2 Review of Literature

Many species of higher plants, including most crops, are subjected to growth inhibition under high-sodium chloride conditions. The salt-induced inhibition of plant growth, so-called salt stress, is caused not only by osmotic effects on water uptake but also by variable effects on plant cell metabolism under salt stress. While the first component can bring about water deficit, the excess of a specific ion can cause toxicity and or induce nutritional disorders. Natural boundaries imposed by soil salinity also limit the caloric and the nutritional potential of agricultural production. These constraints are most acute in areas of the world where food distribution is problematic because of insufficient infrastructure or political instability (Yokoi *et al*., 2002). Water and soil management practices have facilitated agricultural production on soils marginalized by salinity but additional gain by these approaches seems problematic. On the horizon, are crop improvement strategies that are based on the use of molecular marker techniques and biotechnology can be used in conjunction with traditional breeding efforts (Hussain *et al*., 2010).

Nearly one billion hectare of land is affected by salinity or sodicity out of 13 billion hectares of land on the earth. India, for instance has about 7 million hectare of cultivable land affected by salinity. In Tamil Nadu, area affected by salinity is one lakh hectares. The districts where the problem soils are most prevalent are Chengalpattu, Salem, Tanjavur, Trichy, North Arcot, Thirunelveli, Dharmapuri and Ramanathapuram (Vadivel *et al*., 2001).

Almost three quarters of the surface of the earth is covered by salt water and so it is not surprising that salts affect a significant proportion of the world's land surface. These salt affected areas fall broadly into two categories: sodic and saline. Sodic soils are dominated by excess sodium ion exchange sites and a high concentration of carbonate/bicarbonate anions; they have a high pH (greater than 8.5 and perhaps up to 10.8) with a high sodium absorption ratio (SAR) and poor soil structure. Saline soils are again generally dominated by sodium ions, but with the dominant anions being chloride and sulphate; pH values and SARs are much lower and electrical conductivities higher (>4 dS m^{-1}) than in sodic soils.

Salt-affected soils contain sufficient concentrations of soluble salts to reduce the growth of most plant species (Flowers and Flowers, 2005).

2.1. Drought and Salinity

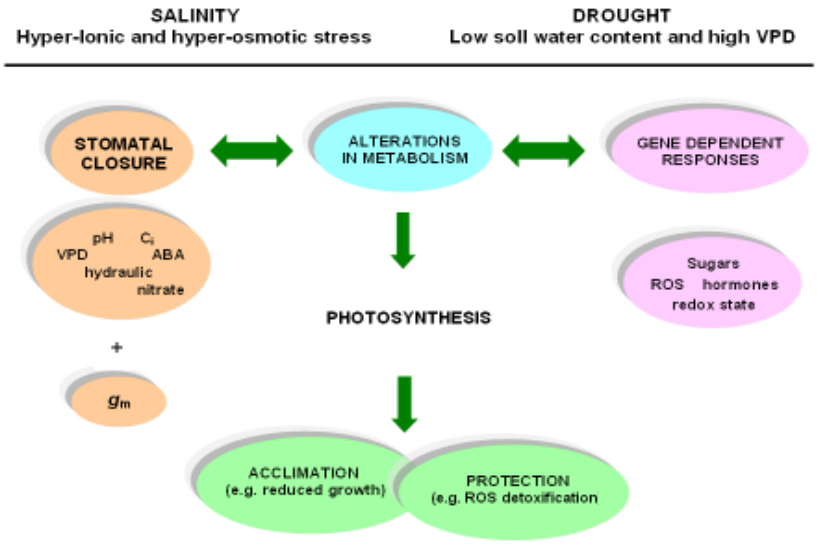
Early responses to water and salt stress have been considered mostly identical (Munns, 2002). Drought and salinity share a physiological water deficit that affects, more or less intensely, all plant organs. However, under prolonged salt stress, plants respond in addition to dehydration to hyper-ionic and hyper-osmotic stress. Leaf tissue water deficit per cell can be triggered not only by low soil water content but also by high vapour pressure deficit of the atmosphere. In addition to alterations in photosynthesis and cell growth, both stresses when slowly imposed often induce osmotic adjustment which is considered an important mechanism to allow the maintenance of water uptake and cell turgor under stress conditions. The effects of drought and salinity on photosynthesis range from the restriction on carbondioxide diffusion into the chloroplast, via limitations on stomatal opening mediated by shoot- and root-generated hormones and on the mesophyll transport of carbondioxide, to alterations in leaf photochemistry and carbon metabolism. These effects were found to vary according to the intensity and duration of the stress as well as with the leaf age (older leaves are more affected by drought and accumulate higher amounts of salt) and the plant species (Lawlor and Cornic, 2002; Munns, 2002; Chaves *et al*., 2003; Flexas *et al*., 2004; Galme's *et al*., 2007).

Under salinity, in addition to water deficits, plants endure salt-specific effects. Salt response follows a biphasic model, with current metabolic data indicating an early similarity with drought, whereas in the long-term, plants are responding to ion toxicity. There are species-specific responses to salt. Some plants are able to prevent salt entry (salt exclusion at the whole plant or the cellular level) or to minimize its concentration in the cytoplasm (by compartmentalizing salt in the vacuoles), thus avoiding toxic effects on photosynthesis and other key metabolic processes. When those processes do not exist or are insufficient, it was shown that sodium chloride at a concentration above 100 mM severely inhibited many enzymes (including photosynthetic ones) (Munns *et al*., 2006).

The enzymes that require potassium as a cofactor and the primary metabolites linked to aminoacid and nitrogen or carbohydrate and polyol metabolism in plants increase when affected by salt. The compatible solutes play a role in osmotic adjustment, membrane and protein protection or scavenging of reactive oxygen species (ROS) and of excess accumulated ammonium ions. In salt affected plants, a depletion of organic acids is observed which might be involved in compensating for ionic imbalance (Sanchez *et al*., 2007). There is closure of stomata and there is impairment of photosynthetic apparatus in response to leaf turgor decline, to high vapour pressure deficit in the atmosphere or to root generated chemical signal.

Figure 1

Physiological effects of drought



Direct effects of drought and salinity on stomata and mesophyll (g_m) conductance as well as on gene expression, resulting in alterations of photosynthetic metabolism and ultimately on plant acclimation (C_i – intracellular CO_2, ABA – Abscisic acid, VPD – vapour pressure deficit, ROS – reactive oxygen species)

Under mild stress, a small decline in stomatal conductance may have protective effects against stress, by allowing plant water saving and improving water-use efficiency by the plant as opined by Flexas *et al*. (2004 and 2007). Although not as straight forward as stomatal conductance measurements,

estimations of mesophyll conductance seem appropriate despite many assumptions involved in the most common methods used (Warren, 2006). These changes in mesophyll conductance may be linked to physical alterations in the structure of the intercellular spaces due to leaf shrinkage (Lawlor and Cornic, 2002) or to alterations in the biochemistry (bicarbonate to carbondioxide conversion) and/or membrane permeability (aquaporins). The physiological effect of drought is illustrated in Figure 1.

Most of the internal resistance to carbondioxide diffusion was found to be in the liquid phase inside cells instead of in intercellular air spaces, i.e. not so much dependent on leaf structure and later studies specifically suggested that mesophyll conductance was depressed under both salt (Centritto *et al*., 2003) and water stress (Flexas *et al*., 2002; Galme's *et al*., 2007). Flexas *et al*. (2006) have shown that germination responds very quickly (within a few minutes) to desiccation after cutting the leaf petiole in air and that reduced germination can be induced by exogenous application of abscisic acid (ABA) to well watered plants. In addition, germination also responds quickly to changes in temperature, light and carbondioxide concentration. All together, these results strongly suggested that germination is regulated biochemically, rather than simply by leaf anatomical traits (Flexas *et al*., 2008).

2.1.1. Halophyte Salt Tolerance

Halophytes are plants that are able to grow in habitats excessively rich in salts, such as salt marshes, sea coasts and saline or alkaline semi deserts. These plants do not have special physiological adaptations that enable them to grow in salt affected soils under seawater irrigation and can produce relatively high consumable biomass in saline areas where non-halophytic species cannot grow nor have low dry matter yields. Therefore, halophytes may be considered as a supplementary feed source under arid and semi-arid conditions (Tawfik *et al*., 2011).

For convenience, plants have been divided into two groups: the salt-sensitive glycophytes and the salt-tolerant halophytes, although in reality one group

merges into the other. The primary environmental factor faced by plants growing in salt marshes and salt deserts is the high concentration of salts that they encounter. For these saline environments (that is excluding the extreme salt deserts), the plant water potential must then be lowered by an equivalent of up to about 500 mM NaCl (Flowers, 1985), which is achieved through adjustment of plant water and solute content. Osmotic adjustment is achieved by compartmentation which takes place in the cytoplasm and the salt necessary for osmotic adjustment is stored in vacuoles (Flowers *et al*., 1986).

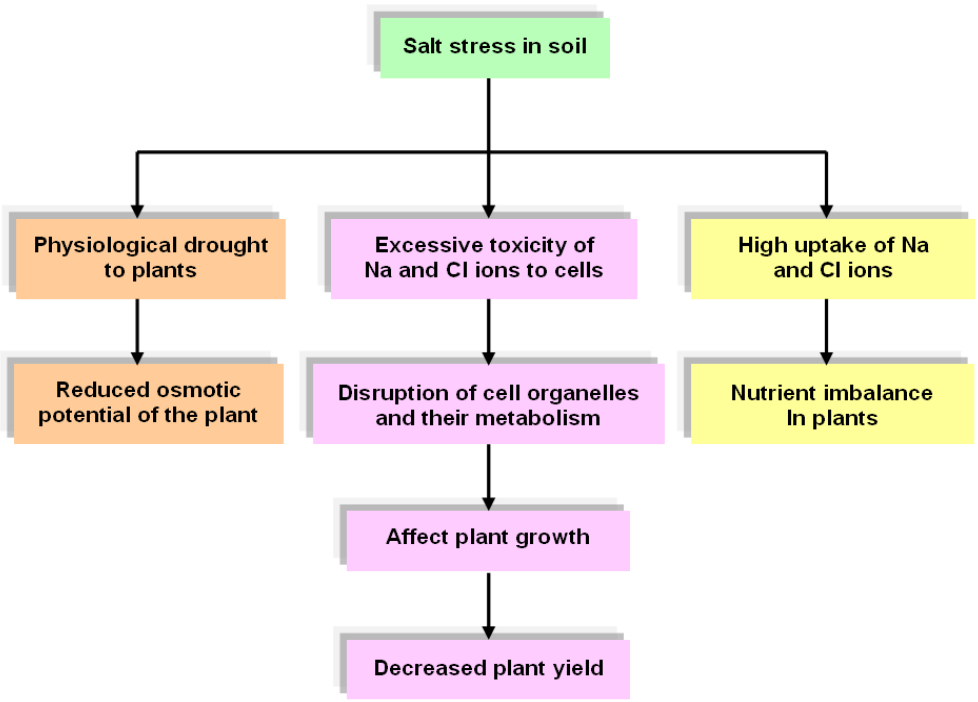
Within the cytoplasm, osmotic adjustment is effected by compatible solutes like organic compounds, such as glycinebetaine, mannitol and proline, which do not damage the metabolism (Rathinasabapathy, 2000). The process of compartmentation requires that halophytes have a mechanism to maintain differences in ion concentration across the membrane that surrounds their vacuoles which depends on membrane structure (Leach *et al*., 1990) and on the proteins that transport ions across membranes. Ions enter plant cells through proteins that form an integral part of cell membranes. These proteins can either form channels through which ions diffuse down electrochemical potential gradients or carriers, where the protein binds an ion on one side of the membrane and releases it on the other side. Both processes are driven by energy-consuming ion pumps. These proteins use the energy stored in ATP to move protons across the membrane generating a difference of hydrogen ion concentration (pH) and electrical potential (DE). It is the difference in electrical potential that drives the inward movement of cations through channels and the difference in hydrogen ion concentration that drives the movement of ions through carriers to which protons and ions bind. In spite of considerable knowledge of the way in which potassium ions crosses membranes, it is not clear how sodium (or chloride) enters plant cells. Although it is generally thought that sodium is ‘mistaken’ for potassium by potassium carriers or channels, it is also possible that sodium enters cells through non-selective cation channels, particularly those activated by glutamate (Demidchik *et al*., 2002; Maser *et al*., 2002).

2.1.2. Glycophytes affected by Salinity

Halophytes, although widespread taxonomically, are relatively rare amongst the 250,000 species of flowering plants and virtually all of our crops are glycophytes. There is, however, considerable variability in the tolerance of these glycophytes to salt. Variation occurs between species and has been quantified for crops (Francois and Maas, 1994; Maas and Hoffmann, 1977) and within species (Bernstein and Ayers, 1953; Datta, 1972; Flowers and Yeo, 1981; Greenway, 1962). Glycophytes, if they are to survive salinity, must adapt to the external salt concentrations; they face the same problems as those faced by halophytes. Salinity has three potential effects on plants: (1) lowering of the water potential, (2) direct toxicity of any sodium and chloride absorbed and (3) interference with the uptake of essential nutrients. The overall impact of salt stress in soil is shown in Figure 2.

Figure 2

Overall impact of salt stress on cultivable soil



The latter might not be expected to have an immediate effect on plant growth as plants have reserves of nutrients that they can mobilise. However, when salt is first encountered by a plant, there are two phases to its response. The first

phase is a response to the changed water relations brought about by the lowering of the external water potential by the salt. These initial effects of salinity (phase 1, due to a change in water potential) are likely to be the same for cultivars of differing salt tolerance. Only as ions are accumulated over time (phase 2) does true difference in salt tolerance appear. Sensitive cultivars accumulate ions more quickly than tolerant cultivars and this ion accumulation leads to leaf death and progressively, death of the plant (Munns, 2002).The adaptations required to survive in salt-affected soils are the same in all plants. Such adaptations are at their most extreme in halophytes, but can be found to differing degrees, in glycophytes. Salt tolerance depends upon: morphology; compartmentation and compatible solutes; regulation of transpiration; control of ion movement; membrane characteristics; tolerating high Na/K ratios in the cytoplasm;salt glands. With so many factors involved, it is to be expected that salt tolerance would depend on the action of many genes.

The direct effects of salt on plant growth are suggested to involve:

(a) Reduction in the osmotic potential of the soil solution that reduces the amount of water available to the plant causing physiological drought (Jahromi *et al*., 2008);

(b) Toxicity of excessive Na and Cl_2 ions towards the cell – the toxic effects include disruption to the structure of enzymes and other macromolecules, damage to cell organelles and plasma membrane, disruption of photosynthesis, respiration and protein synthesis (Feng *et al*., 2002); and (c) Nutrient imbalance in the plant caused by nutrient uptake and/or transport to the shoot leading to ion deficiencies.

To deal with saline soils and minimize crop loss, scientists have searched for new salt-tolerant crop plants and developed salt-tolerant crops through breeding (Adiku *et al*., 2001).

2.1.3. Genetics of Salt Tolerance

The basic resources for biotechnology are genetic determinants of salt tolerance and yield stability. Implementation of biotechnology strategies to achieve this goal requires that substantial research effort be focused to identify salt

tolerance effectors and the regulatory components that control these during the stress episode (Hasegawa *et al*., 2000). Also genetic informations and details about these stress tolerance determinants will be useful for the study of the plant response to salinity. This might reveal how plants sense salt stress, send signals to develop a defensive role and define the metabolic signal pathway outputs that accomplish the processes required for stress survival and alleviation and ultimately the growth in the saline environment (Yokoi *et al*., 2002).

2.2. Adaptive Mechanism of Plants to Salinity Stress

Plants develop a plethora of biochemical and molecular mechanisms to cope with salt stress. Biochemical pathways leading to products and processes that improve salt tolerance are likely to act additively and probably synergistically. Biochemical strategies include (i) selective accumulation or exclusion of ions, (ii) control of ion uptake by roots and transport into leaves, (iii) compartmentalization of ions at the cellular and whole-plant levels, (iv) synthesis of compatible solutes, (v) change in photosynthetic pathway, (vi) alteration in membrane structure, (vii) induction of antioxidative enzymes, and (viii) induction of plant hormones (Parida and Das, 2005).

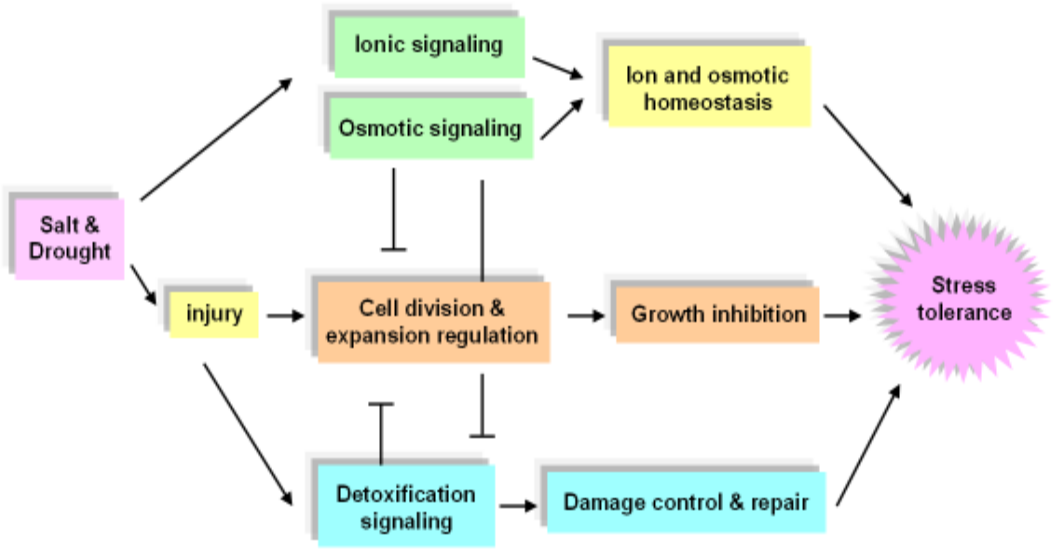
These are extremely complex and an array of mechanism appears to be involved in salt tolerance of plants. Mechanisms for tolerance of the salt specific effects of salinity are of two main types: Those minimizing the entry of salt into the plant and those minimizing the concentration of salt in the cytoplasm. Overall effect of salt stress on plant and their adaptive mechanism can be studied under the following heads:

2.2.1. Osmotic Effect

The inputs for ionic and osmotic signaling pathways are ionic (excess NaCl) and osmotic (e.g., turgor) changes. The output of ionic and osmotic signaling is cellular and plant homeostasis. Direct input signals for detoxification signaling are derived stresses (i.e., injury), and the signaling output is damage control and repair (e.g.) activation of dehydration tolerance genes (Zhu, 2002). Figure 3 presents the functional demarcation of salt and drought stress signaling pathways.

Figure 3

Functional demarcation of salt and drought stress signaling pathways



Effects of salinity on plants are generally summarized as water stress, salt stress and ionic imbalance stress. Water stress (decrease in water potential) arises as a result of evaporation water used by plants and salt accumulation. Excess quantities of soluble salts in soil solution limit the availability of water to plants. Water potential and osmotic potential of plants become more negative with an increase in salinity, whereas turgor pressure increases with increasing salinity. Osmotic potential of cell sap changes as to maintain a constant water potential gradient between leaf and soil. Osmotic effects predominate when plant growth is related to osmotic potential of root medium that contains different salts or combination of salts. Energy expenditure during osmotic adjustment to abiotic stress is one of the main factors for reduced growth (Greenway and Gibbs, 2003).

The maintenance of turgor has been reported to be essential for keeping a normal cell activity which contributes to growth under low water availability. Osmotic adjustment (OA) has been reported to contribute to maintain the turgor pressure and has drawn much attention during the last years. It has been hypothesized that these compounds benefit stressed cells in two ways: by acting as cytoplasmic osmolytes, thereby facilitating water uptake and retention, and by

protecting and stabilizing macromolecules and structure (i.e. proteins, membranes, chloroplast, and liposomes) from damage induced by stress conditions (Martınez *et al*., 2004). Osmotic adjustment (OA) is usually achieved by uptake of inorganic ions i.e. K^+, Na^+ and Cl^- from the soil solution or synthesizing and accumulation of organic compounds as sugars and amino acids, mainly proline (Farouk, 2011). Energy is needed for the synthesis or transport of solutes for osmotic adjustment (Munns, 2002). The excessive accumulation of ions may disrupt the balance of the absorption and the function of other ions in the cell.

In mature leaf, OA plays an important role for plant cell survival, enabling higher stomatal conductance and leaf expansion (Westgate and Boyer, 1985) to sustain photosynthesis under stress conditions. It is accepted that during osmotic adjustment the cells tend to compartmentalize most of the absorbed ions in vacuoles at the same time that they synthesize and accumulate compatible organic solutes in the cytoplasm in order to maintain the osmotic equilibrium between these two compartments (Hasegawa *et al*., 2000). As a consequence of solute accumulation, the osmotic potential of the cell is lowered, which, in turn, attracts water into the cell and thereby, tends to maintain its turgor. In fact, OA is an effective component of salt tolerance, which has a positive direct or indirect effect on plant productivity, because it contributes to the maintenance of turgor and cell volume (Ludlow and Mu-Chow, 1990).

2.2.2. Specific Ion

Detrimental effect of salt may be due to the toxicity of specific ion, elevation of osmotic pressure or the increase in alkalinity which may restrict the availability of water or influence cellular physiology and metabolic pathway. Specific ion toxicity is usually associated with excessive intake of chloride, sodium or other ion and hence might cause nutritional imbalance. One of the most detrimental effects of salt stress is to disrupt the ion homeostasis mechanisms of the plant. In particular, the similar radii of sodium and potassium make it difficult for transport proteins to distinguish between these two ions. Thus under conditions of high external sodium, there is substantial uptake of sodium through potassium transporters or channels (Blumwald *et al*., 2000).

2.2.3. Compartmentalization and Osmolytes

Plants restrict the excess salts in the vacuole or compartmentalize the ions in different tissues to facilitate their metabolic functions (Zhu, 2003). There are several possible strategies that plants could employ to avoid a damaging decrease in the K^+/Na^+ ratio: reduce entry of Na^+ into the cell, remove Na from the cell, or compartmentalize Na into the vacuole where it cannot disrupt cellular function (Surekha *et al*., 2005).

A major category of organic osmotic solutes consists of simple sugars (mainly fructose and glucose),sugar alcohols(glycerol and methylated inositols) and complex sugars (trehalose, raffinose and fructans).Others include quaternary amino acid derivatives (proline, glycine, betaine),tertiary amines(1,4,5,6-tetrahydro-2-methyl-4-carboxyl pyrimidine) and sulfonium compounds (dimethyl sulfonium propionate) (Zhifang and Loescher, 2003).

Proline content increases with increase in salinity as an adaptive change in metabolic pattern. Salt stress is complex and imposes a water stress. This water deficit leads to the formation of Reactive Oxygen Species (ROS) such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen. The activities of antioxidative enzymes such as catalase, ascorbate peroxidase, guaicol peroxidase, glutathione reductase and superoxide dismutase (SOD) increase under salt stress (Mittova *et al*., 2002, 2003).

2.2.4. Oxidative Stress

Salt stress, like other abiotic stresses, can also lead to oxidative stress through the increase in Reactive Oxygen Species(ROS),such as superoxide, hydrogen peroxide and hydroxyl radicals which are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Pastori and Foyer, 2002; Apel and Hirt, 2004).

To overcome the effects of salinity induced oxidative stress, plants make use of a complex antioxidant system, which is composed of low molecular mass antioxidants (glutathione, ascorbate and carotenoids) as well as ROS scavenging enzymes. The SOD may function as a ROS scavenger by converting O_2 to H_2O_2 (Alscher *et al*., 2002). Recent studies have demonstrated that over expression of

mitochondrial Mn-SOD in transgenic *Arabidopsis thaliana* (Wang *et al*.,2004) and chloroplastic Cu/Zn-SOD in transgenic *Nicoyiana tabacum* (Badawi *et al*.,2004) can provide enhanced tolerance to salt stress. Similar results have been found in *Morus alba* (Ramajulu and Sudhakar, 2001), *Triticum aestivum* (Sairam and Tyagi, 2004) and *Lycopersicon sp*. (Mittova *et al*., 2002). Bacterial catalase and Glutathione-S-Transferase / glutathione peroxidase were reported to increase the performance of plants under stress (Roxas *et al*., 2000).

2.2.5. Metabolic Adaptations

Cellular level adaptations are the main responses amenable to molecular analysis and have led to the identification of a large number of genes induced by salt. Functional groups responsible for encoding salt stress proteins are genes for: photosynthetic enzymes, synthesis of compatible solutes, vacuolar sequestering enzymes and radical scavenging enzymes (Kawasaki *et al*., 2001).

High salt (NaCl) uptake competes with the uptake of other nutrient ions, especially potassium, leading to potassium deficiency. Increased treatment of sodium chloride induces increase in sodium and chloride and decrease in calcium, potassium and magnesium levels in a number of plants. Salinity enhances the content of sodium, calcium and chloride and the ratio of K^+/Na^+ decreases in *Vicia faba* and Chikpea (Mudgal *et al*., 2009).

Salinity stress was found to cause an increase in the levels of Na^+ and Cl^- in guava and the highest ion accumulation was found in the leaves followed by the roots. The Ca^{++} levels were stable in the roots but decrease in stems and leaves and the K^+ content was reduced with increased levels of salinity, particularly in the leaves. There was a positive relationship recorded between Na^+ and Cl^- and a negative relationship between Na^+ and K^+ concentration in the roots and leaves (Beckman *et al*., 2008).

Nitrate reductase activity (NRA) of leaves were reported to decrease in many plants under salt stress. The NRA decrease in root and shoot of pea plant led to accumulation of NO^{3-} and NH^{4+} nitrogen (Garg *et al*.,2001).Salinity was found to inhibit nitrogen fixation by reducing nodulation and nitrogenase activity in chickpea (Mudgal *et al*.,2009).

2.3. Effect of Salinity on different growth Stages of Plants

2.3.1. Effect on Germination

Germination is the series of steps that proceed to protrusion of the radical. The phase of germination and seedling growth is critical one. Germination under saline conditions is generally affected due to high osmotic pressure of the solution. This osmotic pressure results in less inhibition of water. Due to capillary rise of salts, the concentration of salts is more at sea depth than at lower levels in soil profile. Salinity has been shown to affect the time and rate of germination (Mudgal, 2004).

Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations were reported to affect seed germination, water deficit, cause ion imbalance of the cellular ions resulting in ion toxicity and osmotic stress (Khan *et al*., 2002; Khan and Panda, 2008).

Salinity can affect germination of seeds either by creating osmotic potential which prevents water uptake, or by toxic effects of ions on embryo viability. Marked reduction in germination of bean (*phaseolus* vulgaris L) exposed to salt stress was reported by Kaymakanova (2009).

Salinity was reported to impair seed germination, reduce nodule formation, retard plant development and reduce crop yield. The plants that grew in saline soils had diverse ionic compositions and a range in concentrations of dissolved salts. These concentrations fluctuated because of changes in water source, drainage, evapotranspiration and solute availability (Jamil *et al*., 2006).

It was reported by Zeynalabedin and Jafari (2002), that salinity has negative relationship with germination, germination rate, root length, shoot length, fresh root weight and fresh shoot weight.

2.3.2. Seedling Growth

In most crops, seedling growth is more sensitive to salt stress than the other growth stages. It is reported that soil salinity causes greater reduction in shoot growth than in root growth (Ramoliya and Pandey, 2003).

Seedling length was found to decrease with increase in salinity indicating the adverse effect of salinity on the growth of bread wheat cultivars. Root and shoot length were found to be decreased significantly by saline irrigation of high electrical conductivity as compared to control. Length of root and shoot were reported to decrease perhaps due to accumulation of ions near the root surface in wheat (Akbarimoghaddam *et al*., 2011). According to Gholipoor *et al*. (2000), salt stress decreased seedling growth in chickpea. Miled *et al*. (2000) indicated that seedling growth as measured by radicle length was severely reduced by NaCl doses higher than 50 mM in rapeseeds. Root and shoot length were decreased by increasing NaCl concentration. Furthermore, the shoots were more sensitive than roots. Saboora and Kiarostami (2006) also observed the same result.

As NaCl concentration increased, it affected shoot and root dry weight. Reduction of dry weights was found to depend on shoot or root lengths (Ghoulam and Fares, 2001). Anuradha and Rao (2001) reported that 24 – epibrassinolide and 28 – homobrassinolide were found to reverse the inhibitory effect on germination and seedling growth due to salinity stress in rice.

2.4. Morphological Characters of Plants

2.4.1. Plant Height

Salt generally alters a wide array of metabolic processes culminating in stunted growth, reduced enzyme activities and photosynthetic carbon metabolism. Metabolic stress caused by sodium chloride may result in decreased plant growth. A remarkable decrease in shoot to root ratio of soyabean was observed with increase in salinity indicating that salinity had more pronounced effect on shoot growth than that of root growth (Dolatabadian *et al*., 2011).

Burman and Kathju (2001) found that plant growth in terms of height decreased with increasing Sodium adsorption ratio (SAR) in all the genotypes of Indian mustard under sodicity stress. Growth of asparagus and tomato was suppressed in the saline soil and strongly suppressed in saline – sodic and sodic soils (Jumberi *et al*., 2002).

2.4.2. Root Length

Root morphology is not only a very important factor for nutrient absorption by roots, but also it is very important for water uptake by roots from saline soils (Schleiff, 2008). Nizam (2011) reported that there were statistically significant differences in root length of perennial ryegrass for various NaCl levels.

Roots are directly exposed to the saline environment. Root growth was reported to be affected less at lower salinity levels (Dai *et al*., 2009). Alshammary *et al.* (2004) reported that plant roots are the first organ to become exposed to salinity and root growth is particularly sensitive and is rapidly reduced or prevented by salinity.

Khan *et al*. (2003) indicated that there were significant differences in absolute and relative root lengths among 100 maize accessions and the reduction in root length was due to increased salt concentration.

2.5. Growth Parameters of Plants

2.5.1. Leaf Area

Leaf area is one of the best parameters to represent the effect of salt stress on plants as suggested by Neto and Tabosa (2000). Burman and Kathju (2001) stated that plant growth in terms of leaf area was decreased with increasing Sodium Adsorption Ratio in Indian mustard. Sodium chloride salinity had a major effect on decreasing the leaf area in bitter almond (Najafian *et al*., 2008). Similar results have been reported by Bray and Reid (2002). Increasing salinity significantly decreased leaf area both at vegetative and flowering stages in mustard (Garg *et al*., 2006). It was reported by Vadez *et al*. (2005), that the reduction in leaf area in plants under salinity stress indicated arrest of leaf expansion, which eventually might limit the area available for photosynthesis.

2.5.2. Leaf Characters

The leaf characters and physiological growth attributes may be important criteria for a tolerant variety (Alamgir and Ali, 2006). Salinity stresses significantly accelerated the senescence at reproductive period. Salt-tolerant cultivars could alleviate this harmful effect by maintaining high leaf area index (LAI), leaf area

duration (LAD) and dry matter accumulation. LAI and LAD of salt-sensitive cultivars of winter wheat were larger than salt-tolerant cultivars in control. However, both were decreased rapidly under salt stress condition (Zheng *et al*., 2008). Foliar spray of brassinosteroid showed an improvement in leaf area index in rice (Maibangsa *et al*., 2000).

2.5.3. Total Dry Matter Production

Salinity is one of the major abiotic stresses affecting plant productivity. Salinity decreases plant dry matter and leaf area (Amirjani, 2011) and finally decreases crop yield. Garg *et al*. (2006) reported that increasing salinity due to saline water irrigation reduced the shoot dry matter progressively. The decrease in seed yield was 16.6 % and shoot dry weight was 23.9 % at the highest level of salinity (10 dSm^{-1}) as compared to control plants.

2.6. Physiological and Biochemical Parameters

2.6.1. Chlorophyll Content

Photosynthesis, together with cell growth, was found to be one among the primary processes to be affected by drought or by salinity (Munns *et al*., 2006) and was found to seriously affect leaf photosynthetic machinery (Ort, 2001). Chlorophyll pigment is responsible for photosynthesis. Destruction of chlorophyll under salt stress is deleterious to plant productivity.

Plants respond to water scarcity and salinity in such a way that depending upon the severity of stress, osmotic compounds build up and have a function in sustaining tissue metabolic activity. It also includes synthesis of compatible solutes as well as adjustments in ion transport which eventually leads to restoration of cellular homeostasis, detoxification and therefore survival of plant under stress as observed by Chaves *et al*. (2009).

Net photosynthesis, transpiration rate and stomatal conductance were found to be significantly affected by salt stress due to changes in chlorophyll content and chlorophyll fluorescence, damage of photosynthetic apparatus and chloroplast structure (Abd El Baki *et al*., 2000; Fidalgo *et al*., 2004; Kao *et al*., 2003; Pinheiro *et al*., 2008). It was reported that both chlorophyll a and chlorophyll b amounts were

decreased with NaCl application in *Zea mays* and *Carthamus tinctorius* plants (Sepehr and Ghorbanli, 2006, Siddiqi *et al*., 2009).

Arshi *et al*. (2002) and Sahoo *et al*. (2001) observed a major decline in chlorophyll content in senna and in rice cultivars under 100 mM NaCl. Albassam (2001) reported that salinity decreased the total chlorophyll content in pearl millet. Similar result was observed by Demiroglu *et al*. (2001) in maize and Pushpam and Rangasamy (2000) in rice cultivars. Suwa *et al*. (2006) reported that the apparent photosynthetic rate remained similar in both control and salt treated plants during the first 4 and 8 days for 50 and 100µmoll^{-1} NaCl treatments, respectively. However, activity in the treated plants declined on the following days and remained lower than the control in tobacco plants.

Sodicity induced changes in total chlorophyll a level was less in sodicity resistant genotypes compared to susceptible Indian mustard (Burman and Kathju, 2001)**.** Maximum chlorophyll content was observed in plants sprayed with brassinosteroid at 1.00 mg/l twice on 25 and 35 DAS in groundnut (Prakash *et al*., 2003). 28 – homobrassinolide application enhancing leaf chlorophyll a, b and total contents and net photosynthesis by 50.3, 32.4, 13.6 and 68.8 % respectively over control in mustard (Hayat *et al*.,2001).

2.6.2. Soluble Protein

Salt stress was found to significantly decrease soluble protein content after exposure to 50,100 and 150mM NaCl and the effect was aggravated with time from 42 to 72 hr in *Phaseolus vulgaris* (Fusun *et al*., 2004). Soybean plants exposed to 100 and 200 mM NaCl showed significant decrease in their protein content by 20.3% and 41.7% (Moussa, 2004). Muthukumarasamy *et al.* (2000) reported remarkable decrease in the protein content of salt stressed radish plants. Similarly the decline in total soluble protein content was shown in *Lycopersicon esculentum*, *Oryza sativa*, *Vicia faba, Amaranthus tricolor* and *Brugiera parviflora* plants under NaCl stress (Al-aghabary *et al*., 2004; Parida and Das, 2005; Parvaiz and Satyavati, 2008; Wang and Nil, 2000).

According to Burman and Kathju (2001), sodicity induced changes in soluble protein content in Indian mustard. There was a remarkable reduction in protein

content. Decreased soluble protein content in salt stress was due to its decreased *de novo* synthesis as there was a marked change in protein synthesizing apparatus of plant tissue which decreased considerably the capacity for protein synthesis. The reduction in soluble protein level with associated increase in free amino acid under salt stress might be attributed both to disruption in protein synthesis as well as enhanced proteolysis. The depression in soluble protein content was less severe when brassinolide was supplemented (Bera *et al*., 2006). Maximum soluble protein content was observed in plants sprayed with brassinosteroid at 1.00 mg l^{-1} twice on 25 and 35 days after sowing (Prakash *et al*., 2003). Maibangsa *et al*. (2000) reported that brassinolide (0.5 ppm) spray increased the photochemical efficiency and RUBISCO activity in rice resulting in high yield.

2.6.3. Nitrate reductase Activity (NR)

Nitrate reductase (NR, E.C.1.6.6.1), the first enzyme in the nitrate assimilation pathway, is a limiting factor of plant growth and development and also it is influenced by a variety of environmental factors (Sengar *et al*., 2008).It was reported that NR activity was found to be increased with exogenous nitrate concentration (Jha *et al*., 2007). The effect of salt stress on nitrate reductase activity might be attributed to inhibition of enzyme induction. Abd El-Baki *et al.* (2000) reported that application of salinity decreased NR activity in maize. Nitrate reductase activity was increased significantly in plants subjected to 100mM NaCl, while it was decreased gradually under 150 and 200 mM treatments compared to control. Decrease in NR activity due to salinity stress was also accompanied with significant decrease in nitrate uptake and total nitrogen content of canola (*Brassica napus* L.) (Bybordi and Ebrahimian,2011). The loading of nitrate into the root xylem was also thought to be a highly salt-sensitive step (Tischner, 2000). The nitrate contents of roots and leaves were decreased by 16.8% and 23.6% respectively at 10mM NaCl compared to the control (Khan and Srivastava, 2000). Foliar application of 0.5ppm brassinosteroid showed higher chlorophyll content, soluble protein content and NRase activity in soybean (Senthil *et al*., 2003).

2.6.4. Gas Exchange Parameter

The reduction in photosynthesis under salt stress was associated with a decrease in stomatal conductance particularly at 10dSm^{-1} salinity level. The

detrimental effects of salt stress on photosynthesis due to stomatal and non stomatal effects are well documented (Garg *et al*., 2001).The amount of photosynthates reaching the growing region would have been decreased because of the inhibition of photosynthesis under salt stress due to stomatal closure or by the direct effect of salt on the photosynthesis apparatus (Garg *et al*., 2006). According to Kerstiens *et al*. (2002), in *Aster tripolium* grown at high salinity, stomatal closure would have been induced by the presence of sodium ions in the apoplast surrounding the guard cells. The occurrence of this system in *Aster tripolium* and not in the closely related glycophyte *A. amellus* suggested that it could be an important factor in the network of physiological attributes required for salt tolerance.

2.6.5. Proline

During osmotic stress, plant cells accumulate low molecular weight osmolytes to prevent water loss and maintain turgor. High level synthesis of osmoprotectants and the key genes encoding enzymes were found to increase proline accumulation (Hong *et al*., 2000). Proline serves as a storage sink for carbon and nitrogen and a free-radical scavenger. It also stabilizes sub cellular structures and buffers cellular redox potential under stress and hence, proline is known as osmo protectants (Murata *et al*., 2000). Proline content was increased with increasing salt concentration in soybean, barley and in sunflower as reported by Fedina *et al*. (2002) and Santos *et al*. (2002).

Evidence supporting the role of proline during salt stress was obtained on the basis of salt tolerance in transgenic tobacco plants with enhanced levels of proline biosynthesis. Transgenic plants grown in 200mM NaCl displayed a 6-fold increase in proline content compared with plants grown in low salinity. Girija *et al*. (2002) reported that proline content was highest in the presence of NaCl in both cotyledons and the embryonic axis of peanut seedlings. Due to salt treatment significant accumulation of proline was observed, which was probably associated with osmotic adjustments and the protection of membrane integrity and reduced peroxidative damage to the lipid membranes in groundnut (Jain *et al*., 2001). According to Aazami *et al*. (2010), an increase in proline content under salinity

stress was probably due to the capacity of some plants to accumulate organic (sucrose, fructose and glucose) and inorganic (Na, K and Cl) metabolites in the cytoplasm to reduce the water potential and change the osmotic gradient, assuring the water flow to the plant and thereby might increase tolerance. The proline content of the roots was increased by treatment with NaCl for two weeks, but the increment was lower than that in the shoots. Further increases in the duration of NaCl treatment beyond two weeks did not result in an appreciable increase in proline content in the shoots or roots in casuarina (Tani and Sasakawa, 2006).

2.6.6. Indole acetic acid (IAA) oxidase Activity

IAA plays a major role in regulating plant growth. It controls vascular tissue development, cell elongation and apical dominance (Wang *et al*., 2001). IAA also responds to salinity in crop plants. However, little information seems to be available on the relationship between salinity stress and IAA levels in plants and the role of IAA in alleviating salt stress (Javid *et al*., 2011). The IAA concentration in leaves of *P. vulgaris was* decreased with the increase in salt concentration from 50 mM to 150mM NaCl (Fusun *et al*., 2004). Vidyavardini (2012), reported that Brassinolide application resulted in reduction in the activity of indole acetic acid oxidase in the two varieties of sorghum plants ('CSH-5' and 'CSH-6') grown in four saline experimental sites in Karaikal compared to the control plants.

2.6.7. Acid phosphatase Activity (AP)

High concentration of salt was reported to cause ion imbalance and hyperosmotic stress to plants due to which it decreased dry matter and leaf area (Amirjani, 2011) and ultimately decreased crop yield. As a consequence of these primary effects, secondary stress such as oxidative damage was noted. Against these abiotic stresses, plants are suggested to adapt themselves by different mechanisms including change in morphological and developmental pattern as well as biochemical responses (Bohnert *et al*., 2005). Adaptation to all these stresses is associated with metabolic adjustments that lead to the modulation of different enzymes (Shinozaki and Shinozaki-Yamaguchi, 1996; Ehsanpour and Amini, 2003). Quantitative and qualitative changes in the activity of enzymes isolated from plants subjected to salt stress have been reported (Yu and Rengel, 1999;

Benavides *et al*., 2002). Acid phosphatase is known to act under salt stress by maintaining a certain level of inorganic phosphate which could be co-transported with H^+ along a gradient of proton motive force. In contrast, a few reports indicate that acid phosphatase activity is independent of phosphate level (Arab and Ehsanpour, 2006).

Abiotic stresses like salt stress was observed to affect the physiological biochemistry of plant cells under *in vivo* and *in vitro* conditions. Salt stress was reported to enhance acid phosphatase activity in wheat (Barrett-Lennard *et al*., 1982). Similar result was reported by Shakeel and Mansoor (2012) where salinity caused increase in AP activity which was increased with increase in the concentration of salt. These findings were also supported by Ahmad and Ebrahimian (2011), who reported that forty five days after salinity induction, NaCl caused a significant increase in AP activity in treated canola plants with 150 and 200 mM NaCl in comparison with control treatment.

2.6.8. Polyphenol oxidase (PPO) Activity

Niknam *et al*. (2006) reported that salt-stress induced PPO activity in calli and seedlings of *Trigonella aphanoneura* and seedlings of *T. foenum-graecum*. Similar result was reported by Aghaleh and Niknam (2009) in soybean. Demir and Kocacaliskan (2001) stated that PPO activities were higher in all the tissues treated with NaCl in bean seedlings. When $CaCl_2$ was combined with NaCl, polyphenol oxidase and catalase activities were higher than in NaCl-stressed plants of *Dioscorea rotundata* (Jaleel *et al*., 2008). Similar results were reported in groundnut (Sulochana *et al*., 2002).

2.6.9. Other Antioxidant Enzymes

An important consequence of salinity stress in plants is the excessive generation of reactive oxygen species (ROS) such as super oxide anion (O_2^-), H_2O_2 and the hydroxyl radicals (OH^-) particularly in chloroplast and mitochondria (Mittler, 2002; Neill *et al*., 2002). Generation of ROS such as super oxide, H_2O_2 and hydroxyl molecules causes rapid cell damage by triggering a chain reaction (Hernandez *et al*., 2001; Imlay, 2003). Plants under stress produce some defense mechanisms to protect themselves from the harmful effect of oxidative stress.

ROS scavenging is one of the common defense responses against abiotic stresses (Vranova *et al.*, 2002). The major ROS scavenging activities include complex non-enzymatic (ascorbate, glutathione, a-tocopherol) and enzymatic responses includes catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), super oxide dismutase (SOD) (Prochazkova *et al*., 2001). It was suggested that antioxidant mechanisms might provide a strategy to enhance salt tolerance in plants. The metabolism of active oxygen species, such as hydrogen peroxide, is dependent on various functionally interrelated antioxidant enzymes, such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase and peroxidase. It was reported that reactive oxygen species, including superoxide and hydrogen peroxide, were elevated with increased salinity, due to the imbalance in the production and destruction of reactive oxygen species (Harinasut *et al*., 2003).

Ascorbate peroxidase and super oxide dismutase, exist in several isoforms and are found in cytosol, mitochondria, plastids etc. (Ishitani *et al*., 1997; Noctor and Foyer, 1998). The increase in enzyme activity with external salinity might be due to increased synthesis of the enzymes. Transgenic plants over expressing ROS scavenging enzymes, such as super oxide dismutase (Alscher *et al*., 2002), ascorbate peroxidase and glutathione S-transferase / glutathione peroxidase (Roxas *et al*., 2000) showed increased tolerance to osmotic and oxidative stresses.

According to Jebara *et al*. (2005), salt treatment generated an activation of SOD and peroxidase activity in bean nodules. Similar result was observed in rice by Tsai *et al*. (2005) and Neto *et al*. (2005) in maize plants.

High salinity reduced SOD activity in both roots (28% in high salinity) and in leaves (16 % at low and 38 % at high salinity), but low salinity led to an increase in root SOD activity up to 2.85 %. In leaves, the peroxidase activity was decreased under salinity and in roots it showed a slight increase of up to 10 % in low salinity and a 29% decrease in high salinity when compared to the control plants as reported by Abdul *et al*.(2007). Similar observations were recorded in wheat (Muthukumarasamy *et al*., 2000; Sreenivasulu *et al*., 2000; Rout and Shaw, 2001; Sairam and Srivastava, 2002) and rice (Pal *et al*., 2004). Higher peroxidase activity

particularly in tolerant cultivar was suggested as an indication to prevent degradation of membrane integrity of the cells against free radicals formed under salt stress in rice varieties (Bera *et al*., 2006). The changes in catalase would vary according to the intensity of stress, time of assay after the stress and induction of new isozymes (Shim *et al*., 2003). Bera *et al*. (2006) explained the positive role of growth regulators in enhancing the peroxidase activity.

2.7. Mineral Nutrition

Increasing salinity progressively and significantly decreased the concentrations of nitrogen, phosphorus and potassium and increased sodium in the shoot tissue. At the salinity level of 0 dSm^{-1}, there was an average decline in the concentration of nitrogen, phosphorus and potassium about 22 % at the flowering stage, whereas sodium concentration was increased by 219 % compared to control in mustard (Garg *et al*., 2006). The maintenance of higher concentrations of nitrogen, phosphorus, potassium and lower concentration of sodium in salt stressed plants was found to be essential to overcome nutrient imbalance and carry out their normal physiological processes. This phenomenon was also observed in Indian mustard. Salt stress conditions are well known to adversely affect N uptake and metabolism in majority of plant species (Burman *et al*., 2002) grown under saline water irrigation.

The detrimental effects of salt on plants are a consequence of both water deficit that results from the relatively high solute concentrations in the soil and a Na^+ specific stress resulting from altered K^+ / Na^+ ratios and Na^+ ion concentrations that are inimical to plants. The alteration of ion ratios in the plant is caused by the influx of Na^+ through pathways that function in the acquisition of K^+. The leaf and root K^+ contents of the transgenic plants grown in 200 mM NaCl were lower than those from plants grown in low salinity. Under high salinity conditions, Na^+ ions might displace K^+ from its carrier binding sites and this competition would have resulted in impaired K^+ uptake and lower K^+ cytosolic concentrations as suggested by Hong- Xia Zhang *et al*. (2001). Jumberi *et al*. (2002) reported that Na concentration in the shoots of the vegetable crops was higher with higher exchangeable sodium percentage in sodic soil.

According to Maeda *et al*. (2005), alleviatory effect of Ca on salt stress might be due to the reduction of sodium absorption and transfer from roots to shoots (Ochiai and Matoh, 2002), the maintenance of an appropriate intracellular concentration of potassium and the improvement of the electro static interactions of Na and Ca in the cell walls and cell membranes (Murata *et al*., 2000). Under salinity stress deposition rates for N, K, P, S, Mg, Zn, Mn and Cu was reduced by 30 – 60%, while Ca deposition was dramatically reduced by 90% in maize (Beatriz and Bernstein, 2005).

2.8. Yield and Yield Components

Under salt stress condition, the onset of flowing is delayed due to the limitations of source size. The quantum of reproductive structure such as number of flowers / panicle is very much reduced. Due to high deposition of salts in tissues, most of the metabolic processes such as synthesis of proteins, amino acids, sugars, starch and other organic compounds are altered. This disturbance in the normal metabolism affects the mobility of metabolites from the site of production to the site of utilization for reproductive growth. Therefore the development of reproductive structures and further maturation processes are very much affected which ultimately diminish the crop yield. Due to imbalance of nutrients under salt stress, hormone synthesis is hampered which may lead to reduction in quantity as well as quality of crop produce (http://agritech.tnau.ac.in/agriculture/ agri_salinity_growth_devep.html). It was reported by Bybordi (2010) that there was a decrease in yield components of canola cultivars due to an increment of salinity stress and there was significant difference among cultivars in aspect of the number of silique in plant, number of seed in silique, 1000 seed weight and seed yield.

Iqbal and Ashraf (2006) found that increase in salinity resulted in significant decrease in grain yield of wheat. Similar result was observed in Indian mustard by Burman and Kathju (2001). Colla *et al*. (2006) reported that the total yield of grafted watermelon plants were reduced in higher salinity level. Pod number per plant and seed yield were significantly increased by 29.1 % over control in homobrassinolide treated mustard plants (Hayat *et al*., 2001).

Wang *et al*. (2001) clearly defined an increase in abscisic acid in response to salinity, and a decline in indole-3-acetic acid (IAA) and salicylic acid (SA). The exogenous application of plant growth regulators, auxins (Khan *et al*., 2004), gibberellins (Afzal *et al*., 2005), cytokinins (Gul *et al*., 2000) produced some benefit in alleviating the adverse effects of salt stress and also improved germination, growth, development and seed yields and yield quality (Egamberdieva, 2009).

2.9. Molecular Cellular Mechanisms for Salt Tolerance

High salinity causes hyperosmotic stress and ion disequilibrium that produced secondary effects or pathologies (Hasegawa *et al*., 2000; Zhu, 2001). Fundamentally, plants cope by either avoiding or tolerating salt stress. That is, plants are either dormant during the salt episode or there must be cellular adjustment to tolerate the saline environment. Tolerance mechanisms can be categorized as those that function to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effects caused by these stresses. The chemical potential of the saline solution initially establishes a water potential imbalance between the apoplast and symplast that leads to turgor decrease, which if severe enough can cause growth reduction. Growth cessation occurs when turgor is reduced below the yield threshold of the cell wall. Cellular dehydration was reported to begin when the water potential difference was greater than could be compensated for by turgor loss (Bohnert *et al*., 2005).

The cellular response to turgor reduction is osmotic adjustment. The cytosolic and organellar machinery of glycophytes and halophytes are suggested to be equivalently Na^+ and Cl^- sensitive; so osmotic adjustment was achieved in these compartments by accumulation of compatible osmolytes and osmoprotectants (Bohnert *et al*., 2005). However, Na^+ and Cl^- are energetically efficient osmolytes for osmotic adjustment and are compartmentalized into the vacuole to minimize cytotoxicity as reported by Blumwald *et al*. (2000) and Niu *et al*. (2005). Since plant cell growth occurs primarily because of directional expansion mediated by an increase in vacuolar volume, compartmentalization of Na^+ and Cl^- facilitates osmotic adjustment that is essential for cellular development. Movement of ions into the vacuole might occur directly from the apoplast into the vacuole through

membrane vesiculation or a cytological process that juxtaposes the plasma membrane to the tonoplast. Then compartmentalization could be achieved with minimal or no exposure of the cytosol to toxic ions. However, Hasegawa *et al*. (2000) opined that it is not clear presently the extent to which processes like these contribute to vacuolar ion compartmentalization.

The bulk of Na^+ and Cl^- movement from the apoplast to the vacuole is mediated through ion transport systems located in the plasma membrane and tonoplast. Presumably, tight coordinate regulation of these ion transport systems is required in order to control net influx across the plasma membrane and vacuolar compartmentalization. The SOS signal pathway is a pivotal regulator of, at least some, key transport systems required for ion homeostasis (Sanders, 2000 and Zhu, 2000).

2.9.1. Ion Homeostasis

In salt-affected soil, there are many salt contaminants, especially NaCl which readily dissolves in water to yield the toxic levels of the ions, sodium ion (Na^+) and chloride ion (Cl^-). Also, the water available in the salt-contaminated soil is restricted, inducing osmotic stress (Castillo *et al*., 2007; Pagter *et al*., 2009). Na^+ is a small molecule that is easily absorbed into root tissues of higher plants and transported through out plant organs, leading to toxic ion damage, osmotic stress and nutritional imbalance (Cha-um *et al*., 2007 and Siringam *et al*., 2009). Root tissues are the first barriers which not only select nutrient ions but also protect against toxic ions. Excess Na^+ in plant cells directly damages membrane systems and organelles, resulting in plant growth reduction and abnormal development prior to plant death (Essah *et al*., 2003; Tester and Davenport, 2003; Davenport *et al*., 2005; Quintero *et al*., 2007).

In halophyte species, there are many salt-defense mechanisms including ion homeostasis, osmoregulation, antioxidant and hormonal regulation (Sairam and Tyagi, 2004). Since NaCl is the principal component of soil salinity stress, a research focus has been the transport systems that are involved in utilization of Na^+ as an osmotic solute (Blumwald *et al*., 2000 and Niu *et al*., 2005).

The SOS stress-signaling pathway was identified to be a pivotal regulator of plant ion homeostasis and salt tolerance (Sanders, 2000). The Na^+ K^+ and membrane injury derived from Na^+ toxicity was demonstrated, leading to lower water content and growth inhibition. In addition, accumulation of soluble sugar contents was related to the increase of Na^+ which might play a role as an osmotic adjustment to maintain the water use efficiency in the root cells when exposed to salt stress (Siringam *et al*., 2011).

2.9.2. Ion Transport Systems

H^+ pumps in the plasma membrane and tonoplast energize the solute transport that is necessary to compartmentalize cytotoxic ions away from the cytoplasm and to facilitate the function of ions as signal determinants and he activity of these H^+ pumps was found to be increased by salt treatment that induced gene expression accounting for some of the up regulation (Maeshima, 2000; Maeshima, 2001; Morsomme and Boutry, 2000 and Ratajczak, 2000). The plasma membrane localized H^+ pump is a P-type ATPase and is primarily responsible for the high pH and membrane potential gradient across this membrane (Morsomme and Boutry, 2000). A vacuolar type H^+ - ATPase and a vacuolar pyrophosphatase were reprted to generate the pH and membrane potential across the tonoplast (Drozdowicz and Rea, 2001).

Recently, the plasma membrane H^+ ATPase was confirmed as a salt tolerance determinant based on analyses of phenotypes caused by the semi-dominant aha4-1 mutation (Vitart *et al*., 2001). The mutation to AHA4, expressed predominantly in the roots seemed to cause a reduction in root and shoot growth of plants that are grown on medium supplemented with 75 mM NaCl. Decreased root length of salt treated aha4-1 plants was found to reduce cell length. In NaCl supplemented medium, leaves of aha4-1 plants seemed to accumulate substantially more Na^+ and less K^+ than those of wild type. It was postulated that AHA4 functions in the control of Na^+ flux across the endodermis (Vitart *et al*., 2001).

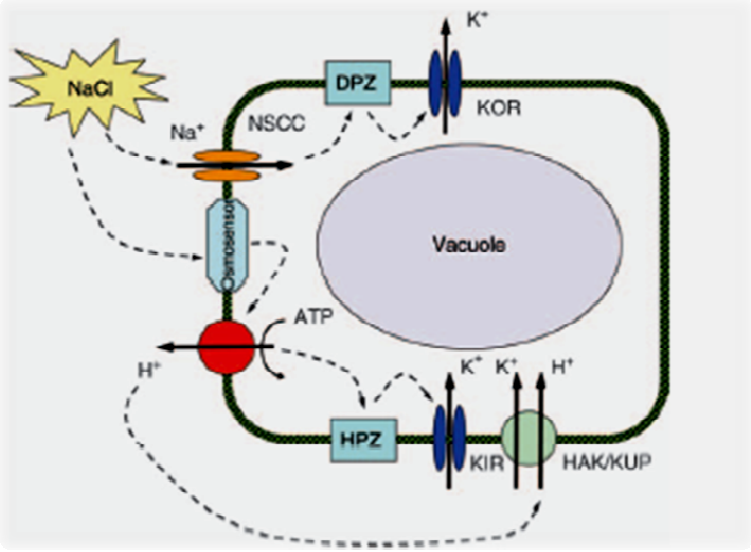
2.9.3. Transmembrane Sodium Movements

Blumwald *et al*. (1987) have claimed the existence of Na^+ /H^+ antiport in tonoplast vesicles of sugar beet based upon the response to Na^+ of pH-dependent

acridine orange fluorescence quenching. The Na^+ effect was increased by Na^+ pretreatment, sensitive to amiloride (an inhibitor of an analogous transporter in various animal systems) and to a number of promising amiloride analogs. Unfortunately, so far there are no reports which have included direct measurement of Na^+ fluxes or confirmation of these results with different probes. At the cellular level, a steady state must be maintained either by the very effective exclusion of Na^+ initially or by the extrusion or turnover of internal pools as even the most easily killed species have significant Na^+ levels in their roots. Though the evidence for the movement of Na^+ mechanism is incomplete, Na^+ movement involves mechanisms other than those mediated by transmembrane transporters; for example, it has been suggested that in unidirectional Na^+ movements, the fluxes may involve vesiculation and turnover of a sub-cytoplasmic compartment (Lazof and Bernstein, 1999). Figure 4 shows the cellular mechanisms involved in the perception of 'ionic' and 'osmotic' components of salt stress.

Figure 4

Cellular mechanisms involved in the perception of 'ionic' and 'osmotic' components of salt stress



NSCC – Non selective cation channels; KOR – outward rectifying K^+ channel ;
KIR – inward rectifying K^+channel; HAK / KUP – potassium transporters ;
DPZ – depoalrization

There are numerous complexities in the study of Na^+ uptake and organismal response, which cloud the interpretation of even apparently straightforward studies.

These include the interactions of Na^+, Ca^{2+}, K^+, membrane surface properties, root cell development and growth. Beyond this, the nature of the transport systems involved in the distribution and compartmentation of Na^+ at the organismal level is largely unknown. Though the potential, integrated system complexity is great, including, at least, sequestration within specific cells and tissues of the root, stem base and leaves and retransport from shoots or sequestered pools to the roots for excretion, it is possible to model the acquisition and allocation of Na without additional basic cellular-level transporting systems as opined by Cramer *et al.*(2007) .

2.9.4. Calcium Signaling and Salt Overly Sensitive (SOS) Signal Transduction Pathway

Three genetically linked Arabidopsis loci (SOS1, SOS2 and SOS3), which are components of a stress-signaling -pathway that controls ion homeostasis and salt tolerance has been identified (Hasegawa *et al*., 2000; Sanders, 2000; Zhu, 2000, 2001). Genetic analysis of Na^+ /Li^+ sensitivity established that SOS1 is epistatic to SOS2 and SOS3 (Zhu, 2000). Results indicated that the SOS signaling pathway regulates Na^+ and K^+ homeostasis and is Ca^{2+} activated (Ishitani *et al*., 2000).The SOS2 serine/threonine kinase (446 amino acids) has a 267 amino acid N-terminal catalytic domain that is similar in sequence to yeast SNF1 (sucrose nonfermenting) kinase and the mammalian AMPK (AMP-activated protein kinase) (Liu *et al*., 2000) which is essential for its salt tolerance determinant function (Zhu, 2000).

Among the SOS signal pathway outputs are transport systems that facilitate ion homeostasis. The plasma membrane sited Na^+ /H^+ antiporter SOS1 is controlled by the SOS pathway at the transcriptional and post-transcriptional level (Guo *et al*., 2001; Zhu, 2001). It was reported by Yokoi *et al*. (2002), that the SOS pathway negatively controls expression of AtNHX family members that are implicated as determinants in the salt stress response.

The experiments of Matsumoto *et al*. (2001) with yeast has provided insight into Ca^{2+} activation of salt stress signaling that controls ion homeostasis and tolerance. Components of the SOS pathway, either SOS3 or upstream elements,

might be associated with an osmotically responsive channel through which Ca^{2+} influx could initiate signaling through the pathway. It has been reported by Hussain *et al*. (2010), that, Ca^{2+} has two roles in salt tolerance, a pivotal signaling function in the salt stress response leading to adaptation and a direct inhibitory effect on a Na^{+} entry system.

2.9.5. Genetic and Physiological Mechanisms that control Stress Tolerance

The physiological mechanisms underlying crop responses to stress and potential biochemical, physiological and architectural modifications that will allow crops to escape, avoid or tolerate stress are the subject of a vast literature. Two general approaches are taken in relation to varietal improvement. The first is the 'empirical' approach that proceeds from genotypic differences associated in the cultivated crop or its wild relatives. The sources of tolerance are identified and then the underlying genetic control is investigated by quantitative trait loci (QTL) analysis in lines segregating for high and low tolerance (Gale, 2003).

The second approach is described as 'ideotype' breeding, in which specific morphologies or physiologies that might be expected to contribute to improved performance under stress are identified in diverse cultivated or wild germplasm and transferred to otherwise adapted varieties. The crossbreeding and marker-aided pyramiding of the underlying alleles is progressed in the same way in both approaches. Drought, salt stress and cold temperature stress are all physiologically linked because all three stress environments result in limiting the crops' physiological access to water and the strategies for improving tolerance are likely to be the same which includes osmotic adjustment, erecting hydrophobic barriers in roots and leaves to retain water (Cattivilli *et al*., 2002).

Although tolerance mechanisms might be expected to overlap, escape or avoidance mechanisms are more likely to be stress specific. For example delay in flowering may escape late season drought but will not help in a chronic saline situation. Deeper roots may be able to reach the last of the water in a drought but would only aggravate salt stress where the salt is being brought to the surface by a rising water table. With this background one would intuitively expect genetic control

to be multigenic and complex, but to overlap somewhat in tolerance to the different stresses (Gale, 2003). Breeding for stress tolerance will proceed more efficiently once it is clear whether, for individual crops and specific stresses; yield potential under stress is controlled by the same genes as yield under optimal conditions. The conclusion will dictate breeding strategy (Nguyen *et al.*, 2001).

2.9.6. Gene Mapping and Marker Development for Genetic Analysis and Marker Aided Selection (MAS) in Breeding

Genetic mapping as a prerequisite to genetic analysis is now part of standard plant breeding. Base molecular maps are now available for most of the crops. Those that are the focus of international effort, e.g. rice, wheat, potatoes, can use the well-developed public maps. Base maps for many of the 'orphan crops', have been made in which there is little international trade. Only a few very minor mandated species remain unmapped. The mapping of quantitative traits where there is often little knowledge of the genetic control in advance of the analysis, such as is usually the case with stress tolerance, is usually carried out by 'QTL mapping'. This requires a scan of the genome, with markers every 10 cm or so to identify those regions where segregation of the trait is associated with segregation for the markers. The reason much denser base maps are needed is that only a subset of the available markers will segregate in any single population. These locations are the basis for establishing a marker aided selection (MAS) breeding programme for tolerance and for eventual map-based cloning of the genes underlying the QTLs. Breeders' markers that are closely linked to the target gene may be derived straight from the base molecular map. Today the ideal marker system will be micro satellites, also known as simple sequence repeats (SSRs), although over the next few years single nucleotide polymorphisms (SNPs), which are more amenable to high throughput methods, will take over as the ideal marker. For rice, which will soon have the benefit of a full genome sequence, markers will never be a problem again. The sequence has been found to contain some 40,000 SSRs8 and SNPs and, base pair deletions or insertions indels, are found in unique sequence at a rate of about 1% 9, which works out at about 24 in every gene (Gale, 2003).

2.9.7. Genomics – the New Genetics

Developments over the past decade, arising particularly from the human genome programme, have led to a new phase of plant genetics. 'Plant genomics' is the application of the newly available vast amounts of genomic DNA sequence, using a range of novel high throughput, parallel and other technologies. In plants a 'whole genome' DNA sequence is available as yet only for arabidopsis, which was 'finished' in 2000. A 'draft' raw almost complete sequence of indica rice has been deposited in the public databases by the Beijing group and a similarly complete sequence of Japonica is available within a private company. The fully annotated public DNA sequence of rice, 88% complete at the moment, will be finished later this year. Undoubtedly more species will follow. Possibly maize will be the next major crop plant to be sequenced, at least for 'gene-rich' regions of the genome (Ku *et al*., 2000).

Technologies which are included under the umbrella of 'genomics' are: automatic DNA sequencing, where one machine can read two million base-pair a day; microarrays and DNA chips where tens of thousands of genes can be scanned for activity levels at the same time; automated genotyping machines that can assay tens of thousands of DNA diagnostic points a day. In fact it will soon be possible to monitor whole genomes for genetic markers or gene expression on single chips. Transformation technologies that allow the facile and efficient genetic modification of almost all crop plants can also be considered genomics technologies which is still in its infancy (Dubcovsky *et al.,* 2001).

2.9.8. Synteny and Comparative Genomics

A second development, which has also emerged over the last decade, is the discovery that gene content and gene order is much more conserved over even quite distantly related species that was previously envisaged. This is known as 'synteny'. Abiotic stress is a major constraint to food production and one that will grow in significance as we approach the increasing world food shortages in the developing world that will characterize the first half of the 21st Century. Aid and technology may be available from the North but the problem is one for the developing world alone. New crop varieties that will produce more in increasingly marginal agricultural environments will be desperately needed (Gale, 2003).