pool, especially proline. It is possible that salinity also affects plasma membrane integrity. Cell walls may be in involved in salt injury, partly by the interaction of Na^+ with the cell wall bound ions such as Ca^{2+}.

Cytokinins are synthesized in the root and are transported to the shoot. ABA is most likely synthesized in the leaves and transported to the root. As salinity stress increases, less cytokinin is found in xylem exudate, while ABA content of the shoot increases. However, the significance of these changes in salinity stress induced damage is not clear. It was once suggested that the reduced cytokinin production by roots under salinity stress was the route of salinity stress. However, more recent work has raised serious questions about the role of cytokinin and ABA as mediators of salt stress.

25.5. SALINITY RESISTANCE

Salinity resistance may arise from (1) resistance to water-stress or osmotic effects of salinity, (2) resistance to salinity-induced ionic toxicity or toxic effects of salinity (Table 25.7), or (3) a combination of both. For example, salinity resistant ecotypes of *Agrostis stolonifera* showed osmoregulation by accumulation of cell-compatible solutes and avoided accumulation of Na^+ and Cl^-, thereby, avoiding ion toxicity. In contrast, salt-resistant lines of wheat were comparable to susceptible ones in terms of osmoregulation and tissue ion concentration (*i.e.*, Na^+ and Cl^- uptake). Obviously, the resistant lines of wheat were more tolerant to ion toxicity. These examples only highlight the importance of resistances to the osmotic and toxic effects of salinity.

25.5.1. Resistance to Salinity-Induced Water Stress

Osmoregulation is common response to salinity stress (Table 25.7). This allows maintenance of turgor and avoids leaf desiccation and other consequences of turgor loss. This is the major mechanism of countering osmotic effects of salinity, *i.e.*, water stress induced by salinity. Proline is an important solute in osmoregulation of halophytes; in some halophytes it may accumulate up to 10-20% of total plant dry weight. *In terms of cell compatibility, proline may be considered as an ideal osmoticum.*

In case of glycophytes, salt resistant and susceptible genotypes may differ in their capacity for osmoregulation. Osmoregulation may be achieved by sugars (*e.g.*, in tomato), various organic acids, glycine-betaine (sugarbeet), myoinositol (tomato), proline (barley, pea) and K^+ ions (sorghum). It has been proposed that in cases like sorghum, where K^+ accumulated in the vacuole is the main cellular osmoticum, accumulation of proline in the cytoplasm maintains a proper osmotic balance between the cytoplasm and vacuole. It is very likely that sugars are an important cellular solute linked with growth cessation under salinity. For example, tomato plants challenged with salt initially wilt, then accumulate mostly sugars, and after a few days resume growth; the accumulated sugars are rapidly consumed, presumably, for growth. If this be the case, such solutes can not be regarded as stable osmoticum for long-term osmoregulation.

The organic solutes involved in osmoregulation may also protect enzymes and cellular membranes from damage due to stress. In addition, factors other than osmoregulation, *e.g.*,

TABLE 25.7
A summary of the different strategies of salinity resistance in halophytes and glycophytes

Feature	*Glycophytes*	*Halophytes*
Resistance to water-stress		
Osmoregulation	Ions (K^+, Na^+, Cl^-) and metabolites	Generally, ions and metabolites; often only metabolites.
Common metabolites (cell compatible)	Sugars, proline, organic acids, glycine-betaine	Usually proline; often glycine-betaine, organic acids, and sugars
Cost of osmoregulation	Energy for ion uptake, compartmentation; Na^+, Cl^- toxicity; competition with growth components	Energy for ion uptake, compartmentation and excretion; competition with growth components
Resistance to salt toxicity		
Salt exclusion (at root or from the shoot)	Many salt-resistant glycophytes; mechanism unknown	In some cases; mechanism unknown
Salt tolerance	Cellular compartmentation (salts accumulated in vacuoles)	Salt excretion by salt glands; cellular compartmentation.

leaf attributes, characteristics of root system, etc., may be involved in relieving salinity-induced water stress. For example, favourable root system should possibly constitute an important component of resistance where salinity is combined with waterlogged conditions.

25.5.2. Resistance to Salinity-Induced Ion Toxicity

It may be emphasized that *enzymes and cellular processes of halophytes are as sensitive to salt as those of glycophytes.* Therefore, ion toxicity avoidance involves a mechanism, which maintains a low nontoxic level of salts in the cytoplasm. This may be achieved in one of the following two ways: (1) ion exclusion and (2) salt tolerance by cellular compartmentation and/or salt excretion (Table 25.7).

25.5.2.1. Ion Exclusion. When some species/genotypes take up smaller quantities of the injurious ions, *e.g.*, Na^+ and Cl^-, so that the concentrations of these ions in their tissues is much lower than those of other species/genotypes, it is known as ***ion*** or ***salt exclusion.*** Salt exclusion at root is an effective mechanism of avoiding ion toxicity. It occurs in some halophytes, and in salt-resistant lines of bajra (*P. glaucum*), rice, soybean, alfalfa and tomato (lines derived from the cross *L. esculentum,* tomato × *Salanum pennellii*). Certain root stocks of grape and citrus, and some cultivars of citrus, *e.g.*, 'Rangpur' lime and 'Cleopatra' mandarin, are good Cl^- excluders. Use of Cl^- excluding root-stocks in commercial grape production has made significant contribution to grape industry.

The mechanism of salt exclusion from the shoot is not well known, especially in cases of glycophytes. It has been suggested that Na^+ efflux from the root or the absorption of salt by specialized xylem parenchyma cells are involved. The reduced salt uptake by some salt resistant genotypes, especially in short-term experiments, may simply be the consequence of their markedly smaller root system. This is because the size of whole root system will be

positively associated with the salt-load on the roots developped by the transpiration-dependent mass flow of ions to the root surface.

25.5.2.2. Salt Tolerance. *Salt tolerance* may be defined as a differential effect on various life processes of the same tissue concentration of salt in different genotypes of a species. There is considerable evidence that genotypes differ in tolerance to the same amount of salt in their tissues. However, the enzymes and cellular processes of halophytes are as sensitive to salt as those of glycophytes. Therefore, an effective mechanism of preventing salt accumulation in cytoplasm, the site of cellular processes, is necessary. Halophytes usually dispose of the excess salt either through specialised salt glands or by cellular compartmentation, *i.e.*, accumulating the salts in vacuoles. In case of glycophytes, cellular compartmentation is envisaged as the mechanism of salt tolerance, but the details of the mechanism are not known.

Response to salinity may change with plant age. At the same time, salt stress increases as the plant continues to grow and transpire under saline conditions. Therefore, the desired adaptive response would be the one in which plants become more resistant with age either due to the plant age or a process of hardening. But there is little information on hardening response to salinity. At the same time, effect of plant age on salinity resistance varies with the crop (Table 25.5).

25.6. GENETICS OF SALINITY RESISTANCE

Genetic variation for salinity resistance exists both among and within species. A reliable estimation of the magnitude of this variability, especially the within species variability, is critical to the success of breeding programmes for salinity resistance. Blum (1988) has argued quite convincingly that the most reliable approach will be to evaluate the extent of yield (or reduction in yield) of genetically different materials under a range of salinity levels.

25.6.1. Interspecific Variation

A considerable variation exists among crops for salinity resistance in terms of both SY_{100} and SY_{50} (Table 25.8). *SY_{100}, threshold salinity*, describes the maximum salinity level at

TABLE 25.8

Differences in the level of salinity resistance of different crops

Crop	*Threshold salinity level (SY_{100})**	*SY_{50}***
Beans	1 dSm^{-1}	4 dSm^{-1}
Onion	1 dSm^{-1}	4 dSm^{-1}
Barley	8 dSm^{-1}	15 dSm^{-1}
Cotton	8 dSm^{-1}	15 dSm^{-1}
Sugarbeet	8 dSm^{-1}	15 dSm^{-1}
Wheat	3 dSm^{-1}	9 dSm^{-1}

* SY_{100}, the maximum salinity level at which there is no reduction in the yield of crop.

** SY_{50}, the salinility level at which there is a 50% reduction in the yield of crop.

which seeds are planted and covered with another layer of cheese cloth. The solution level is raised just sufficiently to wet the cheesecloth. The salt solution is properly aerated.

2. *Seedling Survival and Growth.* In this approach, seeds are germinated under nonsaline conditions, and the seedlings may then be exposed to the saline condition at the desired growth stage. Salinization in hydroponics, which is relatively easier, should be done in a step-wise manner over an interval of several days. Similarly, when plants have to be removed from salinity, it should also be done in a step-wise fashion. Change in salinity level should not be done at the beginning of dark period since at that time seedlings are more sensitive to shock.

The salt solution used in evaluation should represent the salt composition of the soil at the target site. The salts commonly found in saline soils are NaCl, Na_2SO_4, KCl, $MgCl_2$ and $CaCl_2$. Most selection work has been based on NaCl solution, which is usually supplemented with a $CaCl_2$ solution in 2 : 1 ratio.

25.7.2. Estimation of Salinity Resistance and Selection Criteria

Several criteria may be used to assess salinity resistance; some of these criteria are as follows : (1) cell survival, (2) seed germination, (3) dry matter accumulation, (4) leaf death and senescence, (5) leaf ion content, (6) leaf necrosis, (7) root growth, (8) osmoregulation and (9) yield *per se*. The general techniques for their estimation are briefly described and their usefulness as selection criteria in breeding programmes is examined in the following sections.

25.7.2.1. Cell Survival. Cell survival is measured by immersing tissue sections for 24 hr in a solution of known salinity level. The tissue sections are then transferred into a hypertonic glucose solution; the proportion of plasmolysed cells gives the proportion of surviving cells. *This criterion is often regarded as a better index of salt resistance than that based on dry weight or yield, especially when a range of salinity levels is used.*

25.7.2.2. Germination. Seed germination in a saline medium is often used as the sole selection criterion for salt resistance. This surely improves germination under salinity stress. Therefore, *it may be a desirable selection criterion in such species where germination is more salt sensitive than the later stages of plant growth.* But the effect of salinity stress is limited largely to the stage of seed imbibition. Further, there is no good evidence that resistance at germination is associated with resistance of the later stages of growth. Therefore, this measure would not be of value in such species where subsequent stages of plant growth are more sensitive to salt stress than germination.

25.7.2.3. Dry Matter Accumulation. *Seedling or plant dry weight is a good index of salinity resistance as it integrates the various possible effects of responses to salinity.* However, this measurement suffers from the following problems. (1) It is a destructive method hence can not be applied to segregating generations. (2) Genotypic differences in growth potential would confuse this measurement. (3) It is best used when dry weight under both stress and nonstress conditions are determined for all the genotypes; this doubles the work and expenditure.

25.7.2.4. Leaf Death or Senescence. Salinity stress induces leaf death and senescence. This can be estimated visually either (1) as the total dead leaf area or (2) as the number of

dead leaves. The measurements should be made before the onset of natural senescence. This index of salinity resistance can be very effective in separating resistant plants from the population.

25.7.2.5. Leaf Ion Content. In such cases where salinity resistance is based on ion exclusion, the content of concerned ion in plant leaves provides a measure of their salinity resistance. However, such estimations are usually not accessible to many breeders.

25.7.2.6. Leaf Necrosis. Generally, accumulation of specific ions like Cl^-, Na^+ or K^+ causes leaf necrosis. This may be used as a measure of salinity resistance if it is based on ion exclusion.

25.7.2.7. Root Growth. Root growth often expresses well the relative resistance of a plant to mineral toxicity. But there is no conclusive evidence about its usefulness as a criterion of selection for salinity resistance.

25.7.2.8. Osmoregulation. Osmoregulation may be measured as proline or carbohydrate accumulation in response to salinity stress. More simply, it is determined as maintenance of turgor under stress, and is estimated as leaf wilting, desiccation and premature senescence. *This is a highly desirable criterion especially in cases where osmoregulation is found to be important in conditioning salinity resistance.*

25.7.2.9. Yield. *Economic yield should form an important criterion of selection in any index for salinity resistance.* During segregating generations, yield per plant is estimated, while in case of selected lines and germplasm lines yield per unit area, more desirably over a range of salinity levels, is determined. This is particularly desirable in view of the finding that certain wheat varieties resistant to salinity in the vegetative phase were susceptible at reproductive stage, and *vice-versa.*

In conclusion, there is no single criterion for salinity resistance that has been tested through the process of selection leading to the development of a commercial product. Therefore, preferably *more than one feature should be included in the selection criterion, and yield under stress should invariably be included as an important component.*

25.8. SOURCES OF SALINITY RESISTANCE

Salinity resistance may be available in (1) a cultivated variety, (2) germplasm collections, (3) related species, (4) somaclones, or (5) may be generated by transgenes.

25.8.1. Cultivated Varieties

In some crops, salinity resistance may be present in cultivated varieties. For example, when a collection of cultivated and wild accessions of muskmelon were tested, one of the most resistant materials was a cultivar.

25.8.2. Germplasm Collections

It may be expected that salinity resistance will be present in materials adapted to salt-affected areas (Table 25.6). For example, 'Kharchia' wheat adapted to salt-affected areas of Rajasthan was found to be the most salinity resistant.

25.8.3. Related Species

Wild relatives of many crop species are remarkably resistant to salinity. For example, several species of the genus *Elytriga* (*Agropyron*), a relative of wheat, are more resistant than any wheat accession; examples are, *E. elongata* and *E. pontica*. Similarly, *L. cheesmanii, L. peruvianum* and *Solanum pennellii*, relatives of tomato, are remarkably resistant to salinity.

25.8.4. Somaclones

In case of many species, salt resistant variants have been isolated through tissue culture by utilizing somaclonal variation. This aspect is considered in some detail in the next section (25.9.5).

25.8.5. Transgenes

Several transgenes concerned with osmoregulation have been isolated, cloned and some of them have been expressed in plants. This aspect has been considered in some detail in Chapter 24.

25.9. BREEDING APPROACHES FOR SALINITY RESISTANCE

In view of the importance of salinity, considerable efforts have been invested in breeding for salinity resistant varieties. In India, Central Soil Salinity Research Institute (CSSRI), Karnal has the madate for breeding salinity resistant varieties of crop plants. The various approaches for the development of salinity resistant cultivars may be listed as follows: (1) use of resistant root stocks, (2) selection, (3) hybridization, (4) interspecific hybridization, (5) cell selection, and (6) genetic engineering.

25.9.1. Salinity Resistant Root Stocks

This approach is not a breeding but a horticultural strategy. But it does allow cultivation of an otherwise good but salt sensitive variety in a salt-affected area. This approach is obviously applicable to fruit trees. For example, salinity resistant root-stocks have long been used for grapes, and this is considered as a significant contribution to grape industry.

25.9.2. Selection

Selection has been quite effective in the development of salinity resistant lines with improved performance under stress. For example, several salinity resistant rice cultivars have been developed by pureline selection in 'local' varieties adapted to saline areas of several states (Table 25.10).

25.9.3. Hybridization

Intervarietal hybridization is used to combine salinity resistance present in one parent with high yielding ability of the other parent. The segregating generations are generally handled according to the pedigree method. The selection for salinity resistance and yield in the segregating generations may follow one of the following two general approaches.

25.16. SELECTION CRITERIA

Selection for mineral deficiency resistance may be based on (1) visible deficiency symptoms, (2) mineral contents of plant tissues, (3) biochemical assays, and (4) yield; these features are evaluated under the specific deficiency stress.

25.16.1. Visible Deficiency Symptoms

Deficiencies of different elements produce recognizable visible symptoms (Table 25.12). But these symptoms are often affected by plant genotype unrelated to deficiency resistance and by the environment. In addition, they appear only when the mineral concentration is below a certain threshold, while in many marginal situations deficiency would exist without producing visible symptoms. This criterion is commonly used in selection for iron-deficiency resistance as the symptoms are straight forward and they can be enhanced by defoliation. It has also been used for selection in case of Zn and some other elements. Deficiency symptoms can be supplemented or replaced by carefully selected chemical or biochemical tests, but they are far more convenient, easier and cheaper to score.

25.16.2. Mineral Contents of Plant Tissues

The content of concerned element may be determined in the target tissues of plants growing under mineral deficiency. Mineral contents are affected by growth, tissue age, and various environmental factors. Generally, this criterion is regarded as a tool of limited utility in diagnosing mineral-deficiency stress, and for some minerals, *e.g.*, Fe, it is of no value. *Chemical analysis may prove useful in plant selection if various problems of plant age, sampling and the specific mineral are accounted for.*

TABLE 25.12

A brief description of visible deficiency symptoms of different mineral nutrients

Nutrient	*Deficiency symptom*
B	New leaves first affected; leaves crinkled and brittle; stem dark-bronze in colour, hollowed and finally decays
Ca	Young leaves first affected; yellowish specks initially; leaf-tip tightly curled; sticky liquid on dead leaves
Cu	New leaves first affected; yellow to bronze colour first appears at tip
Fe	New leaves first affected; intense yellowing of intervenous tissues, which necrose rarely
K	Older leaves first affected; bronze to yellowish brown necrotic lesions
Mg	Older leaves affected first; light yellow discoloration between veins; margins curled; dark necrotic spots; reddish or purple in colour
Mn	Older leaves first affected; tissue between veins light in colour, later turns necrotic
Mo	Older/younger leaves; mottled and curled; lighter coloured intervenous region
N	Older leaves first affected; general yellowing
P	Older leaves first affected; leaves dark green to dark purple
S	First in new leaves; homogeneous light yellow to yellow colour
Zn	Small yellow new leaves; stem elongation inhibited; rosette-like plants in some cases

homogeneity before the selection is started. Field selection is far more desirable than that in greenhouse mainly due to logistical and economic reasons.

25.17.2. Pots

Pots filled with mineral-deficient soil may be used for selection. Pots are considered most suitable for evaluating seedling responses and are not desirable if seed increase is required. Care should be taken to fill the pots with a homogeneous soil lot with well defined mineral deficiency. If the pots are kept in a greenhouse, a control of temperature is highly desirable.

25.17.3. Hydroponics

These are generally more accurate than pot studies, but it is more limited than the pots in the population size it can handle: therefore, it is rarely used in routine selection work. It can be used to evaluate parents for crosses and the lines selected from the crosses. A certain degree of expertise is required in designing and utilizing such a system for evaluating genotypic differences for mineral deficiency resistance.

25.18. MINERAL TOXICITY RESISTANCE

Mineral toxicity stress is generated by the presence of toxic concentrations of available minerals in the root zone. Acidic soils may contain toxic concentrations of Al and Mn; they are the most important cases of mineral toxicity as they represent about 40% of the arable land world-wide. Breeding for mineral toxicity resistance is much more advanced than that for mineral deficiency resistance.

25.19. MECHANISMS OF TOXICITY AND RESISTANCE

It is not clear if the toxic effects in plants caused by toxic mineral concentrations are common to different minerals and whether cross-resistance to several minerals is possible. Therefore, the toxic effects of different minerals and the mechanisms of resistance therefor are considered separately (Table 25.14).

TABLE 25.14

Toxicity effects and mechanisms of resistance to Al and Mn toxicities

Mineral	*Toxic effects due to*	*Toxicity symptoms*	*Resistance mechanism*
Al	Membrane instability, protein denaturation	Reduced root growth, root discoloration; lack of lateral roots	Increased pH of rhizosphere; exclusion from entering root (Atlas-66 wheat); Al compartmentalized in root, older leaves or within cells
Mn	Reduction in enzyme activities, respiration and ATP levels; increased activities of oxidases	Leaf- chlorosis and necrosis; leaf crinkling and 'cupping' or 'puckering'	Exclusion from shoot due to reduced transport from root, or redestribution in different parts of shoot; tolerance to high Mn concentration (?)

25.19.1. Aluminium

Aluminium (Al) concentration in the soil rarely exceeds 4 ppm, and its interaction with various organic and inorganic components of soil is complex. Generally, Al toxicity may be

investigations on and selection for resistance to specified mineral toxicities/deficiencies. The choice of sand culture or hydroponic system will largely depend on the available expertise and facilities/resources.

25.23. SELECTION CRITERIA

The selection criterion will depend mainly on the type of mineral toxicity for which resistance is being selected as is briefly described below for Al and Mn toxicities.

25.23.1. Al Toxicity

1. Al mainly retards root growth and, as a result, reduces shoot growth. Therefore, ***shoot dry matter*** is often used as a selection criterion in pot and nutrient cultures.
2. ***Root length*** is often evaluated in hydroponic cultures and used as the basis of selection.
3. ***Root weight*** is assayed in hydroponic systems and sometimes used for selection.
4. In addition, ***root deformations and discolourations*** may be evaluated in hydroponics and used for selection.
5. In such cases where Al resistance is based on Al exclusion from root, *e.g.*, in wheat, roots of Al-stressed seedlings are ***stained with haematoxylin***. Roots of resistant seedlings do not take up stain; they are selected and planted in field for generation advance.
6. In field experiments, ***yield*** is ordinarily used as an integrated measure of resistance. For dependable results, yield of each genotype should be evaluated both under Al toxicity stress and nonstress conditions, and the selections are based on high mean performance and low decline due to stress. This, however, can be applied only to the parents to be used in hybridization and to lines evolved from crosses; it can not be applied to segregating generations where individual plant selection has to be done.

25.23.2. Mn Toxicity

Mn toxicity resistance, independent of Al toxicity resistance, has to be evaluated under pot or nutrient culture since in most acid soils both the toxicities are present together. The various selection criteria for Mn toxicity resistance are as follows.

1. Since shoot growth is inhibited before root growth due to Mn toxicity, *shoot growth* estimated as dry matter accumulation or as some other parameter is a very good selection criterion for Mn toxicity resistance.
2. The parents of and the lines isolated from crosses should be evaluated for *shoot growth under both Mn toxicity stress and nonstress conditions.* Selection should be based on mean performance as well as reduction caused by Mn toxicity stress.

25.24. PROBLEMS IN BREEDING FOR MINERAL DEFICIENCY/TOXICITY RESISTANCE

1. Toxicity/deficiency profile varies with the location of problem soil. Therefore, varieties developed for one site may not be suitable for other locations.

2. Al and Mn toxicities occur together in most acid soils. Therefore, breeding for specific resistance to Al or Mn may not serve the purpose.
3. Al and Mn toxicities interact with other mineral nutrients, *e.g.*; Ca, P and Mg. Therefore, proper care has to be exercised during selection and evaluation stages of the breeding programmes.
4. Root observations have to be done in hydroponic systems, which are expensive and require considerable expertise.
5. Final evaluations have to be done under deficiency/toxicity stress as well as nonstress conditions; this doubles the workload and, as a consequence, the expenditure, etc.
6. In case of mineral deficiency, deficiency symptoms develop only when mineral concentration goes down below a threshold level. Chemical and biochemical assays may be used when deficiency symptoms do not develop but these require expertise and money.