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Salinity is one of the most severe problems in worldwide agricultural production. According to the FAO Land and Nutrition Management Service (2008), over 6% of the world’s land is affected by either salinity or sodicity which accounts for more than 800 million ha of land. Salt affected land comprises 19% of the 2.8 billion hectares of arable land on earth and an increase in this menace is posing a serious threat to agriculture globally (Pessarakli and Szabolcs, 1999). Current estimate indicates that 10-35% of the world’s agricultural land is now affected, with very significant areas becoming unsuitable each year. Traditionally, 4 levels of soil salinity based on saline irrigation water have been distinguished, low salinity defined by ECc of less than 0.25 dS m\(^{-1}\); medium salinity by ECc of 0.25 to 0.75 dS m\(^{-1}\); high salinity by ECc of 0.75 to 2.25 dS m\(^{-1}\) and very high salinity with an ECc exceeding 2.25 dS m\(^{-1}\) (US Salinity Laboratory Staff, 1954).

Salinity is one of the most important abiotic stresses, limiting crop production in arid and semi-arid regions, where soil salt contents is naturally high and precipitation can be insufficient for leaching (Zhao et al., 2007). The common cations associated with salinity are Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\) and K\(^+\) while the common anions are Cl\(^-\), SO\(_4^{2-}\), HCO\(_3^-\), CO\(_3^{2-}\) and NO\(_3^-\). Other constituents contributing to salinity in hypersaline soils and water include B, Sr\(^{2+}\), SiO\(_2\), Mo, Ba\(^{2+}\) and Al\(^{3+}\) (Hu and Schmidhalter, 2002). Na\(^+\) and Cl\(^-\) are considered the most important ions as both are toxic to plants and Na\(^+\) in particular causes deterioration of the physical structure of soil (Dubey, 1997; Hasegawa et al., 2000).

2.1 Types and causes of salinity

The particular processes contributing salt combined with the influence of other climatic landscape features and the effects of human activities, determine where salt is likely to accumulate in the landscape (Rengasamy, 2006). Salinity has been classified as “Primary” or “Secondary” salinity by Ghassemi et al. (1995).
Primary salinity results from the accumulation of salts over long periods of time from two natural processes i.e., weathering of rocks containing soluble salts of various types (mainly chlorides of sodium, calcium and magnesium, and to a lesser extent, sulfates and carbonates), and the deposition of oceanic salt carried inland by wind and rain. This type of naturally salt affected areas occurs widely in arid and semiarid areas (Rengasamy et al., 2003). Secondary salinity results from human activities that change the hydrologic balance in the soil between water applied (irrigation or rainfall) and water used by crops (transpiration).

2.2 The effect of salinity on plants

Salinization severely affects the agricultural productivity. The disastrous effects of irrigation-induced soil salinization in the Runn of Kachh represent amongst the most extreme examples in India. In agricultural land, water-logging and salt accumulations affect plant growth adversely to reduce potential crop production. Also salinization of agricultural land at extensive scale causes massive economic loss at the global level (Ghassemi et al., 1995). Salinity directly and indirectly affects the environment by inducing changes in vegetation cover and physical and chemical soil properties. Consequently, loss of biodiversity, shrinkage of wildlife (Barnum, 2005) and ecosystems disruption lead to loss of ecosystem resilience (Barrett- Lennard et al., 2005) that affect local climate, water and mineral cycles. Salts in the soil water may inhibit plant growth for two reasons: (i) Osmotic or water deficit effect of salinity (physiological drought), where the presence of salt in the soil solution reduces the ability of the plant to uptake water and this leads to reduction in growth rate; (ii) Salt specific or ion-excess effect of salinity, where excessive amount of salt entering the plant in its transpiration stream, causing injury to cells in the transpiring leaves and this may cause further reductions in growth (Greenway and Munns, 1980).
According to Dubey (1997) and Yeo (1998) salt causes both ionic and osmotic effects on plants and most of the known responses of plants to salinity are linked to these effects. The general response of plants to salinity is reduction in growth (Romero-Aranda et al., 2001; Ghoulam et al., 2002). The initial and primary effect of salinity, especially at low to moderate concentrations, is due to its osmotic effects (Munns and Termant, 1986; Jacoby, 1994) which is the result of lowering of the soil water potential due to increasing solute concentration in the root zone. At high salinity, some symptoms of plant damage may be recognized such as necrosis and leaf tip burn due to excess Na\(^+\) or Cl\(^-\) ions (Wahome et al., 2001). High ionic concentrations may disturb membrane integrity and function, interferes with internal solute balance and nutrient uptake, causing nutritional deficiency symptoms (Grattan and Grieve, 1999).

Sodium and chloride, usually the most prevalent ions in saline soils or water, account for most of the deleterious effects that can be related to specific ion toxicities (Levitt, 1980). The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties (Bolarin et al., 1991; Ghoulam et al., 2002). Salt stress affects all the major physiological and biochemical processes involved in plant growth and metabolism.

### 2.2.1 Water relations

Osmotic effects of salt on plants are a result of lowering of the soil water potential due to increase in solute concentration in the root zone (Sohan et al., 1999; Romero-Aranda et al., 2001). At very low soil water potentials, this condition interferes with plants ability to extract water from the soil and maintain turgor (Sohan et al., 1999). However, at low or moderate salt concentration (higher soil water potential), plants adjust osmotically (accumulate solutes) and maintain a potential gradient for the influx of water. Salt treatment caused a significant decrease in relative water content (RWC) in sugarbeet varieties (Ghoulam et al., 2002).
indicating a loss of turgor that results in limited water availability for cell extension processes (Katerji et al., 1997). Salt stress caused a significant reduction in all leaf water relation parameters such as water potential, osmotic potential and turgor potential of maize (Nawaz and Ashraf, 2007), rice (Pattanagul & Thitisaksakul, 2008), chickpea (Sheokand et al., 2010) and mungbean (Kabir et al., 2004).

2.2.2 Leaf anatomy

Salinity has been reported to cause anatomical changes in the leaves of a number of plants. Leaves of bean, cotton and Atriplex are reported to show increase in epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter and spongy cell diameter with increasing salinity (Longstreth and Nobel, 1979). In contrast epidermal thickness, mesophyll thickness and intercellular spaces decreased significantly in NaCl treated leaves of the mangrove Brugueira parviflora (Parida et al., 2004). In tomato plants salinity reduced the stomatal density (Romero-Aranda et al., 2001).

2.2.3 Photosynthesis

Growth of plants is dependent on photosynthesis and therefore, environmental stresses affecting growth also affect photosynthesis (Salisbury and Ross, 1992; Dubey, 1997; Taiz and Zeiger, 1998). Studies conducted on different plant species showed that photosynthetic capacity was suppressed by salinity (Kao et al., 2001; Ashraf, 2001; Romero-Aranda et al., 2001). The pronounced salt induced injury symptoms on mungbean (Wahid et al., 2004), rice (Sultana et al., 2001) and maize (Hussein et al., 2007) are enhanced chlorosis, necrosis, while decreased chlorophyll a, b, carotenoid contents and rate of photosynthesis. A positive association between photosynthetic rate and yield under saline conditions has been found in different crops such as Gossypium hirsutum (Pettigrew and
Review of literature

Meredith, 1994) and Asparagus officinalis (Faville et al., 1999). In contrast, there are many studies in which no or little association between growth and photosynthetic capacity is evident, as in Triticum repens (Rogers and Noble, 1992) and Triticum aestivum (Hawkins and Lewis, 1993). The effect of salinity on photosynthetic rate depends on salt concentration and plant species. For instance, in Brugueira parviflora, photosynthetic rate increased at low salinity and decreased at high salinity, whereas stomatal conductance remained unchanged at low salinity and decreased at high salinity (Parida et al., 2004).

According to Yeo et al. (1991) and Alarcon et al. (1994) growth is reduced more rapidly at lower concentrations of sodium in the leaf than is photosynthesis, indicating that plants can withstand a certain loss in photosynthetic rate without any impact on growth. The relationship between photosynthesis and growth of plants under saline conditions is not well understood. Many changes take place in plants in order to enable them to tolerate saline conditions and maintain photosynthetic activity. An understanding of the mechanisms by which salinity affects photosynthesis would help in the improvement of growth conditions and crop yield and would provide useful tools for future genetic tailoring of plants.

2.2.4 Ion levels and nutrient content

High NaCl uptake competes with the uptake of other nutrient ions, such as K⁺, Ca²⁺, Mg²⁺, N and P resulting in nutritional disorders and eventually reduced yield and quality (Grattan and Grieve, 1999). Increased NaCl concentration has been reported to induce increases in Na⁺ and Cl⁻ and decreases in Ca²⁺, K⁺ and Mg²⁺ level in a number of plants like Lycopersicum esculentum (Perez-Afocea et al., 1996), Haloxylon recurvum (Khan et al., 2000), Phaseolus species (Bayuelo-Jiménez et al., 2003), Beta vulgaris (Ghoulam et al., 2002), Vigna radiata (Mohammed, 2007) and Oryza sativa (Sultana et al., 2001). A significant increase in Na⁺ and Cl⁻ content in leaves, stem and root of the mangrove
Salt induced physio-chemical changes in mungbean has been reported without any significant alteration of the endogenous level of $K^+$ and $Fe^{2+}$ but decrease of $Ca^{2+}$ and $Mg^{2+}$ in leaves (Parida et al., 2004).

Under salt stress conditions, the uptake of N by plants is generally inhibited (Feigin et al., 1991; Pardossi et al., 1999; Silveira et al., 2001). An increase in Cl$^-$ uptake and accumulation has been observed to be accompanied by a decrease in shoot $NO_3^-$ concentration in *Solanum melongena* (Savvas and Lenz, 1996) and *Vitis vinifera* (Fisarakis et al., 2001). The nitrate influx rate or the interaction between $NO_3^-$ and Cl$^-$ has been reported to be related to the salt tolerance of the species under investigation. Kafkafi et al. (1992) found that the more salt-tolerant tomato and melon cultivars had higher $NO_3^-$ flux rates than the more sensitive cultivars.

The effect of salinity on P concentration is highly dependent on plant species, plant developmental stage, composition and level of salinity and the concentration of P in the substrate (Grattan and Grieve, 1999). In most cases, salinity decreased the concentration of P in plant tissue (Sonneveld and de Kreij, 1999; Kaya et al., 2001), but the results of some studies indicate salinity either increased or had no effect on P uptake (Ansari, 1990).

Salinity stress has stimulatory as well as inhibitory effects on the uptake of some micronutrients by plants. Nutrient imbalances may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant, or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant’s internal requirement for that essential element (Villora et al., 1997; Grattan and Grieve, 1999).

### 2.2.5 Plant growth

Salt stress (NaCl) caused decreases in seed germination, shoot and root lengths, fresh mass and seedling vigor in mungbean (Misra and Dwivedi, 2004), rice (Sultana et al., 2001),
Salt induced physio-chemical changes in mungbean

wheat (Mahatma et al., 2007), maize (Nawaz and Ashraf, 2007), tomato (Romero-Aranda et al., 2001), sugarbeet (Ghoulam et al., 2002) and cotton (Meloni et al., 2001). It also causes decrease in dry matter, biomass production and height of cereal and pulse crops (Kakkar et al., 2000; Raptan et al., 2001; Rabie, 2005). However, there are differences in tolerance to salinity among species and cultivars as well as among the different plant growth parameters. For instance, Aziz and Khan (2001) found that the optimum growth of Rhizophora mucronata plants was obtained at 50% seawater and declined with further increases in salinity while in Alhagi pseudoalhagi (a leguminous plant), total plant weight increased at Ca-salinity (50 mM NaCl) but decreased at high salinity (100 and 200 mM NaCl) (Kurban et al., 1999). Application of NaCl (ECc 4.0 mS cm\(^{-1}\)) resulted in about 52, 50 and 55 % reduction in total nitrogen contents in mung-bean leaf, root and nodule, respectively (Chakrabarti and Mukherji, 2003). In sugar beet, leaf area, fresh and dry mass of leaves and roots were dramatically reduced at 200 mM NaCl, but leaf number was less affected (Ghoulam et al., 2002). A larger decrease in accumulation of dry matter in shoots than in roots, particularly at high NaCl concentration, was recorded in Vitis vinifera, indicating partitioning of photo-assimilates in favour of roots, for osmotic adjustment under stress (Fisarakis et al., 2001).

### 2.2.6 Carbohydrate metabolism

Various stressful conditions of the environment adversely affect metabolism of sugars and partitioning of photosynthates in growing plants. Contents of soluble sugars increased under salinity (Dubey, 1997), water stress (Foyer et al., 1998) and in chilling environment (Hurry et al., 1995). Furthermore, salt stress reduced total soluble sugars, proteins, free amino acids and nucleic acids in mungbean (Rajan et al., 2000; Chakraborty and Mukherjee,
2003; Mohammed, 2007) and lentil (Zayed and Zeid, 1988; Yupsanis et al., 2001). On the contrary increased accumulation of sugars has been reported in many crop plants (Flowers et al., 1977) and decrease in polysaccharide contents as observed in maize (Khodary, 2004; Abdel-latif, 2007) and rice exposed to salinity due to altered sugar metabolizing enzymes (Djanaguiraman et al., 2006; Pattanagul & Thitisaksakul, 2008). Among the other significant salt stress consequences are changes in contents of sucrose and reducing sugars as well as in sucrose synthase (SS), sucrose phosphate synthase (SPS) and acid invertase activities (Elavumoottil et al., 2003, Kaur et al., 2003). Foyer and Ferrario (1994) reported that the SPS activity seems to be an adaptive control in photosynthesis, assimilate partitioning, carbon export and biomass production under salinity condition. Balibrea (2000) also found a significant positive correlation between SPS activity and sucrose contents of resistant genotype of tomato under salt stress while, it was absent in susceptible genotypes. According to Doehlert and Choureay (1991) the activity of hydrolytic enzymes viz., acid, neutral invertases and SS could be used as indicator for the ability of sink organ to grow and import sucrose under salinity. Salinity causes a reduction in sink activity leading to an increase in sucrose in source leaves and also a decrease in photosynthetic rate by feed back inhibition (Stitt, 1991).

2.2.7 Respiration

Seedling respiration was decreased as salinity level was increased due to the reduction in photosynthesis and increase in respiration per unit of leaf area. It has been reported that increasing salt concentration in growing medium of pea plants increased roots and stems respiration (Livne and Levin, 1966). Nieman (1962) found that growth is inhibited by salt partly due to the diversion of energy from growth to maintenance. The Krebs cycle is composed of a series of enzyme catalyzed reactions and it is of central importance in all
living cells. Pyruvate, the end product of glycolysis is further converted to acetyl coenzyme A, which is the main input of energy in several steps of the Krebs cycle. The reaction is catalyzed by the pyruvate dehydrogenase enzyme complex. Pyruvate dehydrogenase, the first component enzyme of this complex, contributes to linking the glycolytic pathway to the Krebs cycle and releases energy via NADH. Other three important steps in the cycle are conversion of isocitrate to α-keto glutarate catalyzed by isocitrate dehydrogenase, succinate to fumarate catalyzed by succinate dehydrogenase with the generation of energy in the form of FADH$_2$ and malate to oxaloacetate catalyzed by malate dehydrogenase (Voet and Voet, 2004).

2.2.8 Salinity induced DNA, protein and lipid alteration

The effect of salinity stress in plants is mediated at least in part by an enhanced generation of reactive oxygen species (ROS) (Alscher et al., 1997), particularly in chloroplasts and mitochondria (Mittler, 2002). ROS that are also generated as secondary metabolites, endogenously or exogenously, constitute one of the primary causative agents of damage to cellular components such as DNA, proteins and lipids (Lopez et al., 2006). All of the primary components of DNA – the sugar residues, phosphodiester linkages, purine and pyrimidine bases that constitute the structural and functional attributes of the genome, can suffer from damage. The resulting DNA-lesions that vary from innocuous molecular changes to highly mutagenic or genotoxic alterations in the genome lead to genomic instability or genotoxic stress which may result in senescence, aging, change in gene expression, decrease in biodiversity and crop yield. Whereas most of the DNA-damage is repaired, error-prone DNA-repair or DNA-damage beyond repair triggers programmed cell death (Bray & West, 2005). However, less evidence has been given about the relationship between the oxidative stress and the DNA damages induced by salinity in plants. Of the various genotoxicity assays,
comet assay is gaining popularity because the assay is fast and simple to perform. Comet assay mostly carried out employing mammalian cells in vivo and in vitro has been extensively reviewed (Brendler-Schwaab et. al., 2005), but information available with respect to comet assay using plant cells is sparse. In the recent years the studies of plants on DNA level have become unavoidable approach in almost any research in plant genetics and physiology. Nowadays the method of comet assay is gaining enormous popularity due to its broad spectrum of applications. It is extensively used for genotoxicity testing of substances such as industrial chemicals, biocides, agrochemicals, food additives and pharmaceuticals (Brendler-Schwaab et. al., 2005).

Two dimensional protein gel electrophoresis reveals that application of salt to plants brings about a major change in the protein profile (Ramgopal and Carr, 1991). ABA accumulates during NaCl stress and synthesizes a 26 KD protein called osmotin, localized to the vacuole (Singh et al., 1987). LEA (late embryogenesis abundance) proteins, which are ABA-inducible group of proteins, are also induced by salinity (Sairam and Tyagi, 2004). Also three hydroxyl proline containing proteins were identified under NaCl stress and under transcriptional and post transcriptional regulation (Ericson and Alfinito, 1984; Ramgopal, 1987). Most of the salt induced proteins are involved in photosynthesis, photorespiration or oxidative stress pathways (Kim et al., 2005). Salinity is a quantitative trait and arrays of salt induced genes have been isolated from different plant materials (Bray et al., 1993).

Salt stress induces lipid peroxidation through enhanced ROS like O$_2^-$ (superoxide radical), OH (hydroxyl radical) and H$_2$O$_2$ (hydrogen peroxide) production (Liang et al., 2003; Zhang et al., 2006; Shi et al., 2007; Li et al., 2008), thus making the membranes leaky as evinced by increased electrolyte leakage in wheat (Devi et al., 2008), mungbean (Chakraborty and Mukherjee, 2003) and barley (Li et al., 2008). Lipid peroxidation is often used as an indicator of salt-induced oxidative damage (Elkahoui et al., 2005).
Malondialdehyde (MDA) is the decomposition product of polyunsaturated fatty acids in the biomembranes, used as an indicator of lipid peroxidation and tends to show greater accumulation under salt stress (Zhu et. al., 2008).

2.3 Salinity tolerance

Salinity tolerance may be defined as the ability of a plant to grow and complete its life cycle under stressful salt conditions like NaCl or with association of other salts. A variety of mechanisms contribute to salt tolerance (Gorham, 1995).

2.3.1 Morphological basis of salt tolerance

Two things are very important for the adaptation of a species under saline environment, one is control of water loss another is improved ionic balance. Many dicots and chenopods halophytes show succulence and xero-morphism in response of salinity stress during adaptation as NaCl presence caused succulence in cotton, tomato and Salicornia (Blits and Gallagher, 1991). It causes many structural changes as smaller leaves with reduction in number, fewer stomata, thickening of leaf cuticles and earlier lignifications of roots. These adaptations may play an important role in maintaining tissue water contents or succulence but depend on the plant species and type and extent of salinity stress (Poljakoff-Mayber, 1975).

The leaf water contents in wheat are not affected by salinity but in case of radish and sunflower, salinity significantly decreases the leaf water contents (Heikal, 1977). It has also been observed in many crop species that succulence is correlated with increase in total leaf volume (Jennings, 1976), by increasing the cell size, and in this way there is more accumulation of Na\(^+\) and Cl\(^-\) in vacuole and finally vacuole-cytoplasm ratio is increased (Gorham et al., 1985). In some halophytes, special structures can be observed such as salt glands and bladders or trichomes, where excessive salt is accumulated, restricting the
growing cells to exposure to the salts (Flowers *et al.*, 1977; Greenway and Munns, 1980). Salt glands have been found in wild rice (*Porteresia coarctata* Roxb) also (Bal and Dutt, 1986).

2.3.2 Physiological and biochemical basis of salt tolerance

2.3.2.1 Ion homeostasis and salt tolerance

Plants develop various physiological and biochemical mechanisms in order to survive in soils with high salt concentration. Maintaining ion homeostasis by ion uptake and compartmentalization is not only crucial for normal plant growth but is also an essential process for growth during salt stress (Niu Xiaomu *et al.*, 1995; Serrano *et al.*, 1999; Hasegawa, 2013). Irrespective of their nature, both glycophytes and halophytes cannot tolerate high salt concentration in their cytoplasm. Hence, the excess salt is either transported to the vacuole or sequestered in older tissues which eventually are sacrificed, thereby protecting the plant from salinity stress (Reddy *et al.*, 1992; Zhu, 2003).

Major form of salt present in the soil is NaCl, so the main focus of research is to study the transport mechanism of Na\(^+\) ion and its compartmentalization. The Na\(^+\) ion that enters the cytoplasm is then transported to the vacuole via Na\(^+\)/H\(^+\) antiporter. Two types of H\(^+\) pumps are present in the vacuolar membrane: vacuolar type H\(^+\)-ATPase (V-ATPase) and the vacuolar pyrophosphatase (V-PPase) (Dietz *et al.*, 2001; De Lourdes Oliveira Otoch *et al.*, 2001; Wang *et al.*, 2001). Of these, V-ATPase is the most dominant H\(^+\) pump present within the plant cell. During nonstress conditions it plays an important role in maintaining solute homeostasis, energizing secondary transport and facilitating vesicle fusion, while under stressed condition the survivability of the plant depends upon the activity of V-ATPase (Wang *et al.*, 2001). In hypocotyls of *Vigna unguiculata* seedlings and halophyte *Suaeda salsa*, the activity of V-ATPase pump increased when exposed to salinity stress, while V-PPase played a minor role (De Lourdes Oliveira Otoch *et al.*, 2001; Wang *et al.*, 2001).
Increasing evidence demonstrates the roles of a Salt Overly Sensitive (SOS) stress signalling pathway in ion homeostasis and salt tolerance (Hasegawa et al., 2000; Sanders, 2000). The SOS signalling pathway (Figure 1) consists of three major proteins, SOS1, SOS2 and SOS3. SOS1, which encodes a plasma membrane Na\(^+\)/H\(^+\) antiporter, is essential in regulating Na\(^+\) efflux at cellular level. Overexpression of this protein confers salt tolerance in plants (Shi et al., 2000; Shi, 2002). SOS2, which encodes a serine/threonine kinase, is activated by salt stress elicited Ca\(^+\) signals (Liu et al., 2000). The third type of protein is the SOS3 protein which is a myristoylated Ca\(^+\) binding protein, playing an essential role in conferring salt tolerance (Ishitani et al., 2000). SOS2 protein contains a FISL motif (also known as NAF domain), which serves as a site of interaction for Ca\(^{2+}\) binding SOS3 protein (Figure 1). This interaction between SOS2 and SOS3 protein results in the activation of the kinase (Guo et al., 2004). The activated kinase then phosphorylates SOS1 protein thereby increasing its transport activity which was initially identified in yeast (Quintero et al., 2002).

![Figure 1: Model of SOS pathway for salinity stress responses.](image)

Besides conferring salt tolerance it also regulates pH homeostasis, membrane vesicle trafficking and vacuole functions (Quintero et al., 2011; Oh, 2010). Thus with the increase in the concentration of Na\(^+\) there is a sharp increase in the intracellular Ca\(^{2+}\) level which in turn
facilitates its binding with SOS3 protein. Ca\(^{2+}\) modulates intracellular Na\(^{+}\) homeostasis along with SOS proteins. The SOS3 protein then interacts and activates SOS2 protein by releasing its self-inhibition. The SOS3-SOS2 complex is then loaded onto plasma membrane where it phosphorylates SOS1 (Figure 1). The phosphorylated SOS1 results in the increased Na\(^{+}\) efflux, reducing Na\(^{+}\) toxicity (Martínez-Atienza et al., 2007).

Many plants have developed an efficient method to keep the ion concentration in the cytoplasm at a low level. The transport phenomenon is carried out by different carrier proteins, channel proteins, antiporters and symporters. Maintaining cellular Na\(^{+}\)/K\(^{+}\) homeostasis is pivotal for plant survival in saline environments. Plants maintain a high level of K\(^{+}\) within the cytosol of about 100mM and very low concentration of Na\(^{+}\) ion (about 1mM or less), ideal for cytoplasmic enzyme activities. K\(^{+}\) plays a major role in maintaining the turgor within the cell. It is transported into the plant cell against the concentration gradient via K\(^{+}\) transporter and membrane channels. The uptake mechanism is primarily determined by the concentration of K\(^{+}\) available in the soil. During salinity stress, due to increased concentration of Na\(^{+}\) in the soil, Na\(^{+}\) ion competes with K\(^{+}\) for the transporter as they both share the same transport mechanism, thereby decreasing the uptake of K\(^{+}\) (Munns and Tester, 2008; Sairam and Tyagi, 2004).

2.3.2.2 Compatible solute accumulation and osmotic protection

Compatible solutes, also known as compatible osmolytes, are a group of chemically diverse organic compounds that are uncharged, polar, soluble in nature and do not interfere with the cellular metabolism even at high concentration. They mainly include proline (Hoque et al., 2007; Ahmad et al., 2010; Hossain et al., 2011; Nounjan et al., 2012; Tahir et al., 2012), glycine betaine (Khan et al., 2000; Wang and Nii, 2000), sugar (Kerepesi and Galiba, 2000; Bohnert et al., 1995) and polyols (Ford, 1984; Dopp et al., 1985; Ashraf and Foolad,
The concentration of compatible solutes within the cell is maintained either by irreversible synthesis of the compounds or by a combination of synthesis and degradation. As their accumulation is proportional to the external osmolarity, the major functions of these osmolytes are to protect the structure and to maintain osmotic balance within the cell via continuous water influx (Hasegawa et al., 2000).

Amino acids such as cysteine, arginine and methionine, which constitute about 55% of total free amino acids, decrease when exposed to salinity stress, whereas proline concentration rises in response to salinity stress (El-Shintinawy and El-Shourbagy, 2001). Proline supplements enhanced salt tolerance in *Olea europaea* (Ben Ahmed et al., 2010), *Nicotiana tabacum* (Hoque et al., 2008) and *Oryza sativa* (Deivanai et al., 2011) by amelioration of some antioxidative enzyme activities, photosynthetic activity, plant growth and the preservation of a suitable plant water status under salinity conditions.

Glycine betaine is a nontoxic cellular osmolyte that raises the osmolarity of the cell during stress period, playing an important function in stress mitigation. Glycine betaine also protects the cell by osmotic adjustment (Gadallah, 1999), protein stabilization (Makela, 2000), ROS reduction (Ashraf and Foolad, 2007; Saxena et al., 2013) and protects the photosynthetic apparatus from stress damages (Cha-Um and Kirdmanee, 2010). Rahman et al. (2002) reported the positive effect of glycine betaine on the ultrastructure of *Oryza sativa* seedlings when exposed to salt stress.

Polyols are compounds with multiple hydroxyl functional groups available for organic reactions. Sugar alcohols are a class of polyols functioning as compatible solutes, as low molecular weight chaperones and ROS scavenging compounds (Ashraf and Foolad, 2007). They can be classified into two major types, cyclic (e.g., pinitol) and acyclic (e.g., mannitol) which are accumulated within the plant cell when the plant is subjected to salinity stress. These compatible solutes function as a protector or stabilizer of enzymes or membrane...
structures that are sensitive to dehydration or ionically induced damage. It was found that the accumulation of mannitol in both *Arabidopsis* and *Nicotiana tabacum* plants confer salt tolerance, thereby maintaining normal growth and development when subjected to high level of salt stress (Binzel *et al.*, 1988; Binzel *et al.*, 1995).

Accumulations of carbohydrates such as sugars (e.g., glucose, fructose, fructans and trehalose) and starch occur under salt stress (Parida *et al.*, 2004). The major role played by these carbohydrates in stress mitigation involves osmoprotection, carbon storage and scavenging of ROS. It was observed that salt stress increases the level of reducing sugars (sucrose and fructans) within the cell in a number of plants belonging to different species (Kerepesi and Galiba, 2000). Besides being a carbohydrate reserve, trehalose accumulation protects organisms against several physical and chemical stresses including salinity stress, playing an osmoprotective role in physiological responses (Ahmad *et al.*, 2013). Sucrose contents were found to increase in tomato under salinity due to increased activity of sucrose phosphate synthase (Gao *et al.*, 1998). Sugar content, during salinity stress, has been reported to both increase and decrease in various rice genotypes (Alamgir and Yousuf Ali, 1999). In rice roots it has been observed that starch contents decreased in response to salinity while it remained fairly unchanged in the shoot. Decrease in starch contents and increase in reducing and nonreducing sugar content were noted in leaves of *Bruguiera parviflora* (Parida *et al.*, 2004).

Organic acids like pyruvate, citrate, succinate and malate are of fundamental importance at the cellular level due to their participation in several biochemical pathways, involving energy production, formation of precursors for amino acid biosynthesis and modulating adaptation of the plant to the environment. Organic acids have a potential role to play as metabolically active solutes, having a function towards osmotic adjustment and the possible quenching of ill effects arising out of cation excess. These also participate as key
components in the mechanisms adopted by some plants to cope with nutrient deficiencies, metal tolerance and plant microbe interactions operating at the root-soil interface. The accumulation of pyruvate, citrate, fumarate and malate in plant tissues along with their excretion by roots lend support to their involvement in specific physiological functions (Lopez-Bucio et al., 2000).

2.3.2.3 Antioxidant regulation of salinity tolerance

Abiotic and biotic stress in living organisms, including plants, can cause overflow, deregulation, or even disruption of electron transport chains in chloroplasts and mitochondria. Under these conditions molecular oxygen acts as an electron acceptor, giving rise to the accumulation of ROS. Singlet oxygen, the hydroxyl radical, the superoxide radical and hydrogen peroxide are all strongly oxidizing compounds and therefore potentially harmful for cell integrity (Groß et al., 2013). Antioxidant metabolism, including antioxidant enzymes and nonenzymatic compounds, play critical role in detoxifying ROS induced by salinity stress. Salinity tolerance is positively correlated with the activity of antioxidant enzymes, such as

Figure 2: Antioxidant enzymes regulation under salt stress.
superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR) and with the accumulation of nonenzymatic antioxidant compounds (Asada, 1999; Gupta et al., 2005).

Anthocyanin is a flavonoid whose accumulation in plant exposed to salt stress has been largely documented and ascorbate is one of the major antioxidants present within the cell. Exogenous application of ascorbate mitigates the adverse effects of salinity stress in various plant species and promotes plant recovery from the stress (Agarwal and Shaheen, 2007; Munir and Aftab, 2011). Another antioxidant in stress mitigation is glutathione, which can react with superoxide radical, hydroxyl radical and hydrogen peroxide, thereby functioning as a free radical scavenger. It can also participate in the regeneration of ascorbate via ascorbate-glutathione cycle (Foyer et al., 1997). Application of glutathione and ascorbate was found to be effective in increasing the height of the plant, branch number, fresh and dry weight of marigold herbs and flowers, and the content of carbohydrates, phenols, xanthophylls and mineral ion content when subjected to saline condition (Rawia Eid et al., 2011). Many studies have found differences in levels of expression or activity of antioxidant enzymes. These differences in antioxidant activity between genotypes may be due to genotypic differences in degrees of stomatal closure or in other responses that alter the rate of CO₂ fixation and differences that bring into play the processes that avoid photoinhibition and for which the plant has abundant capacity (Munns and Tester, 2008). Roy et al. (2014) in their recent review have argued that there are three main traits in plants, which help them in their adaptation to salinity stress: ion exclusion, tissue tolerance and salinity tolerance. It seems that antioxidants have some role in tissue and salinity tolerance mechanism.
2.3.2.4 Hormone regulation of salinity tolerance

ABA is an important phytohormone whose application to plant ameliorates the effect of stress conditions. Salinity stress causes osmotic stress and water deficit, increasing the production of ABA in shoots and roots (He and Cramer, 1996; Cabot et al., 2009; Cramer and Quarrie, 2002; Kang et al., 2005). The accumulation of ABA can mitigate the inhibitory effect of salinity on photosynthesis, growth and translocation of assimilates (Popova et al., 1995; Jeschke et al., 1997). The positive relationship between ABA accumulation and salinity tolerance has been at least partially attributed to the accumulation of K\(^+\), Ca\(^{2+}\) and compatible solutes, such as proline and sugars, in vacuoles of roots, which counteract with the uptake of Na\(^+\) and Cl\(^-\) (Chen et al., 2001; Gurmani et al., 2011). ABA is a vital cellular signal that modulates the expression of a number of salt and water deficit-responsive genes. ABA treatment induced the expression of MAPK4-like, TIP 1 and GLP 1 genes in wheat and HVP1 and HVP10 for vacuolar H\(^+\)-inorganic pyrophosphatase in *Hordeum vulgare* under salinity stress (Fukuda and Tanaka, 2006; Keskin et al., 2010).

Some other compounds having hormonal properties, such as salicylic acid (SA) and brassinosteroids (BR), also participate in plant abiotic stress responses (Fragnire et al., 2011; Clause and Sasse, 1998). Under salinity stress endogenous level of SA increased along with the increase in the activity of salicylic acid biosynthetic enzyme in rice seedling (Sawada et al., 2006). *Arabidopsis* seedling pretreated with SA showed upregulation of H\(^+\)-ATPase activity, thereby improving K\(^+\) retention during salt stress (Jayakannan et al., 2013). The application of SA also promoted salinity tolerance in barley and mungbean cultivars, as manifested by increases in the chlorophyll and carotenoid contents, enhancing nitrogen and sulfur assimilation, antioxidant metabolism and maintaining membrane integrity, which was associated with more K\(^+\) and soluble sugar accumulation in the root under saline condition (El-Tayeb, 2005; Nazar et al., 2011). The negative effects of salinity may also be mitigated
by BR as its application enhanced the activity of antioxidant enzymes (SOD, POX, APX and GPX) and the accumulation of nonenzymatic antioxidant compounds (tocopherol, ascorbate and reduced glutathione) (El-Mashad and Mohamed, 2012; Anuradha and RamRao, 2001; Krishna, 2003; Ashraf et al., 2010).

2.4 Cultivation under saline conditions

Many plant species that tolerate high levels of salinity have been identified over and have been proposed as alternative crops for cultivation in saline conditions (Aronson, 1989; NAS, 1990; Yensen, 1999). Salinity management is required in most irrigated areas in the semi-arid regions of the world in order to sustain agricultural production. Drainage networks also facilitate the reuse of saline drainage water. Crop cultural practices to mitigate the effects of salinity have also been devised (Pasternak, 1987) and are widely applied.

But as salinity management practices are too expensive for a developing country like India, there is need to develop alternative methods of inducing salt tolerance which are economical and acceptable to the farmers. Plants can cope with the salt toxicity to some extent through physiological adaptation i.e., acclimation and also this ability can be enhanced through the application of some growth regulators and chemical compounds.

2.5 Induction of salt tolerance by exogenous factors

2.5.1 Salicylic acid

Gunes et al. (2007) proposed that salicylic acid acts as an endogenous signal molecule responsible for inducing abiotic stress tolerance in plants. The salicylic acid treatment reduced the damaging action of salinity on seedling growth and accelerated a restoration of growth processes in wheat (Afzal et al., 2005) and maize (Hussein et al., 2007). Salicylic acid solely or in combination with NaCl, induced an increase in relative water content of the barley leaves, increased leaf diffusive resistance, lowered transpiration rate (El-Tayeb, 2005),
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significantly stimulated the activity of Rubisco and also increased chlorophyll and carotenoid contents. Such treatment also decreased the level of soluble sugars, increased the polysaccharide contents, lowered the lipid peroxidation, strongly inhibited Na\(^+\) and Cl\(^-\) accumulation, while stimulated N, Mg, Fe, Mn and Cu accumulation. Salicylic acid improved the concentrations of arginine, lycine, serine, glutamic acid and proline in salt stressed maize plants (Sinha et al., 1993; Khodary, 2004; Gunes et al., 2007; Misra and Saxena, 2009), suggesting that salicylic acid could be used as a potential growth regulator to improve plant salinity stress resistance.

2.5.2 Growth regulators

Foliar spray of indole-3-acetic acid (IAA), gibberellic acid (GA\(_3\)) and kinetin (6-furfuryl aminopurine) growth regulators were able to overcome to a variable extents of adverse effects caused by NaCl stress in Vigna radiata (Chakrabarty and Mukherji, 2002). GA\(_3\) treatment improved the percentage of germination in salt stressed Vigna radiata and Phaseolus aureus (Misra et al., 1996; Rajan et al., 2000; Misra and Dwivedi, 2004), induced a marked increase in the level of pigments and reduction of accumulation of Na\(^+\) and Cl\(^-\) along with the increase of K\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) levels in salt stressed mungbean and wheat (Aldesuquy, 1992; Aldesuquy and Ibrahim, 2001; Abdel Haleem, 2007), partially decreased total polyamine contents and accumulated specific proteins having molecular weight of 21.3 KDa and 99.1 KDa, that might be involved in mungbean for tolerance to salinity (Abdel Haleem, 2007). Kinetin reversed NaCl effects by retarding chlorophyll degradation (Itai and Benzioni, 1976) and enhancing protein synthesis in pea plant (Kahane and Poljakoff-Mayber, 1968). IAA pretreatment totally altered the salt stress induced reduction of CO\(_2\) uptake in mungbean (Chakrabarti and Mukherji, 1994). Treatment with phytohormones decreased
MDA and total peroxide content in salt stressed mungbean leaves by maintaining higher activities of superoxide dismutase, catalase and peroxidase (Chakrabarti and Mukherji, 2003).

2.5.3 Minerals/ Nutrients

Foliar application of nutrient solutions viz., Ca(NO$_3$)$_2$, MnSO$_4$ and K$_2$HPO$_4$ partially altered NaCl-induced detrimental effects on growth in rice by decreasing the nutrient demand and increasing the rate of photosynthesis (Sultana et al., 2001; Colmer et al., 1996; Liu and Zhu, 1997). Yield and yield contributing characters were improved to some extent with the application of increasing levels of potassium in mungbean (Kabir et al., 2004). The addition of calcium either as CaCl$_2$ or CaSO$_4$ increased shoot and root dry weights and leaf chlorophyll contents in snapbeans (Akhavan-Kharazian et al., 1991), increased seedling growth, leaf area, proline and glycinebetaine content, increased superoxide dismutase, ascorbate oxidase and catalase activities in mungbean (Manivannan et al., 2007), and decreased peroxidase activity in the roots of rice seedlings (Lin and Kao, 1999), thus improved oxidative stress condition under salt stress. Manganese was responsible for increasing the photosynthesis in salt-stressed barley plant as Mn$^{2+}$ bind firmly to the lamellae of chloroplasts, affecting the chloroplast structure and photosynthesis (Cramer and Nowak, 1992; Lidon and Teixerira, 2000).

2.5.4 Triazole compounds

Triazole is a pair of isomeric chemical compound having a five membered ring of two carbon atoms and three nitrogen atoms, generally used as antifungal drugs and plant protection fungicides. Triazole compounds improved growth rate in salt stressed plants. Bitertanol (a triazole fungicide) improved the percentage of germination, increased the level of chlorophyll, ascorbic acid, α-tocopherol, sugar and also the activity of amylase and...
peroxidase in salt stressed mungbean seedlings (Rajan et al., 2000). Treatments with paclobutrazol and LAB 150978 increased chlorophyll and carotenoid content in corn and mungbean seedlings respectively (Reddy and Vora, 1986; Saha and Gupta, 1993). Triadimefon and triadimenol treatments increased the nucleic acid contents in the primary leaves of Barley (Foster, 1977). In mungbean, LAB 150978 treatment increased the free amino acid and proline content, which might act as an osmoticum to counteract salinity stress (Saha and Gupta, 1993).

2.5.5 Polyamines, Proline, Glycine-betaine and Arginine

An increase in polyamine levels is believed to be beneficial for stress resistance. Exogenous application of polyamines has often been advocated as a means of ameliorating the detrimental effects of salinity.

The protective potentiality of exogenously applied polyamines, viz., spermidine and spermine, in mitigating NaCl toxicity and inducing short-term salinity tolerance in three indica rice varieties, namely M-1-48 (salt-sensitive), Nonabokra (salt-tolerant) and Gobindobhog (highly sensitive) was observed as the inhibition of root or shoot growth was appreciably reduced, the chlorophyll degradation was prevented, malondialdehyde/H$_2$O$_2$ accumulation or lipoxygenase activity was reduced, inhibited the extent of salt-induced protein carbonylation and enhanced protease and peroxidase activities, and also compensated for the decreased catalase activity (Roychoudhury et al., 2011). Pretreatment with putrescine improved germination percentage in mungbean and wheat (Kakkar and Rai, 1992), increased fresh weight, dry weight and grain yield, prevented degradation of chlorophyll, inhibited the Na$^+$ and Cl$^-$ uptake, accelerated the accumulation of K$^+$, Ca$^{2+}$, Mg$^{2+}$, proline and endogenous putrescine, and altered the reduction of soluble protein, total protein, RNA and DNA contents in rice seedlings (Krishnamurthy, 1991) under salt stress. The adverse effects of salt on the
growth of maize plants were significantly mitigated by the foliar application of glycine-
betaine through improved leaf water potential and leaf turgor potential (Yang & Lu, 2005). 
Furthermore, glycine-betaine has a protective role in preventing cell injury from salt stress as 
has been reported in wheat (Wyn Jones et al., 1984). Exogenously applied proline was found 
to be effective in alleviating the adverse effects of salt stress on seed germination and total 
chlorophyll contents in sorghum (Nawaz et al., 2010). NaCl induced growth inhibition in rice 
seedlings can be partially alleviated by the application of arginine, by maintaining high water 
content (Kakkar et al., 2000).

2.5.6 Hydrogen peroxide (H$_2$O$_2$) and Nitric oxide (NO)

In wheat seedlings under salt stress, addition of H$_2$O$_2$ improved growth and restored 
favorable ionic balance, caused a partial reversal of NaCl induced membrane leakiness, 
increased catalase, ascorbate peroxidase, glutathione reductase activities and also ascorbate 
contents (Devi et al., 2008). Exogenous supplementation of NO as sodium nitroprusside had 
a positive effect on relative water contents in chickpea and maize plants (Sheokand et al., 
2010; Zhang et al., 2006). NO also decreased the H$_2$O$_2$ levels and partially alleviated the 
NaCl induced oxidative damage by increasing superoxide dismutase, dehydro ascorbate 
reductase and catalase activities in chickpea and barley (Sheokand et al., 2010; Li et al., 
2008), ascorbate peroxidase and glutathione reductase activities in wheat (Hai-Hua et al., 
2005) and in barley leaves (Li et al., 2008) under salt stress.

Some findings even suggest that crops like shorgum, soybean and rice can acclimate 
to lethal level of salinity by pretreatment with sublethal level of salinity (Amzallag et al., 
1990; Umezawa et al., 2000; Djanaguiraman et al., 2006).
2.6 Future prospects

Upcoming years in future may incorporate the integrated efforts considering planning of soil and site specific requirements of deploying strategies discussed above to enhance the yield considering sustainable agriculture, incorporating tolerant varieties within the reach of farmers. Attempts have sought and being sought to look for future food security at physiological, biochemical and molecular levels. However, an integrative and feasible management still required to meet with presently available plant preventive strategies for ‘salt amalgamated with stress hindered production’.

Therefore, induction of salt tolerance in economic crops like legumes by application of some alternative method like preconditioning with sublethal stress is the most desired outcome that will be both cost effective and farmer friendly and hence will serve for the welfare of mankind.