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PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR) EFFECT ON PHYSIOLOGICAL PARAMETERS AND MINERAL UPTAKE IN BASIL (*Ociumum basilicm* L.) UNDER WATER STRESS

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ABSTRACT

In order to study the effects of water stress and plant growth promoting rhizobacteria (PGPR) on Proline, soluble carbohydrates, chlorophyll and mineral content in Basil, a field experiment was conducted at the University of Zabol in Iran during 2010 growing season. The experiment laid out as split plot based on randomized complete block design with three replications. Three levels of water stress W_1 = 80 (control), W_2 = 60 and W_3 = 40% of the field capacity (FC) as main plots and four levels of bacterial strain consisting of S_1 = *Pseudomonades* sp, S_2 = *Bacillus lentus*, S_3 = *Azospirillum brasilens,* S_4 = combination of three bacterial and S_5 = control (without use of bacterial) as sub plots. Results showed water stress and different bacterial strain significantly affected on proline and soluble carbohydrate accumulations in leaves of plants. Proline of the S_1 = *Pseudomonades* sp and soluble carbohydrate in S_2 = *Bacillus lentus* plants increased significantly with an increasing of water stress. Chlorophyll content was also increased in all the bacterial strain treatments. Among the bacterial strain, the chlorophyll content of the S_1 and S_4 increased with increasing of water stress. The average concentration of K^+ was higher in S_2 and S_5 bacterial strains in the non-water stress.

Keywords: basil (*Ociumum basilicm* L.), PGPR, physiological parameters, water stress.

INTRODUCTION

Plant tolerance to water stress results from both morphological adaptation and responses at the biochemical and genetic levels. Among the various mechanisms developed by plants to resist water stress, tolerance at the cellular level is essential since it allows tolerant plants to maintain cellular homeostasis. In contrast, sensitive plants suffer rapid irreversible cell damage essentially due to degradation of their membranes [1]. Water stress is the most influential factors affecting crop yield particularly in irrigated agriculture in arid and semi arid regions. it is necessary to get maximum yield in agriculture by using available water in order to get maximum profit form per unit area because existing agricultural land and irrigation water are rapidly diminishing due to rapid industrialization and urban development [2].

In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress [3]. Solinas and Deiana [4] 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. The genus Ocimum (family Labiatae) includes at least 60 species and numerous varieties [5]. 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 [6]. 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 [7].

In many semiarid regions of the world, water stress and infertile soils with low a phosphorus concentration combine to limit crop productivity. In these regions, most aromatic plants are grown under rain fed conditions, where water stress can occur at any time during the growing season. Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that actively colonize plant roots and increase plant growth and yield [8]. The mechanisms by which PGPRs promote plant growth are not fully understood, but are thought to include: (i) the ability to produce phytohormons [9], asymbiotic N_2 fixation [10]. (ii) against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes and/or fungicidal compounds [11], and also (iii) solubilisation of mineral phosphates and other nutrients [12].

Significant increases in growth and yield of agronomical important crops in response to inoculation with PGPR have been reported [13]. Azospirillum, Pseudomonas and Azotobacter strains could affect seed germination and seedling growth [13]. Kloepper *et al*., [14] has been shown that wheat yield increased up to 30% with Azotobacter inoculation and up to 43% with Bacillus inoculation. Strains of Pseudomonas putida and Pseudomonas fluorescens could increase root and shoot elongation in canola [15].

However, information is not available on the PGPR in Basil systems under field conditions. There for, the aim of this studies was to evaluate effect plant growth promoting rhizobacteria (PGPR) and water stress on

osmotic components, chlorophyll content and nutrient uptake in Basil (*Ociumum basilicm* L.)

MATERIALS AND METHODS

Field experiment was conducted at the research farm of Zabol university in Iran (latitude of 30° 54 'N and longitude of 61° 41' E with an elevation of 481 m) in the period of May-July, 2010. The field soil was sandy loam in texture, having pH, 7.4; EC, 1.8 $ds.m^{-1}$; 0.75% of organic carbon; 0.04% N, 6.4 and 185 ppm of available P and K, respectively. Experiment laid out as split plot based on randomized complete block design with three replications. Three levels of water stress W_1 = 80 (control), W_2 = 60 and W_3 = 40% of the field capacity (FC), determined at the 0-15cm soil depth by TDR, as main plots and four levels of bacterial strain consisting of S_1 = Pseudomonades sp, S_2 = Bacillus lentus, S_3 = Azospirillum brasilens, S_4 = combination of three bacterial and S5 = control (without use of bacterial) as sub plots.

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.

Before sowing, the soil was fertilized with N, P and K at rate of 100, 50 and 50kg ha^{-1} as urea, single super phosphate and potassium sulphate, respectively. Half of nitrogen was applied at sowing time and residue at the start of 4 leaves.Seeds were placed at 1-2 cm depth.

Ten mature basil plants were sampled from each treatment for measurements of chlorophyll, proline, soluble carbohydrate, sodium and potassium contents in leaves.

The extracts of mature leaf blades were used to determine soluble carbohydrates [16] and proline [17]. For free proline content, leaf samples were homogenized in 5ml of sulphosalycylic acid (3%) using mortar and pestle. A bout 2ml of extract was taken in test tube and pestle. About 2ml of extract was taken in test tube and to it 2ml of glacia acetic acid and 2ml of ninhydrin reagent were added. The reaction mixture was boiled in water bath at 100°C for 30min. after cooling the reaction mixture; 6 ml of toluene was added and then transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and absorbace read at 520 nm in spectrophotometer against toluene blank.

Statistical analysis

All data were analyzed with SAS Institute Inc 6.12. All data were first analyzed by ANOVA to determine significant ($P \leq 0.05$) treatment effects. Significant differences between individual means were determined using Fisher's protected least significant difference (LSD) test. Data points in the figures represent the means ± SE of three independent experiments at least three replications per cultivar per treatment combination each.

RESULTS AND DISCUSSIONS

Proline, soluble carbohydrates and chlorophyll content

Osmotic adjustment by the Basil (*Ociumum basilicm* L.) with the accumulation of organic solutes might have occurred. A two-way ANOVA indicated a significant main effect of water stress ($P<0.001$) with the proline and soluble carbohydrate contents in plant leaves (Table-1).

Table-1. Results of two-way analysis of variance (ANOVA) of water stress (W) and plant growth promoting rhizobacteria (S) effects and their interaction (S×W) for the variables listed.

Dependent variable	Independent variable (Mean square)					
	Block	**W**	**E_a**	**S**	**S×W**	**E_b**
Proline	1.1251^{ns}	11.456^{**}	0.9956	13.703^{**}	7.7282^{**}	1.231
Soluble carbohydrate	0.0053^{ns}	0.4118^{**}	0.08318	0.1893^{**}	0.4547^{**}	0.0373
chlorophyll	8.626^{*}	13.131^{**}	1.797	5.0057^{*}	5.1362^{*}	2.263
Shoot Na^+	0.000114^{**}	0.0000328^{*}	0.0000025	0.0000123^{ns}	0.0000094^{ns}	0.0000095
Shoot K^+	4243.22^{**}	22060.1^{**}	22060.1	3974.9^{***}	3186.2^{***}	613.9

Number represents *F*-values at 5% level
^{ns} Non-Significant, * and ** significant at $P<0.05$ and $P<0.01$

This tended to occur regardless of bacterial strain. The water stress treatment caused a significant increase in the concentrations of proline and soluble carbohydrate in the leaves of plants in all comparisons (Figures 1 and 2). The greatest accumulations of proline (except in S_1 = *Pseudomonades* sp) and carbohydrate (except in S_2 = *Bacillus lentus*) were observed in W_3 (40% of the field capacity). Proline, sucrose, and other organic sugars in quinoa contribute to osmotic adjustment during stress and protect the structure of macromolecules and membranes during extreme dehydration [18]. Meloni *et al*., [19]

suggest that proline also serves: as an important source of nitrogen in plant metabolism, as a readily available source of energy, and as a reducing agent.

Interaction water stress and bacterial strain were found to be significant for proline and soluble carbohydrate (Table-1). This can be related to the evidence that the bacterial strain supplied to plants interacts with the water stress tolerance of the plants. In this study, water stress or different bacterial strain significantly affected proline and soluble carbohydrate accumulations; whereas, the proline of the S_1 = Pseudomonades sp plants increased significantly with an increase in water stress, along with higher soluble carbohydrate in S_2 = *Bacillus lentus* plants subjected to 60% of the field capacity (Figures 1 and 2).

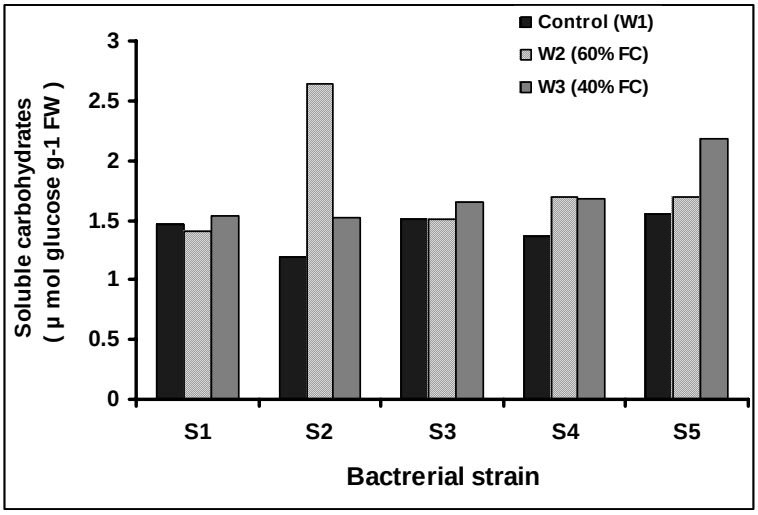

Figure-1. Effect of the water stress and bacterial strain on the soluble carbohydrate content in leaves.

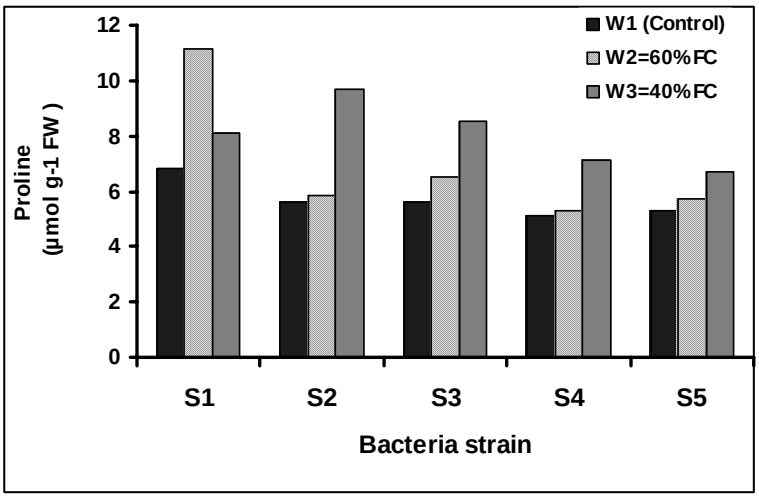

Figure-2. Effect of the water stress and bacterial strain on the proline content in leaves.

The mechanisms of PGPR action are not fully understood but are thought to include: a) the ability to produce plant hormones, such as auxins [20]; b) asymbiotic N_2 fixation [21]; c) solubilization of inorganic phosphate and mineralization of organic phosphate or other nutrients [22] and d) antagonism against phytopathogenic microorganisms by production of siderophores, the synthesis of antibiotics, enzymes or fungicidal compounds and competition with detrimental microorganisms [23].

Data presented in Table-1 showed the effect of water stress, bacterial strain and their interactions on the chlorophyll content in leaves. The obtained data revealed that the concentration of photosynthetic pigment i.e. total chl was higher in plants grown under 40% FC soil moisture level. Chlorophyll content was also increased significantly in all the bacterial strain treatments. Among the bacterial strain, the chlorophyll content of the S_1 = *Pseudomonades* sp and S_4 = combination of three bacterial

plants increased significantly with an increase in water stress (Figure-3).

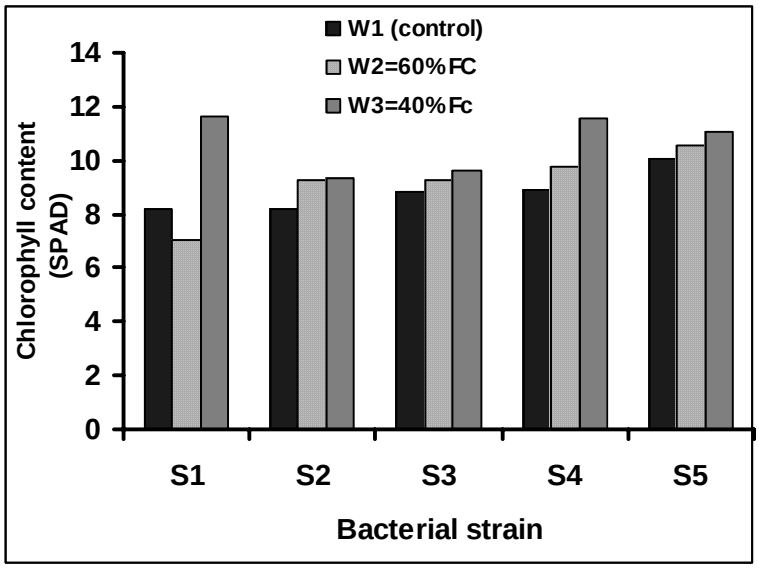

Figure-3. Effect of the water stress and bacterial strain on the chlorophyll content in leaves.

A similar result was reported by Vivas *et al*., [24] who showed that inoculation of bacterial strain increased stomatal conductance and chlorophyll content of lettuce compared to a non- drought control.

Mineral content

Growth and nutrient concentrations usually determine the performance of plants growing in any environment. The effects of water stress and bacterial strains on Na^+ and K^+ uptake per plant in Basil are shown in Table-1. Mineral uptake under water stress treatments in Basil was significantly decreased compared to the non-water stress treatment, interaction between water stress and bacterial strains was found only for potassium content. Treatment with bacterial strains in the non-water stress treatment increased K^+ and Na^+ uptake per plant in Basil (Figures 4 and 5). The average concentration of K^+ was significantly higher in S_2 and S_5 bacterial strains in the non-water stress (Figure-4).

Vivas *et al*., [24] 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-water stress control. Our results as presented above support this conclusion.

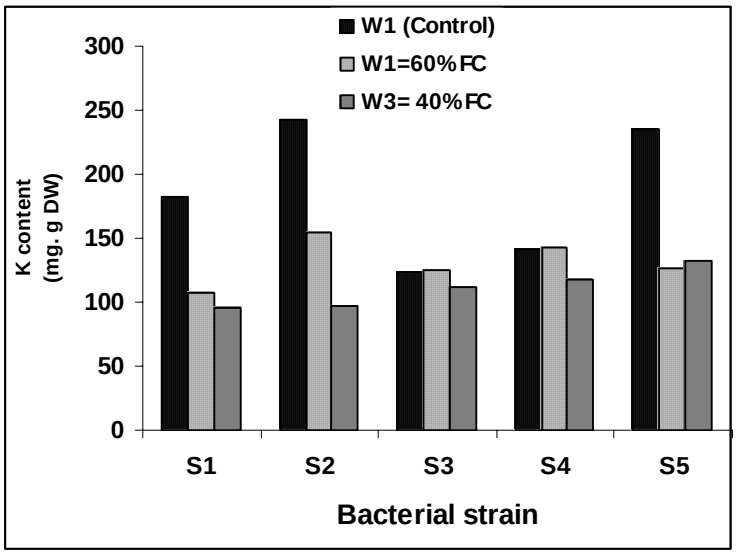

Figure-4. Effect of the water stress and bacterial strain on the potassium content in shoot.

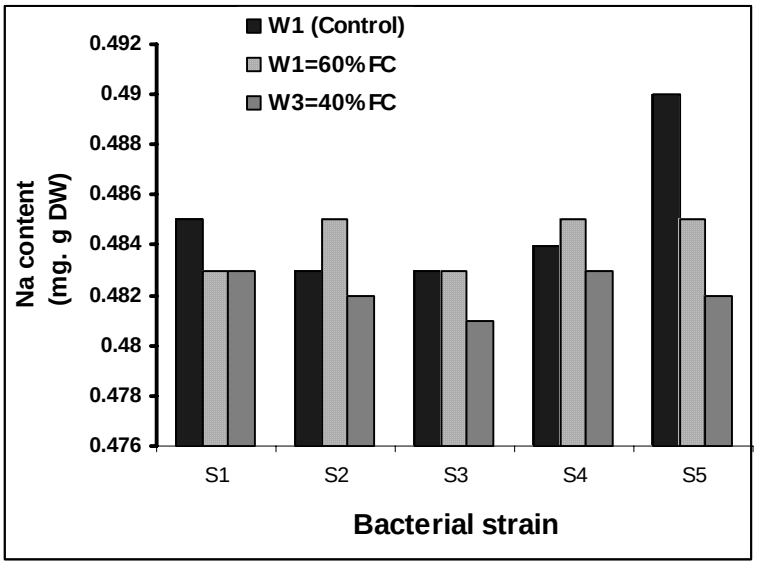

Figure-5. Effect of the water stress and bacterial strain on the sodium content in shoot.

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