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DETERMINATION OF THE EFFECTS OF PGPR ISOLATES AND ALGAE ON PLANT GROWTH IN BROAD BEAN (*Vicia faba* L.) GROWN UNDER WATER STRESS CONDITIONS

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ABSTRACT

In regions exposed to drought stress, the use of bacteria applications to promote yield and quality has increased. This study was carried out to determine the effects of rhizobacteria and algae treatments on some biochemical and physiological properties of broad bean (*Vicia faba* L.) grown under water stress conditions. According to the completely randomized experimental design, the study was carried out in 4 replications in factorial order. In the experiment, the Filiz-99 broad bean variety was used as a plant material. In the study, 4 different biological applications (control, blue-green algae, and 2 different bacteria) and 3 different irrigation levels – 100% (NI), 50% (RI1), and 25% (RI2) – have been applied. In the study, properties such as root and stem length, stem and root fresh weight, stem, and root dry weight, nitrogen balance index, antioxidant, flavonoid, and phenolic properties were examined. Root length changed between 21.37–25.62 cm in bacteria and algae applications, and the highest value was obtained from the B1 application with 25.62 g. At increasing water stress levels, the nitrogen balance index varied in the range of 128.01–77.50%. In bacteria and algae applications, the highest value was obtained from the B1 application. While the phenolic content ranged between 127.53 and 134.31 mg GAE (Gallic Acid Equivalents) g^{-1} with increasing water stress, the highest value among biological applications was B1 application with 138.06 mg GAE g^{-1}. As a result of the interaction of factors, the highest phenolic values were obtained from B1 × RI2 (149.85 mg GAE g^{-1}), B2 × RI2 (137.05 mg GAE g^{-1}), B0 × NI (127.43 mg GAE g^{-1}), and B0 × RI2 (123.69 mg GAE g^{-1}) applications, while the lowest values were obtained from B2 × NI (123.22 mg GAE g^{-1}), Alg × RI2 (126.65 mg GAE g^{-1}), Alg × NI (127.75 mg GAE g^{-1}), and B1 × NI (131.73 mg GAE g^{-1}) applications. In the study, it was determined that bacterial applications were more effective than algae applications.

Key words: rhizobacteria, algae, water stress, broad bean

INTRODUCTION

Broad bean (*Vicia faba* L.), the World's oldest legume plant, began to be cultivated in the early Neolithic period. It is known that broad beans can be grown in large areas ranging from Western Mediterranean countries to India since prehistoric times [Cubero 1973, 1974]. The versatile use of the broad bean plant and its Mediterranean origin have made this product privileged in the Mediterranean basin [Saxena 1991]. Broad bean seeds, which have an important place in human and animal nutrition, are a good source of protein at rates varying between 25% and 35% [Nachi and Le Guen 1996]. Dried broad beans were planted in

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an area of 2,671.497 hectares worldwide, and a total of 5,669,185 tons of products were harvested from this area [FAO 2020]. Broad bean is very resistant to cold compared to beans, cowpea and peas [Vural et al. 2000] , which are considered as dry grains and vegetables in our country, as well as processed in the canned food and food industry. The cultivation expenses of this plant, which has a very important place in human nutrition, are less than other cultivated plants. It is a good pre-plant position in the alternation because it gives early products in the spring months. The green broad bean plant, which has a high nitrogen-fixing ability, is very important in increasing the fertility of the soil [Özdemir 2002]. The rapidly changing world population and the nutritional problem that arises accordingly maintain its importance day by day. In addition, one of the most critical factors emerging as a result of global warming and the resulting climate change is the decrease in water resources and the emergence of drought. Drought is the definition of associating the imbalance in humidity that may occur in a region with the scarcity of water reserves in the same area. According to many researchers, drought is the most important natural disaster among 31 natural disasters. With the narrowing of the leaf area in plants, more development occurs in the roots than in the stem due to the decrease in the transpiration rate. These changes in the roots manifest themselves as better use of water in the environment, and this is one of the morphological changes caused by drought stress. The transmission of compounds that play an active role in the realization of photosynthesis to the root zone of the plant under drought stress causes the rate of photosynthesis to slow down in arid conditions [Pessarakli et al. 2015]. When plants encounter drought stress, they activate their hormone and ion functions. One of these two mechanisms is the increase in abscisic acid (ABA) in plant cells [Kumlay and Eryiğit 2011] and the closure of stomata with drought, while the second ion function is the removal of potassium ions attached to the stomatal cells and the opening of stomata [Munemasa et al. 2015]. It can be said that there is a close relationship between the antioxidant defense mechanisms of plants and their resistance to environmental stresses [Bettaieb et al. 2011, Büyük et al. 2012]. Since the closure of stomata due to drought limits the uptake of carbon dioxide gas (CO_2), the rate of photosynthesis also decreases. In addition, the responses of plants to drought stress may vary depending on their genetic structures in general terms [Chaves et al. 2002]. The fact that there is a symbiotic life between plants and microorganisms such as arbuscular mycorrhizal fungi (AMF) in the soil is known [Tüfenkçi et al. 2012]. Both biotic and abiotic stress significantly affect yield and quality in agricultural production. With the combination of PGPR and AMF in plants, plant growth is promoted, the plant can be protected from stress factors, and the vitality in the soil is preserved [Nadeem et al. 2014]. Kloepper et al. [1980] named it to plant growth promoting rhizobacteria (PGPR). In addition, one of the reasons why these bacteria are called „probiotic rhizobacteria" is that they provide benefits to plants in many ways. These bacteria, which can benefit the plant in many ways, have many features such as fixing nitrogen, dissolving especially phosphorus and heavy metals, producing hormones, increasing mineral-water uptake and enzyme functionality, and also contributing to plant root development [Djordjevic et al. 1987, Ferreira et al. 1987]. Bioagents and biofertilizers used in grain cultivation are important elements in organic agriculture. In this sense, the importance of microorganisms is very clear in the conversion and use of nutrients that are important for plants in organic agriculture.

This study was carried out to determine how PGPR isolates and algae affect the plant in broad bean (*Vicia faba* L.) grown under water stress conditions.

MATERIAL AND METHODS

The experiment was carried out in the fully controlled plant growth cabinet (temperature: 25°C, humidity: 40–50%, photoperiod: 14 h light and 10 h dark, light density: 250 $\mu mol \cdot m^{-1} s^{-1}$) of Van Yuzuncu Yil University, Faculty of Agriculture, Field Crops Department in 2020. In the trial, the Filiz-99 standard broad bean variety (*Vicia faba* L.) obtained from Aegean Agricultural Research Institute was used as seed material. The experiment was conducted with 3 irrigation levels: normal irrigation (NI) and 2 reduced irrigations – 1/2 (RI1) and 3/4 (RI2); and 4 biological applications. Two rhizobacteria (*Azospirillum lipoferum*, 1 × 106 CFU mL^{-1}, B1; *Bacillus megaterium*, 1 × 105 CFU mL^{-1}, B2 rhizobacteria), 1 micro-algae

(*Chlorella saccharophilia*, 2 × 104 CFU mL^{-1}, Algae) were used as biological applications with a control group (control). One of the bacteria used is phosphorus solvent, the other nitrogen fixer, and the other green algae. Since these have different structures and effects from each other, the usage rate for each of them was determined in line with the recommendations of commercial companies. In the study, 1 broad bean variety, 2 rhizobacteria (B1 and B2), 1 algae, and 3 drought types (NI, RI1, and RI2) were applied. Thus, broad bean seeds were sown in 12 pods together with the drought control group. A trial was established with 48 pods out of 4 replications. According to the method stated by Çakmakçı et al. [2014], the broad bean seeds were kept in 70% (v/v) ethanol for 2 minutes and then washed 10 times with distilled water and cleaned from the chemical substance. Broad bean seeds purified with distilled water were dipped in 5% *Chlorella saccharophilia* (blue-green algae) solution and 10 ml L^{-1} *Bacillus megaterium* and *Azospirillum lipoferum* (rhizobacterium) solutions and processed in a rotary mixer (81 rpm) for 20 hours [Çakmakçı et al. 2014]. Potting mortar prepared from soil and perlite (2 : 1 ratio) was filled in 2 L pots, and 3 broad bean seeds were planted in each pot. The analyzes of the potting mortar used in the study were made in the Soil Science and Plant Nutrition laboratory of Van Yuzuncu Yil University, Faculty of Agriculture, and it was determined that the mortar was salt-free, slightly alkaline, and poor in organic matter. By determining the humidity of the working mortar, the pots were watered with 50 cc of life water immediately after sowing the seeds until the emergence time, and this process was terminated with the start of seed emergence. Before the water stress applications were given to the pots, the field capacity and moisture contents were calculated. The process of bringing them to the field capacity was completed by weighing the pots and performing the irrigation processes according to Coşkan and Senyigit [2018] and according to Karagöz et al. [2018]. For the nitrogen and phosphorus requirements of the plants, 40 kg/ha nitrogen and 90 kg/ha phosphorus (P_2O_5) were given to pots as fertilization. Seedling growth was followed for 30 days and on day 30 the plants were exposed to stress conditions. Plants were harvested 15 days after stress treatments.

Morphological measurements made in the plants are as follows. Root length (RL; cm): it was determined by measuring the part from the tip of the root part of the plant to the root collar. Stem length (SL; cm): it was found by measuring the height of the plants from the soil level to the extreme point. Root fresh weight (RFW; g): after the root part of the plants representing the applications was separated, the root wet weights were determined on a precision balance. Stem fresh weight (SFW; g): after the plants representing the applications were cut from the soil level, the stem fresh weights were determined on a precision balance. Root dry weight (RDW; g): after harvest, plant samples were kept in an oven set at 70°C for 48 hours, and root dry weights were calculated. Stem dry weight (SDW; g): after harvest, plant samples were kept in an oven set at 70°C for 48 hours, and stem dry weights were calculated.

Biochemical measurements made in the plant were made as follows. Total flavonoid (mg QE 100 g^{-1}): measurements were made using a chlorophyll and polyphenol meter device (Dualex Scientific + brand, Force-A/France) when the leaf was in a turgor state on the harvest day. In the same leaf sample, the nitrogen balance index (NBI; %) was determined as follows; on the harvest day, when the leaf was in a turgor state, measurements were made using a chlorophyll and polyphenol meter device (Dualex Scientific + brand, Force-A/France). Total phenolic compounds (mg GAE g^{-1}): the Folin-Ciocâlteu spectrophotometric method specified by Heimler et al. [2005] was used to determine total phenolic components. The determination of total antioxidant activity was made according to the method described by Lutz et al. [2011].

Statistical analysis of data. The data obtained in the experiment were subjected to analysis of variance according to the factorial order in the completely randomized experimental design using the Costat (version 6.34) package program. The comparison of the averages was made according to the LSD multiple comparison test.

RESULTS AND DISCUSSION

Seedling length. As seen in Table 1, it was determined that bacteria and algae applications did not have any effect on the stem length of the pod, and stem lengths were found to vary between 31.99–35.08 cm. Contrary to our study, Telek et al. [2019] reported that rhizobacteria had a curative effect on plant height. In the study, the highest stem length value in terms of water stress was obtained from NS as 37.73 cm, and the

Table 1. Some measurements of plant growth parameters

Biologic applications		Reduced irrigation	SL (cm)	RL (cm)	SFW (g)	RFW (g)	SDW (g)	RDW (g)
Control		NI	35.23	23.53	6.67	5.53	0.82	0.66
		RI1	31.20	22.47	3.92	4.94	0.59	0.74
		RI2	29.53	21.97	4.11	5.41	0.63	0.77
		Mean	31.99	22.66 AB	4.90	5.29	0.68	0.72
Bacteria	B1	NI	39.33	24.23	6.48	5.33	0.81	0.62
		RI1	32.80	23.40	3.75	4.38	0.55	0.61
		RI2	25.33	29.23	4.22	5.40	0.53	0.81
		Mean	32.49	25.62 A	4.81	5.04	0.63	0.68
	B2	NI	39.47	24.40	6.69	5.87	0.81	0.60
		RI1	33.40	20.93	5.23	4.77	0.76	0.70
		RI2	30.40	18.77	3.57	4.62	0.46	0.71
		Mean	34.42	21.37 B	5.16	5.09	0.68	0.67
Blue-green algae	Alg	NI	36.90	24.13	7.04	6.18	0.87	0.75
		RI1	34.17	19.63	5.02	5.34	0.73	0.74
		RI2	34.17	29.50	4.86	5.30	0.74	0.85
		Mean	35.08	24.42 AB	5.64	5.61	0.78	0.78
Reduced irrigation (RI) mean		NI	37.73 *a*	24.08	6.72 *a*	5.73	0.83 *a*	0.66
		RI1	32.89 *b*	21.61	4.48 *b*	4.86	0.66 *b*	0.70
		RI2	29.86 *b*	24.87	4.19 *b*	5.18	0.59 *b*	0.78
LSD (5%) BA			ns	4.093 *	ns	ns	ns	ns
LSD (5%) RI			4.558 **	ns	1.026 **	ns	0.146 **	ns
LSD (5%) BA × RI			ns	ns	ns	ns	ns	ns

* There is a statistical difference (5%) between the values shown with different bold and capital letters in the same column
** There is a statistical difference (5%) between the values shown with different lower and italic letters in the same column
RL – root length; SL – stem length; RFW – root fresh weight; SFW – stem fresh weight; RDW – root dry weight; SDW – stem dry weight; B1, B2 – bacteria; Alg – blue-green algae; NI, RI1 and RI2 – reduced irrigation; BA – biologic applications

shortest values (29.86 to 32.89 cm) were taken from RI2 and RI1. It was determined that the interaction of the biological application and water stress factors applied in the experiment was not significant on the stem length of the pod, and stem lengths were determined to vary between 25.33–39.47 cm. Depending on the duration of the stress, the stem length of the plants can change. Depending on the stress conditions, stem growth in plants slows down while root growth continues to reach the water. Root elongation slows down as a result of increases in drought stress, and as a result, decreases in photosynthesis activity occur [Anjum et al. 2011]. Plants with short stems are less affected by drought stress [Shakir et al. 2012]. It was determined that commercial biofertilizers containing *Azoarcus*, *Azospirillum*, and *Azorhizobium* increased root length by 29% and stem length by 65% in wheat plants [Dal Cortivo et al. 2017].

In a previous study, it was reported that the stem length values of the calendula plants varied between 9.34–11.00 cm and 8.58–11.10 cm in bacteria and drought applications [Şelem et al. 2021]. It is thought that the difference between our data and the literature may be due to the plant species.

Root length. In the study, the effect of bacteria and algae applications on the root length of the broad bean was significant. While the longest root length value was obtained from the B1 application with 25.62 cm, the shortest root length value was obtained from the B2 application with 21.37 cm. Commercial biofertilizers containing *Azoarcus*, *Azospirillium*, and *Azorhizobium* were found to increase root length by 29% in wheat plants [Dal Cortivo et al. 2017]. The obtained data are in harmony with the literature. It was determined that water stress levels did not have any effect on root length in pods, and the root lengths were varied between 21.61–24.87 cm. In the study, it was determined that the interaction of biological applications and water stress factors did not affect the root length of the pod, and the root lengths varied between 18.77–29.50 cm. In order for the root system to form in plants in a healthy way, the water required by the plant in the soil must be at the desired level. In addition, an effective root system is accepted as the most important factor protecting plants from drought stress [Blum 2009]. The effect of drought stress on plants may vary depending on the duration and severity of the drought [Abayomi and Abidoye 2009]. Bacterial inoculation increased water availability in plants growing in dry and semi-arid seasons [Heidari and Golpayegani 2012]. In addition, it was observed that micro-algae and bacteria applications produced IAA and increased root growth, thus improving water and nutrient uptake levels [Gururani et al. 2013].

Stem fresh weight. As seen in Table 1, it was determined that bacteria and algae applications did not have any effect on the stem fresh weight, and it was determined that the stem wet weights ranged between 4.81–5.64 g. In stark contrast to our study, Telek et al. [2019] reported that rhizobacteria have an improving share on the shoot fresh weight of the plant. In the same study, it was determined that the water stress levels of the stem fresh weights were significant. The longest stem fresh weight with 6.72 g was obtained from NI application, and the shortest stem fresh weight was 4.19–4.48 from RI2 and RI1. In a previous study, it has been reported that the heaviest and lightest stem age values of calendula were measured as 7.26 g and 3.61 g in drought applications [Şelem et al. 2021]. There is harmony between the results stated in the literature and the obtained values. In the study, it was observed that the interaction of biological applications and water stress factors on stem fresh weight in broad beans was not significant, and stem fresh weights were found to vary between 3.57–7.04 g (Tab. 1). In a study conducted in dry and irrigated conditions on PGPR-treated pea plants, remarkable data were obtained on the stem and root weights of the plant [Glick 1995]. In a greenhouse experiment investigating the mechanism of drought stress in *Phaseolus vulgaris* L. and *Sesbania aculeata species*, it was determined that drought stress decreased compared to the control group [Ashraf and Iram 2005]. As a result of studies on melons, it was reported that drought stress reduced stem weights in plants by 145.65%, and in another study on beans, fresh stem weight decreased by 33% [Yildirim et al. 2020].

Root fresh weight. In the study carried out, it was determined that bacteria and algae applications on root wet weights were insignificant, and root wet weights varied between 5.04–5.61 g. It was determined that water stress levels had no effect on root wet weights, and root wet weights vary between 4.86–5.37 g. It was determined that the interaction of biological applications and water stress factors was not significant on the root wet weight, and the root wet weight was between 4.38–6.18 g (Tab. 1). It was determined that the root fresh weight values of calendula plants varied between 0.89–0.74 g cm and 1.06–0.58 g in bacterial and drought applications [Şelem et al. 2021]. It is thought that the difference between the study results and the literature may be due to the applications and plant species differences. In a study that investigated the effects of drought stress on root fresh weight in bean plants at 3 different irrigation levels (100% full irrigation, 80%, and 60% of field capacity); it was reported that the root fresh weight was decreased as 55% with 60% irrigation level compared to 100% irrigation level [Yildirim et al. 2020].

Stem dry weight. As seen in Table 1, it was determined that algae and bacteria applications did not have any effect on the stem dry weight of the pod, and the stem dry weights varied between 0.67–0.78 g. Contrary to the study results, Oral et al. [2021], in a previous study on soybean, stated that rhizobacteria and drought treatments had a significant effect on the dry weight of the trunk. This situation can be explained as it may be caused by different plant species used in the studies. The effect of water stress levels on stem dry weights were significant, and the highest stem dry weight was obtained from NI with 0.83 g, and the lowest stem dry weight values were obtained from RI2 and RI1 with 0.59, 0.66 g. It was reported that stem dry weight values varied between 1.15–1.08 g cm and 1.27–0.97 g in the bacteria and drought applications in calendula [Şelem et al. 2021]. It is thought that the difference between the obtained data and the literature may be due to the application difference. It was determined that the interaction of biological applications and water stress factors was not significant on the stem dry weight and the stem dry weights varied between 0.60–0.85 g. Oral et al. [2021] in a previous study conducted on soybean, stated that rhizobacteria and drought treatments had a significant effect on the dry weight of the stem.

Root dry weight. As seen in Table 1, it was observed that the biological applications did not have any effect on the root dry weight of the pod and the root dry weights were between 0.67–0.78 g. In the study, it was determined that the water stress levels were not significant on the root dry weight, and the root dry weight ranged between 0.66–0.78 g. In a previous study, in harmony with the study results, it was reported that the root dry weight decreased by 52% with the 60% irrigation level compared to the 100% irrigation level [Yildirim et al. 2020]. It was determined that the interaction due to biological applications and water stress factors wasn't important on root dry weight in pods, and root dry weight varies between 0.60–0.78 g. In a previous study, it was reported that the root dry weight was decreased by 52% with the 60% irrigation level compared to the 100% irrigation level [Yildirim et al. 2020]. In another study, it was stated that root dry values in soybean vary between 0.15 and 0.18 g depending on drought stress [Oral et al. 2021]. It has been reported that the root dry weight values of the calendula plant in drought and bacterial applications varied between 0.17–0.13 g and 0.16–0.14 g [Şelem et al. 2021]. It is thought that the difference between the obtained data and the literature may be due to the application differences in the studies.

Nitrogen balance index. It was determined that biological applications did not have an effect on the nitrogen balance index of the pod, and the nitrogen balance index values varied between 87.77–106.38%. It was determined that the effect of water stress applications on the nitrogen balance index of the pods was significant, the highest value with NI application with 128.01% and the lowest values with RI2 and RI1 applications with 77.50, 83.97% (Tab. 2). In the study, it was observed that the interaction of biological applications and water stress was not important, and the nitrogen fixation index was between 64.76–143.86% (Tab. 2). Contrary to the study results, in a previous study, it was reported that the effect of drought and rhizobacteria interaction on nitrogen balance index in soybean is very important and water stress causes about a 10% decrease in nitrogen balance index value compared to control [Oral et al. 2021]. It is thought that the difference between the literature and the study results may be due to the difference in the applications or plant species used in the studies. In the study, it was determined that there was a decrease in the nitrogen balance index value depending on the increasing drought levels. In harmony with the study results, in a previous study, the effects of drought stress on sorghum were investigated and it was determined that the nitrogen balance in the plant decreased due to drought stress and it caused early ripening in the leaves [Chen et al. 2015]. In a previous study, it was reported that the effect of drought and rhizobacteria interaction on the nitrogen balance index in soybean is very important, and water stress causes about a 10% decrease in nitrogen balance index value compared to control [Oral et al. 2021]. In a study that investigated the effects of drought stress on sorghum, it was determined that the nitrogen balance in the plant decreased due to stress, and it caused early maturation in the leaves [Chen et al. 2015]. Additionally, it was stated that in drought stress conditions, the nutrient intake reflex varies according to the genetic structure of the species and varieties in plants [Sarma and Saikia 2014]. In a similar study, it was determined that, depending on the severity of the drought, there was an increase in nitrogen uptake and a decrease in phosphorus uptake, while there was no effect on potassium uptake. This reaction in plants may differ according to the genus and species of plants [Osakabe et al. 2014].

Antioxidant, phenolic and flavonoid. As seen in Table 2, it was determined that the effects of biological applications and water stress applications on the antioxidant content of the pod were insignificant. It was observed that the values of bacterial and algae applications varied between 89.82–126.82 µmol TE (Trolox Equivalent) g^{-1}, while the values of the water stress applications were in the range of 89.81–121.27 µmol TE g^{-1}. In addition, it was determined that the interaction of biological applications and water stress did not have a significant effect on the antioxidant content of the pod, and the antioxidant values ranged between 52.78–155.46 mg TE g^{-1}. In general, there was an increase in the antioxidant value of the broad

Table 2. The effect of different water treatments and rhizobacteria inoculations on some physiological and biochemical properties of broad bean

Biologic applications		Reduced irrigation	Antioxidant	Phenolic		Flavonoid		NBI	
			(µmol TE g^{-1})	(mg GAE g^{-1})		(mg QE 100^{-1})		(%)	
Control		NI	106.94	127.43	a	7.25		102.53	
		RI1	109.74	125.87	ab	5.69		80.80	
		RI2	52.78	123.69	a	6.89		79.97	
		Mean	89.82	125.66	C	6.61	BC	87.77	
Bacteria	B1	NI	125.93	131.73	b	10.13		143.86	
		RI1	99.07	132.59	ab	9.48		90.79	
		RI2	155.46	149.85	a	11.45		84.50	
		Mean	126.82	138.06	A	10.35	A	106.38	
	B2	NI	63.42	123.22	b	5.69		137.28	
		RI1	137.04	135.4	ab	8.37		76.63	
		RI2	136.11	137.05	a	8.02		64.76	
		Mean	112.19	131.89	B	7.36	B	92.89	
Blue-green algae	Alg	NI	62.96	127.75	b	6.07		128.36	
		RI1	88.88	137.59	ab	6.3		87.67	
		RI2	140.74	126.65	b	3.87		80.78	
		Mean	97.53	130.66	BC	5.41	C	98.93	
Reduced irrigation (RI) mean		NI	89.81	127.53	*B*	7.28		128.01	*A*
		RI1	108.68	132.86	*A*	7.46		83.97	*B*
		RI2	121.27	134.31	*A*	7.56		77.50	*B*
LSD (5%) BA			ns	5.152	*	1.855	*	ns	
LSD (5%) RI			ns	4.462	**	ns		33.676	**
LSD (5%) BA × RI			ns	1.394	***	ns		ns	

* There is a 5% statistical difference between the values shown with different capital letters and bold letters in the same column
** There is a statistical difference (5%) between the values shown with different capital and italic letters in the same column
*** There is a statistical difference (5%) between the values shown with different lowercase letters in the same column
NBI – nitrogen balance index; B1, B2 – bacteria; Alg – blue-green algae; NI, RI1 and RI2 – reduced irrigation

bean depending on the drought levels. In a study, it was reported that antioxidant activity, flavonoid, and polyphenol content increased in buckwheat plants depending on the severity of drought [Hanson et al. 2011]. The literature and the data of the study are in harmony. In addition, it was seen that the free oxygen radicals formed under stress conditions damaged the plant. In the study, it was determined that the effect of biological applications on the phenolic content of the pod was significant, and the highest phenolic value was measured as 138.89 mg GAE g^{-1} from B1, and the lowest phenolic values from B0, B2, and algae applications were measured as 125.66, 131.89 and 130.66 mg GAE g^{-1}, respectively.

In the study, it was determined that the effect of water stress applications on the phenolic content of the pod was significant. While the highest phenolic value was measured as 134.31 mg GAE g^{-1} from RI2, the lowest phenolic value was measured from NI application with 127.53 mg GAE g^{-1}. As seen in Table 2, it was determined that the effect of bacteria and algae on the phenolic content of the pod was significant under different water stress conditions. The highest phenolic values (123.69, 127.43, 137.05, and 149.85 mg GAE g^{-1}) with no statistical difference between them were obtained from the applications of B0 × RI2, B0 × NS, B2 × RI2, and B1 × RI2, respectively. The lowest phenolic values (123.22, 126.65, 127.75, and 131.73 mg GAE g^{-1}) were determined from B2 × NI, Algae × RI2, Algae × NI, and B1 × NI applications, respectively (Fig. 1). In the study, it was observed that the effect of biological applications on the flavonoid content of pods was significant, and the highest flavonoid value was obtained from B1 as 10.35 mg QE 100^{-1}, and the lowest flavonoid values were obtained from algae application with 5.41 mg QE 100^{-1}.

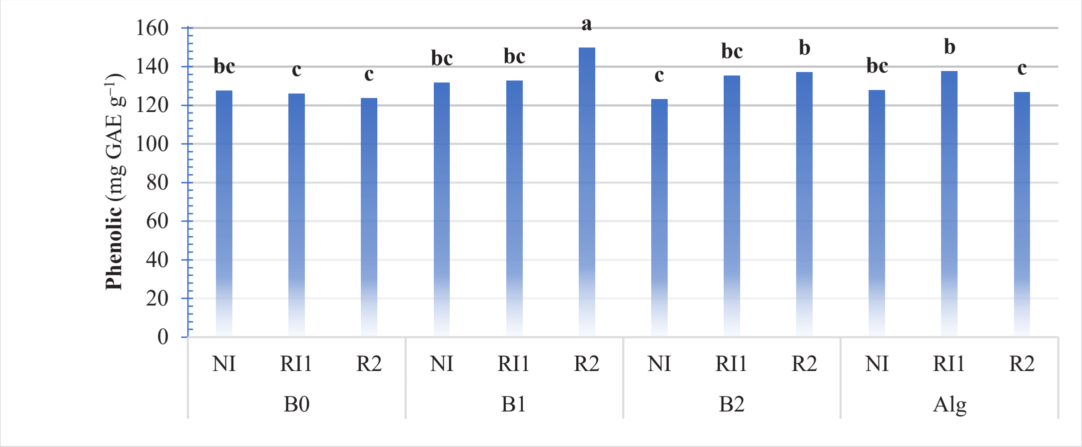

Fig. 1. The comparison of biologic applications on phenolic content of broad bean

It was seen that the phenolic and flavonoid content of broad beans increased due to increased drought stress. It is thought that this increase is due to the adverse environmental conditions, the production of flavanols containing some of the flavonoids which play a role in reducing the damage caused by free radicals and phenolic compounds increases in the endoplasmic reticulum and cytoplasm [Ibrahim and Jaafar 2011]. In the study, it was determined that the effect of water stress applications on the flavonoid content of the pod was not significant, and these values were found to vary between 7.28 and 7.56 mg QE 100^{-1}.

Free oxygen radicals that occur under stress conditions damage the plant. In order to prevent such damages, antioxidant systems are activated in the plant.

The most important antioxidants are vitamins E and C, carotenoids, and glutathione [Rietjens et al. 2002]. It has been found that the ability of plant extracts with high antioxidant potential to scavenge free radicals is related to the amount of phenolic and flavonoid substances they contain. As a result of a study carried out on buckwheat, it was determined that antioxidant activity, flavonoid, and polyphenol content increased depending on the severity of drought [Hanson et al. 2011]. Rhizobium-bacteria association has beneficial properties in terms of plant breeding, increasing productivity, and environmental. In order to bring these benefits to light, it is necessary to increase the number of studies that can be done with different rhizobial isolates and plants [Sharma 2021].

CONCLUSIONS

Drought stress, which occurs due to global warming, is clearly showing its effect on agricultural soils day by day. This situation may cause negativities in world agriculture as well as its effect on food supply. In this sense, it is necessary to take measures to prevent damage to crop production due to drought. Trying alternative methods that encourage plant growth, especially in arid conditions, will be effective in reducing the damage that may occur. With the study, some values in the plant were examined in order to understand to what extent the physiological and biochemical effects of rhizobacteria and blue-green algae, which contribute positively to both the development and growth stages of plants, on water stress conditions on the broad bean plant. In this sense, water stress applications were found to be effective on stem length, stem fresh weight, and stem dry weight of the plant. It has been determined that the effect of *Azospirillum lipoferum* bacteria, one of the bacteria used in the applications, on the properties such as root length, phenolic, and flavonoids in the plant grown under water stress.

As a result, it is seen that bacteria are more effective in reducing water stress in comparison with bacteria and algae. In order for the obtained data to be evaluated better or for the results to be more real, performing such studies in field conditions will provide more accurate results. It is also believed that the study will shed light on the water stress studies that will be carried out in the following years.

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REFERENCES

Abayomi, Y., Abidoye, T. (2009). Evaluation of cowpea genotypes for soil moisture stress tolerance under screen house conditions. Afr. J. Plant Sci., 3(10), 229–237.

Anjum, S.A., Xie, X.-Y., Wang, L.-C., Saleem, M.F., Man, C., Lei, W. (2011). Morphological, physiological and biochemical responses of plants to drought stress. Afr. J. Agric. Res., 6(9), 2026–2032.

Ashraf, M., Iram, A. (2005). Drought stress induced changes in some organic substances in nodules and other plant parts of two potential legumes differing in salt tolerance. Flora, 200(6), 535–546. https://doi.org/10.1016/j.flora.2005.06.005

Bat, M., Tunçtürk, R., Tunçtürk, M. (2019). Kuraklık Stresi Altındaki Ekinezya (*Echinacea purpurea* L.)' da Deniz Yosununun Büyüme Parametreleri, Toplam Fenolik ve Antioksidan Madde Üzerine Etkisi [Effect of seaweed on growth parameters, total phenolic and antioxidant substance in echinacea (*Echinacea purpurea* L.) under drought stress]. Yuzuncu Yil Univ. J. Agric. Sci., 29(3), 496–505 [in Turkish]. https://doi.org/10.29133/yyutbd.532883

Bettaieb, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F., Marzouk, B. (2011). Drought effects on polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. Acta Phys. Plant., 33(4), 1103–1111. http://dx.doi.org/10.1007/s11738-010-0638-z

Büyük, İ., Soydam Aydın, S., Aras, S. (2012). Bitkilerin stres koşullarına verdiği moleküler cevaplar [Molecular responses of plants to stress conditions]. Turk. Hij. Den. Biyol. Derg., 69(2), 97–110 [in Turkish]. https://dx.doi.org/10.5505/TurkHijyen.2012.40316

Blum, A. (2009). Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. Field Crops Res., 112(2–3), 119–123. https://doi.org/10.1016/j.fcr.2009.03.009

Chaves, M.M., Pereira, J.S., Maroco, J., Rodrigues, M.L., Ricardo, C.P.P., Osório, M.L., Carvalho, I., Faria, T., Pinheiro, C. (2002). How plants cope with water stress in the field? Photosynthesis and growth. Ann. Bot., 89(7), 907–916. http://dx.doi.org/10.1093/aob/mcf105

Cubero, J.I. (1973). Evolutionary trends in *Vicia faba* L. Theoret. Appl. Genet., 43(2), 59–65. https://doi.org/10.1007/bf00274958

Cubero, J.I. (1974). On the evolution of *Vicia faba* L. Theoret. Appl. Genet., 45(2), 47–51. https://doi.org/10.1007/bf00283475

Chen, D., Wang, S., Xiong, B., Cao, B., Deng, X. (2015). Carbon/nitrogen imbalance associated with drought-induced leaf senescence in *Sorghum bicolor*. PloS One, 10(8), e0137026. https://doi.org/10.1371/journal.pone.0137026

Çakmakçı, R., Turan, M., Güllüce, M., Sahin, F. (2014). Rhizobacteria for reduced fertilizer inputs in wheat (*Triticum aestivum* spp. vulgare) and barley (*Hordeum vulgare*) on aridisols in Turkey. Int. J. Plant Prod., 8(2), 163–181.

Coşkan, A., Şenyığıt, U. (2018). Farklı Sulama Suyu Düzeyi ve Vermikompost Dozlarının Marul Bitkisinin Mikro Element Alımına Etkileri [Effects of different irrigation water levels and vermicompost doses on micro nutrient uptake of lettuce plant]. Suleyman Demirel University, 1st International Agricultural Structures and Irrigation Congress Special Issue, 348–356 [in Turkish].

Dal Cortivo, C., Barion, G., Visioli, G., Mattarozzi, M., Mosca, G., Vamerali, T. (2017). Increased root growth and nitrogen accumulation in common wheat following PGPR inoculation: assessment of plant-microbe interactions by ESEM. Agric. Ecosys. Environ., 247, 396–408. https://doi.org/10.1016/j.agee.2017.07.006

Djordjevic, M.A., Gabriel, D.W., Rolfe, B.G. (1987). Rhizobium – the refined parasite of legumes. Ann. Rev. Phytopathol., 25(1), 145–168. https://doi.org/10.1146/annurev.py.25.090187.001045

FAO, (2020). http://www.fao.org/faostat/en/#data/QCL/visualize [date of access: 15.12.2021].

Ferreira, M., Fernandes, M., Döbereiner, J. (1987). Role of *Azospirillum brasilense* nitrate reductase in nitrate assimilation by wheat plants. Biol. Fertil. Soil., 4(1), 47–53.

Glick, B.R. (1995). The enhancement of plant growth by free-living bacteria. Canad. J. Microbiol., 41(2), 109–117. https://doi.org/10.1139/m95-015

Gururani, M.A., Upadhyaya, C.P., Strasser, R.J., Yu, J.W., Park, S.W. (2013). Evaluation of abiotic stress tolerance in transgenic potato plants with reduced expression of PSII manganese stabilizing protein. Plant Sci., 198, 7–16. https://doi.org/10.1016/j.plantsci.2012.09.014

Hanson, P., Yang, R.-Y., Chang, L.-C., Ledesma, L., Ledesma, D. (2011). Carotenoids, ascorbic acid, minerals, and total glucosinolates in choysum (*Brassica rapa* cvg. parachinensis) and kailaan (*B. oleraceae* Alboglabra group) as affected by variety and wet and dry season production. J. Food Compos. Anal., 24(7), 950–962. http://dx.doi.org/10.1016/j.jfca.2011.02.001

Heimler, D., Vignolini, P., Dini, M.G., Romani, A. (2005). Rapid tests to assess the antioxidant activity of *Phaseolus vulgaris* L. dry beans. J. Agric. Food Chem., 53(8), 3053–3056. https://doi.org/10.1021/jf049001r

Heidari, M., Golpayegani, A. (2012). Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). J. Saudi Soc. Agric. Sci., 11(1), 57–61. https://doi.org/10.1016/j.jssas.2011.09.001

Ibrahim, M.H., Jaafar, H.Z.E. (2011). Photosynthetic capacity photochemical efficiency and chlorophyll content of three varieties of *Labisia pumila* Benth. exposed to open field and greenhouse growing conditions. Acta Physiol. Plant., 33(6), 2179–2185. https://doi.org/10.1007/s11738-011-0757-1

Karagöz, H., Çakmakçı, R., Hosseinpour, A., Kodaz, S. (2018). Alleviation of water stress and promotion of the growth of sugar beet (*Beta vulgaris* L.) plants by multi-traits rhizobacteria. Appl. Ecol. Environ. Res., 16(5), 6801–6813.

Kumlay, A., Eryiğit, T. (2011). Growth and development regulators in plants: plant hormones. Iğdır Univ. J. Inst. Sci. Tech., 1(2), 47–56.

Kloepper, J.W., Leong, J., Teintze, M., Schroth, M.N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286(5776), 885–886. https://doi.org/10.1038/286885a0

Lutz, M., Henríquez, C., Escobar, M. (2011). Chemical composition and antioxidant properties of mature and baby artichokes (*Cynara scolymus* L.), raw and cooked. J. Food Compos. Anal., 24(1), 49–54. https://doi.org/10.1016/j.jfca.2010.06.001

Munemasa, S., Hauser, F., Park, J., Waadt, R., Brandt, B., Schroeder, J.I. (2015). Mechanisms of abscisic acid-mediated control of stomatal aperture. Curr. Opin. Plant Biol., 28, 154–162. https://doi.org/10.1016/j.pbi.2015.10.010

Nachi, N., Le Guen, J. (1996). Dry matter accumulation and seed yield in faba bean (*Vicia faba* L) genotypes. Agronomie, 16(1), 47–59. Nachi, N.; Le Guen, J. (1996). Dry matter accumulation and seed yield in faba bean (*Vicia faba* L) genotypes. Agronomie, 16(1), 47–59. https://doi.org/10.1051/agro:19960103

Nadeem, S.M., Ahmad, M., Zahir, Z.A., Javaid, A., Ashraf, M. (2014). The role of mycorrhizae and plant growth promoting rhizobacteria (PGPR) in improving crop productivity under stressful environments. Biotechnol. Adv., 32(2), 429–448. https://doi.org/10.1016/j.biotechadv.2013.12.005

Oral, E., Tunçtürk, R., Tunçtürk, M. (2021). The effect of rhizobacteria in the reducing drought stress in soybean (*Glycine max* L.). Leg. Res., 44(10), 1172–1178. https://doi.org/10.18805/LR-631

Osakabe, Y., Osakabe, K., Shinozaki, K., Tran, L.S.P. (2014). Response of plants to water stress. Front. Plant Sci., 5, 86. https://doi.org/10.3389/fpls.2014.00086

Özdemir, S. (2002). Yemeklik Baklagiller [Edible Legumes]. Hasat Yayıncılık Ltd. Şti. İstanbul [in Turkish].

Pessarakli, M., Haghighi, M., Sheibanirad, A. (2015). Plant responses under environmental stress conditions. Adv. Plants Agric. Res., 2(6), 776–286. https://doi.org/10.15406/apar.2015.02.00073

Rietjens, I.M.C.M., Boersma, M.G., Haan, L.D., Spenkelink, B., Awad, H.M., Cnubben, N.H.P., van Zanden, J.J., Woude, H.V.D., Alink, G.M., Koeman, J.H. (2002). The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. Environ. Toxicol. Pharmacol., 11(3), 321–333. https://doi.org/10.1016/S1382-6689(02)00003-0

Sarma, R.K., Saikia, R. (2014). Alleviation of drought stress in mung bean by strain *Pseudomonas aeruginosa* GGRJ21. Plant Soil, 377(1–2), 111–126.

Saxena, M. (1991). Status and scope for production of faba bean in the Mediterranean countries. Options Méditer., 10(1), 5–20.

Sharma, K. (2021). Impact of different rhizobial strains on physiological responses and seed yield of mungbean [*Vigna radiata* (L.) Wilczek] under field conditions. Leg. Res., 44(6), 679–683. https://doi.org/10.15505/LR-4339

Shakir, L., Ejaz, S., Ashraf, M., Qureshi, N.A., Anjum, A.A., Iltaf, I., Javeed, A. (2012). Ecotoxicological risks associated with tannery effluent wastewater. Environ. Toxicol. Pharmacol., 34(2), 180–191. https://doi.org/10.1016/j.etap.2012.03.002

Şelem, E., Nohutçu, L., Tunçtürk, R., Tunçtürk, M. (2021). The effect of plant growth promoting rhizobacteria applications on some growth parameters and physiological properties of marigold (*Calendula officinalis* L.) plant grown under drought stress conditions. Yuzuncu Yıl Univ. J. Agric. Sci., 31(4), 886–897. http://doi.org/10.29133/yyutbd.922874

Telek, Ü., Akıncı, İ.E., Küsek, M. (2019). The effect of rhizobacteria strains on yield and plant characteristics of red hot pepper (*Capsicum annuum* L.). KSU J. Agric. Nat., 22(1), 62–70. https://doi.org/10.18016/ksutarimdoga.vi.448536

Tüfenkçi, Ş., Demir, S., Şensoy, S., Ünsal, H., Durak, E.D., Erdinç, C., Biçer, Ş., Ekincialp, A. (2012). The effects of arbuscular mycorrhizal fungi on the seedling growth of four hybrid cucumber (*Cucumis sativus* L.) cultivars. Turkish J. Agric. Forest., 36(3), 317–327. https://doi.org/10.3906/tar-1012-1608

Vural, H., Eşiyok, D., Duman, İ. (2000) Kültür sebzeleri (sebze yetiştirme) [Cultivated vegetables (growing vegetables)]. Ege Üniversitesi Ziraat Fakültesi Bahçe Bitkileri Bölümü, Bornova-İzmir, 440 [in Turkish].

Yildirim, E., Caşka Kiliçaslan, S., Ekinci, M., Kul, R. (2020). Kuraklık Stresinin Fasulyede Bitki Gelişimi, Bazı Fizyolojik ve Biyokimyasal Özellikler Üzerine Etkisi [The effect of drought stress on plant growth, some physiological and biochemical properties of bean]. Erciyes Univ. J. Institue Sci. Tech., 36(2), 264–273 [in Turkish].