

# Salt stress and phyto-biochemical responses of plants – a review

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## ABSTRACT

The ability of plants to tolerate salts is determined by multiple biochemical pathways that facilitate retention and/or acquisition of water, protect chloroplast functions and maintain ion homeostasis. Essential pathways include those that lead to synthesis of osmotically active metabolites, specific proteins and certain free radical enzymes to control ion and water flux and support scavenging of oxygen radicals. No well-defined indicators are available to facilitate the improvement in salinity tolerance of agricultural crops through breeding. If the crop shows distinctive indicators of salt tolerance at the whole plant, tissue or cellular level, selection is the most convenient and practical method. There is therefore a need to determine the underlying biochemical mechanisms of salinity tolerance so as to provide plant breeders with appropriate indicators. In this review, the possibility of using these biochemical characteristics as selection criteria for salt tolerance is discussed.

**Keywords:** salt stress; proline; chlorophyll; sugar; protein, antioxidants

A wide range of environmental stresses (such as high and low temperature, drought, alkalinity, salinity, UV stress and pathogen infection) are potentially harmful to the plants (Van Breusegem et al. 2001). Salt stress in soil or water is one of the major stresses especially in arid and semi-arid regions and can severely limit plant growth and productivity (Allakhverdiev et al. 2000, Koca et al. 2007).

According to the incapacity to grow on high salt medium, plants have been classified as glycophytes or halophytes. Most plants are glycophytes and cannot tolerate salt stress (Sairam and Tyagi 2004). The deleterious effects of salinity on plant growth are associated with: (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress) or (4) a combination of these factors (Ashraf 1994). During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy and lipid metabolisms are affected. The earliest response is a reduction in the rate of leaf surface expansion followed by cessation of expansion as the stress intensifies but growth resumes when the stress is relieved (Parida and Das 2005).

Resistance to environmental stress occurs when a plant withstands the imposed stress that may arise

from either tolerance or a mechanism that permits escape from the situation. Although whole plant mechanism can contribute to the avoidance of stress during the plants life cycle, tolerance can also occur at the cellular level. Plants are either dormant during the salt episode or there must be cellular adjustment (Yokoi et al. 2002) and the response is species and genotypes dependent and depends on the length and severity of the salinity, the age and stage of development, the organ and the cell type and the sub-cellular compartment. An example of avoidance at the cellular level is the process of osmotic adjustment, where the osmotic potential of the cell is lowered in order to favour water uptake and maintenance of turgor (Bray 1997).

Conventional selection and breeding techniques have been used to improve salinity tolerance in crop plants (Ashraf 2002). The agronomical parameters used for salt tolerance are yield, survival, plant height, leaf area, leaf injury, relative growth rate and relative growth reduction (Ashraf and Harris 2004).

But, the conventional breeding techniques have been unsuccessful in transferring salt tolerance to the target species (Sairam and Tyagi 2004). Many investigators are of the view that plant species should possess distinctive indicators of salt tolerance at whole plant, tissue or cellular level

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(Ashraf 2002, Munns 2002). Nonetheless, parallels have been drawn between different biochemical indicators and plant tolerance. For example, there is strong evidence that glycinebetaine (quaternary ammonium compound) and proline (amino acid) play an adaptive role in mediating osmotic adjustment and protecting the subcellular structures in stressed plants. In a number of studies a positive correlation between the accumulation of these two osmolytes and stress tolerance has been recorded (Yamada et al. 2003, Yang et al. 2003). Due to increased environmental stress the balance between the production of reactive oxygen species (ROS) and the quenching activity of the antioxidants is upset, often resulting in oxidative damage (Spychalla and Desborough 1990). ROS can be important mediators of damage to cell structures, nucleic acids, lipids and proteins (Valko et al. 2006). The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (Halliwell and Gutteridge 1999).

While determining the role of various antioxidants in salt tolerance of tomato, Mittova et al. (2002) demonstrated that higher salt tolerance of wild tomato (*Lycopersicon pennellii*) as compared to cultivated tomato (*Lycopersicon esculentum*) could be correlated with increased activities of SOD (superoxide dismutase), APX (ascorbate peroxidase), and POD (guaiacol peroxidase).

This review provides information on biochemical parameters, which are used as stress indicators at the cellular level. The overproduction of osmoprotectants, increasing expression of antioxidant enzymes helps the plant to withstand the environmental stress.

## SYMPTOMS OF Na<sup>+</sup> ACCUMULATION

Salt stress creates both ionic as well as osmotic stress on plants. These stresses can be distinguished at several levels (Tester and Davenport 2003). The root and shoot growth reduces abruptly in salt sensitive plants and this effect does not appear to depend on salt concentration in the growing tissues, it is rather a response to the osmolarity of the external solution (Munns 2002). Na<sup>+</sup>-specific damage is associated with accumulation of Na<sup>+</sup> in leaf tissues and results in necrosis of older leaves. The time scale over which specific damage is manifested depends on the rate of accumulation of Na<sup>+</sup> in leaves, and on the effectiveness of Na<sup>+</sup> compartmentation

within leaf tissues and cells. The Na<sup>+</sup>-specific effects are superimposed on the osmotic effects of NaCl (Tester and Davenport 2003), and importantly, show greater variation within species than osmotic effect (Munns 2002).

Deficiency of other nutrients in the soil is due to the high concentration of Na<sup>+</sup> that interacts with other environmental factors, such as drought, which exacerbate the problem (Silberbush and Ben-Asher 2001). Besides, high Na<sup>+</sup> hampers the uptake of other nutrients by: (1) Na<sup>+</sup> interfering with transporters in the root plasma membrane, such as K<sup>+</sup>-selective ion channels, and (2) reduction of root growth by high Na<sup>+</sup> concentration (Tester and Davenport 2003). Thus the uptake of water, growth limiting nutrients (such as P, Fe or Zn) and the growth of soil microorganisms, such as mycorrhizal fungi can be inhibited.

Leaves are more vulnerable than roots to Na<sup>+</sup> simply because Na<sup>+</sup> and Cl<sup>-</sup> accumulate to higher levels in shoots than in roots (Tester and Davenport 2003). Though Na<sup>+</sup> is transported to shoots through the rapidly moving transpiration stream in the xylem, it can only return to roots via the phloem. There is limited evidence of extensive recirculation of shoot Na<sup>+</sup> to root, suggesting that Na<sup>+</sup> transport is largely unidirectional and results in progressive accumulation of Na<sup>+</sup> as leaves age (Tester and Davenport 2003).

The high levels of Na<sup>+</sup> or Na<sup>+</sup>:K<sup>+</sup> ratio can disrupt various enzymatic processes in the cytoplasm. K<sup>+</sup> activates more than 50 enzymes and is an essential element in protein synthesis as it binds tRNA to the ribosomes (Blaha et al. 2000). The disruption in protein synthesis appears to be an important cause of damage by Na<sup>+</sup> (Tester and Davenport 2003). Several studies suggest that the plasma membrane may be the primary site of salt injury (Mansour 1997). Nonelectrolytes and water permeability get altered markedly upon salt exposure.

Osmotic damage (i.e. osmotically driven removal of water from cells) could occur as a result of build up of high concentrations (possibly several hundred mmol) of Na<sup>+</sup> in the leaf apoplast, since Na<sup>+</sup> enters leaves in the xylem stream and is left behind as water evaporates (Flowers et al. 1991).

## OSMOLYTES AND OSMOPROTECTANTS

During stress conditions plants need to maintain internal water potential below that of soil and maintain turgor and water uptake for growth

(Tester and Davenport 2003). This requires an increase in osmotica, either by uptake of soil solutes or by synthesis of metabolic (compatible) solutes. To accommodate the ionic balance in the vacuoles, cytoplasm accumulates low-molecular-mass compounds, the compatible solutes because they do not interfere with normal biochemical reactions (Zhifang and Loescher 2003); rather, they replace water in biochemical reactions. With accumulation proportional to the change of external osmolarity within species-specific limits, protection of structures and osmotic balance supporting continued water influx (or reduced efflux) are accepted functions of osmolytes (Hasegawa et al. 2000). While some compatible osmolytes are essential elemental ions, such as K<sup>+</sup>, the majority are organic solutes (Yokoi et al. 2002). However, the solutes that accumulate vary with the organism and even between plant species and a major category of organic osmotic solutes consists of simple sugars (mainly fructose and glucose), sugar alcohols (glycerol and methylated inositols) and complex sugars (trehalose, raffinose and fructans) (Bohnert and Jensen 1996). Others include quaternary amino acid derivatives (proline, glycine betaine,  $\beta$ -alanine betaine, proline betaine, tertiary amines 1,4,5,6-tetrahydro-2-methyl-4-carboxyl pyrimidine), and sulfonium compounds (choline osulfate, dimethyl sulfonium propionate) (Yokoi et al. 2002).

## Carbohydrates

For the various organic osmotica, sugars contribute up to 50% of the total osmotic potential in glycophytes subject to saline conditions (Cram 1976). The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity or drought, despite a significant decrease in net CO<sub>2</sub> assimilation rate (Murakeozy et al. 2003). Carbohydrates such as sugars (glucose, fructose, sucrose, fructans) and starch accumulate under salt

stress (Parida et al. 2002), playing a leading role in osmoprotection, osmotic adjustment, carbon storage, and radical scavenging. A decrease in starch content and an increase in both reducing and nonreducing sugars and polyphenol levels have been reported in leaves of *Bruguiera parviflora* (Parida et al. 2002). In leaves of tomato the contents of soluble sugars and total saccharides are increased significantly, but the starch content is not affected (Khavarinejad and Mostofi 1998). Ashraf and Tufail (1995) determined the total soluble sugar content in five sunflower accessions differing in salt tolerance; the salt tolerant lines had generally greater soluble sugars than the salt sensitive ones (Table 1).

Trehalose, a disaccharide, accumulates under various abiotic stresses and protects membranes and proteins in cells exposed to stress that cause water deficit and reduced aggregation of denatured proteins (Singer and Lindquist 1998). According to Yamada et al. (2003) trehalose has a suppressive effect on apoptotic cell death. There is now conclusive evidence to suggest that trehalose is present in trace amounts in vascular plants, including major crops, but the actual role of this osmolyte in metabolism is still unclear.

## Proteins

Proteins that accumulate in plants under saline conditions may provide a storage form of nitrogen that is re-utilized later (Singh et al. 1987) and may play a role in osmotic adjustment. They may be synthesized *de novo* in response to salt stress or may be present constitutively at low concentration (Pareek-Singla and Grover 1997). It has been concluded that a number of proteins induced by salinity are cytoplasmic which can cause alterations in cytoplasmic viscosity of the cells (Hasegawa et al. 2000).

A higher content of soluble proteins has been observed in salt tolerant cultivars of barley, sunflower,

Table 1. Changes in carbohydrate in response to salinity

Species	Response to salinity	References
<i>Cenchrus pennisetiformis</i>	increase in reducing sugars; decrease in sucrose and starch	Ashraf (1997)
<i>Morus alba</i>	increase in soluble sugars at low salinity; decrease at high salinity	Agastian et al. (2000)
<i>Prosopis alba</i>	increase in soluble carbohydrate	Meloni et al. (2004)
<i>Bruguiera parviflora</i>	increase in sugar; decrease in starch	Parida et al. (2002)
<i>Lepidium crassifolium</i>	increase in soluble sugars	Murakeozy et al. (2003)

finger millet, and rice (Ashraf and Harris 2004). Agastian et al. (2000) have reported that soluble protein increases at low salinity and decreases at high salinity in mulberry cultivars. Although Ashraf and Fatima (1995) 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 (Table 2).

In higher plants, osmotic stress induces several proteins in vegetative tissues, which are related to late-embryogenesis-abundant (LEA) proteins. The correlation between LEA protein accumulation in vegetative tissues and stress tolerance indicates its protective role under dehydration stress (Ingram and Bartels 1996). Engineered rice plants over expressing a barley *LEA* gene, *HVA1*, under the control of rice actin 1 promoter showed better stress tolerance than did the wild type (Xu et al. 1996).

### Amino acids and amides

Amino acids (alanine, arginine, glycine, serine, leucine, and valine, together with the imino acid, proline, and the non-protein amino acids, citrulline and ornithine) and amides (such as glutamine and asparagines) have also been reported to accumulate in plants subjected to salt stress (Mansour 2000). Total free amino acids in the leaves have been reported to be higher in salt tolerant than in salt sensitive lines of sunflower (Ashraf and Tufail 1995), safflower (Ashraf and Fatima 1995), *Eruca sativa* (Ashraf 1994) and *Lens culinaris* (Hurkman et al. 1991).

Proline, which occurs widely in higher plants and accumulates in larger amounts than other amino acids (Abraham et al. 2003), regulates the accumulation of useable N. Proline accumulation

normally occurs in the cytosol where it contributes substantially to the cytoplasmic osmotic adjustment (Ketchum et al. 1991). It is osmotically very active and contributes to membrane stability and mitigates the effect of NaCl on cell membrane disruption (Mansour 1998). Even at supra-optimal levels, proline does not suppress enzyme activity. Maggio et al. (2002) are of the view that proline may act as a signaling/regulatory molecule able to activate multiple responses that are component of the adaptation process. Petrusa and Winicov (1997) demonstrated that salt tolerant alfalfa plants rapidly doubled their proline content in roots, whereas in salt sensitive plants the increase was slow. These results were corroborated by Fougere et al. (1991). However, Aziz et al. (1998) and Parida et al. (2004) report a negative correlation between proline accumulation and salt tolerance in tomato and *Aegiceras corniculatum* respectively.

There are two alternative routes in proline biosynthesis in higher plants: the L-ornithine and the L-glutamate pathways. It is also known that, as in plants, both ornithine and glutamate are precursors of proline biosynthesis in microorganisms and mammals (Figure 1). Delauney et al. (1993) showed that two enzymes: pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR), play major roles in proline biosynthetic pathway (Figure 1). Transgenic tobacco plants over-expressing P5CS have shown increased concentration of proline and resistance to both drought and salinity stresses (Kishor et al. 1995). However, whether proline accumulation in these transgenic plants resulted in increased stress tolerance through osmotic adjustment or other mechanisms is unknown (Sharp et al. 1996).

Transgenic approach to improve plant stress tolerance has appreciable results. Overproduction of proline by genetically manipulated tobacco plant showed tolerance to NaCl (Hong et al. 2000).

Table 2. Changes in soluble protein in response to salinity

Species	Response to salinity	References
<i>Oryza sativa</i>	decrease	Alamgir and Ali (1999)
<i>Vicia faba</i>	decrease	Gadallah (1999)
<i>Amaranthus tricolor</i>	decrease	Wang and Nil (2000)
<i>Bruguiera parviflora</i>	decrease	Parida et al. (2002)
<i>Pancreatium maritimum</i>	increases at low salinity; decrease at high salinity	Khedr et al. (2003)
<i>Arabidopsis thaliana</i>	increase	Quintero et al. (1996)
<i>Fragaria × ananassa</i> cv. <i>Camarosa</i>	increase	El-Baz et al. (2003)

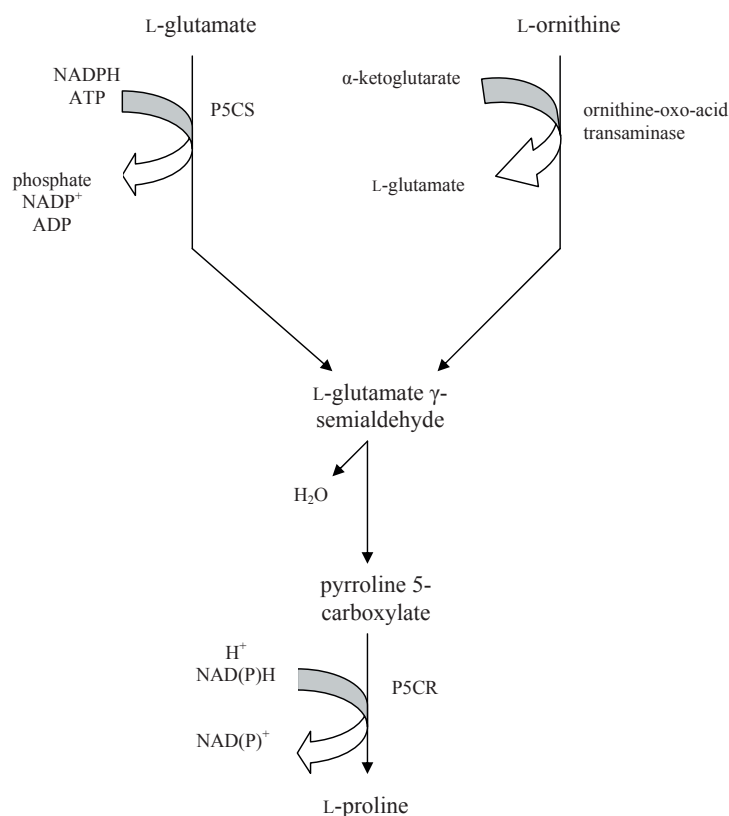


Figure 1. Biosynthetic pathway of proline

Nanjo et al. (2003) demonstrated that introduction of antisense proline dehydrogenase cDNA in *Arabidopsis* overexpresses proline and showed tolerance to freezing temperatures ( $-7^{\circ}\text{C}$ ) as well as salinity (600 mmol NaCl). The *RD29A* promoter-driven *DREB1A* transgenic plants exhibited tolerance to water deficit, salt stress, and freezing temperatures (Kasuga et al. 1999).

However, accumulation of proline in rice plants grown under salt stress was due to salt injury and not as an indication of salt tolerance (Lutts et al. 1999). Similarly, accumulation of proline in two sorghum genotypes contrasting in salt tolerance suggested that proline accumulation was a reaction to salt stress and not a plant response associated with tolerance (De Lacerda et al. 2003). In another experiment, Lutts et al. (1999) showed that under salt stress, higher concentration of proline was accumulated in sensitive rice cultivars than in tolerant genotypes. Currently, there is more evidence supporting the presence of a positive relationship.

Although amides generally accumulate in salt-stressed plants to a lesser extent than do other nitrogen containing compounds (Mansour et al. 2002), concentration of asparagine frequently increases in response to stress (Fougere et al. 1991).

In fact, asparagine accumulated to a greater extent than proline in *Agrostis stolonifera* (Dubey 1997). In barley, NaCl stress enhanced the asparagine pool in roots, xylem sap, and leaf blades of seedlings, whereas glutamine increased only in roots and leaf blades (Yamaya and Matsumoto 1989).

### Quaternary ammonium compounds (QAC)

In several plant species, a positive correlation between leaf osmotic potential and glycinebetaine,  $\beta$ -alaninebetaine and prolinebetaine has been observed. These organic compounds are now known to have osmoprotective effects in the cell as well. Of the QACs in plants subjected to salt stress, glycinebetaine (GB) occurs most abundantly (Mansour 2000). This organic compound is mainly localized in chloroplasts and plays a vital role in chloroplast adjustment and protection of thylakoid membranes, thereby maintaining photosynthetic efficiency and plasmamembrane integrity (Yokoi et al. 2002).

In higher plants, GB is synthesized in chloroplast from serine via ethanolamine, choline, and betaine aldehyde (Rhodes and Hanson 1993). Choline is converted to betaine aldehyde, by choline mo-

noxygenase (CMO), which is then converted to GB by betaine aldehyde dehydrogenase (BADH) (Figure 2). Although other pathways such as direct *N*-methylation of glycine is also known, the pathway from choline to glycine betaine has been identified in all GB-accumulating plant species (Weretilnyk et al. 1989).

GB accumulates in response to stress in many crops, including spinach, barley, tomato, potato, rice, carrot and sorghum (Yang et al. 2003). According to Murata et al. (1992) GB protects the photosystem II (PS II) complex by stabilizing the association of the extrinsic PS II complex proteins under salt stress. The concentrations of GB in species that use it as a compatible osmoticum are variable, for example, in sorghum it is as much as ten-fold than those in maize. GB-deficient genotypes of both species have also been identified. Accumulation of glycinebetaine under saline conditions is also reported to be high in some salt tolerant plants of mulberry but not in sensitive ones (Agastian et al. 2000). Also, glycinebetaine-containing lines of maize exhibited less shoot growth inhibition under saline conditions than deficient lines (Saneoka et al. 1995). The high salt tolerance of a wheat  $\times$  *Lophopyrum elongatum* amphiploid was positively associated with accumulation of GB in young leaves (Colmer et al. 1995). GB content increases during salt stress in a number of plants (Wang and Nil 2000); though it may increase in shoots and may not differ significantly in roots, e.g. *Haloxylon recurvum*.

In many crop plants the natural accumulation of GB is lower than sufficient to ameliorate the adverse effects of dehydration caused by various environmental stresses (Subbarao et al. 2001). Exogenous application of GB to low-accumulating or non-accumulating plants may help reduce adverse effects of environmental stresses (Makela et al. 1998, Yang and Lu 2005). For example, foliar application of GB resulted in a significant improvement in salt tolerance of rice plants (Lutts 2000) and exogenous application of GB on tomato plants subjected to either salt stress or high tem-

peratures resulted in about 40% increase in fruit yield compared with untreated plants (Makela et al. 1998). In a comprehensive study, ameliorating effects of exogenous application of GB on salt-induced shoot- and root growth inhibition and ultra-structural damages were studied in rice seedlings (Rahman et al. 2002), however, there are a few reports suggesting a lack of such positive effects or even apparent negative effects of exogenous GB on plants growing under stress conditions e.g. foliar application of GB did not affect yield components, physiological processes, or endogenous levels of GB in cotton plants grown under drought stress (Meek et al. 2003).

### Polyols

Polyols, the polyhydric alcohols, are among the compatible solutes involved in osmoregulation and are thought to play a role in plant salt tolerance (Bohnert and Shen 1999). They exist in both acyclic and cyclic forms and are widely distributed in the plant kingdom. The most common polyols in plants include acyclic forms, mannitol, glycerol, sorbitol, and cyclic (cyclitols) forms ononitol and pinitol. In general, they are thought to accumulate in the cytoplasm of some halophytes to overcome the osmotic disturbances caused by high concentrations of inorganic ions compartmentalized in vacuoles. Polyols make up a considerable percentage of all assimilated CO<sub>2</sub> as scavengers of stress-induced oxygen radicals (Bohnert et al. 1995). Mannitol, a sugar alcohol that may serve as a compatible solute to cope with salt stress, is synthesized via the action of a mannose-6-phosphate reductase (M6PR) in celery (Zhifang and Loescher 2003) and its accumulation increases when plants are exposed to low water potential. The accumulation is regulated by inhibition of competing pathways and decreased mannitol consumption and catabolism (Stoop et al. 1996). Studies using transgenic tobacco and *Arabidopsis* have shown improved growth of man-

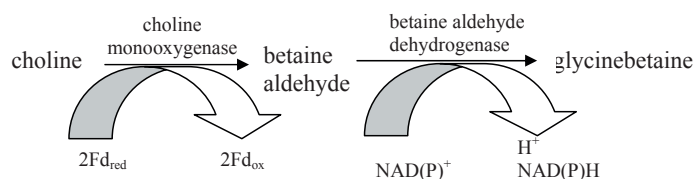


Figure 2. Biosynthetic pathway of glycinebetaine

nitrol accumulating plants under stress (Thomas et al. 1995). Mannitol improves tolerance to stress through scavenging of hydroxyl radicals ( $\text{OH}^\cdot$ ) and stabilization of macromolecular structures. In tobacco, mannitol protects the thiol-regulated enzymes phosphoribulokinase, thioredoxin, ferredoxin and glutathione from  $\text{OH}^\cdot$  (Shen et al. 1997). Abebe et al. (2003), however, state that the amount of mannitol accumulated in response to stress was small and its effect on osmotic adjustment was less than that of other carbohydrates. In contrast to previous approaches that used a bacterial gene to engineer mannitol biosynthesis in plants and other organisms, *A. thaliana*, a non-mannitol-producer, was transformed with the celery leaf *M6PR* gene under control of the *CaMV 35S* promoter. In all independent *Arabidopsis* M6PR transformants, mannitol accumulated in plants in amounts ranging from 0.5 to 6  $\mu\text{mol/g}$  fresh weight. A novel compound, not found in either celery or *Arabidopsis*, 1-*O*- $\beta$ -D-glucopyranosyl-D-mannitol, also accumulates in vegetative tissues of mature plants in amounts up to 4  $\mu\text{mol/g}$  fresh weight but not in flowers and seeds. In the absence of NaCl, all transformants are phenotypically the same as the wild type; however, in the presence of NaCl, mature transgenic plants show a high level of salt tolerance and complete normal development including production of seeds on soil irrigated with 300 mmol NaCl. These results demonstrate a major role of *M6PR* in developing salt-tolerant plants by means of introducing mannitol biosynthesis (Zhifang and Loescher 2003).

## ANTIOXIDANTS

Defence mechanisms against free radical-induced oxidative stress involve: (i) preventative mechanisms, (ii) repair mechanisms, (iii) physical defences, and (iv) antioxidant defences. The plants defend against these reactive oxygen species by induction of activities of certain antioxidative enzymes such as catalase, peroxidase, glutathione reductase, and superoxide dismutase, which scavenge reactive oxygen species (Mittova et al. 2003).

Activities of antioxidative enzymes such as ascorbate peroxidase, glutathione reductase, monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and Mn-SOD increase under salt stress in wheat, while Cu/Zn-SOD remains constant and total ascorbate and glutathione content decrease (Hernandez et al. 2000). In soybean root nodules ascorbate peroxidase, catalase, and

glutathione reductase activities decrease under salt stress, while superoxide dismutase and reduced glutathione increase and malondialdehyde and total protein remain unchanged (Comba et al. 1998). Overexpression of genes leading to increased amounts and activities of mitochondrial Mn-SOD, Fe-SOD, chloroplastic Cu/Zn-SOD, bacterial catalase, and glutathione-S-transferase (GST)/glutathione peroxidase (GPX) can increase the performance of plants under stress (Roxas et al. 2000). Gossett et al. (1994) reported that in cotton (*Gossypium hirsutum* L.) NaCl stress increases the activities of SOD, guaiacol peroxidase, and glutathione reductase and decreases the activities of catalase and ascorbate peroxidase. Salt stress also causes a decrease in total ascorbate, total glutathione, and  $\alpha$ -tocopherol levels in this case. In leaves of rice plant, salt stress preferentially enhances the content of  $\text{H}_2\text{O}_2$  and the activities of SOD, APX, and GPX, whereas it decreases catalase activity (Lee et al. 2001). On the other hand, salt stress has a little effect on the activity levels of glutathione reductase (Lee et al. 2001). Lechno et al. (1997) reported that NaCl treatment increases the activities of the antioxidative enzymes catalase and glutathione reductase and the content of the antioxidants ascorbic acid and reduced glutathione but does not affect the activity of SOD in cucumber plants. In wheat, activities of APX, MDHAR, DHAR, and GR increase in the shoots and decrease in the roots (Meneguzzo and Navarilzzo 1999). The tomato under high salt concentration showed higher antioxidant enzyme activities such as SOD, catalase, ascorbate peroxidase, glutathione reductase, and GST (Rodriguez-Rosales et al. 1999). At higher concentration of NaCl Hernandez et al. (1999) showed that activity of cytosolic CuZn-SOD II, chloroplastic CuZn-SOD II, and mitochondrial and/or peroxisomal Mn-SOD increases with increasing concentration of NaCl (110–130 mmol) in pea.

The non-enzymatic antioxidants viz. vitamin C, vitamin E, carotenoids, lipoic acid and others in the protection against oxidative stress were also reported (Kojo 2004).

## FUTURE PERSPECTIVE

Salt stress causes huge losses of agriculture productivity worldwide. Therefore, plant biologists aimed at overcoming severe environmental stresses needs to be quickly and fully implemented. Together with conventional plant physiology, ge-

netics and biochemical approaches to studying plant responses to abiotic stresses have begun to bear fruit recently. Relevant information on biochemical indicators at the cellular level may serve as selection criteria for tolerance of salts in agricultural crops.

Although there were many transgenic plants with high stress tolerance generated, plant abiotic stress tolerance is a complex trait that involves multiple physiological and biochemical mechanisms and numerous genes. Transgenic plants with commercial value should at the same time retain relatively high productivity and other traits important for agriculture. Moreover, genetic modification should be combined with marker-assisted breeding programs with stress-related genes and QTLs, and ultimately, the different strategies should be integrated, and genes representing distinctive approaches should be combined to substantially increase plant stress tolerance.

## REFERENCES

- Abebe T., Guenzi A.C., Martin B., Cushman J.C. (2003): Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. *Plant Physiol.*, **131**: 1748–1755.
- Abraham E., Rigo G., Szekely G., Nagy R., Koncz C., Szabados L. (2003): Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in *Arabidopsis*. *Plant Mol. Biol.*, **51**: 363–372.
- Agastian P., Kingsley S.J., Vivekanandan M. (2000): Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. *Photosynthetica*, **38**: 287–290.
- Alamgir A.N.M., Ali M.Y. (1999): Effect of salinity on leaf pigments, sugar and protein concentrations and chloroplast ATPase activity of rice (*Oryza sativa* L.). *Bangladesh J. Bot.*, **28**: 145–149.
- Allakhverdiev S.I., Sakamoto A., Nishiyama Y., Inaba M., Murata N. (2000): Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant Physiol.*, **123**: 1047–1056.
- Ashraf M. (1994): Organic substances responsible for salt tolerance in *Eruca sativa*. *Biol. Plant.*, **36**: 255–259.
- Ashraf M. (1997): Changes in soluble carbohydrates and soluble proteins in three arid-zone grass species under salt stress. *Trop. Agric.*, **74**: 234–237.
- Ashraf M. (2002): Salt tolerance of cotton: Some new advances. *Crit. Rev. Plant Sci.*, **21**: 1–30.
- Ashraf M., Fatima H. (1995): Responses of some salt tolerant and salt sensitive lines of safflower (*Carthamus tinctorius* L.). *Acta Physiol. Plant.*, **17**: 61–71.
- Ashraf M., Harris P.J.C. (2004): Potential biochemical indicators of salinity tolerance in plants. *Plant Sci.*, **166**: 3–16.
- Ashraf M., Tufail M. (1995): Variation in salinity tolerance in sunflower (*Helianthus annuus* L.). *J. Agron. Crop Sci.*, **174**: 351–362.
- Aziz A., Martin-Tanguy J., Larher F. (1998): Stress-induced changes in polyamine and tyramine levels can regulate proline accumulation in tomato leaf discs treated with sodium chloride. *Physiol. Plant.*, **104**: 195–202.
- Blaha G., Stelzl U., Spahn C.M.T., Agrawal R.K., Frank J., Nierhaus K.H. (2000): Preparation of functional ribosomal complexes and effect of buffer conditions on tRNA positions observed by cryoelectron microscopy. *Methods Enzymol.*, **317**: 292–309.
- Bohnert H.J., Jensen R.G. (1996): Strategies for engineering water-stress tolerance in plants. *Trends Biotechnol.*, **14**: 89–97.
- Bohnert H.J., Nelson D.E., Jensen R.G. (1995): Adaptations to environmental stresses. *Plant Cell*, **7**: 1099–1111.
- Bohnert H.J., Shen B. (1999): Transformation and compatible solutes. *Sci. Hortic.*, **78**: 237–260.
- Bray E.A. (1997): Plant responses to water deficit. *Trends Plant Sci.*, **2**: 48–54.
- Colmer T.D., Epstein E., Dvorak J. (1995): Differential solute regulation in leaf blades of various ages in salt sensitive wheat and a salt-tolerant wheat × *Lophopyrum elongatum* (Host.) A. Love amphiploid. *Plant Physiol.*, **108**: 1715–1724.
- Comba M.E., Benavides M.P., Tomaro M.L. (1998): Effect of salt stress on antioxidant defence system in soybean root nodules. *Aust. J. Plant Physiol.*, **25**: 665–671.
- Cram W.J. (1976): Negative feedback regulation of transport in cells. The maintenance of turgor, volume and nutrient supply. In: Lüttge U., Pitman M.G. (eds.): *Encyclopaedia of Plant Physiology, New Series*, Vol. 2. Springer-Verlag, Berlin.
- De Lacerda C.F., Cambráia J., Oliva M.A., Ruiz H.A., Prisco J.T. (2003): Solute accumulation and distribution during shoot and leaf development in two sorghum genotypes under salt stress. *Environ. Exp. Bot.*, **49**: 107–120.
- Delauney A.J., Hu C.A., Kishor P.B., Verma D.P. (1993): Cloning of ornithine delta-aminotransferase cDNA from *Vigna aconitifolia* by trans-complementation in *Escherichia coli* and regulation of proline biosynthesis. *J. Biol. Chem.*, **268**: 18673–18678.

- Dubey R.S. (1997): Photosynthesis in plants under stressful conditions. In: Pessarakli M. (ed.): *Handbook of Photosynthesis*. Marcel Dekker, New York: 859–875.
- El-Baz F.K., Mohamed A.A., Aly A.A. (2003): Development of biochemical markers for salt stress tolerance in cucumber plants. *Pak. J. Biol. Sci.*, 6: 16–22.
- Flowers T.J., Hajibagheri M.A., Yeo A.R. (1991): Ion accumulation in the cell walls of rice plants growing under saline conditions: evidence for Oertli hypothesis. *Plant Cell Environ.*, 14: 319–325.
- Fougere F., Le Rudulier D., Streeter J.G. (1991): Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids, and cytosol of alfalfa (*Medicago sativa* L.). *Plant Physiol.*, 96: 1228–1236.
- Gadallah M.A.A. (1999): Effects of proline and glycine-betaine on *Vicia faba* responses to salt stress. *Biol. Plant.*, 42: 249–257.
- Gossett D.R., Millhollon E.P., Lucas M.C. (1994): Antioxidant response to NaCl stress in salt tolerant and salt sensitive cultivars of cotton. *Crop Sci.*, 34: 706–714.
- Halliwell B., Gutteridge J.M.C. (1999): *Free Radicals in Biology and Medicine*. Oxford University Press, Oxford.
- Hasegawa P.M., Bressan R.A., Zhu J.K., Bohnert H.J. (2000): Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 51: 463–499.
- Hernandez J.A., Campillo A., Jimenez A., Alacon J.J., Sevilla F. (1999): Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. *New Phytol.*, 141: 241–251.
- Hernandez J., Jimenez A., Mullineaux P., Sevilla F. (2000): Tolerance of pea plants (*Pisum sativum*) to long-term salt stress is associated with induction of antioxidant defences. *Plant Cell Environ.*, 23: 853–862.
- Hong Z., Lakkineni K., Zhang Z., Verma D.P.S. (2000): Removal of feedback inhibition of 1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiol.*, 122: 1129–1136.
- Hurkman W.J., Rao H.P., Tanaka C.K. (1991): Germin-like polypeptides increase in barley roots during salt stress. *Plant Physiol.*, 97: 366–374.
- Ingram J., Bartels D. (1996): The molecular basis of dehydration tolerance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 47: 377–403.
- Kasuga M., Liu W., Miura S., Yamaguchi-Shinozaki K., Shinozaki K. (1999): Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.*, 17: 287–291.
- Ketchum R.E.B., Warren R.C., Klima L.J., Lopez-Gutierrez F., Nabors M.W. (1991): The mechanism and regulation of proline accumulation in suspension cultures of the halophytic grass *Distichlis spicata* L. *J. Plant Physiol.*, 137: 368–374.
- Khavarinejad R.A., Mostofi Y. (1998): Effects of NaCl on photosynthetic pigments, saccharides, and chloroplast ultrastructure in leaves of tomato cultivars. *Photosynthetica*, 35: 151–154.
- Khedr A.H.A., Abbas M.A., Wahid A.A.A., Quick W.P., Abogadallah G.M. (2003): Proline induces the expression of salt stress responsive proteins and may improve the adaptation of *Pancreaticum maritimum* L. to salt stress. *J. Exp. Bot.*, 54: 2553–2562.
- Kishor P.B.K., Hong Z., Miao G.H., Hu C.A.A., Verma D.P.S. (1995): Overexpression of [delta]-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol.*, 108: 1387–1394.
- Koca M., Bor M., Ozdemir F., Turkan I. (2007): The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ. Exp. Bot.*, 60: 344–351.
- Kojo S. (2004): Vitamin C: Basic metabolism and its function as an index of oxidative stress. *Curr. Med. Chem.*, 11: 1041–1064.
- Lechno S., Zamski E., Telor E. (1997): Salt stress-induced responses in cucumber plants. *J. Plant Physiol.*, 150: 206–211.
- Lee D.H., Kim Y.S., Lee C.B. (2001): The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa*). *J. Plant Physiol.*, 158: 737–745.
- Lutts S. (2000): Exogenous glycine betaine reduces sodium accumulation in salt-stressed rice plants. *Int. Rice Res. Notes*, 25: 39–40.
- Lutts S., Majerus V., Kinet J.M. (1999): NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. *Physiol. Plant.*, 105: 450–458.
- Maggio A., Miyazaki S., Veronese P., Fujita T., Ibeas J.I., Damsz B., Narasimhan M.L., Hasegawa P.M., Joly R.J., Bressan R.A. (2002): Does proline accumulation play an active role in stress-induced growth reduction. *Plant J.*, 31: 699–712.
- Makela P., Jokinen K., Kontturi M., Peltonen-Sainio P., Pehu E., Somersalo S. (1998): Foliar application of glycine betaine – a novel product from sugar beet, as an approach to increase tomato yield. *Ind. Crops Prod.*, 7: 139–148.
- Mansour M.M.F. (1997): Cell permeability under salt stress. In: Jaiwal P.K., Singh R.P., Gulati A. (eds.):

- Strategies for Improving Salt Tolerance in Higher Plants. Oxford and IBH Publishing Co., New Delhi.
- Mansour M.M.F. (1998): Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. *Plant Physiol. Biochem.*, **36**: 767–772.
- Mansour M.M.F. (2000): Nitrogen containing compounds and adaptation of plants to salinity stress. *Biol. Plant.*, **43**: 491–500.
- Mansour M.M.F., Al-Mutawa M.M., Salama K.H.A., Abou Hadid A.F. (2002): Salt acclimation of wheat salt sensitive cultivar by polyamines. In: Ahmad R., Malik K.A. (eds.): *Prospect for Saline Agriculture*. Kluwer Academic Publishers, Dordrecht.
- Meek C., Oosterhuis D., Gorham J. (2003): Does foliar-applied glycine betaine affect endogenous betaine levels and yield in cotton? *Crop Manage.* Available online: doi 10.1094/CM-2003-0804-02-RS
- Meloni D.A., Gulotta M.R., Martínez C.A., Oliva M.A. (2004): The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba*. *Braz. J. Plant Physiol.*, **16**: 39–46.
- Meneguzzo S., Navarilzzo I. (1999): Antioxidative responses of shoots and roots of wheat to increasing NaCl concentrations. *J. Plant Physiol.*, **155**: 274–280.
- Mittova V., Tal M., Volokita M., Guy M. (2002): Salt stress induces up-regulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. *Physiol. Plant.*, **115**: 393–400.
- Mittova V., Tal M., Volokita M., Guy M. (2003): Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. *Plant Cell Environ.*, **26**: 845–856.
- Munns R. (2002): Comparative physiology of salt and water stress. *Plant Cell Environ.*, **25**: 239–250.
- Murakeozy E.P., Nagy Z., Duhaze C., Bouchereau A., Tuba Z. (2003): Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. *J. Plant Physiol.*, **160**: 395–401.
- Murata N., Mohanty P.S., Hayashi H., Papageorgiou G.C. (1992): Glycinebetaine stabilizes the association of extrinsic proteins with the photosynthetic oxygen-evolving complex. *FEBS Lett.*, **296**: 187–189.
- Nanjo T., Fujita M., Seki M., Kato T., Tabata S., Shinzaki K. (2003): Toxicity of free proline revealed in an *Arabidopsis* T-DNA-tagged mutant deficient in proline dehydrogenase. *Plant Cell Physiol.*, **44**: 541–548.
- Pareek-Singla S.L., Grover A. (1997): Salt responsive proteins/genes in crop plants. In: Jaiwal P.K., Singh R.P., Gulati A. (eds.): *Strategies for Improving Salt Tolerance in Higher Plants*. Oxford and IBH Publishing Co., New Delhi.
- Parida A.K., Das A.B. (2005): Salt tolerance and salinity effect on plants: a review. *Ecotoxicol. Environ. Saf.*, **60**: 324–349.
- Parida A.K., Das A.B., Das P. (2002): NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. *J. Plant Biol.*, **45**: 28–36.
- Parida A.K., Das A.B., Mohanty P. (2004): Defense potentials to NaCl in a mangrove, *Bruguiera parviflora*: differential changes of isoforms of some antioxidative enzymes. *J. Plant Physiol.*, **161**: 531–542.
- Petrusa L.M., Winicov I. (1997): Proline status in salt tolerant and salt sensitive alfalfa cell lines and plants in response to NaCl. *Plant Physiol. Biochem.*, **35**: 303–310.
- Quintero F.J., Garcíadeblas B., Rodríguez-Navarro A. (1996): The *SAL1* gene of *Arabidopsis*, encoding an enzyme with 3'(2'),5'-bisphosphate nucleotidase and inositol polyphosphate 1-phosphatase activities, increases salt tolerance in yeast. *Plant Cell*, **8**: 529–537.
- Rahman M.S., Miyake H., Takeoka Y. (2002): Effects of exogenous glycine betaine on growth and ultrastructure of salt-stressed rice seedlings (*Oryza sativa* L.). *Plant Prod. Sci.*, **5**: 33–44.
- Rhodes D., Hanson A.D. (1993): Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, **44**: 357–384.
- Rodríguez-Rosales M.P., Kerkeb L., Bueno P., Donaire J.P. (1999): Changes induced by NaCl in lipid content and composition, lipoxygenase, plasma membrane H<sup>+</sup>ATPase and antioxidant enzyme activities of tomato (*Lycopersicon esculantum* Mill.) calli. *Plant Sci.*, **143**: 143–150.
- Roxas V.P., Lodhi S.A., Garrett D.K., Mahan J.R., Allen R.D. (2000): Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase. *Plant Cell Physiol.*, **41**: 1229–1234.
- Sairam R.K., Tyagi A. (2004): Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.*, **86**: 407–421.
- Saneoka H., Nagasaka C., Hahn D.T., Yang W.J., Premachandra G.S., Joly R.J., Rhodes D. (1995): Salt tolerance of glycinebetaine-deficient and containing maize lines. *Plant Physiol.*, **107**: 631–638.
- Sharp R.E., Boyer J.S., Nguyen H.T., Hsiao T.C. (1996): Genetically engineered plants resistant to soil drying and salt stress: how to interpret osmotic relations. *Plant Physiol.*, **110**: 1051–1053.

- Shen B., Jensen R.G., Bohnert H.J. (1997): Mannitol protects against oxidation by hydroxyl radicals. *Plant Physiol.*, **115**: 527–532.
- Silberbush M., Ben-Asher J. (2001): Simulation study of nutrient uptake by plants from soilless cultures as affected by salinity buildup and transpiration. *Plant Soil*, **233**: 59–69.
- Singer M.A., Lindquist S. (1998): Multiple effects of trehalose on protein folding *in vitro* and *in vivo*. *Mol. Cell*, **1**: 639–648.
- Singh N.K., Bracken C.A., Hasegawa P.M., Handa A.K., Buckel S., Hermodson M.A., Pfankoch F., Regnier F.E., Bressan R.A. (1987): Characterization of osmotin. A thaumatin-like protein associated with osmotic adjustment in plant cells. *Plant Physiol.*, **85**: 529–536.
- Spychalla J.P., Desborough S.L. (1990): Superoxide dismutase, catalase, and alpha-tocopherol content of stored potato tubers. *Plant Physiol.*, **94**: 1214–1218.
- Stoop J.M.H., Williamson J.D., Pharr D.M. (1996): Mannitol metabolism in plants: a method for coping with stress. *Trends Plant Sci.*, **1**: 139–144.
- Subbarao G.V., Wheeler R.M., Levine L.H., Stutte G.W. (2001): Glycine betaine accumulation, ionic and water relations of red-beet at contrasting levels of sodium supply. *J. Plant Physiol.*, **158**: 767–776.
- Tester M., Davenport R. (2003): Na<sup>+</sup> tolerance and Na<sup>+</sup> transport in higher plants. *Ann. Bot.*, **91**: 503–527.
- Thomas J.C., Sepahi M., Arendall B., Bohnert H.J. (1995): Enhancement of seed germination in high salinity by engineering mannitol expression in *Arabidopsis thaliana*. *Plant Cell Environ.*, **18**: 801–806.
- Valko M., Rhodes C.J., Moncol J., Izakovic M., Mazur M. (2006): Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact.*, **160**: 1–40.
- Van Breusegem F., Vranova E., Dat J.F., Inze D. (2001): The role of active oxygen species in plant signal transduction. *Plant Sci.*, **161**: 405–414.
- Wang Y., Nil N. (2000): Changes in chlorophyll, ribulose biphosphate carboxylase-oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. *J. Hortic. Sci. Biotechnol.*, **75**: 623–627.
- Weretilnyk E.A., Bednarek S., McCue K.F., Rhodes D., Hanson A.D. (1989): Comparative biochemical and immunological studies of the glycine betaine synthesis pathway in diverse families of dicotyledons. *Planta*, **178**: 342–352.
- Xu D., Duan X., Wang B., Hong B., Ho T.D., Wu R. (1996): Expression of late embryogenesis abundant protein gene, *HVA1*, from barley confers tolerance to water deficit and salt stress in transgenic rice. *Plant Physiol.*, **110**: 249–257.
- Yamada T., Takatsu Y., Manabe T., Kasumi M., Marubashi W. (2003): Suppressive effect of trehalose on apoptotic cell death leading to petal senescence in ethylene-insensitive flowers of gladiolus. *Plant Sci.*, **164**: 213–221.
- Yamaya T., Matsumoto H. (1989): Accumulation of asparagines in NaCl-stressed barley seedlings. *Ber. Ohara Inst. Landwirtsch. Biol.*, **19**: 181–188.
- Yang X., Lu C. (2005): Photosynthesis is improved by exogenous glycinebetaine in salt-stressed maize plants. *Physiol. Plant.*, **124**: 343–352.
- Yang W.J., Rich P.J., Axtell J.D., Wood K.V., Bonham C.C., Ejeta G., Mickelbart M.V., Rhodes D. (2003): Genotypic variation for glycinebetaine in sorghum. *Crop Sci.*, **43**: 162–169.
- Yokoi S., Quintero F.J., Cubero B., Ruiz M.T., Bressan R.A., Hasegawa P.M., Pardo J.M. (2002): Differential expression and function of *Arabidopsis thaliana* NHX Na<sup>+</sup>/H<sup>+</sup> antiporters in the salt stress response. *Plant J.*, **30**: 529–539.
- Zhifang G., Loescher W.H. (2003): Expression of a celery mannose 6-phosphate reductase in *Arabidopsis thaliana* enhances salt tolerance and induces biosynthesis of both mannitol and a glucosyl-mannitol dimer. *Plant Cell Environ.*, **26**: 275–283.

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