American-Eurasian J. Agric. & Environ. Sci., 11 (3): 445-450, 2011
ISSN 1818-6769

Effect of Biological Fertilizers on Biochemical and Physiological Parameters of Basil (*Ociumum basilicm* L.) Medicine Plant

[1]*Amir Golpayegani and* [2]*Hossein Gholami Tilebeni*

[1]Young Researchers Club, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
[2]Young Researches Club, Gorgan Branch, Islamic Azad University, Gorgan, Iran

Abstract: Basil (*Ocimum basilicum*) is one of the most important medicine plants that its essential (leaf, flower and seed) is used in different medicinal industries. In order to study the effects of salinity stress and plant growth promoting rhizobacteria (PGPR) on the antioxidant status, photosynthesis, mineral content and growth of basil (*Ociumum basilicm* L.). 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, *Pseudomonades* sp. and *Bacillus lentus*, 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 basil plants. Treatment with PGPR strains could alleviate the effect of potentially toxic ions.

Key words: Basil · PGPR · Salinity · Photosynthesis · Mineral uptake · Antioxidant enzyme

INTRODUCTION

In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress [1]. Parida and Das [2] reported that secondary products of plants can be altered by environmental factors and water stress is a major factor affecting the synthesis of natural products. Basil (*Ocimum basilicum,*) is an annual plant belongs to the *Lamiacea* family which has been grown for its essential oil. The essential oil of basil is used to flavor foods, dental and oral products in fragrances and in medicines [3]. The genus *Ocimum* (family Labiatae) includes at least 60 species and numerous varieties [4]. It represents an important source of essential oil used in the food, perfumery and cosmetics industries. Some *Ocimum* species are used in traditional medicine for different applications, especially in many Asian and African countries [5]. The recurring polymorphism determines a large number of subspecies that produce essential oils with varying chemical composition. Some have high camphor content, while others contain citral, geraniol, methylchavicol, eugenol and thymol [6].

Soil salinity limits plant growth and crop production in many parts of the world, particularly in arid and semi-arid areas [7]. Salinity stress also decreases photosynthetic capacity due to the osmotic stress and partial closure of stomata [8]. Plants can suffer from membrane destabilization and general nutrient imbalance [2]. 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 [9]. However, levels of antioxidant enzyme activity and antioxidant concentrations are frequently used as indicators of oxidative stress in plants [5]. Several studies have demonstrated that generation of reactive oxygen species (ROS), such as the superoxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2), alter antioxidant enzymes. Antioxidants are induced in plants in response to stressors such as salinity [10]. A ROS causes oxidative damage to biomolecules such as lipids and proteins and eventually leads to cell death [11]. 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 "-

Corresponding Author: Amir Golpayegani, Young Researchers Club, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.

tocopherol. 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.

Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that can actively colonize plant roots and increase plant growth . 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 . PGPR produce plant growth promoting compounds including phytohormones; auxins, cytokinins and gibberellins, as well as siderophores and antibacterial peptides that inhibit pathogenic strains. 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 , as an increase in *Arabidopsis* growth has been attributed to a number of airborne bacterial chemicals. Bacteria have developed diverse resistance strategies towards toxic minerals. Hasnain and Sabri [12] 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 *Bacillus lentus* and *Pseudomonades* sp and their effect on the antioxidant status and photosynthesis of basil 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 basil. An additional objective was to determine the possible importance of combining *Bacillus lentus* and *Pseudomonades* sp for plant tolerance to soil salinity conditions and to define the possible mechanisms involved.

MATERIALS AND METHODS

Basil (*Ocimum basilicum*) seed were sown to plug plates filled with peat moss and perlite and irrigated with half strength of Hoagland's solution [13]. 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 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 O -K O was applied at 100-80-50 kg ha . The PGPR effect on salinity levels was investigated by using 2 salinity levels (2 and 6 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 108 cells [14]. 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).

Bacterial Culture and Inoculant Preparation: The two strains of plant growth promoting rhizobacteria (PGPR) in these experiments were *Bacillus lentus* and *Pseudomonades* sp. which improve plant growth. Seeds of Basil were washed with distilled water then inoculation was performed by a suspension of any bacteria (108 cfu ml^{-1}) with perlit mixture. There were six rows in each plot. Which the row width and length was 0.3 and 2 meter, respectively.

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), available P content (Lancast) and contents of exchangeable or available K^+ (1 M NH -OAc pH 7, AA, Shimazu 660) . Leaf tissues were separated after harvesting and air-dried at 70°C for 5 days. Dried materials were ground and then digested in H SO for the determination of total nitrogen (Kjeldahl method) or in a ternary solution (HNO :H SO :HClO = 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.

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 . 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 4°C. The supernatant was used for determination of antioxidant enzyme activities of APX [15] and glutathione reductase (GR) [16]. 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 [17] 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 factorial conducted based on RCBD design with three replications. Mean comparisons were conducted using an ANOVA protected least significant difference (LSD) ($P<0.05$) test.

RESULTS AND DISCUSSION

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 basil 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 with control treatment. Under salinity stress, the fresh weight was also increased by 6.8-12.9% in the PGPR strain treatments compared to control treatment. Leaf length and leaf area under non-salinity stress was not significantly different, while leaf area under salinity stress was significantly different. These results agree with Vivas *et al.* [18] 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.

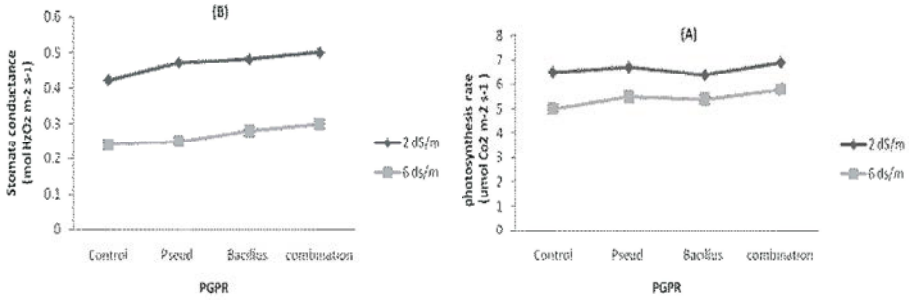

Fig. 1: Effects PGPR on photosynthesis and stomatal conductance of basil leaves under salinity stress.

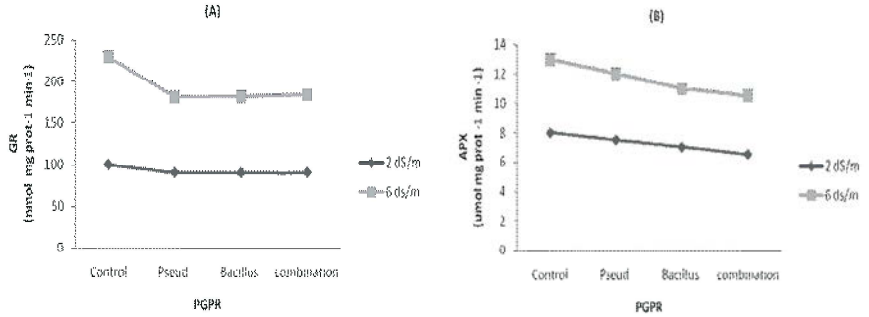

Fig. 2: Effects PGPR on APX and GR activity of basil leaves under salinity stress

Table 1: Effect PGPR on growth, total chlorophyll content of basil plant under salinity stress

Salinity (dS m^{-1})	PGPR	Fresh weight (g $plant^{-1}$)	Dry weigh (g $plant^{-1}$)	Leaf length (cm)	Leaf area (cm^2 $plant^{-1}$)	Total chlorophyll (mg g^{-1})
2	Control	11.2b	1.4c	1.7d	49b	10.8b
	Pseudomonades	11.8b	2.6b	1.4c	51ab	11.9a
	Bacillus lentus	10.2b	2.6b	1.7b	52a	11.9a
	combination	16a	4.5a	2.0a	53a	11.1b
6	Control	6.9cd	0.1d	1f	41d	7.07b
	Pseudomonades	9.7cb	0.3d	1.2e	44c	8.61a
	Bacillus lentus	8.7b	0.5d	1.1e	44c	9.31a
	combination	11.5b	0.7c	1.2e	47b	9.55a
Significance of factors	Block	ns	ns	ns	ns	ns
	Salinity	**	*	*	*	**
	PGPR	*	*	*	*	*
	Interaction	**	**	*	*	*

ns, *and **: not significant, significant at the 5% and 1% probability levels, respectively

Table 2: Effect PGPR on on mineral uptake of basil plant under salinity stress

Salinity (dS m^{-1})	PGPR	T-N	P (mg $plant^{-1}$)	K (mg $plant^{-1}$)	Ca (mg $plant^{-1}$)	Na (mg $plant^{-1}$)
2	Control	23	5.4a	35.43b	13.3b	0.26d
	Pseudomonades	24	6.0a	37.30b	14.9a	0.24c
	Bacillus lentus	25	6.2a	37.7b	15.3a	0.30b
	combination	25	6.3a	41.33a	15.8a	0.32a
6	Control	18	4.1b	19.5d	8.3d	0.21f
	Pseudomonades	19	4.6b	24.30c	9.1c	0.20f
	Bacillus lentus	18	4.6b	27.7c	8.7c	0.22e
	Combination	20	4.8b	28.93c	10.4c	0.23e
Significance of factors	Block	ns	ns	ns	ns	ns
	Salinity	**	**	**	*	*
	PGPR	ns	**	*	*	*
	Interaction	ns	ns	*	*	*

ns, *and **: not significant, significant at the 5% and 1% probability levels, respectively

The photosynthetic rate and stomatal resistance of basil 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.* [19] who showed that inoculation of *Bacillus* sp. and coinoculation of it with *Glomus* sp. both increased stomatal conductance of basil 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.

Minerals Content: The effects of PGPR strains on N, P, K, Ca and Na uptake per plant in lettuce are shown in Table 2. Minerals 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.* [18] 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 strains could improve production of plant growth regulators or increase plant nutrient uptake. Heidari *et al*. [19] reported that inoculation of basil 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 composedn 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 . In contrast with increasing Na content, K content decreased with increasing salinity levels. A similar result was reported in wheat by Grieve and Poss [20] who demonstrated antagonistic absorption between

Na and K under salinity 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, basil plants were treated with PGPR strains followed by measurement of the level of antioxidant activity. The results are presented in Fig. 2. Increasing salinity stress significantly increased enzyme activity, including GR and APX, of basil 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.* [21] also reported that mycorrhizal basil 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 [10]. 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 [22]. Our results as presented above support this conclusion.

REFERENCES

1. 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.
2. Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxic. Environ. Safety, 60: 324-349.
3. Omidbaig, R., 2005. Production and Processing of Medicinal Plants. Vol. 2 Astane Quds Publ. Tehran, pp: 438.
4. Stepien, P. and G. Klobus, 2005. Antioxidant defense in the leaves of C3 and C4 plants under salinity stress. Physiol. Plant, 125: 31-40.
5. Mittler, R., 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci., 7: 405-410.
6. Boojar, 2009. Effect of super absorbent application on antioxidant enzyme activities in canola (*Brassica napus* L.) cultivars under water stress conditions. Am. J. Agric. Biol. Sci., 3: 215-223.
7. 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.
8. Drew, M.C., P.S. Hole and G.A. Picchioni, 1990. Inhibition by NaCl of net CO fixation and yield of 2 cucumber. J. Amer. Soc. Hort. Sci., 115: 472-477.
9. Egamberdiyeva, D., 2007. The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl. Soil. Eco., 36: 184-189.
10. 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.
11. 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.
12. 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- PEG legumes. Faisalabad, Pakistan, pp: 36.
13. Hoagland, D.R. and D.I. Arnon, 1950. A water culture method for growing plants without soil. Calif. Agric. Exp. Stat. Circular, pp: 347.
14. Esfandiari, E., M.R. Shakiba, S.Mahboob, H. Alyari and M. Toorchi, 2007. Water stress, antioxidant enzyme activity and lipid peroxidation in wheat seedling. J. Food Agric. Environ., 5: 149- 153.
15. 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.
16. Goel, A., A.K. Goel and I.S. Sheoran, 2003. Change in oxidative stress enzymes during artificial ageing in cotton (*Gossypium hirsutum* L.) seeds. J. Plant Physiol., 160: 1093-1100.
17. 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.
18. 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.

19. Heidari, M., S.M. Mosavinik and A. Golpayegani, 2011. Plant growth promoting rhizobacteria (PGPR) effect on physiological parameters and mineral uptake in basil (*Ociumum basilicm* L.) under water stress . ARPN J. Agricultural and Biological Sci., 6: 6-11.
20. Grieve, C.N. and J.A. Poss, 2000. Wheat response to interactive effects of boron and salinity. J. Plant Nutr., 23: 1217-1226.
21. 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.
22. 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.