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Alleviation of Drought Stress in Faba Bean Using Humic Acid and Inoculation with Plant Growth Promoting Rhizobacteria

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Abstract

Drought is one of the major limitations to agricultural productivity worldwide and is likely to increase. This work is conducted to study the role of plant growth-promoting rhizobacteria (PGPR) inoculation in combination with humic acid as a foliar application for alleviation of drought stress on faba bean productivity. The inoculation of PGPR with humic acid as a foliar application can save half of the fertilizer requirements as well as reduce crop water requirement to 75% ETC without non significant reduction of faba bean yield. Faba bean inoculated with PGPR combined with humic acid and 50% inorganic NPK had higher microbial enzymes activities, nutrient uptake, and vegetative growth characteristics as well as it had a lower stress indicator (proline content) compared to control. Therefore, it can be relied upon to enhance the growth of faba bean, increase its productivity, while reducing its water requirements, and thus reducing production costs under drought stress.

Keywords: Drought, Faba bean, PGPR, Humic acid, soil microial enzymes.

Introduction

Faba bean (*Vicia faba* L.) is considered the first legume crop in Egypt, where it is the main nutritional source of proteins from plant (Bakry *et al.*, 2011). Egypt ranked fifth order of the ten biggest producers in the world after China, Ethiopia, Australia, and France with 175000 tons.year^{-1} (130952 fed), (FAO, 2018). The total production of faba bean is still insufficient to cover the local consumption so there is a great need to increase our production by expanding through reclaimed areas which represents the hope of cultivated lands and overcoming the deficiency food requirements, as well as, increasing the vertical production through the production of new varieties with high yield potential.

Moreover, freshwater resources for agricultural use are becoming limited due to competition. Drought effect on plant–water potential, enough to interfere with normal functions (Hsiao 2000) changing morphological and physiological features in plants (Rahdari and Hoseini, 2012). The reduction of growth under drought has been studied in numerous crops such as faba bean (Mwanamwenge *et al*, 1999 and Link *et al*, 2007), maize and wheat (Rampino et al., 2006). Water content and fresh weight are common growth parameters that are affected by drought (Jaleel et al., 2009). Also, drought stress affects the transport and availability of soil nutrients, as nutrients are passed to the roots by water. Drought stress decreases nutrient diffusion and mass flow of water-soluble nutrients such as sulfate, nitrate, Ca, Mg, and Si (Barber, 1995; Selvakumar et al., 2012).

Millions of microbes inhabit the plant root system forming a complex ecological community that influences plant growth and productivity through its metabolic activities and plant interactions (Berg, 2009; Lugtenberg and Kamilova, 2009; Schmidt et al., 2014). Changes in the structure of plant-associated bacterial communities in the root zone towards the selection of assemblages that are adapted to abiotic stress, improve the resistance to stress or to promote the health and drought tolerance of plants (Schmidt et al., 2014; Cherif et al., 2015). Worldwide extensive research is being carried out to develop strategies to cope with drought stress through the development of drought-tolerant varieties, shifting the crop calendars, resource management practices, etc. (Venkateswarlu and Shanker, 2009) and most of these technologies are cost intensive. Recent studies indicate that microorganisms can also help plants to cope with drought stress.

Humic acid is a commercial product that contains many elements which improve soil fertility and increase the availability of nutrient elements and consequently affect plant growth. Humic acid particularly is used to remove or decrease the negative effects of chemical fertilizers and some chemicals from the soil. Humic acid is not considered to be a fertilizer but a soil enhancer and improver. It physically modifies the soil, biologically stimulates plant growth, and chemically changes the fixation properties of soil (Kirn et al., 2010). It can significantly reduce water evaporation and increase its use by plants in non-day, arid and sandy soils. Furthermore, they increased the water-holding capacity of soils (khaled and Fawy, 2011). The main objective of the current work was to study the effect of plant growth-promoting rhizobacteria (PGPR) inoculation and yeast or humic acid spraying on squash and beans growth performance, productivity, and yield quality under drought stress.

Materials and Methods

Faba bean seeds

Seeds of faba bean (*Vicia faba*) cv. Hasanobary was obtained from the Agronomy Res. Inst. ARC, Giza, Egypt.

PGPR strains

The PGPR strains namely, *Rhizobium leguminosarum* EMCCN 1014 was used as nitrogen-fixing bacteria for faba bean inoculation. However, *Pseudomonas fluorescens* EMCCN 1067 and *Glomus* sp. were used as potassium and phosphate solubilizers, respectively. The overall biofertilizer strains were obtained from the biofertilization unit, Fac. Agric. Ain Shams Univ., Egypt.

Inorganic fertilizers

Inorganic fertilizers (NPK) were obtained from the local market at Toukh, El-Qalubia, Egypt as ammonium sulphate (20.5% N), calcium superphosphate (15.5 P_2O_5), and potassium sulphate (48% K_2O), respectively.

Foliar spraying

Humic acid (85%) was obtained from Sphinx for International Trade Company, Cairo, Egypt. Humic acid was added as a foliar application at a rate of 500 g/fed. at three times, after 15, 30, and 45 days from sowing.

Experimental soil

Experimental soil was mixed with compost at a rate of (200:1, w: w). the particle size distribution and chemical analysis of the experimental soil are presented in Table (1). Soil analyses were carried out according to methods described by **Page *et al.*, (1982).**

Table 1. Particle size distribution and chemical analysis of the experimental soil.

Property	Values
Soil particles size distribution	
Sand (%)	9.8
Silt (%)	59.1
Clay (%)	31.1
Texture	Silt clay
Chemical analysis	
pH (in soil paste)	7.5
E.C (ds/m)	3.1
Saturation percentage (%)	53
CEC (emol/kg soil)	38.17
$CaCO_3$ (%)	0.70
Organic matter (%)	1.70
Available N (ppm)	17
Available P (ppm)	10.4
Available K (ppm)	182
Available Fe (ppm)	4.1
Available Zn (ppm)	0.5
Available Mn (ppm)	0.1
Available Cu (ppm)	0.2
Soluble cations and anions (meqL^{-1})	
Ca^{++}	10.3
Mg^{++}	8.7
Na^{+}	18.3
K^{+}	1.4

CO_3^{--}	8.8
Cl^-	14.2
SO_4^{--}	15.7

E.C: Electric conductivity, C.E.C: cation exchange capacity

Compost

Plant-animal compost was used as organic manuring at a rate of 5 tons/fed. Some physical and chemical properties of compost are present in Table (2).

Table 2. Physical and chemical properties of used compost in this study.

Parameter	Value
Bulk density (kg/M^3)	670
Moisture content (%)	29
pH	7.4
EC (ds/m)	5.6
Organic matter (%)	47.7
Organic carbon (%)	27.7
Ash (%)	71
Total nitrogen (%)	1.5
C/N ratio	18.4:1
Total phosphourus	0.7
Total potassium	0.8
Fe (ppm)	1400
Zn (ppm)	1.4
Mn (ppm)	300
Cu (ppm)	80

N. D: Not detected

Greenhouse experiment

A greenhouse experiment was layout at a private farm, Marsafa, Benha, El-Qalyubiya governorate, Egypt (Elevation 19 m; 30°27′39″N; 31°11′15″E), during two successive seasons (2019 and 2020) to determine the efficiency of PGPR strains and spraying with humic acid on faba bean growth performance and productivity under different levels of water requirements.

Pots sterilization

Plastic pots (40 cm in diameter; 40 cm wide and 40 cm high) were sterilized by immersing in 5 % formalin solution (38 %) for 15 minutes and covered overnight with plastic sheets, then left to dry in the open air.

Preparation of PGPR inocula

PGPR inocula were prepared using specific media (Allen medium and King's medium) for *R. leguminosarum* EMCCN 1014 and *P. fluorescens* EMCCN 1067 respectively by method described by Abdelrahman *et al* (2021a). Homogenous suspension of cultures containing $2x10^8$ and $9x10^8$ CFU/ml of *R. leguminosarum* EMCCN 1014 and *P. fluorescens* EMCCN 1067 respectively. However homogenous spores suspension of *Glomus sp* containing 250 spores/ml.

The experimental design and treatments

Five treatments were used in this experiment as follows, T1: Chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic acid + 50%NPK, T4: PGPR + Humic acid, and T5: PGPR+50%NPK+Humic acid. These treatments were repeated under three levels of water requirements (100%, 75%, and 50%) and distributed in a randomized complete block design (RCBD) with five replicates for each treatment.

Water requirements

The amount of irrigation water for sunflower crop was applied by flow meter after being calculated according the following equation given by Vcrmciren and Jobling (1980) as follows:

$$IW=\left[\frac{ETo * Kc * Kr * I_1}{Ea1}\right] * 4.2 + LR$$

Where:

IW = Irrigation water applied under drip irrigation system, m^3/fed/irrigation.

ET_0= Reference evapotranspiration (mm/day).

Kc = Crop coefficient.

Kr = Reduction factor (Keller and Karmeli, 1974).

I = Irrigation intervals with drip irrigation system, day.

Ea=Irrigation efficiency of drip irrigation system, %.

LR=Leaching requirement = 10% of the total amount of water, m^3/fed/irrigation.

Cultivation process

Each sterilized plastic pot (40 cm wide and 40 cm high) was filled with (20 kg) of the previously prepared mixture of soil and compost (200:1). Then, the spore suspension of *Glomus* mycorrhizae (250 spores/mL) was added at a rate of 50 mL/pot and mixed thoroughly with soil (in treatemts contained PGPR strains) a week prior cultivation.

Just before cultivation, faba bean seeds, except uninoculated treatments, were coated with cell suspension of mixed PGPR strains using 40% sugar solution at a rate of 500 ml.Kg^{-1} seed. Then, three seeds were sown in each pot. Moreover, the soil of inoculated treatments was inoculated with cell suspension of mixed PGPR strains at a rate of 50 ml.pot^{-1}.

The boost-prepared inocula of PGPR were added after 21 and 55 days after seeding (DAS) at a rate of 50 ml.pot^{-1}. Pots were kept under greenhouse conditions until the end of the experiment and irrigated with water based on the scheme of water requirements. In three equal doses, nitrogen, phosphorus, and potassium were added at a rate of 1.2, 0.6 and 0.9 g.pot^{-1} respectively. In addition, a foliar application of humic acid was applied at a rate of 0.02 g.pot^{-1} at 15-, 30-, and 45-DAS.

Determinations

Microbiological activities

Dehydrogenase, phosphatase, and nitrogenase activities were estimated at 30 and 60 DAS. Dehydrogenase was assayed in the soil according to Hardy *et al*, (1973). The alkaline phosphatase activity was measured according to Tabatabai (1982). Nitrogenase activity in roots was assayed based on the reduction of acetylene to ethylene in quantities by gas chromatography. Acetylene reduction was performed by a modified protocol (Silvester, 1983).

NPK determination

Available forms of nitrogen and phosphorus were determined according to Bremner and Keeny (1965) and Watanabe and Oleson (1965), respectively. While, the soluble potassium was determined according to Jackson (1973).

Proline content assessment

Free proline content was estimated in leaves according to Bates *et al.* (1973). Proline concentration was read spectrophotometrically at 520 nm and calculated using a calibration curve as µmol proline g^{-1} FW.

Faba bean growth, macro element content, and yield determination

A random 3-plant sample was selected to determine faba bean vegetative growth characteristics: plant height, plant fresh weight, shoot dry weights, and No. of branches. At flowering, shoots were dried at 70°C and used for the determination of total nitrogen, phosphorus, and potassium (Chapman and Pratt, 1978; Page *et al*,1982; and A.O.A.C., 2005), respectively. Then N, P, and K uptake were estimated. Pods were harvested at the proper maturity stage, then weighed to estimate plant yield and pod length, and no. of seeds pod^{-1} were estimated. Total nitrogen content was estimated in seeds according to A.O.A.C. (2005), and total crude protein percentage was calculated by multiplying N-values by 6.25.

Statistical analysis

Statistical analysis was carried out according to Snedecor and Cochran (1989). The differences between the mean value of various treatments were compared by Duncan's multiple range test (Duncan's, 1955).

Results and Discussions

Interaction effect of PGPR strains, inorganic fertilizers and/or humic acid on some microbial enzymes movement in faba bean rhizosphere

If we looking for a guide for respiration rate and total microbial activity in soil the dehydrogenase (DH) activity is one of the efficient guides.

Additionally, alkaline phosphatase (AP) and nitrogenase (N-ase) activities are good guides for the mineralization processes of organic phosphorus substrates and as an indication of N_2-fixers activity, respectively. Data in Table (3) shows that DH, AP and N-ase activities in the soil rhizosphere inoculated with PGPR strains relatively increased compared with the control. This increase is caused by the ability of PGPR strains to produce dehydrogenase, nitrogenase, lipases, phosphatases, and proteases (Gupta *et al*, 2015). Through the activity of these enzymes, PGPR play a very significant role in plant growth promotion. Also, Abdel-Rahman *et al* (2021 a, b) reported that soil biological conditions improved after the use of nitrogen-fixing agents and acid phosphate solubilizing bacteria, as an indicator of soil microbial health.

Table 3. Interaction effect of PGPR strains, inorganic fertilizers with/without humic acid on DH activity in faba bean rhizosphere under drought stress.

Treatment	DH activity (TPF/g dry soil/h.)					
	30 days			**60 days**		
Water requirement	**50% ETC**	**75% ETC**	**100% ETC**	**50% ETC**	**75% ETC**	**100% ETC**
T1	31.5f	57.1d	66.2bc	32.1h	51.4f	65.4c
T2	44.4e	62.1c	68.3b	42.6 g	58.2e	65.3cd
T3	32.2f	57.6d	64.5bc	34.1h	56.2d	65.8cd
T4	46.1e	64.3bc	68.1b	43.18g	62.2de	67.7c
T5	54.3d	81.2a	84.3a	43.8g	82.2b	88.3a

T1: chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic+50%NPK, T4: PGPR+Humic and T5: PGPR+50%NPK+Humic, Means with a different superscript litter in the same column are significantly different at ($P<0.05$), ETC:evapo-transpiration of crop (**Water requirement of crop)**

Additionally, all treatments that were treated with humic acid showed increasing in DH and AP compared to non-sprayed ones (Table 3 and 4). Humic acid may enhance the growth of faba bean plants causing an increase in root exudates that are positively related to microbial enzymes activities.

Table 4. Interaction effect of PGPR strains, inorganic fertilizers with/without humic acid on AP activity in faba bean rhizosphere under drought stress.

Treatment	AP activity (µg P -nitrophenol /g dry soil/h.)					
	30 days			**60 days**		
Water requirement	**50% ETC**	**75% ETC**	**100% ETC**	**50% ETC**	**75% ETC**	**100% ETC**
T1	14.1k	19.4gh	21.1de	14.99j	20.29ef	21.99bc
T2	18.0i	20.2fg	22.0bc	18.89i	21.09de	22.89b
T3	16.2j	20.1fg	21.0de	17.09i	20.99de	21.89cd
T4	18.4i	20.3ef	21.7cd	19.29h	21.19de	22.59b
T5	19.3h	22.7ab	23.2ab	20.19fg	23.59ab	24.09a

T1: chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic+50%NPK, T4: PGPR+Humic and T5: PGPR+50%NPK+Humic, Means with a different superscript litter in the same column are significantly different at ($P<0.05$), ETC:evapo-transpiration of crop (**Water requirement of crop)**

Also, Bama *et al,* (2008) found that the humic acid application causes an increase in microbial enzymatic activities. Furthermore, the soil inoculated with PGPR strains combined with NPK 50% gave relatively high activity of DH compared to that treated with a full dose of NPK. Concerning the activity of N-ase, Table 5 shows that by increasing inorganic fertilizers the AP and N-ase activity decrease. This result may be due to the negative effect of nitrogenous inorganic fertilizer on N-ase or AP. These results are in agreement with those obtained by Ayuni *et al,* (2015) and Abdel-Rahman *et al,* (2021b) who found that nitrogenase activity was reduced with the increase of urea -N application. In general, the high application of inorganic nitrogen fertilizers negatively affected nitrogenase activity.

Table 5. Interaction effect of PGPR strains, inorganic fertilizers with/without humic acid on N-ase activity in faba bean rhizosphere under drought stress.

Treatment	N-ase (nmole C2H4/dry soil/h.)					
	30 days			**60 days**		
Water requirement	**50% ETC**	**75% ETC**	**100% ETC**	**50% ETC**	**75% ETC**	**100% ETC**
T1	13.7l	80.2h	142.0e	12.7l	78.3h	141.0e
T2	52.4k	102.6g	145.8e	56.6j	103.6g	144.2e
T3	21.2h	101.3g	261.4d	51.4k	102.3g	280.4d
T4	57.6j	119.7f	357.7c	71.5i	120.7f	358.7c
T5	72.6i	422.5b	539.9a	77.3h	423.5b	542.4a

T1: chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic+50%NPK, T4: PGPR+Humic and T5: PGPR+50%NPK+Humic, Means with a different superscript litter in the same column are significantly different at ($P<0.05$), ETC:evapo-transpiration of the crop (Water requirement of the crop)

Data in Table 3, 4 and 5 showed that three microbial enzymes activities were affected by changes in the ETC (Water requirement). Accordingly, the values of three enzymes in all treatments under 75 and 100 of ETC were higher than that irrigated by a half dose of ETC (50% ETC). The highest value of all microbial enzymes was recorded with plants that were inoculated with PGPR and treated with humic and 50% of NPK under 100% of ETC followed by the same treatment under 75% of ETC.

Generally, DHA, PA, and NA increased with the increase of growth periods to reach their maximum values at 60 DAS (flowering stage). These results may be due to the effect of root exudates which increase during the flowering stage and increase the multiplication rate for different soil microorganisms and their enzymes. There are many reports mention that the three enzymes activities values were higher at the flowering stage (60 DAT) rather than in another growth stage, Shams *et al*, (2013) and Abdel-Rahman *et al*, (2017) found that the densities of microbial enzymes in the rhizosphere were higher at the flowering stage of lettuce and tomato plants than other plant growth stages.

Availability and uptake of N, P and K

PGPR-inoculated soil showed an increase in available and uptake of N, P and K compared to the uninoculated one (Table 6 and 7). Co-inoculation of PGPR with inorganic fertilizer increased the availability of N, P and K in the soil rhizosphere, as is obvious in many studies (Zahir *et al*, 2012, Shedeed *et al*, 2014 and Abdel-Rahman *et al*, 2021b).

Table 6. Interaction effect of PGPR, inorganic fertilizers and/or humic acid on available N, P and K in faba bean rhizosphere.

Treatments	Available – N			Available – P			Available – K		
Water requirement	50% ETC	75% ETC	100% ETC	50% ETC	75% ETC	100% ETC	50% ETC	75% ETC	100% ETC
T1	138fg	165d	170d	105ijk	120ef	154bc	12.2d	16.7b	18.0b
T2	134gh	144f	177c	102jk	117efg	142d	12.8d	14.0cd	16.0bc
T3	128h	140fg	168d	100k	114fgh	122e	11.9d	14.0cd	16.2de
T4	134gh	158e	180c	108hij	116efg	150c	12.0d	14.0cd	16.1bc
T5	139fg	188 b	197a	112ghi	160b	173a	12.0d	18.0b	20.8a

T1: chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic+50%NPK, T4: PGPR+Humic and T5: PGPR+50%NPK+Humic, Means with a different superscript litter in the same column are significantly different at ($P<0.05$), ETC:evapo-transpiration of the crop (Water requirement of the crop)

Treated faba bean by humic acid without inoculation with PGPR gave lower available N, P and K than inoculated ones. Whereas faba bean foliar application with humic acid in combination with PGPR inoculation gave a significant increase in the availability of N, P, and K in comparison with each solely. A similar trend of results was observed in all ETC levels. The positive effect of PGPR and humic acid were confirmed by many reports for instance Vanitha & Mohandass (2014), and Abdelrahman *et al* (2021c) emphasized that humic acid application improves plant physiological processes by enhancing the availability of macro and micronutrients, especially when using in combination with PGPR inoculation.

The highest values of available N, P, and K were obtained when PGPR were applied combined with both 50% of inorganic fertilizers and humic acid under irrigation by 100% of ETC followed by the same treatment under irrigation by 75% of ETC. Respecting N, P, and k uptake, data in Table 7 observed that the records of NPK uptake showed the same trend of results as shown with available NPK. This result may due to the positive relationship between the two parameters.

The increment of available and uptake NPK in inoculated plants may be due to the positive role of inoculation with PGPR strains in improving the availability and absorption of these nutrients. These results are in agreement with those obtained by Reimer *et al*, (2020) and Abdel-Rahman *et al*, (2021a and b) who reported that the use of PGPR strains as bio-inoculants would improve the availability and supply of nutrients from the soil as well as decrease the use of artificial fertilizers, reduce emissions, and encourage sustainable agriculture.

Table 7. Interaction effect of PGPR, inorganic fertilizers and/or humic acid on N, P and K uptake.

Treatments	N mg/plant			P mg/plant			K mg/plant		
Water requirement	50% ETC	75% ETC	100% ETC	50% ETC	75% ETC	100% ETC	50% ETC	75% ETC	100% ETC
T1	**3.99b**	**4.69ab**	**5.01ab**	**0.66fg**	**0.73def**	**0.83b**	**1122.7l**	**1860.7j**	**10740.c**
T2	**4.01b**	**4.81ab**	**5.08ab**	**0.59g**	**0.72def**	**0.79bcd**	**1034.7m**	**7650.2i**	**10651.2d**
T3	**3.98b**	**4.54b**	**4.93ab**	**0.58g**	**0.71def**	**0.75cde**	**119.8n**	**9993.7h**	**10250.7f**
T4	**4.33b**	**4.85ab**	**4.91ab**	**0.64fg**	**0.72def**	**0.81bc**	**1443.8k**	**10250.2g**	**10739.1c**
T5	**4.36b**	**5.13a**	**5.20a**	**0.70ef**	**0.84ab**	**0.92a**	**1860.7j**	**10811.5b**	**10839.1a**

T1: chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic+50%NPK, T4: PGPR+Humic and T5: PGPR+50%NPK+Humic, Means with a different superscript litter in the same column are significantly different at ($P<0.05$), ETC:evapo-transpiration of the crop (Water requirement of the crop)

Growth characteristics

Data in Table (8) showed that faba bean inoculation with PGPR strains has an encouraging effect on faba bean growth characteristics i.e. plant height, plant fresh and dry weight, and the number of branches compared to uninoculated ones.

Also, data showed that plants inoculation with PGPR strains in combination with 50% NPK gave higher records of growth characteristics than that fertilized with the same dose of inorganic fertilizers and sprayed with humic acid. Whilst, the absence of inorganic fertilization led to a decrease in growth characteristics than that fertilized with either full dose or half ones. The lowest values of growth characteristics were observed in faba bean inoculated with PGPR strains combined with foliar spraying by humic acid. Additionally, plants inoculation with PGPR strains combined with 50% NPK and sprayed with humic acid gave the highest records of the above-mentioned parameters under 100% ETC irrigation followed by the same treatment under 75% ETC.

Table 8. Interaction effect of PGPR strains, inorganic fertilizers with/without humic acid on growth characteristics of faba bean under drought stress.

Treatments	Plant height (cm)			No. of branches			Plant fresh weight (g)			Plant dry weight (g)		
Water requirement	50% ETC	75% ETC	100% ETC	50% ETC	75% ETC	100% ETC	50% ETC	75% ETC	100% ETC	50% ETC	75% ETC	100% ETC
T1	74.0^{de}	80.3^{bc}	85.0^{ab}	6.3^{abc}	5.6^{bcd}	6.3^{abcd}	358^{h}	393^{e}	420^{d}	32.0h^{i}	35.5^{ef}	41.0^{bc}
T2	73.3^{ef}	79.6^{c}	81.3^{bc}	5.6^{bcd}	5.6^{bcd}	6.6^{ab}	344^{e}	373^{f}	443^{c}	31.0^{i}	34.0$^{f.g}$	40.0^{c}
T3	69.0^{f}	79.3^{c}	81.3^{bc}	5.0^{cd}	5.3^{bcd}	6.3^{abcd}	334^{i}	370^{fg}	412^{d}	30.4^{i}	34.0^{fg}	38.0^{d}
T4	69.0^{f}	78.3^{cd}	81.3^{bc}	4.3^{d}	5.0^{cd}	5.0^{cd}	328^{i}	362^{gh}	399^{j}	28.0^{j}	33.9^{gh}	37.0^{de}
T5	78.0^{cd}	88.6^{a}	89.3^{a}	5.6^{bcd}	7.0^{ab}	7.6^{a}	358^{h}	485^{b}	531^{a}	32.0^{hi}	42.0^{ab}	42.8^{a}

T1: chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic+50%NPK, T4: PGPR+Humic and T5: PGPR+50%NPK+Humic, Means with a different superscript litter in the same column are significantly different at ($P<0.05$), ETC:evapo-transpiration of the crop (Water requirement of the crop)

The beneficial effect of the used PGPR on the growth characteristics of faba bean may be attributed to their ability to produce IAA, gibberellins, solubilization of phosphate and potassium, their ability to fix atmospheric nitrogen as well as enhancement of root spread and its surface area and growth under drought conditions. The role of PGPR in plant growth, and nutrient management is very well established. PGPR colonize the rhizosphere/endo-rhizosphere of plants and promote the growth of the plants through various direct and indirect mechanisms (Grover *et al*., 2011). Furthermore, the role of microorganisms in the management of biotic and abiotic stresses is gaining importance. The possible explanation for the mechanism of plant drought tolerance induced by rhizobacteria include: the production of phytohormones like abscisic acid, gibberellic acid, cytokinins, and indole-3-acetic acid; ACC deaminase to reduce the level of ethylene in the roots; induced systemic tolerance by bacterial compounds; and bacterial exopolysaccharides (Timmusk and Nevo, 2011; Kim *et al*., 2013; Timmusk *et al.*, 2014). Also, accumulation of osmolytes, exopolysaccharide production, and antioxidant defense were observed. Moreover, induced systemic tolerance has been coined to accommodate the microbial-induced physical and chemical changes in plants, which result in enhanced tolerance to abiotic stresses (Yang *et al.*, 2009).

Plant growth-promoting rhizobacteria (PGPR) strains can increase plant height and fresh and dry weight. The ameliorative effects of PGPR application may be due to the increase of plant cell water potential and decrease the electrolytic leakage and reduces plant sodium ion concentration and increase salicylic acid and gibberellins synthesis (Kang *et al*, 2014).

Proline content of faba bean fruits

Obtained data in **Table (9)** revealed that significant variances in leaves proline content were detected due to drought stress during the study. Herein, the obtained results revealed that leaves proline content significantly increased by decreasing the irrigation water rate as compared to control (100% ETC). Moreover, the most remarkable increase in proline content resulted from the lowest level of water irrigation. Whereas all treatments inoculated with PGPR strains gave higher values of proline content than that uninoculated ones. This result observed the role of inoculation in drought stress decreasing where proline might act as an osmoregulator compound against salinity and drought stress and its accumulation is considered as an adaptive response to stress conditions **Handa *et al.*, (1985)**. Plants adaptation to drought stress is associated with metabolic adjustments that lead to the accumulation of several compatible solute/osmolytes like proline, sugars, polyamines, betaines and other amino acids and water stress proteins like dehydrins (Yancey *et al.*, 1982; Close, 1996). PGPR secrete osmolytes in response to drought stress, which act synergistically with plant-produced osmolytes and stimulates plant growth (Paul *et al.*, 2008).

Table 9. Interaction effect of PGPR strains, inorganic fertilizers with/without humic acid on Proline content in faba bean leaves under drought stress.

Treatments	Proline content (µmol proline g^{-1} FW)		
Water requirement	50% ETC	75% ETC	100% ETC
T1	9.9de	6.4h	2.4j
T2	11.3c	9.9de	6.8gh
T3	11.0c	9.6e	4.3i
T4	12.0b	10.2d	7.1g
T5	16.0a	12.0b	7.8f

T1: chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic+50%NPK, T4: PGPR+Humic and T5: PGPR+50%NPK+Humic, Means with a different superscript litter in the same column are significantly different at (P<0.05), ETC: evapo-transpiration of the crop (Water requirement of the crop)

PGPR *Pseudomonas putida* GAP-P45 inoculation improved plant biomass, relative water content and leaf water potential by the accumulation of proline in plants exposed to drought stress (Sandhya *et al*., 2010). Generally, the high values of proline content were recorded with treatments that were inoculated with PGPR, sprayed with humic acid, and fertilized with 50% NPK. Additionally, El-Meihy (2016) demonstrated the ability of *Pseudomonas flouresence* to synthesize proline in its culture medium at 15.06 µg/mL. When using the proline producer-strain as an inoculum for plants under drought conditions, the proline that is a compatible solute possesses a biosynthetic pathway designated as an osmotic inhibitor in response to osmotic stress.

Faba bean yield

Data in Table 10 showed that the yield, pod length, and the number of seed.pod^{-1} of the treatments that were irrigated by 50% or 75% of ETC and inoculated with PGPR strains significantly increased in comparison with those un-inoculated ones.

Table 10. Interaction effect of PGPR strains, inorganic fertilizers with/without humic acid on faba bean yield under drought stress.

Treatments	Pod length (cm)			No. of seeds/pod			Seeds yield (g)/ plant		
Water requirement	50% ETC	75% ETC	100% ETC	50% ETC	75% ETC	100% ETC	50% ETC	75% ETC	100% ETC
T1	9.7e	13.7cd	16.0bc	3.0e	4.0bc	5.3ab	221.0d	369.3abcd	501.3a
T2	10.3dc	14.0bcd	16.7abc	3.3de	4.0cde	5.6ab	288.0cd	401.0abc	495.7ab
T3	10.3dc	13.7cd	16.0bc	3.3de	4.0cde	4.6abc	266.2cd	389.0abc	469.0ab
T4	10.7de	14.7bc	17.7ab	3.6cd	4.3bc	5.0abc	315.3bcd	408.3abc	498.5a
T5	13.6cd	17.7ab	20.0a	3.6cde	5.6ab	6.0a	340.3bcd	503.3a	516.0a

T1: chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic+50%NPK, T4: PGPR+Humic and T5: PGPR+50%NPK+Humic, Means with a different superscript litter in the same column are significantly different at (P<0.05), ETC:evapo-transpiration of the crop (Water requirement of the crop)

This result is reflecting the role of PGPR inoculation in enhancing plant growth and productivity, especially under drought stress conditions. Many reports confirmed this fact, as reported by Abd Alla et al (2015) and Abdel-Rahman *et al* (2017) who stated that PGPR competitively colonize plant roots and enhance plant growth by several mechanisms that have a positive effect on its production. Enhancing the diversity of PGPR in the rhizosphere along with their colonization ability and mechanism of action would facilitate their wider application in the management of sustainable agricultural crop production. Further, PGPR is being functioned as a connecting link between plants and microbes that could express antagonistic and synergistic interactions with microorganisms and the soil (Shukla, 2019).

Moreover, faba bean inoculation with PGPR strains in combination with 50% NPK gave higher values of the above-mentioned criteria than that foliar sprayed with humic acid in combination with 50% NPK. Also, results indicated that the treatment of faba bean with humic acid, PGPR, and inorganic NPK recorded a significant increase in yield and yield components compared to each one individually. This result shows the synergistic effect of either humic acid or PGPR inoculation in presence of inorganic NKP in increasing the uptake of some nutritional elements, improving plant growth and maximizing plant yield. It is worthy to notice that there are no significant differences between faba bean yield in T4 either in 75% ETC or 100% ETC. Therefore, it could be concluded that the application of PGPR and humic substances could improve plant growth under drought conditions.

Total protein in faba bean seeds

Concerning the total protein in faba bean seeds, data in Table 11 showed that the lowest records of total protein were obtained when plants were irrigated with a rate of 50% ETC. The total protein of plant seeds was increased by increasing the irrigation rate, and this is fat under all treatments.

Table 11. Interaction effect of PGPR strains, inorganic fertilizers with/without humic acid on total protein of faba bean seeds under drought stress.

Treatments	**Total protein (%)**		
Water requirement	**50% ETC**	**75% ETC**	**100% ETC**
T1	20.5e	28.4cd	30.8ab
T2	25.1e	30.1abc	31.3ab
T3	24.8e	29.3bcd	30.7abc
T4	27.1de	30.3abc	31.8ab
T5	27.3de	32.1a	32.5a

T1: chemical fertilization (100% NPK), T2: PGPR+50%NPK, T3: Humic+50%NPK, T4: PGPR+Humic and T5: PGPR+50%NPK+Humic, Means with a different superscript litter in the same column are significantly different at ($P<0.05$), ETC:evapo-transpiration of the crop (Water requirement of the crop)

Moreover, the inoculation of plants with PGPR strains in combination with 50% NPK gave higher values of total protein than that treated with humic acid and fertilized with 50% NPK. This may be due to the act of N_2- fixers in increasing nitrogen uptake (Table 6), which is reflected in total protein. Inoculation of plants with PGPR strains combined with 50% NPK and sprayed with humic acid gave the highest records of total protein under all irrigation rates. Generally, there are no significant differences between the previously mentioned treatment in the plants that were irrigated with 75% ETC and the same treatment under 100%ETC.

Conclusion

Drought stress decreases nutrient diffusion, and nutrient uptake and reduces vegetative growth as well as its effect on plant yield. Drought is one of the main factors that limit the productivity of plants in their natural habitats. In addition, PGPR might alleviate drought effects by increase nutrient uptake by plants from soil and meanwhile reducing inorganic fertilizer requirements. So, this study raises the role of PGPR inoculation in combination with humic acid as a foliar application for alleviating drought stress on faba bean productivity. Form obtained data, it could be concluded that the application of PGPR inocula and humic acid as foliar application can save half of the fertilizer requirements and minimize environmental pollution. Moreover, they can reduce crop water requirement to 75% ETC without nonsignificant reduction of faba bean yield. So using PGPR inoculation in combination with humic acid and 50% inorganic NPK can be reliable to promote plant growth, increase crop production, and decrease water requirements of crop as well as decrease production costs under drought stress. Despite obtained results, using PGPR and anther biological factors in allevaiting drought stress need further studies.

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التخفيف من إجهاد الجفاف في الفول باستخدام حمض الهيوميك والتلقيح بالبكتريا الجذرية المشجعة لنمو النبات

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الجفاف هو أحد القيود الرئيسية للإنتاجية الزراعية في جميع أنحاء العالم ومن المرجح أن يزداد. تم إجراء هذا العمل لدراسة دور التلقيح بالبكتيريا الجذرية **المشجعة** النبات (PGPR) بالاشتراك مع حمض الهيوميك كتطبيق ورقي للتخفيف من إجهاد الجفاف على إنتاجية الفول. اظهرت النتائج انه يمكن أن يؤدي تلقيح PGPR بحمض الهيوميك كتطبيق ورقي إلى توفير نصف متطلبات الأسمدة بالإضافة إلى تقليل متطلبات المياه للمحاصيل إلى 75 ٪ ETCمن المتطلبات المائية دون انخفاض كبير في محصول الفول. الفول الملقح بـ PGPR مع حمض الهيوميك و 50٪ NPKغير العضوي كان له تأثير على نشاط الإنزيمات الميكروبية ، وامتصاص المغذيات ، وخصائص النمو الخضري بالإضافة إلى أنه كان لديه مؤشر إجهاد أقل (محتوى البرولين) مقارنة بالتحكم. لذلك ، يمكن الاعتماد عليها لتعزيز نمو الفول ، وزيادة إنتاجيته ، مع تقليل متطلباته المائية ، وبالتالي تقليل تكاليف الإنتاج في ظل إجهاد الجفاف.