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Physiological Mechanisms Relevant to Genetic Improvement of Salinity Tolerance in Crop Plants

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I. INTRODUCTION

Crop species differ widely in their ability to grow and yield under saline conditions. However, almost all crop plants belong to the glycophytic category, except for a few crop species such as sugar beet, which has halophytic ancestors. By ecological definition, halophytes are the native flora of saline habitats [1]. From a crop improvement perspective, the variability for salinity tolerance within a crop species or among its wild relatives is important. It is also important to understand the physiological mechanisms of salinity tolerance operating within a crop species so that suitable breeding strategies can be developed for improving salinity tolerance. There are several reviews covering the general responses of plants to salinity stress and the mechanisms available in halophytes and glycophytes which allow them to cope with saline habitats [2–7]. However, little attempt has been made to integrate information on these physiological aspects into genetic improvement concepts.

Salinity creates stress by reducing the osmotic potential of the rooting medium and increasing ambient concentrations of ions such as Cl, SO_4, CO_3, HCO_3, Na, Ca, and Mg. Being glycophytes, crop species have no appendages, such as salt glands, bladders, or hairs, that excrete salts absorbed in excess from their shoot tissues. The limited compartmentation ability of the shoot demands strict regulation of ionic delivery to the shoot. Physiological mechanisms controlling salt absorption and distribution in crop plants, and the osmotic adjustment that is essential for turgor-driven water uptake, are covered in this chapter. We specifically address the question of how information on these physiological mechanisms could be utilized in genetic improvement programs as an integrated approach to improving salinity tolerance in a given crop.

II. REGULATION OF ION TRANSPORT

Plants regulate their intracellular ionic composition to maintain a suitable ionic environment for the physiological and biochemical processes that proceed within a cell. This internal environment

needs to be maintained within acceptable limits if plant growth and function are to proceed in saline environments [8]. Salinity under field conditions is characterized by a mixture of salts. However, Na and Cl are predominant in most situations. Therefore, most studies of salinity effects refer to NaCl salinity as a model system, although the effects of all ions that are in excess in a saline environment on nutrient uptake are recognized. Similarly, due to the importance of K in plant nutrition and because the effects of Na on K uptake have been studied extensively, we refer primarily to this interaction in our discussion of ion uptake mechanisms.

A. Regulation at Root Membranes

The concept of dual mechanisms of ion transport is a useful framework for describing ion uptake [9]. At low concentrations of K in the external solution, below 1 m*M*, uptake of K, described by a discrete Michaelis–Menten kinetic equation, is thought to operate at the plasmalemma. We shall call this mechanism 1. At K concentrations in the range 1 to 50 m*M*, mechanism 2 operates. Mechanism 2 is thought to involve diffusive, or at least nonselective ion movement across the plasmalemma with the rate limitation inward from the plasmalemma, probably at the tonoplast [9]. For mechanism 1, there is a high selectivity of the active transport mechanism for K over competing cations such as Na. For mechanism 2, this level of selectivity is not present. Mechanism 1 is not influenced by the concomitant counteranion, but mechanism 2 is. For example, compared with Cl, SO_4 severely depresses K absorption at K concentrations in the range of mechanism 2 but not in the range of mechanism 1. This dual phenomenon of ion uptake has been described for various plant and ionic species (see p. 136 of Ref. 9).

Selective ion transport, at least in the range of mechanism 1, depends on metabolic energy derived from adenosine triphosphate (ATP). This allows charge separation across cell membranes, through primary transport of H^+, thus creating a localized electrochemical gradient for other ions to traverse the membrane [10–12]. Cations move in the opposite direction to H^+ (antiport), while anions are co-transported with it (symport) or move as antiport to OH^- or HCO_3 [13].

Selectivity between ionic species is governed by the particular binding properties of cell membrane constituents. Little is known about this process, due to limited knowledge of plant membrane structure and function [13–15]. Breakthroughs in this regard will allow for an understanding of the molecular basis of ion transport and the effects of salinity on this process. The entry of Na or other ions in excess in the ambient solution can be controlled by this selective binding. An alternative for regulating K/Na levels inside root cells is by means of an outwardly directed Na pump at the plasmalemma [2,16–18].

In most situations, saline or otherwise, Na movement across the plasmalemma into root cells is thought to be passive down an electrochemical gradient [7]. For example, the membrane leakage of Na accounts for the cytoplasmic Na levels found in rice [19]. Jeschke [6] has proposed a model to explain K/Na exchange at the plasmalemma (Figure 1), the components of which are:

1. A proton pump powered by ATP generates an electrical potential difference and proton gradient across the plasmalemma.
2. The electrical charge of H is compensated by an influx of K at a specific site or channel. This site has a lower affinity for Na.
3. The proton gradient provides energy for extrusion of Na from the cytoplasm by a H/N antiport; this site is reported to have a lower affinity for K.

There is variation among crop species in their K/Na exchange capability [6,20]. Barley, wheat, and rye showed efficient K/Na exchange compared to sensitive species such as *Allium cepa* and *Helianthus annuus* [20]. The existence of genotypic differences in this trait within

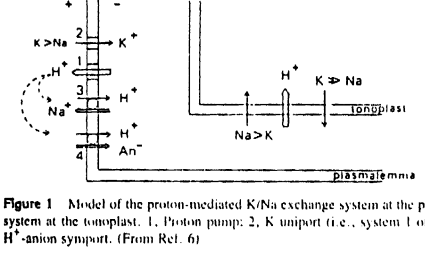


Figure 1 Model of the proton-mediated K/Na exchange system at the plasmalemma and Na/K exchange system at the tonoplast. 1, Proton pump; 2, K uniport (i.e., system I of K influx); 3, H:Na antiport; 4, H^+-anion symport. (From Ref. 6)

crop species and its relation to salinity tolerance is not known. Such information is vital to an evaluation of the potential utility of this trait in genetic improvement programs for salinity tolerance. The relation between K/Na selectivity and salt tolerance has been reviewed [2–4,21–23]. Variation in K/Na exchange suggests at least quantitative differences in membrane properties among different crop species [6]. The general response of many crop plants to a moderate increase in external salinity is increased plant K levels and reduced Na concentrations in tolerant relative to nontolerant genotypes [24–29].

For the high-affinity system mediating K influx (Epstein's mechanism 1), a proton pump appears to be present in the plasmalemma of root cortical cells [6]. However, the graded response of Na-efflux to added K suggests quantitative differences between species, and perhaps among genotypes of a crop species, in the number and efficiency of sites mediating the H/Na antiport [6]. The number of sites for the H/Na antiport needs to be quantified and the existence of genotypic variation within a crop species estimated to determine the feasibility of favorable genetic manipulation of this trait.

At K concentrations above 1 m*M*, in the range of mechanism 2, selectivity is diminished in the face of competition from other ions, such as Na, in the ambient medium. Whether this is due to increased passive movement of all ambient ions across the plasmalemma, down an electrochemical gradient, or less selectivity in an active transport process remains unclear [9]. Eventually, however, if ambient salt concentrations reach high enough levels, membranes would become completely permeable. Information on species or genotypic differences regarding the level at which such physical disruption occurs may also provide a guide to selection for salinity tolerance [30,31].

Most of the kinetic studies referred to above were carried out on tissue previously starved of salts (low salt status). However, as cytoplasmic concentrations of absorbed ions increase, influx rates slow down, indicating a feedback mechanism controlling active influx of ions [13,32]. For example, K concentrations in the cytoplasm of normally growing plants are maintained in the range 90 to 110 m*M* [21]. Although there is considerable speculation on the nature of such feedback mechanisms [13], their further understanding would also assist in selection of genotypes that better control their ion transport processes at the plasmalemma.

B. Intracellular Compartmentation in Roots

Vacuoles occupy more than 80% of a mature root cell's volume and thus provide a means of osmotic regulation for root tissue [33]. This is achieved by compartmentation of inorganic salts,

primarily because these are metabolically inexpensive compared to organic solutes. Salt i move across membranes more easily than do molecules of large molecular weight. There considerable metabolic costs in transporting photosynthates from the shoots for use as osmot in roots [34].

Inorganic ions contribute substantially to osmotic adjustment in root cells of glycophy under saline conditions. However, the amount of osmotic adjustment varies from one spec to another and could be an important factor in determining salinity tolerance. Roots of ma glycophytic crop species contain sustantially higher levels of Na and Cl under saline conditi than do shoots [29,35]. In pigeonpea (*Cajanus cajan*) and its wild relatives, the most toler genotypes retained higher levels of Na and Cl in the roots, and this was associated with salin tolerance in this crop [29,36]. Ability to retain Na and Cl in roots breaks down at a gi concentration, leading to large-scale translocation of these ions to the shoot, with resultant pl mortality. This critical level varies between pigeonpea genotypes and between pigeonpea its wild relatives, and is considered a determinant of the level of salinity tolerance [29].

The cytoplasm shows a strong selectivity for K over Na, Mg over Ca, and P over Cl NO_3 [27,37]. Optimal concentrations for various ions vary in the cytoplasm; thus when k enter the protoplast above this concentration, they may be actively transported through tonoplast into the vacuole. However, these ions could be recovered from the vacuole, depend on the metabolic requirements in other plant parts. Retranslocation of K is one example [6

Vacuoles play an important role in maintaining stable levels of various inorganic ions the cytoplasm, by acting as a storage reservoir for these ions [33]. Under NaCl salinity, and Cl are normally the predominant ions entering the protoplast of root cells. These ions actively pumped into the vacuole after reaching a threshold concentration in the cytopla This would reduce the flow into the xylem of Na and Cl and of other ions associated w salinity (e.g., Ca, Mg, SO_4, CO_3) and thus restrict their translocation to the shoot.

The general hypothesis is that Na and Cl must be excluded from the cytoplasm. Thi based on the sensitivity of enzyme activities to high NaCl levels in vitro [7]. High levels of in the cytoplasm are reported to interfere with K metabolism, resulting in ionic toxicity; bu is not known what Na levels are biochemically compatible with other cytoplasm solutes In corn, cytoplasmic Na concentrations can reach 40 to 70 m*M* under nonsaline conditions [2 but can rise to 140 m*M* under 100 m*M* NaCl external salinity and become toxic to the pl In roots of the halophyte *Triglochin maritima* exposed to 500 m*M* NaCl, the Na/K ratio only 2 in the cytoplasm compared to 15 in the vacuole, although there was approximately m*M* Na in both compartments [38]. Thus the tolerance of the cytoplasm to Na can vary betw species. As long as tissue Na concentration is below the level acceptable for the cytopla more sophisticated compartmentation may not be necessary [7].

There are several factors that could mitigate the adverse effects of excess ions in cytoplasm. One is the type and quantity of organic solutes that could modify the tolerance l of cytoplasm to monovalent cations such as Na. Another is the existence of isoenzymes many enzyme systems, which may have different tolerance thresholds in the cytoplasm. In *mays*, although the total acid phosphatase activity was slightly reduced under salinity, cer isoenzymic forms of acid phosphatase increased in different plant parts [39]. Similarly, relative proportions of malate dehydrogenase isoenzymes were changed during salinity st in pea seedlings [40].

In sunflower, a plastome mutant line that has higher resistance to salinity than that o parental line, reportedly produced a unique isoenzyme of peroxidase under saline conditi [41]. This isoenzyme was found to be resistant to NaCl or Na_2SO_4 salinity up to 1.2% 2.4% respectively, in vitro. Cavalieri and Huang [42] reported that enzymes isolated from r were distinctly more tolerant to Na than those from the shoots; these results might reflect o

differences between shoots and roots and in compartmentation between cytoplasm and vacuole [33]. Another possibility is that certain isoenzymes exist only in certain plant parts; for example, the isoenzyme patterns of shoots could be different from those of roots [43]. Thus the statement which is often made—that "there are no differences in enzyme systems of halophytes and nonhalophytes in their tolerance to monovalent cations in vitro" [3,21,44–46], needs to be reexamined.

Another aspect of the adaptation of higher plants to salinity is compartmentation within the cytoplasm, because the cytosol is particularly sensitive to fluctuating salt levels [35]. For cells involved in salt transport, the rough endoplasmic reticulum (RER) provides a compartment within the cytoplasm in which salt may be sequestered [35]. Substances can be transported symplastically through the RER, via desmotubules. This may also provide a means of ion transfer to vacuoles without disrupting ion concentrations in the cytosol, as RER cisternae may fuse with the tonoplast, releasing their contents into the vacuole [35].

Several hypotheses have been proposed to explain the mode of ion transport from cytoplasm to vacuole through the tonoplast. Pitman and Saddler [16] located an inwardly directed Na pump at the tonoplast that would effectively deplete Na levels in the cytoplasm. Jennings [47] proposed a very similar model for transport of Na from the cytoplasm into the vacuole by means of Na/K exchange. Proton pumps powered by ATP are also thought to play a crucial role in generating the transmembrane electrochemical potential differences required to energize tonoplast ion transport [48,49]. Two types of proton pump are reported to be located in the tonoplast; they are catalyzed by functionally and physiologically distinct phosphohydralases: tp-ATPase and tp-PPase (tonoplast pyrophosphatase) [48].

Exchange of Na and K at the tonoplast can occur only while K remains in the vacuole [6]. Thus distribution of K and Na between vacuole and cytoplasm appears to be crucial for salt tolerance [21,23], and since vacuolar K concentration represents a potential reservoir that could be removed by exchange for Na, the allocation of these ions needs to be regulated. However, the vacuole of root cortical cells is in some respects a dead end; continued selective transport across the root depends on selective transport at the point of entry of salts into the cytoplasm, which depends on the ability of the plasmamembrane to restrict passive influx of sodium and maintain high K/Na selectivity [50]. Thus without control of the quantity of salt that is allowed into the root or that reaches the leaves, intracellular compartmentation either at root cortex or in the shoot would in any case be a very limited option [7]. The vacuole's role may be more in using Na as an osmoticum instead of K and in providing a source of stored K under salinization rather than as part of a selective system of salt transport across the root [50].

C. Regulation of Long-Distance Transport to Shoots

Beyond the plasmalemma, there are several other possible barriers that could minimize transport of excess salts to the shoots. An important one is movement of salts from xylem parenchyma cells into the xylem stream. Evidence favors this process being mediated by active transport [9], with the possibility of further selectivity in ion transport. Xylem parenchyma cells can be differentiated as transfer cells (XPTs) with well-developed wall protuberances adjacent to the bordered pits of xylem vessels in the proximal region of roots and stems. These are reported in *Phaseolus coccineus* [35], *Glycine max* [51], maize [52,53], and squash [54]. These transfer cells accumulate K in the absence of NaCl in the growth medium and Na under saline (NaCl) conditions [51].

A salt-induced formation of wall ingrowths has been reported for xylem parenchyma cells in soybean [51,55] and for the root epidermis cells of *Phaseolus coccineus* [35]. Xylem parenchyma cells and transfer cells are both capable of restricting solutes, particularly Na, by

exchange with K from the transpiration stream [31,56]. These XPTs have been reported to accumulate Na selectively from the transpiration stream and then transfer it to the phloem pathway to be extruded by the roots [57]. In *Lycopersicon*, XPTs in the leaf petiole remove Na from the xylem stream before it enters the leaf lamina [58]. It appears that the entire xylem transport pathway has a backup reabsorption system [6].

The cytoplasm of these transfer cells contains cisternae of RER which increase under NaCl or Na_2SO_4 salinity in *Phaseolus coccineus* hypocotyl and epicotyl [35] and in *Zea mays* [56]. RER could permit a large flow of ions through the cytoplasm of xylem parenchyma cells, assuming that ions are localized mainly in the vacuole [3]. The quantitative significance of this reabsorption process from the xylem in regulating Na-ion transport to the shoot is not known.

The ability of XPTs to absorb Na is finite and could be exhausted rapidly under saline conditions [59]. Some lateral redistribution is possible, however, but this may not be sufficient to prevent Na from eventually reaching the shoot [60]. However, XPTs have a limited capability to store Na, and this Na needs to be removed to the lateral tissue for XPT to continue absorbing Na from the transpiration stream. This Na could be loaded into the phloem and translocated to the roots, where it could either be further compartmentalized or extruded. Such Na extrusion has been reported in *H. vulgare* [16,18,61] and *P. vulgaris* [62]. Thus the practical significance of XPT cells in the basal part of the stem may be limited in controlling Na flow into the shoot to a low degree or a short duration of salinity stress [63,64]. The existence of quantitative variation in XPTs among genotypes in relation to differences in salinity tolerance is not known. Such knowledge is necessary to evaluate the usefulness of this trait from a genetic improvement perspective.

D. Apoplastic Salt Accumulation

Oertli [65] predicted that apoplastic salt load could cause water deficit and turgor loss in leaf cells and proposed it as a mechanism of salinity damage. This concept has received renewed interest [4,66–68]. Under saline conditions, Na and Cl can bypass the ion transport control mechanisms discussed earlier, be carried upward in the xylem stream, and be delivered to the apoplasts of leaf cells [69]. If shoot protoplast accumulates these ions beyond levels that are tolerated in the cytoplasm and its compartmentation capacity of the vacuole, disruption of the metabolic functions due to ionic toxicity would result [70]. On the other hand, a failure to do so would lead to ion accumulation in the apoplast, which could reach very high levels in a short time, as the apoplast occupies only 1% of the cell's volume [65,70]. For instance, even if 90% of the NaCl arriving in the xylem (plants grown at 50 m*M* NaCl external solution) is accumulated in the protoplast, the apoplastic concentrations could reach 500 m*M* within 7 days [70] and cause cell death, although the average tissue Na and Cl concentrations may not reach 100 m*M*. Because of the small apoplast volume, such ion concentrations in the apoplast could occur at overall low tissue concentrations and would thus escape detection in standard tissue analysis [70]. Excessive accumulation of salts in the leaf apoplast would cause turgor loss, stomatal closure, and cell dehydration.

Water deficits in a particular leaf, as opposed to the plant as a whole, could be an inevitable consequence of increasing apoplastic salt load [65] and will occur whenever the rate of arrival of NaCl in the xylem is greater than the rate of accumulation of these ions in leaf cells [67]. Thus arguments that plants have adjusted osmotically to external salinity, which are based on comparisons of solute concentrations in tissue water with external salinity, need to be viewed with caution [71]. The success of a crop species in surviving and reproducing under saline conditions depends considerably on its ability to regulate ion delivery into the xylem stream without causing ion toxicity in leaf protoplasts or apoplastic salt buildup [70]. Genotypes that

could more effectively transfer NaCl from leaf apoplast into leaf cells would be at an advantage. Although this increases their protoplast salt concentrations due to the relative volumes of protoplast and apoplast, this is considered to be less serious than the consequences of apoplastic salt buildup [19,70].

E. Phloem Retranslocation

When Na or Cl levels in the cytoplasm of mesophyll cells reach a tolerance threshold and their compartmentation capacity becomes saturated, additional Na or Cl ions can immediately be transported by intraveinal recycling so as to prevent apoplastic buildup of Na or Cl or ion toxicity in the cytoplasm [64]. Since there is no barrier between the xylem and the leaf apoplast [72], ions can be actively loaded into phloem vessels [73]. This mechanism may play a significant role in the regulation of Na or Cl ions in the shoot [54,62,74]. Based on cytoplasmic Na concentrations, it has been estimated that nearly 25% of the Na entering the leaf can be retranslocated by the phloem [33]. However, phloem loading and retranslocation of Na or Cl is seen as metabolically expensive. Large quantities of Na or Cl in phloem reflects poor control at the root level in regulating ion flow into the xylem. This was found in studies by Lessani and Marschner [75], where phloem translocation of Na or Cl is greatest in sensitive species such as bean, and least in tolerant species such as barley and sugar beet [27].

Among a range of species, there was a significant correlation between a decrease in dry matter production at 100 m*M* NaCl in the medium and Na retranslocation from leaves, particularly, efflux from roots (Figure 2) [75]. If incoming ions are excessive to the shoot's compartmentation ability and the phloem translocation capacity, overloading of Na or Cl ions into the phloem parenchyma transfer cells could occur. This would result in destruction of phloem transfer cells [64,76]. Although phloem retranslocation does contribute to regulation of Na or Cl levels in the shoot, it appears to have a limited role in this regard and thus in determining the level of salinity tolerance. Regulation of Na and Cl levels in the shoot lies primarily with the root's ability to regulate Na or Cl flow into the xylem, rather than the shoot's ability to retranslocate to the root [59].

Availability of sufficient K in growing and expanding regions of the shoot and root is crucial to maintenance of K/Na selectivity and subsequent Na compartmentation in the root cortex. In addition to efficient K/Na selectivity at the plasmamembrane, phloem transport of K reserves within the plant plays an important role in salinity tolerance. Potassium is remobilized from mature leaves by removal of vacuolar K through Na/K exchange at the tonoplast of mesophyll cells. This K is then retranslocated to the growing regions of the root, shoot, and expanding leaves, where there is little vacuolar space and the cytoplasm occupies a major portion of the cell. These growing zones require large quantities of K to meet their demands for osmotic adjustment in the rapidly expanding vacuolar space. Leaves develop and expand close to the shoot apex and derive their mineral nutrient supply from the phloem (which is rich in K), particularly since phloem tissue differentiates prior to xylem elements [77]. With increasing leaf age, minerals are imported mainly by the xylem, which is high in Na levels compared to phloem supply. This Na is compartmentalized through Na/K exchange at the tonoplast; thus K is recovered from the vacuole to provide a major source of K for retranslocation [23].

Nearly 20% of K arriving in the shoot through the xylem could be retranslocated to the growing regions of the root, where high K levels are essential [6]. Such K retranslocation has been reported in barley [78-80], tomatoes, and lupins [6]. The ability to remobilize and retranslocate K into the growing region of the root and shoot plays an important role in Na compartmentation in the root cortex and in maintaining a high K/Na ratio in shoot growing

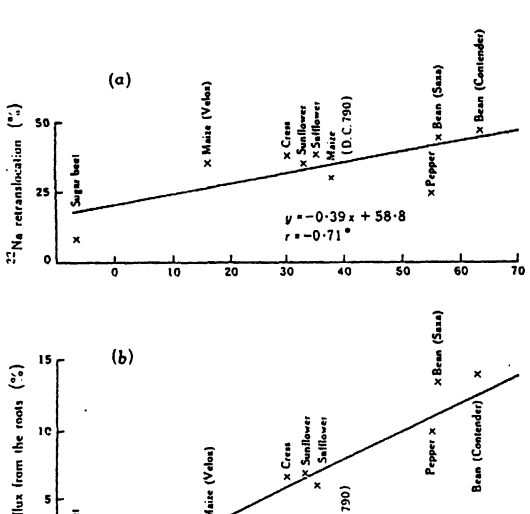


Figure 2 Relationship between decrease in dry matter production and (a) Na retranslocation and (b) efflux of Na from roots, in species differing in their tolerance to salinity. (From Ref. 75)

regions, thus protecting them from Na or Cl toxicity. Most tolerant crop species, such as barley and sugar beet, have a very efficient K recirculation system which is tightly linked to Na regulatory mechanisms. This mechanism may also be important in determining genotypic differences in salinity response.

F. Role of Transpiration

Shoot ion concentrations are a product of transpiration rate, xylem ion concentrations, and growth rate [81]. Under high evapotranspirational demands, transpiration increases, while K/Na selectivity decreases, resulting in increased Na and Cl uptake [82,83]. Alternatively, a reduction in transpiration can decrease ion (Na and Cl) uptake [6,27,60].

There are a number of hypotheses proposed to explain increased xylem sap Na and Cl levels under high evapotranspiration rates in saline growth media. Enhanced water flow interacts

with ion flow across membranes of root cells at more than one site, thus interfering with processes that regulate the balance between ion accumulation in the root cell vacuole and transport to the shoot [6]. Increased water flow due to transpiration promotes passive ion movements where there is no active transport barrier [84].

Water flow can promote the ion flow across the cortex toward a pump that secretes ions into xylem vessels [80]. Ions could either be moved by water along an apoplastic pathway at high concentrations or be coupled to water flow during symplastic passage across the root [85]. High transpiration rates increased Na transport more than K, thus shifting the selectivity toward Na [86,87]. Potassium ions absorbed in roots may be released through Na/K exchange, mainly from vacuoles, for transport to the shoot at times of high evaporative demand [6].

In halophytes, the entry of ions such as Na or Cl into the roots or their release to the xylem sap is tightly regulated at high evapotranspirative demand under saline conditions, thus regulating ion supply to the shoot. Certain morphological features, such as increased width or early development of Casparian strips [88] or formation of a double endodermis [89,90], have been reported to develop under high evaporative demands, thus minimizing the passive influx and bypass flow of Na and Cl ions into the xylem. We are not aware of such anatomical changes reported in any crop species under saline conditions. It may be worthwhile to examine genotypes that show high salinity tolerance for such kinds of adaptive features. Since water use is tightly linked to ion uptake and selectivity, the morphological and physiological traits that increase water use efficiency (WUE) in a given genotype could have a role in determining salinity tolerance [81]. In rice, genotypes that showed higher WUE also have a higher level of salinity tolerance [81].

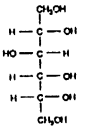
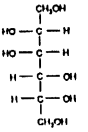
III. ORGANIC SOLUTE ACCUMULATION

A wide variety of organic solutes have been reported to accumulate in plant tissues during water and salt stress conditions and contribute to osmotic adjustment [91]. The chemical nature of the compatible solutes varies from one taxonomic group to another, but most are derivatives of polyols or nitrogen dipoles [27] (Table 1). Osmotic adjustment by the plant promotes turgor maintenance and is thus associated with adaptation to both high soil salinity and low soil moisture [4,92]. Compatible solutes are an important factor in the osmotic balance of the cytoplasm under salt stress [21], where sodium salts are sequestered to play a complementary osmotic role in the vacuole [3,21,23]. However, this is considered to be a halophytic mode of osmoregulation [93], which is energetically more efficient than overall osmoregulation by organic solutes [5,94], which is a common feature of glycophytes [91].

These organic solutes may comprise common metabolites such as sugars, amino acids such as proline [95–98], and organic acids such as proline betaine [99] and other aliphatic quaternary ammonium compounds [100]. There is evidence that solute accumulation is a regulated process and not merely the result of a discrepancy between the sensitivity of the growth process and photosynthesis to stress [101]. Nevertheless, metabolites such as glucose and sucrose accumulate in tissues whose growth has been inhibited by stress [91].

The type of stress would determine which compounds act as osmotic solutes [102]. In grain sorghum, betaine accumulates only under moderate levels of salt stress, not under water stress [102]. However, in crops such as wheat, barley, and rye, betaine accumulates under water stress as well as salinity stress [102]. Nevertheless, salinity is reported to be the more effective stimulator of betaine accumulation [103]. In barley, more glycine betaine is accumulated under gradual stress, but proline is the predominant solute under sudden stress [104].

Table 1 Types of Compatible Solutes That Could Accumulate Under Salinity Stress in Various Plant Species

Solute	Structure	Distribution
D-Sorbitol		Plantaginaceaec Rosaceae
D-Mannitol		Combretaceae Myrsinaceae Rublaceae
D-Pinitol		Leguminoseae Rhizophoraccae Caryophyllaceae
L-Quebrachitol		Euphorblaceae
Glycine betaine	$(CH_3)_3N^{\oplus}CH_2COO^-$	Chenopodlaceae Amaranthaccae Asteraceae Solanaćcae Gramlneae Avicennisceae
β-Alanine betaine	$(CH_3)_3N^{\oplus}CH_2CH_2COO^-$	Plumbaginaccae
Proline		Juncaginaccеae Asteraccae Gramincae
Proline betaine (stachydrine)		Lablaicae Capparidaceae Leguminoscae
3-Dimethylsulfonio propionate	$(CH_3)_2S^{\oplus}CH_2CH_2COO^-$	Asteraccae Gramincae

Source: Ref. 27.

A. Role in Osmoregulation

High concentrations of organic solutes in the cytoplasm could contribute to the osmotic balance when electrolytes are lower in the cytoplasm than in the vacuole [95,105]. These compatible solutes could also act as a nitrogen source [106] or protect membranes against salt inactivation [107,108]. These proposed activities may complement each other within the integrated metabolic and ontogenic pattern of a particular species [109].

Under saline conditions, the large quantity of Na, K, and Cl and other ions that are translocated to the shoot and contribute to the osmotic adjustment are believed to accumulate mainly in the vacuole after reaching threshold levels in the cytoplasm [7]. This concentration of inorganic ions could be considered as a threshold level at which accumulation of such organic solutes as proline, betaine, or other compounds begins in the cytoplasm, thus maintaining the intracellular osmotic balance between cytoplasm and vacuole [102]. For instance, in wheat, proline began accumulating when Na + K exceeded a threshold value of 200 mol/g fresh weight [110]. Also, in grain sorghum, a moderate level of salt stress (0.4 MPa or more) is required to induce a significant betaine concentration [111,112]. Further studies are needed to determine the extent to which this threshold level varies among genotypes of a given species.

The total quaternary ammonium compounds (QACs) in the leaf tissue in wheat species (*Triticum aestivum* and *T. durum*) shows a high positive correlation with salinity treatment [102]. The capacity to accumulate betaine in grasses has been reported to be correlated with basal levels of betaine in unstressed plants [113]. Crops such as oats and rice, which have very low betaine levels under nonsaline conditions, accumulated very little under stress conditions [102].

The relatively small increases in glycine betaine with increasing external salinity, together with the high levels found in many halophytes at very low external salinity, implies that this solute may be redistributed between the vacuole and cytoplasm, depending on tissue electrolyte concentrations [105]. However, in crop plants such as sorghum, it is reported that betaine is relatively nonlabile compared to compounds such as proline [114,115]. A sixfold increase in glycine betaine levels in isolated chloroplasts of spinach under saline conditions was observed, which could account for 36% of the osmotic adjustment in chloroplasts [116].

Proline levels can change quickly in response to abrupt stress, while other organic solutes accumulate more slowly [112]. Thus when stress is applied slowly, less proline accumulates, but the total accumulation of organic solutes remains predictable on the basis of tissue Na and Cl levels [104]. Accumulation of free proline has been correlated with tissue Na concentration in a number of crop species [117–119]. A level of 25 mol proline per gram fresh weight could produce a concentration of 280 *mM* if confined to cytoplasm, thus making a significant contribution to the cytoplasmic solute potential [3].

Proline concentrations were reported to be directly proportional to Na concentrations [120]; each increase in Na concentration is reported to be balanced by an increase in proline concentration equal to about 4% of the rise in Na [121]. This relationship between steady-state proline concentrations and Na levels indicates its role as a cytoplasmic solute [121]. Proline levels for various grasses (*Sorghum bicolor, Agrostis stolonifera, Cynodendactyla, Paspalum vaginatum*, etc.) increased in response to Na accumulation [120]. However, overall proline levels and accumulation rates were highly variable among grasses and therefore are not reliable indicators of relative tolerance levels [120].

In pigeonpea, proline levels increased with increasing external salinity in two genotypes differing in their salt tolerance. The highest proline levels are observed at 10 dS/m, where both genotypes died subsequently [29]. Among the wild species related to pigeonpea, there is a steady increase of proline levels with increasing external salinity in only a few species (Figure 3). There

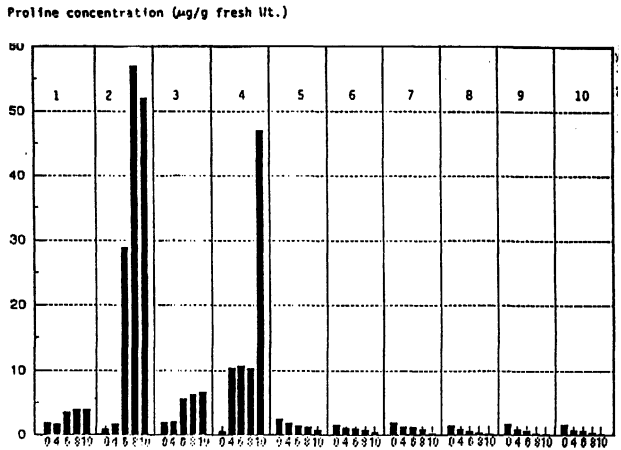


Figure 3 Proline accumulation in the wild relatives of pigeonpea (*Atylosia* sp.) at various salinity level (Leaf samples were collected at 50 days after sowing.) 1, *A. albicans*; 2, *A. sericea*; 3, *A. acutifolia*; 4, *A. lineata*; 5, *A. cajanifolia*; 6, *A. volubilis*; 7, *A. reticulata*; 8, *A. Grandifolia*; 9, *A. goensis*; 10, *A. lanceolata*. (From Refs. 29 and 157.)

was no clear relationship between salinity tolerance and proline accumulation, as proline accumulated to higher levels in both sensitive and tolerant species [29]. Similarly, some tolerant and sensitive species did not accumulate significant levels of proline at any level of external salinity thus defying any simple relationship between proline accumulation and salinity tolerance [29].

It is usually assumed that the cytoplasm comprises about 5% of the cell's volume, proline is accumulated in the cytoplasm, and Na is largely sequestered in the vacuole [121]. Under these conditions, proline alone could merely osmotically balance the Na. However, other ions and organic solutes are also likely to be involved, as field salinity is often caused by a mixture of salts. Thus a variety of ions, particularly K, Mg, or Ca, can accumulate in the cytoplasm under those conditions. Given the wide range of organic solutes that can accumulate in different crop species (Table 1) or even among different genotypes within a crop species, which may have a functionally similar role, it would be unrealistic to expect any direct correlation between salinity tolerance and accumulation of any particular organic solute, either qualitatively or quantitatively.

B. Role in Ion Compartmentation

Compatible solutes or cytosolic solutes could play an important role in regulating intracellular ion distribution under salt stress, thus inducing Na accumulation in the vacuole [120]. External

applied glycine betaine is reported to increase vacuolar Na concentration in barley roots [114]. The salt concentration required for proline accumulation could be the same as is required for salts to be sequestered into the vacuole [112]. The reported threshold of about 200 mol (Na + K/g fresh weight) is only slightly above (Na + K) levels measured in unstressed leaves [112]. In sorghum, proline accumulation seems to be related to total monovalent cation concentration whether either Na or K salts were used in the salinity treatment [111]. An ion pump at the tonoplast could become active at about the same cytoplasmic salt concentration that activates the accumulation of proline or other organic solutes [112].

C. Role in Protecting Enzymes Against Monovalent Cations

Apart from the purpose of osmoregulation, organic solutes can accumulate to protect cell metabolism from the toxic effects of accumulated ions [3,118–120,122]. Pollard and Wyn Jones [123] demonstrated such protection using glycine betaine and, in barley leaves, with the enzyme malate dehydrogenase (decarboxylating). Glycinebetaine has been reported to partially stabilize enzymes and membranes against a range of perturbations [124]. Proline levels up to 600 m*M* did not inhibit enzyme activity in vitro [125]. In barley, 1000 m*M* proline did not inhibit dehydrogenase activity [91]. Polyribosomes are stable in vitro in glycine betaine and proline concentrations up to about 1000 m*M* [126].

Thus the effect of proline and glycine betaine on enzyme systems in the presence of inhibitory ion concentrations may be an expression of a wider role of such compounds in protein stability [91]. Most organic solutes that accumulate under stress conditions are compatible with enzyme activity and continued metabolism [91].

Osmoregulators can not only be compatible with cytoplasmic enzymes, but can either promote or inhibit enzyme activity, depending on the enzyme source [127]. The affinity of phosphoenolpyruvate carboxylase (PEP Case) (extracted from *Cynodon dactylon* and *Sporobolus pungens* grown on saline soil) for PEP was increased by betaine and proline, which resulted in full protection against NaCl inhibition [127]. However, proline did not protect PEP Case against NaCl when it was extracted from *Salsola soda*, although betaine did provide protection [127]. These differences could be due to the existence of isoenzymes.

Although organic-compatible solutes may ameliorate some of the effects of accumulated ions, it seems that ion compartmentation is of greater significance in preserving metabolic activities. In some cases the effects of compatible solutes are apparent only under severe stress and act merely as a survival trait rather than having any beneficial effect on growth during stress [128]. But they may promote growth recovery if these solutes protect enzyme systems against stress-induced degradation, so that they can recommence synthetic function rapidly [91].

D. Metabolic Costs of Organic Solute Accumulation

Despite active accumulation of organic osmotica, there is no evidence of an additional cost, and thus osmotic adjustment exists as an energy-efficient and physiologically effective device for alleviation of drought and salinity stress [129]. However, synthesis of organic molecules such as proline or betaine does put an additional metabolic load on the plant. When sugars are used for osmotic adjustment, they are not available for growth [129]. The accumulation of nonstructural carbon is associated with osmotic adjustment and turgor maintenance [7]. Turner [130] considers that the carbon required for osmotic adjustment would be only a small fraction of that produced by the plant. However, the metabolic cost of storing photosynthate and using it for osmotic adjustment is less than the cost of converting it to new biomass, which the nonstressed plants were better able to do [129]. This explanation was confirmed by the fact

that there was a large increase in the respiration rate accompanied by a rapid increase in le area when stressed plants were irrigated [129].

From the above it appears that a variety of organic solutes accumulate under salinity drought stress conditions. Some of these compounds could be the result of passive accumulati (i.e., due to the general reduction in growth processes). Carbon and nitrogen compounds simply diverted from growth-related activities to produce compounds such as proline, sucros or others as a way of storing them. This avoids the formation of toxic compounds, such ammonia or putricine, from excess nitrogen metabolites. However, there is evidence that solu accumulation is an active process and is very strongly regulated according to immediate pla needs as influenced by external salinity and the plant's ability to regulate ion entry into t transpiration stream. Also, apart from acting as an organic osmoticum in the cytoplasm, the compatible solutes accelerate the compartmentation of Na and Cl into the vacuole, thus playi a significant role in determining the crop species level of salinity tolerance. However, it nee to be realized that organic solute accumulation is only one component in the overall maintenan of a stable internal ionic environment in the cytoplasm, which would ultimately determine survival and production potential of a crop species grown in a saline environment. Thus ability to accumulate organic solutes would have a positive functional role only if a genoty has the "genetic know-how" to regulate ion entry, particularly of Na and Cl, into the transpirati stream.

IV. ORGANISM INTEGRATION

Although various processes that play a role in ionic and osmotic regulation at the whole pla level have being discussed separately, the level of salinity tolerance of a given crop species genotype is the collective expression of a number of processes: influx selectivity, K/Na exchan and Na extrusion, Na compartmentation in the root cortex, Na and Cl regulation at t endodermis, retrieval of Na from the xylem stream by XPT, transpiration efficiency, preventi apoplastic accumulation, phloem retranslocation of Na and Cl, K retranslocation, organic solu accumulation, Na and Cl compartmentation in the leaf, and others. For this reason it is n surprising that no single physiological mechanism/trait shows a clear-cut direct relationship salinity tolerance. Genotypes may differ in one or many processes that regulate entry of Na Cl ions into the plant or qualitative or quantitative differences in the organic solutes. The processes interact at the organism level to determine the ultimate level of tolerance.

V. CONCEPTUAL FRAMEWORK FOR INTEGRATING PHYSIOLOGICAL ASPECTS INTO GENETIC IMPROVEMENT PROGRAMS

There is a substantial amount of information on the physiological responses of crop plants t salinity (i.e., mostly NaCl) stress. A major portion of this information deals merely with the effects of excess salts on various metabolic functions of the plants. As Munns et al. [33] poin out, most of this information describes only the consequences rather than the causes of reduce growth or injury and is thus of limited use for integration into genetic improvement programs. We believe that there is scope for more directed physiological research that would be mor relevant to genetic improvement considerations. Emphasis should be given to understandin the interactions among the many possible processes involved, and thus "organism integration." The two main approaches that we see for achieving this are the "black box" and "physiologica ideotype" approaches.

A. Black Box Approach

The black box approach attempts to proceed from established phenotypic differences (i.e., response to salinity) to the underlying differences in physiological mechanisms contributing to higher levels of tolerance [93,131]. Once a source of a higher level of salinity tolerance is identified in the cultivated species or its wild relatives, the next step would be to transfer this tolerance to agronomically acceptable varieties through a conventional breeding approach. Since salinity tolerance is a complex physiological trait, governed by different genes or groups of genes, the problem is how best to transfer this type of trait or ensemble of traits from the donor parent to the recipient. A black box approach is therefore enhanced by an understanding of the specific physiological traits operating in the donor parent by conducting comparative physiological studies between donor and recipient parents. This will facilitate design of the most appropriate genetic improvement procedures. In particular, simple and effective means of screening segregating populations for salinity tolerance are needed, rather than having to rely on the measurement of growth or yield reduction under given levels of salinity. Identification of the predominant physiological trait or traits responsible for the genotypic differences measured is desirable.

In pigeonpea and its related wild species, there appears to be either a curvilinear or a linear relationship between dry matter and tissue Na or Cl levels ($r^2 = 0.76$; $r^2 = 0.70$; $p < 0.001$; Figure 4a and b). However, this relationship is stronger for Na than for Cl. There is a significant positive linear relationship between tissue Na and Cl levels in both shoots and roots ($r^2 = 0.66$; $p < 0.001$; Figure 4e and f). Although the overall relationship between growth reduction and tissue Na or Cl levels appears to be positive, there is considerable variation among various wild species in the level of ionic tolerance within their tissues. This is indicated by the scatter of points. For instance, for a 50% reduction in growth, tissue Cl levels ranged from <1% to about 4%, and for Na it varied from 0.02% to about 1%. For tissue K levels, we did not find any significant relationship ($r^2 = 0.008$; Figure 4c), however, there is a positive relation between K/Na in shoot and shoot growth ($r^2 = 0.73$; $p < 0.001$; Figure 4d). These data points are also very scattered, which indicates a wide range of variation among species for their optimum K/Na requirements at a given level of growth reduction under salinity. This is not surprising given the complexity of physiological mechanisms operating in Na, K, and Cl regulation and the number of mitigating factors that could change the metabolic tolerance of Na and Cl levels in the tissues.

However, in comparing genotypes that differ in their tolerance, especially among the wild relatives of pigeonpea, we have noticed that the ability to retain higher levels of Na and Cl in the roots could be one of the crucial factors in regulating their levels in the shoot. This regulatory ability breaks down at salinity thresholds that vary across species and genotypes [29,36]. Further studies have shown that this regulatory ability is expressed in the F_1 hybrids of crosses between a tolerant wild relative (*Atylosia albicans*) and a sensitive pigeonpea genotype (ICP 3783) (Figure 5) [36]. Thus this trait is heritable. Further studies are required on the segregating F_2 and F_3 generations, including analysis of the ionic constituents, to establish the inheritance pattern of these physiological traits.

B. Physiological Ideotype and Pyramiding Approach

An *ideotype* is defined as "a hypothetical plant described in terms of traits that are thought to enhance genetic yield potential" [132]. Thus a physiological ideotype for salinity tolerance could be defined in terms of the specific physiological traits that are expected to contribute functionally in maintaining ionic and osmotic relations under saline conditions. As expressed

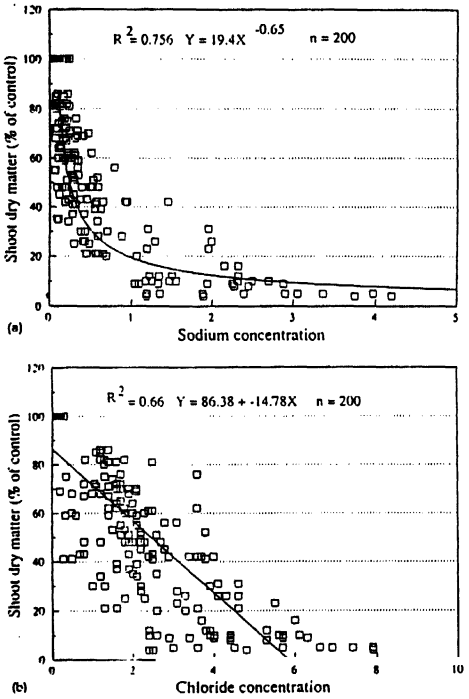


Figure 4 Relationships between shoot dry matter and tissue Na, Cl, K, and K/Na levels (a–d) and between Cl and Na levels in root and shoot (e and f). (Plant samples were collected for growth and chemical analysis at 55 days after sowing; plants were grown at 0-, 4-, 6-, 8-, and 10-dS/m salinity levels.) (From Ref. 157.)

on a relative yield basis, salinity tolerance is the collective expression of a number of physiological traits, as described earlier.

Salinity stress normally varies over time within a crop cycle, from season to season, and from site to site. Different landraces/genotypes/varieties that show a given level of tolerance to salinity are expected to have evolved a variety of mechanisms that contribute to yielding ability under those conditions. For instance, *T. aestivum*, *Secale cereale*, and *Aegilops squarrosa* have an efficient K/Na selectivity character because of the D genome but are less tolerant than crop

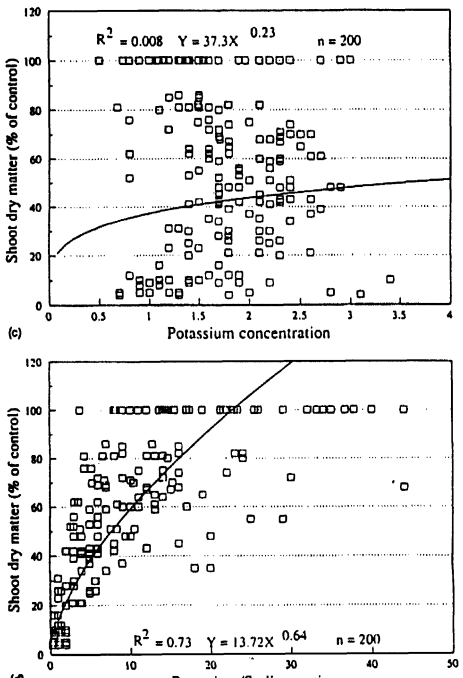
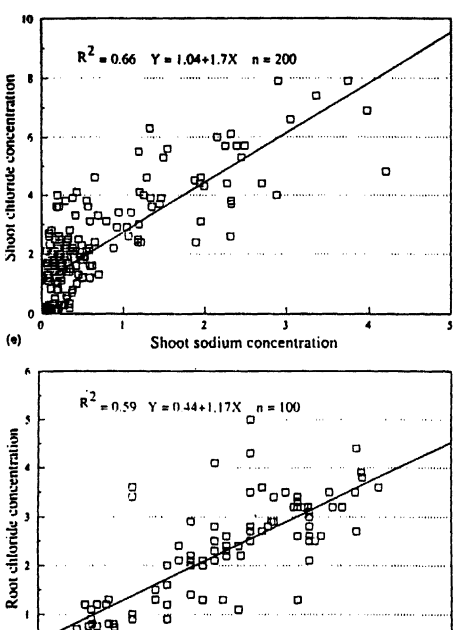


Figure 4 *(Continued)*

species such as *H. vulgare* and *T. durum*, which are less efficient in K/Na selectivity but more efficient in their compartmentation ability [133,134]. Similarly, such differences can be observed among genotypes within a crop species, which is reflected in contradictory reports for various crop species either confirming or disputing a direct correlation between K/Na selectivity and level of salinity tolerance [4].

The underlying philosophy is that although different genotypes may show the same level of tolerance to salinity, they could attain this level of tolerance (i.e., phenotype) through different physiological mechanisms or traits. Lack of sufficient phenotypic variation for salinity tolerance is a serious problem in many crops, and this is particularly so with rice [135]. Even after



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screening the entire world collection of rice germplasm, the most tolerant genotypes would still suffer about a 50% yield reduction at 5 dS/m [135–137]. Conceptually, the physiological approach for improving salinity tolerance in crop plants should be to bring together the relevant traits that would complement each other in a pyramidic manner ("building block" approach) by their selective incorporation into a single genotype or variety under improvement (i.e., optimization of several, probably independent, physiological mechanisms into a single variety) [19].

An analogy can be drawn from disease resistance breeding. In breeding for disease resistance, horizontal resistance (which can be defined as resistance to a number of physiological

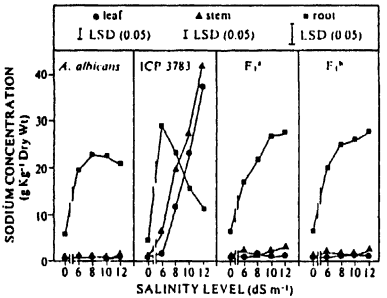

Figure 5 Effect of salinity on tissue Na concentration (g/kg dry weight) of *Atylosia albicans*, *Cajanus cajan* (ICP 3783), and their reciprocal F_1 hybrids (a and b), 75 days after transplanting. Data are means of two replications. (From Ref. 36.)

races of a disease) can be achieved by pyramiding different genes specifically resistant to individual physiological races. This contributes to the stability of a genotype across years in disease-prone environments. The same concept could also be applied to the genetic improvement of salinity tolerance, whereby pyramiding of genes that regulate various specific physiological traits into a single genotype or variety could provide that genotype with the necessary genetic means to respond to various types and levels of salinity stress that it is likely to experience at different locations, sites, and over years. This would contribute to its stability of production as well as widening its adaptability to a greater range of saline environments.

The various steps involved in this kind of approach are:

1. Define the various physiological traits having functional significance in determining the tolerance and productivity of a given crop under saline environments.
2. Establish genetic variability and locate sources of high efficiency for each physiological trait in the germ plasm. Selection should be directed toward the individual components of salinity tolerance on a trait-by-trait basis irrespective of phenotype.
3. Establish the genetic basis for each physiological trait under consideration by studying its inheritance pattern and estimating its heritability, which would determine the feasibility of using that particular trait in a breeding program.
4. Develop restriction fragment length polymorphisms (RFLP) markers if easily identifiable morphological, physiological, or other markers are not readily available for each physiological trait, as this would streamline the selection process of segregating materials in a breeding program.
5. Identify genotypes for each physiological trait which have good combining ability.
6. Incorporate relevant traits into an agronomically acceptable background basis.

Information generated through this exercise could be stored in a database system which would be made available to breeders interested in incorporating salinity tolerance in their breeding programs. This is similar to information databases that are available for morphological

traits from the germ plasm evaluation exercises at CGIAR (Consultative Group for International Agricultural Research) centers.

Selection of traits to be introduced into a given genotype/variety under improvement depends on the target environment in which it will be grown, and the specific traits a particular variety may be lacking. For instance, a variety may be very efficient in Na and Cl compartmentation in the root as well as in the shoot, but may be lacking effective Na or Cl regulation at the plasmalemma. There is evidence of genotypic variation within crop species in Na compartmentation in shoots [19,138,139] and tolerance to high internal Na and Cl levels [69]. In this case, only the trait that is lacking needs to be introduced. Similarly, a given variety may be very efficient in ion regulation, but lacks the necessary genetic means to produce organic solutes.

Development of RFLP markers for each of these physiological components of salinity tolerance could play a crucial role in the incorporation of these physiological traits into a genotype/variety under improvement. Salinity tolerance traits are controlled by a number of genes located throughout the chromosome complement [140]. Each gene of a polygenic system may contribute only a small amount to the trait of interest. Clear dominance is not likely to be exhibited and the phenotype (i.e., the specific trait in this case) would have a large component of environmental variance. All these characteristics conspire to make physiological traits very difficult to analyze. Thus conventional Mendelian methods of analysis, which are suitable for traits controlled by a single or a few genes, cannot be applied to analysis of these physiological traits. This is one reason that physiological traits have not been used extensively in the genetic improvement programs for salinity or drought tolerance, although a number of them having functional significance for determining level of tolerance have been identified [6,141].

With the development of RFLP mapping techniques (for a detailed discussion of RFLP techniques, see Tanksley et al. [142]), it is possible to analyze complex polygenic characters, such as physiological traits, as ensembles of single Mendelian factors. Since RFLP markers can be used to follow simultaneously the segregation of all chromosome segments during a cross, the basic idea is to look for correlations between physiological traits and specific chromosome segments marked by RFLPs. If correlations exist, the inference is that the chromosome segment must be involved in the quantitative trait. The difficult part in this procedure is to establish correlations between the trait and specific chromosome segments. The RFLP markers can easily be scored, but the physiological trait must be characterized in a conventional fashion [142]. Once this most difficult process is completed and specific chromosome segments are implicated in the trait, RFLP markers with a positive effect on a quantitative trait can be selected from a population of plants and incorporated into a single genotype. This is possible because of the ability to score for several RFLP markers simultaneously in a single plant in a manner that is free of environmental influence or gene interactions. Carbon isotope (^{13}C) discrimination, which is an indicator of water use efficiency, could be predicted satisfactorily from three RFLPs in tomato [143]. We have not found any other reports implicating RFLP markers for physiological traits contributing to salinity tolerance. The feasibility of using RFLP markers for physiological traits could bridge the gap between plant physiology and breeding, to facilitate integration of these two disciplines and thus expedite development of varieties that are higher yielding and more stable across environments affected by salinity.

VI. FUTURE OUTLOOK

The past 30 years of research (after the report of dual mechanisms of ion transport by Epstein et al. [144]) on physiological aspects of salinity tolerance has contributed substantially to an

understanding of the mechanisms by which plants cope with excess salts in their habitat. In recent times, efforts have been initiated to identify genes responsible for specific physiological mechanisms. Location of the K/Na selectivity character on the 7a chromosome of the D genome in wheat is one such example [145–147]. Similarly, Na exclusion capability and K/Na discrimination were enhanced in *T. aestivum* by the incorporation of a *Lophopyrum* genome [148]. The K/Na discriminating locus has been located on the 3E chromosome in *Lophopyrum elongatum* [140]. An association with the higher level of salinity tolerance in *Agropyron junceum* has been located in the 5J chromosome [149]. In rice, at least three groups of genes were found to be involved in the inheritance of Na and Ca levels in the plant; Na and Ca levels in shoots and roots were reported to show additive affects with a high degree of heritability [150].

Similarly, Cl translocation is under genetic control [151,152]. Accumulation of organic solutes such as betaine has been reported to be regulated by a limited number of genes [153–156]. Our research with pigeonpea has shown that the higher levels of salinity tolerance, and the associated physiological mechanisms identified in the wild relative *Atylosia albicans*, could be expressed in the reciprocal crosses of F_1 hybrids of this species with the cultivated species (Figure 5) [36]. Information on the genetic control of specific mechanisms is essential for proper integration of physiological research into breeding programs. Recent developments in biotechnology, particularly with genetic markers such as RFLPs, could accelerate this integration of disciplines. Wild relatives have been inadequately explored for their potential to contribute unique physiological mechanisms of salinity tolerance. We hope future efforts would be directed toward generating information on these areas.

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