



Biocompatibility Effect of *Bradyrhizobium japonicum* and *Trichoderma* Strains on Growth, Nodulation and Physiological Traits of Soybean (*Glycine max* L.) under Water Deficit Conditions

Sahar El-Nahrawy¹, Mohssen Elbagory^{1,2} and Alaa El-Dein Omara^{1*}

¹Department of Agricultural Microbiology, Soils, Water and Environment Research Institute, Agricultural Research Center (ARC), Giza 12112, Egypt.

²Department of Biology, Faculty of Science and Arts, King Khalid University, Mohail Assir, KSA.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMB/2020/v20i1130300

Editor(s):

(1) Dr. Veysi Okumus, Siirt University, Turkey.

Reviewers:

(1) Claudia Santos, Federal University of Mato Grosso (Universidade Federal de Mato Grosso), Brazil.

(2) Ngo Nkot Laurette, University of Douala, Cameroon.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/63849>

Original Research Article

Received 10 October 2020
Accepted 17 December 2020
Published 26 December 2020

ABSTRACT

In the future, drought are expected to increase, affecting the productivity of crops sensitive to water scarcity. Through N₂-fixation process, soybean is capable of achieving its nitrogen demands, however, this process is inhibited under drought stress conditions. Therefore, it is vital to find suitable solutions for the agricultural sustainability of soybean.

Under pot experiment, biocompatibility was studied between *Bradyrhizobium japonicum* and *Trichoderma* strains (*Trichoderma viride*, *T. harzianum* and *T. kongii*) for their ability to stimulate the growth, nodulation, N content and photosynthetic pigments of soybean plants under different irrigation intervals (every 2 days (I1), every 4 days (I2), and every 6 days (I3)). The experiment was conducted in summer 2020 with a split-plot randomized complete block design and six replicates. Among the *Trichoderma* strains, *T. harzianum* was the most tolerant to growth and auxin production in the maximum 25% PEG 6000 (poly ethylene glycol) concentration. Also, co-inoculation treatment (irrigation every 6 days and inoculation with *B. japonicum* + *T. harzianum*) recorded an increase rate

reached to 69.4% for shoot length, 102.53% for root length, 79.06% for shoot dry weight, 103.44% for root dry weight and 178.57% for N content compared to control treatment. For physiological traits (chlorophyll a, b, carotenoids and total soluble sugar), there was a significant increase was observed when soybean plants inoculated with *B. japonicum* + *T. harzianum* treatment followed by *B. japonicum* + *T. kongii* treatment, under irrigation every 6 days condition. On the contrary, a decrease was observed in proline content for the same treatments. Thus, an adequate microbial consortium of *Bradyrhizobium* - *Trichoderma*, could represent a promising practical method for increasing the productivity of soybean especially when grown under drought conditions.

Keywords: *Bradyrhizobium japonicum*; *Trichoderma*; soybean; irrigation intervals; physiological traits.

1. INTRODUCTION

In the agricultural sector, plants are often exposed to assorted environmental stresses that significantly affect their growth and productivity. Of these, drought stress is the basic cause of crop loss, detrimental to biomass and yield quality across the world. Traditional agricultural practices are significantly affected by drought due to the climate change, reduction of rainfall, and decrease in soil fertility [1].

To overcome the negative effect of drought stress, it is necessary to look for alternative ways to improve the soil fertility and stimulate the growth of plants. One of these, plant growth-promoting fungi (PGPF) are groups of beneficial microbes that deserve to be highlighted due to positive influences on soil structure and plant productivity [2]. Among the PGPF, the fungal genus *Trichoderma* has been known as eco-friendly biocontrol agent to control plant diseases, enhancing plant growth and also providing tolerance to environmental stresses [3,4]. Furthermore, *Trichoderma* spp. plays important role in releasing some metabolites analogous to phytohormones that enhance growth under drought stress. Due to previous positive effects, *T. harzianum* being the major species used in commercial formulations applied in agricultural areas around the world [5]. In addition, *Trichoderma* are presented in specialized literature as capable of solubilizing phosphates and other nutrients, mainly Fe, Mn, Cu and Zn [6,7], and being a growth and development inducer in important cultivated plants, as recorded in tomato plants inoculated with *T. harzianum* and *T. viride* [6,8], soybean plants inoculated with bradyrhizobia and *T. asperellum* [9], and cucumber plants inoculated with *T. asperellum* or *T. harzianum* [7].

Synergistic consortia of microbes with different metabolic capacities such as N₂ fixation, P-solubilization, and production of phytohormones

can definitely induce better plant development than a single inoculation [5,10]. Legumes are able to establish symbiotic relationships with N₂-fixing bacteria and the inoculation of legume seeds is an important agricultural practice worldwide [2]. Researchers often focus on associations of N₂-fixing bacteria with Plant Growth Promoting Bacteria (PGPB) [11], or Arbuscular Mycorrhizal Fungi (AMF) [12], to improve leguminous plant performance, and reports of N₂-fixing bacteria with PGPF, such as *Trichoderma*, are scarce. There are reports on the benefits of seed inoculation with bradyrhizobia-*Trichoderma* in common bean, soybean, pea, lentil, chickpea, pigeon pea, and clover [9,10,13-15].

In this study, three *Trichoderma* strains (*Trichoderma viride*, *T. harzianum* and *T. Kongii*) were assayed to determine their ability to growth and produce Indole Acetic Acid (IAA) under different concentrations of PEG 6000. Also, biocompatibility was studied between bradyrhizobia (*Bradyrhizobium japonicum* USDA 110) and *Trichoderma* strains to promote the growth and development of soybean plants under different irrigation intervals (every 2 days (I1), every 4 days (I2), and every 6 days (I3)). The results of this study may provide new insights into the combinations of bradyrhizobia and *Trichoderma* with plants, especially in leguminous plants under water deficit conditions.

2. MATERIALS AND METHODS

2.1 Microbial Strains and Growth Conditions

Trichoderma viride, *T. harzianum* and *T. kongii* as well as *Bradyrhizobium japonicum* USDA 110 strains were provided from Bacteriology Laboratory, Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt. Pure cultures of fungi and bacteria used were routinely maintained on Potato Dextrose Agar (PDA) medium [16], and

Yeast Extract Mannitol Agar (YEMA) medium [17], respectively.

2.2 Screening of Microbial Strains for Drought Stress Tolerance

According to [18], different *Trichoderma* and *Bradyrhizobium* strains were studied to determine their growth with different concentrations of polyethylene glycol 6000 (PEG 6000, Sigma Chemical Co., USA), i.e. 0, 5, 10, 15, 20 and 25%. *Trichoderma* strains were cultured in 250-ml Erlenmeyer flasks containing 100 ml of potato dextrose broth medium (PDB) supplemented with different concentration of PEG 6000 then inoculated with 0.5×10^6 spores mL^{-1} and incubated at 30°C for 7 days. On parallel, *B. japonicum* was cultured in 500-ml Erlenmeyer flasks containing 100 ml Yeast Extract Mannitol broth medium (YEMB) medium supplemented with different concentrations of PEG 6000 as mention above then inoculated at 1×10^8 CFU mL^{-1} and incubated at 30°C for 5 days. After incubation, growth was counted by using PDA and YEMA medium and expressed in terms of log₁₀ for *Trichoderma* and *Bradyrhizobium* strains, respectively [19].

2.3 Screening of Microbial Strains for Auxin Production under Drought Stress

The qualitative determination of IAA produced by *Trichoderma* and *Bradyrhizobium* strains was performed using the Salkowski-based colorimetric technique [20]. Under different concentrations of PEG 6000 as mention above, 0.5×10^6 spores mL^{-1} and 1×10^8 CFU mL^{-1} of *Trichoderma* and *Bradyrhizobium* strains were inoculated in 250-mL Erlenmeyer flasks containing 100 ml of PDB and YEMB medium supplied or not (control) with L-tryptophan (100 mg L^{-1}), respectively. The flasks were incubated in a shaker 150 rpm and 30°C for 7 days. Subsequently, 1 mL of centrifuged supernatant was mixed with 4 mL of Salkowski reagent (0.5 mM FeCl_3 and 35% HClO_4), and was left in the room temperature under dark conditions for 30 min. The amount of auxin produced by different microbial strains was read at 535 nm using UV/Visible Spectrophotometer (Bibby Scientific Ltd, Dunmow, Essex. UK, Model 6705). The amount of auxin was calculated against standard concentrations of indole acetic acid [21].

2.4 Experiment Preparation, Inoculation and Planting

The experiment was conducted during summer 2020 in a greenhouse of Bacteriology Laboratory, Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt. Soybean seeds (*Glycine max* L. cv. Giza 111) was obtained from Field Crops Research Institute, Department of Leguminous Crops, Sakha, Agricultural Research Station, Egypt, were previously disinfected with 70% (v/v) ethanol and 3% (v/v) sodium hypochlorite. Four seeds of soybean were sown in plastic pots (5 Kg) containing autoclaved (121 °C; 1.5 Pa; 4 h) loamy soil and simultaneously inoculated with 1.0 mL of bradyrhizobia inoculant (1×10^9 CFU mL^{-1}) or co-inoculated with 1.0 mL of bradyrhizobia inoculant + 1.0 mL of fungal inoculant (1×10^5 spores mL^{-1}) under three irrigation intervals (every 2 (I1), 4 (I2), and 6 (I3) days after 15 days from sowing.

The physicochemical and biological properties of the soil used are: pH, 7.31; EC, 1.77 dSm^{-1} ; organic matter (%), 1.28; particle size distribution sand, silt and clay (%), 47.00, 35.50 and 17.50, respectively; available N (mg Kg^{-1}), 17.95; available P (mg Kg^{-1}), 9.77; available K (mg Kg^{-1}), 321.3. Also, total count of bacteria, 19×10^7 CFU g^{-1} ; total count of fungi, 82×10^4 CFU g^{-1} and total count of actinomycetes 41×10^5 CFU g^{-1} according to [22].

On the seventh day after sowing, two plants remained in each pot. The experiment was conducted as a split-plot randomized complete block design with six replicates as shown in Table 1.

2.5 Measurements

At 60 days after sowing, the shoot and root length were measured with the aid of a measuring tape. Shoot, root and nodules dry weight were determined after drying in a forced aeration oven at 65°C until a constant weight. The nitrogen content in the shoot dry weight was determined by the Kjeldahl method [23].

The content of chlorophyll a, chlorophyll b and carotenoids was determined after extraction of fresh leaf samples with 80% ethanol [24]. The values of chlorophyll a and b were used to determine the total chlorophyll.

Table 1. Treatment used for greenhouse experiment

Main plot (Irrigation intervals)	Treatment
I1	Irrigation every 2 days
I2	Irrigation every 4 days
I3	Irrigation every 6 days
Sub main plot (Inoculation treatments)	
T1	Control
T2	Inoculation with <i>B. japonicum</i> USDA 110
T3	Inoculation with <i>T. viride</i>
T4	Inoculation with <i>T. harzianum</i>
T5	Inoculation with <i>T. Kongii</i>
T6	Inoculation with <i>B. japonicum</i> USDA 110 + <i>T. viride</i>
T7	Inoculation with <i>B. japonicum</i> USDA 110 + <i>T. harzianum</i>
T8	Inoculation with <i>B. japonicum</i> USDA 110 + <i>T. Kongii</i>

Free proline content in the leaves of soybean plants was determined following the method of Bates [25]. Leaf samples 0.5 g were homogenized in 5 mL of sulphosalicylic acid (3%) using mortar and pestle. About 2 mL of extract was taken in test tube and to it 2 mL of glacial acetic acid and 2 ml of ninhydrin reagent were added. The reaction mixture was boiled in water bath at 100°C for 60 min. 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 absorbance read at 520 nm in spectrophotometer against toluene blank. Proline concentration was determined using a calibration curve and expressed as $\mu\text{ mol proline g}^{-1}\text{ FW}$.

Total soluble sugar was estimated using anthrone reagent [26]. Briefly, 0.1 mL of alcoholic leaf extract (80% ethanol) was added to 3 mL freshly prepared anthrone reagent and mixed by vortexing then boiling in a water bath for 10 min, and measured using UV/Visible Spectrophotometer (Bibby Scientific Ltd, Dunmow, Essex, UK, Model 6705) at 620 nm. A calibration curve of glucose was used to quantify soluble sugar in plant samples.

2.6 Statistical Analysis

Data in the present study are represented as mean \pm SD (standard deviation) for screening of microbial strains to growth and auxin production. Further, the analysis of data for various growth parameters in the plant growth experiment was done by analysis of variance (ANOVA), software SPSS 14.0 for windows and the significant differences between the treatments were analyzed by Duncan's multiple range test ($P \leq 0.05$).

3. RESULTS

3.1 Screening of Microbial Strains for Drought Stress Tolerance

Growing the investigated strains of *B. japonicum* and *Trichoderma* strains (*T. viride*; *T. harzianum* and *T. kongii*) on YEM and PDB medium supplemented with PEG 6000 at the levels of 5 to 25%, showed a marked variation among these strains (Table 2). Generally, all tested strains were able to grow in PEG 6000 concentrations at up to 25%. Also, after incubation time for 5 days for *B. japonicum* and 7 days for *Trichoderma* strains, viable cell numbers showed a decrease in the growth with increasing PEG concentrations. It was noticed that *B. japonicum* recorded 3.89 log number at 25% compared to other concentrations of PEG 6000. Whereas, *T. harzianum* was the most tolerant to higher applied PEG 6000 concentrations compared to the other *Trichoderma* strains which recorded 4.54 and 3.09 log number at 20 and 25%, respectively.

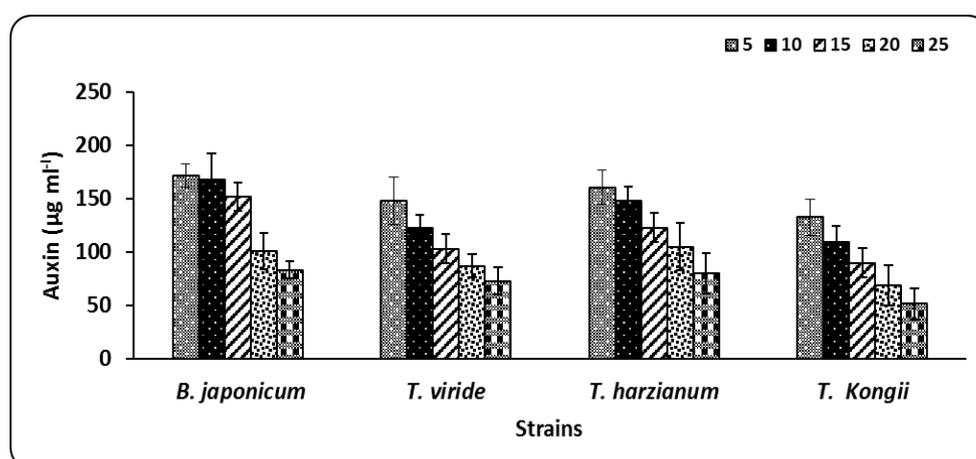
3.2 Auxin Production by Microbial Strains under Drought Stress

To determine the ability of studied microbial strains to assist plant to tolerant drought stress, auxin production assay was carried out. Pink color formed was observed in *B. japonicum* and *Trichoderma* strains (*T. viride*; *T. harzianum* and *T. Kongii*) indicating that these strains were able to produce auxin under different concentrations of PEG 6000 (Fig. 1). Among all *Trichoderma* strains, *T. harzianum* attained 160.32, 148.21, 122.77, 105.00 and 80.26 $\mu\text{g mL}^{-1}$ at 5, 10, 15, 20 and 25% PEG 6000, respectively. But, *B. japonicum* attained 171.89 $\mu\text{g mL}^{-1}$ at the lowest concentration (5%) and 83.23 $\mu\text{g mL}^{-1}$ at the highest concentration (25%).

Table 2. Effect of different concentration of PEG 6000 on growth CFU (Log 10) mL⁻¹ of *B. japonicum* and *Trichoderma* strains

Conc. of PEG (%) in the medium	<i>B. japonicum</i>	<i>Trichoderma viride</i>	<i>Trichoderma harzianum</i>	<i>Trichoderma kongii</i>
0 (control)	7.52 ± 0.51	4.55 ± 0.22	5.01 ± 0.38	4.75 ± 0.18
5	7.73 ± 0.67	7.79 ± 0.32	6.97 ± 0.36	6.49 ± 0.27
10	7.34 ± 0.34	7.93 ± 0.28	7.83 ± 0.30	7.23 ± 0.15
15	5.96 ± 0.37	5.11 ± 0.21	4.37 ± 0.42	4.01 ± 0.19
20	4.32 ± 0.45	3.18 ± 0.36	4.54 ± 0.47	3.88 ± 0.32
25	3.89 ± 0.38	2.08 ± 0.13	3.09 ± 0.29	1.42 ± 0.25

Values are presented as the mean ± SD with n = 3.

**Fig. 1. Auxin production by *B. japonicum* and *Trichoderma* strains under different concentration of PEG 6000 (5, 10, 15, 20 and 25 %)**

Values are presented as the mean ± SD with n = 3

3.3 Promotion of Soybean Growth

3.3.1 Shoot length, root length, shoot dry weight and root dry weight

The influence of single and co- inoculation with *B. japonicum* and *Trichoderma* strains on plant growth promotion of soybean (Giza 111) were evaluated under different irrigation intervals every 2, 4 and 6 days (Table 3 and Fig. 2). After 60 days from sowing, results showed that shoot length, root length, shoot dry weight and root dry weight were significantly increased in the case of co-inoculation treatments compare to single inoculation treatments under water stress (Table 3). A decrease in the growth promotion of soybean plants was observed with increasing of irrigation intervals (main plot treatments). But, for sub main treatments, significant effect was observed when soybean seeds inoculated with *B. japonicum* + *T. harzianum* which attained 32.54 cm; 12.39 cm; 5.30 g and 1.29 g for shoot length, root length, shoot dry weight and root dry weight compared to other inoculation treatments, respectively (Table 3).

The interaction effect between the different irrigation intervals and the inoculation treatments showed significant effects for shoot length, root length, shoot dry weight and root dry weight of soybean plants (Fig. 2). At 60 days, treatment T7 (irrigation every 6 days and inoculation with *B. japonicum* + *T. harzianum*) recorded high values 30.43 cm, 11.20 cm, 4.96 g and 1.18 g for shoot length, root length, shoot dry weight and root dry weight compared to other treatments under the same conditions, respectively.

3.3.2 Nodules dry weight and nitrogen content

After 60 days from sowing, results of single and/or dual inoculation with *B. japonicum* and *Trichoderma* strains on nodules dry weight (mg plant⁻¹), and nitrogen content (mg plant⁻¹) of soybean plants grown under different irrigation intervals are presented in (Tables 4, 5 and Fig. 3). For irrigation intervals, a decrease in nodules dry weight and nitrogen content of soybean plants was observed with increasing of irrigation periods. Nodules dry weight increased due to

positive inoculation, therefore, nodule dry weight (177.81 mg plant⁻¹), showed significant increase in seeds inoculation of *B. japonicum* + *T. harzianum*, followed by *B. japonicum* + *T. kongii* (165.39 mg plant⁻¹), and *B. japonicum* (158.89 mg plant⁻¹), over un-inoculated control. Similar trend was also exhibited in nitrogen content (Table 4). Our findings for the interaction effect indicated that there was a statistically significant positive relationship ($p \leq 0.05$) between irrigation intervals and inoculation treatments for nodules dry weight (Table 5). Data showed that an

increase in nodules dry weight was observed with *B. japonicum* + *T. harzianum* treatment resulted 194.77, 182.00 and 156.66 mg plant⁻¹ followed by *B. japonicum* + *T. kongii* treatments resulted 181.84, 168.66 and 145.66 mg plant⁻¹ for 2, 4 and 6 days (irrigation intervals), respectively. Regarding nitrogen content, there was an increase with co-inoculation treatment which recorded high values 39.00, 35.33 and 34.66 mg plant⁻¹ for *B. japonicum* + *T. harzianum*, *B. japonicum* + *T. kongii* and *B. japonicum* + *T. viride*, respectively (Table 5).

Table 3. Effect of different irrigation intervals and different inoculation with *B. japonicum* and *Trichoderma* strains on shoot length (cm), root length (cm), shoot dry weight (g) and root dry weight (g) of soybean plants

Treatment	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
Main plot (Irrigation intervals)				
I1	30.31 a	11.03 a	4.91 a	1.12 a
I2	28.07 b	9.62 b	4.56 b	1.01 b
I3	26.05 c	8.56 c	4.21 c	0.89 c
F. test	**	**	**	**
Sub main plot (Inoculation)				
Control	20.11 h	6.65 g	3.21 e	0.68 g
<i>B. japonicum</i>	30.81 c	10.92 c	5.00 b	1.12 c
<i>T. viride</i>	25.79 g	8.09 e	4.18 d	0.85 e
<i>T. harzianum</i>	27.85 d	8.60 d	4.53 c	0.89 d
<i>T. Kongii</i>	25.11 e	7.66 f	4.06 d	0.79 f
<i>B. japonicum</i> + <i>T. viride</i>	31.71 b	11.73 b	5.13 b	1.21 b
<i>B. japonicum</i> + <i>T. harzianum</i>	32.54 a	12.39 a	5.30 a	1.29 a
<i>B. japonicum</i> + <i>T. Kongii</i>	31.22 bc	11.85 b	5.08 b	1.23 b
F. test	**	**	**	**

I1 (every 2 day), I2 (every 4 day), and I3 (every 6 day); Mean values are significant at $P \leq 0.05$

Table 4. Effect of different irrigation intervals and different inoculation with *B. japonicum* and *Trichoderma* strains on nodules dry weight (mg plant⁻¹) and nitrogen content (mg plant⁻¹) of soybean plants

Treatment	Nodules dry weight (mg plant ⁻¹)	Nitrogen content (mg plant ⁻¹)
Main plot (Irrigation intervals)		
I1	90.51 a	34.89 a
I2	84.12 b	30.04 b
I3	72.04 c	25.20 c
F. test	**	**
Sub main plot (Inoculation)		
Control	0.0 d	18.97 f
<i>B. japonicum</i>	158.89 c	34.83 c
<i>T. viride</i>	0.0 d	20.58 e
<i>T. harzianum</i>	0.0 d	22.05 d
<i>T. Kongii</i>	0.0 d	19.77 ef
<i>B. japonicum</i> + <i>T. viride</i>	155.71 c	40.67 b
<i>B. japonicum</i> + <i>T. harzianum</i>	177.81 a	43.64 a
<i>B. japonicum</i> + <i>T. Kongii</i>	165.39 b	39.83 b
F. test	**	**

I1 (every 2 day), I2 (every 4 day), and I3 (every 6 day); Mean values are significant at $P \leq 0.05$

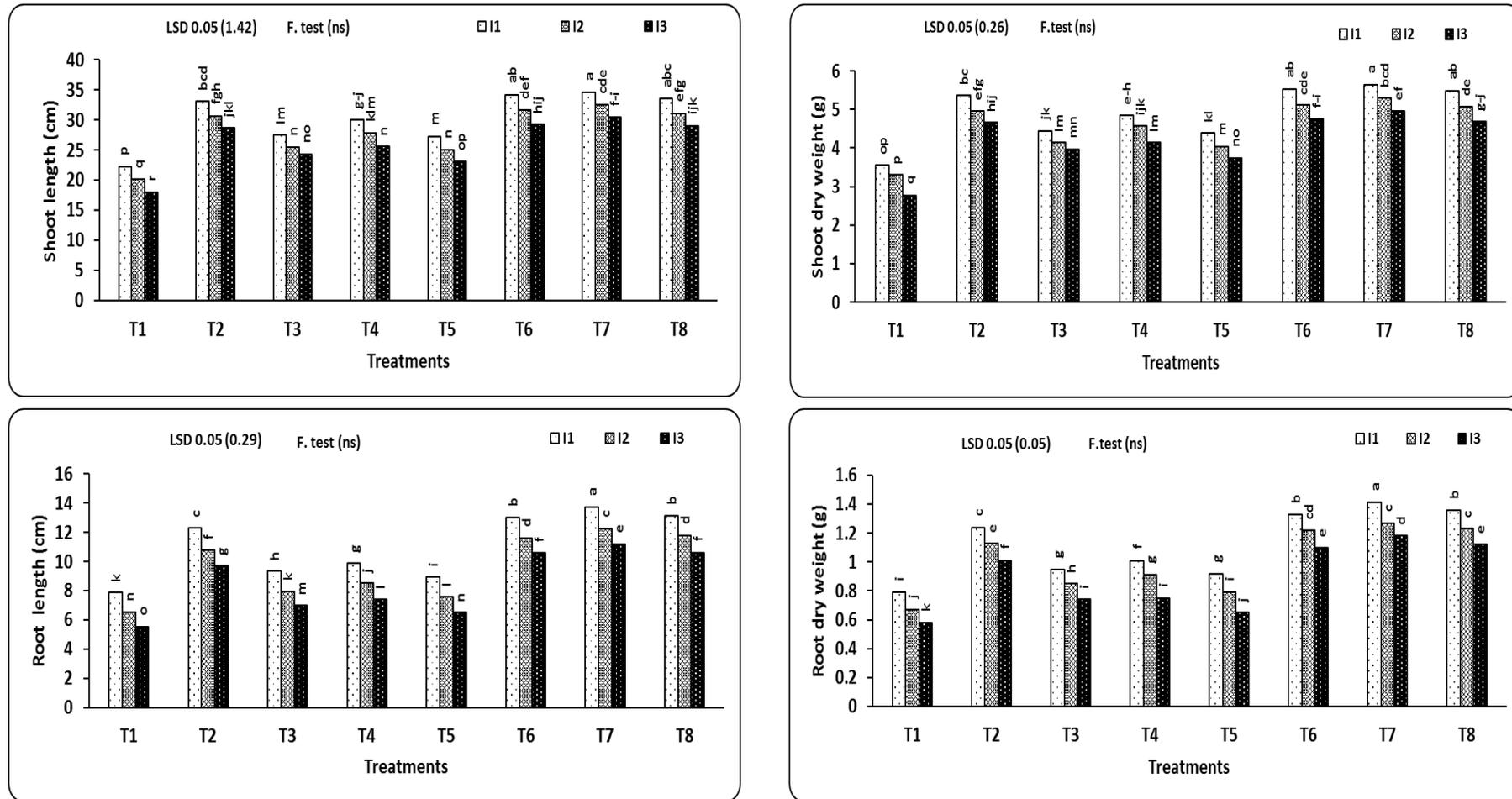


Fig. 2. Interaction effect between different irrigation intervals and different inoculation with *B. japonicum* and *Trichoderma* strains on shoot length (cm), root length (cm), shoot dry weight (g) and root dry weight (g) of soybean plants. T1: Control; T2: *B. japonicum*; T3: *T. viride*; T4: *T. harzianum*; T5: *T. Kongii*; T6: *B. japonicum* + *T. viride*; T7: *B. japonicum* + *T. harzianum*; and T8: *B. japonicum* + *T. Kongii*. I1 (every 2 day), I2 (every 4 day), and I3 (every 6 day); Mean values are significant at $P \leq 0.05$

Table 5. Effect of the interaction between different irrigation intervals and different inoculation with *B. japonicum* and *Trichoderma* strains on nodules dry weight (mg plant⁻¹) and nitrogen content (mg plant⁻¹) of soybean plants

Inoculation	Irrigation intervals		
	I1	I2	I3
Nodules dry weight (mg plant⁻¹)			
Control	0.00 i	0.00 i	0.00 i
<i>B. japonicum</i>	175.00 c	163.00 e	138.66 h
<i>T. viride</i>	0.00 i	0.00 i	0.00 i
<i>T. harzianum</i>	0.00 i	0.00 i	0.00 i
<i>T. Kongii</i>	0.00 i	0.00 i	0.00 i
<i>B. japonicum</i> + <i>T. viride</i>	172.48 cd	159.33 ef	135.33 h
<i>B. japonicum</i> + <i>T. harzianum</i>	194.77 a	182.00 b	156.66 f
<i>B. japonicum</i> + <i>T. Kongii</i>	181.84 b	168.66 d	145.66 g
F. test	**	**	**
Nitrogen content (mg plant⁻¹)			
Control	23.91 j	19.00 m	14.00 p
<i>B. japonicum</i>	39.83 de	34.66 f	30.00 g
<i>T. viride</i>	25.41 hi	20.66 kl	15.66 o
<i>T. harzianum</i>	26.84 h	22.00 k	17.33 n
<i>T. Kongii</i>	24.31 ij	19.33 lm	15.66 o
<i>B. japonicum</i> + <i>T. viride</i>	46.36 b	41.00 d	34.66 f
<i>B. japonicum</i> + <i>T. harzianum</i>	47.92 a	44.00 c	39.00 e
<i>B. japonicum</i> + <i>T. Kongii</i>	44.51 c	39.66 de	35.33 f
F. test	NS	NS	NS

I1 (every 2 day), I2 (every 4 day), and I3 (every 6 day); Mean values are significant at $P \leq 0.05$



Fig. 3. Nodules formed on soybean plant root inoculated by *B. japonicum* and *Trichoderma* strains under irrigation intervals stress (every 6 days), a: control; b: *B. japonicum*; c: *B. japonicum* + *T. viride*; d: *B. japonicum* + *T. harzianum* and e: *B. japonicum* + *T. Kongii*

3.3.3 Pigments content

There was a significant influence between irrigation intervals (main plot) and inoculation treatments (sub plot) for pigments content of soybean leaves (chlorophyll a, b and total chlorophyll as well as carotenoids). Among irrigation intervals (I₁, I₂ and I₃), the findings showed that the highest values were obtained from soybean plants treated with irrigation every 2 days (I₁) which recorded 1.19, 0.67, 1.86 and 0.54 mg g⁻¹ FW, while the lowest ones

were obtained from soybean plants treated with irrigation every 6 days (I₃) which recorded 1.06, 0.57, 1.63 and 0.48 mg g⁻¹ FW for chlorophyll a, b and total as well as carotenoids, respectively (Table 6). Consequently, the combination with *B. japonicum* + *T. harzianum* treatment resulted an increase in pigments content recording 1.38, 0.84, 2.23 and 0.65 mg g⁻¹ FW for chlorophyll a, b and total as well as carotenoids, compared to other inoculation treatments under study, respectively (Table 6).

In addition, there was a significant interaction ($P \leq 0.05$) of irrigation intervals with inoculation treatments (Fig. 3). Under soybean plants treated with irrigation every 6 days (I₃), it was observed that *B. japonicum* + *T. harzianum* treatment (T7) resulted 1.45, 0.82, 2.17 and 0.63 mg g⁻¹ FW followed by *B. japonicum* + *T. kongii* treatments (T8) resulted 1.29, 0.79, 2.08 and 0.60 mg g⁻¹ FW for chlorophyll a, b and total as well as carotenoids, compared to other inoculation treatments under study, respectively (Fig. 4).

3.3.4 Proline and total soluble sugar content

Soybean plants exposed to irrigation intervals and treated with single or dual inoculation treatments were significantly differed ($p \leq 0.05$) in proline and total soluble sugar (TSS) content (Tables 7, 8).

Proline content under different irrigation intervals (every 2 days (I₁), 4 days (I₂), and 6 days (I₃)) was increase 5.99, 6.52 and 6.76 $\mu\text{mol g}^{-1}$ FW, while TSS decreased 3.43, 3.28 and 2.96 $\mu\text{g g}^{-1}$ FW, respectively. Also, proline content decreased due to positive inoculation by *B. japonicum* + *T. harzianum* recording 3.66 $\mu\text{mol g}^{-1}$ FW. On the contrary, led to an increase in TSS recording 4.02 $\mu\text{g g}^{-1}$ FW, compared to other inoculation treatments (Table 7).

For interaction effect, inoculation treatment by *B. japonicum* + *T. harzianum* recorded low values 2.62, 3.82 and 4.55 $\mu\text{mol g}^{-1}$ FW for proline content, however, recorded high values 4.18, 4.05 and 3.83 $\mu\text{g g}^{-1}$ FW for TSS content at different irrigation intervals 2, 4 and 6 days, compared to control and other inoculation under study, respectively (Table 8).

4. DISCUSSION

The beneficial plant-microbe interactions in the rhizosphere are important for maintaining plant health and soil fertility [14]. One of these microbes is fungi which have been widely used not only in biocontrol of plant pathogens and enhancing plant growth but also providing tolerance to environmental stresses [27].

In our study, screening of microbial strains was accomplished on the basis of their viable cell numbers and auxin production. Viable cell numbers of the tested strains showed survived poorly in the medium with increasing PEG concentrations (Table 2). Also, all strains were able to grow in all tested PEG concentrations at up to 25%. Therefore, *T. harzianum* was the most tolerant to higher applied PEG 6000 concentrations compared to the other *Trichoderma* strains and these results may be due to an alteration of synthesis patterns of lipopolysaccharides and protein subjected to adaptation of drought stress [28-30].

Table 6. Effect of different irrigation intervals and different inoculation with *B. japonicum* and *Trichoderma* strains on Chlorophyll a, Chlorophyll b; Total chlorophyll and carotenoid of soybean plants

Treatment	Ch a	Ch b	Total Ch	Caro.
	mg g ⁻¹ FW			
Main plot (Irrigation intervals)				
I1	1.19 a	0.67 a	1.86 a	0.54 a
I2	1.13 b	0.64 b	1.77 b	0.51 b
I3	1.06 c	0.57 c	1.63 c	0.48 c
F. test	**	**	**	**
Sub main plot (Inoculation)				
Control	0.88 f	0.42 f	1.30 f	0.38 e
<i>B. japonicum</i>	1.17 c	0.68 c	1.86 c	0.55 c
<i>T. viride</i>	0.98 d	0.49 d	1.48 d	0.43 d
<i>T. harzianum</i>	1.01 d	0.51 d	1.52 d	0.44 d
<i>T. Kongii</i>	0.92 e	0.44 e	1.37 e	0.40 e
<i>B. japonicum</i> + <i>T. viride</i>	1.28 b	0.78 b	2.06 b	0.59 b
<i>B. japonicum</i> + <i>T. harzianum</i>	1.38 a	0.84 a	2.23 a	0.65 a
<i>B. japonicum</i> + <i>T. Kongii</i>	1.35 a	0.84 a	2.20 a	0.64 a
F. test	**	**	**	**

Ch a: Chlorophyll a; Ch b: Chlorophyll b; Total Ch: Total chlorophyll; Caro.: carotenoid; I1 (every 2 day), I2 (every 4 day), and I3 (every 6 day); Mean values are significant at $P \leq 0.05$

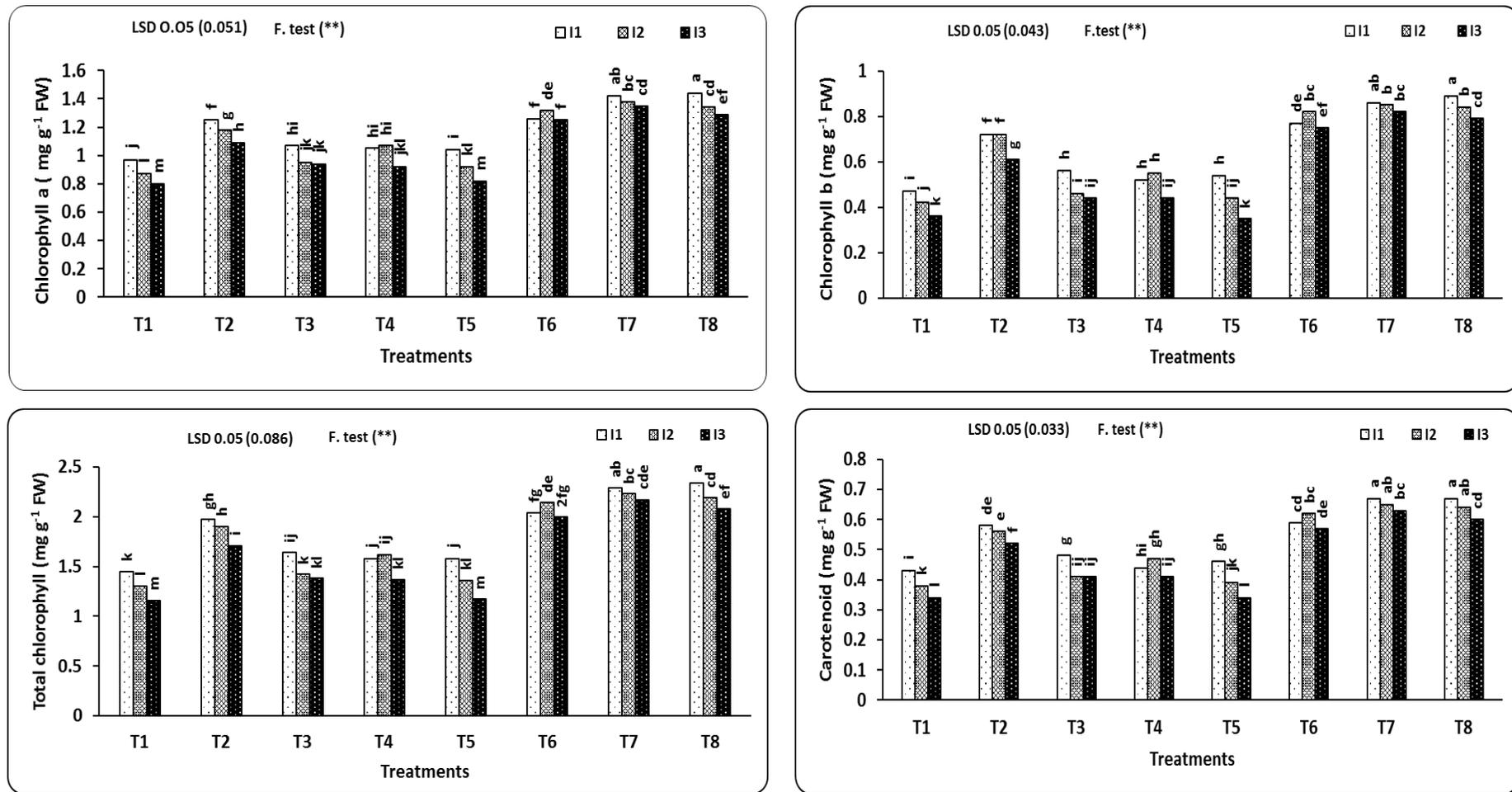


Fig. 4. Interaction effect between different irrigation intervals and different inoculation with *B. japonicum* and *Trichoderma* strains on Chlorophyll a, Chlorophyll b; Total chlorophyll and carotenoid (mg g⁻¹ FW) of soybean plants. T1: Control; T2: *B. japonicum*; T3: *T. viride*; T4: *T. harzianum*; T5: *T. Kongii*; T6: *B. japonicum* + *T. viride*; T7: *B. japonicum* + *T. harzianum*; and T8: *B. japonicum* + *T. Kongii*. I1 (every 2 day), I2 (every 4 day), and I3 (every 6 day); Mean values are significant at P ≤ 0.05

Table 7. Effect of different irrigation intervals and different inoculation with *B. japonicum* and *Trichoderma* strains on proline and total soluble sugar content of soybean plants

Treatment	Proline ($\mu\text{ mol g}^{-1}\text{ FW}$)	Total soluble sugar ($\mu\text{g g}^{-1}\text{ FW}$)
Main plot (Irrigation intervals)		
I1	5.99 c	3.43 a
I2	6.52 b	3.28 b
I3	6.76 a	2.96 c
F. test	**	**
Sub main plot (Inoculation)		
Control	7.97 a	1.82 f
<i>B. japonicum</i>	5.84 e	3.41 c
<i>T. viride</i>	7.62 b	2.90 e
<i>T. harzianum</i>	6.37 d	3.25 d
<i>T. Kongii</i>	7.46 b	2.80 e
<i>B. japonicum</i> + <i>T. viride</i>	6.78 d	3.81 b
<i>B. japonicum</i> + <i>T. harzianum</i>	3.66 f	4.02 a
<i>B. japonicum</i> + <i>T. Kongii</i>	5.70 e	3.77 b
F. test	**	**

I1 (every 2 day), I2 (every 4 day), and I3 (every 6 day); Mean values are significant at $P \leq 0.05$

Table 8. Effect of the interaction between different irrigation intervals and different inoculation with *B. japonicum* and *Trichoderma* strains on proline and total soluble sugar content of soybean plants

Inoculation	Irrigation intervals		
	I1	I2	I3
Proline ($\mu\text{ mol g}^{-1}\text{ FW}$)			
Control	7.56 cde	8.06 abc	8.29 a
<i>B. japonicum</i>	5.44 ij	5.74 i	6.33 g
<i>T. viride</i>	8.18 ab	7.69 bcd	7.00 f
<i>T. harzianum</i>	5.81 hi	6.64 fg	6.65 fg
<i>T. Kongii</i>	6.89 f	7.54 de	7.95 a-d
<i>B. japonicum</i> + <i>T. viride</i>	6.30 gh	7.11 ef	6.94 f
<i>B. japonicum</i> + <i>T. harzianum</i>	2.62 m	3.82 l	4.55 k
<i>B. japonicum</i> + <i>T. Kongii</i>	5.17 j	5.54 ij	6.38 g
F. test	**	**	**
Total soluble sugar ($\mu\text{g g}^{-1}\text{ FW}$)			
Control	2.25 m	1.91 n	1.30 o
<i>B. japonicum</i>	3.18 gh	3.58 e	3.47 ef
<i>T. viride</i>	3.16 gh	2.87 jk	2.68 kl
<i>T. harzianum</i>	3.49 ef	3.35 fg	2.92 ij
<i>T. Kongii</i>	3.11 hi	2.77 jk	2.53 l
<i>B. japonicum</i> + <i>T. viride</i>	4.07 ab	3.87 bcd	3.51 ef
<i>B. japonicum</i> + <i>T. harzianum</i>	4.18 a	4.05 abc	3.83 d
<i>B. japonicum</i> + <i>T. Kongii</i>	4.00 a-d	3.86 cd	3.47 ef
F. test	**	**	**

I1 (every 2 day), I2 (every 4 day), and I3 (every 6 day); Mean values are significant at $P \leq 0.05$

Among all *Trichoderma* strains, *T. harzianum* attained 160.32, 148.21, 122.77, 105.00 and 80.26 $\mu\text{g mL}^{-1}$ at 5, 10, 15, 20 and 25% PEG 6000, respectively. But, *B. japonicum* attained 171.89 $\mu\text{g mL}^{-1}$ at the lowest concentration (5%) and 83.23 $\mu\text{g mL}^{-1}$ at the highest concentration (25%) for auxin production (Fig. 1). This observation indicates superior performance of

B. japonicum with *T. harzianum* in auxin production and may be contribute to improved nutrition of plants growing under drought conditions [4,31-33].

Our findings suggest that inoculation in combination with *B. japonicum* and *T. harzianum* may help reducing detrimental effects of drought

in soybean plants. Herein, the length and dry weight of shoot and root decreased remarkably under drought stress (Table 3 and Fig. 2), and these results found compatible with [34], dissecting in tomato plants. Similarly, Hassan [35], also suggested that *Trichoderma* spp. significantly increased height, root length and root dry weight in millet plant. Also, significant increases in height; stem diameter; dry weight of shoots and roots of cowpea plants due to co-inoculated with *Bradyrhizobium* and *T. asperelloides* T02 [4]. Therefore, these results may be featured to varied growth support mechanisms; like upgraded nutrient bio-availability through solubilization and chelation of minerals and boosted nutrient uptake efficacy [36]. Additionally, phytohormones such as auxin and gibberellin are generated in *Trichoderma* exposed plants and these molecules have the capability of strengthening the development of the plants under harsh conditions [37].

On the other hand, the formation of nodules and N₂ fixation depends on species of bradyrhizobia and their susceptibility to the detrimental effects of drought. For example, slow-growing rhizobia generally thought to survive desiccation better than fast-growing rhizobia [38]. Furthermore, rhizobia from wild legumes of semiarid land displayed a variable growth pattern, production of extracellular polysaccharide (EPS), IAA, siderophores, and phosphate solubilization activity [39]. So far, the effect of water stress on symbiosis is the concern; it affects nodule establishment, C and N metabolism, nodule O₂ permeability, nitrogenase activity, and total plant N₂ fixation ability [40,41]. From our results, soybean seeds inoculation with *B. japonicum* + *T. harzianum*, showed significant increase in nodule dry weight and nitrogen content (Tables 4, 5). There are several reports on the benefits of co-inoculation with bradyrhizobia and *Trichoderma*. Mendes [4], who reported that cowpea plants inoculated with bradyrhizobia and *T. asperelloides* T02 displayed significant increases in nodule dry weight (63%) when compared to cowpea plants inoculated only with bradyrhizobia.

Another plant response to drought stress is changes in photosynthetic pigment contents. It is well known that drought stress suppresses the pigments in the photosynthetic apparatus in an array of plant species. In parallel, the values represented in Table 6 and Fig. 4 show that chlorophyll and carotenoid synthesis were

negatively affected due to the drought. Loss of chlorophyll content under irrigation intervals is known a primary cause of inactivation of photosynthesis [42]. Therefore, carotenoids support photo-protection of chlorophyll molecules and possess an antioxidant effect by scavenging reactive oxygen species (ROS) [43]. In our results, root colonization with *B. japonicum* + *T. harzianum* alleviates the drought stress caused affects by improving pigment contents in soybean. This result overlaps with the reports of [44], who suggest increased chlorophyll content in the drought tolerant *T. hamatum* DIS 219b colonized seedlings. Additionally, Harman [45], reported that *T. harzianum* strain T22 raised leaf greenness in maize, which improves the vigor with plenty of carbon source for plant development. Also, Mendes [4], showed that the maximum chlorophyll a and b were recorded in the cowpea plants co-inoculated with bradyrhizobia and *T. asperelloides* T02, and this parameter was 40 and 33% higher compared to cowpea inoculated only with bradyrhizobia , respectively.

Soybean plants exposed to irrigation intervals (I1, I2 and I3) and treated with *B. japonicum* + *T. harzianum* was significantly differed in proline and total soluble sugar content (Tables 7, 8). Indeed, it is believed that *Trichoderma* spp. has capacity to extract more water from the rhizosphere. In a similar research, it was shown that symbiotic plants spend significantly less water than non-symbiotic plants [27]. Martínez-Medina [46], demonstrated that *T. hamatum* boosts nutrient uptake and takes water from deeper soil to increase water potential. From our results, *B. japonicum* + *T. harzianum* treatment may easily help plant to reduce the proline content that accumulates under water deficit conditions resulting in osmotic adjustment, free radical scavenging and stabilization of subcellular structures in plant cells and mitigation the harmful impacts of water deficit [47,48].

5. CONCLUSION

Under water deficit conditions, the study showed that *Trichoderma* promotes positive effects on soybean nodulated by *Bradyrhizobium* and acts as stimulators of plant growth and development vegetative growth, N content and photosynthetic pigments. Thus, an adequate microbial consortium of *Bradyrhizobium* - *Trichoderma*, like *T. harzianum*, could represent a promising practical method for increasing the productivity of soybean and other agronomically important

legumes, especially when grown under water deficit conditions.

ACKNOWLEDGEMENT

Thanks to all staff members and colleagues in The Bacteriology Research Laboratory, Sakha Agricultural Research Station, Kafr El-Sheikh, Egypt for their valuable cooperation which made completion of this work possible.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- Saba H, Vibhash D, Manisha M, Prashant KS, Farhan H, Tauseef A. *Trichoderma*—a promising plant growth stimulator and biocontrol agent. *Mycosphere*. 2012;1, 3(4):524-31.
- Figueiredo MD, Bonifacio A, Rodrigues AC, de Araujo FF. Plant growth-promoting rhizobacteria: Key mechanisms of action. In *Microbial-mediated Induced Systemic Resistance in Plants*. 2016;23-37.
- Guler NS, Pehlivan N, Karaoglu SA, Guzel S, Bozdeveci A. *Trichoderma atroviride* ID20G inoculation ameliorates drought stress-induced damages by improving antioxidant defence in maize seedlings. *Acta Physiologiae Plantarum*. 2016;1, 38(6):132.
- Mendes JB, da Costa Neto VP, de Sousa CD, de Carvalho Filho MR, Rodrigues AC, Bonifacio A. *Trichoderma* and bradyrhizobia act synergistically and enhance the growth rate, biomass and photosynthetic pigments of cowpea (*Vigna unguiculata*) grown in controlled conditions. *Symbiosis*. 2020;3:1-1.
- Rubio MB, Hermosa R, Vicente R, Gómez-Acosta FA, Morcuende R, Monte E, Bettiol W. The combination of *Trichoderma harzianum* and chemical fertilization leads to the deregulation of phytohormone networking, preventing the adaptive responses of tomato plants to salt stress. *Frontiers in Plant Science*. 2017;2(8):294.
- Li RX, Cai F, Pang G, Shen QR, Li R, Chen W. Solubilisation of phosphate and micronutrients by *Trichoderma harzianum* and its relationship with the promotion of tomato plant growth. *PLoS One*. 2015;25, 10(6):e0130081.
- Chen SC, Zhao HJ, Wang ZH, Zheng CX, Zhao PY, Guan ZH, Qin HY, Liu AR, Lin XM, Ahammed GJ. *Trichoderma harzianum*-induced resistance against *Fusarium oxysporum* involves regulation of nuclear DNA content, cell viability and cell cycle-related genes expression in cucumber roots. *European Journal of Plant Pathology*. 2017;1,147(1):43-53.
- Ekundayo EA, Ekundayo FO, Osinowo IA. Antifungal activities of *Trichoderma viride* and two fungicides in controlling diseases caused by *Sclerotium rolfsii* on tomato plants; 2015.
- Chagas LF, Junior AF, Fidelis RR, de Carvalho Filho MR, de Oliveira Miller L. *Trichoderma asperellum* efficiency in soybean yield components. *Comunicata Scientiae*. 2017;8(1):165-9.
- Babu S, Prasanna R, Bidyarani N, Nain L, Shivay YS. Synergistic action of PGP agents and *Rhizobium* spp. for improved plant growth, nutrient mobilization and yields in different leguminous crops. *Biocatalysis and Agricultural Biotechnology*. 2015;1,4(4):456-64.
- Rodrigues AC, Vendruscolo CT, da Silveira Moreira A, Santana MV, de Paula Oliveira JE, Bonifacio A, Figueiredo MA. *Rhizobium tropici* exopolysaccharides as carriers improve the symbiosis of cowpea-*Bradyrhizobium-Paenibacillus*. *African Journal of Microbiology Research*. 2015; 16,9(37):2037-50.
- Rego A, Diop I, Sadio O, Sylva MC, Agbangba CE, Touré O, Kane A, Neyra M, Ndoye I, Wade TK. Response of cowpea to symbiotic microorganisms inoculation (*Arbuscular mycorrhizal* Fungi and *Rhizobium*) in cultivated soils in Senegal. *Universal Journal of Plant Science*. 2015; 3(2):32-42.
- Alcántara C, Thornton CR, Pérez-de-Luque A, Le Cocq K, Pedraza V, Murray PJ. The free-living rhizosphere fungus *Trichoderma hamatum* GD12 enhances clover productivity in clover-ryegrass mixtures. *Plant and Soil*. 2016;1, 398(1-2):165-80.
- Mweetwa AM, Chilombo G, Gondwe BM. Nodulation, nutrient uptake and yield of common bean inoculated with *Rhizobium* and *Trichoderma* in an acid soil. *Journal of Agriculture Science*. 2016;8:61-71.
- Jagadeesh V, Patta S, Triveni S, Keshavulu K, Rani KJ, Raghavendra K. Effect of biological seed coating on pigeon

- pea seedling vigour. International Journal of Current Microbiology and Applied Sciences. 2017;6(8):843-54.
16. Okon Y, Albrecht SL, Burris RH. Methods for growing *Spirillum lipoferum* and for counting it in pure culture and in association with plants. Applied and Environmental Microbiology. 1977; 33(1):85-8.
 17. Vincent JM. A manual for the practical study of the root- nodule bacteria. "IBP Handbook No. 15. Black well Sci., Pul. Oxford and Edinburgh. 1970;54-5
 18. Jain AK, Singh OP, Ruhela AK. Influence of polyethylene glycol on biomass of *Trichoderma* species. Advances in Plant Sciences. 2009;22(1):33-34.
 19. Allen ON. Experiments in soil bacteriology. Burgess Publishing Co.; 1950.
 20. Gordon SA, Weber RP. Colorimetric estimation of indoleacetic acid. Plant Physiol. 1951;26(1):192–195.
 21. Qi W, Zhao L. Study of the siderophore-producing *Trichoderma asperellum* Q1 on cucumber growth promotion under salt stress. Journal of Basic Microbiology. 2013;53(4):355–364.
 22. Allen ON. Experiments in soil Bacteriology. University of Wiscosin second printing. 1959;202.
 23. Bremner JM. Total nitrogen. In: black CA. Methods of soil analysis chemical and microbiological properties. Madison: American Society of Agronomy. 1965;Part 2:1149–1178.
 24. Lichtenthaler HK, Wellburn AR. Determination of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochemical Society Transactions. 1983;11:591–592.
 25. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water-stress studies. Plant and Soil. 1973;39: 205–207.
 26. Ibragimova MV, Rumyantseva ML, Onishchuk OP, Belova VS, Kurchak ON, Andronov EE, Dzyubenko NI, Simarov BV. Symbiosis between the root-nodule bacterium *Sinorhizobium meliloti* and alfalfa (*Medicago sativa*) under salinization conditions. Microbiology. 2006;1,75(1):77-81.
 27. Chepsergon J, Mwamburi L, Kassim MK. Mechanism of drought tolerance in plants using *Trichoderma* spp. International Journal of Science and Research. 2014; 3:1592-5.
 28. Armada E, Roldan A, Azcon R. Differential activity of autochthonous bacteria in controlling drought stress in native *Lavandula* and *Salvia* plants species under drought conditions in natural arid soil. Microbial Ecology. 2014;67:410–420.
 29. Omara A, El-Gaafarey T. Alleviation of Salinity Stress Effects in Forage Cowpea (*Vigna unguiculata* L.) by *Bradyrhizobium* sp. Inoculation. Microbiol. Res. J. Inter. 2018;23(3):1-16. DOI: 10.9734/MRJI/2018/40727
 30. Hafez E, Omara AE, Ahmed A. The coupling effects of plant growth promoting rhizobacteria and salicylic acid on physiological modifications, yield traits, and productivity of wheat under water deficient conditions. Agronomy. 2019;9(9):524.
 31. Raheem A, Shaposhnikov A, Belimov AA, Dodd IC, Ali B. Auxin production by rhizobacteria was associated with improved yield of wheat (*Triticum aestivum* L.) under drought stress. Archives Agron. Soil Sci. 2018;64(4):574–587. Available:https://doi.org/10.1080/03650340 .2017.1362105
 32. Yasmin H, Nosheen A, Naz R, Bano A, Keyani R. L-tryptophan assisted PGPR-mediated induction of drought tolerance in maize (*Zea mays* L.). Journal of Plant Interactions. 2017;12 (1):567–578. Available:https://doi.org/10.1080/17429145 .2017.1402212
 33. Kumari S, Vaishnav A, Jain S, Varma A, Choudhary DK. Induced drought tolerance through wild and mutant bacterial strain *Pseudomonas simiae* in mung bean (*Vigna radiata* L.). World Journal of Microbiology and Biotechnology. 2016;32:4. DOI:10.1007/s11274-015-1974-3
 34. Mastouri F, Björkman T, Harman GE. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. Phytopathology. 2010;100(11): 1213-21.
 35. Hassan MM, Daffalla HM, Modwi HI, Osman MG, Ahmed II, Gani ME, Abdel El Gabar EB. Effects of fungal strains on seeds germination of millet and *Striga hermonthica*. Universal Journal of Agricultural Research. 2014;2(2):83-8.
 36. Doni F, Isahak A, Zain CR, Yusoff WM. Physiological and growth response of rice plants (*Oryza sativa* L.) to *Trichoderma* spp. inoculants. AMB Express. 2014;1, 4(1):45.

37. Rawat L, Singh Y, Shukla N, Kumar J. Salinity tolerant *Trichoderma harzianum* reinforces NaCl tolerance and reduces population dynamics of *Fusarium oxysporum* f. sp. *ciceri* in chickpea (*Cicer arietinum* L.) under salt stress conditions. Archives of Phytopathology and Plant Protection. 2013;1,46(12):1442-67.
38. Zahran HH. Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. Journal of Biotechnology. 2001;4,91(2-3):143-53.
39. Bhargava Y, Murthy JS, Kumar TR, Rao MN. Phenotypic, stress tolerance and plant growth promoting characteristics of rhizobial isolates from selected wild legumes of semiarid region, Tirupati, India. Advances in Microbiology. 2016;21,6(1):1-2.
40. Zahran HH. Legume-Microbe interactions under stressed environments. In Microbes for Legume Improvement. 2017;301-339.
41. Sadowsky MJ. Soil stress factors influencing symbiotic nitrogen fixation. In Nitrogen Fixation in Agriculture, Forestry, Ecology, and the Environment. 2005;89-112.
42. Shukla N, Awasthi RP, Rawat L, Kumar J. Biochemical and physiological responses of rice (*Oryza sativa* L.) as influenced by *Trichoderma harzianum* under drought stress. Plant Physiology and Biochemistry. 2012;1,54:78-88.
43. Behera RK, Mishra PC, Choudhury NK. High irradiance and water stress induce alterations in pigment composition and chloroplast activities of primary wheat leaves. Journal of Plant Physiology. 2002; 1,159(9):967-73.
44. Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL, Bailey BA. The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. Journal of Experimental Botany. 2009;1,60(11):3279-95.
45. Harman GE, Petzoldt R, Comis A, Chen J. Interactions between *Trichoderma harzianum* strain T22 and maize inbred line Mo17 and effects of these interactions on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola*. Phytopathology. 2004;94(2):147-53.
46. Martínez-Medina A, Alguacil MD, Pascual JA, Van Wees SC. Phytohormone profiles induced by *Trichoderma* isolates correspond with their biocontrol and plant growth-promoting activity on melon plants. Journal of Chemical Ecology. 2014;1, 40(7):804-15.
47. Hafez EM, Alsohim AS, Farig M, Omara AE, Rashwan E, Kamara MM. Synergistic effect of Biochar and plant growth promoting Rhizobacteria on alleviation of water deficit in rice plants under salt-affected soil. Agronomy. 2019;9(12):847.
48. Omara A, Elbagory M. Enhancement of plant growth and yield of wheat (*Triticum aestivum* L.) under drought conditions using plant-growth-promoting bacteria. Annual Research and Review in Biology. 2018;28:1–18.

© 2020 El-Nahrawy et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/63849>