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# Salt tolerance and physiological response of plants to salinity: A Review

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**Abstract:-**

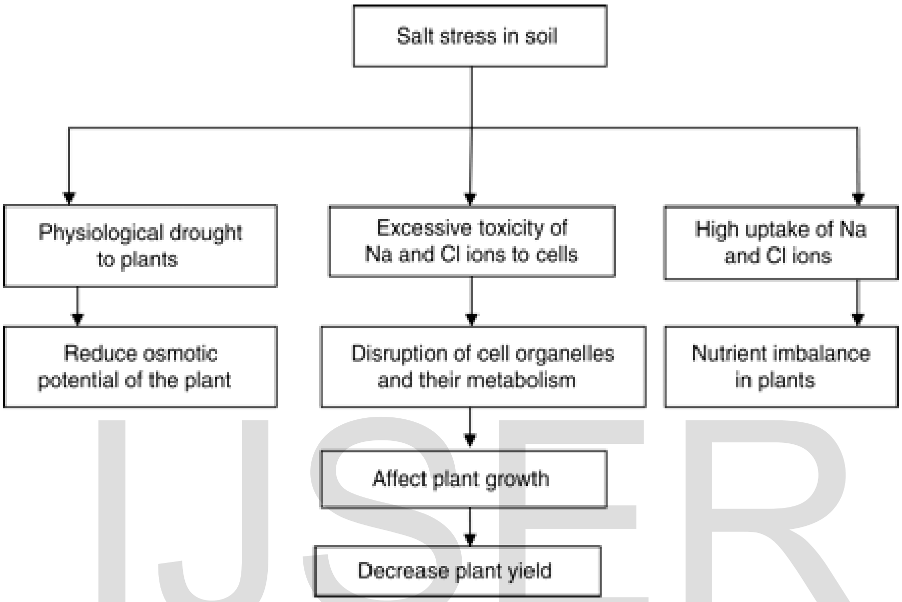
Large areas of land throughout the world cannot be used for food production because of the limitations imposed by natural or man-made environmental stresses. Therefore, the study of stress phenomena and related tolerance mechanisms is becoming more important. The purpose of this review is to summarize the advances and prospects for the genetic improvement of salt tolerance in cultivated crops. The genetic approach to the salinity problem is fairly new, but has generated considerable interest worldwide. This approach provides exciting prospects for increasing productivity in salt- affected area. Salinity stress response is multigenic, as a number of processes involved in the tolerance mechanism are affected, such as various compatible solutes/osmolytes, polyamines, reactive oxygen species and antioxidant defence mechanism, ion transport and compartmentalization of injurious ions. Processes such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set are adversely affected by high salt concentration, ultimately causing diminished economic yield and also quality of produce. In this review, we have discussed about production of salt-tolerant plants through genetic engineering. Future prospects and concerns, along with the importance of novel techniques, as well as plant breeding.

**Keywords:** Salt tolerance, Halophytes, Compatible solutes; Ion homeostasis, genetic engineering, transgenic plants, osmotic adjustmement, seedling growth.

## Introduction:

Soil salinity has been a major concern to global agriculture throughout human history (Lobell *et al.*, 2007). In recent times, it has become even more prevalent as the intensity of land use increases globally (Haque, 2006). It is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semi- arid areas (Giri *et al.*, 2003; Al-Karaki, 2006). Saline soils occupy 7 % of the earth's land surface (Ruiz-Lozano et al.,2001) and increased salinization of arable land will result in to 50 % land loss by the middle of the 21st century (Wang *et al.*, 2003). Plants are subjected to various abiotic stresses such as low temperature, salt, drought, floods, heat, oxidative stress and heavy metal toxicity during their life cycle. Among all this, salinity is the most typical abiotic stress (Mahajan and Tuteja 2005). According to an FAO survey (2008), it is expected that over 800 million ha will be affected by salinity in the near future, making it a major constraint to food production for a steadily increasing population. Rapidly shrinking agricultural land, due to industrialization and/or habitat use is a major threat to sustainable food production. World population is increasing at an alarming rate and is supposed to reach nine billion by 2050, but our food production is limited (Varshney *et al.*, 2011).In light of all this, it is almost imperative to raise salt tolerant plants to effectively use salt affected agricultural land for sustainable crop production. Efforts to improve crop performance under environmental stresses have not been that fruitful because the fundamental mechanisms of stress tolerance in plants remain to be completely understood.

The most important aspect of plant responses leading to salt stress tolerance is the regulation of uptake and distribution of $Na^+$ ions (Tester and Davenport, 2003). Along osmotic homeostasis, maintenance of ionic homeostasis is an important strategy for achieving enhanced tolerance to environmental stresses (Sun *et al.*, 2009).Salinity is one of the major severe abiotic factors affecting crop growth and productivity (Munns and Tester 2008). Salt's negative effects on plant growth have initially been associated with the osmotic stress component caused by decreases in soil water potential and, consequently, restriction of water uptake by roots.



**Source - (Evelin *et al.*, 2009)**

Zhang *et al.*, 2012 have summarized 2171 salt-responsive proteins from proteomic analysis of 34 different plant species (19 crops and other plants) and functionally categorized these proteins as follows; photosynthesis, carbohydrate and energy metabolism, metabolism, stress and defence, transcription, protein synthesis, protein folding and transport, protein degradation, signalling, membrane and transport, cell structure, cell division/differentiation and fate, miscellaneous, and unknown function. Transgenic development is another straight forward technology to improve crop yield in abiotic stress affected land (Roy and Basu, 2009). The development of tolerant crops by genetic engineering, on the other hand, requires the identification of key genetic determinants underlying stress tolerance in plants and introducing these genes into crops. Development of transgenic plants tolerant to specific abiotic stresses is the straight-forward solution to improve crop productivity under unfavourable environments. The promising transgenic can be maintained *in vitro* through repeated subculture (Roy and Mandal, 2006). Those transgenic also can be mass-multiplied using tissue culture technique (Roy and mandal, 2011).

## Classification of salt-affected soil:-

Soils are considered saline if they contain salt in a concentration sufficient to interfere with the growth of most crop species (US Salinity Laboratory, 1969).Saline and sodic soils can significantly reduce the value and productivity of affected land. Soil salinity and related problems generally occur in arid or semiarid climates where rainfall is insufficient to leach soluble salt from the soil or where surface or internal soil drainage is restricted. Salinity problems can also occur on irrigated land, particularly when using brackish or saline water for irrigation. Ions most commonly associated with soil salinity include the anions: chloride (Cl-), sulfate ($SO_4$), carbonate ($CO_3$) bicarbonate ($HCO^{3-}$) and sometimes nitrate ($NO^{-3}$) and the cations sodium (Na+), calcium (Ca++), magnesium (Mg++) and sometimes potassium (K++). Salt-affected soils are divided into three groups depending on the amounts and kind of salt present. The classification depends on total soluble salts (measured by electrical conductivity (E.C.), soil pH and exchangeable sodium percentage).

**Classification of salt- affected soil: (Table 1)**

| Classification | Electrical conductivity (mmhos/cm) | Soil pH | Exchangeable Sodium (%) |
|---|---|---|---|
| **Saline** | > 4.0 | < 8.5 | < 15 |
| **Sodic** | < 4.0 | > 8.5 | > 15 |
| **Saline-sodic** | > 4.0 | < 8.5 | > 15 |

**Source: Cardon and Mortvedt, 2001 > = greater than, < = less than.**

**Distinguishing features of saline and sodic soils: - (Table-2)**

| Characteristics | Saline soils | Sodic soils |
|---|---|---|
| **1. Chemical** | a. Dominated by neutral soluble salts consisting of chlorides and sulphates of sodium, calcium and magnesium. | a. Appreciable quantities of neutral soluble salts generally absent. Measurable to appreciable quantities of salts capable of alkaline hydrolysis, e.g. $Na_2CO_3$, present. |
| | b. pH of saturated soil paste is less than 8.2. | b. pH of the saturated soil paste is more than 8.2. |
| | c. An electrical conductivity of the saturated soil extract of more than 4 dS/m at 25 °C is the generally accepted limit above which soils are classed as ‘saline’. | c. An exchangeable sodium percentage (ESP) of 15 or more is the generally accepted limit above which soils are classed as ‘sodic’. Electrical conductivity of the saturated soil extract is generally less than 4 dS/m at 25 °C but may be more if appreciable quantities of $Na_2CO_3$ etc. are present. |
| | d. There is generally no well-defined relationship between pH of the saturated soil paste and exchangeable sodium percentage (ESP) of the soil or the sodium adsorption ratio (SAR) of the saturation extract. | d. There is a well defined relationship between pH of the saturated soil paste and the exchangeable sodium percentage (ESP) of the soil or the SAR of the saturation extract for an otherwise similar group of soils such that the pH can serve as an approximate index of soil sodicity (alkali) status. |

| | | |
|---|---|---|
| | e. Although Na is generally the dominant soluble cation, the soil solution also contains appreciable quantities of divalent cations, e.g. Ca and Mg. | e. Sodium is the dominant soluble cation. High pH of the soils results in precipitation of soluble Ca and Mg such that their concentration in the soil solution is very low. |
| | f. Soils may contain significant quantities of sparingly soluble calcium compounds, e.g. gypsum. | f. Gypsum is nearly always absent in such soils. |
| **2. Physical** | a. In the presence of excess neutral soluble salts the clay fraction is flocculated and the soils have a stable structure. | a. Excess exchangeable sodium and high pH result in the dispersion of clay and the soils have an unstable structure. |
| | b. Permeability of soils to water and air and other physical characteristics are generally comparable to normal soils. | b. Permeability of soils to water and air is restricted. Physical properties of the soils become worse with increasing levels of exchangeable sodium/pH. |
| **3. Effect on plant growth** | In saline soils plant growth is adversely affected: | In sodic soils plant growth is adversely affected: |
| | a. chiefly through the effect of excess salts on the osmotic pressure of soil solution resulting in reduced availability of water; | a. chiefly through the dispersive effect of excess exchangeable sodium resulting in poor physical properties; |
| | b. through toxicity of specific ions, e.g. Na, Cl, B, etc.; | b. through the effect of high soil pH on nutritional imbalances including a deficiency of calcium; |
| | | c. through toxicity of specific ions, e.g. Na, $CO_3$, Mo, etc. |
| **4. Soil improvement** | Improvement of saline soils essentially requires removal of soluble salts in the root zone through leaching and drainage. Application of amendments may generally not be required. | Improvement of sodic soils essentially requires the replacement of sodium in the soil exchange complex by calcium through use of soil amendments and leaching and drainage of salts resulting from reaction of amendments with exchangeable sodium. |
| **5. Geographic distribution** | Saline soils tend to dominate in arid and semi-arid regions. | Sodic soils tend to dominate in semi-arid and sub-humid regions. |
| **6. Ground-water quality** | Groundwater in areas dominated by saline soils has generally high electrolyte concentration and a potential salinity hazard. | Groundwater in areas dominated by sodic soils has generally low to medium electrolyte concentration and some of it may have residual sodicity so has a potential sodicity hazard. |

**Causes of Soil salinization-**

A significant decline in soil quality has occurred throughout the entire world as a result of adverse changes in its physical, chemical and biological properties. Salinization consists of an accumulation of water soluble salts in the soil. These salts include the ions potassium (K+), magnesium (Mg2+), calcium (Ca2+), chloride (Cl-), sulphate ($SO_4^{2-}$), carbonate ($CO_3^{2-}$), bicarbonate ($HCO_3^-$) and sodium (Na+). Sodium accumulation is also called sodification. High sodium contents result in destruction of the soil structure which, due to a lack of oxygen, becomes incapable of assuring plant growth (Freire, 2009).

## Natural soil salinization and Sodification factors-

The natural factors influencing soil salinity are:-

- Geological phenomena which increase the salts concentration in groundwater and consequently in the soil;
- Natural factors capable of bringing groundwater containing elevated salt contents to the surface;
- Infiltration of groundwater in below sea-level zones (micro-depressions with reduced or absent drainage);
- Drainage of waters from zones with geological substrates capable of liberating large amounts of salts;
- Action of winds, which, in coastal zones, can transport moderate amounts of salts to the interior.

The salinization process occurs in soils situated in regions of low rainfall and which have a water-bearing stratum near the surface. In coastal zones, salinization could be associated with the over-exploitation of ground waters due to the demand induced by increased urbanization, or by industry and agriculture.

## Secondary factors leading to soil salinization and sodification:-

The most influential anthropogenic factors are:

- Irrigation with water containing elevated salt contents;
- rise in phreatic water level due to human activities (infiltration of water from unlined channels and reservoirs, irregular distribution of irrigation water, deficient irrigation practices, inadequate drainage);
- use of fertilizers and other production factors, namely for intensive agriculture in land with low permeability and reduced possibilities for leaching;
- Irrigation with residual waters with high salt contents;
- Elimination of residual waters with high salt contents by way of the soil;
- Contamination of the soil with industrial water and sub-products with high salt contents.

Thus salinization of a soil depends on the quality of the water used for irrigation, on the existence and level of natural and/or artificial drainage of the soil, on the depth of the water-bearing stratum and on the original concentration of salt in the soil profile.

## Correcting salt-affected soils-

Salt-affected soils can be corrected by:

- **Improving drainage –**
  In soils with poor drainage, deep tillage can be used to break up the soil surface as well as clay pans and hardpans, which are layers of clay or other hard soils that restrict the downward flow of water.
- **Leaching-**
  Leaching can be used to reduce the salts in soils. You must add enough low-salt water to the soil surface to dissolve the salts and move them below the root zone. The water must be relatively free of salts (1,500 - 2,000 ppm total salts), particularly sodium salts.

- **Reducing evaporation-**

Applying residue or mulch to the soil can help lower evaporation rates.

- **Applying chemical treatments-**

Before leaching saline-sodic and sodic soils, you must first treat them with chemicals, to reduce the exchangeable sodium content. To remove or exchange with the sodium, add calcium in a soluble form such as gypsum.

- **A combination of these methods.**

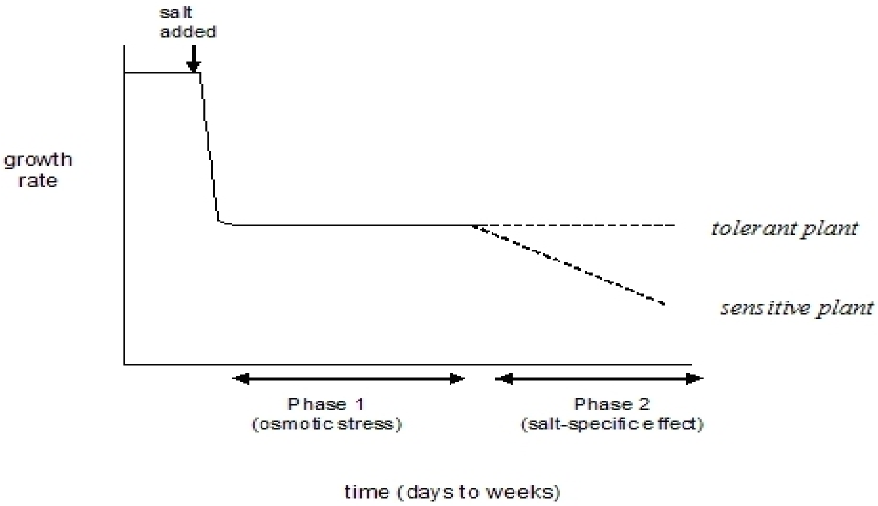
## SALINITY STRESS AND PLANT DEVELOPMENT:-

### Growth Stage Response:-

A crop is more sensitive during one stage than another; there is an opportunity to regulate the salinity of irrigation water during the season to minimize salt injury at the sensitive stage. The plant's ability to respond to salt stress depends on the genes that are functioning at the stage of development during which the stress occurs (Epstein and Rains. 1987.) Sugar beet, barley, and cotton are among the most salt-tolerant agricultural crops, but each is relatively sensitive during germination or early seedling growth (Ungar 1974, Bernstein and Hayward 1958). On the contrary, corn, pea, gram, and beans are more sensitive during later stages of development (S.S. Piruzyan 1959). Rice is tolerant during germination (Pearson *et al.*, 1966) and becomes very sensitive during the seedling stage and again somewhat sensitive during fertilization of florets (M.T. Kaddah *et al.*, 1973). Corn is more salt sensitive during emergence and seedling growth but becomes more salt tolerant by the flowering stage (E.V. Maas *et al.*, 1983). Salt resistance is low in young tomato plants, becomes much higher by the bud stage, and decreases during flowering (E.B. Dumbroff *et al.*, 1977).

**(Fig-1)** Degree of salt stress in rice affect the crops differently. (Mishra *et al.*,1988)

Plants undergo characteristic changes from the time salinity stress is imposed until they reach maturity (Munns, 2002a). Munns (2002a, 2005) developed the concept of the 'two-phase growth response to salinity'. The first phase of growth reduction happens quickly (within minutes) after exposure to salinity. This response is due to the osmotic changes outside the root causing changes in cell-water relations (osmotic effect). The osmotic effect initially reduces the ability of the plant to absorb water. The second much slower effect, taking days, weeks or months is the result of salt accumulation in leaves, leading to salt toxicity in the plant, primarily in the older leaves (i.e. salt-specific effect).



**(Fig-2)** Schematic illustration of the two-phase growth response to salinity for genotypes that differ in the rate at which salt reaches toxic levels in leaves (Munns, 2005).

Although salinity effects occur at almost all growth stages, including germination, seedling, vegetative and mature stages of field-grown cotton, it is generally believed that germination and young seedling stages are more sensitive to salinity stress than other stages (Ahmad *et al.*, 2002). Under salt stress, plant growth, nutrient absorption and metabolism, protein synthesis and water absorption are greatly altered, making it difficult for plants to fully utilize nutrients (Pessarakli, 2001; Ella and Shalaby, 1993).

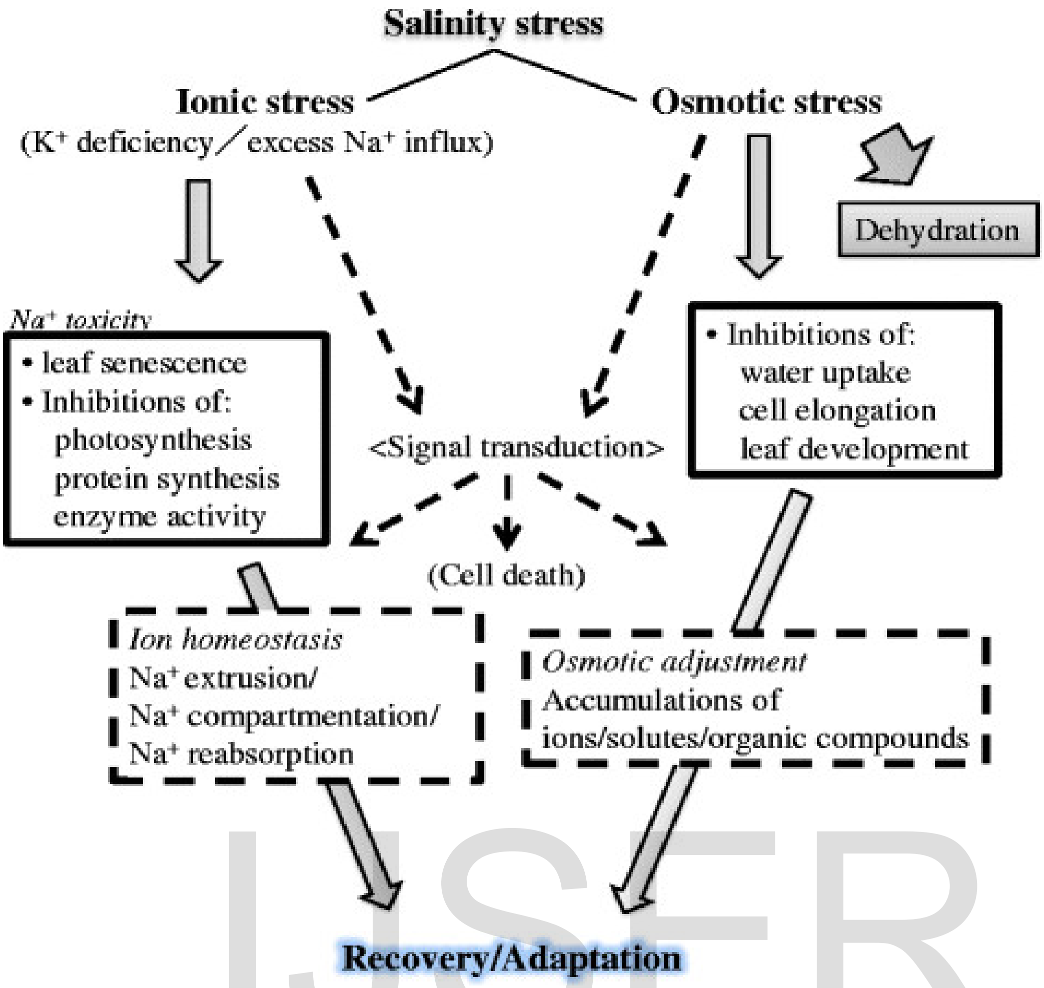
## Salt tolerance Mechanisms in plants:-

Salt tolerance may vary considerably with genetic traits. A plant species' tolerance for salinity will be overridden by a sudden exposure to salinity, even if the species is a halophyte (Albert, 1975). Probably the most suitable response to measure is growth or yield, especially at moderate salinities (Allen, Chambers, & Stine, 1994). Salt tolerance, in fact, can be usually assessed as the percent biomass production in saline versus control conditions over a prolonged period of time (this usually correlates with yield) or in terms of survival, which is quite appropriate for perennial species (R. Munns, 2002). Salinity tolerances may increase or decrease depending on the plant species and/or environmental factors. For some species, salt sensitivity may be greatest at germination, whereas for other species, sensitivity may increase during reproduction (Howat, 2000; Marschner, 1986). Plants have evolved several mechanisms to acclimatize to salinity. It is possible to distinguish three types of plant response or tolerance: (R Munns & Tester, 2008)-

a) The tolerance to osmotic stress

b) The $Na^+$ exclusion from leaf blades

c) Tissue tolerance



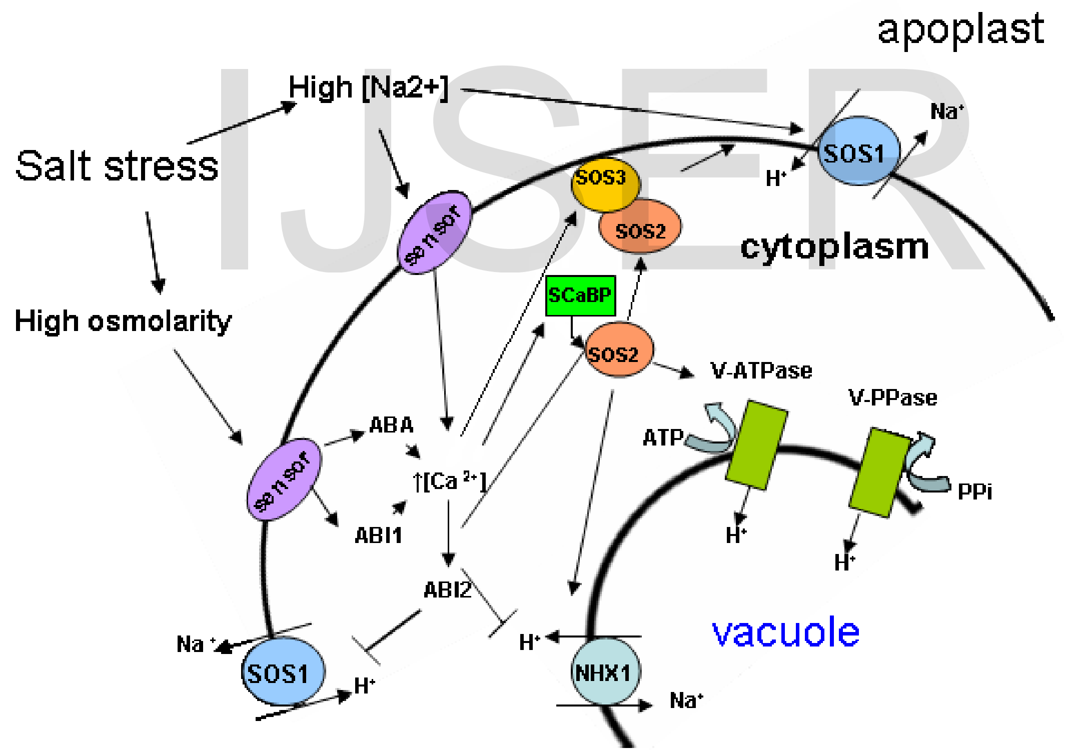
**(Fig-3)** Source- (Horie et al., 2012)

## Osmotic Adjustment:

Osmotic tolerance involves the plant’s ability to tolerate the drought aspect of salinity stress and to maintain leaf expansion and stomatal conductance (Rajendran, Tester, & Roy, 2009). If the accumulation of salts overcomes the toxic concentrations, the old leaves die (usually old expanded leaves) and the young leaves, no more supported by the export of photosynthates, undergo a reduction of growth and new leaves production. The mechanisms involved in osmotic tolerance is related to stomatal conductance, water availability and therefore to photosynthetic capacity to sustain carbon skeletons production to meet the cell's energy demands for growth have not been completely unravelled, it has been demonstrated that the plant’s response to the osmotic stress is independent of nutrient levels in the growth medium (Hu, Burucs, von Tucher, & Schmidhalter, 2007). In response to osmotic stress, plants produce osmolytes like glycine betaine, trehalose or proline, which protect them from dehydration or protein denaturation. However, oxidative stress-an outcome of ionic stress lead to the production of different enzymatic or non-enzymatic antioxidants, which protect plants from harmful effects of reactive oxygen species (Shao *et al.*, 2007).

## Salt (Na+) exclusion:-

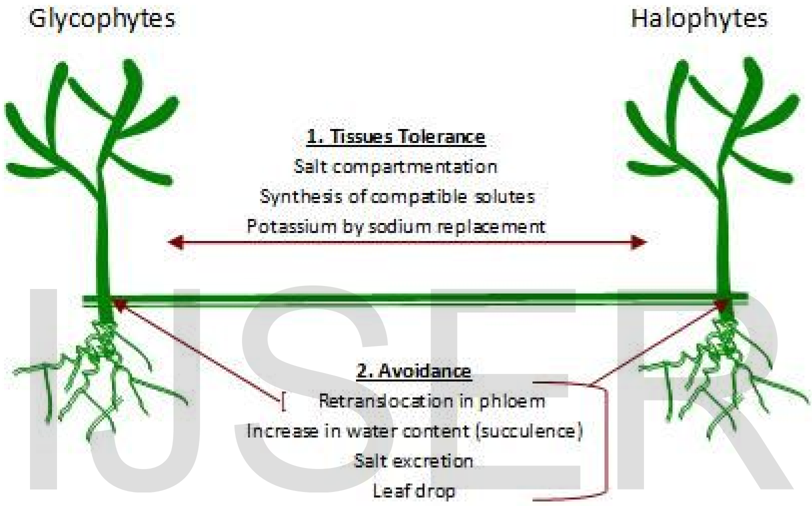
In the majority of plant species grown under salinity, Na+ appears to reach a toxic concentration before Cl− does, and so most studies have concentrated on Na+ exclusion and the control of Na+ transport within the plant (R Munns & Tester, 2008).It involves the ability to reduce the ionic stress on the plant by minimizing the amount of Na$^+$ that accumulates in the cytosol of cells, particularly those in the transpiring leaves. Na+ exclusion from leaves is associated with salt tolerance in cereal crops including rice, durum wheat, bread wheat and barley (Richard A. James, Blake, Byrt, & Munns, 2011). Exclusion of Na+ from the leaves is due to low net Na+ uptake by cells in the root cortex and the tight control of net loading of the xylem by parenchyma cells in the stele (Davenport, James, Zakrisson-Plogander, Tester, & Munns, 2005). Excess Na$^+$ and high osmolarity are separately sensed by unknown sensors at the plasma membrane level, which then induce an increase in cytosolic [Ca$^{2+}$]. This increase is sensed by SOS3 which activates SOS2. The activated SOS3-SOS2 protein complex phosphorylates SOS1, the plasma membrane Na$^+$/H$^+$ antiporter, resulting in the efflux of Na$^+$ ions. SOS2 can regulate NHX1 antiport activity and V-H$^+$-ATPase activity independently of SOS3, possibly by SOS3-like Ca$^{2+}$-binding proteins (SCaBP) that target it to the tonoplast. Salt stress can also induce the accumulation of ABA, which, by means of ABI1 and ABI2, can negatively regulate SOS2 or SOS1 and NHX1. (Adapted from Silva & Gerós (2009).



**(Fig-4) Signalling pathways responsible for Na+ extrusion in Arabidopsis under salt stress. Silva & Gerós (2009).**

## Tissue tolerance:-

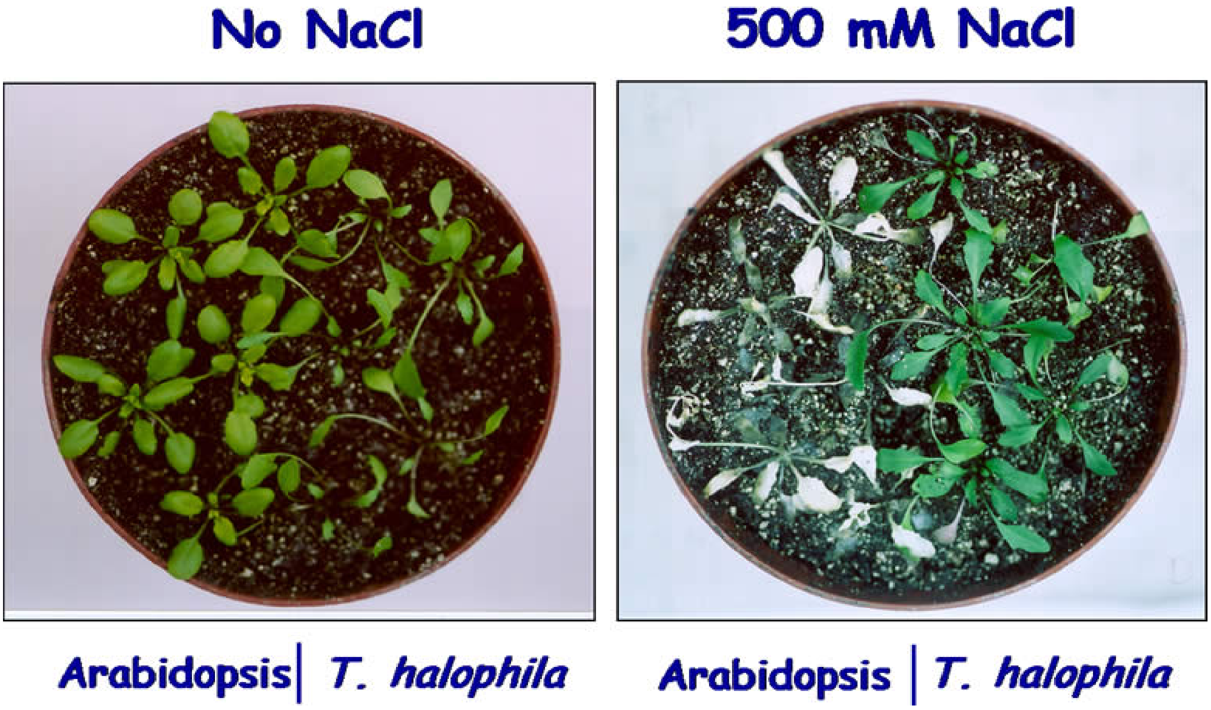
Tissue tolerance entails an increase of survival of old leaves. It requires compartmentalization of Na+ and Cl− at the cellular and intracellular level to avoid toxic concentrations within the cytoplasm, especially in mesophyll cells in the leaf (R Munns & Tester, 2008) and synthesis and accumulation of compatible solutes within the cytoplasm. The function of the compatible solutes is not limited to osmotic balance. Compatible solutes are typically hydrophilic, and may be able to replace water at the surface of proteins or membranes, thus acting as low molecular weight chaperones (Hasegawa, Bressan, Zhu, & Bohnert, 2000). Among the best known compatible solutes, proline and glycine betaine (GB) have been reported to increase greatly under salt and drought stresses (R. Munns, 2002; Sakamoto & Murata, 2002) and constitute the major metabolites found in durum wheat under salt stress, as in other *Poaceae* (Ashraf & Foolad, 2007; Carillo, Mastrolonardo, Nacca, & Fuggi, 2005; Sairam & Tyagi, 2004).



**(Fig-5)** Adaptive strategies for salt tolerance in plants.

## Halophytes as a tool for salt control:

It is of interest to show the great importance of halophytes in friendly, safety cleaning the soil from salinity. Most of the plants cannot tolerate high salt concentrations of the soil and cannot be grown on a salt affected land (Glenn and Brown, 1999). Some of the plants have the ability to grow under salinity due to the presence of different mechanisms in them for salt tolerance such plants are known as salt resisting plants, salt tolerating plants or halophytes (Flowers *et al.*, 1986).Salt tolerating plants represent only 2% of terrestrial plant species but they represent a wide diversity of plant forms (Glenn and Brown, 1999). In many halophytes, the optimum salinity for growth is shifted to levels of salt at which most plants and all crop plants experience severe reductions in growth and yield (Greenway, H. and Munns, R. 1980.). As a result, halophytes provide the opportunity to compare growth at low and high levels of salinity to distinguish responses that are truly adaptive from those that are the result of lesions or other types of damage.



**(Fig-6)**

Research with halophytic species has provided glimpses of these adaptive components, but has been limited by the lack of molecular genetics in any of the species studied. With the advent of molecular genetics in *Arabidopsis*, functional studies have identified genetic elements and pathways that alter stress sensitivity in this glycophyte. Using information from studies in *Arabidopsis*, we can now determine if these components (salt and osmotic tolerance determinants) and mechanisms (the associated regulatory pathways) are part of the adaptations used by *Thellungiella halophila* (salt stress), a naturally salt tolerant, genetically tractable relative of *Arabidopsis* (Zhu, 2001).

**(Fig-7)** *Opuntia humifusa*

**(Fig-8)** *Ilex glabra*

**(Fig-9)** *Rosa carolina*

**(Fig-10)** *Lathyrus maritimus*

**(Fig-11)** *Oenthera biennis*

**(Table-3) Salt tolerant plants list -**

| ***Betula papyrifera*** | **Paper birch** |
|---|---|
| ***Celtis occidentalis*** | Hackberry |
| ***Juniperus virginiana*** | Eastern red cedar |
| ***Nyssa slyvatica*** | Black tupelo |
| ***Pinus rigida*** | Pitch pine |
| ***Populus deltoides*** | Eastern cottonwood |
| ***Quercus alba*** | White oak |
| ***Quercus palustris*** | Pin oak |
| ***Quercus rubra*** | Red oak |
| ***Quercus stellata*** | Post oak |
| ***Amelanchier arborea*** | Common serviceberry |
| ***Aronia arbutifolia*** | Red chokeberry |
| ***Cephalanthus occidentalis*** | Buttonbush |
| ***Gaylussacia baccata*** | Black huckleberry |
| ***Ilex glabra*** | Inkberry |
| ***Myrica pensylvanica*** | Bayberry |
| ***Prunus maritima*** | Beachplum |
| ***Rosa carolina*** | Pasture rose |
| ***Salix discolor*** | Pussy willow |
| ***Sambucus canadensis*** | Black elderberry |
| ***Vitis labrusca*** | Fox grape |
| ***Eupatorium album*** | White thoroughwort |
| ***Lathyrus maritimus*** | Beach pea |
| ***Opuntia humifusa*** | Eastern prickly pear |
| ***Oenthera biennis*** | Common evening primrose |

**(Source -Green belt native plant centre, city of New York )**

## Genetic Modifications of plants to make them salt tolerant:-

Abiotic stresses adversely affect growth and productivity and trigger a series of morphological, physiological, bio- chemical and molecular changes in plants. When a plant is subjected to abiotic stress, a number of genes are turned on, resulting in increased levels of several metabolites and proteins, some of which may be responsible for conferring a certain degree of protection to these stresses (Burke *et al* ., 2006).The adaptive physiological and biochemical responses of a plant to salinity are controlled by genes that encode salt tolerance mechanisms (Casas *et al.*, 1992). Since salinity tolerance is a complex trait, it is most likely controlled by interactions of hundreds of salt responsive genes (Sahi *et al.*, 2006, Winicov, 1998).

Plants recognise a salinity stress and condition adaptive response mechanisms (Hasegawa & Bressan, 2000). Reported responses involve many molecular processes such as ion homeostasis (membrane proteins involved in ionic transport), osmotic adjustment and water regime regulation (osmolytes), as well as scavenging of toxic compounds (enzymes; Benke *et al.*, 2010, Blumwald *et al.*, 2004). The regulatory molecules conditioning these responses have been found to be cellular signal pathway components and transductors of long distance response co-ordination such as hormones, mediators, transcription factors and regulatory genes (Mishra *et al.*, 2006). The expression of such genetic regulators during plant stress has been studied at the transcriptional level (Fernandez *et al.*, 2008, Hasegawa & Bressan, 2000). Consequently, abiotic stress-inducible genes have been classified into two categories; 1) those that directly protect against environmental stress; and 2) those that regulate gene expression and signal transduction against stress response (Hasegawa & Bressan, 2000, Kawaura *et al.*, 2008, Mishra *et al.*, 2006, Popova *et al.*, 2008, Ueda *et al.*, 2002).

## Molecular mechanisms under lying regulation of cellular Na$^+$ ion homeostasis during plant growth in salt:-

Accumulation of Na$^+$ in the cell cytoplasm of plants is toxic because of its adverse effects on cellular metabolism and ion homeostasis. In order to avoid the harmful effects of salt stress on growth and development, plants have developed mechanisms to maintain low levels of salt in the cytoplasm. One mechanism involves removal of Na$^+$ from the cytoplasm by transport into the vacuole or out of the cell. This transport is catalyzed by Na$^+$/H$^+$ exchangers (antiporters), membrane proteins localized in the vacuolar (tonoplast) and plasma membrane, respectively( Figure 12 ). Na$^+$/H$^+$-exchange activity is driven by the electrochemical gradient of protons (H$^+$) generated by the H$^+$-pumps in the plasma membrane (H$^+$-ATPase) or the tonoplast (H$^+$-ATPase and H$^+$-pyrophosphatase). Our studies are focused on understanding the role and regulation of these transport proteins in the plant's response to salt stress.

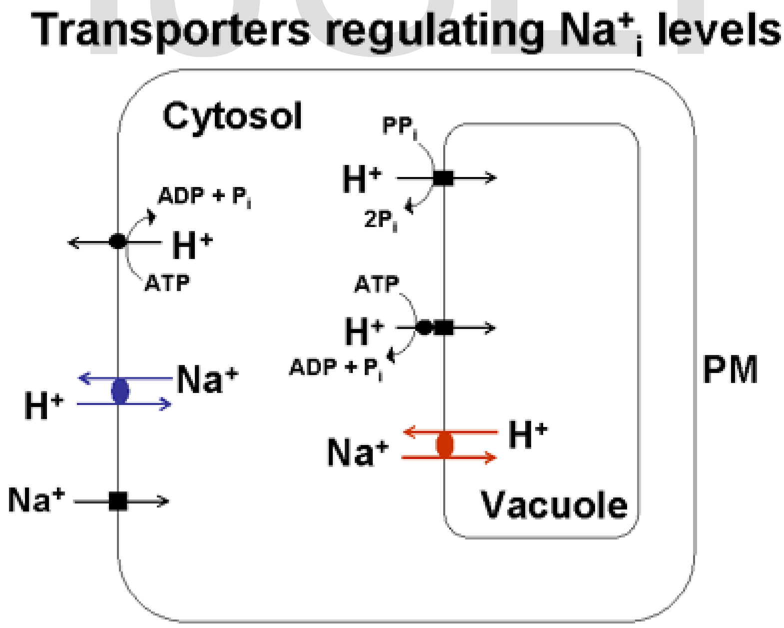
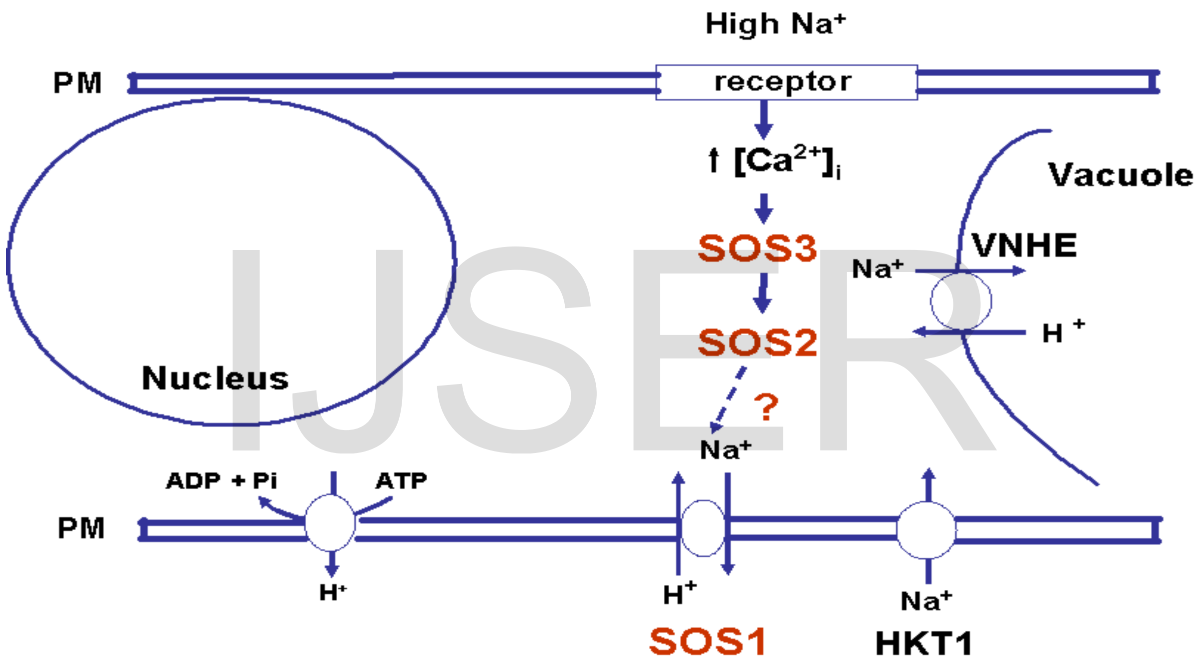


**Figure 12 – model of plant cell**

Recently, much has been learned about signal transduction pathways that regulate plant ion homeostasis during salt stress (Zhu, J.-K. 2002.). In a genetic screen designed to identify components of the cellular machinery that contribute to salt tolerance in *Arabidopsis thaliana*, three Salt-Overly-Sensitive genes (*SOS1*, *SOS2*, and *SOS3*) were found to function in a common pathway (Wu *et al.*,1996;Liu *et al.*, 1997;Zhu *et al.*,1998;Zhu *et al.*,2000). (Fig-13).Loss-of-function mutations in *SOS1* render plants extremely $Na^+$-sensitive, and overexpression of *SOS1* in Arabidopsis improves salt tolerance (Shi *et al* .,2003). As was found for mutations in *SOS1*, mutations in *SOS3* or *SOS2* cause plant hypersensitivity to $Na^+$ . *SOS3* encodes an EF-hand type calcium-binding protein that may sense calcium changes elicited by salt stress (Zhu *et al.*, 1998.) Calcium, together with SOS3, can activate SOS2, a serine/threonine protein kinase .( Zhu, 2000) *SOS1* encodes a 127 kilo dalton membrane protein with 12 putative membrane-spanning domains and a long hydrophilic tail at the C-terminal end of the protein . The predicted membrane-spanning domains in the SOS1 protein display significant similarity to domains of plasma membrane-localized $Na^+/H^+$ exchangers from animal, bacterial, and fungal cells. Salt stress up-regulation of *SOS1* is partly under control of the SOS3/SOS2 regulatory pathway. (Shi *et al.*, 2000). Comparing $Na^+/H^+$ exchange activity in plasma membrane-enriched vesicles isolated from wild-type and *sos* mutant plants, we have shown that SOS1 is a $Na^+/H^+$ exchanger -- exchange activity was significantly reduced in plasma membrane-enriched vesicles isolated from *sos1* plants relative to activity in vesicles isolated from wild-typeplants.



**(Figure 13 – SOS model).**

the transport activity of SOS1 is post-translationally activated by SOS2/SOS3 as mutations in *SOS2* and *SOS3* led to reductions in plasma membrane $Na^+/H^+$ exchange activity, and transport in these mutants could be restored by adding constitutively active recombinant (activated) SOS2 protein kinase *in vitro*.( Qiu *et al.*, 2002). Salt tolerance in plants appears to be a developmentally regulated process and the tolerance of the plants at one stage of development is not always correlated with tolerance at other stages. (Greenway, H. and Munns, R. 1980).For example, in tomato, barley, corn, rice and wheat, salt tolerance tends to increase with the age of the plant (Foolad, M.R.2004).Twenty-five years ago Emanuel Epstein (1980) described the technical and biological Constraints to solving the problem of salinity. Two basic genetic approaches that are currently being used to improve stresss tolerance include:

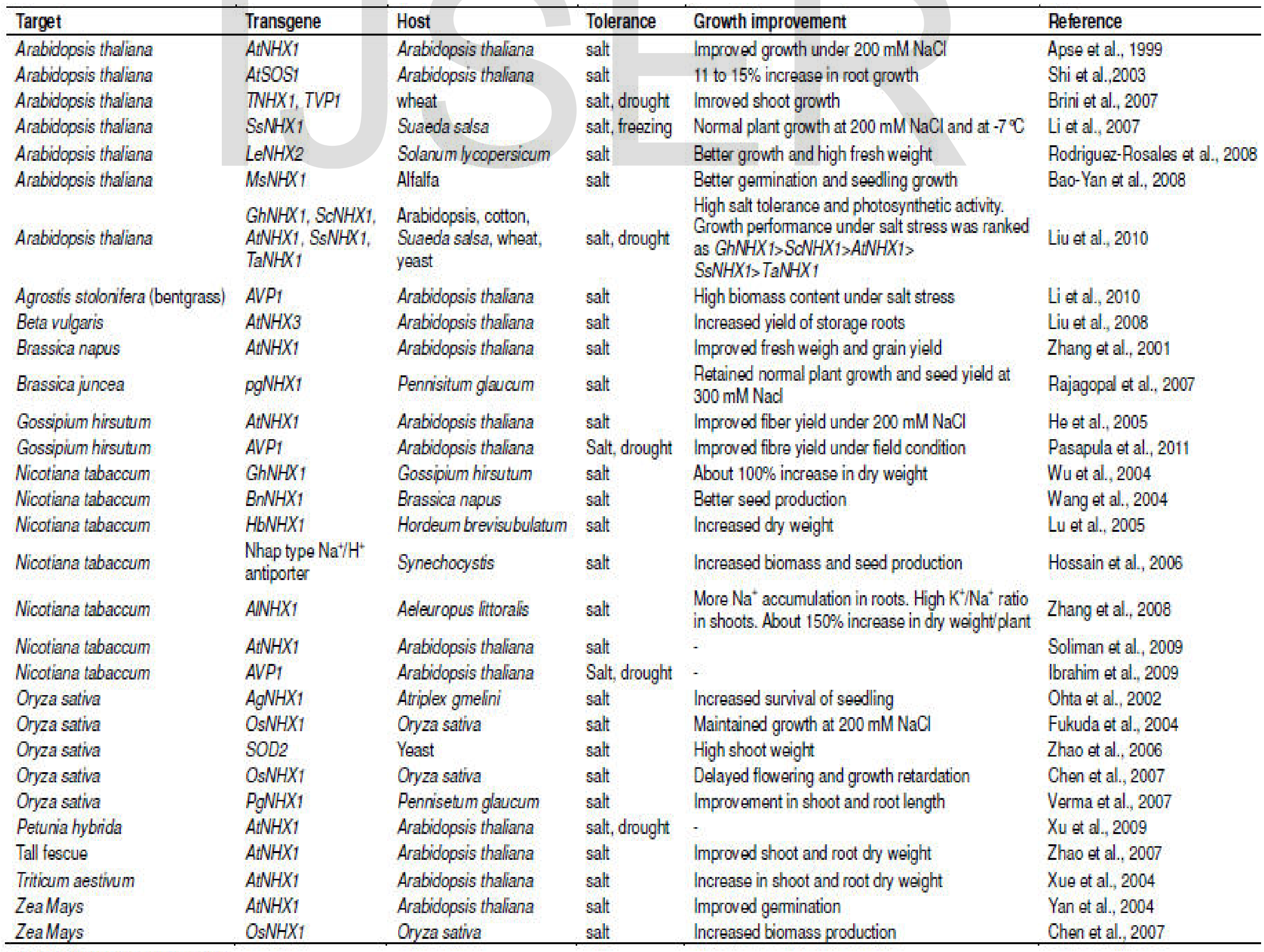
(i) Exploitation of natural genetic variations, either through direct selection in stressful environments or through the mapping of quantitative trait loci (QTLs – regions of a genome that are associated with the variation of a quantitative trait of interest) and

(ii) Generation of transgenic plants to introduce novel genes or to alter expression levels of the existing genes to affect the degree of salt stress tolerance. (Flowers 2004, Lindsay,.*et al.* 2004).

QTLs associated with salt tolerance at the germination stage in barley (Mano, Y. and Takeda, K. (1997) tomato (Foolad, M.R. *et al*, .1999) and Arabidopsis (Quesada, V. *et al.*, 2002) were different from those QTLs associated with salt tolerance at the early stage of growth; the plants selected by their ability to germinate at high salinity did not display similar salt tolerance during vegetative growth. The development of molecular biology techniques has enabled the development of DNA markers that can be used to identify QTLs. The use of QTLs has improved the subsequent marker-assisted selection.( Foolad, M.R.2004).

efficiency of selection, in particular for those traits that are controlled by several genes and are highly influenced by environmental factors (Flowers, T.J. 2004). There is considerable evidence to support the view that salt tolerance and its sub-traits are determined by multiple QTLs and that both additive and dominance effects are important in the inheritance of many of the traits associated with salt tolerance (Gregorio, G.B.*et al.*, 2002)The development of high-density DNA maps that incorporate microsatellite markers, RFLP (restriction fragment-length polymorphisms) and AFLP (amplified fragment-length polymorphisms), and advances in marker-assisted selection techniques will facilitate pyramiding traits of interest to attain substantial improvement in crop salt tolerance.

**Table 1.** List of abiotic stress tolerant transgenic plants with $Na^+/H^+$ antiporters and related genes.



| Target | Transgene | Host | Tolerance | Growth improvement | Reference |
|---|---|---|---|---|---|
| *Arabidopsis thaliana* | *AtNHX1* | *Arabidopsis thaliana* | salt | Improved growth under 200 mM NaCl | Apse et al., 1999 |
| *Arabidopsis thaliana* | *AtSOS1* | *Arabidopsis thaliana* | salt | 11 to 15% increase in root growth | Shi et al.,2003 |
| *Arabidopsis thaliana* | *TNHX1, TVP1* | wheat | salt, drought | Imroved shoot growth | Brini et al., 2007 |
| *Arabidopsis thaliana* | *SsNHX1* | *Suaeda salsa* | salt, freezing | Normal plant growth at 200 mM NaCl and at -7 °C | Li et al., 2007 |
| *Arabidopsis thaliana* | *LeNHX2* | *Solanum lycopersicum* | salt | Better growth and high fresh weight | Rodriguez-Rosales et al., 2008 |
| *Arabidopsis thaliana* | *MsNHX1* | Alfalfa | salt | Better germination and seedling growth | Bao-Yan et al., 2008 |
| *Arabidopsis thaliana* | *GhNHX1, ScNHX1, AtNHX1, SsNHX1, TaNHX1* | Arabidopsis, cotton, *Suaeda salsa*, wheat, yeast | salt, drought | High salt tolerance and photosynthetic activity. Growth performance under salt stress was ranked as *GhNHX1>ScNHX1>AtNHX1> SsNHX1>TaNHX1* | Liu et al., 2010 |
| *Agrostis stolonifera* (bentgrass) | *AVP1* | *Arabidopsis thaliana* | salt | High biomass content under salt stress | Li et al., 2010 |
| *Beta vulgaris* | *AtNHX3* | *Arabidopsis thaliana* | salt | Increased yield of storage roots | Liu et al., 2008 |
| *Brassica napus* | *AtNHX1* | *Arabidopsis thaliana* | salt | Improved fresh weigh and grain yield | Zhang et al., 2001 |
| *Brassica juncea* | *pgNHX1* | *Pennisitum glaucum* | salt | Retained normal plant growth and seed yield at 300 mM Nacl | Rajagopal et al., 2007 |
| *Gossipium hirsutum* | *AtNHX1* | *Arabidopsis thaliana* | salt | Improved fiber yield under 200 mM NaCl | He et al., 2005 |
| *Gossipium hirsutum* | *AVP1* | *Arabidopsis thaliana* | Salt, drought | Improved fibre yield under field condition | Pasapula et al., 2011 |
| *Nicotiana tabaccum* | *GhNHX1* | *Gossipium hirsutum* | salt | About 100% increase in dry weight | Wu et al., 2004 |
| *Nicotiana tabaccum* | *BnNHX1* | *Brassica napus* | salt | Better seed production | Wang et al., 2004 |
| *Nicotiana tabaccum* | *HbNHX1* | *Hordeum brevisubulatum* | salt | Increased dry weight | Lu et al., 2005 |
| *Nicotiana tabaccum* | Nhap type $Na^+/H^+$ antiporter | *Synechocystis* | salt | Increased biomass and seed production | Hossain et al., 2006 |
| *Nicotiana tabaccum* | *AlNHX1* | *Aeleuropus littoralis* | salt | More $Na^+$ accumulation in roots. High $K^+/Na^+$ ratio in shoots. About 150% increase in dry weight/plant | Zhang et al., 2008 |
| *Nicotiana tabaccum* | *AtNHX1* | *Arabidopsis thaliana* | salt | - | Soliman et al., 2009 |
| *Nicotiana tabaccum* | *AVP1* | *Arabidopsis thaliana* | Salt, drought | - | Ibrahim et al., 2009 |
| *Oryza sativa* | *AgNHX1* | *Atriplex gmelini* | salt | Increased survival of seedling | Ohta et al., 2002 |
| *Oryza sativa* | *OsNHX1* | *Oryza sativa* | salt | Maintained growth at 200 mM NaCl | Fukuda et al., 2004 |
| *Oryza sativa* | *SOD2* | Yeast | salt | High shoot weight | Zhao et al., 2006 |
| *Oryza sativa* | *OsNHX1* | *Oryza sativa* | salt | Delayed flowering and growth retardation | Chen et al., 2007 |
| *Oryza sativa* | *PgNHX1* | *Pennisetum glaucum* | salt | Improvement in shoot and root length | Verma et al., 2007 |
| *Petunia hybrida* | *AtNHX1* | *Arabidopsis thaliana* | salt, drought | - | Xu et al., 2009 |
| Tall fescue | *AtNHX1* | *Arabidopsis thaliana* | salt | Improved shoot and root dry weight | Zhao et al., 2007 |
| *Triticum aestivum* | *AtNHX1* | *Arabidopsis thaliana* | salt | Increase in shoot and root dry weight | Xue et al., 2004 |
| *Zea Mays* | *AtNHX1* | *Arabidopsis thaliana* | salt | Improved germination | Yan et al., 2004 |
| *Zea Mays* | *OsNHX1* | *Oryza sativa* | salt | Increased biomass production | Chen et al., 2007 |

'-' Means information is not available.

## Achievements:-

The first attempt to evaluate the inheritance of salt tolerance was made by Lyon (1941). Heterosis was apparent under saline (NaCl) conditions in the elongation of stems in hybrids of L. Esculentum produced with three wild species ( *L. Cheesmanii* , *L. peruvianum* , *and L. Pennellii =Solanum pennellii* ) by Tal and Shannon 1983). It has been generally recorded that salinity adversely affects seedling growth and some relevant metabolic processes of glycophytic plants (Shaddad & Zidan, 1989; Hampson & Simpson, 1990; Zidan & Al-Zahran 1994).Several transgenic approaches have shown enhanced salinity tolerance in transgenic rice, sweet potato, tobacco and *Arabidopsis thaliana* plants that over express polyamine biosynthetic enzyme genes (Roy and Wu 2001; Kasukabe *et al.*, 2004; 2006; Wi *et al.*, 2006). One of the most common stress responses in plants is over production of different types of compatible organic solutes (Serraj and Sincdair 2002; Azevedo Neto *et al.*, 2004). Glenn *et al.*, 1999) have engineered transgenic *Arabidopsis* plants that overexpress *AtNHX1*, a vacuolar $Na^+/H^+$ antiport, which allowed the plants to grow in 200 mM NaCl. Zhang and Bhumwald (2001) reported the genetic modification of tomato plants to overexpress the *Arabidopsis thaliana AtNHX1* antiport, which likewise allowed those plants to grow in the presence of 200 mM NaCl. Xue *et al.* (2004) generated transgenic wheat expressing a vacuolar $Na^+/H^+$ antiport gene *AtNHX1*. The transgenic wheat lines exhibited improved biomass production. Transformation of tobacco with *GhNHX1* from cotton conferred salt tolerance and transgenic plants showed about 100% increase in plant dry weight (Wu *et al.*, 2004).

## Conclusions:-

Salt tolerance is a complex trait in plants, but molecular and genetic approaches are beginning to characterize the diverse biochemical events that occur in response to salt stress. In the short term, it will remain a challenge to manipulate the essential protective mechanisms in plants and to utilize our biochemical knowledge for optimal molecular engineering of salt tolerance in plants. Research on the physiology of salt tolerance has demonstrated that the overall trait is determined by several sub-traits, any of which can in turn be determined by several genes. With the recognition that the enhanced expression of a number of functionally related genes may be required for optimal improvements in salt tolerance, molecular engineering has been expanded to include proposals for multiple gene transfers to enhance salt tolerance. An equally promising approach to manipulating many genes may emerge as we learn more about the specificity of signalling pathways that turn on transcription of related genes that counteract salt stress at the cellular level. Overall, we are likely to see continued significant progress in our understanding and ability to modify salt tolerance by molecular engineering using both model and crop plants based on knowledge of how salinity affects plant bio-chemistry and physiology through gene expression.

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