such as  $\text{K}^+$  and  $\text{Ca}^{2+}$  are greatly reduced by salt stress, which eventually adversely affects the salt tolerance of the plants under conditions of induced low nutrient availability.<sup>79</sup>

The role of K/Na selectivity in plants under saline conditions has been discussed by many workers.<sup>38,80,81</sup>  $\text{K}^+/\text{Na}^+$  selectivity during influx and by K/Na exchange, or during xylem release and by reabsorption of  $\text{Na}^+$  from the xylem sap has been extensively studied.<sup>81</sup> In addition, there is good evidence that preferential K retranslocation occurs. Furthermore, the K/Na for nonhalophytes should in general, be  $>1$  for normal functioning of all the metabolic processes in the plant.<sup>82</sup>

In a study with *Agropyron* spp., it was demonstrated that *A. elongatum* was considerably more salt tolerant than *A. intermedium*.<sup>83</sup> The difference in their salt tolerance was shown to be due to a higher rate of uptake of  $\text{K}^+$  in *A. elongatum* when grown in saline solution culture. Flowers et al.<sup>38</sup> suggested that a preference for  $\text{K}^+$  at high salt concentrations is a useful attribute in salinity stress and a good criterion for selection for salt tolerance.<sup>54</sup>

Both  $\text{K}^+$  and  $\text{Ca}^{2+}$  are required in the external growth medium to maintain the selectivity and integrity of the cell membrane.  $\text{Ca}^{2+}$  and  $\text{K}^+$  play similar roles for selective transport of ions across membranes.<sup>84,85</sup> As a consequence, high Na/K and Na/Ca ratios in saline environments may impair the selectivity of root membranes and account for passive accumulation of  $\text{Na}^+$  in the roots and shoots.<sup>86</sup> Other physiological mechanisms such as stomatal movement, photosynthesis, and transpiration also are affected by the low Na/K ratio. Bernstein<sup>87</sup> noted that the amount of calcium in normal soils that is adequate for normal plant

growth becomes inadequate under saline conditions because of its interaction with other toxic ions in the soil. The effect of  $\text{Ca}^{2+}$  deficiency under saline conditions was assessed in beans by LaHaye and Epstein.<sup>88</sup> They observed that at 50 mol  $\text{m}^{-3}$  NaCl in the growth medium, the growth rate of beans declined and the Na content in the leaves was increased when the external Na/Ca ratio was 17. Increasing the  $\text{Ca}^{2+}$  concentration in the saline solution disturbed the uptake of  $\text{Na}^+$  by the bean roots. At Na/Ca = 5, there was no entry of  $\text{Na}^+$  into the bean leaves.

The effect of a low Na/Ca ratio in the growth medium was studied in rice.<sup>89</sup> Addition of  $\text{Ca}^{2+}$  into the saline medium slightly increased growth and  $\text{K}^+$  uptake. Similar results in rice were recorded by Muhammad et al.<sup>90</sup> in relation to Na/Ca and Na/K ratios of the saline environment. By contrast, Yeo and Flowers<sup>91</sup> found no effect of varying Na/Ca ratios from 5 to 500 on growth or Na content of rice shoots. Similarly Ashraf et al.<sup>72,78</sup> found no difference in  $\text{K}^+$  or  $\text{Ca}^{2+}$  uptake in selected salt-tolerant lines of three leguminous and seven grass species under constant Na/Ca or Na/K ratios.

In light of these examples, it can be argued that Na/K or Na/Ca ratios in the external environment do not have the same effects in different species. High K/Na selectivity, which was suggested as a selection criterion for salt tolerance in glycophytes,<sup>37,54</sup> may be suitable in many species, but not in all.

## VII. THE GENETIC BASIS OF SALINITY TOLERANCE

The genetic foundation work of Mendel<sup>92</sup> in devising two laws of inheritance, and their rediscovery by Bateson<sup>93</sup> had little effect initially on the practices of plant breeding. This certainly does not seem as surprising to us today as it did to earlier geneticists. This is due to the fact that almost all economic useful characters, with few exceptions, with which the plant breeder has to deal are polygenic in inheritance and the genotypes for these characters cannot be measured because they are highly modified by environment, unlike that of Mendelian characters. It also has been noted that due to the absence of an

appropriate measure, the Mendelian approach is inapplicable to the study of polygenic inheritance. For the measurement of polygenic variation, we have to rely on various statistical methods because the various effects and inter-relationships of polygenes can be inferred only from the statistical properties such as the mean, variance, covariance, etc. of a breeding population. By using these approaches, significant improvements in yield, quality, and other desirable traits of crops have been achieved by plant breeders<sup>94,95</sup> over the past 50 years. However, most of the improvements have been achieved under conditions of optimal input of water and mineral nutrients. Under these conditions, there may have been unconscious selection of those individuals that are relatively inefficient in the use of water and nutrients when supplies of them are limited.<sup>96,97</sup> By contrast, very little attention has been given to the adaptation of crops by genetic means to unfavorable soil conditions such as low or high mineral nutrient contents, waterlogging, drought stress, salt stress, or extremes of pH. There are relatively few cases when biometrical genetic methods have been used to assess patterns of inheritance and genetic architecture in crops or in wild species in response to soil stress conditions, and even fewer that have examined these features of crop salinity tolerance. This is despite the fact that as long ago as 1945 Lyon<sup>98</sup> examined the inheritance of salt tolerance in tomato. He showed that *Lycopersicon pimpinellifolium* was less sensitive than *L. esculentum* to  $\text{Na}_2\text{SO}_4$ , and  $F_1$  hybrids between these two species were identical in salt tolerance to the sensitive parent, indicating a single major gene controlling the presence or absence of tolerance, with sensitivity being dominant.

Later, Stroganov<sup>41</sup> used conventional selection and breeding techniques for the development of salt tolerance in cotton. Increased tolerance was achieved by crossing salt-tolerant selections from a salt-affected field with a population from a nonsaline field. In this way, he was able to improve both cotton yield (about 7 to 43%) and fiber quality.

Two soybean varieties, Lee and Jackson, are known to be salt tolerant and salt sensitive, respectively, with the difference being due to  $\text{Cl}^-$  exclusion in cv. Lee.<sup>57</sup> Genetic analysis of the character showed that cv. Lee possessed a single dominant

gene responsible for  $\text{Cl}^-$  exclusion that is absent from cv. Jackson. Salt induces male sterility in rice, and from conventional selection and breeding techniques, Akbar and Yabuno<sup>99</sup> concluded that resistance to sterility was a dominant character controlled by at least three pairs of genes.

## A. Heritable Variation

The magnitude of the genetic component of the observed phenotypic variation is of fundamental importance for selection to be effective. The heritable variation is the total variation resulting from segregation of genotypes in a population. If the whole heritable variation is additive, and nonadditive variance is absent or very low among the genes controlling a character, there would be a rapid response to selection and a maximum genetic advance through continued selection than if the nonadditive variance were higher among the genes.

Estimation of heritable variation, and more particularly its genetic components in a breeding population, is of great practical value for plant breeders. Detailed methods for estimating heritable variation have been described by Falconer<sup>100</sup> and Lawrence<sup>101</sup> and the reader is referred to these works.

The total variance of a character that can be observed by the breeder is the phenotypic variance ( $V_p$ ), which itself has two components of variance due to genetic difference (i.e., the genetic variance,  $V_G$ ) and variance due to environmental factors (i.e., environmental variance,  $V_E$ ).

Thus,  $V_p = V_G + V_E$  and  $V_G = V_A + V_D + V_I$ ; where  $V_A$  = additive genetic component,  $V_D$  = dominance variance, and  $V_I$  = interaction variance, i.e., epistasis.

The extent to which phenotypes are determined by their genotypes is known as broad-sense heritability ( $h_B^2$ ) and is expressed as  $V_G/V_p$ . The extent to which phenotypes are determined by genes with additive effects on the phenotype transmitted from the parents is expressed as  $V_A/V_p$ . This ratio is known as narrow-sense heritability ( $h_N^2$ ).

The values of narrow-sense heritability estimate that proportion of the phenotypic differences that is due to additive genetic effects and hence it reflects the degree of resemblance be-

tween parents and offspring. This is the parameter that a plant breeder requires to evaluate his breeding material. If the value of  $h_N^2$  is  $>0.3$  for a character, a reasonably rapid response to selection would be expected.

Despite the importance of knowing the genetic basis of phenotypic variability for any breeding program, there are remarkably few data concerning it in the case of salt tolerance. Hunt<sup>102</sup> showed that salt tolerance in *Agropyron intermedium* was highly heritable with a parent-progeny correlation coefficient ( $r$ ) of 0.83. Similarly, salt tolerance in alfalfa also was shown to be highly heritable ( $h_N^2 = 0.41$ ) by Noble et al.<sup>103</sup> when plants within a single cultivar were selected at the adult stage at high salt concentration. In the same crop, Allen et al.<sup>104</sup> improved the salt tolerance at the germination stage through recurrent selection and again found the character highly heritable (broad-sense heritability = 50%). Ashraf et al.<sup>77,105</sup> estimated narrow-sense heritabilities in a range of grass and leguminous forage species (Table 4). Their heritability estimates were obtained from female parent-progeny regression, and from response to selection (realized heritability). The values in all species were quite high. Such

**TABLE 4**  
**Narrow-Sense Heritability Estimates**

Species	NaCl test concentration (mol m <sup>-3</sup> )	Heritability (2b)
<i>Lolium perenne</i>	275	0.33
<i>Dactylis glomerata</i>	225	0.32
<i>Agrostis stolonifera</i>	250	0.28
<i>A. castellana</i>	250	0.26
<i>Holcus lanatus</i>	250	0.19
<i>Festuca rubra</i>	300	0.44
<i>Puccinellia distans</i>	425	0.72
<i>Brassica napus</i> (forage rape)	200	0.74
<i>Trifolium alexandrinum</i>	225	0.50
<i>Medicago sativa</i>	250	0.52
<i>T. pratense</i>	200	0.98

*Note:* These estimates are based on female parent-progeny regression for seedling root growth of seven grass and seedling shoot growth of four dicotyledonous forage species when grown at appropriate NaCl concentrations in 1/10 strength nutrient solution

After References 30 and 77.

high heritability estimates suggest that improvement in salt tolerance could be obtained through further selection and breeding in all the species examined. Similarly, variation in NaCl tolerance in sorghum<sup>106</sup> has been shown to have a significant additive genetic component (Table 5).

Heritability in a wild population of *F. rubra* from a salinized pasture was found to be quite high.<sup>22</sup> The genetic basis of NaCl tolerance has been assessed further in wild populations of *F. rubra*, and in *Holcus lanatus*, *Agrostis stolonifera*, and *D. glomerata* from salt marsh, sea spray zone, and inland habitats. Narrow-sense heritability estimates were high for all populations, except for one *F. rubra* population from a salt marsh and a single population of *A. stolonifera* from a spray zone. The low narrow-sense heritability estimates for these two populations suggest that additive genetic variation for NaCl tolerance had largely become fixed in these populations as a consequence of many generations of strong directional selection.

**TABLE 5**  
**Estimation of Components of Variation for Root Length of Eight Lines of *Sorghum bicolor***

Components	NaCl (100 mol m <sup>-3</sup> )
E	5.33 ± 1.41
D	32.64 ± 4.21
H <sub>1</sub>	59.54 ± 9.72
H <sub>2</sub>	37.26 ± 8.46
F	24.92 ± 9.99
h	10.39 ± 5.67
(H <sub>1</sub> /D) <sup>1/2</sup>	1.35
H <sub>2</sub> /4H <sub>1</sub> ( $\bar{v}$ )	0.16
1/2 F/[D(H <sub>1</sub> - H <sub>2</sub> )] <sup>1/2</sup>	0.46
[(4D H <sub>1</sub> ) <sup>1/2</sup> + F]/[(4D H <sub>1</sub> ) <sup>1/2</sup> - F]	1.79
Narrow-sense heritability	0.51
Broad-sense heritability	0.82

Note: Components were measured in 100 mol m<sup>-3</sup> NaCl following diallele cross.

After Azhar, F. M. and McNeilly, T., *Plant Breed.*, 101, 114, 1988. With permission.

## B. Number of Genes

Knowledge of the number of genes and their mode of action controlling the expression of the

quantitative characters that he/she desires to change also is valuable information for the breeder. This is because the same amount of genotypic variance can be produced by a few genes, each having a large effect, or by several genes, each having a relatively small effect. However, the immediate response to selection, the potential for further response, and the maximum genetic advance attainable through continued selection will be different in these two cases.<sup>107</sup> Thoday and Thompson<sup>108</sup> considered that most quantitative characters are such that the majority of variations can be attributable to a few major genes. In such a situation, the plant breeder should speed initial response to selection by tracking individual genes. By contrast, if quantitative traits are controlled by many genes, plant breeders may have to rely on greater gains over a longer time span, rather than smaller gains in the short term through recurrent selection.<sup>109</sup>

An example of salt tolerance as a quantitative trait being subject to major influence by a single gene was reported by Abel and Mackenzie<sup>57</sup> in soybean. They observed that the high salt tolerance of cv. Lee was due to the presence of a single dominant gene (Ncl), whereas a recessive gene (ncl) was responsible for controlling salt sensitivity in cv. Jackson.

In another study concerning salt-induced, delayed-type panicle sterility in rice, Akbar and Yabuno<sup>110</sup> noted that different rice varieties performed differently when subjected to salinity at the flowering stage. The F<sub>1</sub> generation produced by crossing a relatively salt-tolerant cv. Johna 349 and a relatively salt-sensitive cv. Magnolia was highly salt tolerant. The patterns of inheritance of the character salt-induced, delayed-type panicle sterility were determined in the F<sub>2</sub> generation of the back-cross population derived from cv. Magnolia × cv. Johna 349 crosses. It was found that resistance to sterility was a dominant character and at least three pairs of genes were estimated to be involved.

Apart from these two crops, the number of genes controlling salt tolerance in other important crops has yet to be determined. There is a great need for further studies on a more comprehensive scale to determine the number of genes controlling salt tolerance in different crops. By knowing the number of genes, a plant breeder can use his breed-

ing material in a better way and can use appropriate breeding programs to improve a character.

### C. Selection and Breeding for Salt Tolerance

There is now good evidence that soil scientists have devised various reclamation measures and management practices to make the salt-affected land fit for agriculture. However, these methods are expensive and are not always a practical solution to the problem of soil salinity. A biological approach to overcoming salinity stress has gained considerable attention in view of a large amount of experimental evidence about the evolution of highly salt-tolerant wild populations of a small number of species.<sup>22,30</sup>

Considerable improvements have been achieved in the past few decades in a number of agronomic traits in many crop species, e.g., in economic yield, disease resistance, drought resistance, and quality, through a combination of artificial selection and improved cultural practices. For example, in the U.S. in the 50-year period from 1931–1980, yields of maize and sorghum increased by 325 and 320%, respectively.<sup>111</sup> Similarly, in the U.K. in the period from 1968–1979, the average wheat yield increased by about 41%. These increases are largely due to the introduction of new varieties.<sup>112</sup> The efficacy of artificial selection also can be assessed from a long-term selection experiment carried out in Illinois for the improvement of grain oil and protein content in maize.<sup>113</sup> It was possible to increase the oil content of maize from about 5 to about 15% after 50 cycles of selection.

Such achievements have relied solely on assessment of the phenotypic expression of the features involved. Knowledge of the underlying physiological mechanisms producing those phenotypes was not clearly known and clearly unnecessary. A similar approach has been used with some success in the past to distinguish between salt-tolerant and salt-sensitive plants of wheat and barley<sup>114,115</sup> and is currently being used elsewhere for the improvement of salt tolerance in a range of crop species.

## VIII. SOURCES OF GENETIC VARIATION

The basic features of evolution in natural conditions and under domestication are the same. Both have two basic requirements:

1. There must be genetically stable variation for the desired character in a population
2. There must be a means to select individuals possessing the best expression of the character

Plant breeders have at their disposal a number of sources of useful variation that may be exploited to improve salt tolerance in important crop species.

### A. Screening of Local/Exotic Germplasm-Intercultivar Variation

Local/exotic accessions of most major crops species are available for screening. Much of this variability will be the outcome of previous natural or unconscious artificial selection. The most tolerant accessions desired from them are multiplied either for further selection or for direct use on salt-affected soils. For example, Dewey evaluated 25<sup>116</sup> and 60<sup>117</sup> strains of *Agropyron desertorum*, respectively, for ability to survive at different salt concentrations but found very few relatively tolerant strains. Shannon,<sup>118</sup> working with *A. elongatum*, screened 32 accessions in a range of salt treatments, and he identified 7 highly tolerant accessions. Qureshi et al.<sup>119</sup> found considerable variation in salt tolerance in 12 spring wheat cultivars, and they were able to identify 3 cultivars/lines that were relatively highly salt tolerant at different growth stages. Kingsbury and Epstein<sup>114</sup> screened 5000 accessions of spring wheat and found only a small number that survived to produce an economic yield at a high salt concentration. Azhar and McNeilly<sup>120</sup> reported considerable variation in a sample of 51 accessions of *Sorghum bicolor*. Recently, Ashraf and Waheed<sup>121</sup> screened 148 accessions of lentil from which they identified 5 lines that are highly tolerant to NaCl.

## B. Screening of Variable Material of a Single Cultivar — Intravarietal Variation

Large numbers of genotypes from genetically variable material of either naturally outcrossing species, or produced by artificial crossing in species that are predominantly inbreeding, have been screened and tolerant individuals identified. The origin of this variability is difficult to know; the important fact is that it is present. Epstein and Norlyn<sup>115</sup> achieved a significant improvement in salt tolerance of barley after a single cycle of selection, when selection was carried out on variable Composite Cross material. Their selected lines survived until maturity and gave a reasonable seed yield when irrigated with undiluted seawater. Salt tolerance in an alfalfa (*M. sativa* L.) cultivar was increased through recurrent selection at the adult stage,<sup>103</sup> and alfalfa salt tolerance at the germination stage was improved after five cycles of mass selection.<sup>104</sup>

In parallel studies, Ashraf et al.<sup>30,77</sup> screened 10,000 to 20,000 seeds of variable material of 7 grass and 3 leguminous species and identified small numbers of tolerant individuals in each species with an overall selection intensity <1% in all cases. The progeny of the selected tolerant lines produced greater biomass under saline conditions than their respective unselected base populations (Figures 2 and 3). Al-Khatib et al.<sup>150</sup> using the method of Ashraf et al.,<sup>77</sup> has markedly improved the salt tolerance of two alfalfa cultivars through recurrent selection at the seedling stage.

In maize, salt tolerance was improved by screening 10,000 seeds of a salt-sensitive cultivar Akbar at 180 mol m<sup>-3</sup> NaCl in nutrient solution.<sup>122</sup> The progeny of the salt-tolerant selection line produced significantly greater biomass than the unselected base population when tested over a range of NaCl concentrations. In a similar experiment, 10,000 seeds from F<sub>2</sub> discard material from a wheat-breeding program were screened for salinity tolerance in sand irrigated with nutrient solution with NaCl + CaCl<sub>2</sub> added to give an EC of 36 dS/m. A total of 98 seeds germinated and produced healthy seedlings, of which only 6 survived to maturity and produced seed. These six plants constituted a salt-tolerant selection line that was equal under glasshouse conditions to the salt-tolerant cv. LU 26S in dry weight and seed yield.

## C. Interspecific Hybridization

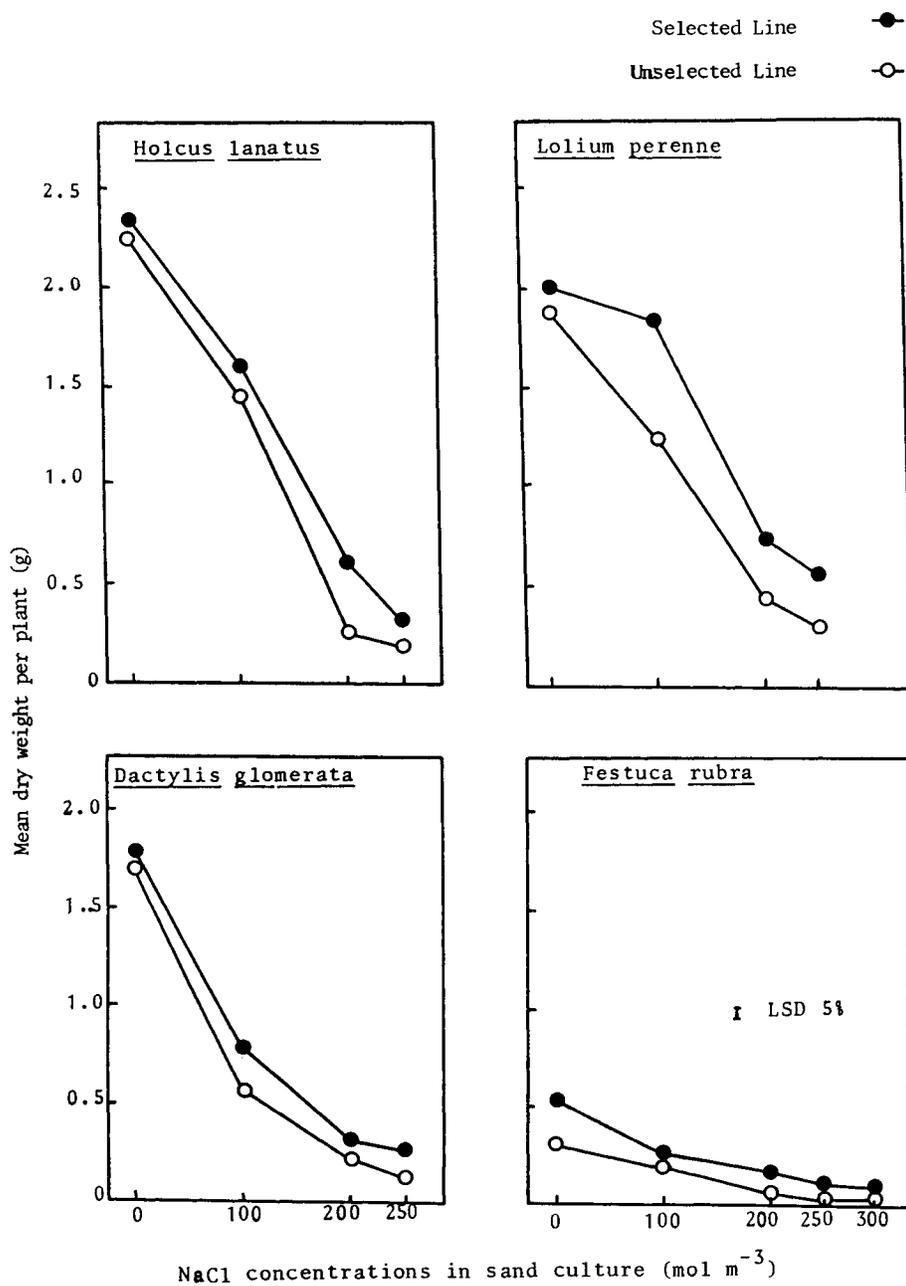
Salt tolerance of some crop species has been enhanced by transferring genes from highly tolerant wild relatives to cultivated species by conventional breeding techniques. This was attempted by Lyon,<sup>98</sup> who found that *Lycopersicon pimpinellifolium* was relatively more tolerant to Na<sub>2</sub>SO<sub>4</sub> than *L. esculentum*. Interspecific crosses and their F<sub>1</sub> hybrids exhibited the characteristics of the sensitive parent. Rush and Epstein<sup>123</sup> assessed the salt tolerance of the wild *L. cheesmanii* and the cultivated *L. esculentum* and found the former to be relatively highly salt tolerant. The two species are interfertile, and some highly tolerant plants have been selected from F<sub>2</sub> progeny of the interspecific cross. These plants also had acceptable fruit size, whereas those of *L. cheesmanii* averaged about 1 cm in diameter.

## D. Intergeneric Hybridization

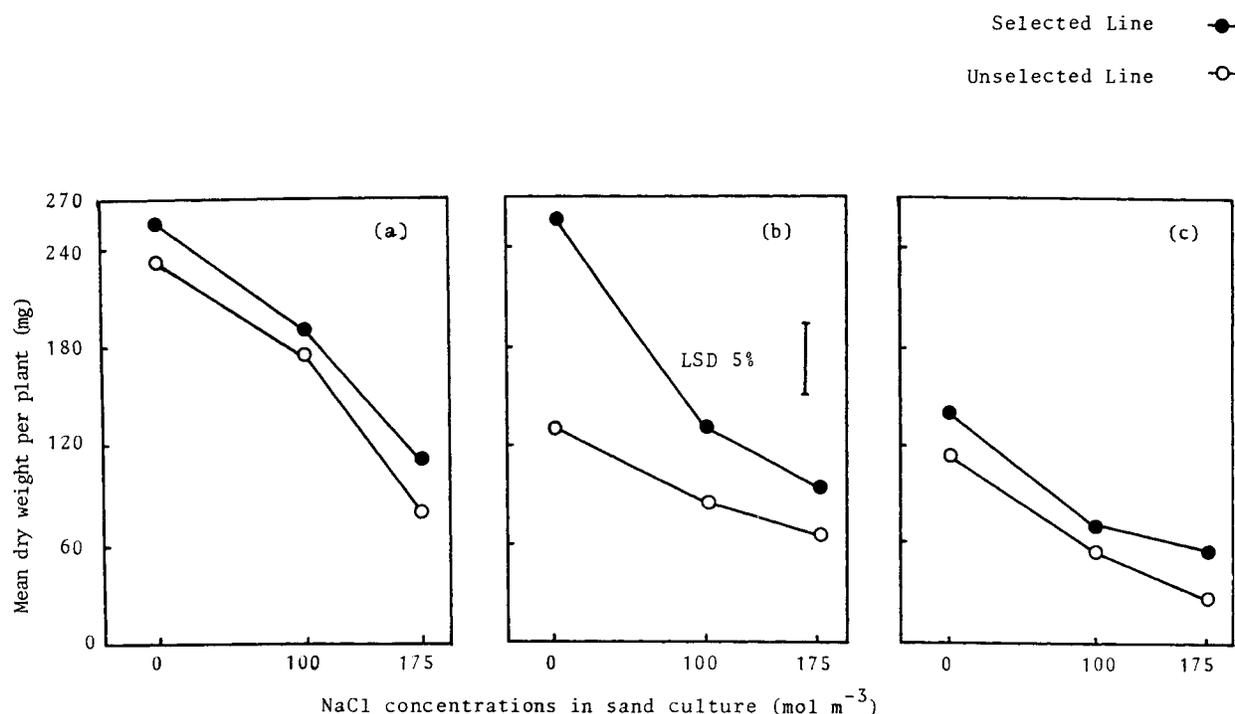
Attempts have been made to hybridize spring wheat with the highly salt-tolerant wild grass, *Thinopyrum bessarabicum*.<sup>124</sup> The resultant hybrid was significantly more tolerant than the wheat parent, but data about seed yield from these crosses under saline conditions are not yet available. At the Nuclear Institute for Agriculture and Biology, Faisalabad, attempts have been made to hybridize the highly salt-tolerant *Leptochloa fusca* with rice and wheat, and a program of hybridization of the salt-tolerant *Sclerophyllum coarctatum* and *Oryza sativa* also is under way at IRRI.<sup>27</sup> The results of these operations are not yet complete.

## E. Induced Mutations

The conventional methods for developing better crop varieties/lines to get the desired combination of characters are still being used extensively and are valid for crops where appropriate genetic variability is available. However, if the genes for a new character do not exist or if the variability has already been exhausted through selection, then the variability may be sought using mutagens, which may be chemicals or radiation. Modern methods such as protoplast fusion



**FIGURE 2.** Mean plant dry weight of selected and unselected lines of four grass species after 7-weeks growth in sand culture at differing NaCl concentrations.



**FIGURE 3.** Mean dry weights of plants from selected and unselected lines of three legume species [*Trifolium alexandrinum* (a), *Medicago sativa* (b), *Trifolium pratense* (c)] after 4 weeks of growth in sand culture at different NaCl concentrations.

and recombinant DNA techniques are available but their use for development is unknown to date. However, the use of mutagens in developing salt-tolerant rice mutants has been shown to be successful.<sup>125</sup> Variability for salt tolerance in the salt-sensitive cv. Taichung 65 was created by treating fertilized egg cells with different doses of N-methyl-N-nitrosourea (MNU). In M<sub>3</sub>, two salt-tolerant mutants were detected that had 83 and 90% survival at the seedling stage in 0.5% NaCl.

Scientists at the Nuclear Institute of Agriculture and Biology, Faisalabad, Pakistan, have produced some rice mutants with gamma radiation. Of these, two mutants, NIAB Rice-1 and PSR 1-84, have surpassed the existing salt-tolerant varieties, Pokkali and Johna 349, in yield under saline conditions.<sup>28</sup>

## F. Somaclonal Variation

Cell and tissue culture by itself is a mutagenic process and gives rise to somaclones, and thus is an important source of somaclonal variation. The method seems to be very promising as each cell is

a potential plant, and large numbers of cultivated cells can be screened to select cells tolerant/resistant to a stress. The role of cell and tissue culture techniques in the development of salt-tolerant lines has been emphasized by many workers. For instance, Zenk<sup>126</sup> isolated a salt-tolerant line from haploid cells of *Nicotiana sylvestris* that had 50% relative (percent of control basis) growth at 1% NaCl, whereas the nonselected cells did not survive at the same salt level. Later, in 1975, Dix and Street<sup>127</sup> selected lines of *N. sylvestris* and *Capsicum annum* at lethal concentrations of NaCl. These lines were resistant to 1 and 2% NaCl. Similarly, Nabors et al.<sup>128</sup> were able to isolate cell lines of tobacco (*N. tabacum*) that showed tolerance first to 0.16% NaCl and then the tolerance was further enhanced to 0.52% NaCl. Hasegawa et al.,<sup>129</sup> working with the same species, selected a cell line that was tolerant to 1% NaCl. However, the salt tolerance of the lines was lost when grown under nonsaline conditions. Cell lines of *M. sativa*<sup>76</sup> and *O. sativa*<sup>130</sup> also have been selected and were shown to be tolerant to high salt concentrations. Lines of the former species were tolerant to 1% NaCl, whereas those of the latter to 2%

NaCl. A somaclonal variant of rice for Al toxicity and salt species stress through somatic cell culture has been reported at IRRI.<sup>131</sup> The data described above refer to cell lines that show resistance/tolerance to salinity. However, there are very few studies that report the regeneration of plants from the salt-tolerant cells. Nabors et al.<sup>132</sup> were able to regenerate tobacco plants from NaCl-tolerant cells; they found that F<sub>1</sub> and F<sub>2</sub> plants showed high tolerance to NaCl compared to plants regenerated from nontolerant cells. Similar success has been achieved by Stavarek et al.,<sup>133</sup> who have regenerated hundreds of plants from salt-tolerant cells of *M. sativa*.

In view of the above-mentioned studies, selection through cell and tissue culture seems to be very rapid. However, no salt-tolerant crop variety through this method is known yet, particularly in cereals.

It seems somewhat remarkable that selection for salinity tolerance using single cells resulted in salinity tolerance in such a complex organization in the many thousands of cells that the whole plant represents. It is even more remarkable in light of the opinion of Shannon<sup>118</sup> and many others that salinity tolerance is a whole plant, and not a single cell, phenomenon.

## IX. CRITERIA FOR SELECTION

For a successful breeding program, it is crucial to consider a single selection criterion rather than a set of characters. However, it is amply clear from the literature that plant physiologists/biochemists have in the past recommended many selection criteria such as proline, betaine, glycine-betaine, pinitol, sugar accumulation, Na/K or Na/Ca ratios, etc. The problem is that these parameters do not show any consistent relationship with salt tolerance in different crop species, and even more so because physiological mechanisms in many cases vary from variety to variety within a single species.<sup>37</sup>

Work done on heavy metal tolerance of a number of grass species clearly shows that selection need not necessarily involve an understanding of the mechanism or physiological basis of tolerance. For this character, even after more than 20 years of study, the mechanism of heavy metal

tolerance is still not clearly known, although it is controlled by a small number of genes or possibly a single major gene in some species. Characters like salt tolerance or drought tolerance, which are controlled by many genes, suggest that the underlying physiological mechanism(s) of tolerance could be very complex. However, for heavy metal-tolerance, root length differences have been widely used as a reliable selection criterion to distinguish between heavy metal-tolerant and -sensitive populations and even to distinguish between individuals within a population.<sup>134-137</sup>

Root length differences also have been used with considerable success to detect salt-tolerant plants in a number of grass species.<sup>15,18,21-23,30</sup>

Root growth does not however appear to be a useful criterion in some leguminous and other dicotyledonous species, although Ab-Shukor et al.<sup>138</sup> successfully distinguished salt-tolerant and salt-sensitive natural populations of *Trifolium repens* on the basis of root-growth tests. Ashraf et al.<sup>72</sup> successfully used shoot-length measurements for assessment of tolerance; in other crops, selection based on whole plant performance provided a means of selecting for salt tolerance such as in rice,<sup>27</sup> millet,<sup>139</sup> and wheat.<sup>140</sup> Such procedures may well be applicable to other crop species.

## X. BREEDING METHODS

Several breeding methods have been described by Allard<sup>141</sup> for both self-pollinated and cross-pollinated crops. The use of specific method depends on two main factors: (1) the breeding system of crops; and (2) the objective for which breeding is to be carried out, whether it is for quality, yield, or resistance to stresses, etc.

### A. Normal Techniques

For breeding in self-fertilizing crops, various methods are available such as pure line breeding, mass selection, pedigree method, bulk method, backcrossing, etc.

Pure line breeding is a very successful means of selecting new varieties from a genetically variable original population, bearing in mind that all

of the plants of such populations are homozygous. Pure line breeding is concerned with selecting a large number of individual plants and comparing the performance of their progeny in different field trials. The progeny that excels in yield is selected as a new variety. By contrast, in mass selection, the progeny of many pure lines constitute a new variety. These two methods have been used successfully in wheat for selecting salt-tolerant individuals from  $F_2$  variable material from the former Plant Breeding Institute Cambridge (U.K.). In this study, only six plants were identified that were tolerant to salt at all different stages of the plant life cycle.<sup>146</sup>

The pedigree method is most common for breeding self-pollinated crops. In this method, superior types are selected in successive segregating generations and records are kept of the pedigree of each of the progenies. This method has been used at IRRI for breeding rice for salinity stress.<sup>27</sup> Some salt-tolerant varieties such as Pokkali, Johna 349, Nona Bokra, Kalarata, and Damodar have been bred with parents having superior characteristics. The resultant progenies have been screened at high salt concentration (EC<sub>e</sub> 8 dS/m). In this way, five highly salt-tolerant strains have been developed.

Although the plant breeder in the pedigree method can use selection expertise to a greater extent than in any other valid method available for breeding self-pollinated crops, the limitation of the method lies in the fact that the plant breeder has to handle large amounts of material. To overcome this problem, plant breeders sometimes prefer to use the bulk method rather than the pedigree method. In the bulk method, the hybrids are grown in bulk without keeping a record of the ancestry of parents and their families, and selection is practiced on the basis of the performance of individuals rather than of the progenies of the selected plants.

The backcross method has been widely used by plant breeders in self-pollinated crops for transferring particular genes to material, generally an existing variety that is deficient in a particular characteristic. This method is of particular significance in disease and pest resistance breeding. Repeated backcrosses are made to the desirable parent, and selection is practiced in each backcrossed generation to ensure com-

plete transfer of the requisite characteristics from the donor parent. For instance, the small fruit size of the salt-tolerant hybrid from crossing the wild tomato *Lycopersicon cheesmanii* and the cultivated tomato *L. esculentum* was improved by successive backcrossing to the cultivated tomato parent.<sup>123</sup> The hybrid between spring wheat and the highly salt-tolerant wild grass *T. bessarabicum* was highly salt tolerant.<sup>124</sup> However, the hybrid has very poor grain yield.<sup>152</sup> Here, yield may be improved by repeatedly backcrossing it with spring wheat and selecting for both yield and salinity tolerance in the backcross progeny.

For cross-pollinated crops, there are many breeding methods that are extensively used by plant breeders. The most important are mass selection, backcrossing, hybridization of inbred lines to produce hybrid varieties, recurrent selection, and the development of synthetic varieties. Mass selection and recurrent selection have been widely used in recent years for the development of salt tolerance in a range of forage and grain crops. For instance, the mass selection approach was used in barley<sup>115</sup> (self-pollinated crop) for the development of a salt-tolerant line from variable material, Composite Cross XXI. Ashraf et al.<sup>30,77</sup> used the mass selection method to select small numbers of salt-tolerant individuals out of 10,000 to 20,000 seedlings at lethal levels of NaCl from a range of forage and grass species. A recurrent selection strategy was used by Noble et al.<sup>103</sup> for breeding alfalfa for salt tolerance. They practiced selection in a single cultivar of alfalfa for three consecutive cycles and as a result of this they were able to develop a highly salt-tolerant line. Very recently, Ashraf and McNeilly<sup>122</sup> produced a highly salt-tolerant line after only two cycles of selection in maize.

Despite conventional plant breeding methods, some recent developments such as cell and tissue culture, protoplast fusion, and recombinant DNA techniques also have been suggested as very efficient and rapid means to improve a character in a crop species. With cell and tissue culture methods, we can study how plants tolerate stress and then selection can be practiced to select genetically superior plants. The use of tissue culture in development of salt tolerance in a number of species was described earlier.

## B. Protoplast Fusion

In certain species, there is evidence that with protoplast fusion new combinations of nuclear genes from two somatic cells can be made. Protoplast fusion also can be practiced in hybrids containing new combinations of nuclear and organelle genes. The use of this approach to develop salt tolerance is identified and this variation is not exploitable by the cultivated plants by sexual hybridization either *in vivo* or *in vitro*.<sup>142</sup> This method has many beneficial aspects. For instance, if two species are sexually incompatible, fertile amphidiploid somatic hybrids can be developed that can be vegetatively propagated. Success in this method is well evident with a few dicotyledonous species such as potato, tobacco, Petunia, and carrot. The technique is generally not as successful with monocots as with dicots. A major problem is the regeneration of plants from protoplasts or cell cultures. The culture technique has some disadvantages so that induced phenotypic mutations may not maintain their stability after successive generations.

This technique is still in its infancy and the extent of its applicability in plant breeding is not yet known.

## C. Recombinant DNA Techniques

A promising new approach, recombinant DNA technology, introduces a gene into a plant cell, which results in enhanced variability. This method involves isolating DNA from a genetically marked species and transferring that marker to a second species where it is both transmitted and expressed. Generally, microorganisms such as viruses and bacteria are used as vectors to introduce the desirable gene carrying the appropriate genetic information into plant cells. It has been anticipated<sup>142</sup> that appropriate vectors and techniques will possibly be developed for the introduction and expression of foreign or altered genes into a plant cell in the very near future. We can consider it an excellent achievement if whole plants containing altered DNA are obtained. A well-known example of such an achievement is the development of a new vector system for enhancing stress tolerance in plants by Dr. J. Kjosbakken and A. Dandekar in California.

It has been observed that gall-forming bacteria, *Agrobacterium tumefaciens*, are potential vectors for transferring the useful stress-tolerance gene, *osm*, to leguminous plants. Proline has received considerable attention as an important osmoticum produced in plants under salt or drought stress. It is known that substrates used in its synthesis are produced in the bacteroids of leguminous root nodules. Recently, recombinant DNA plasmids have been developed that are able to transfer and replicate in root nodule bacteria.<sup>143</sup>

Another technique is the microinjection of DNA from the nucleus of one species into the nucleus of another. Despite its considerable practical value, it has a great limitation in screening and isolating cell lines, which are genetically transformed by such injections.<sup>144</sup>

Recombinant DNA technology may enable genetic engineers to incorporate a single desirable gene in contrast to conventional plant breeding wherein both desirable and undesirable genes are inserted. Gene manipulation technologies are generally somewhat further from application than culture technologies. In order to achieve a useful genetic transfer, protoplast or cell regeneration must take place. This has been found successfully in very few species.<sup>145</sup> Another problem with recombinant DNA techniques is that most traits, particularly those relating to stress tolerance, are polygenic so that the utilization of this technique may be more limited than current expectations.

Another problem is that gene transcription and translation are much more highly complex in eukaryotes than in prokaryotes, and a proportion of eukaryotic DNA does not code for protein and thus it is difficult to determine its function. The great disadvantage of all the modern genetic transfer techniques lies in the fact that character improvement is carried out at the cellular level and the expression of the desired character may not appear in the whole plant. Experience to-date with salt tolerance studies in a range of crop species shows that salt tolerance of most crop species differs from stage to stage.

## XI. CONCLUSIONS

Although variability in salinity tolerance is important for species to exploit saline habitats,

other prevailing environmental factors also are involved that may prevent this possibility due to the inability of selection to promote the evolution of resistant individuals. Nonetheless, it would appear that variability in salinity tolerance is more widely available than generally appreciated and it can be exploited to enhance the salinity tolerance of a considerable number of crop plants. Because the reclamative and preventative measures for transforming salt-affected lands into arable land are very expensive, an evolutionary approach to overcoming salinity problems thus seems to be the only economic and efficient alternative, certainly for developing countries. In fact, considerable success has been achieved in the past in evolving several tolerant/resistant cultivars for other stresses such as aluminum toxicity, drought, waterlogging, and low mineral nutrients.

However, for successful selection, a few points should be contemplated. First, selection pressure used should be very high as this will eliminate all but the most salt-tolerant individuals from within a variable population. Second, the imposition of high selection pressure allows selection of very few individuals with considerably high tolerance. Experience to-date shows that high selection pressures allowing <1% survival have successfully separated highly salt-tolerant lines of several crop species. Third, a large number of plants, as many as can be reasonably handled, should be screened. The author has been able to screen up to 30,000 seeds of some species, allowing the development of highly salt-tolerant lines of several crops. In addition to conventional selection and breeding techniques, various modern genetic engineering techniques such as tissue culture, protoplast fusion, and recombinant DNA techniques may have a role in the improvement of crop salinity tolerance. Their value is, however, as yet unknown, but they will not completely replace conventional plant breeding procedures.

## ACKNOWLEDGMENTS

The author wishes to thank Dr. T. McNeilly and Professor A. D. Bradshaw (FRS), Department of Environmental and Evolutionary Biology, University of Liverpool, for kindly reading

the manuscript thoroughly and providing some very constructive comments.

## REFERENCES

1. **Dudal, R.**, Inventory of the major soils of the world with special reference to mineral stress, in *Plant Adaptation to Mineral Stress in Problem Soils*, Wright, M. J., Ed., Cornell University, Agricultural Experiment Station, Ithaca, NY, 1976, 3.
2. **Christiansen, M. B.**, World environmental limitations to food and fiber culture, in *Breeding Plants for Less Favourable Environments*, Christiansen, M. B. and Lewis, C. F., Eds., John Wiley & Sons, New York, 1982, 1.
3. **Wittwer, S. H.**, Future technological advances in agriculture and their impact on the regulatory environment, *Bioscience*, 29, 603, 1979.
4. **Eckholm, E. P.**, Salting the earth, *Environment*, 17, 9, 1975.
5. **Massoud, F. I.**, Salinity and alkalinity as soil degradation hazards, FAO/UNDP Expert Consultation on Soil Degradation, FAO, Rome, 1974, 21.
6. **Carter, V. G. and Dale, T.**, *Topsoil and Civilization*, University of Oklahoma Press, Norman, 1974.
7. **Gelburd, D. E.**, Managing salinity — lessons from the past, *J. Soil Water Conserv.*, July–August, 329, 1985.
8. **Lewis, L. N.**, A vital resource in danger, *Calif. Agric.*, 38, 2, 1984.
9. **Malik, K. A., Aslam, Z., and Naqvi, M.**, *Kallar Grass — A Plant for Saline Land*, The Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan, 1986.
10. **Nace, R. L.**, Water management, agriculture, and ground-water supplies, in *Water and Agriculture*, Hockensmith, R. D., Ed., Publ. No. 62, American Association for the Advancement of Science, Washington, D. C., 1960, 43.
11. **Malloch, A. J. C.**, Vegetation of the maritime clifftops of the Lizard and Land's End Peninsulas, West Cornwall, *New Phytol.*, 70, 1155, 1971.
12. **Goldsmith, F. B.**, The vegetation of exposed seacliffs at South Stack, Anglesey. II. Experimental studies, *J. Ecol.*, 61, 819, 1973.
13. **Ashraf, M., McNeilly, T., and Bradshaw, A. D.**, Tolerance to sodium chloride and its genetic basis in natural populations of four grass species, *New Phytol.*, 104, 725, 1986.
14. **Ashraf, M., McNeilly, T. and Bradshaw, A. D.**, Tolerance of *Holcus lanatus* L. and *Agrostis stolonifera* L. to soil salinity and soil spray, *Plant Soil*, 96, 77, 1986.
15. **Wu, L.**, The potential for evolution of salinity tolerance in *Agrostis stolonifera* L. and *Agrostis tenuis* Sibth., *New Phytol.*, 80, 471, 1981.
16. **Aston, J. L. and Bradshaw, A. D.**, Evolution in closely adjacent populations. II. *Agrostis stolonifera* in maritime habitats, *Heredity*, 21, 649, 1966.

17. **Tiku, B. L. and Snadydon, R. W.**, Salinity tolerance within the grass *Agrostis stolonifera* L., *Plant Soil*, 35, 421, 1971.
18. **Ahmad, I. and Wainwright, S. W.**, Tolerance to salt, partial anaerobiosis and osmotic stress in *Agrostis stolonifera*, *New Phytol.*, 79, 605, 1977.
19. **Humphreys, M. O.**, The genetic basis of tolerance to salt spray in populations of *Festuca rubra* L., *New Phytol.*, 91, 287, 1982.
20. **Watt, T. A.**, The effects of salt water and soil type upon the germination, establishment, and vegetative growth of *Holcus lanatus* L. and *Lolium perenne* L., *New Phytol.*, 94, 275, 1983.
21. **Hannon, N. J. and Bradshaw, A. D.**, Evolution of salt tolerance in coexisting species of grass, *Nature*, 220, 1342, 1968.
22. **Venables, A. V. and Wilkins, D. A.**, Salt tolerance in pasture species, *New Phytol.*, 80, 613, 1978.
23. **Khan, A. H. and Marshall, C.**, Salt tolerance within populations of chewing fescue (*Festuca rubra* L.), *Commun. Soil Sci. Plant Anal.*, 12, 1271, 1981.
24. U.S. Salinity Laboratory Staff, *Diagnosis and Improvement of Salinity and Alkali Soils*, U.S. Dept. Agric. Handbook, 1954, 60.
25. **Maas, E. V. and Hoffman, G. J.**, Crop salt tolerance-current assessment, *J. Irrig. Drainage Div. Am. Soc. Civ. Eng.*, 103, 115, 1977.
26. **Francois, L. E., Danovan, T. and Maas, E. V.**, Salinity effects on seed yield, growth, and germination of grain sorghum, *Agron. J.*, 76, 714, 1984.
27. **Akbar, M., Gunawardena, I. E., and Ponnampereuma, F. N.**, Breeding for soil stresses, in *Progress in Rainfed Lowland Rice*, IRRI, Philippines, 1986, 263.
28. **Anon.**, *Fifteen Years of NIAB*, Nuclear Institute for Agriculture and Biology, Faisalabad, Pakistan, 1986.
29. **McNeilly, T., Ashraf, M. and Veltkamp, C.**, Leaf micromorphology of sea cliff and inland plants of *Agrostis stolonifera* L., *Dactylis glomerata* L. and *Holcus lanatus* L., *New Phytol.*, 106, 261, 1987.
30. **Ashraf, M., McNeilly, T., and Bradshaw, A. D.**, The potential for evolution of salt (NaCl) tolerance in seven grass species, *New Phytol.*, 103, 299, 1986.
31. **Symeonidis, L., McNeilly, T., and Bradshaw, A. D.**, Interpopulation variation in tolerance to cadmium, copper, lead, nickel, and zinc in nine populations of *Agrostis capillaris* L., *New Phytol.*, 101, 207, 1985.
32. **Walley, K., Khan, M. S. I. and Bradshaw, A. D.**, The potential for evolution of heavy metal tolerance in plants. I. Copper tolerance in *Agrostis tenuis* Sibth., *Heredity*, 32, 309, 1974.
33. **Ranwell, D. S.**, *Coastal Marshes in Perspective*, Regional Studies Group Bulletin, No. 2, 1.
34. **Wu, L., Bradshaw, A. D., and Thurman, D. A.**, The potential for evolution of heavy metal tolerance in plants. III. The rapid evolution of copper tolerance in *Agrostis stolonifera*, *Heredity*, 34, 165, 1975.
35. **Levitt, J.**, *Responses of Plants to Environmental Stresses*, Vol. 2, 2nd ed., Academic Press, New York, 1980.
36. **Maas, E. V. and Nieman, R. H.**, Physiology of plant tolerance to salinity, in *Crop Tolerance to Suboptimal Land Conditions*, ASS, CSA, SSSA, Madison, WI; a Special Publ. No. 32, 1978, 277.
37. **Greenway, H. and Munns, R.**, Mechanisms of salt tolerance in nonhalophytes, *Annu. Rev. Plant Physiol.*, 31, 149, 1980.
38. **Flowers, T. J., Troke, P. F., and Yeo, A. R.**, The mechanism of salt tolerance in halophytes, *Annu. Rev. Plant Physiol.*, 28, 89, 1977.
39. **Wyn Jones, R. G.**, Salt tolerance, in *Physiological Processes Limiting Plant Productivity*, Johnson, C. B., Ed., Butterworths, London, 1981, 271.
40. **Cram, W. J.**, Negative feedback regulation of transport in cells. The maintenance of turgor, volume, and nutrient supply, in *Transport in Plants II Part A, Cells, Encyclopaedia of Plant Physiology, New Series*, Luttge, U. and Pitman, M. G., Eds., Springer-Verlag, Berlin, 1976, 284.
41. **Strogonov, B. P.**, Physiological basis of salt tolerance of plants (as affected by various types of salinity), Adak, Nauk. SSSR., Translated from Russian, Israel, Progr. Sci. Transl., Jerusalem, 1964.
42. **Salisbury, F. B. and Ross, C. W.**, *Plant Physiology*, 3rd ed., Wadsworth Publishing Co., Belmont, CA, 1985.
43. **Allaway, W. G.**, Accumulation of malate in guard cells of *Vicia faba* during stomatal opening, *Planta*, 110, 63, 1973.
44. **Osmond, C. B.**, Ion absorption and carbon metabolism in cells of higher plants, in *Encyclopaedia of Plant Physiology, New Series*, Vol. 24, Luttge, U. and Pitman, M. G., Eds., Springer-Verlag, Berlin, 1976, 347.
45. **Bernstein, L. and Ayers, A. D.**, Salt tolerance of five varieties of carrots, *Proc. Am. Soc. Hort. Sci.*, 61, 360, 1953.
46. **Hsiao, T. C.**, Plant responses to water stress, *Annu. Rev. Plant Physiol.*, 24, 519, 1973.
47. **Rains, D. W.**, Salt tolerance — new developments, in *Advances in Food Producing Systems for Arid and Semi-Arids*, Academic Press, New York, 1981, 431.
48. **Storey, R., Ahmad, N., and Wyn Jones, R. G.**, Taxonomic and ecological aspects of the distribution of glycinebetaine and related compounds in plants, *Oecologia*, 27, 319, 1977.
49. **Hanson, A. D. and Nelsen, C. E.**, Betaine accumulation and (<sup>14</sup>C) formate metabolism in water stressed barley leaves, *Plant Physiol.*, 62, 305, 1978.
50. **Wyn Jones, R. G. and Storey, R.**, 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, 1978.
51. **Ashraf, M.**, The effect of NaCl on water relations, chlorophyll, and protein and proline contents of two

- cultivars of blackgram (*Vigna mungo* L.), *Plant Soil*, 119, 205, 1989.
52. **Moftah, A. E. and Michel, B. E.**, The effect of sodium chloride on solute potential and proline accumulation in soybean leaves, *Plant Physiol.*, 83, 238, 1987.
  53. **Wyn Jones, R. G.**, An assessment of quaternary ammonium and related compounds as osmotic effectors in crop plants, in *Genetic Engineering of Osmoregulation*, Rains, D. W., Valentine, R. C., and Hollaender, A., Eds., Plenum Press, New York, 1980, 155.
  54. **Wyn Jones, R. G., Gorham, J. and McDonnell, E.**, Organic and inorganic solute contents as selection criteria for salt tolerance in the Triticeae, in *Salinity Tolerance in Plants — Strategies for Crop Improvement*, Staples, R. C. and Toenniessen, G. H. Eds., John Wiley & Sons, New York, 1984, 189.
  55. **Waisel, Y.**, *Biology of Halophytes*, Academic Press, New York, 1972.
  56. **Lagerwerff, J. V. and Eagles, H. E.**, Osmotic and specific effects of excess salt on beans, *Plant Physiol.*, 36, 472, 1961.
  57. **Abel, G. H. and Mackenzie, A. J.**, Salt tolerance of soybean varieties (*Glycine max* (L.) Merrill) during germination and later growth, *Crop Sci.*, 4, 157, 1964.
  58. **Abel, G. H.**, Inheritance of the capacity for chloride inclusion and chloride exclusion by soybeans, *Crop Sci.*, 9, 697, 1969.
  59. **Greenway, H.**, Plant response to saline substrates. VII. Growth and ion uptake throughout plant development in two varieties of *Hordeum vulgare*, *Aust. J. Biol. Sci.*, 18, 763, 1965.
  60. **Rush, D. W. and Epstein, E.**, Genotypic responses to salinity: differences between salt sensitive and salt tolerant genotypes of tomato, *Plant Physiol.*, 57, 162, 1976.
  61. **Greenspan, H. and Kessler, B.**, Sodium fluxes in excised *Citrus* roots under steady state condition with particular reference to salinity resistance, *J. Exp. Bot.*, 21, 360, 1970.
  62. **Lauchli, A. and Epstein, E.**, Mechanisms of salt tolerance in plants, *Calif. Agric.*, 38, 18, 1984.
  63. **Ashraf, M., Bokhari, M. H., and Karim, F.**, Salinity effects on some cultivars/lines of mash (*Vigna mungo* (L.) Hepper) at two growth stages, *Biologia*, 34, 81, 1988.
  64. **Ashraf, M., McNeilly, T., and Bradshaw, A. D.**, The potential for evolution of tolerance to NaCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and seawater in four grass species, *New Phytol.*, 112, 245, 1989.
  65. **Collander, R.**, Selective absorption of cations by higher plants, *Plant Physiol.*, 16, 691, 1941.
  66. **Winter, E.**, Salt tolerance of *Trifolium alexandrinum* L. III. Effects of salt on ultrastructure of phloem and xylem transfer cells in petioles and leaves, *Aust. J. Plant Physiol.*, 9, 239, 1982.
  67. **Winter, E. and Preston, J.**, Salt tolerance of *Trifolium alexandrinum*. IV. Ion measurement by x-ray microanalysis in unfixed, frozen hydrated leaf cells at various stages of salt treatment, *Aust. J. Plant Physiol.*, 9, 251, 1982.
  68. **Thomson, W. W.**, The structure and function of salt glands, in *Plants in Saline Environments*, Poljakoff-Mayber, A. and Gale, J. Eds., Ecological Studies 15, Springer-Verlag, New York, 1975, 118.
  69. **Lauchli, A.**, Salt exclusion: an adaptation of legumes for crops and pastures under saline conditions, in *Salinity Tolerance in Plants — Strategies for Crop Improvement*, Staples, R. C. and Toenniessen, G. H., Eds., John Wiley & Sons, New York, 1984, 171.
  70. **Jacoby, B.**, Function of bean roots and stems in sodium retention, *Plant Physiol.*, 39, 445, 1964.
  71. **Winter, E. and Lauchli, A.**, Salt tolerance of *Trifolium alexandrinum* L. I. Comparison of the salt response to *T. alexandrinum* and *T. pratense*, *Aust. J. Plant Physiol.*, 9, 221, 1982.
  72. **Ashraf, M., McNeilly, T., and Bradshaw, A. D.**, Response and ion uptake of selected salt tolerant and unselected lines of three legume species, *New Phytol.*, 104, 463, 1986.
  73. **Hannon, N. J. and Barber, H. N.**, The mechanism of salt tolerance in naturally selected populations of grasses, *Search*, 3, 259, 1972.
  74. **Van Steveninck, R. F. M., Van Steveninck, M. E., Stelzer, R., and Lauchli, A.**, Studies on the distribution of Na<sup>+</sup> and Cl<sup>-</sup> in two species of lupin (*Lupinus luteus* and *Lupinus angustifolius*) differing in salt tolerance, *Physiol. Plant.*, 56, 465, 1982.
  75. **Lessani, H. and Marschner, H.**, Relation between salt tolerance and long-distance transport of sodium and chloride in various crop species, *Aust. J. Plant Physiol.*, 5, 27, 1978.
  76. **Croughan, T. P., Stavarek, S. J., and Rains, D. W.**, Selection of a NaCl tolerant line of cultured alfalfa cells, *Crop Sci.*, 18, 959, 1978.
  77. **Ashraf, M., McNeilly, T., and Bradshaw, A. D.**, Selection and heritability of salt tolerance in four forage species, *Crop Sci.*, 227, 232, 1987.
  78. **Ashraf, M., McNeilly, T., and Bradshaw, A. D.**, Patterns of ion distribution in selected NaCl tolerant and normal lines of four grass species, *Biol. Plant.*, 32, 302, 1990.
  79. **Kuiper, P. J. C.**, Functioning of plant cell membranes under saline conditions: membrane lipid composition and ATPases, in *Salinity Tolerance in Plants — Strategies for Crop Improvement*, Staples, R. C. and Toenniessen, G. H., Eds., John Wiley & Sons, New York, 1984, 77.
  80. **Rains, D. W.**, Salt transport by plants in relation to salinity, *Annu. Rev. Plant Physiol.*, 23, 367, 1972.
  81. **Jeschke, W. D.**, 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*, Staples, R. C. and Toenniessen, G. H., Eds., John Wiley & Sons, New York, 1984, 37.
  82. **Wyn Jones, R. G., Brady, C. J., and Speirs, J.**, Ionic and osmotic regulation in plants, in *Recent Advances in the Biochemistry of Cereals*, Laidman, D. L. and

- Wyn Jones, R. G., Eds., Academic Press, London, 1979, 63.
83. **Elzam, O. E. and Epstein, E.**, Salt relations of two grass species differing in salt tolerance. I. Growth and salt content at different salt concentrations. II. Kinetics of the absorption of  $K^+$ ,  $Na^+$ , and  $Cl^-$  by their excised roots, *Agrochimica*, 13, 190, 1969.
  84. **Wyn Jones, R. G. and Lunt, O. R.**, The function of calcium in plants, *Bot. Rev.*, 33, 407, 1967.
  85. **Fageria, N. K.**, Ionic interactions in rice plants from dilute solutions, *Plant Soil*, 70, 309, 1983.
  86. **Kramer, D., Lauchli, A., Yeo, A. R., and Gullasch, J.**, Transfer cells in roots of *Phaseolus coccineus*: ultrastructure and possible function in exclusion of sodium from the shoot, *Ann. Bot.*, 41, 1031, 1977.
  87. **Bernstein, L.**, Effects of salinity and sodicity on plant growth, *Annu. Rev. Phytopathol.*, 13, 295, 1975.
  88. **LaHaye, P. A. and Epstein, E.**, Calcium and salt toleration by bean plants, *Physiol. Plant.*, 25, 213, 1971.
  89. **Kawasaki, T. and Moritsugu, M.**, Effect of calcium on salt injury plants. II. Barley and rice, *Ber. Ohara Inst. Landwirtsch Biol. Okayama Univ.*, 17, 73, 1978.
  90. **Muhammad, S., Akbar, M. and Neue, H. U.**, Effect of Na/Ca and Na/K ratios in saline culture solution on the growth and mineral nutrition of rice (*Oryza sativa* L.), *Plant Soil*, 104, 57, 1987.
  91. **Yeo, A. R. and Flowers, T. J.**, The absence of an effect of the Na/Ca ratio on sodium chloride uptake by rice (*Oryza sativa* L.), *New Phytol.*, 99, 81, 1985.
  92. **Mendel, G.**, Experiments in plant hybridization, in *Classical Papers in Genetics*, Peters, J. A., Ed., Prentice-Hall, Englewood Cliffs, NJ, 1959.
  93. **Bateson, W.**, *Mendel's Principles of Heredity*, University Press, Cambridge, 1909.
  94. **Harlan, J. R.**, Genetic resources in wild relatives of crops, *Crop Sci.*, 16, 329, 1976.
  95. **Sprague, G. F., Alexander, D. E., and Dudley, J. W.**, Plant breeding and genetic engineering: a perspective, *Bioscience*, 30, 17, 1980.
  96. **Engibous, J. C.**, Implications of fertilizer shortages for crop yield and quality, in *International and National Outlook — the Necessity for Efficient Nutrient Utilization*, Usherwood N. R. and Doll, E. C., Eds., Special Publ. 25, American Society for Agronomy, Madison, WI, 1975, 37.
  97. **Pimentel, D., Hurd, L. E., Bellotti, A. C., Forster, M. J., Oka, I. N., Sholes, O. D., and Whitman, R. J.**, Food production and the energy crisis, *Science*, 182, 443, 1973.
  98. **Lyon, C. B.**, Responses of two species of tomatoes and their  $F_1$  generation to sodium sulphate in the nutrient medium, *Bot. Gaz.*, 103, 107, 1941.
  99. **Akbar, M. and Yabuno, T.**, Breeding for saline-resistant varieties of rice. IV. Inheritance of delayed type panicle sterility induced by salinity, *Jpn. J. Breed.*, 27, 237, 1977.
  100. **Falconer, D. S.**, *Introduction to Quantitative Genetics*, Longman, London, 1981.
  101. **Lawrence, M. J.**, The genetical analysis of ecological traits, in *Evolutionary Ecology*, Shorrocks, B., Ed., Blackwell, Oxford, 1984, 27.
  102. **Hunt, O. J.**, Salt tolerance in intermediate wheatgrass, *Crop Sci.*, 5, 407, 1965.
  103. **Noble, C. L., Halloran, G. M., and West, D. W.**, Identification and selection for salt tolerance in lucerne (*Medicago sativa* L.), *Aust. J. Agric. Res.*, 35, 239, 1984.
  104. **Allen, S. K., Dobrenz, A. K., Schonhorst, M. H., and Stones, J. E.**, Heritability of NaCl tolerance in germinating alfalfa seeds, *Agron. J.*, 77, 90, 1985.
  105. **Ashraf, M., McNeilly, T. and Bradshaw, A. D.**, Heritability of sodium chloride tolerance in seven grass species, *Euphytica*, 35, 935, 1986.
  106. **Azhar, F. M. and McNeilly, T.**, The genetic basis of variation for salt tolerance in *Sorghum bicolor* (L.) Moench. seedlings, *Plant Breed.*, 101, 114, 1988.
  107. **Burton, G. W.**, Quantitative inheritance in grasses, *Proc. 6th Int. Grassland Cong.*, p. 277, 1952.
  108. **Thody, J. M. and Thompson, J. N., Jr.**, The number of segregating genes implied by continuous variation, *Genetica*, 46, 335, 1976.
  109. **Comstock, R. E.**, Quantitative genetics in maize breeding, in *Maize Breeding and Genetics*, Walden, D. B., Ed., John Wiley & Sons, New York, 1978, 191.
  110. **Akbar, M. and Yabuno, T.**, Breeding for saline-resistant varieties of rice. III. Responses of  $F_1$  hybrid to salinity in reciprocal crosses between Johna 349 and Magnolia, *Jpn. J. Breed.*, 25, 216, 1975.
  111. **Poehlman, J. M.**, *Breeding Field Crops*, AVI Publishing, Westport, CT, 1978.
  112. **Silvey, V.**, The contribution of new wheat, barley, and oat varieties to increasing yield in England and Wales, 1947–1978, *J. Natl. Inst. Agric. Bot.*, 15, 399, 1981.
  113. **Woodworth, C. M., Leng, E. R., and Jugenheimer, R. W.**, Fifty generations of selection for protein and oil in corn, *Agron. J.*, 44, 60, 1952.
  114. **Kingsbury, R. W. and Epstein, E.**, Selection for salt-resistant spring wheat, *Crop Sci.*, 24, 310, 1984.
  115. **Epstein, E. and Norlyn, J. D.**, Seawater based crop production; a feasibility study, *Science*, 197, 249, 1977.
  116. **Dewey, D. R.**, Salt tolerance of twenty five strains of *Agropyron*, *Agron. J.*, 52, 631, 1960.
  117. **Dewey, D. R.**, Breeding crested wheatgrass for salt tolerance, *Crop Sci.*, 2, 403, 1962.
  118. **Shannon, M. C.**, Testing salt tolerance variability among tall wheatgrass lines, *Agron. J.*, 70, 719, 1978.
  119. **Qureshi, R. H., Ahmad, R., Ilyas, M., and Aslam, Z.**, Screening of wheat (*Triticum aestivum* L.) for salt tolerance, *Pak. J. Agric. Sci.*, 17, 19, 1980.
  120. **Azhar, F. M. and McNeilly, T.**, Variation for salt tolerance in *Sorghum bicolor* (L.) Moench. under hydroponic conditions, *J. Agron. Crop Sci.*, 159, 269, 1987.
  121. **Ashraf, M., and Waheed, A.**, Screening of local/exotic accessions of lentil (*Lens culinaris Medic.*) for salt tolerance at two growth stages, *Plant Soil*, 128, 167, 1990.

122. **Ashraf, M. and McNeilly, T.**, Improvement of salt tolerance in maize (*Zea mays* L.), *Plant Breed.*, 104, 101, 1990.
123. **Rush, D. W. and Epstein, E.**, Breeding and selection for salt tolerance by the incorporation of wild germplasm into a domestic tomato, *J. Am. Soc. Hortic. Sci.*, 106, 699, 1981.
124. **Forster, B. P., Gorham, J., and Miller, T. E.**, Salt tolerance of an amphiploid between *Triticum aestivum* and *Agropyron junceum*, *Plant Breed.*, 98, 1, 1987.
125. **Ashraf, M.**, Induced Variability for Salinity and Aluminium Tolerance by N-Methyl N-Nitrosourea Treatment of Fertilized Egg Cell of Rice, Ph.D. thesis, University of the Philippines at Los Banos, Philippines, 1984.
126. **Zenk, M. H.**, Haploids in physiological and biochemical research, in *Haploids in Higher Plants*, Kasha, K. J., Ed., University of Guelph Press, Guelph, Canada, 1974, 339.
127. **Dix, P. J. and Street, H. E.**, Sodium chloride-resistant cultured cells lines from *Nicotiana sylvestris* and  *Capsicum annum*, *Plant Sci. Lett.*, 5, 231, 1975.
128. **Nabors, M. W., Daniels, A., Nadolny, L., and Brown, C.**, Sodium chloride tolerant lines of tobacco cells, *Plant Sci. Lett.*, 4, 155, 1975.
129. **Hasegawa, P. M., Bressan, R. A., and Handa, A. K.**, Growth characteristics of NaCl-selected and non-selected cells of *Nicotiana tabacum* L., *Plant Cell Physiol.*, 21, 1347, 1980.
130. **Croughan, T. P., Stavarek, S. J., and Rains, D. W.**, *In vitro* development of salt resistant plants, *Environ. Exp. Bot.*, 21, 317, 1981.
131. International Rice Research Institute (IRRI), Annual Report for 1982, P.O. Box 933, Manila, Philippines, 1983.
132. **Nabors, M. W., Gibbs, S. E., Bernstein, C. S., and Meis, M. E.**, NaCl-tolerant tobacco plants from cultured cells, *Z. Pflanzenphysiol. Bd.*, 97, 13, 1980.
133. **Stavarek, S. J., Croughan, T. P., and Rains, D. W.**, Regeneration of plants from long-term cultures of alfalfa cells, *Plant Sci. Lett.*, 19, 253, 1980.
134. **Bradshaw, A. D. and McNeilly, T.**, *Evolution and Pollution*, Edward Arnold, London, 1981.
135. **Wilkins, D. A.**, A technique for the measurement of lead tolerance in plants, *Nature*, 180, 37, 1957.
136. **McNeilly, T. and Bradshaw, A. D.**, Evolutionary processes in populations of copper tolerant *Agrostis tenuis* Sibth., *Evolution*, 22, 108, 1968.
137. **Gregory, R. P. G. and Bradshaw, A. D.**, Heavy metal tolerance in populations of *Agrostis tenuis* Sibth. and other grasses, *New Phytol.*, 64, 131, 1965.
138. **Ab-Shukor, N. A., Kay, Q. O. N., Stevens, D. P., and Skibinski, D. O. F.**, Salt tolerance in natural populations of *Trifolium repens* L., *New Phytol.*, 109, 483, 1988.
139. **Ashraf, M. and McNeilly, T.**, Salinity effects on five lines/cultivars of pearl millet (*Pennisetum americanum* L.), *Plant Soil*, 103, 13, 1987.
140. **Ashraf, M. and McNeilly, T.**, Variability in salt tolerance of nine spring wheat cultivars, *J. Agron. Crop Sci.*, 160, 14, 1988.
141. **Allard, R. W.**, *Principles of Plant Breeding*, John Wiley & Sons, New York, 1960.
142. **Hanson, M. R.**, Cell culture and recombinant DNA methods for understanding and improving salt tolerance of plants, in *Salinity Tolerance in Plants — Strategies for Crop Improvement*, Staples, R. C. and Toenniessen, G. H., Eds., John Wiley & Sons, New York, 1984, 335.
143. **Valentine, R. C.**, Genetic engineering of salinity-tolerant plants, *Calif. Agric.*, 38, 36, 1984.
144. **Epstein, E. and Rains, D. W.**, Advances in salt tolerance, *Plant Soil*, 99, 17, 1987.
145. **Evans, D. A.**, Agricultural applications of plant protoplast fusion, *Biotechnology*, 1, 253, 1983.
146. **Ashraf, M. and McNeilly, T.**, A potential source of variation for salt tolerance in spring wheat, *Hereditas*, 115, 115, 1991.
147. **Maas, E. V.**, Crop tolerance to saline sprinkling water, *Plant Soil*, 89, 273, 1985.
148. **Dobrenz, A. K., Schonhorst, M. H., Stone, J. E., Thompson, R. K., Allen, S., and Smith, D.**, AZGERM SALT-I Nondormant alfalfa germplasm, *Crop Sci.*, 23, 807, 1983.
149. **Rana, R. S.**, Wheat variability for tolerance to salt affected soils, in *Genetics and Wheat Improvement, Proc. 1st Natl. Semin. on Genetics and Wheat Improvement*, Gupta, A. K., Ed., IBH Publishing Co., Ludhiana, India, 1977, 180.
150. **Al-Khatib, M., McNeilly, T., and Collins, J. C.**, The potential of selection and breeding for improved salt tolerance in lucerne (*Medicago sativa* L.), *Euphytica*, 65, 43, 1993.
151. **Ashraf, M.**, personal communication.
152. **Qureshi, R. H.**, personal communication.