

Salinity Stress and its management in plants: Review article

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ABSTRACT

Anthropogenic activities have significantly contributed for adding stresses to the ecosystem. These activities are responsible for the deterioration of biodiversity and species extinction at the world level. Both abiotic and biotic stresses generate free radicals which are harmful for human as well as plant body. Recently a new group of plant hormones known as brassinosteroids (BRs) have been reported to be implicated in plant growth and development, stress management and other physiological activities. Another class of compounds known as polyamines (PAs) have also been regarded as an integral component of defense system in plants as well as animals.

SALINITY STRESS

Salinity is one of the major agricultural constraint limiting plant growth and development in most parts of the world (Gao *et al.*, 2008). Nearly 10 % of the total land surface is salt affected and an estimated 10 mha of agricultural land is lost annually due to salinisation and water logging (Monirifar and Barghi, 2009). In India, about 6.73 mha of land area is affected with soil salinity (Mandal *et al.*, 2009). Naturally occurring salinisation is primarily caused by elevation of capillary water level and subsequent evaporation of saline ground water leaving the salts on the soil surface. Man made salinisation is more common in irrigated areas of arid regions which are more susceptible to salinisation (Supper, 2003).

Accumulation of water soluble salts, especially sodium chloride (NaCl), sodium carbonate (Na₂CO₃) and partially calcium chloride (CaCl₂) results in saline soils (Nawaz *et al.*, 2010). In saline soils high concentration of NaCl results in severe competition between Na⁺ and K⁺ ions which disturb the common transport system of plant roots. The presence of Ca²⁺ ions further deteriorate the condition by enhancing the K⁺/Na⁺ intracellular accumulation in plant systems (Maathuis *et al.*, 1996). Salinity stress not only has damaging effect on plants but also increases pH level of soils. Moreover, high salt concentration also leads to the deterioration of soil structure and hinders desirable air water balance essential for biological processes occurring at plant roots (Nawaz *et al.*, 2010).

Effects of salinity stress

Salinity stress mainly affects through ion imbalance and hyperosmotic stress in plant systems which in turn leads to the production of reactive oxygen species (ROS) (Ahmad *et al.*, 2009). Salt stress causes adverse effects on plants due to osmotically induced water stress, specific ion toxicity, ion imbalance and oxidative stress (Munns, 2002). ROS such as superoxide (O₂^{•-}), hydrogen peroxide (H₂O₂), hydroxyl radicals (OH[•]) and singlet oxygen (¹O₂) are produced under salt stress. ROS are well known to cause oxidative damages to lipids, carbohydrates, DNA which ultimately results in cell death (Sairam and Tyagi, 2004). Although ROS are produced under normal conditions in

chloroplasts and peroxisomes through photorespiration in light and in mitochondria during darkness respectively, but during stressful conditions, their concentration gets increased many folds and causes harmful effects (Ahmad *et al.*, 2009).

Reactive oxygen species (ROS) produced under salt stress are known to attack polyunsaturated fatty acids giving rise to lipid hydroperoxides, increasing leakiness and causes secondary damage to membrane protein, DNA and RNA (Ahmad *et al.*, 2009). Physiological processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are also adversely affected by high salt levels (Sairam and Tyagi, 2004). Disturbed ability of water uptake and reduced growth of plant roots has also been observed under NaCl stress (Nawaz *et al.*, 2010). More recently the disturbed chemical potential between apoplast and symplast leads to decrease in turgour pressure and reduction in plant growth has been reported by Hussain *et al.* (2010).

Reduced growth of two maize cultivars (cv. Golden and C-20) was also observed by Nawaz and Ashraf (2007). Reduction in net carbon dioxide assimilation under salinity stress has been observed by Saffan (2008).

Abass and Latif (2005) reported significant reduction in the growth of *Corchorus olericius* seeds treated with 1000, 3000 and 5000 ppm of NaCl. Significant reduction in germination percentage, germination rate, biomass of four vegetable species namely *Brassica campestris*, *Amaranthus paniculatus*, *Beta vulgaris*, *Brassica oleracea capitata* under salinity stress was also recorded by Jamil *et al.* (2006). At a concentration of 150 mM NaCl, there was a reduction in fresh weights of hypocotyls of *Jatropha curcas* plants (Gao *et al.*, 2008). Similarly, Shahbaz and Ashraf (2007) observed a significant reduction in the shoot fresh and dry biomass of two wheat cultivars (S-24 and Mh-97) under salinity stress. Suspension in the growth processes of bean plants, with increasing salinity level was recorded by Stoeva and Kaymakanova (2008).

Decreased overall yield of rice crop was observed by Joseph *et al.*, (2010) under salt stress. Reduced seedling growth in *Glycine max*, *Phaseolus vulgaris*, *Phaseolus adenanthus*, *Vigna unguiculata*, *Mucuna poggei* under NaCl stress had also been reported by Taffouo *et al.* (2009). A decrease in leaf area, fresh and dry biomass of shoots was observed by Houimli *et al.* (2008) in *Capsicum annum* under salt stress. Similarly, Bhaskar *et al.* (2009) also observed decline in seed germination of *B. juncea* growing under NaCl stress. Reduced wheat seedlings growth under salt stress was also recorded by Hameed *et al.* (2008). Significant reduction in the chlorophyll pigments and water relations of *Panicum miliaceum* was reported by Sabir *et al.* (2009).

Two species of *Salicornia* (*persica* and *europaea*) were treated with different concentrations of NaCl (0, 85, 170, 340 and 510 mM) showed that fresh and dry weight of both species increased significantly at 85 and 170 mM and decreased at higher concentrations. Salinity increased proline content in both species as compared to control. Moreover there was an increase in activities of antioxidant enzymes like SOD, CAT and GPOX (Aghaleh *et al.*, 2010). Similarly, Gorai *et al.* (2010) observed that NaCl stress (300 and 500 mM) caused a significant reduction in growth allocation of different tissues in *Phragmites australis*. Seeds of four lettuce (*Lactuca sativa*) varieties (Romaine, Augusta, Vista, Verte) differing in their salt sensitivity were sown at 0, 50, 100 and 150 mM NaCl. There

was reduction in germination percentage with increasing salt concentration, with more decrease in vista and verk.

SALINITY STRESS AND ITS MANAGEMENT

Salt stress causes oxidative damages usually by the formation of ROS directly or through its involvement in redox reactions. The ROS like OH^{\cdot} , H_2O_2 and O_2^{\cdot} causes oxidative damages to the metabolic machinery of plants. Although, ROS are produced under normal conditions in chloroplast and mitochondria but during biotic and abiotic stresses, their level gets enhanced and their concentration is not detoxified appropriately, thus causing oxidative stress (Chen and Schopfer, 1999). Plants have developed unique protection mechanism to negate the effects of oxidative stress. This protection mechanism is constituted by defense system consisting of antioxidants and antioxidative enzymes. Moreover, certain compatible solutes called osmolytes are also actively involved in NaCl stress amelioration.

Various antioxidant enzymes involved in oxidative stress management are catalase (CAT), superoxide dismutase (SOD), guaiacol peroxidase (GPOX), peroxidase (POD), glutathione reductase (GR), ascorbate peroxidase (APOX) (Chen and Schopfer, 1999). Among antioxidant enzymes, SOD constitutes the first line of defense against ROS. It dismutates superoxide anion to hydrogen peroxide (H_2O_2). Thereafter, harmful H_2O_2 is catalysed by CAT, POD and APOX to H_2O and O_2 . Under stressful conditions, enhancement in SOD activity of *Spirulina plantensis* had been observed by Choudhary *et al.* (2006). Similarly, Gratao *et al.* (2008) also reported an enhancement in the activities of CAT and SOD in tomato plants growing under salinity conditions.

Hernandez and Almansa (2002) observed an increase in the activities of SOD and GR in *Pisum sativum* under salinity stress. Seedlings of *Jatropha curcas* exposed to salt stress showed increased activities of SOD and POD at 150 and 200 mM NaCl stress (Gao *et al.*, 2008). Similarly, Esfandiari *et al.* (2007) also recorded enhanced activity of CAT in two wheat cultivars (Alvand and Sardari) subjected to 50 mM NaCl stress. Improved activities of SOD, CAT and POX in *Cassia angustifolia* under salt stress had been reported by Agarwal and Pandey (2004). However decreased activities of APOX and GR were recorded in *Zea mays* seedlings under high salt concentration (Arora *et al.*, 2008).

Besides antioxidant enzymes, plants respond to high salt concentration *via* antioxidants like ascorbic acid, glutathione (GSH), vitamin E, carotenoids (CAR) and flavonoids (FLA).

Ascorbic acid (ASA) is a water soluble antioxidant which helps in scavenging ROS (Smirnoff, 1996). Exogenous application of ASA to the root medium of *Lycopersicon esculentum* seedlings had been observed to remarkably increase seedling survival along with treatment of 300 mM NaCl (Shalta and Neumann, 2001). Exogenous application of ASA increased growth and yield of canola plants growing under salinity stress (Sakr and Arafa, 2009). Pretreatment of sorghum seedlings to glycinebetains or ascorbic acid had been observed to alleviate the inhibitory effects of salinity stress (Arafa *et al.*, 2007). Similarly, Al-Hakimi and Hamada (2001) observed that presoaking of *Triticum aestivum* grains with ASA could counteract the adverse effects of NaCl stress.

Glutathione (GSH) is a non sulphur containing tripeptide acting as a storage and transport form of reduced sulphur. GSH is a water soluble low molecular weight antioxidant playing central role in ascorbate glutathione cycle (Tausz *et al.*, 2004). GSH has been shown to reduce oxidative stress by scavenging $O_2^{\bullet-}$ and H_2O_2 (Cozatl and Sanchez, 2006). Soaking of *Brassica napus* seeds with reduced GSH nullified the inhibitory effects of salinity stress on growth parameters like shoot fresh and dry weight (Kattab, 2007). Similarly, Ruiz and Blumwald (2002) observed that there was an induction of cysteine and GSH synthesis in *Brassica napus* during salt stress.

Carotenoids are lipid soluble antioxidants playing an important role in plant metabolism as well as in oxidative stress tolerance (Ahmad *et al.*, 2009). They protect photosynthetic apparatus against various harmful stresses (Strzalka *et al.*, 2003). Similarly Mane *et al.* (2010) reported an enhancement in the content of carotenoids in *Cymbopogon nardus* growing under salinity stress suggesting its role in alleviating salinity stress. There was an increase in β -carotene content of *Chlorella vulgaris* subjected to salinity stress (Hiremath and Mathad, 2010).

Besides antioxidants and antioxidant enzymes certain osmolytes like proline, glycinebetains, mannitol and sorbitol also get accumulated to overcome the ill effects of salt stress (Sairam and Tyagi, 2004). *Oryza sativa* plants were shown to accumulate proline under NaCl stress (Garcia *et al.*, 1997). Alamgir *et al.* (2007) also observed enhancement in proline accumulation under salinity stress in rice seedlings.

The active involvement of plant growth regulators *viz.* auxins, jasmonates, brassinosteroids and polyamines in key physiological processes have been widely accepted (Shi *et al.*, 2010). In the last few decades the application of brassinosteroids as a unique agricultural tool to enhance resistance potential of plants against abiotic and biotic stresses is on the rise.

BRASSINOSTEROIDS

Brassinosteroids (BRs) are growth promoting phytohormones found at low concentration in pollen, seeds and young vegetative tissues throughout the plant kingdom. They are found in gymnosperms, monocotyledons, dicotyledons and in algae. The endogenous level of BRs varies from tissue to tissue and with the age of the plant. Highest concentration of BRs is found in pollen and immature seeds (Khirpach *et al.*, 2000). They are found at nanomolar and micromolar concentrations and have been reported to regulate large number of physiological activities such as cell division, seed germination and vascular differentiation (Clouse and Sasse, 1998).

PHYSIOLOGICAL ACTIVITIES OF BRASSINOSTEROIDS

Cell division and cell expansion

BRs have been recorded for stimulating the cell division at nanomolar concentrations. Fathutdinova *et al.* (2002) observed an increase in the mitotic rate of wheat roots after treatment with 24-epibrassinolide (EBL). Stimulation of cell division by 50% in cultured parenchyma cells of *Helianthus annuus* was observed by Clouse and Zuerk (1991) by nM application of BRs along with auxins and cytokinins. The growth induced by BRs is due to cell division and cell elongation (Clouse and Sasse, 1998). Pronounced elongation of hypocotyls, epicotyls and peduncle of dicots, as well as coleoptiles and mesocotyls of monocots has been observed upon nM to μ M application of BRs (Clouse,

1996). Cell expansion induced by BRs has been attributed to proton extrusion and hyperpolarisation of cell membranes in the asymmetric expansion of the joint pulvini of rice lamina (Cao and Chen, 1995).

Seed germination

Ali *et al.* (2005) recorded significant enhancement in percentage seed germination of *Cicer arietinum* seeds treated exogenously with homobrassinolide (HBR) at 10^{-8} M concentration. Similarly, Sharma and Bhardwaj (2007) recorded promotion of seed germination by exogenous application of BRs in *Brassica juncea*. Improved seed germination by application of BRs was also reported in *Eucalyptus camaldulensis* by Sasse *et al.* (1995).

Vegetative growth

Chon *et al.* (2000) reported that pre treatment of rice caryopsis with brassinolide (BL) could increase leaf sheath when grown in light. Malabadi and Nataraja (2010) showed the role of EBL in micropropagation of orchids. EBL application had also been observed to enhance root growth in *Hordeum vulgare* (Kartal *et al.*, 2009).

Vascular differentiation

The requirement of auxins and cytokinins for the initiation of xylem development both *in vitro* and *in vivo* has been widely understood (Fukuda, 1997). Similarly, BRs have also been recorded to play significant role in vascular differentiation. Clouse and Zurek (1991) observed that nM concentration of exogenous BL in *Helianthus annuus* could increase differentiation of treachery elements.

Brassinosteroid and stress management

BRs have been reported to play an important role in abiotic and biotic stress management. BRs are now regarded as an important component of plant stress response, due to their capacity to regulate antioxidative defense system.

BRs application has been effectively used for ameliorating the adverse effects of salt stress in plants. Anuradha and Rao (2003) observed that BRs could significantly reduce the inhibitory effects of salt stress in rice plants by improving the pigment levels and nitrate reductase activity. Application of BRs had been observed to relieve the salinity induced inhibition of seed germination and seedling growth in barley (Kulaeva *et al.*, 1991) and rice (Anuradha and Rao, 2003).

Arora *et al.* (2008) had reported that application of 28-HBR could alleviate oxidative stress in salt treated maize plants. Seedlings of *Brassica juncea* cv. varuna showed decreased activities of nitrate reductase, carbonic anhydrase and chlorophyll content under salinity stress. However presoaking treatment of seeds with HBR was observed to reduce salinity stress by enhancing the activities of nitrate reductase, carbonic anhydrase and chlorophyll contents (Hayat *et al.*, 2007). Exogenous application of EBL was found to alleviate inhibitory effects of salt stress on shoot growth parameters and relative water content in *Capsicum annum* (Houimli *et al.*, 2008). Similarly, Anuradha and Rao (2001) reported that BRs application in *Oryza sativa* growing under salinity stress could reverse the inhibitory effects on seed germination and seedling growth and also improve the level of nucleic acid and compatible solutes.

Exogenous application of BRs was observed to reduce the ill effects of salinity stress in rice seeds (Rao *et al.*, 2002). BRs have also been observed to ameliorate the impact of salt stress on growth by restoring pigment levels and enhancing nitrate reductase activity (Anuradha and Rao, 2001). BRs enhanced the activities of APOX, CAT and SOD in *Brassica juncea* under salt stressed and control conditions (Sirhindi *et al.*, 2009). Improvement of membrane stability index and water content in *B. juncea* under NaCl stress was observed by Ali *et al.* (2008). More recently, Divi *et al.* (2010) reported that salt tolerance in *Arabidopsis* mutants deficient in ABA, ethylene, jasmonic acid and salicylic acid when treated with exogenous BRs could be significantly enhanced.

POLYAMINES

Polyamines (PAs) are the low molecular weight polycations found ubiquitously in all living organisms and functioning in a wide range of biological processes (Takahashi and Kakehi, 2009). The main PAs found in plant cells are diamine putrescine (Put), triamine spermidine (Spm) and cadaverine (Cad) and tetramine spermine (Spm). They are aliphatic compounds which are positively charged at physiological pH. This property allows PAs to interact with negatively charged biomolecules such as DNA, RNA, proteins and phospholipids, thus involved in the regulation of physical and chemical properties of membrane, nucleic acid structure and function and modulations of enzyme activities. PAs have been shown to play important role in physiological processes such as cell growth and development and management of various environmental stresses (Gill and Tuteja, 2010). PAs are regarded as a class of growth regulators due to their active involvement in various physiological activities which are described as below:-

PAs are involved in many developmental processes such as cell division, embryogenesis, reproductive organ development, root growth and abiotic stresses (Sawhney *et al.*, 2003). Put has been shown to influence somatic embryogenesis and plant regeneration in *Pinus gerardiana* (Malabadi and Nataraja, 2010). Similarly, Sacramento *et al.* (2004) observed that in the cultures of *Grateloupia*, Spm promoted the maturation of cystocarp to the eventual liberation of spores from aseptic fertile explants. Exogenous application of PAs to *Citrus reticulata* plants conferred dehydration tolerance *via* modulation of antioxidant capacity and stomatal response (Shi *et al.*, 2010).

Takahashi and Kakehi (2009), observed that Spd is essential for the survival of *Arabidopsis* embryos. Similarly, Savarin *et al.* (2010) proved that PAs act as potential regulators of the complex dynamical properties of anionic microtubules. Foliar application of Put at 100 and 200 ppm on *Syngonium* plants had significantly increased all growth characters like plant height, stem diameter, number of leaves, leaf area, fresh and dry weights of roots and leaves (Quesni *et al.*, 2010). Cotton seeds soaked with Spm at different concentrations showed an enhancement in germination percentage, vegetative and flowering stages. In addition to this hypocotyls and epicotyls length of seedlings were significantly enhanced. Moreover, increased level of PAs at flowering stages had also been observed to play important role in reproductive organ development (Abd El-Wahed, 2006).

Zeid (2004) reported that exogenous Put application (10^{-2} mM) in *Phaseolus vulgaris* L. cv Giza 6 results in increasing seed germination up to 88% and this effect was probably due to the activation of amylase and protease enzymes. Ndayiragije and Lutts (2006) observed that exogenous application of Put reduced sodium and chloride accumulation in NaCl treated calli of salt sensitive rice cultivar I Kong Pao. Amelioration of salinity stress by increased level of endogenous PAs in leaf tissue of sunflower exposed to 50, 100 and 150 mM NaCl was reported by Mutlu and Bozcuk (2007). Exogenous Spm cures hypersensitivity of a Spm deficient mutant of *Arabidopsis thaliana* to high salt stress (Yamaguchi *et al.*, 2006).

Foliar spray of Put to *Pisum sativum* growing under salinity stress results in the improvement of growth parameters (Hussein *et al.*, 2006). Enhanced expression of arginine decarboxylase (ADC) gene, polyamine biosynthesis enzymes (arginine decarboxylase and ornithine decarboxylase) with rise in Put content was recorded in apple callus under salt stress (Liu *et al.*, 2007).

Effect of salt stress on PA metabolism was examined in two rice cultivars I Kong Pao (IKP), salt sensitive and Pokkali salt resistant. Salt stress was observed to increase the concentration of Put in a salt resistant Pokkali variety, however decreased concentration of Put was observed in the salt sensitive IKP variety, suggesting a protective function of Put in response to NaCl stress (Quinet *et al.*, 2010). Similar observations were recorded by Tassoni *et al.* (2008) that with an increase in salt concentration in *Arabidopsis*, Spm was the most abundant PA suggesting its important role in tolerance of salt stress.

Recently pretreatment with 1 mM Spd, Spm or Put has been used to prevent salt induced K^+ leakage in the mature root zone of hydroponically grown maize and *Arabidopsis* (Pandolfi *et al.*, 2010). Exogenous application of Put and Spd were able to ameliorate salt stress in barley seedlings by maintaining root tonoplast integrity (Zhao and Qin, 2004). Exogenous application of Put had been recorded to alleviate salinity stress by maintaining high K^+ level and lowering Na^+ in *Brassica juncea* leaves (Lakra *et al.*, 2006).

Jiu Ju *et al.* (2007) observed that exogenous Spd application in cucumber seedlings during salinity stress results in increased content of endogenous Spd and Spm. Besides it also increased SOD, POD and CAT activities and decreased ROS production rate, H_2O_2 and malondialdehyde (MDA) contents and electrolyte leakage and thus significantly promoted root growth. Duan *et al.* (2008) also reported that exogenous Spd application to *Cucumis sativus* growing under salinity stress could enhance short term salinity tolerance.

When seeds of *Amaranthus*, green gram, green peas, spinach and chickpea were grown in MS medium supplemented with various concentrations of NaCl, only spinach and Amaranthus were found to be salt tolerant at 50 mM NaCl. However when seeds were inoculated in MS medium supplemented with different concentrations of NaCl and Spd, all the seeds showed germination. Thus Spd was shown to enhance salinity tolerance by increasing seed germination percentage (Rebecca *et al.*, 2010). Similarly, Chattopadhaya *et al.* (2002) reported that exogenous application of Spd and Spm could significantly prevent the leakage of electrolytes and amino acids from roots and

shoots induced by salinity stress in *Oryza sativa*. Ali (2000) observed that presoaking of *Atropa belladonna* plants with Put reduced the net accumulation of sodium and chloride under salinity stress.

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