Response of crops to salinity under Egyptian conditions: a review

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Abstract

An option to cope with higher population densities in developing countries is to increase productivity of cultivated land. This makes also understanding of environmental stress phenomena and related tolerant mechanisms more important in countries such as Egypt.

Soil salinity is one of the major problems for agriculture in semi arid regions. In Egypt, plants are subjected to extreme climatic factors such as high temperatures and drought. Under these conditions, dissolved salts may accumulate in soils because of the insufficient leaching of ions. An accumulation of salt in upper soil layers may be also due to a unsuitable irrigation management.

Challenges faced by crop plants cultivated in the presence of excess salt are disturbance of osmotic regulation, ion imbalance, and oxidative stress, which impair plant metabolism and growth.

Only a few crop species and genotypes are adapted to saline conditions. The response of crops to salinity is a complex phenomenon and involves changes in plant morphology and physiology. In this review parameters associated with whole plant response to salt stress are described with special emphasis on findings in northern Africa.

Keywords: antioxidative enzymes, compatible osmolytes, halophytes, phenylurea, proline, salt tolerance

Zusammenfassung

Pflanzliche Reaktionen auf Salzstress unter Bedingungen in Ägypten: ein Review-Artikel

Die Steigerung der landwirtschaftlichen Produktion ist eine Möglichkeit, den Nahrungsmittelbedarf einer wachsenden Bevölkerung zu decken. In Ländern wie Ägypten erfordert dies ein besseres Verständnis physiologischer Reaktionen von Pflanzen auf Stress durch Wassermangel oder Versalzung sowie die Klärung kausaler Ursachen für Toleranz gegenüber diesen Stressfaktoren.

Bodenversalzung ist eines der größten Probleme in der Landwirtschaft unter semi-ariden Bedingungen. Dafür ist neben Hitze und Trockenheit ist auch ein ungeeignetes Bewässerungsmanagement verantwortlich.

Versalzung führt bei Pflanzen zu Störungen in der Osmoregulierung, des Ionen-Gleichgewichtes und zu oxidativem Stress, welche den Stoffwechsel und das Wachstum der Pflanzen beeinträchtigen.

Nur wenige Pflanzenspezies und Genotypen haben sich salinen Wachstumsbedingungen angepasst und reagieren mit vielfältigen Veränderungen in Morphologie und Physiologie auf Salzstress. In dem vorliegenden Review-Artikel werden die Reaktionen von Pflanzen auf Salzstress beschrieben, wobei schwerpunktmäßig Forschungsergebnisse aus dem nordafrikanischen Raum berücksichtigt wurden.

Schlüsselwörter: Antioxidative Enzyme, Halophyten, Osmolyten, Phenylharnstoff, Prolin, Salztoleranz

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1 Salinity in Egyptian agriculture

The agriculture in Egypt depends on irrigation mainly from the river Nile and in some places from underground water. The principal irrigation water resources are the Nile and the water gathered in its dams, particularly the High Dam of Aswan. The Egypt annual water quota from the Nile is 55.5 billion m³ per year (Ezzat, 1993). The irrigated area amounts to about 7.5 millon feddan (1 feddan = 0.42 ha) (EL-Zanaty, 1995). With less than 200 mm precipitation per year, rainfall is negligible as a water source for agriculture, except for a small area along the Mediterranean Coast in the Western Desert and the Sinai.

Egypt is a country with about 5000 years of experience in irrigation. Nevertheless, the country's economy suffers from severe salinity problems as due to irrigation with low quality water and poor drainage systems 33 % of the cultivated land are already salinized. Overcoming salt stress is a main issue in these regions to secure crop productivity (Ghassemi et al., 1995, El Raey, 1997).

Unsuitable irrigation management, especially the use of salty sea and lake water is a main reason for the increase of soil salinity in which sodium chloride (NaCl) is the dominant salt (EL-Assioti, 1992). Processed wastewater may provide an alternative water source for irrigation, but needs to be analyzed for contaminants before application in order to avoid environmental pollution (Abou Hadid, 2003). Water losses which exceed precipitation are further reasons for increasing the salt concentration in the upper soil layer.

2 Potential biochemical indicators for salinity tolerance in plants

For overcoming salt stress, plants have evolved complex mechanisms that contribute to the adaptation to osmotic and ionic stress caused by high salinity. During the onset and development of salt stress within a plant, all the major processes such as compatible osmolytes, protein synthesis, and lipid metabolism are affected. The resistance or sensitivity to salt stress depends on the species, the genotype and the development age of the plants.

2.1 Compatible osmolytes

Compatible osmolytes (class of small molecules) are potent osmoprotectants that play a role in counteracting the effects of osmotic stress. Proline, glycinebetaine, polyamines, and carbohydrates have been described as being effective against salt stress (Hare and Cress, 1997). These compatible osmolytes show rapid and quantitatively important changes in their concentration following variation in salinity (Fathi-Ettai and Prat, 1990). Proline accu-

mulation is one of the most frequently reported modifications induced by water and salt stress in plants and is often considered to be involved in stress resistance mechanisms. Its possible roles have been attributed to stabilizing the structure of macromolecules and organelles through stabilizing proteins and membranes against the denaturating effect of high concentrations of salts and other harmful solutes (Munns, 2002). The role of accumulated proline as an osmoprotectant during osmotic stress was studied in the north African region for wheat (*Triticum aestivum*) by Al-Hakimi and Hamada (2001) and for tomato (*Solanum lycopersicum*) by Aziz et al. (1999).

Exogenous addition of proline was very effective in counteracting the effect of salt. EL-Enany (1995) reported, that addition of proline (100 mg/l) to the medium containing 100 and 150 mM NaCI counteracted the inhibitory effect of NaCl and enhanced shoot regeneration, especially at high NaCI levels in tomato cell cultures. The higher levels of proline served presumably as a compatible osmotic buffer in the cytoplasm against high vacuolar ion concentrations. In addition, the amelioration effects of proline and glycinebetaine on plant growth, stability of leaf membranes, leaf relative water content, chlorophyll content and leaf osmotic potential of bean plants (Vicia faba) grown under salt stress with NaCl and CaCl2 were studied by Gadallah (1999). The results of this study indicated, that proline and glysinebetaine application (8.7 and 8.5 µM applied three times in 5 day intervals by foliar spraying) reduced membrane injury, increased K+ uptake and chlorophyll content, and improved plant growth. The role of proline as part of salt-stress signaling in the desert plant Pancratium maritimum was examined by Khedr et al. (2003). Salt-stress resulted in a decrease in the amount of ubiquitin-conjugates (small protein targeting damaged proteins for degradation via the proteasome), particularly in the roots. This effect was reversed by exogenous proline. It is concluded that proline improves the salt-tolerance of Pancratium maritimum L. by protecting the protein turnover machinery against stress-damage and up-regulating stress protective proteins.

A number of nitrogen containing compounds accumulates in plants exposed to saline stress. The most frequently accumulating nitrogen containing compounds include amino acids, amides, proteins, quaternary ammonium compounds and polyamines. The specific nitrogen containing compounds that accumulate in saline environments is plant-specific. Osmotic adjustment, protection of cellular macromolecules, storage of nitrogen, maintenance of cellular pH, detoxification of the cells, and scavenging of free radicals are proposed functions of these compounds. The accumulation of nitrogen containing compounds is usually correlated to salt tolerance (Mansour, 2000).

The most abundant amino acids (cysteine, arginine,

methionine) constitute to about 55 % of the total free amino acid content, and their content is reduced in NaCl-treated wheat plants. In contrast, the content of valine, isoleucine, aspartic acid, and proline is increased in response to NaCl stress. NaCl-treated wheat seedlings showed a 1.6-fold increase in total free amino acids compared to the control (El-Shintinawy and El-Shourbagy, 2001).

Artemisia (*Artemisia monosperma*) is native wild plant used as an anthelmintic in Egypt. The effect of different concentrations of NaCl on growth, mineral ions and organic solutes were investigated by Zayed (2006). Increased salt stress decreased dry matter biomass, length of shoot and root. Hereby, the osmotic potential of NaCl had a great effect on the shoot/root ratio. Additionally, the proline, saccharide, and soluble protein content increased in the shoot sap of these plants. Oxalic and citric acid increased up to 8.5 and 12 times compared to the control samples.

Salt tolerance of maize (*Zea mays*) is known to change with the growth stage, identifying the multiple parameters associated with salt tolerance is important for evaluating maize genotypes and improving their tolerant. Two maize cultivars, salt sensitive Tri hybrid 321 and salt tolerant Giza 2, were studied to evaluate their adaptation to NaCl imposition at cell and whole plant level (Mansour et al., 2005). Changes in growth and mineral content of roots and shoots, glycinebetaine and free proline levels in shoots and plasma membrane permeability were measured. NaCl treatment increased the plasma membrane permeability in both cultivars. Na+ exclusion from the shoot was not correlated with salt tolerance. The proline and glycinebetaine accumulation in the shoot was a possible indicator for salt tolerance in the maize genotypes.

For durum wheat (*Triticum durum*) the soluble sugar content was proved to be a better marker for drought tolerance than the proline content (Al Hakimi et al., 1995).

Carbohydrate changes under salt stress are of particular importance because of their direct relationship with biochemical processes such as photosynthesis and respiration. Among the soluble carbohydrates, sucrose and fructose have a potential role in adaptation to salt stress. The findings of Aly et al. (2003) indicated that the total soluble carbohydrate content might be a useful trait to select salt-tolerant maize genotype.

Some crops, such as barley, sugar cane, date palm and sugar beet can be grown on saline soils. The biochemical responses of sugar beet plants grown under salt stress (control, 3000 and 6000 ppm) in a sandy soil were investigated in a pot experiment (Eisa and Ali, 2003). The results showed a strong positive correlation between shoot osmotic potential and sucrose concentration in the roots. Increasing NaCl levels resulted in a significant increase of the Na content in both shoots and roots. The K⁺ content in the shoot sharply decreased whereas the K⁺ content in

roots was not significantly influenced by increasing NaCl levels. Under saline conditions, the results indicated also a negative correlation between shoot osmotic potential and shoot Na⁺ content. Sugar beet obviously actively takes up higher amounts of Na⁺ and utilizes it for regulation of the osmotic potential in leaves. Sucrose played a main role in the regulation of the root osmotic potential followed by K, glucose and Na (Eisa and Ali, 2003).

2.2 Enzymes

Unfavorable environmental conditions such as low temperature, drought, salinity, led in higher plants to an increased level of reactive oxygen species such as superoxide, hydrogen peroxide, and the hydroxyl radical. To reduce the deleterious effects of oxidative stress, plant cells possess an antioxidative system consisting of low molecular-weight antioxidants, such as ascorbate, α -tocopherol, glutathione and carotenoids, as well as antioxidative enzymes. These include superoxide dismutase for scavenging the superoxide radicals and other key enzymes, like ascorbate peroxidase, and glutathione reductase (Scandalios, 1997). Several studies demonstrated that salt-tolerant species increase their antioxidant enzyme activities and antioxidant content in response to salt stress, while saltsensitive species do not (Ashraf and Harris, 2004, Khan et al., 2002, Shalata et al., 2001).

Salt stress enhanced the catalase activity in seedlings of chickpea (*Cicer arietinum*) (El-Kady et al., 1982). Other studies showed a positive correlation between the activity of antioxidative enzymes and stress tolerance under salt stress in onion (*Allium cepa*) (Abd El-Baky et al., 2003) and in common bean (Jebara et al., 2005). However, severe salt-stress resulted in an inhibition of the antioxidative enzymes catalase and peroxidase. Salt-stress also upregulated several dehydrin proteins (Khedr et al., 2003).

2.3 Proteins

Salinity stress may lead to an accumulation/depletion of metabolites, resulting in temporal changes in the level of cellular proteins.

El-Shintinawy and El-Shourbagy (2001) reported, that an addition of 2 μ M thiamine alleviated the effects of NaCl on the protein profile. The results showed that after addition of thiamine, the 24 kDa protein, which disappeared with NaCl treatment, has been initiated again. Moreover, thiamine treatment stimulated the accumulation of the 20 kDa protein.

Hassanein (1999) stated that peanut plants (*Arachis hypogaea*) grown under NaCl showed induction (127 and 52 kDa) or repression (260 and 38 kDa) in the synthesis of few polypeptides. In addition, 9 different esterase isoenzymes

were detected in embryos of seeds germinated in 105 mM NaCl, whereas only 5 of them were detected in the embryos of untreated seeds. The ability of peanut to grow at high concentrations of NaCl may be due to the alteration in gene expression. El-Farash et al. (1993) studied the expression of 12 different proteins bands, which were induced in salt stressed tomato plants. They reported that the expression of these proteins was genetically regulated, depending on the salt concentration. Gomaa (1995) attributed the increase of protein content in plant leaves under saline condition in addition to yeast inoculation (used as bio-fertilizer) to the growth hormone produced by the yeast strain.

2.4 Molecular mechanisms

Bacterial studies shed light on the mechanisms of cellular adaptation to osmotic stress. The osmoregulatory genes which control the production of osmoprotective molecules have been isolated and manipulated in different species of micro-organisms. Abd El-Halim and Abd El-Salam (1985) transferred osmoregulatory genes from barley and yeast into *Azotobacter chrococcum*.

Recently, most of the research and field trials in Egypt are being carried out by the Agricultural Genetic Engineering Research Institute (AGERI) in Cairo. The investigations aim at enhancing osmotic stress tolerance in wheat and tomato grown in Egyptian. This will be achieved by over-expressing the key regulatory enzymes of proline biosynthesis and sulfur assimilation pathways. AGERI scientists established a transformation and regeneration system for wheat and transformed a number of genes that have been reported to affect drought and salt tolerance. The mtlD gene (from Escherichia coli, which accumulates mannitol), the HVA1 gene (from barley, which confers delayed leaf wilting), and the fructan gene (from Bacillus subtilis, which plays a role in osmotic adjustment) were all transferred into wheat. Under laboratory conditions, the transformed lines were expressing the genes and proteins and appear to be more salt tolerant than the control plants. Transgenic plants harboring genes for the biosynthesis of mannitol, proline, ononitol, trehalose, betaine and fructan have shown a significant improvement in tolerance to water stress (Moghaieb et al., 2000).

3 Amelioration of salinity

An increasing awareness of environmental issues make a better use of natural resources, a reduced use of chemicals and the efficient input of irrigation water important goals of sustainable agriculture in Egypt. Different strategies to overcome the deleterious effects of salinity on plantswere investigated.

3.1 Bio-fertilization with yeast

An improved salinity and tolerance of crop plants by using bio-fertilizers was investigated in several studies. Gomaa and Gaballah (2004) studied the effect of salt stress on four Egyptian maize varities (Giza 2, One Way Cross 10, One Way Cross 129 and Three Way Cross 352) grown under different salinity levels in relation to bio-fertilization with yeast (*Rhodotorula glutinis*). The results showed that bio-fertilization alleviated adverse effects of high levels of salinity and plants accumulated more polyamines than those, which received no bio-fertilizer, especially at high salinity levels. For all examined varieties, bio-fertilization influenced the plant K-content. The inoculation of bean seeds with yeast under saline conditions increased the percentage of germination compared to non inoculated seeds (Gaballah and Gomaa, 2004).

3.2 Vitamin C

The supply of ascorbic acid (vitamin C) to tomato seedling might decrease the synthesis of active oxygen species and thereby increase resistance to salt stress (Shalata and Neumann, 2001).

3.3 Salicylic acid

Salicylic acid is an endogenous growth regulator, which participates in the regulation of physiological processes in plants. The application of salicylic acid to crop plants can affect their salt tolerance.

The influence of combined salicylic acid and NaCl treatments on the activity of ribulose 1,5- bisphosphate carboxylase, the content of photosynthetic pigments (chlorophylls a, b and carotenoids), the rate of ¹⁴CO₂- fixation and sugars level of maize were determined by Khodary (2004). NaCl significantly reduced all parameters. Foliar application of salicylic acid counteracted the deleterious effects of NaCl on maize and enhanced the salt tolerance in terms of improving the measured plant parameters. Salicylic acid obviously stimulated salt tolerance of maize by activating photosynthetic activity (Khodary, 2004).

El-Tayeb (2005) studied the effect of seed treatments with NaCl (0, 50, 100, 150 and 200 mM) and 1 mM salicylic acid on barley (*Hordeum vulgare*) before sowing. Increasing NaCl levels reduced germination rate, growth parameters, and the content of K, Ca, P and insoluble sugars in shoots and roots of 15-day old seedlings. The content of photosynthetic pigments (Chl a, b and carotenoids) decreased with increasing NaCl concentration. In comparison, the content of Na, soluble sugars, soluble proteins, free amino acids including proline, the lipid peroxidation level and the peroxidase activity increased in shoots and

roots with increasing NaCl rates. Electrolyte leakage from plant leaves was found to increase with higher salinity. The treatment with salicylic acid increased the relative water content, fresh and dry weight, photosynthetic pigments, insoluble saccharides, the P content and the peroxidase activity in the stressed seedlings.

3.4 Phenylurea

Ali and Abbas (2003) studied the adverse effect of salt stress on germination, antioxidant enzymes, phenolic compounds, flavonoids and the possibility of using phenylurea to alleviate the deleterious effect of salinity in barley plants. The results showed that the application of phenylurea decreased the activity of peroxidase and indoleacetic acid oxidase and increased the activity of polyphenol oxidase. This led to a decrease in total phenolic compounds and flavonoids and consequently increased the growth rate. NaCl stress in barley seedlings caused an increase in total phenolic compounds and flavonoids and enhanced the activity of peroxidase and indoleacetic acid oxidase which led consequently to a decrease of the growth rate. Phenylurea application is likely to be a commercially viable way for reducing the effect of salinity stress compared with synthetic application of cytokinin.

3.5 Root exudates

Root exudates of three weed plants, jungle rice (*Echinochloa colona*), cocklebur (*Xanthium strumarium*) and purslane (*Portulaca oleracea*), were collected and concentrated and used as foliar spray into NaCl-stressed soybean (*Glycine max*) seedlings (Moussa, 2004). For the treatment with root exudates of jungle rice the highest content of protein, oil, NO₃, P, K, and Mg was found and the highest nitrate reductase activity and translocation efficiency (¹⁴CO₂-assimilates). Additionally, this treatment showed the lowest content of free proline, Na⁺ and Cl⁻ ions, total alkaloids, and protease activity.

3.6 Micro-organisms

Rhizosphere bacteria such as *Azotobacter*, *Arthrobacter* and *Streptomyces* exert strong resistance beneficial effects on plant growth and integrity by nutrient dissolution, nitrogen–fixation, and the production of plant hormones (El-Shanshoury, 1991).

Magda et al. (2003) Stated that applying *Azotobacter chroococcum* and/or *Streptomyces niveus* to maize plants grown under NaCl, influenced the content of total-soluble sugars, total free amino acids, proline total soluble proteins, DNA and RNA in shoots and roots resulting in a higher salt tolerance of the plants. This response was more evident

when both micro-organisms were applied.

The use of micro-organisms to increase the salt tolerance of maize was also studied by Hamdia et al. (2004). Two maize cultivars (cv. 323 and cv. 324) with different tolerances to salt stress were used. The mechanisms of maize salt tolerance and the role of inoculation by Azospirillum in elevating salinity stress conditions were examined. Maize cv. 323 was mostly sensitive to salinity, while cultivar 324 was more resistant. In the tolerant cv. 324, soluble and total saccharides, soluble protein in shoots and total protein in roots increased under salinity stress. The sensitivity of cv. 323 was associated with a depletion in saccharides and proteins. Proline accumulation was higher and detected earlier at a lower salinity concentration in the salt sensitive cv. 323 compared to the salt tolerant cv. 324. A sharp reduction in the activity of nitrate reductase and nitrogenase in shoots and roots of both cultivars was induced by salinity stress. When salt stressed maize was inoculated with Azospirillum, the proline concentration declined significantly. Furthermore, Azospirillum inoculation stimulated the activities of nitrate reductase and nitrogenase in shoots and roots of both cultivars.

3.7 Ca application

Abdel-Kader and Saleh (2002) studied the effect of external Ca applications on mustard plants (Sinapis alba) under salt stress (100 mM NaCl) in relation to ion uptake, proline, chlorophyll a, b, protein and abscisic acid concentrations. The CaCl2 treatment was found to protect the mustard plants against the adverse effect of NaCl salinity. Salinity caused a significant decrease in Ca²⁺ and K⁺ uptake and decreased the content of chlorophyll a, b, accompanied by significant increases in Na⁺ uptake, and proline and abscisic acid concentrations. Salinity induced the appearance of new protein bands in these studies. This could be attributed to the effect of CaCl2 by decreasing the abscisic acid concentration, synthesis of new polypeptides and increasing the K⁺ and Ca²⁺ uptake. There is the possibility that abscisic acid can interact with other hormones in order to coordinate whole plant response to salinity stress (Abdel-Kader and Saleh, 2002).

3.8 Cultivation of Halophytes

Halophytes are plants, which tolerate excessive NaCl concentrations in the soil water. Growing tomatoes in saline soil after the wild grown halophyte *Zygophyllum coccinium* resulted in higher tomato yields, as the pre-crop reduced soil salinity to a tolerable level for the tomato plants. In these experiments foliar application of NPK further decreased salinity stress of the tomato plants (Shaaban et al., 2004).

Conclusion

This brief review of publications about salinity shows different strategies of plants to adapt to soil salinity. This knowledge is a key to develop practical strategies for coping with salt stress.

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