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SALT TOLERANCE OF CROP PLANTS

Hamdia, M. A [1] and Shaddad, M. A. K. [2]

[1] *Botany Department, Faculty of Science, El-Minia University, El-Minia, Egypt*
[2] *Botany Department, Faculty of Science, Assiut University, Assiut, Egypt*

*Email- hamdia10@yahoo.com

Several environmental factors adversely affect plant growth and development and final yield performance of a crop. Drought, salinity, nutrient imbalances (including mineral toxicities and deficiencies) and extremes of temperature are among the major environmental constraints to crop productivity worldwide. Development of crop plants with stress tolerance, however, requires, among others, knowledge of the physiological mechanisms and genetic controls of the contributing traits at different plant developmental stages. In the past 2 decades, biotechnology research has provided considerable insights into the mechanism of biotic stress tolerance in plants at the molecular level. Furthermore, different abiotic stress factors may provoke osmotic stress, oxidative stress and protein denaturation in plants, which lead to similar cellular adaptive responses such as accumulation of compatible solutes, induction of stress proteins, and acceleration of reactive oxygen species scavenging systems. Recently, the authores try to improve plant tolerance to salinity injury through either chemical treatments (plant hormones, minerals, amino acids, quaternary ammonium compounds, polyamines and vitamins) or biofertilizers treatments (Asymbiotic nitrogen-fixing bacteria, symbiotic nitrogen-fixing bacteria and mycorrhiza) or enhanced a process used naturally by plants to minimise the movement of Na+ to the shoot, using genetic modification to amplify the process, helping plants to do what they already do - but to do it much better."

key words: Salt tolerances, plants, phytohormones, biofertilizers, molecular genetic, polyamines, minerals

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Introduction

Several environmental factors adversely affect plant growth and development and final yield performance of a crop. Drought, salinity, nutrient imbalances (including mineral toxicities and deficiencies) and extremes of temperature are among the major environmental constraints to crop productivity worldwide. It is estimated that less than 10% of the world's arable lands may be free of major environmental stresses (Dudal 1976). Tolerance to biotic stresses is very complex at the whole plant and cellular levels (Foolad et al. 2003a; Ashraf and Harris 2004; Munns and Tester 2008, Grewal 2010). This is in part due to the complexity of interactions between stress factors and various molecular, biochemical and physiological phenomena affecting plant growth and development (Zhu 2002). Currently, there are no economically viable technological means to facilitate crop production under stress conditions. However, development of crop plants tolerant to environmental stresses is considered a promising approach, which may help satisfy growing food demands of the developing and under-developed countries. Development of crop plants with stress tolerance, however, requires, among others, knowledge of the physiological mechanisms and genetic controls of the contributing traits at different plant developmental stages. In the past 2 decades, biotechnology research has provided considerable insights into the mechanism of biotic stress tolerance in plants at the molecular level. High salt stress disrupts homeostasis in water potential and ion distribution, (Hasegawa et al. 2000; and Rontein et al. 2002). For example, though stress tolerance mechanisms may vary from

species to species and at different developmental stages (Hamdia and Shaddad (1996), Ashraf 1994; Floolad, 1999 b; Floolad and lin 2001, Jenkins et al, 2010), basic cellular responses to a biotic stresses are conserved among most plant species (Zhu 2001a; b and Zhu 2002)). Furthermore, different abiotic stress factors may provoke osmotic stress, oxidative stress and protein denaturation in plants, which lead to similar cellular adaptive responses such as accumulation of compatible solutes, induction of stress proteins, and acceleration of reactive oxygen species scavenging systems (Zhu 2002). Water stress-induced leaf abscission is one of the most important drought resistance   mechanisms that plants have developed during evolution (Hamdia, 2008, Chalk and Alves (2010)

Understanding plant salt tolerance

One of the most common stress responses in plants is overproduction of different types of compatible organic solutes (Serraj and Sincdair 2002; Azevedo Neto et al. (2004). Compatible solutes are low molecular weight, highly soluble compounds that are usually nontoxic at high cellular concentrations. Generally, they protect plants from stress through different courses, including contribution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins (Yancey et al. 1982; Bohnert and Jensen, 1996) . Furthermore, because some of these solutes also protect cellular components from dehydration injury, they are commonly referred to as osmoprotectants. These solutes include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alaninebetaine, prolinebetaine, choline *O*-sulfate, hydroxyprolinebetaine, and

pipecolatebetaine (Rhodes and Hanson, 1993). Although much effort has been devoted to genetically engineer plants for over-production of various osmoprotectants, there has been little success in achieving the desired protective levels of these osmolytes in plants (discussed below). Alternatively, in some plants increased resistance to a biotic stresses has been achieved by exogenous application of various organic solutes (Hamdia and El-Anany 1998). This approach, which may significantly contribute to increased crop production in stress environments, however, has not received sufficient consideration in the literature. In this article, the roles of glycine betaine and proline as well as their exogenous applications in increasing plant stress tolerance, in particular in response to drought, salt, and temperature stresses, are reviewed and discussed.

The distribution of homeostasis occurs at both the cellular and the whole plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death. To achieve salt tolerance, three interconnected aspects of plant activities are important. First, damage must be prevented or alleviated. Second, homeostatic conditions must be re-established in the new, stressful environment. Third, growth must resume, albeit at a reduced rate (Borsani 2003; Zhu et al. 2005). Hamdia (1994) show that salinity stress affected growth, the chlorophylls contents, saccharides, nitrogen content and some minerals (Na^+, K^+, Ca^{++}, Mg^{++}, and P) in cucumber plants (*Cucumis sativus*). Spraying the shoot system with sodium pyruvate greatly ameliorated the adverse effect of NaCl salinity. This counteraction was associated with an increase in the contents of saccharides, protein, Ca^{++} and p. De Pascale et al. (2005) examine the influence of residual soil salinity on growth, yield and mineral composition of cauliflower and broccoli. Koyro (2006) study the physiological response of I (L.) to

the four major constraints of salinity (leaf gas exchange, water (and ion) relations and osmotic adjustment in hydroponically grown plants exposed to 6 weeks of various salt levels. Growth strongly depressed net photosynthesis and water use efficiency was affected by high salinity. Leaf water potential and osmotic potential decreased after salinity stress was imposed. The concentration of Ca^{++}, K^+, Mg^{++} and NO_3 were decreased.

Moussa (2004) state that root exudates of three weed plants, jungle rice, cocklebur and purslane, were use as foliar spray into NaCl stressed soybean seedlings to test their possible ameliorative effects on NaCl induced injury. Exudates of jungle rice roots exhibited highest level of kinetin and gibberellic acid and lowest concentration of indole acetic acid. Cocklebur root exudated contained highest level of IAA or ABA while purslane one showed amount of GA_3 or kinetin. NaCl imposition decrease protein content oil, K^+ Mg^+, P and NO_3, whereas increased praline contents, allakaloids, Na^+ and Cl^-. Shaddad et al. (2006) show that cv. Giza 168 was more salt tolerant and cv. Sohag was salt sensitive even at low salinity levels. There is a difference in growth criteria of the two wheat cultivars were mirrored by evident variations in absorption and distribution of Na+ and K+ among the different organs of the two wheat cultivars. This was observed as a marked and progressive increase in the catalase, peroxidase and ascorbate peroxidase which consequently maintained. The MDA concentration was comparable to the control value.

Meloni et al (2008) test the hypothesis that *Schinopsis quebracho Colorado* is a glycophytic that shows different salt tolerance responses with development stage. proline content, total soluble carbohydrates and Na+, K+ and Cl- concentarions in leaves and roots of seedlings, and the cholorophyll concentration and relative water content of leaves

were measured. Seedling growth decrease drastically with increasing salinity. An increase in NaCl from 0 to 100 mM^{-1} also significantly reduced the leaf relative water content by 22% and increased the praline concentration by 60% in roots. In contrast total soluble carbohydrates were showed a sodium exclusion capacity. S. quebracho Colorado was more tolerant to salinity during germination and seedling phase. Hamed and Ashraf (2008) and Hameed et al (2010) state that the shoot dry matter was slightly affected by varying levels of salt in the ecotype of *Cynodon dactylon* (L.) which collected from the salt Range and study anatomical adaptation. However, in contrast, its root weight was markedly increased. On the other hand, the ecotype from the salt Range accumulated relatively less amount of Na^+ in the shoot than did in that from Faisalsbad, shoot or root K^+ and Ca^{++} contents varied inconsistently in both ecotypes under salt stress. All photosynthetic parameters, leaf water potential and osmotic potential, and chlorophyll content in both ecotypes were adversely affected by salt stress. The salt tolerant ecotype accumulated significantly higher organic osmotic (total free amino acids, praline, soluble proteins soluble sugars) under saline conditions than its in tolerant counterpart. Overall the salt tolerant ecotype of C. dactyl on from salt Range showed high salt tolerant but to its restricted uptake of Na^+ accompanied by an increased uptake of K^+ and Ca^{++} in the roots as well as shoot sue to its higher photosynthetic capacity and accumulation of organic osmotica such as free amino acids and proline under saline conditions.

Mechanisms of Alleviating Salt Injury

The Role of Biofertilizers

Soil infertility in arid zones is often due to the presence of large quantities of salt, and the introduction of plants capable of surviving under

these conditions (salt-tolerant plants) is worth investigating (Delgado et al. 1994). Under agricultural condition, the amount of nitrogen removal is greater than the nitrogen input. To maintain soil fertility, nitrogen can be returned to the soil through biological nitrogen fixation (BNF) or by nitrogen fertilizer applications. Under agricultural condition, the amount of nitrogen removal is greater than the nitrogen input. To maintain soil fertility, nitrogen can be returned to the soil through biological nitrogen fixation (BNF) or by nitrogen fertilizer applications (Zahran 1991; Katerji et al. 2000; Hamdia et al, 2005). Asymbiotic nitrogen-fixing bacteria, including the genus *Azospirillum* are considered to be among the most important micro-organisms that play a significant role in soil fertility in tropical and temperate regions of the world. *Azospirillum* strains are commonly isolated worldwide from the rhizosphere of a diverse range of cereals, including corn, millet, sorghum, and wheat. Although more than a decade has passed since the beginning of intensive worldwide *Azospirillum* inoculation experiments to improve crop productivity of non-leguminous plants, there is still considerable and continued interest in testing the efficiency of *Azospirillum* strains for their ability to enhance and/or improve yields under salt stress ((Neyra et al. 1995; Hamdia and El-Komy 1998; Hamdia et al. 2000, Hamdia et al, 2004). The *Rhizobium*-legume symbiosis in arid ecosystems is particularly important for locations where the area of saline soils is increasing and becoming a threat to plant productivity. Legumes, which are usually present in arid ecosystems, may adapt to fix more N_2 under saline conditions than legumes grown in other habitats (Perssarakli et al. 1989; Zahran 1991; Katerji et al. 2000; Hamdia 2005). It has been argued that drought tolerance in plants can be enhanced by P and K nutrition. Application of mycorrhiza improves plant growth and nutrient acquisition and thus protects plants against salt stress in arid and semi-arid areas (Al-Karaki 2000). Therefore, the importance of inoculation of soil and plants with the beneficial microorganisms that enhance plant nutrition has been increased (Bashan, 1990; El-Rewainy 1994; Sarwar et al. 1998). Hamdia et al. (2000a) and El- Komy et al. (2003) showed that plant bacterization had stimulatory effect on amino acids accumulation compared with control uninoculated plants up to −1.2 MPa salinity level. Thus *Azospirillum* inoculation plays an important role in protein biosynthesis either directly through fixation of nitrogen or indirectly by enhancing soil N uptake.

However, plant inoculation with *Azospirillum* resulted in significant increases in shoot and root total N-yield up to −0.9 and −1.2 MPa salinity levels, respectively. Increased total N-yield at such high salinity levels could be attributed to factors other than N_2-fixation, since nitrogen fixation was completely inhibited at such NaCl levels (Hamdia and El-Komy 1998).

The Role of Phytohormones in Alleviating Salt Stress.

Phytohormones such as IAA, gibberellic acid (GA_3), and kinitin are known to be involved in the regulation of plant response to salinity stress and counteract the adverse effect of stress conditions (Itai *et al*., 1978, Walker and Dumbroff 1981;Hamdia, 1991, Hamdia and Brakat 1999, Hamdia, 1998; Cassin, 2001 a and b). Bejaoui (1985) stated that the effect of exogenously applied GA_3 or IAA in alleviation of salt stress might be through the activation of a specific enzyme, which participates in RNA and protein synthesis.

Kaya et al. (2009) show that plant hormones can be defined as organic substances that are produced in one part of plant and translocated to another parts, where at very low concentration, they stimulate physiological response. Plant hormones are natural

products and when they are synthesized chemically they are called plant growth regulators. Plants are usually subjected to environmental factors such as drought or high soil and water salinity. The reduction in plant growth exposed to saline environments could be due to either the effects of specific c ions on metabolism, or adverse water relations. Different strategies are being employed to maximize plant growth under saline conditions. One of them is to produce salt tolerant genotypes of different crops. Attempts to improve tolerance to salinity through conventional plant breeding methods are time consuming and laborious, and rely on existing genetic variability. In addition, many other attempts have been made to overcome this disorder, including proper management and exogenous application of plant growth regulators. In this context, the levels of gibberellic acid (GA), abscisic acid (ABA), indoleacetic acid (IAA), cytokinins (CK), jasmonates (JA) and triazoles under salt stress are being discussed in this review.

a. Indole acetic acid

Numerous studies have demonstrated improvement in seed germination of different plant species under both normal and stress conditions in response to priming with plant growth hormones or other organic substances (Ashraf and Foolad, 2005 and Shaddad et al. 2008). In wheat, while seed germination decreased with increasing levels of salinity, the adverse effect of salinity was alleviated by soaking seed with IAA or NAA (Balki and Padole 1982, Gulnaz et al. 1999). Fukuda and Tanaka (2006) state that the effects of ABA, auxin, and gibberellin on the expression of two genes (*HVP1* and *HVP10*) for vacuolar H^+-inorganic pyrophosphatase (EC 3.6.1.1) and one (*HvVHA-A*) for the catalytic subunit (subunit A) of vacuolar H^+-ATPase (EC 3.6.1.3) by quantification of the transcript levels, to identify the hormones responsible for regulating the expression of

these genes in barley (*Hordeum vulgare* L.) in response to environmental changes such as salt stress. ABA markedly induced the expression of *HVP1* and slightly increased the expression of *HVP10* and *HvVHA-A*. In contrast, 2,4-D only increased the expression of *HVP1*, and GA_3 had no significant effects on any gene. The maximum level of *HVP1* transcripts in response to these hormones was also much higher than the levels of *HVP10* and *HvVHA-A* transcripts. In addition, we also analyzed the expression of one gene (*HvNHX1*) for vacuolar Na^+/H^+ antiporter, and *HvNHX1* expression changed in a pattern similar to that of the *HVP1* expression. Furthermore, treatment with ABA and 2,4-D increased Na^+/H^+ antiport activity and proton-translocating activities by H^+-PPase and H^+-ATPase in tonoplast vesicles, and treatment with ABA also increased the amount of V-PPase protein of tonoplast vesicles. These results suggest that the hormones ABA and 2,4-D regulate the expression of the H^+-pump and Na^+/H^+ antiporter genes and are thus important effectors that regulate the expression of *HVP1* and *HvNHX1*. Akbari et al. (2007) showed that increasing NaCl concentrations of NaCl reduced germination percentage, radicle length, hypocotyls length, seedling fresh and dry weight and hypocotyl dry weight and hypocotyl dry weight. Auxin increased hypocotyls length, seedling fresh and dry weigh and hypocotyls dry weight, but did not influence on seed germination percentage and radical length of the three cultivars of wheat plants.

b. Gibberellic acid

Hamdia and El-Komy (1998) and Hamdia et al. (2000) stated that fresh and dry mass of maize plants were significantly increased by *Azospirillum* inoculation at the lower concentrations of NaCl. These investigations have also show that maize plants inoculated with *Azospirillum* or GA_3 application at different NaCl levels significantly increased the

chlorophyll content. Hegazi (1983), attributed the significant increases in growth parameters of wheat by *Azospirillum* inoculation to general improvement of the physiological status including the plant chlorophyll content. Earlier, Rice (1982) attributed the beneficial effects of some N_2 fixing bacteria on rice plants as compared with urea-treated plants, to the enhanced chlorophyll content. Radi et al. (2006) stat that pre-soaking wheat (Sakha 92) seeds in GA_3 increased the germination potential especially at moderate salinization levels. On the other hand, ABA did not increase the germination potential of the tested seeds at all salinization levels. Fresh and dry matter yields of wheat seedlings decreased at high salinization levels and 90 mM NaCl represented the threshold of salinity. Pre-soaking wheat seeds in GA_3 caused marked increases in fresh and dry matter yields of the salinized seedlings. While ABA treatment resulted in non-significant changes in dry matter production of stressed seedlings, the fresh matter production at most salinization levels tended to increase. GA_3 or ABA treatment generally increased the soluble carbohydrates and total protein contents of salt stressed seedlings. The stimulatory effect of NaCl salinity on protein synthesis was additionally enhanced as a result of GA_3 at all salinization levels or due to ABA treatment especially at high salt levels. The stimulatory effects of salt stress on the accumulation of free amino acids other than proline in the seedlings increased, with the application of GA_3, while the activated synthesis of proline was partially attenuated. Application of GA_3 was sufficient to attenuate partially the stimulatory effect of NaCl supply on proline biosynthesis, while ABA treatment failed to play this role. Shaddad et al. (2006) the mechanism of salt tolerance of two wheat cultivars, *Giza 168*, *Sohag 3* and interaction effect of salinity and GA_3 treatments have been evaluated. The data showed that *cv. Giza 168* was more salt tolerant and *cv. Sohag 3* was salt sensitive even at low salinity

levels. This was based on the differences in growth criteria of the two tested wheat cultivars under the influence of salinity. While dry matter yield of root, stem and leaf remained mostly unchanged at mild salinity in *cv. Gize 168,*those of *cv. Sohag 3* were markedly affected even at low salinity. These differences in growth criteria of the two wheat cultivars were mirrored by evident variations in absorption and distribution of Na^+ and K^+ among the different organs of the two wheat cultivars .Generally, Na^+ content in *cv. Giza 168* was lower than *cv. Sohag 3,* while the amount of Na^+ distributed similarly between roots, stem and leaves for *cv. Giza 168*. On the other hand, the it distributed unequally in *Sohag 3 cv.*. An opposite pattern has been obtained in K^+ where its amount was much higher in *cv. Giza 168* than *cv. Sohag 3*, whatever the plant organ tested. Consequently, K^+/Na^+ ratio was much more higher in *cv. Giza 168* than *Sohag 3, w*hich indicated the superiority of salt tolerance of *cv. Giza 168,* as compared with the more salt sensitive *cv. Sohag 3*. This was observed as a marked and progressive increase in catalase, peroxidase and ascorbate peroxidase which consequently maintained. The concentration of MDA (as an indicator of free radical damage to cell membrane) was approximately comparable to the control value, especially at mild salinity in *cv. Gize 168*. On other hand, salt sensitive *cv. Sohag 3* failed to enhance the activity of peroxidase and ascorbate peroxidase with a consequently increased MDA content.

Shaddad et al. (2006, 2008) revealed that the two wheat cultivars (Sohag 3 and Giza 186) accumulate carbohydrate and proline content by salinity stress in different organs, except Giza 168 stem, where carbohydrates were significant declined by salinity stress. Soluble protein content varied not only between the two wheat cultivars but also between the different plant organs. In cv. Sohag, the

soluble protein content in root and stem decreased decreased slightly by increasing salinity in the soil especially at the higher salinization level. Proline concentration in root, stem and leaf of both cultivars was significantly increased with increasing salinity in soil. This accumulation was higher in salt sensitive cv. Sohag 3, compared to the salt tolerant cv. Giza 168, especially at higher salinity level. GA_3 treatments improved the growth criteria, crop yield of the two wheat cultivars. This was judged with the observable increase in protein content in the different organs of both cultivars.

Shah (2007) study the effect of gibberellic acid (GA_3) on growth, physiology and yield of salt-stressed mustrsd (Brassica Juncea L. Czern & Coss)) cv. *Varuna* plants were studied. The stressed imprse by 25 or 50 mM NaCl reduced *substantially* leaf area, dry mass, leaf cholorophyll content, stomstoal conductance and net photosynthetic rate 50 days after emergence. At harvest, although other yield components were generally reduced, total seed protein content showed a significant increase. Furthermore, the response was more pronounced at higher concentration NaCl (50 mM) applied. On the contrary, the application of 10^{-5} GA_3 appeared to mitigate the adverse effects of salinity stress on the overall performance and productivity of mustard.

Munns (2002) reported that salinity reduces the ability of plants to take up water, and this quickly causes reductions in the growth rate, along with a suite of effects identical to those caused by water stress. Later, there may be a salt-specific and additional effect on growth: if excessive amounts of salt enter the plant they will eventually rise to toxic levels in the older transpiring leaves, and reduce the photosynthetic capacity of the plant. Salinity can therefore affect growth via changed water relations, hormonal balance, or carbon supply, the relative importance of each process depending on the time scale of the response. Chakrabarti and Mukherji (2003) stated that Efficiency of pretreatment as foliar spray of indole-3-acetic acid, gibberellic acid and kinetin, each ranging from 0.1 to 10.0 μM concentration, in restoring the metabolic alterations imposed by NaCl salinity was investigated in *Vigna radiata* (L.) Wilczek. Glycolate oxidase, superoxide dismutase, catalase and peroxidase activities increased under stress in leaves and roots also. Malondialdehyde content and total peroxide content also increased under stress. All the three hormones used were able to overcome to variable extents the adverse effects of stress imposed by NaCl to these parameters.

Weathers et al. (2005) stated that Few studies have focused on the effect of a broad range of phytohormones on growth and secondary metabolism of a single hairy root species. We measured growth, development, and production of the antimalarial drug, artemisinin, in *Artemisia annua* hairy roots in response to the five main hormones: auxins, cytokinins, ethylene, gibberellins (GA), and abscisic acid (ABA). Single roots grown in six-well plates in medium B5 with 0.01 mg l^{-1} (0.029 μ*M*) GA_3 produced the highest values overall in terms of the number of lateral roots, length of the primary root, lateral root tip density, total lateral root length, and total root length. When the total root lengths are compared, the best conditions for stimulating elongation appear to be: GA 0.01 mg l^{-1} (0.029 μ*M*) > ABA 1.0 mg l^{-1} (3.78 μ*M*)=GA 0.02 mg l^{-1} (0.058 μ*M*). Bulk yields of biomass were inversely proportional to the concentration of each hormone tested. All cultures provided with ABA yielded the highest amount of biomass. Both 6-benzylaminopurine and 2-isopentenyladenine inhibited root growth, however, only 2-isopentenyladenine stimulated artemisinin production, more than twice that of the B5 controls,

and more than any other hormone studied. These results will prove useful in increasing hairy root growth and artemisinin production.

c. Cytokinis

Iqbal et al (2006) stat that cytokinins are often considered abscisic acid (ABA) antagonists and auxins antagonists/synergists in various processes in plants. Seed enhancement (seed priming) with cytokinins is reported to increase plant salt tolerance. It was hypothesized that cytokinins could increase salt tolerance in wheat plants by interacting with other plant hormones, especially auxins and ABA. The present studies were therefore conducted to assess the effects of pre-sowing seed treatment with varying concentrations (100, 150 and 200 mg l^{-1}) of cytokinins (kinetin and benzylaminopurine (BAP)) on germination, growth, and concentrations of free endogenous auxins and ABA in two hexaploid spring wheat (Triticum aestivum L.) cultivars. The primed and non-primed seeds of MH-97 (salt-intolerant) and Inqlab-91 (salt-tolerant) were sown in both Petri dishes in a growth room and in the field after treatment with 15 dS m−1 NaCl salinity. Both experiments were repeated during 2002 and 2003. Among priming agents, kinetin was effective in increasing germination rate in the salt-intolerant and early seedling growth in the salt-tolerant cultivar when compared with hydropriming under salt stress. Thus, during germination and early seedling growth, the cytokinin-priming induced effects were cultivar specific. In contrast, kinetin-priming showed a consistent promoting effect in the field and improved growth and grain yield in both cultivars under salt stress. The BAP-priming did not alleviate the inhibitory effects of salinity stress on the germination and early seedling growth in both cultivars. The increase in growth and grain yield in both cultivars was positively correlated with leaf indoleacetic acid concentration and negatively with ABA

concentration under both saline and non-saline conditions. The decrease in ABA concentration in the plants raised from kinetin-primed seeds might reflect diminishing influence of salt stress. However, the possibility of involvement of other hormonal interactions is discussed.

d. Salycilic acid

Salicylic acid is (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. At the same time at present considerable interest has been aroused by the ability of SA to produce a protective effect on plants under the action of stress factors of different abiotic nature. Thus convincing data have been obtained concerning the SO induced increase in the resistance of wheat seedlings to salinity (Shakirova and Bezrukova 1997), and water deficit (Bezrukova et al. 2001), Tari et al. (2002) study long-term incubation of tomato plants in low concentration of salicylic acid enabled plants to tolerate salt stress caused by 100 mM NaCl. Na$^+$ ions accumulated in the leaf tissues of treated plants and functioned as osmolytes without the well-known detrimental effects of the excess sodium.

Sakhabutdinova et al. (2003) investigated the effect of salicylic acid (SA) on plant resistance to environmental stress factors. Treatment of wheat plants with 0.05mM SA increased the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth. Phytohormones are known to play a key role in plant growth regulation. It was found that the SA treatment caused accumulation of both ABA and IAA in wheat seedlings. However, the SA treatment did not influence cytokinin content. We suppose, that the protective and growth promoting effects of SA are due to the phenomenon described above. The SA treatment reduced the damaging action of salinity and water deficit on seedling growth and accelerated a

restoration of growth processes. Treatment with SA essentially diminished the alteration of phytohormones levels in wheat seedlings under salinity and water deficit. The SA treatment prevented the decrease in IAA and cytokinin content completely which reduced stress-induced inhibition of plant growth. Also, high ABA levels were maintained in SA treated wheat seedlings which provided the development of antistress reactions, for example, maintenance of proline accumulation. Thus protective SA action includes the development of antistress programs and acceleration of normalization of growth processes after removal stress factors

The Role of Minerals

Minerals are considered as an important role in the mechanisms plant tolerance to salinity stress (Hamdia, 1993)

a. Calcium and Magnesium

Hamdia (1993a) study alleviation of NaCl injury on pigment, saccharide and mineral centents of wheat plants by exogenous application of $CaCl_2$ or KCl. Tuna et al. (2007) report_that the supplemental calcium sulphate added to nutrient solution containing salt (NaCl; 75 mM significantly improved growth and physiological variables affected by salt stress. Plant growth, fruit yield, and membrane permeability, leaf K^+, Ca^{++} and N in tomato plants. The effect of supplemental CaSO4 in mainting membrane permeability, increasing of Ca^{++}, N, and K^+ and reducing Na^+ concentration in leaves could offer an economical and simple solution to tomato crop production problems caused by high salinity. Igbal et al. (2006) show that whether salt tolerance could be improved in spring wheat (Triticum aestivum L.), the present study was performed by soaking the seeds of two cultivars, namely MH-97 (salt sensitive) and Inqlab-91 (salt tolerant), for 12 h in distilled water or 100 mol/m3 $CaCl_2$, KCl, or

NaCl. Primed seeds from each treatment group and non-primed seeds were sown in a field in which NaCl salinity of 15 dS/m was developed. Priming of seeds with $CaCl_2$, followed by priming with KCl and NaCl, was found to be effective in alleviating the adverse effects of salt stress on both wheat cultivars in terms of shoot fresh and dry weights and grain yield. Priming with $CaCl_2$ alleviated the adverse effects of salt stress on hormonal balance in plants of both cultivars. In MH-97 plants, $CaCl_2$ pretreatment considerably reduced leaf abscisic acid (ABA) concentrations and increased leaf free salicylic acid (SA) concentrations under both saline and non-saline conditions. In contrast, in the Inqlab-91 plant, $CaCl_2$ increased free indoleacetic acid (IAA) and indolebutyric acid (IBA) content. However, priming of seeds with $CaCl_2$ did not alter free polyamine levels in either cultivar, although spermidine levels were considerably lower in plants raised from seeds treated with $CaCl_2$ for both cultivars under saline conditions. Priming with KCl increased growth in Inqlab-91 plants, but not in MH-97 plants, under saline conditions. The salinity induced reduction in auxins (IAA and IBA) was alleviated by NaCl priming in both cultivars under saline conditions. However, NaCl increased leaf free ABA content and lowered leaf SA and putrescine levels in Inqlab-91 plants under saline conditions. In conclusion, although all three priming agents (i.e. $CaCl_2$, KCl, and NaCl) were effective in alleviating the adverse effects of salt stress on wheat plants, their effects on altering the levels of different plant hormones were different in the two cultivars. (Managing editor: Ping He)

b. Nitrogen, phosphurs and potassium

Hamdia (1993) stated that salinity inhibited growth, and affects the contents of cholorophylls, carotenoids, saccharides, amino acids, proteins, DNA and RNA in broad bean plants. Foliar application of

NaH₂PO4 and NaNO₃ greatly ameliorated the adverse effects of NCl. The counteraction was associated with an increase in contents of saccharides, proteins, DNA and RNA.

Hamdia et al. (2000) evaluate the possible role of inoculation by *Azospirillum lipoferum* or *Bacillus polymexa* or foliar P and K fertilization for improving salinity tolerance of maize plants. Maize plants were inoculated or treated by KH_2PO_4 and grown under (NaCl) stress. The effect of bacterial inoculation and foliar fertilization on the growth and other physiological parameters of Stalinized plants were detected. In most cases, either inoculation with any of the N_2-fixers or spraying with KH_2PO_4 resulted in an increase in fresh and dry matter as well as water content and enhanced plant mineral nutrition exhibited by increased Mg/K and decreased P/K, Ca/K and Na/K ratios.Potassium is known to improve resistance of plants to environmental stress (Wu et al. 2009). Report of Sellstedt et al. (1993) indicates that K can apparently alleviate the effects of water shortage on symbiotic N_2 fixation of *V. faba* and *P. vulgaris*. The response of 0.8 or 0.3 mM K^+ allowed nodulation and subsequent nitrogen fixation of *V. faba* and *P. vulgaris* under a high-water regimen (field capacity to 25% depletion). It was also shown that the symbiotic system in this legume is less tolerant to limiting K supply than are the plants themselves. Species of legumes vary in the type and quantity of the organic solutes, which accumulate intracellularly in leguminous plants under water stress. This could be a criterion for selecting drought-tolerant legume-*Rhizobium* symbioses that are able to adapt to arid climates. Wu et al. (2009) study the interactive effect of potassium and sodium on root growth and expression of K/Na transporter genes in rice.

Exogenous application of proline

Exogenous application of proline can play an important role in enhancing plant stress tolerance. This role can be in the form of either osmoprotection (Wyn Jones and Gorham 1983; Handa et al. 1986, Hamdia, 1987) or cryoprotection (Snngstad et al. 1990 and Santarius 1992). For example, in various plant species growing under saline conditions, exogenously-supplied proline provided osmoprotection and facilitated growth (Csonka and Hanson 1991) and Yancey, 1994). In rice, exogenous application of 30 mM proline counteracted the adverse effects of salinity on early seedling growth, though higher concentrations of proline resulted in reduced growth (Roy et al. 1993). Exogenous application of proline to stressed plants of the halophyte *Allenrolfea occidentalis* increased their growth and halted increased production of ethylene due to salt- or drought-stress (Chrominski et al. 1989). Proline can also protect cell membranes from salt-induced oxidative stress by enhancing activities of various antioxidants (Yan et al. 2000). For example, growth of tobacco suspension cells under salt stress was promoted by exogenous application of 10 mM proline, which was proposed to be due to proline action as a protectant of enzymes and membranes (Okuma et al. 2000). In soybean cell cultures maintained under salt stress, exogenous application of proline increased activities of superoxide dismutase and peroxidase, which normally contribute to increased salt tolerance (Yan et al. 2000 and Hua and Guo 2002).In barley embryo cultures under saline conditions, exogenous application of proline resulted in a decrease in Na^+ and Cl^- accumulations and an increase in growth (Lone et al. 1987). Such ameliorative effects of proline were indicated to be due to plasma membrane stabilization (Mansour 1998).

Ashraf and Foolad (2006) stat that glycine betaine (GB) and proline are two major organic

osmolytes that accumulate in a variety of plant species in response to environmental stresses such as drought, salinity, extreme temperatures, UV radiation and heavy metals. Although their actual roles in plant osmotolerance remain controversial, both compounds are thought to have positive effects on enzyme and membrane integrity along with adaptive roles in mediating osmotic adjustment in plants grown under stress conditions. While many studies have indicated a positive relationship between accumulation of GB and proline and plant stress tolerance, some have argued that the increase in their concentrations under stress is a product of, and not an adaptive response to stress. In this article, we review and discuss the evidence supporting each of these arguments. As not all plant species are capable of natural production or accumulation of these compounds in response to stress, extensive research has been conducted examining various approaches to introduce them into plants. Genetically-engineered plants containing transgenes for production of GB or proline have thus far faced with the limitation of being unable to produce sufficient amounts of these compounds to ameliorate stress effects. An alternative "shot-gun" approach of exogenous application of GB or proline to plants under stress conditions, however, has gained some attention. A review of the literature indicates that in many, but not all, plant species such applications lead to significant increases in growth and final crop yield under environmental stresses. In this review article, numerous examples of successful application of these compounds to improve plant stress tolerance are presented. However, to streamline useful and economic applications of these compounds, further investigations are needed to determine the most effective concentrations and number of applications as well as the most responsive growth stage(s) of the plant. All these factors may vary from species to species. Furthermore, a better understanding of the mechanisms of action of exogenously applied GB and proline is expected to aid their effective utilization in crop production in stress environments.

Exogenous application of GB

In many crop plants the natural accumulation of GB is lower than sufficient to ameliorate the adverse effects of dehydration caused by various environmental stresses (Wyn Jones and Story 1981; Yancey 1994; Subbarao et al. 2001). Exogenous application of GB to low-accumulating or non-accumulating plants may help reduce adverse effects of environmental stresses (Agboma et al 1997 a & b; Makela et al. 1998 a; Yang and Lu 2005). Externally-applied GB can rapidly penetrate through leaves and be transported to other organs, where it would contribute to improved stress tolerance (Makela et al. 1998 b). The efficiency of penetration can be improved by using various surfactants such as kinetic, lus-50 and sito+ (Subbarao et al., 2001). Furthermore, because naturally produced GB does not normally break down in plants (Bray et al. 2000), it can easily be collected as a relatively inexpensive by-product from high-producing plants such as sugar beets (Rhodes et al 1998 and Yang et al. 2003). This may make extraction and exogenous application of GB an economically feasible approach to counteract adverse effects of environmental stresses on crop productivity.

In addition to its direct protective roles, either through positive effects on enzyme and membrane integrity or as an osmoprotectant, GB may also protect cells from environmental stresses indirectly via its role in signal transduction. For example, GB may have a role in Na^+/K^+ discrimination, which substantially or partially contributes to plant salt tolerance. Ion homeostasis in plants is governed by various membrane transport systems. Recently, significant progress has been made in the

characterization of cation transporters that maintain ion homeostasis during salt stress in plants, of which SOS (salt overly sensitive) is a novel signaling pathway (Chinnusamy et al. 2005). This pathway is somewhat regulated by MAP kinases, expressions of which are highly affected by GB. Also, some physiological studies of GB-treated turfgrass and Arabidopsis plants indicate that GB up-regulates expression of many genes ($\sim$360 genes), of which more than 6% are known to be related to signal transduction (John 2002). Examples are lipoxygenase, monodehydroascorbate reductase, osmotin, putative receptor kinase, calmodulin, protein kinase, and receptor protein kinase. These and other evidence have led some investigators to suggest that GB contributes to plant salt tolerance through its role in signal transduction and ion homeostasis (John 2002; Yilmaz 2004). However, knowledge of how GB affects expression of genes responsible for, or related to, plant salt tolerance is scarce. Elucidation of the roles of GB in regulating genes of signaling pathways used by plants to respond to environmental stresses may lead to devising approaches to improve plant stress tolerance

Exogenous application of polyamines:

Polyamines are ubiquitous low-molecular-weight aliphatic amines that are involved in regulation of plant growth and development (Martin-Tanguy 2001). Because of their polycationic nature at physiological pH, polyamines are able to interact with proteins, nucleic acids, membrane phospholipids and cell wall constituents, thereby activating or stabilizing these molecules. The most commonly found polyamines in higher plants, the diamine putrescine (Put), the triamine spermidine (Spd) and the tetraamine spermine (Spm) may be present in the free, soluble conjugated and insoluble bound forms. Soluble conjugated polyamines are those which are covalently conjugated to small molecules such as phenolic compounds, and insoluble bound polyamines are those which are covalently bound to macromolecules such as nucleic acids and proteins. Polyamines are ubiquitous low-molecular-weight aliphatic amines that are involved in regulation of plant growth and development (Martin- Tanguy 2001). Because of their polycationic nature at physiological pH, polyamines are able to interact with proteins, nucleic acids, membrane phospholipids and cell wall constituents, thereby activating or stabilizing these molecules. The most commonly found polyamines in higher plants, the diamine putrescine (Put), the triamine spermidine (Spd) and the tetraamine spermine (Spm) may be present in the free, soluble conjugated and insoluble bound forms. Soluble conjugated polyamines are those which are covalently conjugated to small molecules such as phenolic compounds, and insoluble bound polyamines are those which are covalently bound to macromolecules such as nucleic acids and proteins.
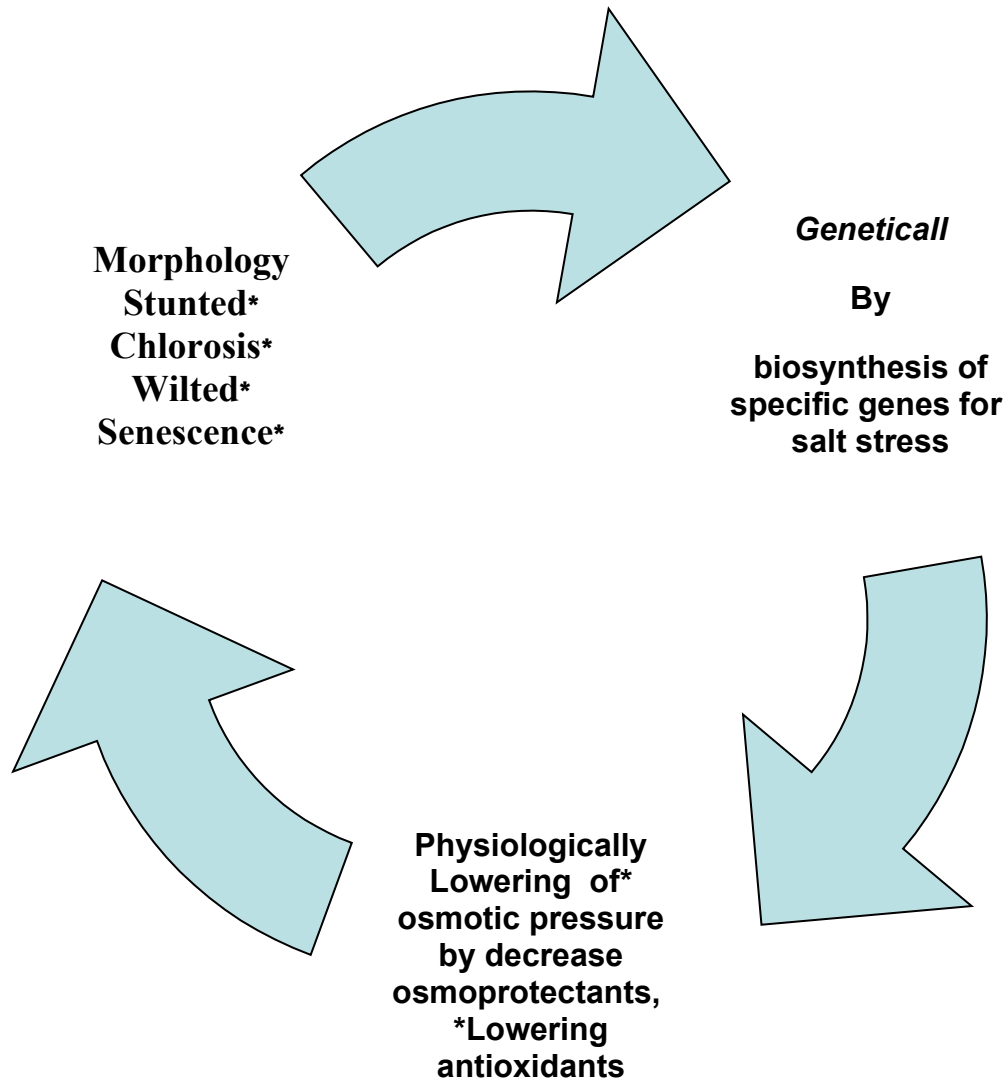
Geneticall

By

biosynthesis of specific genes for salt stress

Morphology Stunted* Chlorosis* Wilted* Senescence*

Physiologically Lowering of* osmotic pressure by decrease osmoprotectants, *Lowering antioxidants

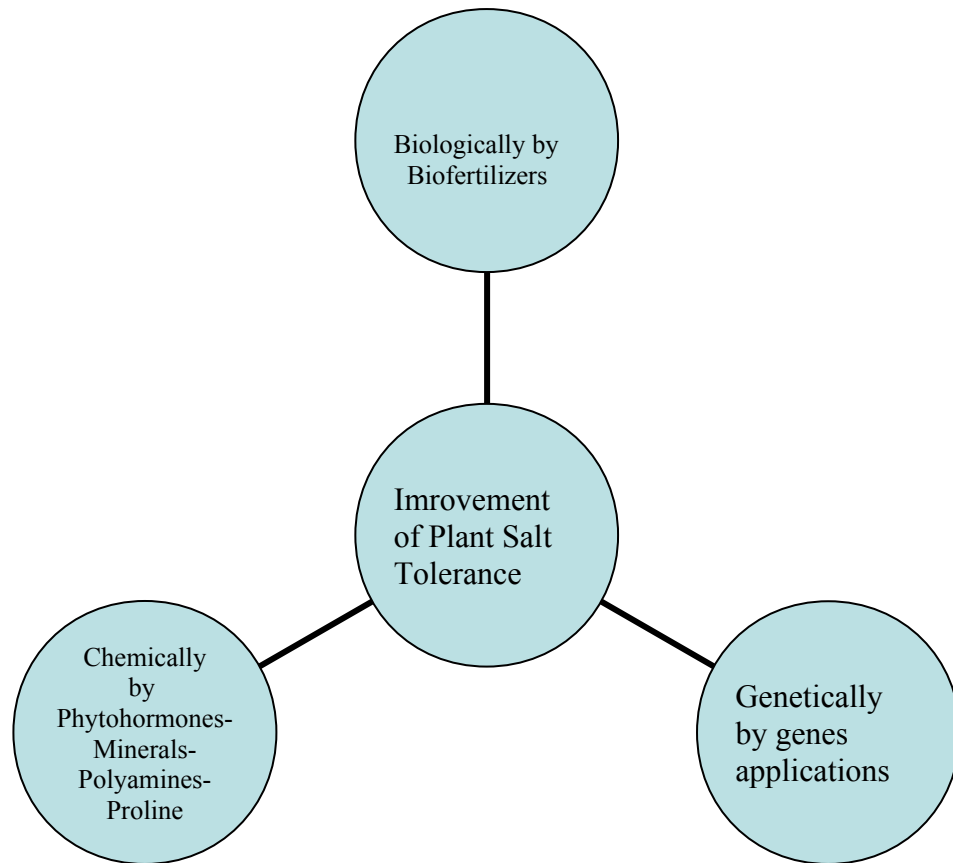**Fig. 1:** Digram illustrated the deleterious effect of salt stress on plants.

Fig. 2 : Digram showed the directions of improving plant salt tolerance.

Numerous attempts have been made to improve the salinity tolerance of a variety of crops by traditional breeding programs, but commercial success has been limited thus far. In recent years, some methods, such as transgenic approaches and exogenous polyamine application, have been directed towards the agricultural use of polyamine ability for enhancing the salinity tolerance of plants. Several transgenic approaches have shown enhanced salinity tolerance in transgenic rice, sweet potato, tobacco and *Arabidopsis thaliana* plants that overexpress polyamine biosynthetic enzyme genes (Roy and Wu 2001; Kasukabe et al. 2004; 2006; Wi et al. 2006). However, the complex regulation of polyamine

synthesis in relation to endogenous substrate availability, developmental stages and organ-specific parameters has handicapped the achievement of practical transgenic results (Ndayirgije and Lutts 2006). Alternatively, exogenous polyamine application is a convenient and effective approach for enhancing salinity tolerance of crops and eventually improving crop productivity under high salinity. Indeed, exogenous polyamine application has been successfully used for enhancing salinity tolerance of plants (Chattopadhayay et al 2002; Verma and Mishra 2005). Among the three major polyamines, Spd has in many cases been more closely associated with stress tolerance in plants (Shen et al. 2000). With respect to salinity tolerance, however, the available data are contradictory. Roy and Wu (2001) reported that overexpression of ADC transgenes in rice resulted in enhanced salinity tolerance along with an increase in endogenous concentrations of Put, but not Spd and Spm. Verma and Mishra (2005) found that salinity-caused reduction in seedling growth of *Brassica juncea* was alleviated by exogenous Put. In contrast, in rice shoots, Krishnamurthy and Bhagwat (1989) observed that salinity caused excessive accumulation of Put, with little change in Spd and Spm content in salinity-sensitive cultivars, whereas in salinity-tolerant cultivars, the same stress induced a remarkable increase in Spd and Spm content and a decrease in Put. Chattopadhayay et al (2002) found that salinity-induced injury of rice plants was greatly mitigated by Spd or Spm added to the salinized nutrient solution. In contrast, it was recently reported that Put, Spd and Spm did not alleviate salinity-induced growth suppression in rice (Ndayiragije and lutts 2006).

Thus, involvement of polyamines in salinity tolerance of plants appears inconclusive.

Dudan et al. (2007) state that we investigated the effects of short-term salinity stress and spermidine application to salinized nutrient solution on polyamine metabolism and various stress defense reactions in the roots of two cucumber (*Cucumis sativus* L.) cultivars, Changchun mici and Jinchun No. 2. Seedlings grown in nutrient solution salinized with 50 mM NaCl for 8 d displayed reduced relative water content, net photosynthetic rates and plant growth, together with increased lipid peroxidation and electrolyte leakage in the roots. These changes were more marked in cv. Jinchun No. 2 than in cv. Changchun mici, confirming that the latter cultivar is more salinity-tolerant than the former. Salinity stress caused an increase in superoxide and hydrogen peroxide production, particularly in cv. Jinchun No. 2 roots, while the salinity-induced increase in antioxidant enzyme activities and proline contents in the roots was much larger in cv. Changchun mici than in cv. Jinchun No. 2. In comparison to cv. Jinchun No. 2, cv. Changchun mici showed a marked increase in arginine decarboxylase, ornithine decarboxylase, *S*-adenosylmethionine decarboxylase and diamine oxidase activities, as well as free spermidine and spermine, soluble conjugated and insoluble bound putrescine, spermidine and spermine contents in the roots during exposure to salinity. On the other hand, spermidine application to salinized nutrient solution resulted in alleviation of the salinity-induced membrane damage in the roots and plant growth and photosynthesis inhibition, together with an increase in polyamine and proline contents and antioxidant enzyme activities in the roots of cv. Jinchun No. 2 but not of cv. Changchun mici. These

results suggest that spermidine confers short-term salinity tolerance on cucumber probably through inducing antioxidant enzymes and osmoticants.

The role of vitamins

Shaddad et al. (1990) show that pyridoxine and ascorbic acid could be alleviated the salt injury of broad bean plant. Hamdia (2000) studies on the influence of biotin or pyridoxine in amelioration the effect of salinity in lupine plants on growth and metabolities of zea mays plant. Vitamine C is a universal reductant and antioxidant of plants. It is found at concentration 1-2 Mm in legume nodules (Matamoros et al. 1999) and is positively correlated with metabolic for the operation of the ASG-GSH pathways, but it also has beneficial effects that do not require the presence of APX, ASC can directly scavenge ROS. It is also involved in hydroxylation of praline, regulation of the cell cycle and numerous fundamental processes of plant growth and development (Noctor and Foyer 1998). Exogenous application of vitamine C to plant could lead to growth stimulation through the activation of some enzymatic reaction. Alquraing (2007) and Azooz and Al-Ferdan (2009) determine the influence of vitamine C pre-treatment seed subjected to saline stress during germination through growth crieteria, compatible solutes and adaptive mechanisms of the antioxidant.

The role of molecular genetic in increasing salt tolerance

Munns and Tester (2008) study the physiological and molecular mechanisms of tolerance to osmotic and ionic components of salinity stress are reviewed at the cellular, organ, and whole-plant level. Plant growth responds to salinity in two phases: a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves. Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, Na^+ or Cl^- exclusion, and the tolerance of tissue to accumulated Na^+ or Cl^-. Our understanding of the role of the *HKT* gene family in Na^+ exclusion from leaves is increasing, as is the understanding of the molecular bases for many other transport processes at the cellular level. However, we have a limited molecular understanding of the overall control of Na^+ accumulation and of osmotic stress tolerance at the whole-plant level. Molecular genetics and functional genomics provide a new opportunity to synthesize molecular and physiological knowledge to improve the salinity tolerance of plants relevant to food production and environmental sustainability. Chehab et al (2009) study changes in carbohydrate composiotion were investigated at the end of two important table olive cultivars "Meski" and Picholine" grown in Tunisia under different irrigation regimes

Miguel et al (2009) During the last years, our understanding of the mechanisms that control plant response to salt stress has been steadily progressing. Pharmacological studies have allowed the suggestion that the cytoskeleton may be involved in regulating such a response. Nevertheless, genetic evidence establishing that the cytoskeleton has a role in plant tolerance to salt stress has not been reported yet. Here, we have characterized *Arabidopsis* T-DNA mutants for genes encoding proteins orthologous to prefoldin (PFD) subunits 3 and 5 from yeast and mammals. In these organisms, PFD subunits, also known as Genes Involved in Microtubule biogenesis (GIM), form a heterohexameric PFD complex

implicated in tubulin and actin folding. We show that, indeed, PFD3 and PFD5 can substitute for the loss of their yeast orthologs, as they are able to complement yeast *gim2Δ* and *gim5Δ* mutants, respectively. Our results indicate that *pfd3* and *pfd5* mutants have reduced levels of α- and β-tubulin compared to the wild-type plants when growing under both control and salt-stress conditions. In addition, *pfd3* and *pfd5* mutants display alterations in their developmental patterns and microtubule organization, and, more importantly, are hypersensitive to high concentrations of NaCl but not of LiCl or mannitol. These results demonstrate that the cytoskeleton plays an essential role in plant tolerance to salt stress.

Yang et al (2009) reported that plants overexpressing *AtNHX1* or *SOS1* have significantly increased salt tolerance. To test whether overexpression of multiple genes can improve plant salt tolerance even more, we produced six different transgenic *Arabidopsis* plants that overexpress *AtNHX1*, *SOS3*, *AtNHX1 + SOS3*, *SOS1*, *SOS2 + SOS3*, or *SOS1 + SOS2 + SOS3*. Northern blot analyses confirmed the presence of high levels of the relevant gene transcripts in transgenic plants. Transgenic *Arabidopsis* plants overexpressing *AtNHX1* alone did not present any significant increase in salt tolerance, contrary to earlier reports. We found that transgenic plants overexpressing *SOS3* exhibit increased salt tolerance similar to plants overexpressing *SOS1*. Moreover, salt tolerance of transgenic plants overexpressing *AtNHX1 + SOS3*, *SOS2 + SOS3*, or *SOS1 + SOS2 + SOS3*, respectively, appeared similar to the tolerance of transgenic plants overexpressing either *SOS1* or *SOS3* alone.

Moller et al (2009) stated that soil salinity affects large areas of cultivated land, causing significant reductions in crop yield globally. The Na^+ toxicity of many crop plants is correlated with overaccumulation of Na^+ in the shoot. We have previously suggested that the engineering of Na^+ exclusion from the shoot could be achieved through an alteration of plasma membrane Na^+ transport processes in the root, if these alterations were cell type specific. Here, it is shown that expression of the Na^+ transporter HKT1;1 in the mature root stele of *Arabidopsis thaliana* decreases Na^+ accumulation in the shoot by 37 to 64%. The expression of *HKT1;1* specifically in the mature root stele is achieved using an enhancer trap expression system for specific and strong overexpression. The effect in the shoot is caused by the increased influx, mediated by HKT1;1, of Na^+ into stelar root cells, which is demonstrated in planta and leads to a reduction of root-to-shoot transfer of Na^+. Plants with reduced shoot Na^+ also have increased salinity tolerance. By contrast, plants constitutively expressing *HKT1;1* driven by the cauliflower mosaic virus 35S promoter accumulated high shoot Na^+ and grew poorly. Our results demonstrate that the modification of a specific Na^+ transport process in specific cell types can reduce shoot Na^+ accumulation.

Other Ways for increasing salt tolerannce

Tester (2009) said that an international team of scientists has developed salt-tolerant plants using a new type of genetic modification that could help make salt-tolerant cereal crops a reality.

The research team - based at the University of Adelaide's Waite Campus in South Australia -used a

new GM technique to contain salt in parts of the plant where it does less damage.

The work was led by researchers from the Australian Centre for Plant Functional Genomics and the University of Adelaide's school of agriculture, food and wine, in collaboration with scientists from the department of plant sciences at Britain's University of Cambridge.

Professor Mark Tester, who works for both Australian institutions, said the team modified genes around the plant's water conducting pipes (xylem) so that salt was removed from the transpiration stream before it got to the shoot. This kept salt in the form of sodium ions (Na+) out of the leaves of a model plant species.

"This reduces the amount of toxic Na^+ building up in the shoot and so increases the plant's tolerance to salinity," Tester said. "In doing this, we've enhanced a process used naturally by plants to minimise the movement of Na^+ to the shoot. We've used genetic modification to amplify the process, helping plants to do what they already do - but to do it much better."

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