Annual Review & Research in Biology
3(4): 422-454, 2013

SCIENCEDOMAIN *international*
www.sciencedomain.org

Biochemical and Molecular Basis of Varietal Difference in Plant Salt Tolerance

Aryadeep Roychoudhury[1*] and Mayukh Chakraborty[1]

[1]Post Graduate Department of Biotechnology, St. Xavier's College (Autonomous), 30, Mother Teresa Sarani, Kolkata – 700016, West Bengal, India.

Authors' contributions

This review work was carried out by the two authors. Author AR made an exhaustive study of the subject matter and drafted the entire manuscript. Author MC managed the proper citations of the references, their arrangements and proofreading tasks. Both the authors read and approved the final manuscript.

Review Article

Received 27th March 2013
Accepted 27th May 2013
Published 15th June 2013

ABSTRACT

World agriculture is facing a lot of challenges like producing 70% more food for an additional 2.3 billion people by 2050. However, the productivity of crops is not increasing in parallel with the food demand. The lower productivity is attributable to various abiotic stresses, of which increased soil salinity is one of the foremost causes. The negative effect of salinity is caused by Na^+ and Cl^- ions producing the critical conditions for plant survival. The obvious outcome of salinity includes membrane damage, nutrient imbalance, altered levels of growth regulators, enzymatic inhibition and improper metabolic functions, including photosynthesis which ultimately leads to plant demise. Crops vary significantly in their threshold limits of salt tolerance. A well-focused approach combining the molecular, physiological, biochemical and metabolic aspects of salt tolerance is essential to alleviate the drastic effects of salinity and develop salt-tolerant crop varieties. The exploitation of genetic differences of available germplasm has the greatest significance, because it helps to identify the genotypes performing well even under saline conditions. Screening of crops for tolerance can strengthen the breeding programs by identifying genotypes with high salt tolerance and yield potential. This strategy involves comparative investigation of various morphological, physiological, biochemical, enzymatic and ionic responses, together with the study of differential expression pattern of genes/proteins concerned with salt tolerance at different developmental stages under salt stress in salt-sensitive and salt-tolerant

**Corresponding author: Email: aryadeep.rc@gmail.com;*

cultivars. If the response is greater in the salt-tolerant line, it suggests a role in stress tolerance. Many pitfalls are associated with such approach, i.e., climatic variation, varied physiochemical properties of soil and amount of precipitation, which reduces the effectiveness of screening under field conditions. Even then, such studies can readily identify the salt-tolerant genotype, leading to the identification of novel genes or markers contributing towards salt tolerance, when overexpressed in the susceptible cultivars. The tolerant genotypes, thus screened can be recommended for cultivation in marginal salt-affected areas. This review focuses on the biochemical and molecular basis of varietal differences in salt tolerance among the cultivars of various plant species.

Keywords: Biochemical response; molecular regulation; salinity stress; salt tolerance; varietal difference.

1. INTRODUCTION

Soil salinization is one of the serious forms of soil degradations, which can arise from natural causes and human-mediated activity, such as irrigation in arid and semi-arid regions. Approximately 20% of the irrigated lands in the world are presumably affected by soil salinization [1]. More than 800 million hectares of land throughout the world are salt-affected. This amount accounts for more than 6% of the world's total land area. Increased salinization of arable land is expected to cause 30% land loss within the next 25 years and up to 50% by the year 2050 [2]. Coastal salinity and accumulation of salts in the irrigated land primarily decrease crop yield. Particularly in tropical Asia, because annual precipitation does not exceed evapotranspiration, soluble salts tend to accumulate and build-up in the soil of arid and semi-arid regions, instead of being leached, and can reach the levels inhibitory to plant growth and development. Intensive irrigation in such areas further complicates the problem, leaving behind huge salt deposits after evaporation, leading to secondary salinization and alkalinization. Of the 1,500 million ha of land farmed by dryland agriculture, 32 million ha (2%) are affected by secondary salinity. Of the current 230 million ha of irrigated land, 45 million ha (20%) are salt affected [3].

The term "salinity" represents all the problems of the soil accumulating excessive salts over long periods of time. Such soil can be categorized into sodic (or alkaline) and saline soils [4]. Sodic soils having a poor soil structure, generally spread over arid and semi-arid regions, retaining high concentrations of Na^+ at the exchangeable site of clay particles in the soil, which shows high pH (greater than 8.5) with a high exchangeable sodium percentage (ESP >15) [4]. Saline soils can be generally found in arid regions, estuaries, and coastal fringes, which are dominated by Na^+ ions with electrical conductivity of the extract (EC_e) more than 4 dS m^{-1} or that corresponds to approximately 40 mM NaCl and generates an osmotic pressure of approximately 0.2 MPa [4,5] or gives an Ece exceeding 4 mmhos cm^{-1} at 25ºC, in the water-saturated soil paste extract. Moreover, saline soils exhibit ESP of <15 and much lower pH values than the sodic soils [4]. Rainwater contains 6-50 mg Kg^{-1} of NaCl, the concentration decreases with distance from the coast.

We do not know yet what genetic make-up distinguishes some plants or varieties from others with respect to stress tolerance or stress resistance. The extensive genetic diversity for salt tolerance that exists in plant taxa is distributed over numerous genera [6]. Plants are traditionally classified as halophytes or glycophytes based on their capacity to grow on high salt medium. A principal difference between the two is the capacity of the former to survive

salt shock. Some halophytes can withstand salts (more than twice the concentration of sea water) because of very special anatomical and morphological adaptations or avoidance mechanisms. This greater capacity allows halophytes to more readily establish metabolic steady state for growth in saline environment. Halophytes are able to maintain Na^+ and Cl^- exclusion at higher salt concentration than glycophytes, e.g., *Hordeum marinum* excludes both Na^+ and Cl^- until at least 450 mM NaCl. For optimal growth, halophytes require electrolyte (typically Na^+ and Cl^-) concentrations higher or much higher than plants in non-saline soils. Mechanisms for salt tolerance are of two main types - those minimizing the entry of salt into the plant, and those minimizing the concentration of salt in the cytoplasm. Halophytes have both types of mechanisms - they 'exclude' the salt, as well as effectively compartmentalize in vacuoles the salt that gets in. This allows them to grow for long periods of time in saline soil. Many glycophytes respond to relatively low salt concentrations (below about 6,000 mg L^{-1}, or roughly 100 mM) by "salt exclusion," particularly through low rates of net transport of sodium or chloride, or both, from root to shoot. Most of these salt-excluding glycophytes cannot adjust osmotically to the low external water potential by increased synthesis of organic solutes and therefore suffer from a decrease in turgor. Hence salinity may induce an osmotic stress in this kind of glycophyte. While some glycophytes 'exclude' salt, but unable to compartmentalize it, others cannot even 'exclude' so that salt concentrates to toxic levels in transpiring leaves. Plants survive because of osmotic adjustment (OA) through intracellular compartmentation that partitions toxic ions away from the cytoplasm through energy-dependent transport into the vacuole [7]. A summarized difference between halophytes and glycophytes is given in Table 1A.

Research of recent decades has established that most halophytes and glycophytes tolerate salinity by rather similar strategies often using analogous tactical processes (Table 1B). With their high level of salt tolerance, halophytes would appear to be the plants of choice to search for tolerant genes and study salt tolerance mechanisms. However, glycophytic plant cells could be adapted to tolerate high concentrations of salts that would kill unadapted cells. Therefore, the difference in salt tolerance between halophytes and glycophytes appears to be quantitative rather than qualitative; the basic stress protection mechanisms are probably conserved in all plant species and ubiquitous. The differences in salt sensitivity/ tolerance may have resulted from differences in regulatory circuits or from gene alleles coding for key salt tolerance effectors. Many of the molecular entities that mediate ion homeostasis and salt stress signaling are similar in all plants. *Arabidopsis thaliana* is treated as a model genetic organismal system for dissection of plant salt stress response and cellular tolerance mechanism. However, *A. thaliana* is a true glycophyte. It does not undergo a full life cycle even at moderate salinity (~100 mM NaCl). Therefore, studies on *A. thaliana* reveal little information on salt tolerance, but a great deal on stress. The halophyte *Mesembryanthemum crystallinum* has emerged as a model system for understanding the molecular response to salt stress. This plant switches from C_3 photosynthesis to crassulacaean acid metabolism (CAM) in response to salt or drought stress. However, salt tolerance in *M. crystallinum* is tightly linked to succulence and CAM and it may prove difficult to transfer halotolerance mechanisms into crops that show neither of these traits. More recently, another halophyte, *Thellungiella halophila* (salt cress), closely related to and sharing many of the advantages of *A. thaliana* as experimental system, has been proposed as a model system for studying salt resistance in plants [8,9].

Table 1A. Comparison between halophytes and glycophytes

	Halophytes	Glycophytes
1.	Plants adapted to saline habitat.	Plants adapted to sweet water.
2.	Tend to take up Na^+ in the shoot more rapidly so that the roots typically have much lower NaCl concentration than the rest of the plant.	Restrict ion movement to the shoot by attempting control of ion influx into root xylem.
3.	**a)** Salt exclusion is the most adaptive feature regulating the internal salt load. **b)** More responsive Na^+ partitioning and more effective capacity to co-ordinate this partitioning with the processes controlling growth, cell expansion in growing tissues, turgor in differential organs and ion flux across plasma membrane. **c)** The ability to take up and confine sodium to leaves lowers the osmotic potential of aerial plant parts; this facilitates water uptake and transport as well as lowers the metabolic cost for osmolyte production.	Most of them have a poor ability to exclude salt, and it concentrates to toxic levels in the transpiring leaves. Even if they exclude salt, they are unable of proper partitioning of Na^+ in aerial plant parts; hence cannot uptake water.
4.	The cytotoxic ions like Na^+ and Cl^- are compartmentalized into the vacuole and used as osmotic solutes.	No such mechanism. Na^+ and Cl^- both are highly toxic.
5.	More efficient performances of a few basic tolerance mechanisms.	Usually suffer severe salt injury and damages with growth inhibition and nutritional disorders.

Table 1B. Are halophytes and glycophytes really different?

1.	Amidst the above differences, there actually remains a continuous spectrum between extreme halophytes and extreme glycophytes. All plants have in their genomes genes for salt tolerance. Without adaptation, the salt tolerant genes may not be properly expressed to confer tolerance. Salt tolerant genes in halophytic species may have evolved from genes in glycophytes that were adapted to low levels of salt stress, so that there is no clear boundary line between halophytes and glycophytes.
2.	Glycopyhtes also do have salt tolerance machinery that may not be operating effectively in salt unadapted conditions. Adaptation can increase salt tolerance, e.g., some tobacco cells can tolerate up to 500 mM salt after salt adaptation, while normal cells cannot grow even in 100 mM. Therefore, the difference between halophytes and glycophytes appear to be quantitative rather than qualitative, and the basic salt tolerance mechanisms are probably conserved in all plant species.
3.	Thus, better responsiveness to salinity is not unique to halophytes; glycophytes also show salt tolerance provided that the stress imposition is gradual. The question remains as to whether particular biochemical mechanisms in halophytes are better activated or are pre-activated, allowing a more rapid and successful overall response to salinity stress. Because of the diversity of halophytic species, there is no simple answer to this question.

2. PLANT RESPONSE TO SALT STRESS

Salt stress studies fall into four main categories: (i) physiology of salt toxicity and tolerance- This includes cellular, metabolic and whole plant responses to salt, (ii) mechanisms of salt transport across cellular membranes and over long distances - this includes characterization of various ion transporters involved in salt uptake, extrusion, compartmentalization, and in the control of long distance transport, (iii) survey of salt stress-inducible genes by using gene chips and cDNA microarrays, (iv) mutational analysis of salt-tolerant determinants and salt stress signaling [10]. The response can occur in two distinct phases through time. In the first (osmotic) phase that starts immediately after salt concentration around the roots increases to a threshold level, the rate of shoot growth or the rate of expansion of growing leaves declines dramatically. A reduction in the leaf area development relative to root growth would decrease the utilization of water by the plant, allowing it to conserve soil moisture and prevent build up of salt concentration in the soil. The growth of roots is enhanced at the cost of shoot length, and the roots probe deeper into the soil for search of more water, since water deficit accompanies ionic toxicity. The second (ion-specific) phase of response starts when salt accumulates to toxic levels in the old leaves. In case the death rate of older leaves supersedes the rate of formation of young leaves, photosynthetic capacity fails to provide carbohydrate resources for the newly emerging leaves, retarding their growth. Although for most species, Na^+ appears to reach a toxic concentration before Cl^-, for some species like soybean, citrus and grapevine, Cl^- is regarded to be more toxic. Salt tolerance may be defined as sustained growth of plants in a highly saline environment. It is usually assessed either as the percent biomass production in saline versus control conditions over prolonged period of time or in terms of survival [11].

3. VARIETAL DIFFERENCES IN SALT TOLERANCE AMONG PLANT SPECIES

Plant species differ greatly in their salt tolerance mechanism, as reflected by their overall physiological, biochemical and molecular responses, which we focus mainly in this review. In case of cereals, rice (*Oryza sativa*) is the most sensitive and barley (*Hordeum vulgare*) the most tolerant. Bread wheat (*Triticum aestivum*) is moderately tolerant and durum wheat (*T. turgidum* ssp. *durum*) is less so. Despite vast efforts, rice remains one of the most salt-sensitive crop plants and sensitivity depends on the nature and concentration of salts, soil pH, water regime, plant growth, duration and exposure to salt as well as to temperature. Secondary salinization is also a serious problem since rice grows in swamps and fresh water marshes. Rice and most grain crops show stress symptoms and reduced yield even when the ECe is lower than 4.0 dS m^{-1} (~40 mmol L^{-1} NaCl). Rice generally tolerates salinities ranging between 1.9–3 dS m^{-1} [12], which is comparable to a concentration of around 20–30 mM NaCl. The salinity threshold for rice is thus 3.0 dS m^{-1} with a 12% reduction in yield, per dS m^{-1}, beyond this threshold [13]. Rice is considered sensitive to salinity, particularly during early vegetative and later at reproductive stages. Rice also exhibits genetic variability in their sensitivity to salt stress, vegetative growth of some cultivars showing surprisingly high resistance to soil salinity [14]. Among the low-land rice genotypes, the Indica varieties Pokkali and Nonabokra are regarded as highly salt-tolerant ecotypes on the basis of various physiological parameters. These two varieties are well known salt-tolerant donors in classical breeding. Salt tolerance of these varieties is principally due to additive gene effects [15]. Pokkali, a notable tolerant local land race from Southern India (Kerala), is grown in marshy areas that are close to the sea, often in conjunction with prawn farming. In recent years, another variety Oormundakon has been identified as a probable salt-tolerant donor in breeding purposes. Other salt-tolerant cultivars include Matla, Jarava and Dee-geo-woo-gen

(DGWG). All these tolerant varieties are low yielding. On the other hand, majority of high yielding cultivars like M-1-48, IR-29, IR-72, IR-36, Jaya and Taichung Native-1 (TN-1) are relatively sensitive to salinity as low as 50 mol m^{-3}, and the internal salt concentration rises even greater than this. Salt-resistant varieties have been developed by crossing with Pokkali. Pokkali has, in fact, often been taken as a check in several national and international programs aimed at evolving genotypes combining high salt tolerance and high crop yield. The improved cultivars developed by Central Soil Salinity Research Institute (CSSRI), Karnal, are CSRI1, CSR3, CSR10, CSR13 and CSR27. CSR27 has been bred through crossing Nonabokra and IR-5657-33-2 rice types. CSR27 variety shows high tolerance to sodic (pH 9.6–9.9) and saline (ECe up to 9 dS m^{-1}) soils. Another cultivar developed at CSSRI is CSR10 for sodic and inland saline soils. It is derived from the cross M40-431-24-114 and Jaya. CSR10 can withstand highly deteriorated alkaline (pH 9.8–10.2) and inland saline soil (ECe 6–10 dS m^{-1}) conditions under transplanted irrigated management system. Other varieties developed in India are Damodar, Dasal and Getu (renamed as CRS3). The former two are moderately salt-tolerant while Getu is the most tolerant. The CSA University of Agriculture and Technology, Kanpur, has developed another salt-resistant variety Usar1. In case of wheat, the most tolerant variety is Kharchia 65, a selection from local land race of Rajasthan, and most of the resistant varieties have been developed using this variety as the base material. Other salt-tolerant varieties include CSW540-2, KRLI-4, KRL3-4 and KRL19. High yielding wheat variety HD2009, showing moderate salt tolerance has been generated by Indian Agricultural Research Institute (IARI), New Delhi. The International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Hyderabad, has developed a salt-tolerant pigeon pea genotype ICPL227, while CSSRI has developed a salt-tolerant mustard cultivar called CS52. Salt tolerance is known to vary differently with growth and development for different salt-tolerant varieties and the differences are not prominent at the seedling stage. Salt concentration in leaves reaches toxic levels more quickly in salt-sensitive than in salt-tolerant varieties, due to differences in the ability of roots to exclude salt from the xylem sap flowing to the shoot [16].

The variation in salinity tolerance in dicotyledonous species is even greater than in monocotyledonous species. Some legumes are very sensitive, even more sensitive than rice; the alfalfa or lucerne (*Medicago sativa*) is very tolerant, and halophytes like *Artiplex* continue to grow well at salinities greater than that of seawater. Many dicotyledonous halophytes require a quite high concentration of NaCl (100-200 mM) for optimal growth. In case of tomato, *Lycopersicon cheesmanii* (Galapagos ecotype) was shown to be far more salt-tolerant than the *L. esculentum*. The former could survive in full strength seawater nutrient solution while *L. esculentum* could not in most cases withstand levels higher than 50% seawater. Growth rates were reduced in both species under saline conditions but *L. esculentum* was more severely affected [17].

We next discuss the factors leading to the varietal differences in the rate of accumulation of NaCl in leaves. These factors could be attributed to the differences in one or more of the following: i) the rate of NaCl uptake by the plant, ii) the partitioning of this NaCl between the root and the shoot, iii) the rate of export in the phloem, and iv) growth rate. In studies of *Casuarina* and citrus genotypes, the more salt-tolerant genotype had lower xylem concentrations of both Na^+ and Cl^-. For a crop species adapted to salinity, NaCl concentration in the xylem was no more than 2% of the external NaCl concentration. In lupin, a relatively salt-tolerant species, the xylem concentrations of Na and Cl were about 8 mol m^{-3} at 100 mol m^{-3} NaCl, and in barley and cotton, which are more salt-tolerant, the xylem NaCl concentration was a little lower, namely 5% of the external concentration. In a comparison of two *Casuarina* species, the genotype with higher transpiration rate accumulated the most

NaCl in the shoot [16]. In salt-tolerant species, the phloem transport is relatively insignificant; in barley it was calculated to be about 10% of the import [18]. In the salt-sensitive lupin, it was almost equal to the import. Shoot growth rate regulates varietal difference in the sense that if one variety has a lower shoot growth rate than another, then for a given rate of NaCl transport, it would have a proportionately higher concentration of NaCl in leaves. Other possible causes of varietal differences most likely involve apoplastic ion transport properties across root, water-use efficiency, cellular compartmentation and tissue tolerance (apoplast/protoplast balance). The differences in salt tolerance among rice cultivars can be caused by differential compartmentalization of Na^+ in the shoot. Within the shoot, Na^+ is buffered mainly by the intracellular compartmentalization, which supplements the primary response of reducing Na^+ influx into the shoot and effectively buffers the apoplastic fluid in tolerant cultivars leading to robust stress response and effective survival. Entry of Na^+ into the shoot is restricted in a Ca^+ dependent manner by altering the apoplastic transpirational bypass flow. This modulation actually operates in both salt-sensitive and salt-tolerant cultivars, but with differing efficiencies and set points. In a comparison of two barley varieties, the sodium pump was more efficient in the less salt-tolerant variety [19]. Calcium could affect the activity of this pump, and there are varietal differences in calcium binding as well [20]. Varietal differences have also been observed in the flux of Na across the root of wheat [21] and in the cellular flux of Na^+ to the xylem [19], but they were higher in the more tolerant variety. Earlier results [22] showed that less Na^+ was taken up into the cytosol of salt-tolerant Pokkali than into salt-sensitive BRRI Dhan29. The K^+-selective channels do not contribute to the Na^+ uptake in Pokkali, whereas they are the major pathways for Na^+ uptake in BRRI Dhan29 along with non-selective cation channels. However, non-selective cation channels seem to be the main pathways for Na^+ uptake in Pokkali. Comparisons were carried out in two varieties of durum wheat (*Triticum turgidum*) L. subsp. *durum*, known to differ in salt tolerance and Na^+ accumulation; the relatively salt-tolerant landrace line 149 and the salt-sensitive cultivar Tamaroi [23]. The major differences in Na^+ transport between the genotypes were (1) the rate of transfer from the root to the shoot (xylem loading), which was much lower in the salt-tolerant genotype, and (2) the capacity of the leaf sheath to extract and sequester Na^+ as it entered the leaf. The genotypes did not differ significantly in unidirectional root uptake of Na^+. Vacuolar sequestration could play a significant role as suggested by salt tolerance conferred on overexpression of the vacuolar Na^+/H^+ antiporter in rice [24]. There is often a negative correlation between Na^+ and Cl^- concentration in the leaves and plant growth or survival. At higher salinity, salt-tolerant varieties have higher organic salt content and lower uptake and accumulation of Na^+ and Cl^- than the less tolerant ones. With many species, varietal differences may exist in the rate of accumulation of Na^+ and Cl^- in the roots and leaves. It is generally known that the maintenance of low cytosolic Na^+ concentration and K^+/Na^+ homeostasis is an important aspect of salinity tolerance and that the salt-tolerant lines show higher K^+/Na^+ levels [25]. Higher accumulation of Na^+ in salt-sensitive rice variety M-1-48 were shown due to salt stress, while the salt-tolerant variety Nonabokra had higher K^+ levels with lesser Na^+ levels [26]. The leaves and roots of the more salt-tolerant genotype usually have lower internal Na^+ and Cl^- concentration for the same time of exposure to NaCl than the salt-sensitive ones. The leaf Na concentration required to cause 50% reduction in chlorophyll also differs between rice varieties, with the tolerant varieties suffering less chlorophyll damages [26].

It has also been suggested that the two most likely factors causing varietal differences in salt tolerance are varietal differences in the rate of salt transport to the shoots and in leaf expansion rates. Water relations change dramatically when plants are exposed to NaCl, accounting for the rapid reduction in leaf growth rate. The rate of leaf expansion is controlled by the water status of the root rather than the shoot or leaf. The varietal difference in leaf

water relations generally develop with time. The more tolerant varieties tend to have lower leaf NaCl concentrations and hence lower leaf osmotic pressure. In a comparison of five *Citrus* varieties, the turgor pressure increased with time in the more salt-sensitive varieties (which took up more Cl^-), and decreased with time in the more tolerant ones [27]. Comparison of the seedlings of two citrus rootstocks, the salt-tolerant Cleopatra mandarin and the salt-sensitive Carrizo citrange showed that the tolerant genotype excluded more Cl^-, i.e., it absorbed lower amounts of Cl^- per volume of water. Cleopatra also possessed a less efficient root system for water uptake and a higher shoot-to-root ratio. Overall, Cl^- absorption is linked to water use and that further tolerance in Cleopatra is mostly conferred by superior root resistance to Cl^- uptake [28]. In case of wheat, it has been shown that the varieties differ in the rate at which leaves die. For the sensitive variety, the rate at which leaves die overtakes the rate of leaf expansion. In the moderately tolerant variety, enough leaves survive the vegetative phase of growth to allow a continued growth of the ear (which excludes NaCl more effectively than the leaves). In the tolerant variety, only a few leaves are dead at ear emergence, and the plant yield is better. The rate of senescence is higher in the more salt-sensitive genotypes, because salt concentration in leaves rise more rapidly, with sooner leaf death [16]. The differential upregulation of the osmolytes, polyamines (PAs), antioxidants (enzymatic and non enzymatic) and abscisic acid (ABA) or the differential expression level of transcription factors and/or target genes and proteins cumulatively account for the varietal difference in salt tolerance mechanism of various plant species. The salt-tolerant species have been frequently employed to isolate genes involved in conferring salt tolerance, and thereby gain an understanding of the mechanisms that distinguish them from their salt-sensitive counterparts.

Many components of signaling pathways have been implicated in plant responses to salinity, inferred by a range of approaches such as transcriptomics and reverse genetics. Invoking the adaptive relevance of a particular response to Na^+ in a plant that is poorly adapted to salinity is cumbersome. These approaches could be strengthened by comparing the responses in salt-tolerant and salt-sensitive lines – if the response is greater in the tolerant line, it suggests a role in stress tolerance; however, if the response is smaller, it is not related to tolerance, but is a downstream response to the stress. The comparative responses of some of these stress-related components are discussed in the following sections.

4. BIOCHEMICAL BASIS OF VARIETAL DIFFERENCE

4.1 Compatible Solutes

A related strategy for the plant cells to regain turgor and resume growth during ionic stress is to accumulate compatible osmolytes, the process termed as OA. The osmolytes reduce the osmotic potential of the cytosol to facilitate water uptake and retain water. These compounds can accumulate to high levels without apparently interfering with the normal intracellular metabolism. Osmoprotectants may preserve cell integrity in various ways. The simplest way would be to prevent ion entry into the sensitive parts of the plant or to enhance ion exclusion from them. Compatible solutes are polar, highly soluble and typically hydrophilic so that their protective function might be in maintaining the hydration sphere of proteins under water stress conditions. High concentrations of these substances protect proteins from misfolding and hence act as low molecular weight chaperones, stabilize some macromolecules or molecular assemblies, reduce the inhibitory effects of ions on enzyme activity to increase their thermal stability, and prevent dissociation of enzyme complexes. They alleviate the

toxic effect of reactive oxygen species (ROS) generated by salt stress, act as scavengers of hydroxyl radicals (extremely toxic, short lived active oxygen species), thus preserving either enzyme activity or membrane integrity. Some of the common osmolytes triggered during salinity stress include reducing sugar and major carbohydrates like sucrose, fructose, glucose; sugar alcohols (pinitol, ononitol, cyclitol); polyols (either straight chain compounds like adonitol, sorbitol and mannitol or cyclic polyols as myo-inositol); complex sugars (trehalose, raffinose and fructans); total free amino acids especially proline (Pro) and glycinebetaine (GB); organic acids like lactate, malate, citrate, succinate, fumarate, benzoate, salicylate, malonate and γ-amino butyric acid (GABA); free ammonia and quaternary ammonium compounds (β-alanine-betaine, proline-betaine and hydroxyproline-betaine); tertiary sulfonium salts (dimethylsulfoniopropionate, choline o-sulfate) etc.

In many halophytes, Pro or GB occurs at sufficiently high concentrations in leaves (over 40 mM) to contribute an osmotic pressure over 0.1 MPa in the cell as a whole. In glycophytes, the concentrations of compatible solutes that accumulate are not so high, on the order of 10 mM, but if partitioned exclusively in the cytoplasm, they could generate a significant osmotic pressure and thus function as osmolytes [5]. In case of five sunflower accessions, the salt-tolerant lines had generally greater soluble sugars than the salt-sensitive ones [29]. Considerable variations in the accumulation of soluble sugars in response to salt stress are evident at both interspecific or intraspecific levels and even among lines, all of which are salt-tolerant. Salinity exerted a distinctly differential effect on fructose-1, 6-bisphosphatase isolated from salt-sensitive and salt-tolerant rice varieties. Cytosolic and chloroplastic isoforms of the enzyme from salt-sensitive rice seedlings exhibited decreased catalytic activity during growth in the presence of salt. Furthermore, chloroplastic fructose 1, 6-bisphosphatase purified from salt-sensitive (*O. sativa* cv. IR-26) and from the wild halophytic rice *Porteresia coarctata* differed in their *in vitro* salt tolerance property. A higher content of soluble proteins (accumulating under saline conditions in the cytoplasm and providing a storage form of nitrogen) has been observed in salt-tolerant cultivars of barley, sunflower, finger millet, and rice [30]. The soluble protein increases at low salinity and decreases at high salinity in mulberry cultivars [31]. Although it was found that salt-tolerant and salt-sensitive accessions of safflower did not differ significantly in leaf soluble proteins, there are reports of decrease in soluble protein content in response to salinity [32]. The inhibitory effect of increasing concentration of NaCl on *in vitro* enzymatic activity could be prevented by preincubation of the enzyme with a number of osmolytes with an effectiveness in the order polyol > sugars [33]. Total free amino acids in the leaves are reported to be higher in salt-tolerant than in salt-sensitive lines of sunflower [29], safflower [32], *Eruca sativa* [34] and *Lens culinaris* [35]. Pro has been reported to activate other mechanisms, such as the formations of strong H-bonded water around protein for protecting protein structures and scavenger of free radicals [36]. The Pro concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants under stress in many plant species such as rice [37], alfalfa [38], maize [39,40], pigeon pea [41] and potato [42]. It was found that salt-tolerant alfalfa plants rapidly doubled their Pro content in the roots, whereas in salt-sensitive plants, the increase was slow [38]. Similar results were also reported in alfalfa by other groups [43]. In case of pea, the tolerant genotypes (climax and samarinazard) accumulated high ratios of Pro and low concentration of Na in their leaves than salt-sensitive (ambassidar and PF-400) pea genotypes. The high ratios of leaf Pro and low Na in tolerant genotypes showed that there was a strong correlation between salt tolerance and OA. The findings suggested that salt tolerance potential of pea genotypes was highly associated with leaf Pro contents and Na concentration [44]. However, a negative correlation between Pro accumulation and salt tolerance in tomato and *Aegiceras corniculatum* respectively were reported [45, 46]. The accumulation of Pro in two sorghum genotypes contrasting in salt

tolerance suggested that Pro accumulation was a reaction to salt stress and not a plant response associated with tolerance [47]. In another experiment, it was shown that under salt stress, higher concentration of Pro was accumulated in sensitive rice cultivars than in tolerant genotypes [48]. Pro contents in salt-tolerant rice varieties KDML105 and Sangyod were higher than those in salt-sensitive varieties, Pathumthani 1 and Black Sticky [49]. The three salt-tolerant wheat genotypes Sarsabz, Lu-26s and KTDH-22, showed higher grain yield under salinity stress with lesser Na content in their leaves and also had higher Pro accumulation [50]. It was observed that salt-tolerant ecotypes of *Agrostis stolonifera* accumulated more Pro in response to salinity than did salt-sensitive ecotypes [51]. Relatively salt-tolerant plants of *Brassica juncea* showed a higher degree of OA in the leaves and a higher critical point concentration of NaCl, at which the endogenous level of free Pro rose sharply, than did the relatively salt-sensitive genotypes [52]. Higher Pro accumulation was found in salt-tolerant *B. juncea* plants with better growth than the control [53]. The activities of Pro biosynthetic enzymes, pyrroline-5-carboxylate reductase and ornithine aminotransferase increased considerably in *B. juncea*, but to a large extent in tolerant lines, under salt stress [54]. In contrast, the activity of Pro degrading enzyme, Pro oxidase, decreased under salt stress in the leaf tissues of all the lines of *B. juncea*. The lower expression of a gene encoding Pro dehydrogenase (*PDH*) contributed to the higher salt tolerance of *Thellungiella halophila*, compared with its salt-sensitive relative *Arabidopsis thaliana* [55]. In case of maize, the comparatively salt-tolerant genotype PEHM 3 recorded higher contents of Pro, GB, total soluble sugars, K^+ and Ca^{2+} than the salt-susceptible genotype Navjot, which recorded higher Na^+ content and Na^+/K^+ and Na^+/Ca^{2+} ratios than PEHM 3 [56]. In contrast, more Pro accumulation in salt-sensitive species of tomato than in tolerant wild relatives was reported [57]. Working with soybean, it was shown that the Pro content could not be used as a sensitive indicator of salt stress [58]. Similarly, a negative relationship was reported between Pro accumulation and salt tolerance in *Vigna mungo* [59]. In rice, the salt-resistant cultivars, Nonabokra and IR-4630 accumulated less Pro in their leaves than the salt-sensitive I Kong Pao and IR-31785 [48,60]. A similar negative relationship between Pro accumulation and salt tolerance in tomato was observed [45]. It seems probable that Pro as indicator is unspecified for salt/drought tolerance. Some tolerant plants have an alternative defense mechanism as a major route, so that Pro is a minor or unchanged parameter. Pro level is very low, yet the plants can survive in salt stress. In contrast, in some other plant species, Pro enrichment in a high concentration is a major OA for survival under salt stress [61]. The salt tolerance of (wheat x *Lophopyrum elongatum*) amphidiploids was related to accumulation of asparagine and GB in young leaf blades [62]. Accumulation of GB under saline conditions was reported to be high in some salt-tolerant grasses but not in salt-sensitive grasses [61, 63, 64, 65, 66]. Accumulation of GB under saline conditions was also high in salt-tolerant plants of mulberry but not in sensitive ones [31]. Also, GB-containing lines of maize exhibited less shoot growth inhibition under saline conditions than deficient lines [67]. In contrast, accumulation of choline and betaine in response to salt stress was found to be more pronounced in salt-sensitive than in salt-tolerant lines of *Trifolium alexandrinum* [68]. Furthermore, no relationship was noted between GB accumulation and salt tolerance of species of the genera *Triticum*, *Agropyron* and *Elymus* [69]. In view of these reports, it is evident that the occurrence of GB accumulation is widespread but sporadic, since in some plant species it occurs in large quantity, whereas in some others its occurrence is not to a great extent. Thus, to relate the accumulation of GB with plant salt tolerance requires more experimental evidence [70]. Information, in general, regarding the physiological role of polyols in plants under saline conditions is insufficient and even less is known about the relative responses of closely related salt-tolerant and salt-sensitive cultivars. However, there is growing understanding of the genetic and biochemical basis of polyol accumulation. In the halophyte *M. crystallinum*,

the gene encoding the enzyme myo-inositol-*O*-methyl transferase is upregulated after salt stress [71,72]. Furthermore, in the same species, increased phloem transport of myo-inositol and reciprocal increased transport of Na^+ and inositol to leaves was observed under salt stress, showing an interdependence of Na^+ uptake and changes in the distribution of myo-inositol within the plant [73]. Comparative targeted metabolic profiling revealed marked differences in the metabolite composition between salt-sensitive and salt-tolerant soybean varieties [74]. The secondary metabolites, for example, isoflavones and saponins, were also used as markers to discriminate between closely related soybean genotypes. Genistin and group B saponins were identified as the key secondary metabolites correlated with salt tolerance.

4.2 Polyamines (PAs)

PAs are low molecular weight aliphatic amines or polycations found in all cells that play a protective role during salinity stress. Their positive charge at physiological pH enables them to interact electrostatically with polyanionic macromolecules and phospholipid head groups of the membranes within the cell. Thus, they can stabilize membrane systems protecting them from oxidative damages during salt stress; the potency of this action is suggested to be of the order: Spm > Spd > Put. In plants, the diamine putrescine (Put) is synthesized from arginine by arginine decarboxylase (ADC) followed by its conversion to triamine spermidine (Spd), catalyzed by spermidine synthase (SPDS) or S-adenosylmethionine decarboxylase (SAMDC) and finally to tetramine spermine (Spm) by spermine synthase (SPMS). During salt stress, PA contents and enzyme activities have been reported to increase or decrease dependent on tissues, species, salt concentration and duration of the experiment. PA degradation is catalyzed by either diamine oxidase (DAO) for Put or polyamine oxidase (PAO) for Spd and Spm. Exogenous PA application, which is expected to increase the levels of an endogenous PA, is considered as a convenient and effective strategy to enhance tolerance against salt stress.

In only a few studies has the accumulation of PAs been compared in closely related salt-tolerant and salt-sensitive species or cultivars. Interestingly, the accumulation was found to be higher in salt-sensitive than in salt-tolerant lines of rice [75] and tomato [62]. In the salt-tolerant rice cultivar Co43, foliar application of Put inhibited the Na^+ and Cl^- uptake, and accelerated the accumulation of K^+, Ca^{2+}, Mg^{2+}, Pro and endogenous Put in the leaves of salt-stressed plants. Furthermore, Put application prevented the degradation of chlorophyll and inhibited the reductions of soluble protein, total protein, RNA and DNA contents, and elevated their concentrations in the leaves of plants exposed to salinization. Exogenous supply of Put on salt stressed plants considerably increased the shoot growth (fresh weight and dry weight) and grain yield, ameliorating NaCl toxicity [76]. Under saline conditions, the salt-tolerant rice cultivars (AU1, Co43, and CSC1) maintained a high level of higher PAs, like Spd and Spm, whereas salt-sensitive rice cultivars (Co36, CSC2, GR3, IR20, TKM4 and TKM9) maintained only a high level of Put [77]. The salt-tolerant and salt-sensitive rice varieties have different basal levels of ADC activity in shoots and roots in each cultivar. Salt-tolerant cultivars accumulated less Put content than salt-sensitive cultivars after salinization, comparable with smaller reduction of growth, development and survival rate [78]. The significant increase in Put level in the salt-sensitive cultivars could be due to the differential uptake of ions, resulting in changes in cell pH and ionic balance. Either there is an enhancement of Put synthesis, or alternately an inhibition of Spd/Spm synthesis by the inhibition of SAMDC activity. While Spd and Spm contents in the shoot system of salt-tolerant variety increased with increasing stages of growth, there was no significant change in stressed salt-sensitive cultivars. Higher levels of Spd and Spm were shown in the salt-

tolerant rice variety Nonabokra than the salt-sensitive M-1-48 and the aromatic Gobindobhog rice varieties during salinity stress [26]. The expression of SAMDC gene and protein was constitutive in Nonabokra, whereas it was inducible only by salt (200 mM NaCl, 16 h) in the susceptible varieties M-1-48 and Gobindobhog. Both Spd and Spm could recuperate all the three rice varieties M-1-48, Nonabokra and Gobindobhog from salt-induced damages to different degrees. The salt injuries, encountered in M-1-48 and Gobindobhog, both of which showed greater susceptibility to salinity stress, were more pronouncedly alleviated and counteracted by the PAs than the salt-tolerant Nonabokra [79]. The reversal of inhibitory effect of salinity stress was conferred by preventing growth inhibition or various forms of cellular damages, maintaining proper K^+/Na^+ balance or triggering the level of osmolytes and activity of antioxidant enzymes. Exogenous Spd can be applied as short term pretreatment, prior to the introduction of salt stress, in order to elevate salt tolerance of rice, particularly in the salt-tolerant cultivar Pokkali [80]. Two rice cultivars differing in their salt tolerance to long term salt stress (for 7, 14 and 21 days) were examined earlier [81]. The ADC (and hence Put levels) and SAMDC activities diminished in both cultivars as a consequence of salt treatment. It was proposed that ADC induction and subsequent Put accumulation occur rapidly after an osmotic shock, but not when increasing accumulation of salt occur within the plants under weeks of salt treatment. Put was probably used for higher PA biosynthesis, when plants were exposed to long term salt stress. However, SPDS activity was reduced in the salt-tolerant cultivar (Giza), but not in the salt-sensitive cultivar (El Paso), while no PAO activity was detected. During the salinization period, Put and Spd levels decreased in both the cultivars, although less dramatically in Giza. The Spm accumulation seemed to be a general trend in the long term stress response of rice; the changes observed in Spm levels were consequences of salt build up in the leaf, and that the increased content of this tetramine in the shoot did not improve tolerance, if salt accumulation was not avoided. Moreover, Spm accumulation occurred in both the varieties, suggesting that Spm accumulation is not a salt tolerance trait. Practically undetectable *SAMDC* transcripts in M-1-48 and Gobindobhog rice seeds could be seen under control conditions, induced only after exogenous ABA (50 µM, 6 h) treatment, whereas they were expressed at a much higher level even in dry and water-imbibed seeds of Nonabokra (constitutive expression), and lesser induced by ABA [82]. The SAMDC protein expression also showed similar pattern. The expression of the *SAMDC1* gene in rice seedlings was dramatically induced by salinity and drought. The transcript levels of *SAMDC1* in the two rice varieties differing in salt tolerance was found to be higher in the salt-tolerant rice variety than in the salt-sensitive one, and occurred more quickly when both the varieties were exposed to low concentrations of NaCl stress (0–150 mM) [83]. Salinity induced either the stimulation of Spd and Spm synthesis or inhibition of PAO in salt-tolerant cultivars, which stabilizes membrane systems during metabolic adaptation to salinity. The importance of ionic and osmotic components of salt stress on modification of free PA level was studied in the seedlings of sensitive (IKP) and tolerant (Pokkali) cultivars of rice [84]. Under isoosmotic concentrations of NaCl or PEG, it was found that Put have differential role in non-photosynthetic organs versus photosynthetic organs, because it accumulated to high amounts in the roots of Pokkali in comparison to IKP, whereas, an opposite trend was recorded in the shoots. It was concluded that in rice roots, Put accumulation under salt stress occurred in a time of hours, supporting a role of Put in short term salt stress response. Salinity stress-induced higher loss of K^+ ion and sharp inhibition of plasma membrane-bound H^+-ATPase activity could be more effectively overcome in salt-sensitive rice cultivars by exogenously supplied Spd [85]. While Spd and Spm synthesis in the salt-tolerant variety is stimulated to stabilize membrane systems, there is no significant change in stressed salt-sensitive cultivars. Salinity stress increased the superoxide and H_2O_2 production, particularly in *Cucumis sativus* cv. Jinchun No. 2 roots, while the salinity-induced increase in antioxidant enzyme activities and Pro

contents in the roots was higher in cv. Changchun mici than in cv. Jinchun No. 2 [86]. A marked increase in ADC, ODC, SAMDC and DAO activities, as well as free Spd and Spm, soluble conjugated and insoluble bound Put, Spd and Spm contents were also noted in the roots of Changchun mici than Jinchun No. 2, under salt stress. Changes in PA contents have also been reported in response to different stresses [87], indicating that PA accumulation is a non-specific response to salt stress.

Recent studies were conducted to see whether interaction between PAs and ROS may determine the extent of genotypic variation in salinity tolerance [88], by using barley genotypes contrasting in salinity tolerance. Application of hydroxyl radicals-generating Cu^{2+}/ascorbate mixture induced transient Ca^{2+} and K^+ fluxes in barley roots. Put and Spm alone induced only transient Ca^{2+} efflux and negligible K^+ flux. However, both Put and Spm strongly potentiated hydroxyl radicals-induced K^+ efflux and respective non-selective current. This synergistic effect was much more pronounced in a salt-sensitive cultivar Franklin as compared to a salt-tolerant TX9425. As retention of K^+ under salt stress is a key determinant of salinity tolerance in barley, it was suggested that the alteration of cytosolic K^+ homeostasis, caused by interaction between PAs and ROS, may have a substantial contribution to genetic variability in salt sensitivity in this species.

4.3 Antioxidants

The exposure of plants to high salinity leads to the generation of ROS such as singlet oxygen, superoxide anion radicals, hydroxyl radicals, H_2O_2 and O_3. Both superoxide and hydroperoxyl radicals undergo spontaneous dismutation to produce H_2O_2. Plants possess both enzymatic and non enzymatic antioxidants for the scavenging of ROS. The common ROS-scavenging enzymes are the superoxide dismutase (SOD), catalase (CAT), peroxidases (POXs) including glutathione POXs. The multiple (three) isoforms of the enzyme SOD (mitochondrial Mn-, chloroplastic Fe- and cytosolic/chloroplastic/peroxisomal-Cu/Zn-SODs) catalyzes the dismutation or disproportionation of two superoxide radicals to H_2O_2 and oxygen. The CAT can decompose the H_2O_2 formed into H_2O and O_2 within the peroxisome. The scavenging of H_2O_2 in other cell compartments (cytosol, vacuole, cell wall and extracellular space) depends on distinct POXs, such as guaiacol peroxidases (GPX) and ascorbate peroxidases (APX). The ascorbate (ASC)-glutathione cycle which utilizes reduced glutathione (GSH) as an electron donor to regenerate ASC from its oxidized form dehydroascorbate (DHA) is considered the main pathway of free radical removal. This cycle is catalyzed by a set of four enzymes, APX, monodehydroascorbate reductase (MDHAR), GSH-dependent dehydroascorbate reductase (DHAR) and glutathione reductase (GR), acting in an orchestrated manner [89]. The non-enzymatic components involve a network of low molecular mass antioxidants with high reducing potentials like ASC, cysteine, non-protein thiols, GSH, tocopherols, β-carotene, phenolic compounds, anthocyanins, flavonoids, tannins and lignin precursors.

Genetic differences in salinity tolerance are not necessarily due to differences in the ability to detoxify ROS. Several studies have found differences in the levels of expression or activity of antioxidant enzymes, these differences are sometimes associated with the more tolerant genotype, and sometimes with the more sensitive genotype. The differences in antioxidant activities between genotypes may be due to genotypic differences in degrees of stomatal closure or in other responses that alter the rate of CO_2 fixation, differences that bring into play the processes that avoid photoinhibition. Increase in activities of SOD, APX, CAT and GR under salinity and comparatively higher activity in tolerant wheat genotypes has been reported. In addition, comparatively higher Cu/Zn-SOD, Fe-SOD, APX and GR activity was

found in chloroplastic fraction in tolerant wheat genotypes in response to salt stress [90]. The NaCl-induced enhanced mRNA expression and activity of Mn-SOD, APX, GR and MDHAR in tolerant pea cv. Granada was reported, while in salinity-sensitive cv. Chillis, no significant changes in activity and mRNA levels of the above enzymes were observed [91]. Similar result was also obtained for foxtail millet [92] under salt stress. Significant genotypic variation was observed in the responses in terms of salt tolerance in salt-tolerant KRL19 and salt-sensitive WH542 genotypes of wheat. Upon desalinization, the recovery was more in KRL19 as compared to WH542. The salt tolerance of KRL19 could be ascribed to higher K^+/Na^+ ratio, carotenoid and ASC content. With salt stress, the extent of enhancement of Pro was more profound in the tolerant cultivar [93]. The antioxidant systems SOD, GSH, GR, APX and carotenoids were considered as selection criteria for salt tolerance in *Sorghum*. Particularly the increase in GR and APX activity were highly pronounced in the most tolerant genotypes at 50 mM NaCl than in sensitive genotypes. The increase in enzyme activity might be due to increasing the synthesis of the enzyme or an increased activation of constitutive enzyme pool [94]. Comparing the mechanisms of antioxidant production in salt-tolerant and salt-sensitive plants, a decline in SOD activity and an increase in POX activity was reported in the salt-sensitive rice varieties, Hitomebore and IR-28, in response to salt stress [95]. These salt-sensitive varieties also showed an increase in lipid peroxidation and electrolyte leakage as well as Na^+ accumulation in the leaves under saline conditions. In contrast, two salt-tolerant rice varieties, Pokkali and Bankat, showed differing protective mechanisms against ROS under salt stress. The cultivar Pokkali showed only a slight increase in SOD, but a slight decrease in POX activity, and almost unchanged lipid peroxidation, electrolyte leakage and Na^+ accumulation under saline conditions. In contrast, cv. Bankat showed Na^+ accumulation in leaves and symptoms of oxidative damage similar to the salt-sensitive cultivars. The basal activities of the antioxidative enzymes viz., SOD, CAT, POX, APX and GR were significantly higher in the leaves of all the resistant rice cultivars as compared to the sensitive ones [96]. The various enzymatic and non-enzymatic components of antioxidant system showed differential responses in the leaves of salt-tolerant and salt-sensitive cultivars of rice during salinity stress. With increasing salinity, the activities of SOD and GR enhanced in all the tolerant cultivars while declined in the sensitive cultivars. The magnitude of increase in the activities of CAT and POX was more pronounced in the sensitive cultivars than in the tolerant cultivars. The amount of ASC content and GSH were higher in the leaves of the tolerant cultivars than that of the sensitive cultivars under saline conditions. It was inferred that leaves of salt-tolerant cultivars tend to attain greater capacity to perform reactions of antioxidative pathway under saline conditions to combat salinity-induced oxidative stress. In cotton, significantly higher constitutive concentrations of CAT and α-tocopherol were found in salt-tolerant than in salt-sensitive lines [97,98]. Salt stress caused a considerable increase in the activities of POX and GR in the salt-tolerant cultivars, whereas the activities of these enzymes remained unchanged or decreased in the salt-sensitive cultivars. The salt-tolerant cultivars also had a lower oxidized/reduced ASC ratio and a higher reduced/oxidized glutathione ratio than the salt-sensitive lines under saline conditions. Lipid peroxidation in the salt-sensitive lines increased more than in the salt-tolerant lines under salt stress. Confirming this relationship at the cellular level in cotton, higher SOD and GR activities were found in the cultured cells of salt-tolerant cv. Dhumad than in those of moderately-tolerant (H-14) or salt-sensitive (RAhs-2) cultivars in saline medium [99]. In the leaves of salt-stressed maize plants, the SOD, APX, GPX and GR activities increased with time; this increase was more pronounced in the salt-tolerant than in the salt-sensitive genotype [100]. In salt-stressed roots of the salt-tolerant genotype, SOD and CAT activities decreased and APX, GPX and GR activities remained unchanged. In the roots of the salt-sensitive genotype, salinity reduced the activity of all studied enzymes, indicating a decreased GSH turnover rate and a less active ASC and GSH in roots. The

change in POX activity in response to salinity is not a reliable criterion for screening for tolerance in *Brassica* species [101,102]. The amount of SOD activity in the roots of the tolerant genotype Quantum was greater than the sensitive genotype Fornix of *Brassica napus* L during NaCl stress. In shoot, the SOD activity induced in both genotypes up to 100 mM concentration; increase in salt concentration from 100 to 150 mM caused decrease in SOD activity in the shoot of both genotypes but decrease in salt-sensitive genotype was greater than the salt-tolerant genotype [103]. Recently, while assessing the enzymatic and non-enzymatic antioxidant responses of Carrizo citrange, a salt-sensitive citrus rootstock to different levels of salinity, the adverse effect on the growth of citrus rootstock was mainly due to a cellular intoxication by Cl^- and not to the salt-induced oxidative stress [104]. The possible involvement of the antioxidant system in the salt tolerance of cultivated tomato (*Lycopersicon esculentum*) and its wild salt-tolerant relative *Lycopersicon pennellii* has also been assessed [105]. In the latter species, the constitutive level of lipid peroxidation and activities of CAT and GR were lower, whereas the activities of SOD, APX and DHAR were inherently higher than those in the cultivated tomato species. Working with the same two species of tomato, high salt tolerance of the wild salt-tolerant species was found to be due to maintenance of high SOD to APX activity [106]. In another study [107], the better protection of the wild salt-tolerant tomato (*L. pennellii*) root plastids from salt-induced oxidative stress was correlated with increased activities of SOD, APX and GPX. The salt-tolerant *Plantago maritima* showed a better protection mechanism against oxidative damage caused by salt stress by its higher induced activities of antioxidant enzymes than the salt-sensitive *P. media*. Activities of SOD, CAT and GR decreased in *P. media* with increasing salinity. While four SOD activity bands were identified in leaves of *P. maritima*, only two bands were observed in *P. media*. While five POX activity bands were identified in the leaves of *P. maritima*, only two bands were determined in *P. media*. The MDA level in the leaves increased under salt stress in *P. media* but showed no change and decreased in *P. maritima* [108]. The flavonoids, including the nonenzymatic components such as flavonols, flavonones, proanthocyanidins and anthocyanins function as antioxidants by scavenging reactive oxygen intermediates and harmful lipid peroxy free radicals and inhibiting lipoprotein oxidation or lipid peroxidation of cell membranes induced by reactive oxygen radicals [109]. The higher levels of the two antioxidants, anthocyanin and cysteine were reported in the salt-tolerant rice, Nonabokra compared to the salt-sensitive variety M-1-48, following salinity stress [26]. Different antioxidative capacities, both in the apoplast and in the symplast of pea leaves, contributed to a better protection against salt stress in relatively salt-tolerant cultivars. Salt-induced oxidative damage led to necrotic lesions in the minor veins of pea leaves, as oxidative stress was higher in the apoplasts [110]. The GSH/GSSG ratio declined under salt stress, and there was no GR activity in the apoplasts; the sensitive cultivar Lincoln was more affected than the tolerant cv. Puget with reduction in growth response. Continuous exposure to salt stress in rice seedlings made them more tolerant when the GSH/GSSG levels returned to normal values after an initial decline [111]. Under short-term NaCl stress, the salt-tolerant rice Pokkali showed higher activity of CAT and enhanced levels of antioxidants like ASC and GSH than the sensitive cultivar Pusa Basmati 1 [112]. The results with two rice varieties Lunishree and Begunbitchi showed decreased ASC content under salt stress except in lower concentrations of NaCl in Lunishree, allowing better antioxidant protection as reported for other plants. The increase in ASC levels in lower NaCl concentrations in Lunishree depended both on the rate of its synthesis and regeneration. Increased GSH content in the stressed and recovered roots of Lunishree, whereas its decreased content in Begunbitchi reflected, at least partially, the increased demand of GSH in ROS detoxification in Lunishree compared to Begunbitchi. The GR activity showed a greater decline in Begunbitchi than in Lunishree under NaCl stress, while constitutive levels of GR were higher in Lunishree than in Begunbitchi [113]. The higher activity of SOD, APX,

CAT and GR, and comparatively lower $O^{2\cdot-}$, H_2O_2 and lipid peroxidation were observed in the maize genotype PEHM-3 compared to Navjot, where salinity induced decrease in carotenoids was more [56]. The accumulation of the total flavonoid contents in salt-stressed seedlings of the salt-tolerant rice varieties KDML105 and Sangyod were higher than those in salt-sensitive varieties Pathumthani 1 and Black Sticky [49]. The modifications of flavonoid structure i.e., glycosylation, prenylation and methylation could affect their antioxidant properties; thus they may help inhibit lipid peroxidation in stressed-plants [114,115,116]. The variation in esterase activity was noted at the tillering stage of callus-regenerated plants of salt-tolerant rice Pokkali and salt-sensitive M-I-48, Annapoorna and Jyothi rice genotypes [117].

4.4 ABA

The phytohormone ABA is the common mediator in plants in response to any form of abiotic stress. ABA can control the salt stress-induced water deficit of the plant by reducing transpiration, via effects on stomatal closure through guard cell depolarization and alterations of guard cell turgor and volume, driven by cation and anion effluxes. Intercellular or endogenous ABA concentration in leaf can increase 10-50 folds within a few hours of the onset of water deficit, caused by high osmoticum, high NaCl or drying. An important part of the physiological response to ABA is achieved through de novo gene expression, which has been mostly identified by microarray experiments. At the molecular level, ABA is the common regulator in stress signal transduction pathways, controlling either the ion channels or changes in the expression of many stress-inducible genes that ultimately translate to major changes in proteome expression [118].

The salt-tolerant varieties seem to have a higher ability for ABA synthesis than the sensitive ones. This agrees with reported results comparing sensitive/tolerant cultivars of different species [119,120]. Only a minor increase in ABA level in the roots of salt-stressed TN-1 (salt-sensitive) seedlings was noted [15]. The salt-tolerant varieties Pokkali and Nonabokra exhibited a considerably larger, more rapid and also less transient increase in ABA contents. Of the two salt-tolerant varieties, Nonabokra consistently accumulated the highest levels of endogenous ABA. During osmotic shock (150 mM NaCl), the peak ABA concentration was 30 folds higher for Nonabokra and six folds higher for Pokkali, as compared to TN-1. It was suggested that although salt-tolerant rice cultivars showed higher increases of ABA than the sensitive cultivars, there was not a direct correlation between absolute ABA content and degree of tolerance. Therefore, the importance of both the rate of ABA increase as well as the absolute ABA levels was pointed out. Exogenous ABA (100 µM, 48 h) treatment to rice seedlings led to the maximum accumulation of Put and Spd, as well as total soluble PAs in the aromatic rice variety Gobindobhog, as compared to the salt-sensitive variety M-1-48 and salt-tolerant variety Nonabokra [121]. The maximum PA accumulation was suggested as a means to shield off ABA-induced stress injuries in Gobindobhog, which suffered the maximum damages due to lipid hydroperoxidation and free radical generation. In rice, ABA contents increased in a semi salt-tolerant variety, but only marginal changes were measured in salt-tolerant and salt-sensitive ones [122]. They also reported that exogenous ABA treatments improved tolerance to salinity in sensitive but not in tolerant rice cultivars. The tolerance of the salt-sensitive IR-29 to saline stress was generally improved by ABA treatment and leaf Na^+ content reduced to their respective control treatment [123]. This ABA effect was evident in IR-29 with low tolerance, as their ability to recover from stress increased up to seven fold. Independent of the saline treatment, the absolute endogenous leaf ABA content in sensitive variety was significantly more than the tolerant one. However,

upon stress, the increase in endogenous ABA synthesis was higher in tolerant than in sensitive varieties. These data further suggested that there was differential sensitivity to ABA in the tolerant and sensitive cultivars and the enhanced concentrations at tolerant levels acted primarily to maintain root and shoot growth during salt stress. Moreover, the differences in the level of tolerance to saline stress are related to their differential capacity of ABA synthesis under stress conditions. The higher salt tolerance of *Brassica napus* compared with the salt-sensitive *B. carinata* is due to its lower ABA accumulation under salt stress [124]. ABA concentrations significantly increased in resistant maize leaves under salt stress, which may contribute to acidifying the apoplast, which in turn is a prerequisite for growth [125]. Mutants of *Arabidopsis* with reduced ABA content or sensitivities are more tolerant to salt stress during germination [126]. This is probably because salt stress increases ABA level in the wild type, which is inhibitory to germination. Salinity induced increase in ABA concentration is also correlated with inhibition of leaf expansion in different species [124,127,128]. The increases in ABA concentration in the leaf expansion zones correlate with reduction in leaf expansion rate, though the extent of reduction is genotype-specific [129]. The salt stress response was examined based on the method of ABA application in four potato genotypes of varying salt stress resistance [130] - the sensitive ABA-deficient mutant and its normal sibling, a resistant genotype line 9506, and commercial cultivar Norland of moderate resistance. Under a single dose, growth rate increased in all genotypes under salt stress, whereas slowly increasing multiple ABA applications generally maintained stable growth rates except in the ABA-deficient mutant, where there was an upward growth trend. The percentage in root water content enhanced only under slowly-increasing multiple ABA doses in two genotypes, whereas none of the single-dose treatments induced any change. The single ABA dose enhanced vertical growth, whereas the slowly increasing multiple ABA dose applications enhanced lateral shoot growth. It was indicated that the method of ABA application regime in itself can alter plant responses under salt stress in resistant and sensitive potato lines, and that certain application regimes may reflect responses to elevated endogenous levels of ABA.

5. MOLECULAR BASIS OF VARIETAL DIFFERENCE

Changes in gene expression occur in plants following an exposure to salinity stress. A number of salt-responsive genes have been isolated and characterized, which include the late embryogenesis abundant (*lea*) genes like wheat *Em*, rice *Rab16A* and *Osem*, barley *HVA1* and *HVA22* etc, genes for membrane transporters like carriers, channels, symporters and antiporters, genes encoding proteins for ion homeostasis like Na^+/H^+ antiporter involved in vacuolar sequestration of toxic ions etc. Micro- and macroarray-based transcriptional profiling has provided quantitative information about the expression levels of a large number of genes simultaneously. In higher plants, different EST/cDNA collections have been employed for transcriptional profiling to identify genes whose expression levels change in response to salt. It appears that overall tolerance to high salt levels is due to effectors that directly modulate stress etiology or attenuate stress effects and due to regulatory molecules that are involved in stress perception, signal transduction and modulation of the effectors' functions. Further progress on the transcript changes in response to salt application has been made using the comparative genomics approach. Comparative stress genomics essentially means that various commonalities and differences in expression patterns of different genes relative to populations that differ in stress tolerance are scored. This approach appears highly valuable for unveiling the key genetic contributors to the complex physiological processes involved in salt tolerance trait [8].

The identification of salt-stress-specific changes in gene expression can be achieved by comparing gene expression in non-induced and stress-induced tissues or by comparing different genotypes such as contrasting cultivars [131]. A fewer number of genes was induced by 250 mM NaCl stress in *Thellungiella halophila* (salt cress, a wild salt-tolerant relative of *Arabidopsis*), in contrast to *Arabidopsis*, indicating that the salt stress tolerance may be due to the constitutive overexpression of many genes that function in stress tolerance and that are only stress-inducible in *Arabidopsis* [132]. A larger spectrum of gene expression changes noted between *Atnhx1* knockout mutant and wild type plants [133] showed that salt-sensitive and salt-tolerant *Arabidopsis* phenotypes differ markedly in the expression of their genetic machinery. The transcript regulation was examined in response to high salinity in the salt-tolerant rice cultivar Pokkali and salt-sensitive rice cultivar IR-29, using a microarray of 1,728 cDNAs from different libraries obtained from salt-stressed tissues [134]. The gene expression in response to salt in contrasting rice plants was both qualitative as well as quantitative. The transcript levels of a large number of genes (including those associated with detoxification, stress response, signal transduction, etc.) undergo pronounced changes upon subjecting the seedlings of salt-tolerant rice Dee-geo-woo-gen to salt stress [135]. The response of salt stress in IR-29 and salt tolerant FL478 line was investigated using microarray [136]. Salinity stress induced a large number of genes involved in the flavanoid biosynthetic pathway in IR-29 but not in FL478. The three types of proteins, identified as an ABA-responsive His-rich, 40 kDa protein and group 2 and group 3 LEA proteins coordinately accumulated to higher levels in roots from both salt-tolerant varieties Pokkali and Nonabokra, compared with TN-1 [15]. Nonabokra exhibited a less pronounced induction of ABA-responsive proteins, while Pokkali showed a greater induction of the same proteins. The ABA-induced dehydrin levels were associated with varietal differences and salt tolerance. In a comparative analysis of gene expression in *Lophopyrum* and wheat, a set of 11 mRNAs was found to be induced to higher levels in the roots from the halotolerant wheat relative and the amphidiploids, both upon osmotic shock and in response to exogenous ABA [137,138]. Sets of proteins present at different levels in tolerant versus sensitive genotypes have also been detected in a salt-tolerant barley cultivar [139, 140]. The *oslea3* gene expression was compared for the salt-tolerant variety Pokkali and the salt-sensitive cultivar TN-1. The maximal mRNA levels were found in roots of the tolerant variety, also declining less rapidly upon sustained salt shock, concomitant with a delayed drop in shoot water content. DNA blot analysis indicated the existence of a small *oslea3* gene family in rice with an equal gene number in both ecotypes. The results suggested that a differential regulation of *oslea3* expression is an aspect of the varietal differences in salt stress tolerance [141]. The variation in the expression pattern of the group 2 *lea* gene (encoding dehydrin protein) *Rab16A* was detected in the three varieties M-1-48, Nonabokra and Gobindobhog. The transcript level was the maximum in Nonabokra even under control condition, which was very slightly triggered upon salt stress. In case of M-1-48 (salt-sensitive variety) and Gobindobhog (salt sensitive aromatic variety), much lesser transcript level could be detected and that too only after salt exposure [26]. Similar result was also obtained previously [142]. The polypeptide band of 21 kDa, that cross-reacted with the anti-dehydrin antiserum was confounded in Nonabokra at a high level both under control (untreated) and salt-treated conditions, indicating that the RAB16A (dehydrin) protein is expressed constitutively in Nonabokra. In M-1-48 and Gobindobhog, only salt treatment could produce the protein in sufficient and detectable amounts. A positive correlation exists between the expression pattern of the ABA-inducible transcription factors and salt tolerance in rice. This was also corroborated by earlier observations [142,143]. The rice trans-acting factor OSBZ8, which targets the regulation of *Rab16A* gene, was found to exhibit salinity stress-inducible accumulation in salt-sensitive rice cultivars and constitutive expression in salt-tolerant cultivars, indicating a positive role of OSBZ8 towards salt tolerance in the vegetative tissues

of rice. The salt-responsive proteins in the roots of the salt-tolerant rice variety, Pokkali and the salt-sensitive varieties IR-64 and IR-29 were also studied by a proteomic approach. Among the salt responsive proteins identified were an ABA and stress responsive protein (ASR1), APX and caffeoyl CoA O-methyltransferase (CCOMT). The CCOMT was markedly upregulated by salt stress in Pokkali, but changed little in IR-29. CCOMT is involved in suberin and lignin biosynthesis and increased lignification might help to reduce the bypass water flow that allows Na^+ ions to enter rice roots via an apoplastic route [3, 144, 145]. A comparative investigation of transcript regulation was undertaken in the roots of a salt-tolerant FL478 and salt sensitive IR-29 rice varieties [146]. Though the genes encoding aquaporins, a silicon transporter, and N transporters were induced in both the cultivars, the transcripts for cation transport proteins including OsCHX11, OsCNGC1, OsCAX, and OsTPC1 showed differential regulation between the cultivars. The encoded proteins participated in reducing Na^+ influx, reduced Na^+ translocation to the shoot, lowering the tissue Na^+/K^+ ratio and limiting the apoplastic bypass flow in the roots of FL478, the tolerant variety. Comparative proteomic and transcriptomic analyses between fragrant Thai jasmine rice cultivar, KDML105, sensitive to salinity and the salt tolerant cultivar Pokkali were performed [147]. For Pokkali, the upregulation of nine identified salt-induced proteins (involved in photosynthesis, photorespiration and the oxidative stress detoxification system) was related to the increase in abundance of the respective mRNA transcripts. In contrast, although mRNA transcripts encoding all ten identified proteins could be detected in KDML105, only three differential proteins spots were detected in the proteomic analysis. This indicated that although KDML105 contains elevated transcript level of genes needed for salt tolerance, the posttranscriptional mechanisms controlling protein expression levels were not as efficient as in Pokkali, indicating targets for future genetic improvement. The elevated levels of proteins play important roles in conferring tolerance to salt stress in the rice cultivar Pokkali by providing more efficient metabolic readjustment, thus leading to higher photosynthetic efficiency and hence a better growth performance. Although KDML105 expressed genes for several proteins needed for salt tolerance, it was unable to induce these stress-responsive processes at both transcriptional and post-transcriptional levels as effectively as Pokkali. Soluble proteins extracted from leaves of two wheat species (*Triticum durum* sensitive cv. Ben Bachir and *Triticum aestivum* tolerant cv. Tanit) differing in their sensitivity were analyzed by 2-dimensional gel electrophoresis (2-DE) in order to detect NaCl-induced changes. The greatest alterations in the polypeptide profiles following salt stress were found in the most sensitive cultivar; among the 12 spots (molecular mass 15-31 kDa) specifically considered in the acidic region of the gel, 11 declined and even disappeared in the leaf proteins of the NaCl-sensitive variety, while in the tolerant species, two more new polypeptides were induced by NaCl [148]. When the effect of salt stress on the polypeptide levels was examined using 2-DE in roots of two contrasting wheat (*T. durum*) cultivars, i.e., sensitive cv. Ben Bachir and tolerant cv. Chilli, the net synthesis of a 26 kDa polypeptide was significantly changed in the tolerant cultivar [149]. A comparative proteomic analysis of salt response was performed in the roots of wheat cultivars Jing-411 (salt-tolerant) and Chinese Spring (salt- sensitive), subjected to a range of salt stress concentrations (0.5%, 1.5% and 2.5%) for two days [150]. Comparative analysis through 2-DE maps showed that 41 differentially expressed protein spots (DEPs) were salt-responsive with significant expression changes in both varieties under salt stress, and 99 (52 in Jing-411 and 47 in Chinese Spring) were variety-specific. Only 15 and 9 DEPs in Jing-411 and Chinese Spring, respectively, were upregulated in abundance under all three salt concentrations. Some salt responsive DEPs, such as guanine nucleotide-binding protein subunit β-like protein, RuBisCO large subunit-binding protein subunit α and pathogenesis related protein 10, were upregulated significantly in Jing-411 under all salt concentrations, whereas they were downregulated in salinity-stressed Chinese Spring. The transcripts of K^+-

transporters (HvHAK1 and HvAKT1), vacuolar H^+-ATPase and inorganic pyrophosphatase (HvHVA/68 and HvHVP1) were more abundant in the shoots of the salt-tolerant barley cultivar K305 than in shoots of the salt-sensitive cultivar I743 [151]. Expression of HvHAK1 and Na^+/H^+ antiporters (HvNHX1, HvNHX3 and HvNHX4) was higher in roots of K305 than in I743 with prolonged exposure to salt. Taken together, these results suggested that the better performance of K305 compared to I743 during salt stress was related to its greater ability to sequester Na^+ into sub-cellular compartments and/or maintain K^+ homeostasis. The severe growth reduction in I743 was attributed to the elevated levels of Na^+ in shoots. The proteome response of a salt-sensitive (Line 527) and a salt-tolerant (Afzal) barley genotype to prolonged salt treatment was earlier compared [152]. Upregulation of proteins which involved in ROS scavenging, signal transduction, protein processing and cell wall might increase plant adaptation to salt stress. The upregulation of the three of four antioxidant proteins (thioredoxin, methionine sulfoxide reductase and DHAR) in the susceptible genotype Line 527 suggested a different tolerance mechanism (such as tissue tolerance) to tolerate a salinity condition in comparison with the salt-tolerant genotype. The gene expression profile was also compared between a salt-tolerant (Prasad) and susceptible variety (Lepakshi) of foxtail millet in response to salt stress [153]. Using cDNA-AFLP, they identified 27 non-redundant differentially expressed cDNAs that are unique to salt-tolerant variety which represent different groups of genes involved in metabolism, cellular transport, cell signaling, transcriptional regulation, mRNA splicing, seed development and storage, etc. The expression patterns of seven out of nine such genes showed a significant increase of differential expression in tolerant variety after 1 h of salt stress in comparison to salt-sensitive variety as analyzed by quantitative Real time-PCR.

Several studies on the genetic variability across rice cultivars suggested that the activity and composition of root plasma membrane transporters could underlie the observed cultivar-specific salinity tolerance in rice. Concentrations well over 200 mM NaCl completely repress enzyme activity in vitro. The enzymes in the halophytes are not more tolerant of salt *in vitro* than the corresponding enzymes in non halophytes, suggesting that compartmentation of Na^+ are an essential mechanism in all plants. Thus, differences in the expression levels of *AtNHX1* or *AtAVP1* may affect the potential to sequester Na^+ in vacuoles of the leaves. Increased efficiency of intracellular compartmentation may explain differences in salinity tolerance between closely related species. This hypothesis is supported by the findings of a much greater salt stress-induced Na^+/H^+ antiporter activity in the salt-tolerant species *Plantago maritima* than in the salt-sensitive species *Plantago media* [154]. The differences in the expression of the transcript for OsHKT-type transporter (homologous to the wheat K^+/Na^+-symporter HKT1) in time and space in salt-tolerant Pokkali and salt-sensitive IR-29 rice varieties was suggested as a factor that may distinguish salt stress-sensitive and stress-tolerant lines and correlated with a component of ion homeostasis [155]. The expression level of the gene encoding OsAKT1 homologous to inward-rectifying potassium channels of the AKT/KAT subfamily during salt stress was compared in rice lines showing different salinity tolerance [156]. In the salt-tolerant, sodium-excluding varieties Pokkali and BK, OsAKT1 transcripts disappeared from the exodermis in plants treated with 150 mM NaCl for 48 h, but OsAKT1 transcription was not repressed in these cells in the salt-sensitive, sodium-accumulating variety IR-29. Significantly, all the lines were able to maintain potassium levels under sodium stress conditions, while sodium concentrations in the leaves of IR-29 increased 5-10-fold relative to the sodium concentration in BK or Pokkali. The divergent, line-dependent and salt-dependent regulation of this channel did not significantly affect potassium homeostasis under salinity stress. Rather, repression in Pokkali/BK and lack of repression in IR-29 correlated with the overall tolerance character of these lines. A higher ATPase activity was found in a low Na^+ accumulating line of *Trifolium alexandrinum*

than in a high Na^+ accumulating line [157]. In wheat, a significant increase in the activity of plasma membrane (PM)-ATPase in the shoots of the salt-tolerant line and a marked decrease in that of salt-sensitive line due to salt stress were found [158]. In contrast, in roots, an increase in PM-ATPase activity due to salt stress was observed in the salt sensitive line but not in the salt-tolerant line. However, V-ATPase activities in shoots and roots were not significantly different between the lines differing in salt tolerance. These results are in agreement with those for carrot [159] and mung bean [160]. Studies were conducted on the transcriptome of a wild tomato genotype, *Solanum pimpinellifolium* PI365967 that is significantly more salt-tolerant than the cultivated tomato, *Solanum lycopersicum* Moneymaker [161]. The salt overly sensitive (SOS) pathway was found to be more active in PI365967 than in Moneymaker, coinciding with relatively less accumulation of Na^+ in shoots of PI365967. A gene encoding salicylic acid-binding protein 2 (SABP2) was induced by salinity only in PI365967, suggesting a possible role for salicylic acid signaling in the salt response of PI365967. The fact that two genes encoding lactoylglutathione lyase were salt-inducible only in PI365967, together with much higher basal expression of several glutathione *S*–transferase (*GST*) genes, suggested a more effective detoxification system in PI365967. The specific downregulation in PI365967 of a putative high-affinity nitrate transporter, known as a repressor of lateral root initiation, may explain the better root growth of this genotype during salt stress. In a recent observation [162], it was suggested that the salt-tolerant genotypes of *Brassica spp.,* like CS52 and CS54 showed higher induction of genes encoding components of SOS pathway, viz., *SOS1* (a plasma membrane bound Na^+/H^+ antiporter), *SOS2* (Ser/Thr protein kinase that activates *SOS1*), *SOS3* (calcium bindng protein that activates SOS2) and vacuolar antiporter *NHX-1*, as compared to the salt-susceptible genotypes Varuna and T9. This resulted in restricted uptake of toxic Na^+, and efficient Na^+ exclusion and sequestration system, which was manifested in lesser reduction in tissue K content and higher K/Na ratio under salt stress, paving the way for better ion homeostasis and salinity tolerance in salt-tolerant *Brassica* genotypes. In case of mulberry, salt stress resulted in a much decrease in growth, dry matter and leaf area, as well as total polypeptide profiles in the susceptible cultivar ATP, than the tolerant cultivar S1 [163].

6. QUANTITATIVE TRAIT LOCI (QTLs)

Salt tolerance and its sub-traits might be determined by multiple gene loci. Using molecular marker technology, it is now feasible to analyze the quantitative traits, such as salt tolerance and identify the chromosomal regions associated with such character, known as quantitative trait loci (QTLs) Mapping of quantitative trait loci (QTLs) can enable dissection of the genetic control of each tolerance mechanism, opening up the possibility of future efforts to increase the selection efficiency in the breeding program, and develop varieties with improved salinity tolerance by precisely transferring QTLs into popular varieties and pyramiding multiple relevant QTLs for a particular stress-prone environment. This will lead to map based cloning opening a new avenue for genetic manipulations using the real candidate genes rather than using some non-specific general abiotic stress-response genes. A number of mapping studies have identified QTLs associated with salinity tolerance in rice [164,165]. Several QTLs controlling tolerance traits, including major QTLs for shoot K^+ concentration on chromosome 1 (*qSKC-1*) and shoot Na^+ concentration on chromosome 7 (*qSNC-7*) were identified [166]. The *SKC1* gene was subsequently cloned and found to encode a sodium transporter that helps control K^+ homeostasis under salt stress [167]. In Pokkali, a major QTL associated with the Na–K ratio and seedling-stage salinity tolerance, named *Saltol*, was identified on chromosome 1, along with a number of minor QTLs on other chromosomes. The Pokkali-derived QTLs were characterized for seedling stage salinity tolerance in preparation for use in marker-assisted breeding [168]. A Nonabokra derived variety CSR27

was used for mapping six QTLs for salt tolerance at seedling stage [169]. In an intergeneric cross of tomato, QTLs were found associated with fruit yield in plants growing under saline conditions [170]. QTL associated with tolerance vary with the stage of plant development. However, the fact that a QTL represents many, perhaps hundreds, of genes remains a problem to finding key loci within a QTL.

7. CONCLUSION AND FUTURE PERSPECTIVES

No well-defined indicators are available to facilitate the improvement in salinity tolerance of agricultural crops through breeding. In the past few decades, plant breeders have successfully improved salinity tolerance of some crops through conventional selection and breeding techniques, with relatively little direct input from physiologists or biochemists. There is a consensus among scientists that selection is more convenient and practicable if the plant species under test possesses distinctive indicators of salt tolerance, whether at the whole plant, tissue, or cellular level. Thus, there is a pressing need to unravel the cellular mechanisms and underlying biochemical mechanisms of salinity tolerance, so as to give meaningful advice to plant breeders. Several physiological responses that are candidate indicators to salinity differ qualitatively or quantitatively between salt-tolerant and sensitive species or lines. Yet, it has not proved possible to find any sensitive criterion that could reliably be used by breeders to improve salt tolerance. This is partly because its physiology is so complex that in addition to variation among species, in many cases it varies also from cultivar to cultivar within a single species and with physiological age. Furthermore, salinity tolerance is unlikely to be determined by a single gene or gene product [171], but probably results from the expression of a number of genes, the importance of which is dependent upon their interaction with other salt tolerance genes and the external salt concentration [172]. The mechanism for salinity tolerance becomes even more complicated when response to salinity stress of a plant species varies with the growth stage of its life cycle. In order to develop practicable strategies for selecting salt-tolerant lines/genotypes of potential crops, it is necessary to gain detailed information on whether changes in physiological/biochemical parameters due to salt stress are attributable to detrimental effects of salt stress, or are components of the adaptation mechanism. Furthermore, it would be much more valuable if biochemical indicators, as indicated in this review, are specified for individual species rather than generalized for all species. Plants respond to salinity stress in part by modulating gene expression, which ultimately leads to the restoration of cellular homeostasis. The signal transduction pathways mediating these adaptations can be examined by combining forward and reverse genetic approaches with molecular, biochemical and physiological studies [30].

Naturally salt-tolerant species are now being promoted in agriculture, particularly to provide forage, medicinal plants, aromatic plants [173] and for forestry [174]. At the extreme, plants that can grow productively at very high salt levels could be irrigated with brackish water or seawater [175]. Although plants that put resources into developing salt-tolerance mechanisms (e.g., the production of compatible solutes, antioxidants or stress-tolerant proteins to maintain osmotic balance is an energetic cost) may do so at the expense of other housekeeping vital functions, many salt-tolerant plants show optimal growth in saline conditions [176] and salt marshes and have high productivity [176,177].

Recent work provides indications (through the abundance of stress-regulated and transcriptional- and translational-associated genes in salt stress libraries) that cells undergo an adjustment to reprogram their metabolism to survive under stress conditions, and thus genes involved in this reprogramming may as well turn out to be important players in

controlling salt stress response. For the discovery of novel salt-stress tolerance-related genes, comparative genomics is clearly turning out to be an important tool. Using comparative genomics approach, there are several attempts to unravel how Pokkali, Nonabokra, *Porteresia* and other related rice types are endowed with high salt tolerance. Novel salt-stress-tolerant genes thus emerging from different directions of research need to be validated in future attempts.

It is conceivable that approaches like genomic-scale expressed sequence tag (EST) and genome sequencing, and cDNA microarray analyses that are now under way hold promise to rapidly isolate and identify all candidate genes of the 'osmome', 'xerome' or 'thermome' — the gene complement essential for tolerance of osmotic potential, desiccation or temperature stresses. The large datasets generated by these efforts will be integrated and comparisons will be made between different cellular and glycophytic, halophytic and xerophytic plant models to identify the cellular tolerance mechanisms, as indicated in the present review. Mining of these data will supply a systematic agenda for functional analysis with the use of tagged mutant collections, complementation and overexpression tests, accompanied by microarray analyses to reveal hierarchical relationships between specific signaling components and downstream effector genes [178]. The functional determination of all genes that participate in salinity stress adaptation or tolerance reactions are expected to provide an integrated understanding of the biochemical and physiological basis of salt stress responses in plants. Understanding specific protein–protein interactions will require the construction of protein-linkage maps using yeast two-hybrid technologies. Approaches with proteomics will be necessary to clarify the structural predictions of genome sequence information and to assess the protein modifications and protein–ligand interactions that are relevant to stress tolerant phenotypes [178]. Identification of specific genes that are up or down regulated will provide a specific focus for transformation, although choosing the key genes for tolerance is currently far from happening. Transgenic technology will undoubtedly continue to aid the search for the cellular mechanisms that underlie tolerance, but the complexity of the trait is likely to mean that the road to engineering such tolerance into sensitive species will be long. In the meantime, it would be expedient to continue to invest in other avenues such as the manipulation of ion excretion from leaves through salt glands and the domestication of halophytes [179]. Armed with such information from established models, it will be possible to rationally manipulate and optimize tolerance traits for improved crop productivity against salinity stress in the coming future.

Overall, our review highlights the fact that varietal differences in salt tolerance in different plant species can be attributed to the differential accumulation of stress-related metabolites at the biochemical level as well as differential transcript accumulation and protein expression at the molecular level, with the tolerant varieties showing comparatively higher and constitutive level, while the susceptible varieties with lower and inducible expression system [26,142].

ACKNOWLEDGEMENTS

Financial support from Science and Engineering Research Board (SERB), Government of India through the research grant (SR/FT/LS-65/2010) to Dr. Aryadeep Roychoudhury is gratefully acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yeo AR. Predicting the interaction between the effects of salinity and climate change on crop plants. Sci Hortic. 1999;78:159–74.
2. Roy Choudhury A, Das K, Ghosh S, Mukherjee RN, Banerjee R. Transgenic plants: benefits and controversies. J Bot Soc Bengal. 2012;66:29-35.
3. Roychoudhury A, Datta K, Datta SK. Abiotic stress in plants: from genomics to metabolomics. In: Tuteja N, Gill SS, Tuteja R, editors. Omics and plant abiotic stress tolerance, Bentham Science Publishers; 2011a.
4. IRRI. Stress and disease tolerance: 1. Breeding for salt tolerance in rice. Available: http://www.knowledgebank.irri.org/ricebreedingcourse/bodydefault.htm#breeding for salt tolerance.htm; 2011.
5. Munns R, Tester M. Mechanisms of salinity tolerance. Annu Rev Plant Biol. 2008;59:651-81.
6. Greenway H, Munns R. Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol. 1980;31:149–90.
7. Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ. Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol. 2000;51:463–99.
8. Bressan RA, Zhang C, Zhang H, Hasegawa PM, Bohnert HJ, Zhu JK. Learning from the *Arabidopsis* experience. The next gene search paradigm. Plant Physiol. 2001;127:1354–60.
9. Zhu JK. Plant salt tolerance. Trends Plant Sci. 2001;6:66-71.
10. Xiong L, Zhu JK. Molecular and genetic aspects of plant responses to osmotic stress. Plant Cell Environ. 2002;25:131–39.
11. Munns R. Comparative physiology of salt and water stress. Plant Cell Environ. 2002;25:239-50.
12. Grattan SR, Zeng L, Shannon MC, Roberts SR. Rice is more sensitive to salinity than previously thought. Calif Agric. 2002;56:189–95.
13. Maas EV. Crop salt tolerance. In: Tanji KK, editor. Agricultural Salinity Assessment and Management, ASCE Manuals and Reports on Engineering. No. 71. American Society of Civil Engineers, New York; 1990.
14. Yeo AR, Flowers TJ. Varietal differences in the toxicity of sodium ions in rice leaves. Physiol Plant. 1983;59:189–95.
15. Moons A, Bauw G, Prinsen E, Montagu MV, Straeten DVD. Molecular and physiological responses to abscisic acid and salts in roots of salt-sensitive and salt-tolerant indica rice varieties. Plant Physiol. 1995;107:177–86.
16. Munns R. Causes of varietal differences in salt tolerance. Proceedings of the International Congress of Plant Physiology, New Delhi; 1988.
17. Rush DW, Epstein E. Genotypic responses to salinity: differences between salt-sensitive and salt-tolerant genotypes of the tomato. Plant Physiol. 1976;57:162-66.
18. Munns R, Fisher DB, Tonnet ML. Na^+ and Cl^- transport in the phloem from leaves of NaCl-treated barley. Aust J Plant Physiol. 1986;13:757–66.
19. Wolf O, Jeschke WD. Sodium fluxes, xylem transport of sodium, and K/Na selectivity in roots of seedlings of *Hordeum vulgare*, c.v. California Mariout and *H. distichon* cv. Villa. J Plant Physiol. 1986;125:243-56.

20. Lynch J, Lauchli A. Salt stress disturbs the calcium nutrition of barley (*Hordeum vulgare* L.). New Phytol. 1985;99:345-54.
21. Davis RF. Sodium fluxes in intact roots of wheat varieties differing in salt tolerance. In: Cram WJ, Janacek K, Bybova R, Sigler K, editors. Membrane transport in plants. Academia, Prague; 1984.
22. Kader MA, Lindberg S. Uptake of sodium in protoplasts of salt-sensitive and salt-tolerant cultivars of rice, *Oryza sativa* L. determined by the fluorescent dye SBFI. J Exp Bot. 2005;56:3149-58.
23. Davenport R, James RA, Zakrisson-Plogander A, Tester M, Munns R. Control of sodium transport in durum wheat. Plant Physiol. 2005;137:807-18.
24. Ohta M, Hayashi Y, Nakashima A, Hamada A, Tanaka A, Nakamura T, Hayakawa T. Introduction of a Na^+/H^+ antiporter gene from *Atriplex gmelini* confers salt tolerance to rice. FEBS Lett. 2002;532:279–82.
25. Chattopadhyay MK, Tiwari BS, Chattopadhyay G, Bose A, Sengupta DN, Ghosh B. Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa*) plants. Physiol Plant. 2002;116:192–9.
26. Roychoudhury A, Basu S, Sarkar SN, Sengupta DN. Comparative physiological and molecular responses of a common aromatic indica rice cultivar to high salinity with non-aromatic indica rice cultivars. Plant Cell Rep. 2008a;27:1395-410.
27. Walker RP, Torokfalvy E, Grieve AM, Prior LD. Water relations and ion concentrations of leaves on salt-stressed citrus plants. Aust J Plant Physiol. 1983;10:265-77.
28. Moya JL, Gómez-Cadenas A, Primo-Millo E, Talon M. Chloride absorption in salt-sensitive Carrizo citrange and salt-tolerant Cleopatra mandarin citrus rootstocks is linked to water use. J Exp Bot. 2003;54:825-33.
29. Ashraf M, Tufail M. Variation in salinity tolerance in sunflower (*Helianthus annuus* L.). J Agron Soil Sci. 1995;174:351–62.
30. Ashraf M, Harris PJC. Potential biochemical indicators of salinity tolerance in plants. Plant Sci. 2004; 166: 3–16.
31. Agastian P, Kingsley SJ, Vivekanandan M. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica. 2000;38:287–90.
32. Ashraf M, Fatima H. Responses of some salt tolerant and salt sensitive lines of safflower (*Carthamus tinctorius* L). Acta Physiol Plant. 1995;17:61–71.
33. Ghosh S, Bagchi S, Lahiri Majumder A. Chloroplast fructose-1,6-bisphosphatase from *Oryza* differs in salt tolerance property from the *Porteresia* enzyme and is protected by osmolytes. Plant Sci. 2001;160:1171-81.
34. Ashraf M. Organic substances responsible for salt tolerance in *Eruca sativa*. Biol Plant. 1994;36:255–59.
35. Hurkman WJ, Rao HJ, Tanaka CK. Germin-like polypeptides increase in barley roots during salt stress. Plant Physiol. 1991;97:366–74.
36. Alia, Mohanty P, Matysik J. Effect of proline on the production of singlet oxygen. Amino Acids. 2001;21:195-200.
37. Shereen A, Ansari RU, Yamin S, Raza S, Mumtaz S, Khan MA, et al. Physiological responses of rice (*Oryza sativa* L) to saline stress. Pak J Bot. 2007;39:2527-534.
38. Petrusa LM, Winicov I. Proline status in salt tolerant and salt sensitive alfalfa cell lines and plants in response to NaCl. Plant Physiol Biochem. 1997;35:303-10.
39. Sharp RE, Wu Y, Voetberg GS, Saab IN, LeNoble ME. Confirmation that abscisic acid accumulation is required for maize primary root elongation at low water potentials. J Exp Bot. 1994;45:1717-43.
40. Cha-um S, Kirdmanee C. Effect of osmotic stress on proline accumulation, photosynthetic abilities and growth of sugarcane plantlets (*Saccharum officinarum* L.). Pak J Bot. 2008;40:2541-52.

41. Waheed A, Hafiz IA, Qadir G, Murtaza G, Mahmood T, Ashraf M. Effect of salinity on germination, growth, yield, ionic balance and solute composition of pigeon pea (*Cajanus cajan* (L.) Millsp.). Pak J Bot. 2006;38:1103-17.
42. Rahnama H, Ebrahimzadeh H. The effect of NaCl on proline accumulation in potato seedlings and calli. Acta Physiol Plant. 2004;26:263-70.
43. Fougere F, Le Rudulier D, Streeter JG. Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids, and cytosol of alfalfa (*Medicago sativa* L.). Plant Physiol. 1991;96:1228-36.
44. Shahid MA, Pervez MAI, Ashraf MY, Ayyub CM, Ashfaq M, Mattson NS. Characterization of salt tolerant and salt sensitive Pea (*Pisum sativum* L.) genotypes under saline regime. Pak J Life Soc Sci. 2011;9:145-52.
45. Aziz A, Martin-Tanguy J, Larher F. Stress-induced changes in polyamine and tyramine levels can regulate proline accumulation in tomato leaf discs treated with sodium chloride. Physiol Plant. 1998;104:195–202.
46. Parida AK, Das AB, Mohanty P. Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: differential changes of isoforms of some antioxidative enzymes. J Plant Physiol. 2004;161:531–42.
47. De Lacerda CF, Cambraia J, Oliva MA, Ruiz HA, Prisco JT. Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. Environ Exp Bot. 2003;49:107–120.
48. Lutts S, Majerus V, Kinet JM. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. Physiol Plant. 1999;105:450–58.
49. Chutipaijit S, Cha-um S, Sompornpailin K. Differential accumulations of proline and flavonoids in indica rice varieties against salinity. Pak J Bot. 2009;41:2497-506.
50. Khan MA, Shirazi MU, Khan MA, Mujtaba SM, Islam E, Mumtaz S, et al. Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). Pak J Bot. 2009;41:633-38.
51. Ahmad I, Wainwright SJ, Stewart GR. The solute and water relations of *Agrostis stolonifera* ecotypes differing in their salt tolerance. New Phytol. 1981;87:615–29.
52. Jain S, Nainawatee HS, Jain HK, Chowdhury JB. Proline status of genetically stable salt-tolerant *Brassica juncea* L. somaclones and their parent cv. 'Parkash'. Plant Cell Rep. 1991;9:684–87.
53. Kirti PB, Hadi S, Chopra VL. Seed transmission of salt tolerance in regenerants of *Brassica juncea* selected *in vitro*. Cruciferae Newsletter. 1991;85:14–5.
54. Madan S, Nainawatee HS, Jain HK, Chowdhury JB. Proline and proline metabolizing enzymes in *in vitro* selected NaCl-tolerant *Brassica juncea* L. under salt stress. Ann Bot. 1995;76:51–7.
55. Kant S, Kant P, Raveh E, Barak S. Evidence that differential gene expression between the halophyte *Thellungiella halophila*, and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na uptake in *T. halophila*. Plant Cell Environ. 2006;29:1220-34.
56. Kholová J, Sairam RK, Meena RC, Srivastava GC. Response of maize genotypes to salinity stress in relation to osmolytes and metal-ions contents, oxidative stress and antioxidant enzymes activity. Biol Plant. 2009;53:249-56.
57. Tal M, Katz A, Heiken H, Dehan K. Salt tolerance in the wild relatives of the cultivated tomato: proline accumulation in *Lycopersicon esculentum* Mill., *L. peruvianum* Mill. and *Solanum pennellii* Cor. treated with NaCl and polyethylene glycol. New Phytol. 1979;82:349–60.
58. Moftah AB, Michel BB. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiol. 1987;83:283–86.

59. 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. 1989;119:205–10.
60. Lutts S, Kinet JM, Bouharmont J. Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. Plant Growth Regul. 1996;19:207–18.
61. Wyn Jones RG, Storey R. Betaines. In: Paleg LG, Aspinall A, editors. The physiology and biochemistry of drought resistance in plants. Academic Press, Sydney; 1981.
62. Colmer TD, Epstein E, Dvorak J. Differential solute regulation in leaf blades of various ages in salt sensitive wheat and a salt-tolerant wheat x *Lophopyrum elongatum* (Host) A. Love amphiploid. Plant Physiol. 1995;108:1715–24.
63. Grieve CM, Maas EM. Betaine accumulation in salt stressed sorghum. Physiol Plant. 1984;61:167–71.
64. Sakamoto A, Murata N. The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant Cell Environ. 2002;25:163-71.
65. Hanson AD, Grumet R. Betaine accumulation: metabolic pathways and genetics. In: Key JL, Kosuge T, editors. Cellular and Molecular Biology of Plant Stress, A.R. Liss, New York; 1985.
66. Rhodes DP, Rich J, Myers AC, Rueter CC, Jamieson GC. Determination of betaines by fast atom bombardment mass spectrometry: Identification of glycinebetaine deficient genotypes of *Zea mays*. Plant Physiol. 1987;84:781–88.
67. Saneoka H, Nagasaka C, Hahn DT, Yang WJ, Premachandra GS, Joly RJ, et al. Salt tolerance of glycinebetaine-deficient and containing maize lines. Plant Physiol. 1995;107:631–38.
68. Varshney KA, Gangwar LP, Goel N. Choline and betaine accumulation in *Trifolium alexandrinum* L. during salt stress. Egyptian J Bot. 1988;31:81–6.
69. Wyn Jones RG, Gorham J, McDonnell E. Organic and inorganic solute contents as selection criteria for salt tolerance in the Triticeae. In: Staples R, Toennissen GH, editors. Salinity tolerance in plants: strategies for crop improvement, Wiley, New York; 1984.
70. Mansour MMF. Nitrogen containing compounds and adaptation of plants to salinity stress. Biol Plant. 2000;43:491–500.
71. Vernon DM, Bohnert HJ. A novel methyl transferase induced by osmotic stress in the facultative halophyte *Mesembryanthemum crystallinum*. EMBO J. 1992;11:2077–85.
72. Nelson DE, Rammesmayer G, Bohnert HJ. Regulation of cell specific inositol metabolism and transport in plant salinity tolerance. Plant Cell. 1998;10:753–64.
73. Nelson DE, Koukoumanos M, Bohnert HJ. Myo-inositol-dependent sodium uptake in ice plant. Plant Physiol. 1999;119:165–72.
74. Wu W, Zhang Q, Zhu Y, Lam HM, Cai Z, Guo D. Comparative metabolic profiling reveals secondary metabolites correlated with soybean salt tolerance. J Agric Food Chem. 2008;56:11132-38.
75. Katiyer S, Dubey RS. Salinity-induced accumulation of polyamines in germinating rice seeds differing in salt tolerance. Trop Sci. 1990;30:229–40.
76. Krishnamurthy R. Amelioration of salinity effect in salt tolerant rice *(Oryza sativa* L.) by foliar application of putrescine. Plant Cell Physiol. 1991;32:699-703.
77. Krishnamurthy R, Bhagwat KA. Polyamines as modulators of salt tolerance in rice cultivars. Plant Physiol. 1989;91:500–4.
78. Chattopadhyay MK, Gupta S, Sengupta DN, Ghosh B. Expression of arginine decarboxylase in seedlings of indica rice (*Oryza sativa* L.) cultivars as affected by salinity stress. Plant Mol Biol. 1997;34:477–83.

79. Roychoudhury A, Basu S, Sengupta DN. Amelioration of salinity stress by exogenously applied spermidine or spermine in three varieties of indica rice differing in their level of salt tolerance. J Plant Physiol. 2011b;168:317–28.
80. Saleethong P, Sanitchon J, Kong-ngern K, Theerakulpisut P. Pretreatment with spermidine reverses inhibitory effects of salt stress in two rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. Asian J Plant Sci. 2011;10:245-54.
81. Maiale S, Sánchez DH, Guirado A, Vidal A, Ruiz OA. Spermine accumulation under salt stress. J Plant Physiol. 2004;161:35–42.
82. Roychoudhury A, Basu S, Sengupta DN. Comparative expression of two abscisic acid-inducible genes and proteins in seeds of aromatic indica rice cultivar with that of non-aromatic indica rice cultivars. Indian J Exp Biol. 2009a;47:827-33.
83. Li Z-Y, Chen S-Y. Differential accumulation of the S-adenosylmethionine decarboxylase transcript in rice seedlings in response to salt and drought stresses. Theor Appl Genet. 2000;100:782–8.
84. Lefevre I, Gratia E, Lutts S. Discrimination between the ionic and osmotic components of salt stress in relation to free polyamine level in rice (*Oryza sativa* L.). Plant Sci. 2001;161:943–52.
85. Roy P, Niyogi K, Sengupta DN, Ghosh B. Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H^+-ATPase in salt-tolerant and salt-sensitive rice cultivars. Plant Sci. 2005;168:583–91.
86. Duan JJ, Li J, Guo SR, Kang YY. Exogenous spermidine affects polyamine metabolism in salinity-stressed *Cucumis sativus* roots and enhances short-term salinity tolerance. J Plant Physiol. 2008;165:1620-35.
87. Kakkar RR, Rai VR. Polyamines under salt stress. In: Jaiwal PK, Singh RP, Gulati A, editors. Strategies for Improving Salt Tolerance in Higher Plants, Oxford and IBH Publishing Co., New Delhi; 1997.
88. Velarde-Buendía AM, Shabala S, Cvikrova M, Dobrovinskaya O, Pottosin I. Salt-sensitive and salt-tolerant barley varieties differ in the extent of potentiation of the ROS-induced K^+ efflux by polyamines. Plant Physiol Biochem. 2012;61:18-23.
89. Roychoudhury A, Basu S. Ascorbate-glutathione and plant tolerance to various abiotic stresses. In: Anjum NA, Umar S, Ahmad A, editors. Oxidative stress in plants causes, consequences and tolerance, IK International Publishing House Pvt. Ltd.; 2012.
90. Sairam RK, Tyagi A. Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci. 2004;86:407–20.
91. Hernandez JA, Jimenez A, Mullineaux P, Sevilla F. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. Plant Cell Environ. 2000;23:853-62.
92. Sreenivasulu N, Grimm B, Wobus U, Weschke W. Differential response of antioxidant compounds to salinity stress in salt-tolerant and salt-sensitive seedlings of foxtail millet (*Setaria italica*). Physiol Plant. 2000;109:435-42.
93. Mandhania S, Madan S, Sheokand S. Differential response in salt tolerant and sensitive genotypes of wheat in terms of ascorbate, carotenoids proline and plant water relations. Asian J Exp Biol Sci. 2010;1:792-7.
94. Hefny M, Abdel-Kader DZ. Antioxidant-enzyme system as selection criteria for salt tolerance in forage Sorghum genotypes (*Sorghum bicolor* L. Moench). In: Ashraf M, Ozturk M, Athar Habib-ur-Rehman, editors. Salinity and water stress; 2009.
95. Dionisio-Sese ML, Tobita S. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 1998;135:1-9.
96. Chawla S, Jain S, Jain V. Salinity induced oxidative stress and antioxidant system in salt-tolerant and salt-sensitive cultivars of rice (O*ryza sativa* L.). J Plant Biochem Biotechnol. 2013;22:27-34.

97. Gossett DR, Millhollon EP, Lucas MC. Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci. 1994;34:706–14.
98. Gossett DR, Banks SW, Millhollon EP, Lucas MC. Antioxidant response to NaCl stress in a control and a NaCl-tolerant cotton line grown in the presence of paraquat, buthionine sulfoxime, and exogenous glutathione. Plant Physiol. 1996;112:803–9.
99. Garratt LC, Janagoudar BS, Lowe KC, Anthony P, Power JB, Davey MR. Salinity tolerance and antioxidant status in cotton cultures. Free Radic Biol Med. 2002;33:502–11.
100. de Azevedo Neto AD, Prisco JT, Eneas-Filho J, de Abreu CEB, Gomes-Filho E. Effect of salt stress on antioxidative enzymes and lipid peroxidation in leaves and roots of salt-tolerant and salt-sensitive maize genotypes. Environ Exp Bot. 2006;56:87-94.
101. Stevens HC, Calvan M, Lee K, Siegel BZ, Siegel SM. Peroxidase activity as a screening parameter for salt stress in *Brassica* species. Phytochemistry. 1978;17:1521–5.
102. Siegal SM, Chen J, Kottenmeier W, Clark K, Siegel BZ, Chang H. Reduction in peroxidase in *Cucumis*, *Brassica* and other seedlings cultured in saline waters. Phytochemistry. 1982;21:539–42.
103. Jalali-e-Emam SMS, Alizadeh B, Zaefizadeh M, Zakarya RA, Khayatnezhad M. Superoxide dismutase (SOD) activity in NaCl stress in salt-sensitive and salt-tolerance genotypes of Colza (*Brassica napus* L.). Middle-East J Sci Res. 2011;7:7-11.
104. Arbona V, Flors V, Jacas J, Garcia-Agustin P, Gomez-Cadenas A. Enzymatic and non-enzymatic antioxidant responses of Carrizo citrange, a salt sensitive citrus rootstock, to different levels of salinity. Plant Cell Physiol. 2003;44:388–94.
105. Shalata A, Tal M. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. Physiol Plant. 1998;104:169–74.
106. Mittova V, Volokita M, Guy M, Tal M. Activities of SOD and the ascorbate-glutathione cycle enzymes in subcellular compartments in leaves and roots of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. Physiol Plant. 2000;110:42–51.
107. Mittova V, Guy M, Tal M, Volokita M. Response of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-dependent oxidative stress: increased activities of antioxidant enzymes in root plastids. Free Rad Res. 2002;36:195–202.
108. Sekmen AH, Türkan I, Takio S. Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive *Plantago media*. Physiol Plant. 2007;131:399-411.
109. RoyChoudhury A, Roy C, Sengupta DN. Transgenic tobacco plants overexpressing the heterologous *lea* gene *Rab16A* from rice during high salt and water deficit display enhanced tolerance to salinity stress. Plant Cell Rep. 2007;26:1839-59.
110. Hernández JA, Ferrer MA, Jiménez A, Ros-Barceló A, Sevilla F. Antioxidant systems and $O_2^{\cdot-}/H_2O_2$ production in the apoplast of *Pisum sativum* L. leaves: its relation with NaCl-induced necrotic lesions in minor veins. Plant Physiol. 2001;127:817–31.
111. Fadzilla NM, Finch RP, Burdon RH. Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. J Exp Bot. 1997;48:325-31.
112. Vaidyanathan H, Sivakumar P, Chakrabarty R, Thomas G. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) - differential response in salt-tolerant and sensitive varieties. Plant Sci. 2003;165:1411-8.
113. Khan MH, Panda SK. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol Plant. 2008;30:81-9.

114. Heim KE, Tahliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002;13:572-84.
115. Caturla N, Vera-Samper E, Villalain J, Reyes Mateo C, Micol V. The relationship between the antioxidant and antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. Free Rad Biol Med. 2003;34:648-62.
116. Potapovich AI, Kostyuk VA. Comparative study of antioxidant properties and cytoprotective activity of flavonoids. Biochemistry. 2003;68:514-9.
117. Swapna TS. Salt stress induced changes on enzyme activities during different developmental stages of rice (*Oryza sativa* Linn.). Indian J Biotechnol. 2003;2:251-8.
118. Roychoudhury A, Paul A. Abscisic acid-inducible genes during salinity and drought stress. In: Berhardt LV, editor. Advances in Medicine and Biology. Vol 51. Nova Publishers; 2012.
119. Chen S, Li J, Wang T, Wang S, Polle A, Hüttermann A. Osmotic stress and ion-specific effects on xylem abscisic acid and the relevance to salinity tolerance in poplar. J Plant Growth Regul. 2002;21:224-33.
120. Lee TM, Lur HS, Chu C. Role of abscisic acid in chilling tolerance of rice (*Oryza sativa* L.) seedlings: I. Endogenous abscisic acid levels. Plant Cell Environ. 1993;16:481-90.
121. Roychoudhury A, Basu S, Sengupta DN. Effects of exogenous abscisic acid on some physiological responses in a popular aromatic indica rice compared with those from two traditional non-aromatic indica rice cultivars. Acta Physiol Plant. 2009b;31:915–26.
122. Bohra JS, Dörffling H, Dörffling K. Salinity tolerance of rice (*Oryza sativa* L.) with reference to endogenous and exogenous abscisic acid. J Agron Crop Sci. 1995;174:79-86.
123. Saeedipour S. Salinity tolerance of rice lines related to endogenous abscisic acid (ABA) level synthesis under stress. Afr J Plant Sci. 2011;5:628-33.
124. He T, Cramer GR. Abscisic acid concentrations are correlated with leaf area reductions in two salt-stressed rapid-cycling *Brassica* species. Plant Soil. 1996;179:25–33.
125. Zörb C, Geilfus CM, Mühling KH, Ludwig-Müller J. The influence of salt stress on ABA and auxin concentrations in two maize cultivars differing in salt resistance. J Plant Physiol. 2013;170:220-4.
126. Koornneef M, Reuling G, Karssen CM. The isolation and characterization of abscisic acid-insensitive mutants of *Arabidopsis thaliana*. Physiol Plant. 1984;61:377–83.
127. Cramer GR. Is an increase in ABA concentration the cause of growth inhibition in salt-stressed plants? Plant Physiol. 1994;105:S71.
128. Montero E, Cabot C, Poschenrieder C, Barcelo´ J. Relative importance of osmotic-stress and ion-specific effects on ABA-mediated inhibition of leaf expansion growth in *Phaseolus vulgaris*. Plant Cell Environ. 1998;21:54–62.
129. Cramer GR, Quarrie SA. Abscisic acid is correlated with the leaf growth inhibition of four genotypes of maize differing in their response to salinity. Funct Plant Biol. 2002;29:111–5.
130. Etehadnia M, Waterer DR, Tanino KK. The method of ABA application affects salt stress responses in resistant and sensitive potato lines. J Plant Growth Regul. 2008;27:331–41.
131. Grover A, Kapoor A, Satya Lakshmi O, Agarwal S, Sahi C, Katiyar-Agarwal S, et al. Understanding molecular alphabets of the plant abiotic stress responses. Curr Sci. 2001;80:206-16.
132. Taji T, Seki M, Satou M, Sakurai T, Kobayashi M, Ishiyama K, et al. Comparative genomics in salt tolerance between *Arabidopsis* and *Arabidopsis*-related halophyte salt cress using *Arabidopsis* microarray. Plant Physiol. 2004;135:1697–709.

133. Sottosanto JB, Gelli A, Blumwald E. DNA array analyses of *Arabidopsis thaliana* lacking a vacuolar Na/H antiporter: impact of AtNHX1 on gene expression. Plant J. 2004;40:752–71.
134. Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, et al. Gene expression profiles during the initial phase of salt stress in rice. Plant Cell. 2001;13:889–905.
135. Shiozaki N, Yamada M, Yoshiba Y. Analysis of salt-stress inducible ESTs isolated by PCR-subtraction in salt-tolerant rice. Theor Appl Genet. 2005;110:1177–86.
136. Walia H, Wilson C, Condamine P, Liu X, Ismail AM, Zeng L, et al. Comparative transcriptional profiling of two contrasting rice genotypes under salinity stress during the vegetative growth stage. Plant Physiol. 2005;139:822–35.
137. Gulick PJ, Dvorak J. Coordinate gene response to salt stress in *Lophopyrum elongatum*. Plant Physiol. 1992;100:1384-88.
138. Galvez AF, Gulick PJ, Dvorak J. Characterization of the early stages of genetic salt-stress responses in salt-tolerant *Lophopyrum elongatum*, salt-sensitive wheat, and their amphidiploid. Plant Physiol. 1993;103:257-65.
139. Ramagopal S. Differential mRNA transcription during salinity stress in barley. Proc Natl Acad Sci USA. 1987;84:94-8.
140. Hurkman WJ, Fornari CS, Tanaka CK. A comparison of the effect of salt on polypeptides and translatable mRNAs in roots of a salt-tolerant and a salt-sensitive cultivar of barley. Plant Physiol. 1989;90:1444-56.
141. Moons A, De Keyser A, Van Montagu M. A group 3 LEA cDNA of rice, responsive to abscisic acid, but not to jasmonic acid, shows variety-specific differences in salt stress response. Gene. 1997;191:197-204.
142. Mukherjee K, Roy Choudhury A, Gupta B, Gupta S, Sengupta DN. An ABRE-binding factor, OSBZ8, is highly expressed in salt tolerant cultivars than in salt sensitive cultivars of indica rice. BMC Plant Biol. 2006;6:18.
143. Roychoudhury A, Gupta B, Sengupta DN. Trans-acting factor designated OSBZ8 interacts with both typical abscisic acid responsive elements as well as abscisic acid responsive element like sequences in the vegetative tissues of indica rice cultivars. Plant Cell Rep. 2008b;27:779–94.
144. Salekdeh GH, Siopongco J, Wade LJ, Ghareyazie B, Bennett J. Proteomic analysis of rice leaves during drought stress and recovery. Proteomics. 2002a;2:1131-45.
145. Salekdeh GH, Siopongco J, Wade LJ, Ghareyazie B, Bennet J. A proteomic approach to analyzing drought and salt responsive in rice. Field Crops Res. 2002b;76:199-219.
146. Senadheera P, Singh RK, Maathuis FJM. Differentially expressed membrane transporters in rice roots may contribute to cultivar dependent salt tolerance. J Exp Bot. 2009;60:2553–63.
147. Jankangram W, Thammasirirak S, Jones MG, Hartwell J, Theerakulpisut P. Proteomic and transcriptomic analysis reveals evidence for the basis of salt sensitivity in Thai jasmine rice (*Oryza sativa* L. cv. KDML105). Afr J Biotechnol. 2011;10:16157-66.
148. Ouerghi Z, Remy R, Ouelhazi L, Ayadi A, Brulfert J. Two-dimensional electrophoresis of soluble leaf proteins isolated from two wheat species (*Triticum durum* and *Triticum aestivum*) differing in sensitivity towards NaCl. Electrophoresis. 2000;21:2487-91.
149. Majoul T, Chahed K, Zamiti E, Ouelhazi L, Ghrir R. Analysis by two-dimensional electrophoresis of the effect of salt stress on the polypeptide patterns in roots of a salt-tolerant and salt-sensitive cultivar of wheat. Electrophoresis. 2000;21:2562-65.
150. Guo G, Ge P, Ma C, Li X, Lv D, Wang S, et al. Comparative proteomic analysis of salt response proteins in seedling roots of two wheat varieties. J Proteomics. 2012;75:1867-85.

151. Ligaba A, Katsuhara M. Insights into the salt tolerance mechanism in barley (*Hordeum vulgare*) from comparisons of cultivars that differ in salt sensitivity. J Plant Res. 2010;123:105-18.
152. Fatehi F, Hosseinzadeh A, Alizadeh H, Brimavandi T, Struik PC. The proteome response of salt-resistant and salt-sensitive barley genotypes to long-term salinity stress. Mol Biol Rep. 2012;39:6387-97.
153. Jayaraman A, Puranik S, Rai NK, Vidapu S, Sahu PP, Lata C, et al. (2008) cDNA-AFLP analysis reveals differential gene expression in response to salt stress in foxtail millet (*Setaria italica* L.). Mol Biotechnol. 2008;40:241-51.
154. Staal M, Maathuis FJM, Elzenga JTM, Overbeek JHM, Prins HBA. Na^+/H^+ antiport activity in tonoplast vesicles from roots of the salt-tolerant *Plantago maritima* and the salt-sensitive *Plantago media*. Physiol Plant. 1991;82:179-84.
155. Golldack D, Su H, Quigley F, Kamasani UR, Muñoz-Garay C, Balderas E, et al. Characterization of a HKT-type transporter in rice as a general alkali cation transporter. Plant J. 2002;31:529-42.
156. Golldack D, Quigley F, Michalowski CB, Kamasani UR, Bohnert HJ. Salinity stress-tolerant and -sensitive rice (*Oryza sativa* L.) regulate AKT1-type potassium channel transcripts differently. Plant Mol Biol. 2003;51:71-81.
157. Parihar JS, Singh D, Baijal BD. Effect of salt stress on ATPase, Na^+ and K^+ uptake in berseem (*Trifolium alexandrinum* L.). Acta Bot Indica. 1990;18:51–4.
158. Ayala F, Ashraf M, O'Leary W. Plasma membrane H^+-ATPase activity in salt-tolerant and salt-sensitive lines of spring wheat (*Triticum aestivum* L.). Acta Bot Neerl. 1997;46:315–24.
159. Colombo R, Cerana R. Enhanced activity of tonoplast pyrophosphatase in NaCl-grown cells of *Daucus carota*. J Plant Physiol. 1993;142:226–9.
160. Nakamura Y, Kasamo K, Shimosato N, Sakata M, Ohta E. Stimulation of the extrusion of protons and H^+-ATPase activities with the decline in pyrophosphate activity of the tonoplast in intact mung bean roots under high salt stress and its relation to external levels of Ca^{2+} ions. Plant Cell Physiol. 1992;33:139–49.
161. Sun W, Xu X, Zhu H, Liu A, Liu L, Li J, et al. Comparative transcriptomic profiling of a salt-tolerant wild tomato species and a salt-sensitive tomato cultivar. Plant Cell Physiol. 2010;51:997–1006.
162. Chakraborty K, Sairam RK, Bhattacharya RC. Differential expression of salt overly sensitive pathway genes determines salinity stress tolerance in *Brassica* genotypes. Plant Physiol Biochem. 2012;51:90-101.
163. Jyothsna Kumari G, Giridara Kumar S, Thippeswamy M, Annapurnadevi A, Thimma Naik S, Sudhakar C. Effect of salinity on growth and proteomic changes in two cultivars of mulberry (*Morus alba* L.) with contrasting salt tolerance. Indian J Biotechnol. 2007;6:508-18.
164. Singh RK, Gregorio GB, Jain RK. QTL mapping for salinity tolerance in rice. Physiol Mol Biol Plants. 2007;13:87–99.
165. Haq TU, Gorham J, Akhtar J, Akhtar N, Steele KA. Dynamic quantitative trait loci for salt stress components on chromosome 1 of rice. Funct Plant Biol. 2010;37:634–45.
166. Lin HX, Zhu MZ, Yano M, Gao JP, Liang ZW, Su WA, et al. QTLs for Na^+ and K^+ uptake of the shoots and roots controlling rice salt tolerance. Theor Appl Genet. 2004;108:253–60.
167. Ren ZH, Gao JP, Li LG, Cai XL, Huang W, Chao DY, et al. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nature Genet. 2005;37:1141–6.
168. Thomson MJ, de Ocampo M, Egdane J, Rahman MA, Sajise AG, Adorada DL, et al. Characterizing the *Saltol* quantitative trait locus for salinity tolerance in rice. Rice. 2010;3:148-160.

169. Ammar MHM, Singh RK, Singh AK, Mohapatra T, Sharma TR, Singh NK. Mapping QTLs for salinity tolerance at seedling stage in rice (*Oryza sativa* L). African Crop Sci Conference Proceedings. 2007;8:617-20.
170. Breto MP, Asins MJ, Carbonell EA. Salt tolerance in *Lycopersicon* species. 3. Detection of quantitative trait loci by means of molecular markers. Theor Appl Genet. 1994;88:395-401.
171. Cheeseman JM. Mechanism of salt tolerance in plants. Plant Physiol. 1988;87:547–50.
172. Shannon MC. Breeding, selection and the genetics of salt tolerance, In: Staples RC, Toenniessen GH, editors. Salinity tolerance in plants. Strategies for crop improvement, Wiley, New York; 1984.
173. Qadir M, Tubeileh A, Akhtar J, Larbi A, Minhas PS, Khan MA. Productivity enhancement of salt-affected environments through crop diversification. Land Degrad Dev. 2008;19:429–53.
174. Marcar NE, Crawford DF. Trees for saline landscapes. CSIRO Publishing: Melbourne; 2004.
175. Rozema J, Flowers T. Crops for a salinized world. Science. 2008;322:1478–80.
176. Flowers TJ, Colmer TD. Salinity tolerance in halophytes. New Phytol. 2008;179:945–63.
177. Colmer TD, Flowers TJ. Flooding tolerance in halophytes. New Phytol. 2008;179:964–74.
178. Cushman JC, Bohnert HJ. Genomic approaches to plant stress tolerance. Curr Opin Plant Biol. 2000;3:117-24.
179. Flowers TJ. Improving crop salt tolerance. J Exp Bot. 2004;55:307-19.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=239&id=9&aid=1471