PHYSIOLOGICAL REACTION OF BEAN PLANTS (*PHASEOLUS VULG.* L.) TO SALT STRESS

M. Kaymakanova[1*] and N. Stoeva[1]

[1]Agricultural University, 12, Mendeleev Str., Plovdiv 4000, Bulgaria

Summary. The physiological responses of three different bean cultivars (cv. Lody, cv. Gina and cv. Tara) to salt stress were studied under laboratory conditions. The plants were grown in pots as hydroponic cultures in a half-strength Hoagland nutrient solution. The plants were treated for 7 days with NaCl and Na_2SO_4 (100 mM), starting at the appearance of the first trifoliate leaf unfolded. It was established that the applied dose of both salt types caused stress in the young bean plants, which found expression in the suppression of growth and photosynthesis activity. The bean cultivars showed different reaction to salinity and the type of salt. It was evident that cv. Lody was most sensitive to salt stress. The applied Na_2SO_4 caused stronger inhibition in all cultivars than those treated with NaCl. The amount of proline in the tissues of the salt-treated plants increased, while the cell water potential was reduced.

***Key words*:** bean, growth, leaf-gas exchange, proline, salt stress, water potential.

Abbreviations: E – transpiration rate; g_s – stomatal conductance; LP – lipid peroxidation; MDA – Malondialdehyde; NADP – nicotinamiddinucleotide phosphate; PAR – Photosynthetic activity radiation; P_N – net photosynthetic rate; ROS- Reactive oxygen species; TBA - Thiobarbituric acid.

*Corresponding author, e-mail: mira_4u@abv.bg

INTRODUCTION

For centuries, agriculture in arid and semi-arid environments has faced an increase in soil salinity. Salinity is one of the most important abiotic stress factors limiting plant growth and productivity (Khan and Panda, 2008). Salinity affects almost every aspect of the physiology and biochemistry of plants and significantly reduces yield. High exogenous salt concentrations affect seed germination, water deficit, cause ion imbalance of the cellular ions resulting in ion toxicity and osmotic stress (Khan et al. 2002; Khan and Panda, 2008).

The most important process that is affected in plants, growing under saline conditions, is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular CO_2 concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophylls and carotenoids (Stepien and Klobus, 2006). Salinity reduces the ability of plants to utilize water and causes a reduction in growth rate, as well as changes in plant metabolic processes (Munns, 2002).

Osmotic effects are due to salt-induced decrease in the soil water potential. Salinity results in a reduction of K^+ and Ca^{2+} content and an increased level of Na^+, Cl^- and SO_4^{2-}, which forms its ionic effects (Mansour et al., 2005). Reduction in biomass, photosynthetic capacity changes in leaf water potential and leaf turgor have been reported to have a cumulative effect attributed to salinity stress (Tourneux and Peltier, 1995; Gamma, 2007), it is also clear that several soil and other environmental factors do influence plant growth under salinity conditions.

Characters like yield, survival, vigor, leaf damage and plant height, have been the most commonly used criteria for identifying salinity tolerance (Shannon, 1984; Gamma, 2008). Other indices of tolerance have also been proposed that are based on specific physiological characteristics - accumulation of specific ions in shoots or leaves, or production of a specific metabolite (Gamma, 2008). Alternatively, relative growth rate (RGR) has been used as a relative basis on which to compare growth rates of plants.

A large number of plant species accumulate proline in response to salinity stress and that accumulation may play a role in combating salinity

stress. However, data do not always indicate a positive correlation between the osmolyte accumulation and the adaptation to stress (Asharf and Harris 2004; Mansour, 2000; Mansour et al., 2005).

Salt stress induces cellular accumulation of damaging active oxygen species, which can damage membrane lipids, proteins and nucleic acids (Mittler, 2002; Grant and Loake, 2000). Lipid peroxidation, induced by free radicals, is also important in membrane deterioration (Hernandez at al., 2000; Demiral and Turkan, 2005; Mandhania et al., 2006; Khan and Panda, 2008).

In most cases, salinity problems are linked to an excess of NaCl in the irrigation water, but sometimes other salts like Na_2SO_4 are present. There are few studies on the effect of Na_2SO_4 on plant growth.

The objective of the present study was to evaluate the physiological responses of three bean cultivars to equimolar concentrations of two salt types - NaCl and Na_2SO_4.

MATERIALS AND METHODS

Bean seeds (*Phaseolus vulgaris* L.,) cultivars Lody, Gina and Tara, were surface-sterilized with in 0.5 % NaOCL (sodium hypochlorite) solution for 1 min and then washed thoroughly in sterilized water. The seeds were germinated in the dark at 26 ^oC in vermiculite media. After that, they were transferred in pots filled with half-strength Hoagland nutrient solution and grown in a growth chamber under controlled environmental conditions. The conditions maintained during the experiments were the following: light duration - 14 h, light intensity (PAR) 250 μmol m^{-2} s^{-1}, temperature – 22 ± 2 ^oC and relative air humidity – 60 ± 5 %. At the appearance of the first trifoliate leaf, an experimental design with three treatments was arranged as such cultivars:

- control – plants, supplied with ½ of Hoagland solution;
- plants, supplied with ½ of Hoagland solution enriched with 100 mM NaCl;
- plants, supplied with ½ of Hoagland solution enriched with 100 mM Na_2SO_4;

Treatment of plants with salts continued for 7 days.

The fresh and dry weight of roots, shoots, and leaves, and the leaf area (electronic area meter – NEO-3, TU-Sofia) were measured.

The net photosynthesis rate, transpiration rate and stomatal conductance of the youngest fully developed intact leaves were measured with a portable infrared gas analyzer *LCA-4* (*Analytical Development Company Ltd,.* Hoddesdon, England), equipped with a *PLCB-4* chamber. The measurements were made in the chamber (giving 11 cm^2 leaf area) under irradiance of 800 μmol (PAR) m^{-2} s^{-1}, temperature of 26±2 °C, external CO_2 concentration of 400 μmol mol^{-1}, and relative air humidity of 70 %.

The water potential (Ψ) in leaves was measured with a pressure chamber (ELE-International, England*)*.

The level of lipid peroxidation in the root tissues was determined as 2-thiobarbituric acid (TBA) reactive metabolites chiefly malondialdehide (MDA) accumulation as described (Heath and Packer, 1968).

Proline was extracted in 3 % sulphosalicylic acid and was determined calorimetrically according to the method of Bates et al. (1973).

RESULTS AND DISCUSSION

The summary of the analysis of variance for the biometrical parameters (Table 1) studied during the experiment showed that salinity stress had adverse effect on the biomass yield and leaf area of the three cultivars. Differences in shoot dry weight were highly significant depending on the salinity type of and cultivars. The root length in all cultivars was reduced as a result of the salt stress and this was most evident in the plants from cultivar Lody treated with Na_2SO_4. The relative growth rates (RGR) in the three cultivars were almost similar under non-saline conditions but declined considerably after salt treatment (Fig. 1).

Reduction of the biomass in beans under saline condition was indicative of several growth limitations. Salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot/root ratio. Some authors reported that in Phaseolus vulgaris salinity reduced shoot and root weights (Brougnoli and Lauteri, 1991; Gamma, 2008).

Salt stress in beans caused significant differences in gas exchange

Table 1. Effect of equimolar NaCl and Na_2SO_4 treatments on biometrical parameters of control and salt-treated plants. FMpl – fresh mass of plants (g), DMpl - dry mass of plants, h- steam height (cm), l- root length (cm) and LA – leaf area (cm^2). Each value is the mean ± SD (n=3) of three independent experiments with three replicates of measurements.

Parameters	l cm (root)	h cm (steam)	$FM_{pl\,(g)}$	$DM_{pl\,(g)}$	LA (cm^2)
Control Lody	16.73 ± 0.15	24.80± 0.20	8.972± 0.15	1.192± 0.06	300± 7.57
100 mM NaCL	13.07± 0.21	16.77± 0.25	6.033± 0.54	0.728± 0.06	241± 2.89
100 mMNa_2SO_4	11.37 ± 0.21	13.33± 0.32	4.610± 0.29	0.583± 0.04	228± 7.64
Control Gina	15.73± 0.31	21.73± 1.38	7.998± 0.12	1.021± 0.06	259± 27.54
100 mM NaCL	12.80± 0.20	18.20± 0.66	6.171± 0.17	0.656± 0.04	227± 21.94
100 mM Na_2SO_4	10.80± 0.40	15.30± 0.56	4.517± 0.12	0.570± 0.01	201± 7.64
Control Tara	12.87± 0.50	16.17± 0.25	6.529± 0.52	0.822± 0.07	290± 11.55
100 mM NaCL	10.63± 0.25	13.07± 0.42	4.118± 0.39	0.537± 0.05	250± 0.00
100 mM Na_2SO_4	9.37± 0.25	12.03± 0.21	3.144± 0.14	0.396± 0.01	225± 11.55

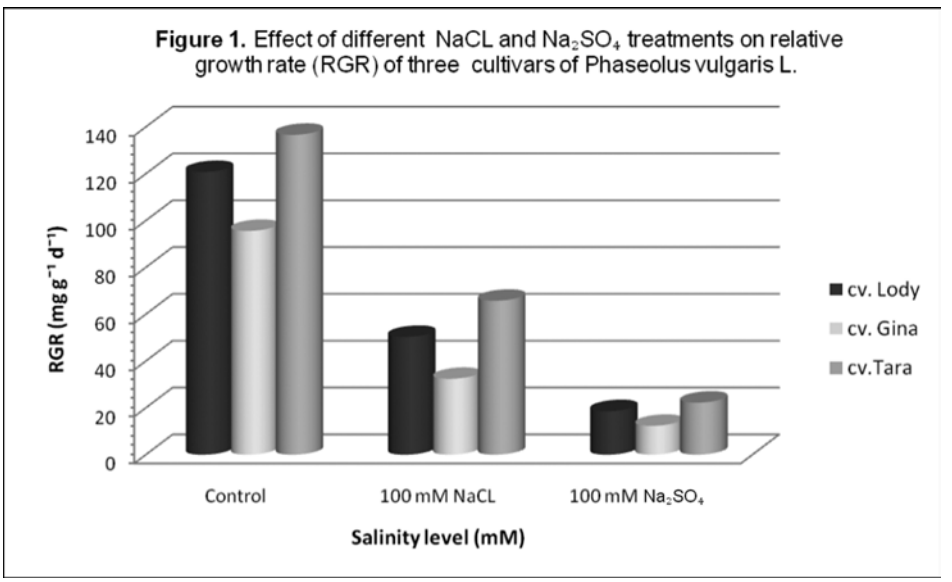

Figure 1. Effect of different NaCL and Na_2SO_4 treatments on relative growth rate (RGR) of three cultivars of Phaseolus vulgaris L.

parameters in the plants of three cultivars (Fig. 2). On the 7th day of treatment P_N was reduced to 70 % (cv. Lody and Gina – 100 mM NaCl) and 60 % (cv. Tara) in comparison with the control. The second salt (100 mM Na_2SO_4) decreased P_N to 40 % and 20 % compared to the control plants.

The decline in photosynthesis observed in case of salinity could be attributed to stomata factors. During salt stress the concentration of CO_2 in chloroplasts decreased because of the reduction in stomata conductance, in spite of the apparent stability of CO_2 concentration in intercellular spaces (Tourneux and Peltier, 1995). Brougnoly and Lauteri (1991) also indicated that reduced photosynthetic carbon assimilation was attributed to reduced stomata conductance. This tendency, with respect to the water use efficiency, observed in the leaves suggests that the non-stomata factors, in addition to the stomata ones, affected photosynthesis. Limited CO_2 fixation due to stress conditions leads to a decrease in carbon reduction by the Calvin cycle and decrease in oxidizes NADP+ to serve as an electron acceptor in photosynthesis (Khan and Panda, 2008).

The data with respect to the transpiration intensity (E) in the control and salt-treated plants followed the same tendency as photosynthesis (Fig. 2).

After 7 days of salt treatment, E had fallen by 51,58 and 54 % in comparision with the control plants (cv. Lody, cv. Gina, cv. Tara - 100 mM NaCl). The results show, that the transpiration rate was inhibited to a greater extent by the second salt - Na_2SO_4 - 68, 64 and 57 % relation to the control plants (cv. Lody, cv. Gina, cv. Tara), respectively. The data also show that the inhibition was weakest in the plants from cultivar Tara.

Salinity strongly decreased stomata conductivity (g_s), which reduced transpiration rate (E). Since transpiration rate followed the same trend as that in photosynthesis, it is clear that the reduction in photosynthesis has the same effects on both stomata and transpiration as the three are integral elements of the photosynthetic apparatus of plants (Gamma, 2007).

Generally, transpiration rate tended to decline with increasing salinity. This may be due to the fact that lowered water potentials in the root can trigger a signal from root to shoot (such as abscisic acid, which has been suggested to be the operating mechanism (Zhang and Davies, 1991). However an alternative hypothesis could be that the inhibition of photosynthesis caused

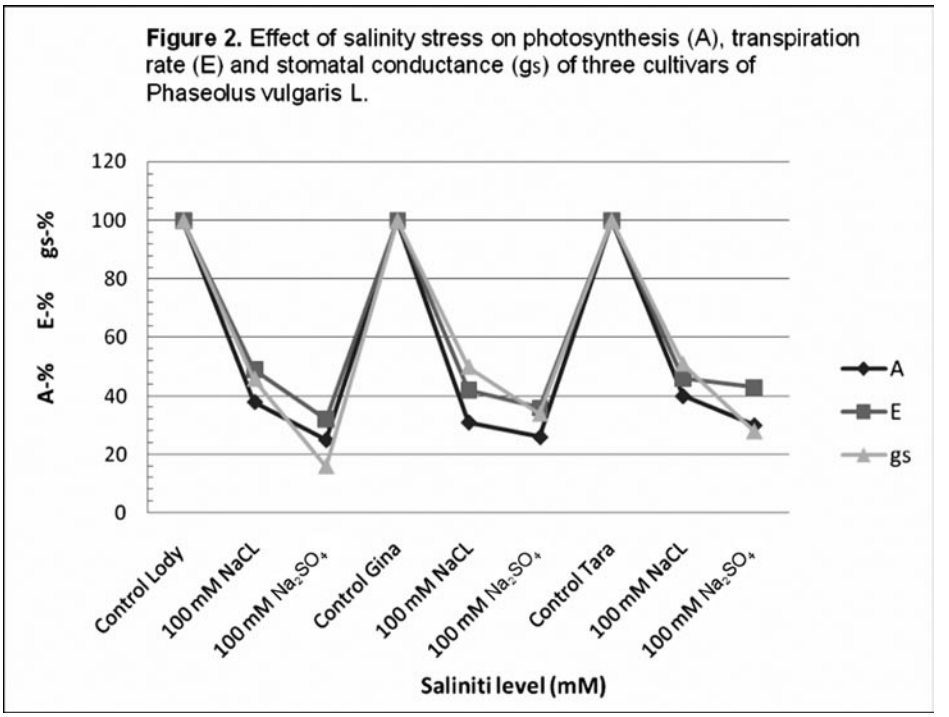

Figure 2. Effect of salinity stress on photosynthesis (A), transpiration rate (E) and stomatal conductance (gs) of three cultivars of Phaseolus vulgaris L.

by salt accumulation in the mesophyll produces an increase in intercellular CO_2 concentration, which reduces the stomatal aperture (Josefa, 2003).

It was also observed that the stomata conductance of young bean plants declined with salinity and was very low in cv. Lody in Na_2SO_4 salinity.

Water status is highly sensitive to salinity and therefore is dominant in determining the plant responses to stress (Stepien, 2006). The results in Fig. 3 showed that water potential (Ψ) decreased considerably in the 100 mM sodium chloride- and sulphate-treated plants because salinity increased cellular water loss.

The results obtained during the experiments, showed that with respect to the water potential the three cultivars responded differently to the chloride and sulfate stress. In the plants treated with 100 mM NaCl the water potential inhibition was about 200 %. The inhibition value was considerably lower in the case of the second treatment with 100 mM Na_2SO_4, where cultivar Lody proved to be most sensitive, and Ψ was 300 %, compared to the non-treated plants.

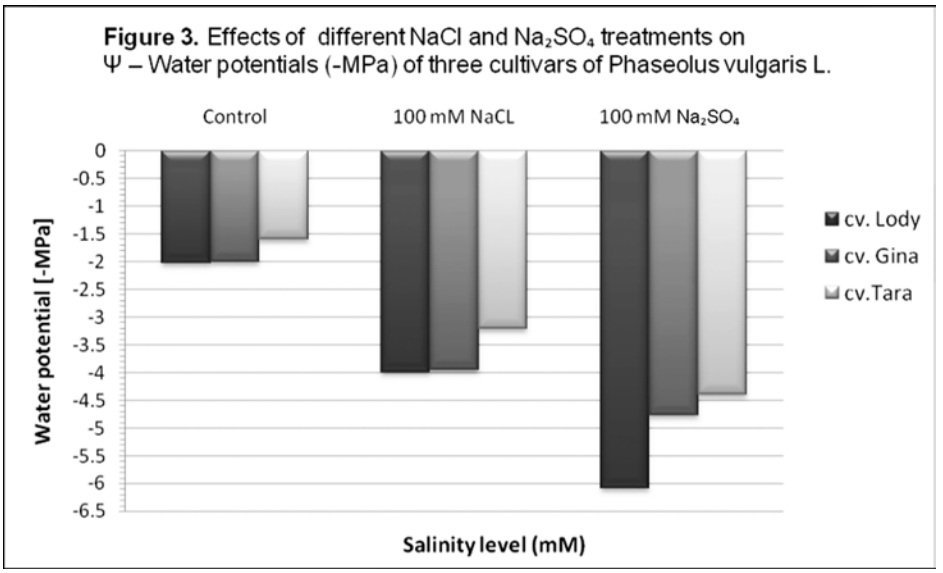

Figure 3. Effects of different NaCl and Na_2SO_4 treatments on Ψ – Water potentials (-MPa) of three cultivars of Phaseolus vulgaris L.

The reduced water potential could be explained by the fact that during stress carbon allocation, osmotic adjustment and accumulation of soluble sugars compete with other sinks and can affect growth (Gamma, 2007). Generally, there is substantial evidence that glycophytic as well as halophytic species adjust to high salt concentrations by lowering tissue osmotic potentials with an increase of inorganic ions accumulation in tissues (Cachorro et al., 1995).

Proline (Pro) accumulation is an important physiological index for plant response to salt stress (Shi and Yin,1993), as well as to other types of stress. Salinity increased markedly the Pro content in different salt sensitive and tolerant species/cultivars: with greater Pro accumulation in salt tolerant ones, which is supposed to correlate with the adaptation to salinity (Ashraf and Harris, 2004; Mansour at al, 2005). Our results (Fig. 4) implicate that NaCl and Na_2SO_4 stress increases Pro accumulation (3-3.5 times compared to the control) in the shoots of plants, strongly in cv. Tara, when the Ψ was higher. The result demonstrates that sulfate stress can cause higher accumulation of proline than the chloride type. This fact suggests that the induction of proline synthesis is related not only to changes in the water potential and to the salinity type – chloride and sulfate, but also resulted

from metabolism interruption by high-stress intensity or from an adaptive response with special physiological function.

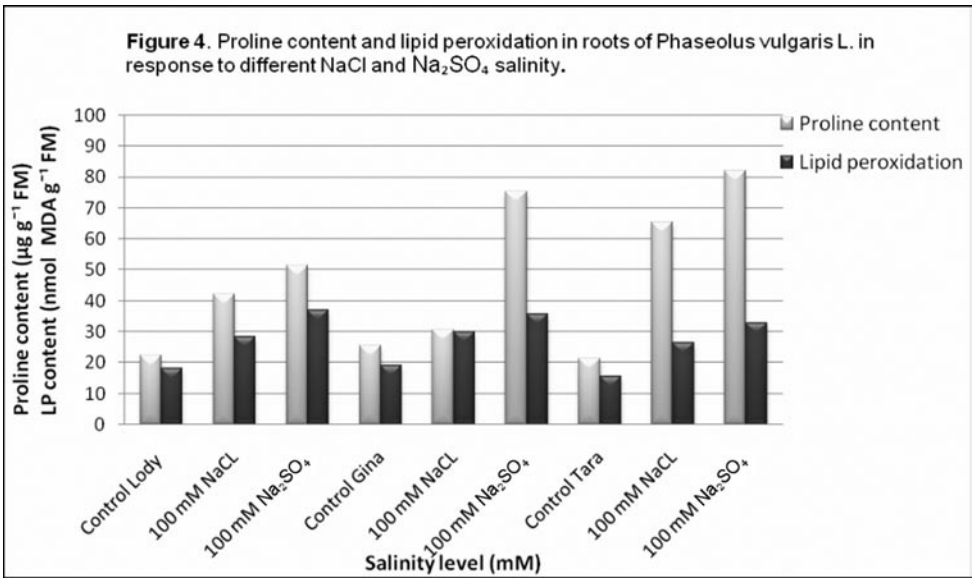

Figure 4. Proline content and lipid peroxidation in roots of Phaseolus vulgaris L. in response to different NaCl and Na_2SO_4 salinity.

The increased levels of proline, under salt stress, have been reported in two wheat cultivars by Khatkar and Kuhad (2000). It was suggested that proline accumulation may be caused by increased proteolysis or by decreased protein synthesis. The higher concentration of proline under salt stress is favorable to plants as proline participates in the osmotic potential of leaf and thus in the osmotic adjustment. Besides the role of osmolyte, proline can also confer enzyme protection and increase membrane stability under various condition. Proline accumulation may also help in nonenzymic free radical detoxifications (Durgaprasad et al. 1996; Khan et al., 2002).

Lipid peroxidation measured as the amount of tiobarbituric acid reactive substance or malondialdehide is produced when polyunsaturated fatty acids in the membrane undergo oxidation by the accumulation of free oxygen radicals. As lipid peroxidation is the symptom mostly ascribed to oxidative damage, it is often used as an indicator of increased damage (Hernandez et al., 2000; Khan and Panda, 2008). The results reported in this paper showed that the degree of accumulation of MDA was higher in the roots of cv. Lody compared to cv. Tara and Gina, indicating higher rate of lipid peroxidation

in cv. Lody due to salt stress (Meloni et al, 2003; Bor et al., 2003).

In conclusion, salinity (NaCl and Na_2SO_4) induced a decrease in CO_2 fixation, due probably to both stomatal and non-stomatal limitations, however, with a differential sensitivity to the salt source. The different sensitivity of Pn to the salt source can be attributed, at least in part, to a direct ionic effect.

The growth processes in the bean plants were suppressed which was a result of the disturbed osmotic processes and the toxic effect of Cl^- , SO_4^{2-} and Na^+. The equimolar concentrations of NaCl and Na_2SO_4 suppressed, each to a different extent, the physiological processes in the plants. The weaker toxic effect in the plants treated with NaCl was probably a result, on the one hand, of the weaker inhibition of the water potential and the stomata closure, and on the other hand, of the lower concentration of Na^+ in the tissues of the plants treated with chloride, compared to that in the plants treated with Na_2SO_4.

However, to evaluate physiological and morphological responses of adapted bean cultivars to salinity stress, we suggest more robust methodologies, in terms of time and resources, for screening bean for salinity tolerance. These include physiological markers such as shoot and root dry weight, relative growth rate (RGR), photosynthesis rate, lipid peroxidation and proline content as essential parameters for screening for salinity.

References

Ashraf, M., P.J. S.Haris, 2004. Pontential biochemical indicators of salinity tolerance in plants. Plant Sci., 166, 3-16.

Bates, L.S., R.P. Walden, I.D. Tears, 1973. Rapid termination of free proline for water stress studies. Plant Soil, 39, 205-208.

Bor, M., F. Ozdemir, I. Turkan, 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet Beta vulgaris L. and will beet beta maritime L. Plant Sci., 64,77-84.

Cachorro, P., R. Martinez, A. Ortiz, A. Cerda, 1995. Abscisic acid and osmotic relations in Phaseolus vulgaris L. under saline conditions.

Plant Sci., 95,29-32.

Gama P.B., S.Inanaga, K.Tanaka, R. Nakazawa, 2007. Physiological response of common bean (Phaseolus Vulg. L.) seedlings to salinity stress. African J. of Biotechnology, vol.6 (2), 79-88.

Garsia-Sanchez, F.,J.L. Jufon, M. Carvaial, J.P. Syverstem,2002. Gas exchange, chlorophyll and nutrient contents in relation to Na+ and Cl- accumulation in 'Sunburst' mandarin grafted on different rootstocks. Plant Sci., 162, 705-712.

Grant, J.J., J.G. Loake, 2000. Role of reactive oxygen intermediate and cognate redox signalling in diseance resistance. Plant Physiol, 124, 21-29.

Demiral, T. I.,Turkan, 2005. Comperative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Env. Exp. Bot., 53, 247-257.

Durgapras, K.M.R.,M. Muthukumarasamy,R.Paneerselvam,1996.Changes in protein metabolism induced by salinity in soybean seedlings. Ind. J. Plant Physiol., 198-101.

Heath, R., L. Packer, 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys., 125 189-198.

Hernandez, J.A., P. Mullineaux , F. Sevilla, 2000 .Tolerance of pea (*Pisum sativum L.*) to long term stress is associated with induction of antioxidant defences. Plant Cell Environ., 23, 853-862.

Josefa, M. N., C. Gariddo, V. Martinez. M. Carvajal, 2003. Water relations and xylem transport of nutrients in pepper plants grown under two different salt stress regimes. Plant Growth Regul., 41, 237-245.

Khan, M.H., S.K. Panda, 2008. Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physyol Plant, 30, 91-89.

Khan, M.H., L.B.Singha, S.K.Panda, 2002. Changes in antioxidant levels in Oriza sativa L. roots subjected to NaCl-salinity stress. Acta Physyol Plant , 24, 145-148.

Khatkar, D., M.S. Kuhad, 2000. Short-term salinity induced changes in two wheat cultivars at different growth stages. Biol. Plant., 43(4), 629-632.

Mandhania, S., S. Madan, V. Sawhney, 2006. Antioxidant defense mechanism under salt stress in wheat seedlings. Biol. Plant, 227, 227-231.

Mansour, M. M. F., 2000. Nitrogen containing compounds and adaptation of plants to salinity stress, Biol. Plant., 43, 491-500.

Mansour, M.M., F.Z. Salama., M. Ali, A. F. Abou Hadid, 2005. Cell and plant responses to NaCl in Zea Mays L. cultivars differing in salt tolerance. Gen. Appl. Plant Physiol., 31(1-2), 29-41.

Meloni, D.A., A.A.Oliva, Z.A. Martinez, J. Cambraia, 2003. Photosynthesis and activity of superoxid dismutase,peroxidase and glutathione reductase in cotton under salt stress. Environ. Exp. Bot., 49, 69-76.

Misra, A.N., S.M. Sahu, M. Misra, P. Singh , N. Das, M. Kar, P. Sahu, 1997. Sodium chloride induced changes in leaf growth and pigment and protein contents in two rice cultivars. Biol. Plant., 39, 257-262.

Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7, 405-410.

Munnas, R. C., 2002. Comparative physiology of salt and water stress. Plant Cell Environ., 25, 239-250.

Shannon, M., 1984. Breeding, selection and the genetics of salt tolerance. In Salt tolerance in Plants. Strategies for Crop Improvement, eds. Staples RC, GH Toenniessen, Wiley, New York, pp. 300-308.

Stepien, P., G. Klobus, 2006. Water relations and photosynthesi in Cucumis sativus L. leaves under salt stress. Biologia Plantarum, 50(40), 610-616.

Tourneux, C., G Peltier, 1995. Effect of water deficit on the photosynthetic oxygen exchange measured using $^{18}O_2$ and mass spectrometry in Solanum tuberosum leaf disks. Planta, 195, 570-577.

Zhang, J. W.J. Davies, 1991. Antitranspirant activity in xylem sap of maize plants. J. Exp. Bot., 42, 317-321.