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Plant Growth Promoting Rhizobacteria Effect on Antioxidant Status, Photosynthesis, Mineral Uptake and Growth of Lettuce under Soil Salinity

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Abstract: This study was carried out to investigate the effects of plant growth promoting rhizobacteria (PGPR) on the antioxidant status, photosynthesis, mineral content and growth of lettuce (*Lactuca sativa* L.) under different soil salinity conditions. Increasing salinity in the soil decreased plant growth, photosynthesis, stomatal conductance, chlorophyll content, and mineral uptake compared to soil without salinity. Inoculation with two PGPR strains, *Serratia* sp. and *Rhizobium* sp., into saline soils alleviated the salinity effects on the antioxidant enzymes ascorbate peroxidase (APX) and glutathione reductase (GR), along with those on photosynthesis, mineral content and growth. As a result, an increase in salinity in the soil caused a physiological response or disorder in lettuce plants. Treatment with PGPR strains could alleviate the effect of potentially toxic ions.

Key words: Lettuce, PGPR, salinity, photosynthesis, mineral uptake, antioxidant enzyme

INTRODUCTION

Soil salinity limits plant growth and crop production in many parts of the world, particularly in arid and semi-arid areas[1]. Salinity stress also decreases photosynthetic capacity due to the osmotic stress and partial closure of stomata[2]. Plants can suffer from membrane destabilization and general nutrient imbalance[3,4]. Salt stressed plants accumulate various molecules found in organic matter such as proline, glucose, glycine betaine etc. in the cell membrane for osmoregulation to occur thereby protecting enzyme activity[5]. However, levels of antioxidant enzyme activitiy and antioxidant concentrations are frequently used as indicators of oxidative stress in plants[6]. Several studies have demonstrated that generation of reactive oxygen species (ROS), such as the superoxide radical ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet-}$) and hydrogen peroxide (H_2O_2), alter antioxidant enzymes. Antioxidants are induced in plants in response to stressors such as salinity[7,8]. A ROS causes oxidative damage to biomolecules such as lipids and proteins and eventually leads to cell death[9]. To protect against oxidative stress, plant cells produce both antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POX) and catalase (CAT), and non-enzymatic antioxidants such as ascorbate, glutathione and "-tocopherol[6,9]. Ascorbate peroxidase (APX) is part of the scavenging cycle and catalyzes the reaction of ascorbic acid with H_2O_2, while glutathione reductase (GR) catalyzes the regeneration of ascorbic acid[10].

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that can actively colonize plant roots and increase plant growth[11]. These PGPR can prevent the deleterious effects of phytopathogenic organisms and stressors from the environment. The *Bacillus* sp. strains enhance soybean nodulation and growth under low temperature stress[12]. PGPR produce plant growth promoting compounds including phytohormones; auxins, cytokinins and gibberellins[13], as well as siderophores[14], and antibacterial peptides that inhibit pathogenic strains[15]. It has been recently shown that plants will respond to rather unconventional bacterial signal compounds, such as quorum sensing molecules and volatile compounds. Bacterial volatiles may have a significant role in plant growth promotion[16], as an increase in *Arabidopsis* growth has been attributed to a number of airborne bacterial chemicals. Bacteria have developed diverse resistance strategies towards toxic

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minerals. Hasnain and Sabri[17] reported that inoculation of wheat with *Pseudomonas* sp. stimulated plant growth by reduction of toxic ion uptake, increases in auxin contents and formation of stress-specific proteins in plants under stress caused by the toxic ion.

Little is known about the co-inoculation of *Serratia* sp. and *Rhizobium* sp. and their effect on the antioxidant status and photosynthesis of lettuce under different conditions of soil salinity. In this paper, we report a detailed study of the effect of long-term stress due to salinity on the mineral content, photosynthesis and antioxidant level of lettuce. An additional objective was to determine the possible importance of combining *Serratia* sp. and *Rhizobium* sp. for plant tolerance to soil salinity conditions and to define the possible mechanisms involved.

MATERIALS AND METHODS

Plant growth and experimental design: Lettuce seeds (*Lactuca sativa* L. cv. Cheongchima) were sown to plug plates filled with peat moss and perlite (2:1, v/v) and irrigated with half strength of Hoagland's solution[18]. They were germinated in a greenhouse under natural light conditions, a daytime temperature of about 28°C and relative humidity of 65-70%. Twenty days after sowing, seedlings were transplanted into sterilized pots (17 cm diameter and 15 cm deep) containing 2 kg of sterilized soil for 2 hr at 130 °C, one seedling per pot. The soil used was Typic Endoaquepts (USDA, Inceptisols). The soil characteristics were pH (1:5 water) 6.5, EC 1.50 dS m^{-1}, organic matter 15 g kg^{-1}, total nitrogen 1.6 g kg^{-1}, CEC: Ca 4.9, K 1.5 and Na 0.4 $cmol^{+}$ kg^{-1}. A basal fertilizer N-P_2O_5-K_2O was applied at 100-80-50 kg ha^{-1}. The PGPR effect on salinity levels was investigated by using 2 salinity levels (1.5 and 7.0 dS m^{-1}). Saline solution was applied only once at the beginning. The pots with the salinity treatment were equilibrated for 7 days before transplanting seedlings. A sterilized vinyl bag was put underneath each pot to collect excess water due to drainage. This water was reapplied to the respective pot. One day after transplanting, one seedling was inoculated with 1 mL of inoculum containing approximately 10^8 cells[19]. The temperature in the greenhouse was maintained at 28 ± 2°C with a relative humidity of 65% and a 16 hr photoperiod created by using supplemental lighting from high-pressure sodium lamps. All plants were harvested 30 days after transplanting. The photosynthesis and stomatal conductance of plants was measured using a Li-Cor 6400 (Li-Cor Inc, Lincoln, Nebraska, USA) before harvesting the plants. To analyze antioxidative enzymes, fresh leaves were harvested 30 days after transplanting and then stored immediately into a deep-freezer (-80 °C). The experiment was structured following a randomized complete block design (RCBD) with four replications.

Bacterial culture and inoculant preparation: The two strains of plant growth promoting rhizobacteria (PGPR) in these experiments were *Serratia proteamaculans* ATCC35475 (SP) and *Rhizobium leguminosarum* bv. viciae 128C56G (RL), which improve plant growth and/or nitrogen fixation in legume plants[12,20]. The RL strain originated from Nitragin Inc., Milwakee, WI, USA. Two PGPR strains were cultured in LB medium and incubated on an orbital shaker at 150 rpm for 48 h at 27ºC. The cells in cultured bacterial broth were collected by centrifugation at 2,822 x g for 15 min at 4 °C and washed with sterilized tap water. The pelleted cells was resuspended with sterilized tap water and then the cells were adjusted to about 10^8 cells mL^{-1}, based on an optical density OD_{620} = 0.08[19]. One mL of inoculum was applied to each seedling.

Inorganic elements and chlorophyll content: To analyze mineral elements, soil samples were collected before the experiment and air-dried for chemical analysis. Soil samples were sieved (2 mm screen) and analyzed for the following: pH (1:5 water extraction), organic matter content (Wakley and Black method[21]), available P content (Lancast[22]) and contents of exchangeable or available K^+ (1 M NH_4-OAc pH 7, AA, Shimazu 660)[23]. Leaf tissues were separated after harvesting and air-dried at 70°C for 5 days. Dried materials were ground and then digested in H_2SO_4 for the determination of total nitrogen (Kjeldahl method[22]) or in a ternary solution (HNO_3:H_2SO_4:$HClO_4$ = 10:1:4 with volume) for the determination of P, K, Ca and Na. Chlorophyll was extracted by 80% acetone (v/v) and its contents were determined at 663 nm and 645 nm by a Hitachi U-2000 dual length spectrophotometer[24].

Antioxidant activity: To determine the levels of antioxidant enzymes fully expanded leaves were homogenized in 50 mM phosphate buffer (pH 7.5) containing 1.0% (w/v) polyvinyl-pyrrolidone (PVP), 0.1 mM EDTA and 0.5% (v/v) Triton X-100[25]. For ascorbate peroxidase (APX) assay, leaves were homogenized in 50 mM phosphate buffer (pH 7.0) containing 5 mM ascorbate and 1 mM EDTA. The homogenate was filtered through four layers of muslin cloth and centrifuged at 12,000x g for 10 min. All assays were conducted at 4ºC. The supernatant was used for determination of antioxidant

enzyme activities of APX[26] and glutathione reductase (GR)[27]. The oxidation rate of ascorbate was estimated by following the decrease in absorbance at 290 nm for 3 min. All spectrophotometric analyses were conducted on a Shimaszu (UV-Vis 1600, Japan) spectrophotometer. Protein contents were determined according to the Bradford[28] method using bovine serum albumin (BSA) as a standard.

Statistical analysis: All data were analyzed statistically by an analysis of variance using CoStat software (CoHort Software, Monterey, USA). Salinity and PGPR treatments were tested in an experiment using a randomized complete block model with four replications. Mean comparisons were conducted using an ANOVA protected least significant difference (LSD) ($P<0.05$) test.

RESULTS AND DISCUSSIONS

Plant growth and photosynthesis: Results of the measurements of growth response and total chlorophyll content are given in Table 1. Plant growth was significantly increased by inoculation with PGPR. The fresh and dry weight of lettuce under non-salinity stress was increased by 13.0 and 13.0% in the RL, and in the combined treatment by 13.2 and 12.3%, respectively, in comparison to the control treatment. Under salinity stress, the fresh weight was also increased by 6.8-12.9% in the PGPR strain treatments compared to the control treatment. Leaf length and leaf area under non-salinity stress was not significantly different, but leaf area under salinity stress was significantly different. These results agree with Vivas et al.[29] who reported that the shoot and root growth of lettuce inoculated by *Bacillus* sp. under drought stress conditions were increased compared to the control. The reduction of plant growth caused by salinity stress is the most common phenomenon of plants under stress, although measurement of stress indicators might not be significant. This is understandable since the reduction of plant growth is the result of the alteration of many physiological activities in the plant, such as photosynthetic activity, mineral uptake and antioxidant activity. Chlorophyll content was also increased significantly in all the PGPR strain treatments. The photosynthetic rate and stomatal resistance of lettuce plants exposed to salinity is presented in Figure 1. Photosynthetic rate and stomatal resistance were not significant under non-salinity stress, but under salinity stress they were. It was especially evident with respect to stomatal conductivity. A similar result was reported by Vivas et al.[29] who showed that inoculation of *Bacillus* sp. and coinoculation of it with *Glomus* sp. both increased

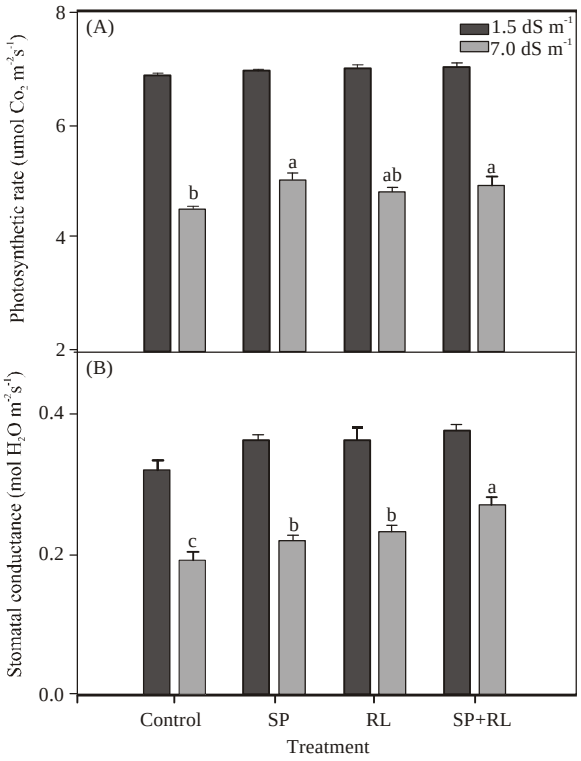

Fig. 1: PGPR effects on photosynthesis and stomatal conductance of lettuce leaves under salinity stress. Means with the same letters are not significantly different at $P<0.05$ when compared by LSD. Treatment means are with ±S.E. of four replications

stomatal conductance of lettuce compared to a non-drought control. Inoculation with PGPR strains increased plant growth compared to the non-inoculated control treatment. In this study the inoculation with PGPR strains under soil salinity conditions did improve plant growth compared to the non-inoculated control.

Mineral content: The effects of PGPR strains on N, P, K, Ca and Na uptake per plant in lettuce are shown in Table 2. Mineral uptake under salinity stress treatment in lettuce was significantly decreased compared to the non-salinity stress treatment, but an interaction between salinity and strains was not found. Treatment with PGPR strains in the non-salinity stress treatment increased P (11.1-16.6%), K (10.5-16.9%) and Na (13.7-17.2%) uptake per plant in lettuce. Vivas et al.[29] reported similar results. The N, P and K concentrations in lettuce inoculated by *Bacillus* sp. under drought stress conditions were increased by about 5, 70 and 50%, respectively, compared to the non-salinity stress control. This means that PGPR

Table 1: PGPR effect on yield, total chlorophyll content and growth of lettuce grown for 4 weeks under salinity stress

Salinity (dS m^{-1})	Strain	Fresh weight (g $plant^{-1}$)	Dry weight (g $plant^{-1}$)	Leaf length (cm)	Leaf area (cm^2 $plant^{-1}$)	Total chlorophyll (mg g^{-1})
1.5	Control	16.9b	1.06b	13.8	496	15.8b
	SP	18.0ab	1.15ab	14.1	518	16.9a
	RL	19.1a	1.19a	14.6	522	16.9a
	SP+RL	19.1a	1.20a	14.5	538	16.1b
7.0	Control	13.2c	0.89	12.2	419c	11.5b
	SP	14.3b	0.93	12.5	440bc	12.7a
	RL	14.1b	0.93	13.0	447ab	13.1a
	SP+RL	14.9a	0.96	13.3	470a	13.2a
Significance of factors						
	Salinity	***	***	***	***	***
	Strains	**	**	**	**	**
	Interaction	ns	ns	ns	ns	*

*, ** and *** significant at 95%, 99% and 99.9% confidence and ns not significant
SP, *Serratia proteamaculans*; RL, *Rhizobium leguminosarum*

Table 2: PGPR effect on mineral uptake of lettuce grown for 4 weeks under salinity stress

Salinity (dS m^{-1})	Strain	T-N	P	K	Ca	Na
				(mg $plant^{-1}$)		
1.5	Control	23	5.4b	50.1b	13.3	2.9b
	SP	24	6.0a	55.4ab	14.9	3.3a
	RL	25	6.2a	56.9a	15.3	3.3a
	SP+RL	25	6.3a	58.6a	15.8	3.4a
7.0	Control	18	4.1b	27.0	8.3b	26.7
	SP	19	4.6a	28.0	9.1ab	26.9
	RL	18	4.6a	27.6	8.7b	26.9
	SP+RL	20	4.8a	29.5	10.4a	26.8
Significance of factors						
	Salinity	***	***	***	***	***
	Strains	ns	***	*	ns	*
	Interaction	ns	ns	ns	ns	ns

* and *** significant at 95% and 99.9% confidence and ns not significant
SP, *Serratia proteamaculans*; RL, *Rhizobium leguminosarum*

strains could improve production of plant growth regulators or increase plant nutrient uptake. Hasnain and Sabri[17] reported that inoculation of wheat with *Pseudomonas* sp. stimulated plant growth by reduction in toxic ion uptake, increase in auxin content and formation of stress-specific proteins in plants under stress caused by the toxic ions. The application of inoculum composed of *Arthrobacter* sp. and *Flavobacterium* sp. increased the uptake of P, Ca, Cl and Ni and decreased Pb content in barley plants under field stress conditions[30]. In contrast with increasing Na content, K content decreased with increasing salinity levels. A similar result was reported in wheat by Grieve and Poss[31] who demonstrated antagonistic absorption between Na and K under salinity

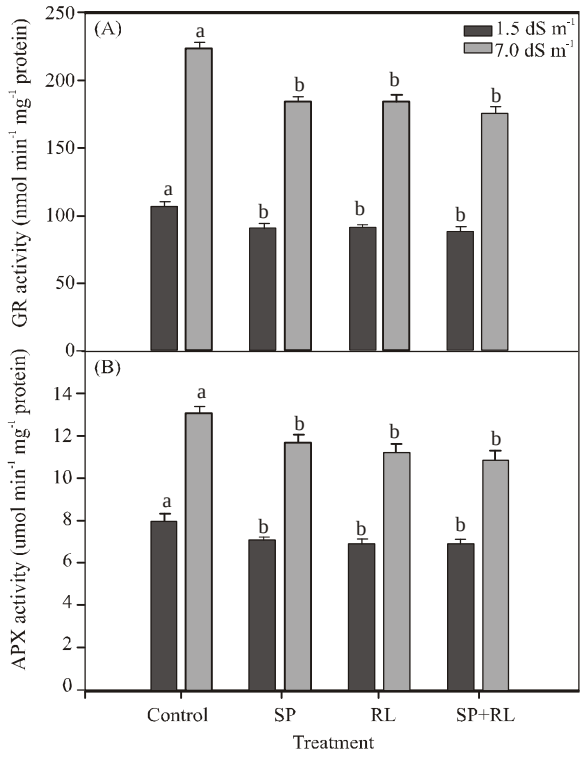

Fig. 2: PGPR effects on APX and GR activity of lettuce leaves under salinity stress. Means with the same letters are not significantly different at P<0.05 when compared by LSD. Treatment means are with ±S.E. of four replications.

stress conditions. Under soil salinity stress, P and Ca uptake per plant in all PGPR treatments was increased compared to the non-salinity stress control. This also means that a PGPR treatment under salinity stress conditions could alleviate the inhibition of plant growth.

Antioxidant activity: To understand the protective action of antioxidants against salinity stress, lettuce plants were treated with PGPR strains followed by measurement of the level of antioxidant activity. The results are presented in Figure 2. Increasing salinity stress significantly increased enzyme activity, including GR and APX, of lettuce leaves compared to the control in the experiment. Inoculation with PGPR strains under salinity stress decreased enzyme activity with increasing salinity stress. It is interesting to note that though a significant interaction was found, treatment with PGPR strains tended to reduce the salinity stress effect on the activity of these two enzymes. Ruiz-Lozano et al.[32] also reported that mycorrhizal lettuce plants showed increased superoxide dismutase (SOD) activity under drought stress and this was correlated to plant protection against drought. Stress resistance in plants has been related to more effective antioxidant systems[8]. Detoxification of cellular H_2O_2 through the activity of the Asada-Halliwell scavenging cycle is an important element of plant defense mechanisms against ROS[33]. Our results as presented above support this conclusion.

REFERENCES

1. Shannon, M.C., 1984. Breeding, Selection, and the Genetics of Salt Tolerance. In: Salinity Tolerance in Plants: Strategies for Crop Improvement. John Wiley & Sons, New York, USA, pp. 231-254.
2. Drew, M.C., P.S. Hole and G.A. Picchioni, 1990. Inhibition by NaCl of net CO_2 fixation and yield of cucumber. J. Amer. Soc. Hort. Sci., 115: 472-477.
3. Hasegawa, P.M., R.A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Physiol. Plant Mol. Biol., 51: 463-499.
4. Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxic. Environ. Safety, 60: 324-349.
5. Munns, R. and A. Termaat, 1986. Whole-plant responses to salinity. Aust. J. Plant Physiol., 13: 143-160.
6. Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7: 405-410.
7. Shalata, A. and M. Tal, 1998. The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt tolerant relative *Lycopersicon pennellii*. Physiol. Plant., 104: 169-174.
8. Bor, M., F. Özdemir and I. Türkan, 2003. The effect of salt stress on lipid peroxidation and antioxidants in leaves of sugar beet *Beta vulgaris* L. and wild beet *Beta maritime* L. Plant Sci., 164: 77-84.
9. Del Rio L.A., F.J. Corpas, L.M. Sandalio, J.M. Palma and J.B. Barroso, 2003. Plant peroxisomes, reactive oxygen metabolism and nitric oxide. IUBMB Life, 55: 71-81.
10. Smirnoff, N., 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol., 186: 69-74.
11. Kloepper, J.W. and M.N. Schroth, 1978. Plant growth-promoting rhizobacteria on radishes. IV. International Conference on Plant Pathogenic Bacteria. Angers France, 2: 879-882.
12. Bai, Y., X. Zhou, and D.L. Smith, 2003. Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. Crop Sci., 43: 1774-1781.

13. Garcia de Salamone, I.E., R.K. Hynes and L.M. Nelson, 2001. Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can. J. Microbiol., 47: 404-411.
14. Castignetti, D. and J. Smarrelli, 1986. First year field performance of spruce seedlings inoculated with plant growth promoting rhizobacteria. Can. J. Microbiol., 39: 1084-1088.
15. Maurhofer, M., C. Keel, U. Schnider, C. Voisard, D. Hass and G. Defago, 1992. Influence of enhanced antibiotic production in *Pseudomonas fluorescens* strain CHAO on its disease suppressive capacity. Phytopathology, 82: 190-195.
16. Ryu, C.M., M.A. Farag, C.H. Hu, M.S. Reddy, H.X. Wei, P.W. Pare and J.W. Kloepper, 2003. Bacterial volatiles promote growth in *Arabidopsis*. Proceedings of the National Society of America, 100: 4927-4932.
17. Hasnain, S, and A.N. Sabri, 1996. Growth stimulation of *Triticum aestvum* seedlings under Cr-stress by nonrhizospheric *Pseudomonas* strains. In: Abstract Book of 7th Int. Symp. On Nitrogen Fixation with Non-legumes. Faisalabad, Pakistan, pp. 36.
18. Hoagland D.R. and D.I. Arnon, 1950. A water culture method for growing plants without soil. Calif. Agric. Exp. Stat. Circular, 347.
19. Bhuvaneawari, T.V., B.G. Turgeon and W.D. Bauer, 1980. Early events in the infection of soybean (*Glycine max* L. Merr.) by *Rhizobium japonicum* I. location of infectable root cells. Plant Physiol., 66: 1027-1031.
20. Noel, T.C., C. Sheng, C.K. Yost, R.P. Pharis and M.F. Hynes, 1996. *Rhizobium leguminosarum* as a plant growth-promoting rhizobacterium: direct growth promotion of canola and lettuce. Can. J. Microbiol., 42: 279-283.
21. Allison, L.E., 1965. Organic carbon. In: Methods of Soil Analysis. Part ¥±, Black C.A. (Eds.), Am. Soc. Agron. Inc. Publ., Madison, USA, pp. 1367-1376.
22. RDA (Rural Development Administration, Korea), 1988. Methods of Soil Chemical Analysis. National Institute of Agricultural Science and Technology, RDA, Suwon, Korea.
23. Richards, J.E. and T.E. Bates, 1989. Studies on the potassium-supplying capacities of southern Ontario soils. ¥². Measurement of available K., Can. J. Soil Sci., 69: 597-610.
24. Arnon, D.K., 1949. Copper enzymes in isolated chloroplasts. Phenoloxidase in *Beta vulgaris*. Plant Physiol., 24: 1-15.
25. Anderson, J.A., 2002. Catalase activity, hydrogen peroxide content and thermotolerance of pepper leaves. Sci. Hort., 95: 277-284.
26. Chen, G.X. and K. Asada, 1989. Ascorbate peroxidase in tea leaves: occurrence of two isozymes and the differences in their enzymatic and molecular properties. Plant Cell Physiol., 30: 987-998.
27. Rao, M.V., G. Paliyath and D.P. Ormrod, 1996. Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol., 110: 125-136.
28. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
29. Vivas, A., A. Marulanda, J.M. Ruiz-Lozano, J.M. Barea and R. Azcon, 2003. Influence of a *Bacillus* sp. on physiological activities of two arbuscular mycorrhizal fungi and on plant responses to PEG-induced drought stress. Mycorrhiza, 13: 249-256.
30. Kozhemyakov, A.P., I.I. Chernyae, A.A. Belimov, A.M. Kunakova, E.V. Fomenko, L.Y. Yudkin, V.V. Stepanok, G.Y. Rabinovich and I.V. Chernenok, 1995. Effect of inoculation with nitrogen fixing bacteria on heavy metals and radionucleotide uptake by the plants grown in contaminated soils. In: Nitrogen fixation: Fundamentals and Applications, Proc. 10th Int. Congr. On Nitrogen Fixation, St. Petersburg, 1995. Eds. Tikhonovich et al., Kluwer Academic Publishers, Dordrecht, pp. 765.
31. Grieve, C.N. and J.A. Poss, 2000. Wheat response to interactive effects of boron and salinity. J. Plant Nutr., 23: 1217-1226.
32. Ruiz-Lozano, J.M., C. Collados, J.M. Barea and R. Azcón, 2001. Cloning of cDNAs encoding SODs from lettuce plants which show differential regulation by arbuscular mycorrhizal symbiosis and by drought stress. J. Exp. Bot., 52: 2241-2242.
33. Lee, D.H. and C.B. Lee, 2000. Chilling stress-induced changes of antioxidant enzymes in the leaves of cucumber: in gel enzyme activity assays. Plant Sci., 159: 75-85.