Chapter 13

Morphological, Physiological, and Biochemical Modulations in Crops under Salt Stress

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Abstract Crop plants are affected by biotic and abiotic stresses (including salinity) and such stresses may affect the growth and yield of these crop plants seriously. High temperature (due to climate change) has also changed the pattern of precipitation and caused rise in sea level. These two factors have impacted soil salinization.

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To address such problems naturally, the crop plants adapt themselves by different mechanisms including changes in morphological, physiological, and biochemical processes. Both ions including sodium and chloride are the main ions, that become the reason for many physio-biochemical modulations inside plant tissues, in a similar way, chloride ion is the most dangerous because NaCl releases around 60% more ions in soil comparatively with Na_2SO_4. An extra amount of such types of salts increases the osmotic potential in soil matrix consequently the water absorbance by plants is reduced that leads towards physiological stresses or drought. This increase of Cl^- relates to salt tolerance that is linked to plant growth, water use efficiency, and transpiration. Increasing salinity in the nutrient solution reduces growth directly and restricts leaf and root mineral fixing. In this chapter, we have discussed insights into various kinds of morphological, physiological, anatomical, and biochemical modulations in plants caused by abiotic stresses especially salinity. In the era of climate change, plant scientists should focus on each shotgun approaches as well as long-term genomic techniques to enhance salt tolerance in commercially important crops to ensure food security and sustainable productivity.

Keywords High temperature · Soil salinization · Morphological modulations · Physico-chemical modulations · Anatomical modulations · Salt tolerant genotypes

13.1 Introduction

In current environmental conditions, saline soils are increasing day by day. Agricultural productivity is greatly degraded by the accumulation of salts in underground water. Quality of water resources and soil fertility are the vital components that fulfill the food, feed, and fiber demand of ever-growing global population. Salinity restricts the production of nearly over 6% of the world's land and 20% of the irrigated land (15% of total cultivated areas) (Munns 2005) and adversely affects agricultural production throughout the world. Many fertile lands are converted into salt affected ones because of underprivileged practices, e.g., irrigation with underground brackish water. Salinization of good arable land results in immense socio-economic losses. The loss of excellent natural resource is another problem because the population depends upon its livelihood on these lands, which are gradually declining through the spread of salinity (Acosta-Motos et al. 2017; Hammad et al. 2018). Salinity is considered as one of the major threats to crop plants which leads to lessen economic productivity by affecting different plant morpho-physiological and biochemical processes (Qazizadah 2016). Salinity tolerance can be elevated as a useful tool to characterize plants in different categories. The plants having the capability to endure high salt concentration and maintain their growth under saline conditions are characterized as halophytes. However, the plant species which face loss in their morphological, physiological, and metabolic processes are identified as glycophytes. The mechanisms adopted by halophyte species to combat salt stress and toxic ions like Na^+ ion toxicity include restriction of Na^+ ions entry into root cells, prohibiting Na^+ ions from leaves surface, movement of Na^+ ions from transpirational xylem stream to roots, compartmentation and sequestering of Na^+ ions in vacuoles and Na^+ exclusion through salt glands (Sun et al. 2009).

Halophytes can uphold their stomatal conductance to a level, that implement minimum effects on photosynthesis; in glycophytes, these effects are more noticeable (López-Climent et al. 2008). Halophytes exhibit high water use efficiency and low internal CO_2 concentration related to glycophytes. Solute accumulation is more prominent in halophytes than glycophytes. Due to various salinity tolerating mechanisms, a lower level of Na^+ and Cl^- ions is observed in halophytic cytoplasm and chloroplast. Halophytes exhibit lower Na^+ ion concentration in roots than upper plant parts (Sun et al. 2009). Sodium partitioning is significantly associated with plant metabolic processes and ion flux at both cellular and whole plant level (Park et al. 2016). Halophytes maintain their survival efficiently in salt shocks. These plants maintain their metabolic activities in a controlled way under saline conditions (Yang and Guo 2018).

The higher salt concentration impose disturbance at the plant cellular level and increase the salt level in soil solution decreases the plant's water uptake ability and results in water scarcity conditions (Munns 2005; Stępień and Kłbus 2006; El Sabagh et al. 2019). Salinity and drought induce similar adverse effects on plant growth; however, salinity have more severe effects on ionic balance in cell through vacuole compartmentation. This high ionic concentration possess restrictions in regular enzymatic activities. As a consequence of this increased ionic concentration in cytoplasm, enzymatic activities become ceased and the plant bends towards decline (Munns 2005). Salt tolerant genotypes regulate toxic ion accumulation (Ryu and Cho 2015) and translocation of these ions in the other plant parts. There are many factors affecting the plant's response to salinity but, irrigation system, edaphic and climatic conditions are the most important ones (Yang and Guo 2018).

13.2 Morphological Responses to Salt Stress

Salinity effects biomass and morphological traits of plants. Roots, shoots, and leaves are the part of plants that are severely affected by salinity. Salt stress in the root zone of plant reduces its root length (Ryu and Cho 2015). Reduced root conductivity is also associated with salt stress (Park et al. 2016). More than 90% of water transport from root to shoot is observed to be declined with increased salt stress. Reduced root hydraulic variables are induced by low soil osmotic potential and high root suberization because of salinity (Rizwan et al. 2015). The morpho-physiological characteristics of roots are directly related to the ability to exclude Cl^- from shoots (Farooq et al. 2017). Size of the root system determines the Cl^- uptake by the plant, which accumulates in the leaves and causes salt stress symptoms (Kaleem et al. 2018). Chloride uptake by roots and accumulation in leaves are linked to transpiration rate and water absorption, respectively. So, root morphological characters, which regulate water use, are of utmost importance in inducing salt tolerance (Farooq et al. 2017). Shoot to root ratio is an important and crucial factor in determining salt tolerance. Salinity also limits the shoot elongation and due to this reason plant canopy volume reduces (Kaleem et al. 2018).

Hence, salt stress adversely affects shoot growth and development. Along with shoot and root, salinity also induced its effects on leaves in terms of reduction in number of leaves, leaf area, and increased necrosis percentage (García-Sánchez et al. 2005). Necrosis/leaf burn symptoms, growth reduction (Rizwan et al. 2015), and leaf drop are identified at medium and high salinity. Sodium accumulation, as a result of salinization induces necrosis in old leaves, which initiate from tips and margins and then extend towards the leaf base. It also decreases the life span of leaves, net productivity, and crop yield (Craine 2005). Biomass reduction and foliar damage become more noticeable with the passage of time, with increasing the salinity levels.

Salinity reduces cell division and thus fresh matter production. As under saline condition, photosynthetic rate imbalances, so production of biomass is greatly decreased (Ryu and Cho 2015). Shoot dry weight decreases significantly with the increase in salinity. A reduction in whole plant dry weight, plant height, root length, root dry weight, and number of nodules was recorded in different crops (Sharifi et al. 2007). Moreover, significant reduction was observed in seed yield per plant, with the increase in salinity (Ashraf et al. 2005). A decreasing trend of plant height and total biomass was also observed in Juncus species (Greenwood and MacFarlane 2009). A salt tolerant plant species show a strong rooting system, which is helpful in osmotic adjustment under stress conditions (Yang and Guo 2018). A stronger rooting system increases the water availability by penetrating deep, below the salinity zone. The direct effect of salinity on roots is its growth retardation by interfering with mineral nutrition of the plant. Under saline growing conditions, decreased concentration of oxygen and internal ethylene accumulation increases, which ultimately leads to decline in root growth and elongation (Ashraf et al. 2005).

13.3 Limitations in Seed Germination, Survival %, and Growth Rate under Salt Stress

Seed germination is significantly affected by increasing salinity in the growing media (Othman et al. 2006). Halophytes and glycophytes vary significantly in their seed germination response to high salinity level. Halophytes follow a characteristic pattern by resisting salinity through normal germination at initial and low salinity levels but afterwards a rapid decreasing trend is observed with rise in salinity level. However, glycophytes show a gradual decrease in germination with rising salinity level. The low solute potential of growing media, because of increased salinity level results in reduced imbibition rate of seeds. This situation negatively affects the metabolic processes within seed like enzymatic imbalances (Ryu and Cho 2015), modifications in Nitrogen metabolism, alterations in plant growth regulator levels, and decline in reserves utilization. The food reserves present in seeds vary significantly in response to salinity. Lipid contents decline while sugar level rises (Yang and Guo 2018). The increment in sugar level is contributed by lipid metabolism and starch and protein breakdown (Acosta-Motos et al. 2017). Salinity cause accumulation of soluble sugars, free protein, and soluble proteins in seeds. These compounds also perform osmotic adjustment and prove to be beneficial for developing embryo.

Plant growth under saline conditions can be determined in terms of relative growth rate (RGR), net assimilation rate on a leaf weight basis (NAR (w)), leaf weight ratio (LWR), and nutrient uptake and utilization. It was found that salt treatment induced negative effects on both RGR and NAR (w), whereas LWR showed no definite trend. Net assimilation rate is correlated with relative growth rate (Nguyen et al. 2015). Salinity induces a negative effect on root mass fraction (RMF) and increase stem mass fraction (SMF) (Acosta-Motos et al. 2017).

13.4 Physiological Responses to Salinity Stress

Salinization disrupts the physiological processes of the plant because of ion toxicity and osmotic effect (Syvertsen and Levy 2005). Salinity proved to be a prominent threat for crop productivity by limiting plant growth processes through increased ion toxicity, inefficiency of photosynthesis, respiration rate, transpiration rate (Yuan 2006), stomatal conductance, membrane instability and permeability, decreased biosynthesis of chlorophyll, nitrite, and nitrate reductase activity

Salinity significantly lowers the stomatal conductance, photosynthesis, transpiration (Liu et al. 2006; Parida and Das 2005), and relative water content (Naumann et al. 2007). Salinity tolerant plant species show higher photosynthetic capacity and more accumulation of organic osmolytes (Hameed and Ashraf 2008). Under salinity conditions, closing of stomata results in decreased photosynthesis as well as transpiration. Chloride accumulation causes the reduction of net assimilation of CO_2 (Nguyen et al. 2015). Sodium is found to have more prominent effect in the reduction of these attributes, which accumulate in excessive amounts under saline conditions. Lower photosynthetic activity is contributed by reduced rubisco activity (Nguyen et al. 2015).

At moderate salinity levels, photosynthesis rate increases, as observed in sugar beet and eggplant. Higher photosynthesis rate is linked to higher stomatal conductance, which results in higher net assimilation of CO_2. This results in higher biomass production and crop yield (Farooq et al. 2017). Gas exchange is inversely related to the concentration of sodium and chloride ions due to salinization. CO_2 assimilation rate is associated with ion toxicity and water relation (Park et al. 2016). Increased sodium concentration leads to lower nitrate reductase activity, photosystem II and chlorophyll degradation (Kaya et al. 2020). It also results in cell membrane leakage due to the replacement of Ca^{2+} with Na^+ (Manchanda and Garg 2008).

Diffusion limitation in respect of mesophyll and stomatal conductance, contribute to photosynthesis inhibition under salt stress. Stomatal limitation with stomatal closure, non-stomatal limitation, or both limitations with stomatal closure at low tissue salt concentration and a disturbance of photosynthetic activity at high tissue salt concentration can be identified for salt-induced low photosynthesis rate. A significant correlation exists between stomatal conductance and photosynthesis rate (Yu et al. 2020). Maintenance of net photosynthetic rate, stomatal conductance, and elevated chlorophyll concentration are considered prerequisite for inducing salinity tolerance.

Elevated CO_2 levels interact with salinity in various ways. In olive (*Olea europea*), elevated CO_2 levels strongly affect the photosystem II and Chlorophyll content, under salinization. Elevated CO_2 does not interfere with Na^+ and Cl^- concentration in leaves and roots of tolerant cultivar whereas decreased these toxic ion accumulations in salt sensitive olive cultivar. Elevated CO_2 increased water use and reduces toxic ion uptake, but not significantly affected plant growth (Yang et al. 2005). Plant growth, shoot/root ratio, net gas exchange, water use, and root Ca^{2+} experience a decreasing trend with the increasing salinity while root N increases.

13.5 Cellular Responses to Salt Stress

Plants tolerate adverse effects of salinity by adjusting their biochemical and molecular processes, accordingly (García-Caparrós and Lao 2018). The plant mechanisms, which contribute to salinity tolerance include ion inclusion or exclusion, controlled uptake and transport of ions into shoots, ion compartmentalization at cellular and whole plant level, compatible solutes synthesis, modifications in photosynthetic mechanism, alterations in membrane structures, and accumulation of antioxidative enzymes (Arbona et al. 2005) and plant hormones (Parida and Das 2005).

Cell division is one of the various metabolic processes which faces serious irregularities. Precisely, it affects the leaf anatomy by inducing epidermal and mesophyll cell thickness, increment in palisade cell length and diameter, spongy cell diameter, reduction in intercellular space, changes in mitochondria and vacuole, decline in plant leaf area, and stomatal density (Yang and Guo 2018).

Ion compartmentalization at cellular level is an essential adaptive mechanism for plant species, to regulate their metabolic activities under salt stress (Parihar et al. 2015). Salt tolerant plant species retain toxic levels of ions in vacuoles and inhibit their interference with cytoplasmic metabolic activities. This adaptation leads to plant survival in adverse conditions. It is known as portioning or compartmentalization of toxic ions. It is independent of the membrane potential (positive or negative) inside the membrane (Farooq et al. 2017). The Na^+ and Cl^- restriction in vacuole induce higher concentrations of K^+ and organic osmolytes in cytoplasm in order to adjust osmotic pressure of the ions in the vacuole (Ashraf et al. 2005). Sodium compartmentation results in vacuolar alkalization, which is found to be partially associated with Na^+/H^+ antiporter activity. The restriction of toxic concentration of sodium ions in vacuole is regulated by salt inducible enzyme Na^+/H^+ antiporter (Padan and Landau 2016). Vacuolar Na^+/H^+ antiporters utilize the proton gradient, produced by H^+-adenosine triphosphatase (H^+-ATPases) and H^+-inorganic pyrophosphates (H^+-PPases). So, salt stress tolerance of plant species is based on the coordinated performance of Na^+/H^+ antiporters, H^+-ATPases, and H^+-PPases (Padan & Landau 2016). The Na^+/H^+ antiporter activity increases with the addition of sodium ions which is very significantly noted in tolerant plant species (Parihar et al. 2015).

13.6 Salinity-Induced Ion Toxicity and Nutrient Imbalance

Ion toxicity leads to reduction in growth due to adverse effects on some essential physiological and biochemical processes (Sun et al. 2009). Chloride, sodium, boron, lithium, etc., are the major ions which interfere with the metabolic processes of the plant when accumulated in excessive amount. As a result of salinization, plants differ in their ion uptake mechanism due to some multiple adaptations to toxic ions operating concurrently within a specific plant. Salt tolerance of a plant species refers to its ability to restrict translocation of toxic ions in shoots (Yu et al. 2020). This ability is regulated by cell specificity of tissues, morphological features, and water use efficiency (Nguyen et al. 2015). These adaptive mechanisms alter the plant response to salinity by inflicting characteristic modifications at both cellular and whole plant level.

Excessive accumulation of sodium and chloride ions results in reduced growth and nutrient imbalance (Liu et al. 2006). High sodium concentration in leaves is found to have a negative effect on net CO_2 assimilation, as indicated in some citrus species (Mishra and Tanna 2017). Chloride ion in high concentration imposes toxic effect on photosynthesis. The chloride uptake is significantly determined by shoot to root ratio through passive transport.

The nutrient imbalance occurs due to high ratios of Na^+/Ca^+, Na^+/K^+, Na^+/Mg^+, Cl^-/NO_3^-, and $Cl^-/H_2PO_4^-$, which leads to reduced yield and growth. The increment in the uptake of Na^+ has an antagonistic effect on the Ca^+ and K^+ uptake. However, Ca^+ and K^+ are of key importance to membrane integrity and proper functioning (Acosta-Motos et al. 2017). Plants adopt a mechanism to maintain adequate Ca^+ and K^+ concentration at cellular level, under saline conditions. Ca^+/Na^+ ratio is maintained in the saline growing media by increasing Ca^+ concentration in order to increase salt tolerance.

13.7 Potassium/Calcium Ions

Under salt stress K^+ concentration decreases (Acosta-Motos et al. 2017). In the presence of excessive Na^+, K^+ leaches out from soil exchange complex. So, a competition develops between Na^+ and K^+ at soil–root interface. Na^+/K^+ ratio can be considered as a source to observe salt tolerance of plant species (Willadino and Câmara 2005). In order to maintain adequate K^+ concentration, plants utilize low affinity and high affinity channels for K^+ uptake. Three low affinity channels, i.e., inward rectifying channels (KIRC), K^+ outward rectifying channels (KORC), and voltage independent cation channels (VIC), and two high affinity transporters are identified for maintaining cellular K^+/Na^+ ratio. The higher K^+ concentration is related to higher biomass production and thus salt tolerant plant species have the ability to retain more concentration of potassium (Ashraf et al. 2005).

Calcium role is significant in new cell wall synthesis, especially the middle lamella, which provides separation in neighboring cells, spindle formation during cell division, and regulating cell membrane integrity (Kaleem et al. 2018). In germinating seeds, calcium concentration with the passage of time decreases with respect to elevated levels of salt stress. Salt tolerant species experience more accumulation of K^+ and Ca^{2+}, which helps in maintaining optimal growth (Ryu and Cho 2015). Salinity tolerant grass species *Cynodon dactylon* (L.) exhibited this attribute through less Na^+ accumulation in roots and more accumulation of K^+ and Ca^{2+} in roots as well as leaves (Hameed and Ashraf 2008).

13.8 Magnesium, Nitrogen, and Nitrate Ions

Salt affected trees have low leaf Mg^{2+} concentrations because of low Mg^{2+} concentration in the exchange complex. Increasing Ca^{2+} (by addition of Ca^{2+} as gypsum $CaSO_4$) implements an antagonistic effect by displacing Mg^{2+} from the soil complex hence reduce Mg^{2+} concentration. Moreover, high sodium accumulation shows an antagonistic relationship with nitrogen (N), potassium (K^+), and manganese (Mn) uptake. Salinization results in high concentration of phosphorous and low Mg content (Min et al. 2014). However, the N and Ca^{2+} percentages in the roots do not vary significantly with the increase in salinity while a significant reduction in leaf N and Ca^{2+} concentrations is observed in salinity affected seedlings. Chloride uptake negatively influences the N uptake, while positively affects the concentration of Ca^{2+} and Na^+. Salinity interferes with the translocation mechanism of these elements. These findings displayed that under saline conditions, leaf mineral contents are least affected by root mineral status.

An inhibitory effect is observed on NO_3 (Sun et al. 2009) and phosphorous uptake, due to accumulation of high concentration of Cl^- ions under saline conditions. High leaf chloride contents, as a result of increased salinity caused reductions in chlorophyll contents, decreased the photosynthesis rate (Sun et al. 2009), and induced bleaching or bronzing of in leaves while high leaf sodium contents lowered the gas exchange rates. Salinity also induced progressive depletions of carbohydrates in leaves and roots thus inhibiting root growth and development (Iqbal et al. 2006).

13.9 Micronutrients

The micronutrient (Cu, Fe, Mn, Mo, and Zn) availability is affected by salinity as evidenced in saline and sodic soils. However, plant type, plant tissue, growing conditions, micronutrient concentration, salinity level, and its composition determine the effect of salt stress on the availability of micronutrients (Acosta-Motos et al. 2017). Manganese deficiency is reported in barley, with increasing salinity level, which can be compensated by manganese addition in soil. In contrast, few reports also suggested no effect and increased manganese concentration in plant shoots. Similarly, contrasting opinions are given by scientists, related to Zn, Fe, Mo, and Cu concentration. At different salinity levels, plants respond by decreasing the concentration of molybdenum, magnesium, iron, and zinc in their leaves. Copper is reported to remain unaffected under salt stress (Yang and Guo 2018).

13.10 Toxic Ions Inclusion and Exclusion Mechanism Under Salinity Stress

Salt exclusion at whole plant level involves ion partitioning, which constrains the salt movement towards shoots (Sun et al. 2009). Cellular level exclusion involves the inhibition of entry of toxic ions into the cell or directs their outward flow if get entered. Exclusion mechanism may also involve toxic ion extrusion through salt glands present on the leaf surface (Ashraf et al. 2005). Toxic ion exclusion is an adaptive strategy by plants experiencing salt stress. Roots maintain salinity levels by extrusion to soil or transport to shoots via xylem transpirational stream (Davenport et al. 2005). Phloem is reported as a chief source of toxic ion transport from shoots to roots. As Na^+ and Cl^- are the major toxic ions salt tolerant species are either Cl^- excluder or Na^+ excluder (Farooq et al. 2017). High concentration of Na^+ and Cl^- ions were recorded in shoots of salt tolerant species. Plant's ability to exclude sodium or chloride ions decreases with the increase in salinity level (Ghosh et al. 2016).

Some halophytes and some salt tolerant plant species like barley are also identified as Na^+ accumulators, which are signified by Na^+ concentration in their roots (Ghosh et al. 2016). The ionic distribution trend and vacuolar compartmentalization determine the extent of these strategies in glycophytes (Yang and Guo 2018). Sodium accumulation under salinity can also be explained through Na^+-ATPases concept. The absence of Na^+-ATPases (García-Sánchez et al. 2005) restrict the efflux of Na^+. The sodium efflux occurs against electrochemical gradient, which requires energy. Consequently, the sodium efflux decreases and sodium accumulates in plant tissues (Ashraf et al. 2005). Exclusion of 97% of Na^+ present in the soil at root surface is considered essential for all plants to maintain safe Na^+ level in shoots (Acosta-Motos et al. 2017).

13.11 Salinity and Biochemical Attributes

13.11.1 Leaf Pigments

Leaf pigments experience noticeable changes in their concentrations under salinity. Carotenoid contents are reported to decline in response to salinity however anthocyanin pigment increased as a result of salt stress. A decreasing trend of total chlorophyll, as well as Chl a and Chl b has been reported as a result of salinity, which can be contributed by the absolute concentration of chloride and/or sodium in the leaves. However, the increase in chlorophyll a, b, and total chlorophyll was reported on weight basis. As salinity causes a reduction in leaf size therefore one gram of salt affected plant showed a greater number of leaves (Weisany et al. 2011).

13.11.2 Sugars, Protein, and Lipid

Under saline conditions, sugar concentration becomes high in mesophyll cells of leaves, which induces feedback inhibition to photosynthesis process. The disturbance in normal sugar utilization process causes the increment in sugar concentrations in growing tissues (Skorupa et al. 2019). The concentration of total soluble carbohydrates increased in the leaves and roots of the seedlings, grown under saline conditions (NaCl or Cl is osmotic condition). The increment in carbohydrate level may be contributed by high chloride concentration in plant tissue or starch degradation as salt tolerant species have less starch accumulation (Poór et al. 2011). The carbohydrate accumulation rate varies among salt tolerant species (Skorupa et al. 2019). A decline in starch content and starch phosphorylase activity and increment in reducing, non-reducing, and sucrose phosphate synthase activity is observed under saline conditions. Soluble protein decreases in response to salinity (Park et al. 2016). Lipids play a key role in the protection of delicate organs. Energy is stored in the form of lipids and is a constituent of cellular membranes. Lipids play a significant role in inducing tolerance against stress conditions. Lipid content declines at higher levels of salinity.

13.11.3 Osmoprotectants

Physiological tolerance involves compartmentation and osmotic adjustment, utilizing inorganic and organic compounds (Acosta-Motos et al. 2017). This osmotic adjustment in plants occurs through the accumulation of compatible solutes which may be inorganic like essential elemental ions such as K^+ and organic compounds including sugars (glucose and fructose, mainly), sugar alcohols (glycerol, methylated inositol), and complex sugars (trehalose, raffinose, and fructans) (Zhu et al. 2016). Other important compounds include quaternary amino acid derivatives (proline, glycinebetaine, β alaninebetaine, and proline betaine), tertiary amines (1,4,5,6-carboxyl pyrimidine), and sulfonium compounds (choline-o-sulfate, dimethyl sulfonium propionate) (Ashraf et al. 2005). The compatible solutes lower the osmotic potential of the cell and maintain water status of plant (Parihar et al. 2015). They are hydrophilic in nature and act to replace water molecules present on the surface of proteins, protein complexes, and membranes and thus serve as an osmoprotectant (Zhu et al. 2016). They do not disturb the enzymatic activity of the cell and pH of the cytosol. These are produced as a result of some specific modifications in biochemical reactions, which occur only under stress conditions (Parida and Das 2005).

Compatible solutes regulate the enzyme activity under salt stress and do not interfere with the metabolic activities of the cell even if accumulated in high concentration. These organic solutes protect proteins and ribosome structures from the adverse effects of a toxic concentration of ions. These organic compounds mainly accumulate in leaves, with maximum concentration in salt tolerant species. These osmoprotectants scavenge the reactive oxygen species (ROS), which poses damage to cell functioning (Ashraf and Foolad 2005).

The osmolyte synthesis is related to basic metabolic activities. The amino acid biosynthesis pathways lead to origin of proline (glutamic acid), aspartate (ecotine), glycinebetaine (choline metabolism), and pinitol (myo-inositol synthesis). Mass action to the same extent can be regarded as a mechanism, adopted by osmolytes for regulating cytoplasmic osmotic potential (Parihar et al. 2015).

13.11.4 Glycinebetaine

The accumulation of nitrogen-containing compounds varies with plant species under salt stress (Grieve et al. 2007). These compounds perform the specific functions of osmotic adjustment, cell macromolecule protection, nitrogen storage, buffering, cell detoxification, and ROS scavenging to induce salt tolerance in plant species (Parida and Das 2005). Glycinebetaine (GB) (a quaternary compound) is identified as one of the prominent compounds, which is utilized for osmotic adjustment (Yang et al. 2005). Glycinebetaine restores the integrity of thylakoid membrane in salt and drought stress (Ashraf and Foolad 2005). It improves salt tolerance by protecting photosynthetic protein complexes (Nguyen et al. 2015) and reducing lipid peroxidation of cellular membranes. Glycine, betaine indirectly enhances the photosynthetic activity of plant cells, experiencing salt stress by regulating photosynthetic machinery by concentrating in chloroplast, regulating chloroplast metabolism, and protecting thylakoid membranes. It stabilizes the extrinsic proteins of PSII and hence increases the efficiency of PSII under salt stress (Yang and Guo 2018).

13.12 Free Amino Acids, Total Soluble Proteins, and Proline

Accumulation of some free amino acids and GB in osmoregulation is found to be a prominent stress tolerant strategy in plants. Amides (glutamine and asparagines) accumulation is also reported in plants experiencing salt stress (Cui et al. 2018). A considerable accumulation of proline, asparagine, and glutamine is also reported in cultivars of strawberry grown under salinity (Keutgen and Pawelzik 2008). The most prominent amino acid which accumulates under salt stress conditions in plants is proline. Proline accumulation because of salinity stress increased its importance as a compatible solute, which aids in osmotic adjustment (Cui et al. 2018). Salt tolerant plant species show maximum amino acid accumulation in leaves, as observed in Sunflower, Safflower, *Eruca sativa*, *Lens culinaris,* and *Phragmites australis* (Parida and Das 2005).

Proline accumulation, under salt stress is more profound in monocots. However, proline accumulation was insignificant in salt stressed barley seedlings (Parihar et al. 2015). Proline is not accumulated specifically for salinity stress, but also occur under drought stress. Proline synthesis is contributed by low water potential of growing media. Proline regulates the membrane stability and alleviates the salinity effects on cell membrane disruption by controlling the osmotically active useable N accumulation. Proline enhances the salt tolerance by protecting the protein turnover machinery against stress damage and up-regulating stress protective proteins in *Pancratium maritimum* L..

Few reports also displayed the opposite picture, i.e., more proline accumulation in salt sensitive varieties of tomato as compared to tolerant species (Yu et al. 2020). An inverse relation is reported between proline accumulation and salt tolerance in *Vigna mungo (*Win and Oo 2017*)*. Soluble proteins also play a key role in osmotic adjustment during salt stress. Salt tolerant species show high content of soluble protein, as evidenced in barley (El-Esawi et al. 2018), Sunflower (Zeng et al. 2016), rice (Ghosh et al. 2016), and finger millet (Acosta-Motos et al. 2017). However, a decreasing trend of soluble protein was also noted with the increase in salinity (Mishra and Tanna 2017). Increased level of protein synthesis in response to salt stress is found in many plant species. For example, osmotins and dehydrins, regulate the protein structure and activity. Late embryogenesis proteins (LEA) are synthesized excessively under salt stress which safeguards the adverse osmotic effects of NaCl (Saha et al. 2016).

Proline accumulation can be considered as a representative of severe saline conditions. Soluble protein accumulation cannot be considered as related to salt tolerance mechanism (Sun et al. 2009). Proline stabilizes subcellular structures, scavenge ROS, and protect cell membranes by stimulating antioxidant activity (Kaleem et al. 2018). It may be an inhibiting agent for plant growth if high concentration of proline is applied. It is of utmost importance to optimize the effective concentration of exogenously applied proline. High proline concentration applied reduced seedling growth and lowered leaf Na^+/K^+ ratio (Saha et al. 2015). The exogenously applied proline concentration varies with the plant species and plant developmental stage (Cui et al. 2018). Proline is found to have a protective role in photosynthesis by provision of regenerated NADP through transcriptional activation of the NADPH-dependent P5C-Synthetase. This NADP provision prevents photo-inhibition.

13.12.1 Polyamines

Accumulation of polyamines like putrescene, spermidine, spermines, etc., is also related with plants growing under saline conditions. The major role of polyamines is in cell elongation, root formation (Saha et al. 2015), cell division, organogenesis, and plant senescence. However, polyamines contribute little to osmotic adjustment. Extent of polyamine accumulation varies in a single species. In *Brassica compestris*, small alterations in polyamine level are noticed upon exposure to long-term salinity stress, whereas plant experienced significant increment in polyamine level and enzymatic activities when subjected to a short duration of salt stress (Ke et al. 2018). More polyamine accumulation was observed in salt sensitive cultivars of rice and tomato. The salt tolerant plant species synthesize excessive concentration of polyamines such as putrescene and spermine (Sequera-Mutiozabal et al. 2017). However, increased concentration of putrescene and tyramine was observed in roots of salt sensitive plant species of rice (Ghosh et al. 2016).

Polyols contribute to osmoregulation in salt stress. These are classified as acyclic (manitol) and cyclic (pinitol) (Parida and Das 2005). The high concentration of ions in vacuole results in osmotic disturbance. Polyols concentrate in cytoplasm to counteract these changes. The salt tolerating ability of tobacco is found to be related to high accumulation of polyols (Saha et al. 2015).

13.13 Summary and Future Research Prospects

Salinity is restraining the crop yield by causing modulations at molecular, cellular, morphological, physiological, and biochemical levels. It is depicted that plants try to cope with salt stress by regulating nutrient uptake, maintaining water status, osmotic adjustment, and through antioxidant defensive system. All these mechanisms are species-dependent. Salinity is limiting the plant growth, production, and quality not only in agronomic crops but in horticultural crops too. So, there is a dire need to improve the salt tolerance potential of crops. Plant scientists should focus on each shotgun approaches as well as long-term genomic techniques to enhance salt tolerance in commercially important crops to ensure food security and sustainable productivity. Identification of tolerant genotypes by using various physiological and biochemical indicators of stress tolerance, exogenous application of stress-inducing compounds like silicon, new generation growth hormones, and growth promoting microbes are the potential strategies to mitigate salinity-induced drastic effects of product quality and quantity within shorter span on time.

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