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DIFFERENTIAL RESPONSE TO SEA SALT SALINITY BY NITRATE AND ANTIOXIDANT SYSTEM IN SIX SOYBEAN VARIETIES

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ABSTRACT. Six varieties of soybean (*Glycine max* L.) plants were grown for 30 days under three levels of sea salt salinity (0.0, 8.0 and 16.0 mS/cm^2) for studying the effect of sea salt on uptake of nitrate and response of the antioxidant system for these salinity doses. Salt treatments resulted in a gradual decline in nitrate uptake by increasing sea salt concentration, which mean that this will bring negative consequences on nitrogen assimilation. However, salt treatments induced the accumulation of hydrogen peroxide and glycinebetaine in the leaves of all soybean verities as an adaptive strategy to cope with salt stress. On the other hand, there was a differential response in phenolic compounds among soybean verities as a function of salt concentration and the studied variety, which means there has a decline in phenolics under salt stress in the varieties Crawford, G21, G22 and G83, but in contrary in G35 and G82, phenolics has accumulated in response to salinity. Isozymes electrophoretic banding showed changes in peroxidase activity with sea salt, however superoxide dismutase showed stability in number and intensity of bands with salt treatments. Esterase enzyme was more sensitive to salinity and showed a gradual decline in activity by increasing salt concentration.

Key words: Soybean; Salinity; Antioxidants; Phenolics; Isozymes.

INTRODUCTION

Soybean (*Glycine max* L.) is one of the economically most important crops, which is used as a food because of the high protein content. The oil content of soybean seeds ranges from 17 to 24%, with 85% poly unsaturated fatty acids compressing two essential fatty acids (linoleic and linolenic acid), which are not synthesized by the human body (Balasubramaniyan and Palaniappan, 2003).

Nitrate (NO_3^-) is the most important source of nitrogen for most crop plants, and often confines plant growth (Meloni *et al*., 2004) and its uptake is mediated by different transport systems. Nitrate uptake is the first step of nitrogen metabolic

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pathway and is considered a key step of nitrogen metabolism. However, absorption of nitrate by the plant root is limited by high salinity in soil or nutrient solution (Yaoa *et al*., 2008). Some studies qualified uptake reduction to the antagonistic effect between Cl^- and NO_3^- or reduced water uptake (Abdelgadir *et al*., 2005). But, until now, there are few reports on the effect of sea salt salinity on the endogenous nitrate content of crop plants.

Hydrogen peroxide (H_2O_2) is a non-radical form of ROS which is considered for long time as a toxic cellular metabolite, but it is toxic only at high concentrations (Uchida *et al*., 2002). In contrast, it has proposed that exogenous application of H_2O_2 former to salt exposure induced salinity tolerance through the activation of enzymatic antioxidant defense system (Uchida *et al*., 2002). Szechynska-Hebda *et al*. (2012) reported that H_2O_2 is the most stable and viable molecule among ROS in signal transduction. Consequently, H_2O_2 is a likely signaling molecule that contributes to the cross-tolerance phenomenon, which mean that exposure of plants to one stress, as H_2O_2, may offer protection against another stress, such as salinity (Neill *et al*. 2002).

Glycinebetaine (GB) is a major organic and water soluble compatible solute that accumulate in a variety of plant species in response to abiotic stresses to maintain normal metabolism during stress (Ashraf and Foolad, 2007). Also, GB stabilizes the structure and activity of enzymes and maintains the integrity of membranes against the damaging effects of various abiotic stresses (Gorham, 1995). Enzymes responsible for GB synthesis are found in chloroplast stroma and their activity is increased in response to salinity (Chen and Murata, 2002).

Phenolic compounds stimulated in response to biotic/abiotic stresses and play an important role in adsorbing and neutralizing free radicals, quenching singlet oxygen, or decaying peroxides (Ksouri *et al*., 2007). Salinity stress induces interference in the metabolic processes resulting in an increase in phenolics (Ayaz *et al*., 2000). Higher activity of phenolics might be due to its high hydrogen-donating ability and radical stabilization than other antioxidant molecules (Rice-Evans *et al*., 1996).

Isozymes are biologically active different forms of the enzyme encoded by different genes in the same organism. Peroxidases (EC 1.11.1.7) are involved in responses to biotic and abiotic stresses, the biosynthesis of lignin (Lagrimini *et al*., 1987) and subrin (Espiele *et al*., 1986), scavenging of ROS, chlorophyll degradation and senescence (Yamaguchi and Watada, 1991). Superoxide dismutase (EC 1.15.1.1) catalyses the disproportion of two superoxide radical to molecular oxygen and H_2O_2 (Scandalios, 1993). Esterase (EC 3.1.1.1) are lipolytic enzymes catalyze the hydrolysis of triacylglycerols into

fatty acids, diacylglycerol, monoacylglycerol, and glycerol (Arpigny and Jaeger, 1999). Isozymes activity is a potential biomarker of plants under salt stress. Thus, the present study was designed to establish the impact of sea salt stress on phenolic compounds, nitrate and hydrogen peroxide contents of different soybean varieties. In addition, the variation among these varieties in their expression to some selected isozymes as biological markers with salt stress was elucidated.

MATERIALS AND METHODS

Seeds of six varieties of soybean (*Glycine max* L. Merrill) were kindly provided by Agricultural Research Center (ARC) El-Dokki, Giza, Egypt. The obtained varieties are named Crawford, Giza 21, Giza 22, Giza 35, Giza 82 and Giza 83. The seeds were surface sterilized with 0.5% sodium hypochlorite for 5 minutes and washed three times with tap water then rinsed with deionized water. The seeds were sown in plastic pots of 45 cm diameter and 25 cm depth containing 8 kg clay sandy soil (2:1 w/w) and irrigated with tap water. At full emergence, after five days, the pots received sea salt solutions (EC = 0.0, 8.0 and 16.0 mS/cm^2) prepared by dissolving the sea salt in tap water. The pots were arranged in spilt plot design in a randomized complete blocks arrangement with three replications. The plants were left to grow under the natural day/night conditions (14h light/ 10h dark) at 28±2°C in the greenhouse of Botany Department, Faculty of Science, Tanta University, during the summer season of 2014. The pots were irrigated whenever needed with the salt solutions to the end of the growth period (30 days). After that, fresh samples were harvested for the measurement of hydrogen peroxide and isozymes, some samples were dried for the measurement of nitrate and glycinebetaine contents.

The nitrate content in leaf powders was determined according to the method adopted by Cataldo *et al*. (1975) and expressed as µmol/g d.wt using standard curve by KNO_3. Hydrogen peroxide (H_2O_2) content was determined using the method given by Velikova *et al*. (2000) and the amount of H_2O_2 was calculated using the extinction coefficient (0.28 μM^{-1} cm^{-1}) and expressed as nmol/g f.wt. Glycinebetaine (GB) content of the leaf powdered tissues was measured by the method of Grieve and Grattan (1983) and the concentration of GB was calculated by using standard graph developed with different concentrations of GB and expressed as µg/g d.wt. Total phenolic content was estimated quantitavely using the method described by Jindal and Singh (1975) and calculated as mg/g d.wt using standard curve prepared by gallic acid.

Isozyme-PAGEs were prepared by extracting 0.2 g fresh leaves of 30-day-old seedlings according to El-Fadly *et al*. (1990). Esterases (EST) were estimated by the method of Soltis *et al*. (1983) using α-naphthyl acetate for α-esterase and β-naphthyl acetate for β-esterase. Superoxide dismutase (SOD) was stained with the method of Beauchamp and Fridovich (1971) using nitroblue tetrazolium (NBT), TEMED and riboflavine. Peroxidase (POD) enzyme was visualized in the gel using *o*-dianisidine as a hydrogen donor according to the protocol adopted by Suranto (2001).

RESULTS AND DISCUSSION

Salinity stress is accomplished by disturbance in metabolic processes, plasma membrane permeability and ion uptake. *Fig. 1* illustrate the response of nitrate content of six soybean varieties to three levels of sea salt salinity (0.0, 8.0 and 16.0 mS/cm^2). Nitrate content was extensively reduced in the presence of sea salt in all the treated soybean varieties, which mean that nitrate influx reduced by salt stress . The maximum decline was observed at the highest salinity level (16 mS/cm^2) when compared to control treatments. The lowest nitrate content was recorded in soybean cultivar G22 under the control treatment, however the highest one was recorded in G83 variety. The most pronounced reduction in nitrate content as a result of the highest salinity level was obtained by Crawford variety, but the least reduction was obtained by G83 one.

Our results are in accordance with those of Silveira *et al*. (1999), who reported reduction of nitrate uptake by salt stressed plants, as salinity stress interferes with nitrogen processing and utilization. Frechill *et al*. (2001) stated that saline conditions can affect the different steps of nitrogen metabolism such as uptake, reduction and assimilation, which may be responsible for the observed reduction in plant growth rate. Salinity interferes nitrate uptake at two levels: a) direct antagonism of chloride with nitrate b) and changing plasma membrane integrity (Cramer *et al*., 1985). However, Abdelgadir *et al*. (2005) showed that the inhibition of NO_3^- absorption in tomato as a result of salt stress was more strongly related to reduced water uptake than to Cl^- antagonism. Niu *et al*. (1995) reported that salinity might stimulate alternations in either influx or efflux of nutrient ions such as Ca^{2+}, K^+ and mainly NO_3^- from vacuole to cytosol and from cytosol to the external medium.

Fig. 2 represent the changes in hydrogen peroxide (H_2O_2) content of six soybean varieties grown under three levels of sea salt salinity. There was a significant variation between soybean varieties in H_2O_2 content under control treatment (0.0 mS/cm^2). The least H_2O_2 content was recorded by Crawford variety and the highest one was recorded by G83 one. H_2O_2 content showed a peak by the elevation of salinity level in all varieties, except G21, which showed a reverse trend. The most pronounced H_2O_2 content was achieved by the highest salinity level (16 mS/cm^2) in G83 variety.

These results are compatible with those of Fadzilla *et al*. (1997), who reported an increase of H_2O_2 production in rice plants in response to salt stress. Also, Weisany *et al*. (2012) stated that H_2O_2 content of soybean leaves was considerably increased with increasing salinity. They attributed the increase in H_2O_2 to the decrease in water potential which might have limited H_2O_2 diffusion from the place of its

generation (leaves) to the other plant organs. Vranova *et al.* (2002) demonstrated that excess H_2O_2 in the cell can accelerate processes like Haber-Weiss/Fenton reaction, resulting in the generation of hydroxyl radicals (OH^-) that can cause lipid peroxidation. Another possible reason for increasing of H_2O_2 under salt stress is the increased peroxisomal photorespiration reactions and β-oxidation of fatty acids (Noreen and Ashraf, 2009). Excess H_2O_2 generation during salt stress has its deleterious effects on plant cells and organelles as mitochondria, plasma membrane and chloroplasts. So, plants have developed an effective strategy for eliminating the excessive amounts of H_2O_2 through an effective scavenging system to prevent its toxic effects on biomembranes and biomolecules.

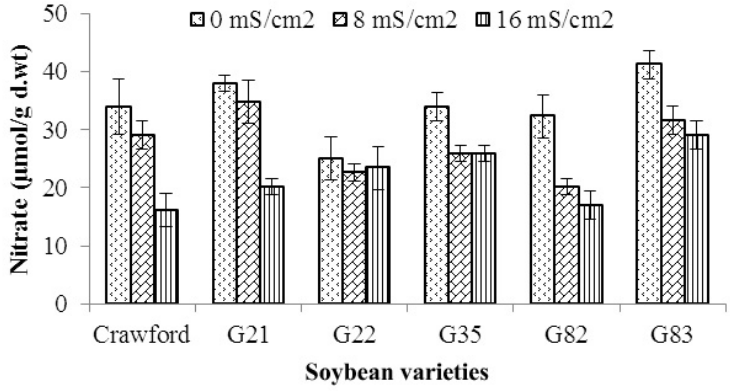


Figure 1 - Nitrate content of six soybean varieties grown under three levels of sea salt salinity for 30 days

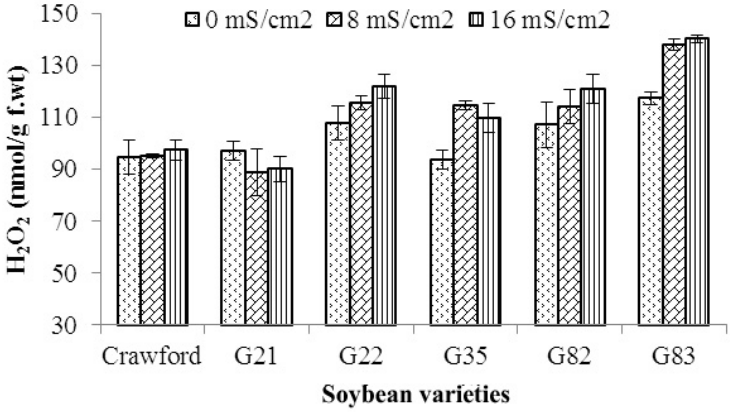


Figure 2 - H_2O_2 content of six soybean varieties grown under three levels of sea salt salinity for 30 days

As shown in *Fig. 3*, glycinebetaine (GB) content of soybean plants was increased in all varieties by increasing salinity level. Where salt treatments provoked an increase in the level of GB content with the increment in salinity level. The highest GB content was recorded as a result of 16 mS/cm^2 sea salt treatment in all soybean varieties. However, the highest GB level under control conditions was exhibited by G82, followed by G83, and the lowest one was exhibited by Crawford and G21 varieties.

Glycinebetaine is a quaternary ammonium compound that occurs in a wide range of plants and performs a significant role in plants subjected to environmental stresses. The increased production of GB improves plant tolerance to various abiotic stresses without strong phenotypic changes (Wani *et al*., 2013). GB acts as osmoprotectant by stabilizing quaternary structures of proteins against the adverse effects of high salinity, and efficiently protects various components of the photosynthetic machinery such as Rubisco and photosystem II from salt induced inactivation or damage (Papageorgion and Murata, 1995). Wani *et al*. (2013) reviewed that GB plays an adaptive function in mediating osmotic adjustment and protecting the sub-cellular structures in stressed plants, protect the transcriptional and translational machineries and act as a molecular chaperone in the refolding of enzymes.

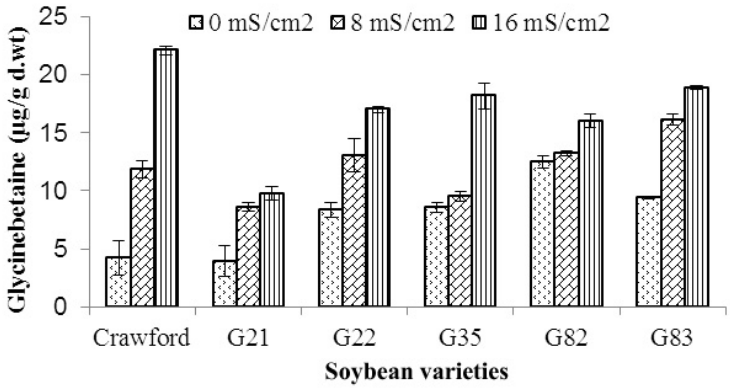


Figure 3 - Glycinebetaine content of six soybean varieties grown under three levels of sea salt salinity for 30 days

The total content of phenolic compounds in six soybean varieties under the effect of three sea salt salinity levels is shown in *Fig. 4*. The highest phenolic content under the control treatment was exhibited by G22 variety, however the lowest one was exhibited by G35 and G82 varieties. Addition of sea salt resulted in a variable effect on soybean varieties, where the treatment with sea salt had resulted in the decline of

phenolics compared to the control treatments of soybean varieties Crawford, G21, G22 and G83. On the other hand, sea salt treatments had resulted in the increment of phenolic content higher than that of the control in soybean varieties G35 and G82.

The accumulation of phenolic compounds after salinity stress have been reported in the earlier work of Elhaak *et al*. (2014). Also, Sadak (2015) reported that the exposure of sunflower to sea salt salinity have resulted in the accumulation of phenolic compounds. Rivero *et al*. (2001) attributed the massive accumulation of phenolic compounds in response to salinity stress to the activation of phenylalanine ammonia lyase (PAL). These phenolic compounds play an important role in scavenging free radicals, where its antioxidative properties arise from their elevated reactivity as hydrogen or electron donors enabling them to stabilize and delocalize the unpaired electrons (Huang *et al*., 2005). Also, phenolics act as antioxidants by inhibiting enzymes involved in radical generation (Castellano *et al*., 2012).

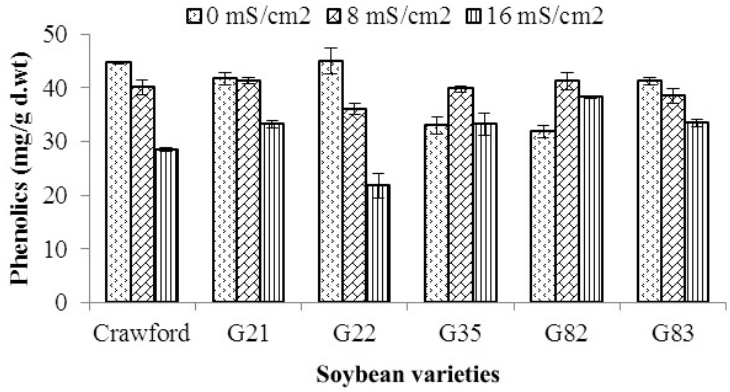
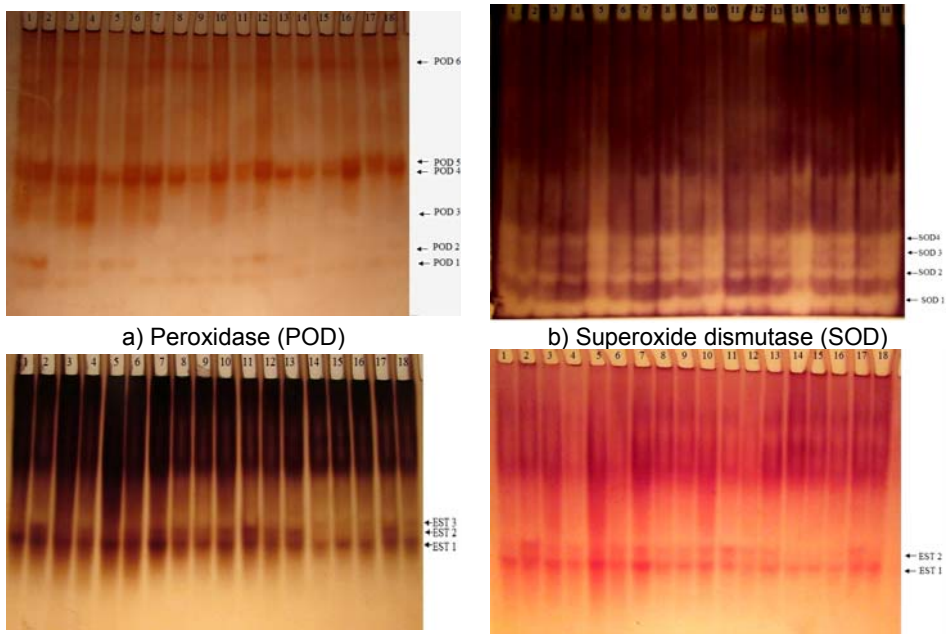


Figure 4 - Phenolic compounds content of six soybean varieties grown under three levels of sea salt salinity for 30 days

Activity analysis of soybean varieties under sea salt stressed treatments stained onto gels detected variation of peroxidase (POD), superoxide dismutase (SOD) and α and β esterases (EST) isozymes as biochemical markers in response to salinity stress (*Fig. 5*). Enzymes encoded by different alleles of a distinct locus or by separate loci commonly show different electrophoretic mobilities due to variations in the amino acids sequence of the enzyme molecules, which in turn dependent on the sequences of nucleotides in DNA (Micales *et al*., 1992). POD isozymes pattern (*Fig. 5a*) reflected six different isoforms (POD1-6) in the studied soybean varieties under different levels of sea salt salinity. POD4 and POD6 were commonly found in all treatments

with the same intensity. However, POD1 was not detected in G82 and G83 under control treatment, but detected in all soybean varieties under 8.0 and 16.0 mS/cm^2. POD2 isoform is highly affected by salt stress, where it is present in all varieties in the unstressed treatment, but disappeared under salinity from all soybean varieties, except G82 and G83 at 8.0 mS/cm^2 and G83 at 16.0 mS/cm^2. POD3 isoform was more sensitive, that it completely disappeared in salt treatments. POD5 isoform is common in all varieties under control and salt treatments, except G82 variety at 0.0 mS/cm^2 salt level.



a) Peroxidase (POD) b) Superoxide dismutase (SOD)

c) α-Esterase (α-EST) d) β-Esterase (β-EST)

Lanes: (1) Crawford, (2) G21, (3) G22, (4) G35, (5) G82, (6) G83, (7) Crawford+8 mS/cm^2, (8) G21+8 mS/cm^2, (9) G22+8 mS/cm^2, (10) G35+8 mS/cm^2, (11) G82+8 mS/cm^2, (12) G83+8 mS/cm^2, (13) Crawford+16 mS/cm^2, (14) G21+16 mS/cm^2, (15) G22+16 mS/cm^2, (16) G35+16 mS/cm^2, (17) G82+16 mS/cm^2 and (18) G83+16 mS/cm^2.

Figure 5 - Zymogram of four enzymes in the leaves six soybean varieties grown under three levels of sea salt salinity for 30 days

The pattern of SOD isozymes on native PAGE gels of soybean varieties (*Fig. 5b*) under three levels of sea salt salinity (0.0, 8.0 and 16.0 mS/cm^2) revealed four isoforms designated as SOD1-4. The SOD1 isoform was the highly expressed, while SOD3 isoform was the weakest. From the provided zymogram, soybean SOD was shown to be non-sensitive to salinity and all the bands were expressed with the same intensity.

This result is compatible with the findings of Shao *et al*. (2015), who reported that the leaves of *Andrographis paniculata* showed no obvious band intensity changes of SOD and CAT with salt stress. Also, Luna *et al*. (1985) concluded that SOD activity in maize was not affected by drought stress. Nevertheless, it has been reported by Turhan *et al*. (2008) that unchanged or enhanced enzyme activities were observed in salt-tolerant cultivars to reduce stress severity thus allowing cell growth, and POD activity was found to be higher, providing protection against the oxidative stress.

Zymogram of α-Esterase (α-EST) electrophoretically detected three isoforms in soybean leaves under salinity treatments (*Fig. 5c*). The zymogram showed that, α-EST1 is common in all varieties at all salinity levels, but its intensity decrease gradually by increasing salt concentration. α-EST2 is found in G21, G35 and G83 varieties under non-saline conditions, however 8 mS/cm^2 salinity level induced the expression of this isoform in all varieties. Also, 16 mS/cm^2 salinity level resulted in switching off α-EST2 isoform gene and switching on α-EST3 isoform gene in the different soybean varieties. Also, the electrophoretic profile of β-Esterase (β-EST) isozyme showed two isoformes of this enzyme with the commonness of β-EST1 in soybean varieties at the various salinity levels (*Fig. 5d)*. However, β-EST2, like α-EST2, is found only in G21, G35 and G83 varieties only at the non-saline treatment. β-EST2 was induced by 8.0 mS/cm^2 salinity level at all soybean varieties, but it was disappeared by 16 ms/cm^2 treatment from soybean varieties except Crawford and G82 varieties. So, salt stress induced negative effects on esterases in the number and intensities of the bands. Our results are incompatible with those of Mohamed (2005), who reported induction of esterases expression in maize plants exposed to NaCl salinity. Also, Hassanein (1999) concluded that salinity increase esterase isoenzymes in peanut and the number of esterase isoenzymes increase by increasing NaCl concentration. On the other hand, Swapna (2003) reported that salinity stress decrease esterase activity in rice plant at seedling stage. Radic and Pevalek-Kozlina (2010) reported that low salt stress induced EST activity in the leaves of *Centaurea ragusina*, while the higher concentrations decline it. In general, the change in the isozyme profile through the induction of new isozymes or the disappearance of existing bands play an important role in the cellular defense against oxidative stress caused by salt stress.

CONCLUSION

Overall, the results of this study showed that all the studied soybean varieties are mild-salt tolerant. Furthermore, our results proved that salinity stress resulted in negative consequences on nitrogen metabolism

due to diminution of nitrate uptake, nevertheless it has enhanced the accumulation of the compatible osmolyte glycinebetaine and the antioxidant phenolic compounds. Also, salinity caused pronounced shift in the activity of peroxidase and esterase isozymes, but has a non-significant effect of superoxide dismutase.

REFERENCES

Abdelgadir E.M., Oka M., Fujiyama H., 2005 - Characteristics of Nitrate Uptake by Plants Under Salinity. J. Plant Nutr., 28: 33-46.

Arpigny J.L., Jaeger K.E., 1999 - Bacterial lipolytic enzymes: classification and properties. Biochemical J., 343: 177-183.

Ashraf M., Foolad M.R., 2007 - Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot., 59 (2): 206-216.

Ayaz F.A, Kadioglu A., Turgut R., 2000 - Water stress effects on the content of low molecular weight carbohydrates and phenolic acids in *Ctenanthe setosa* (Rosc.) Eichler. Can. J. Plant Sci., 80: 373-378.

Balasubramaniyan P., Palaniappan S.P., 2003 - Field crops. An overview. Principles and practices of agronomy. pp 45-46. Agrbios, India.

Beauchamp C., Fridovich I., 1971 - Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels. Anal. Biochem., 44: 276-287.

Castellano G., Tena J., Torrens F., 2012 - Classification of phenolic compounds by chemical structural indicators and its relation to antioxidant properties of *Posidonia oceanic* (L.) Delile. MATCH Communications in Mathematical and in Computer Chemistry, 67: 231-250.

Cataldo D.A., Haroon M., Schrader L.E., Youngs V.L., 1975 - Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Communication Soil Science Plant Analysis, 6 (1): 71-80.

Chen T.H., Murata N., 2002 - Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Current Opinion in Plant Biology, 5: 250-257.

Cramer G.R., Lauchli A., Polito V.S., 1985 - Displacement of Ca^{2+} by Na^{+} from the plasmalemma of root cells. A primary response to salt stress? Plant Physiol., 79: 207-227.

EL-Fadly G., Sidaros S., Deif A.A., 1990 - Effect of boistin on gene expression and yield components of faba bean (*Vicia faba*) infected with broad bean strain virus. Proc. 3th Conf. Agric. Dev. Res. Faculty of Agriculture, Ain shams University, Cairo, Egypt.

Elhaak M.A., Abo-Kassem E.M., Saad-Allah K.M., 2014 - Effect of the combined treatment with sodium and calcium chlorides on the growth and medicinal compounds of *Cichorium intybus*. International Journal of Current Microbiology and Applied Sciences, 3 (7): 613-630.

Espiele K.E., Franeesei V.R., Kolattnkndy P.E., 1986 - Immunocytochemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound healing potato tuber tissue. Plant Physiol., 81: 487-492.

Fadzilla N.M., Finch R.P., Burdon R.H., 1997 - Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. J. Exp. Bot., 48: 325-331.

Frechill S., Lasa B., Ibarretxe L., Lamsfus C., Aparicio Trejo P., 2001 - Pea responses to saline stress is affected by the source of

nitrogen nutrition (ammonium or nitrate). Plant Growth Regul., 35: 171-179.

Gorham J., 1995 - Betaines in higher plants: biosynthesis and role in stress metabolism. Amino acids and their derivatives in higher plants, pp.171-203. Wallsgrove R.M. (Eds). Cambridge University Press, Cambridge.

Grieve C.M., Grattan S.R., 1983 - Rapid assay for the determination of water soluble quaternary ammonium compounds. Plant Soil, 70: 303-307.

Hassanein A.M., 1999 - Alteration in protein and esterase peanut in response to salinity stress. Biol. Plantarum, 42: 241-248.

Huang D., Ou B., Prior R.L., 2005 - The chemistry behind antioxidant capacity assays. J. Agr. Food Chem., 53: 1841-1856.

Jindal K.K., Singh R.N., 1975 - Phenolic content in male and female *Carica papaya*: A possible physiological marker for sex identification of vegetable seedlings. Physiol. Plantarum, 33: 104-107.

Ksouri R., Megdiche W., Debez A., Falleh H., Grignon C., Abdelly C., 2007 - Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. Plant Physiol. Biochem., 45 : 244-249.

Lagrimini L.M., Burkhart W., Moyer M., Rothestein S., 1987 - Molecular cloning of complementary DNA encoding the lignin forming peroxidase from tobacco: molecular analysis and tissue - specific expression. Proceedings of the National Academy of Sciences of the United States of America, 84: 7542-7546.

Luna M., Badiani M., Felice M., Artemi F., Germanni G., 1985 - Selective enzyme inactivation under water stress in maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) seedlings. Environ. Exp. Bot., 25:153-156.

Meloni D.A., Gulotta M.R., Martinez C.A., Oliva M.A., 2004 - The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba*. Braz. J. Plant Physiol., 16 (1): 39-46.

Micales J.A., Bonde M.R., Peterson G.L., 1992 - Isozyme analysis in fungal taxonomy and molecular genetics. Handbook of applied mycology, pp57-79. Arora D K, Elander R.P., Mukerja K.G. (Eds.) Fungal biotechnology. Marcel Dekker Inc., New York.

Mohamed A.A., 2005 - Two-dimensional Electrophoresis of Soluble Proteins and Profile of Some Isozymes Isolated from Maize Plant in Response to Nacl. Res. J. Agric. & Biol. Sci., 1 (1): 38-44.

Neill S., Desikan R., Hancock J., 2002 - Hydrogen peroxide signaling. Current Opinion in Plant Biology, 5: 388-395.

Niu X., Bressan R.A., Hasegawa P.M., Pardo J.M., 1995 - Ion homeostasis in NaCl stress environments. Plant Physiol., 109: 735-742.

Noreen Z., Ashraf M., 2009 - Assessment of variation in antioxidative defense system in salt treated pea (*Pisum sativum*) cultivars and its putative use as salinity tolerance markers. J. Plant Physiol., 166: 1764-1774.

Papageorgion G.C., Murata N., 1995 - The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving photosystem II complex. Photosynth. Res., 44: 243-252.

Radić S., Pevalek-Kozlina B., 2010 - Differential esterase activity in leaves and roots of *Centaurea ragusina* L. as a consequence of salinity. Period. Biol., 112 (3): 253-258.

Rice-Evans C.A., Miller N.J., Paganga G., 1996 - Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Bio Med., 20: 933-56.

Rivero R.M., Ruiz J.M., Garcia P.C., Lopez-Lefebre R., Sanchy E., Romero L., 2001 - Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and water melon plants. Plant Sci., 160: 315-321.

Sadak M.S., 2015 - Pre-sowing Seed Treatment with Proline Improves some Growth, Biochemical aspects, yield quantity and quantity of two sunflower cultivars grown under seawater salinity stress. Scientia Agriculturae, 9 (1): 60-69.

Scandalios J.G., 1993 - Oxygen stress and superoxide dismutase. Plant Physiology, 101: 712-726.

Shao Y., Gao J., Wu X., Li Q., Wang J., Ding P., Lai X., 2015 - Effect of salt treatment on growth, isoenzymes and metabolites of *Andrographis paniculata* (Burm. f.) Nees. Acta Physiologiae Plantarum, 37: 1-12.

Silveira J.A., Cardoso B., Melo A.R., Viegas R., 1999 - Salt-Induced Decrease In Nitrate Uptake And Assimilation In Cowpea Plants. Revista Brasileira de Fisiologia Vegetal, 11 (2): 77-82.

Soltis D.E., Haufler C.H., Dartow D.C., Gastony G.J., 1983 - Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. American Fern Journal, 73 (1): 9-27.

Suranto., 2001 - Isozyme studies on the morphological variation of Ranunculus nanus populations. Agrivita, 23 (2): 139-146.

Swapna T.S., 2003 - Salt Stress Induced Changes on Enzyme activities during Different Developmental Stages of Rice (*Oryza sativa* Linn.). Indian Journal of Biotechnology, 2: 251-258.

Szechynska-Hebda M., Skrzypek E., Dąbrowska G., Wędzony M., van Lammeren A., 2012 - The effect of endogenous hydrogen peroxide induced by cold treatment in the improvement of tissue regeneration efficiency. Acta Physiologiae Plantarum, 34: 547-560.

Turhan E., Gulen H., Eris A., 2008 - The activity of antioxidative enzymes in three strawberry cultivars related to salt-stress tolerance. Acta Physiologiae Plantarum, 30: 201-208.

Uchida A., Jagendorf A.T., Hibino T., Takabe T., 2002 - Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Science, 163: 515-523.

Velikova V., Yordanov I., Edreva A., 2000 - Oxidative stress and some antioxidant systems in acid rain-treated bean plants. Plant Science, 151: 59-66.

Vranova E., Inze D., Breusegem F., 2002 - Signalling transduction during oxidative stress. Journal of Experimental Botany, 53: 1227-1236.

Wani S.H., Singh N.B., Haribhushan A., Mir J.I., 2013 - Compatible Solute Engineering in Plants for Abiotic Stress Tolerance - Role of Glycine Betaine. Current Genomics, 14: 157-165.

Weisany W., Sohrabi Y., Heidari G., Siosemardeh A., Golezani K.G., 2012 - Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). Plant Omics Journal, 5 (2): 60-67.

Yamaguchi N., Watada A.E., 1991 - Regulated chlorophyll degradation in spinach leaves during storage. Journal of the American Society for Horticultural Science, 116: 58-62.

Yaoa J., Shia W.M., Xua W.F., 2008 - Effects of Salt Stress on Expression of Nitrate Transporter and Assimilation-Related Genes in Tomato Roots. Russian Journal of Plant Physiology, 55 (2): 232-240.