Journal of Plant Physiology and Breeding

2022, 12(2): 59-70 ISSN: 2008-5168

Research paper

Effect of nitrogen-fixing bacteria and mycorrhiza on biochemical properties and absorption of essential elements in green pea (*Pisum sativum* L.) under water deficit stress

Mehdi Ghasembaghlou*, Mohammad Sedghi, Raouf Seyed Sharifi, and Salim Farzaneh

Received: June 6, 2022 Accepted: September 3, 2022
Department of Agronomy and Plant Breeding, University of Mohaghegh Ardabili, Ardabil, Iran
*Corresponding author; Email: gasembagloomehdi@yahoo.com

Abstract
Drought stress is a critical abiotic stress that has a negative effect on plant productivity. Plant growth-promoting rhizobacteria (PGPR) such as *Azotobacter* and *Azospirillium* positively affect plant physiology, especially under drought stress. The recent study aimed to examine the effects of *Mycorrhiza* fungi and PGPR on the activity of antioxidant enzymes and the amount of nutrient absorption under water deficit conditions. A factorial experiment was performed based on a randomized complete block design with three replications. Factors were irrigation (regular irrigation, water deficit at the grain filling stage, water deficit at the flowering stage, no irrigation) and Mycorrhiza fungi and PGPR (*Azotobacte rcoroccum*, *Azospirillum lipoferum*, *Mycorrhiza arbuscular*as, *Azotobacter coroccum* + *Azospirillum lipoferum*, *Azotobacte rcoroccum* + *Mycorrhiza arbuscular*, *Azospirillum lipoferum* + *Maycorrhiza arbuscular*, *Azotobacte rcoroccum* + *Azospirillum lipoferum* + *Mycorrhiza arbuscular*, *no* inoculation). Drought stress decreased chlorophyll content and absorption of elements and increased proline, catalase, and peroxidase activity. Also, biofertilizers reduced the amount of proline, catalase, and peroxidase. They increased the amount of chlorophyll content and absorption of elements.

Keywords: antioxidant enzyme; biofertilizer; drought; nutrients

How to cite: Ghasembaghlou M, Sedghi M, Seyedsharifi R, and Farzaneh S, 2022. Effect of nitrogen-fixing bacteria and mycorrhiza on biochemical properties and absorption of essential elements in green pea (*Pisum sativum* L.) under water deficit stress. Journal of Plant Physiology and Breeding 12(2): 59-70.

Introduction

As critical abiotic stress, water deficit stress causes severe changes in plant metabolism. Moreover, by increasing the atmospheric CO2 concentration due to climate change, the rate of photosynthesis and water consumption efficiency is increased. Although this phenomenon improves crop performance, it negatively affects plant productivity (Shirinbayan *et al*. 2019). The occurrence of high temperatures during the reproductive growth stage (besides drought) is harmful to many important vegetable crops, such as tomatoes, peppers, beans, and sweet corn (Ray 2015). The frequency of drought periods can decrease vegetable yield and quality, however, dry matter content in some crops increases by deficit water stress (Nemeskeri *et al*. 2019). The plant's response to existing stress depends on its severity, duration, and development stage.

Green pea (*Pisums sativum* L.) efficiently utilizes the spring precipitation. However, its flowering and seed development coincides with the dry June and July environment, which requires irrigation. Irrigation programming and the water requirement depend on the plant varieties' water use and drought tolerance (Zaman 2012).

The moisture content and biomass are mainly affected by drought stress in plants (Kamara *et al*. 2009). Drought stress has a negative influence on nutrient uptake and translocation as the soil nutrients mobility depends on water flow. Therefore, soil water limitations reduce the absorption of nutrients (Atouei *et al*. 2019). Moreover, drought stress causes the production of oxygen free radicals, which can damage the plant defense system and increase reactive oxygen species (ROS) (Farooq *et al*. 2014).

Inoculation of seeds by plant growth-promoting rhizobacteria (PGPR) might be a sustainable method to alleviate the effect of drought stress in crop production. PGPR readily colonizes the root rhizosphere and establishes facultative and obligate associations with host plants. More frequently, these interactions enhance crop productivity and ameliorate the biotic/abiotic stresses through known and unknown mechanisms (Vurukonda *et al*. 2016; Barnawal *et al*. 2017; Forni*et al*. 2017).

Some bacteria such as Azotobacter, Bacillus, Klebsiella, Pseudomonas, Azospirillum, and Serratia are effective microorganisms (Parray*et al*. 2016), and these PGPRs have helpful effects on plant growth and development via direct or indirect mechanisms (Ngumbi and Kloepper 2016; Enebe and Babalola 2018). Direct interference of PGPRs with plants can be in nutrient uptake, phytohormones and siderophoresynthesis, and enhancement of antioxidant systems (Kang *et al*. 2014; You and Chan 2015; Parray*et al*. 2016). Oxidative damage occurs when the production of ROS disrupts and destroys lipid membranes and chlorophyll (Meher *et al*. 2018). Kang *et al* (2014) demonstrated that inoculation of soybean seeds with *Pseudomonas putida* reduced the antioxidant activity compared to the control under water stress.

Since water availability for crop production has become a significant problem in agricultural ecosystems, especially in arid and semi-arid areas, this experiment aimed to determine the efficacy of biofertilizers in inducing tolerance in the green pea plants through studying the antioxidant systems and mineral uptake under water deficit stress.

Materials and Methods

This research was conducted in Goomand, East Azarbaijan Province, Iran (semi-arid climate, 38° 6′ N, 46° 27′ E) during the 2018-2019 growing season. The average annual temperature and average annual rainfall for this region are 19.5 °C and 342 mm, respectively and the soil texture is clay-loam. The experiment was conducted as factorial arranged in a randomized complete block design with three replications. Factors were irrigation regimes at four levels, including full irrigation (I_1, without stress as the control), water deficit at the grain filling stage (I_2), water shortage at the flowering stage (I_3) and without irrigation (I_4), and biofertilizers consisted of bacteria and Mycorrhiza inoculation: *Azotobacter coroccum* (F_1), *Azospirillum lipoferum* (F_2), *Mycorrhiza arbuscular* (F_3), *Azotobacter coroccum* + *Azospirillum lipoferum* (F_4), *Azotobacter coroccum* + *Mycorrhiza arbuscular* (F_5), *Azospirillum lipoferum* + *Maycorrhiza arbuscular* (F_6), *Azotobacter coroccum* + *Azospirillum lipoferum* + *Mycorrhiza arbuscular* (F_7) and a non-inoculated control (F_8).

Inoculated green pea (*Pisum sativum* L.) seeds

(Emraz cultivar) were sown in 3×2 m^2 plots on April 4 in both years, in which rows and plant spacing was 25 and 5 cm, respectively. Seed inoculation with *Azotobacter coroccum* and *Azospirillum lipoferum* was performed using 100 mL of inoculant (2 * 108 CFU mL^{-1}) per 50 kg of seeds, which were homogenized and kept in the shade for 30 min. *Mycorrhiza arbuscular* was mixed with field soil. *Pisums sativum* seeds were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA).

Proline content was measured according to Bates *et al* (1973). A 0.04 g of fresh leaves were homogenized in 100 ml of 3% sulfosalicylic. Then, 2 ml of the prepared solution was added to 2 ml of ninhydrin, 2 ml of pure acetic acid, and 64 ml of toluene. After forming two layers, the proline content was estimated by a Hitachi spectrophotometer (Japan) at 520 nm. The data was recorded as µmol per g fresh weight (µmol g^{-1} FW). Catalase activity was assessed in the leaves with a method described by Sinha (1972). A 1.5 mL of the reaction mixture including 1 mL phosphate buffer (0.01 M, pH 7.0), 0.4 mL distilled water, and 0.1 mL of centrifugation supernatant was prepared. By adding 0.5 mL H_2O_2 (320 mM) the reaction started, and then, incubated at 25 °C at different time intervals. The reaction was stopped by adding 2 mL of dichromate: acetic acid reagent (1:3 ratio). The tubes were instantly placed and kept in a boiling water bath for 20 minutes and then, centrifuged for 15 minutes (1500 g). Absorbance was read at 570 nm with a spectrophotometer. The enzyme activity was reported as nmol H_2O_2 consumed min^{-1} mg^{-1} protein. Peroxidase activity was measured according to Chance and Maehly (1955). The reaction mixture included 3,3'-diaminobenzidine-tetra hydrochlorides dehydrate solution containing 0.1% (w/v) gelatin, 150 mM Na-phosphate-citrate buffer (pH 4.4), and 0.6% H_2O_2. Absorbance was read for 5 min at 465 nm by a spectrophotometer (PowerWave XS, BioTek, USA). The chlorophyll index was measured from the youngest fully developed leaf with a chlorophyll meter (CCM-200 plus).

Total N in the shoots was measured by Kjeldahl digestion of ground and dried samples (Anonymous 1980). The phosphorus content was measured by vanadomolybdate phosphoric acid method (Jackson 1973), and the potassium concentration was assessed by a flame photometer.

Analysis of variance was performed after verifying the assumptions of normality and homoscedasticity. Data were analyzed with SAS 9.1 software. The means were compared using Duncan's multiple range test at $p \leq 0.05$.

Results and Discussion

Proline content

The interaction of drought stress and biofertilizers was significant for the proline content (Table 1). The highest amount of proline (2.97 µmol g^{-1} FW) was obtained in the control (without the biofertilizer and irrigation). The lowest amount of proline (0.69 µmol g^{-1} FW) was obtained in the combined treatment of three biofertilizers and normal irrigation (Table 2). Reduction in proline destruction, promoting its synthesis from glutamate, or increasing protease activity may be the reasons for the accumulation of proline under stress (Sharma and Kuhad 2006). The regulation of

proline metabolism by ABA (Schutz and Fangmeir 2001) and high-energy compounds in photosynthesis that stimulate proline synthesis also may contribute to proline accumulation. Proline accumulates in the cell under stress conditions because it protects cytosolic enzymes and cell structure (Fang and Xiong 2015). PGPRs increase phosphorus absorption and other elements necessary for plant growth and development and protect plants from stress (Gilik *et al*. 2001). Plants inoculated with biofertilizers have better water relations and balanced nutrient status than non-inoculated ones (Lozano-Ruiz 2003).

Catalase activity

The highest activity of catalase (0.70 nmol min^{-1} mg^{-1} protein) was observed in the combination of three microorganisms at non-irrigated conditions, and the lowest activity (0.14 nmol min^{-1} mg^{-1} protein) was obtained in the combined treatment of all three fertilizers at normal irrigation conditions (Table 3).

CAT removes the hydrogen peroxide (H_2O_2) in the plant peroxisomes (Agarwal and Pandey 2004). El Negar *et al*. (2017) reported that under control conditions (no stress) and irrigation level of 75% field capacity (moderate stress), H_2O_2 concentration was not considerably increased in the quinoa cultivars. However, at severe drought conditions, the concentration of H_2O_2 increased, and quinoa enhanced the catalase synthesis to scavenge it.

Table 1. Analysis of variance for the studied traits of green peas under different levels of water deficit and biofertilizers

Treatments	df	Mean squares						
		Proline	CAT	POX	Chlorophyll	N	P	K
Replication	2	0.009^{ns}	0.0001^{ns}	0.001^{ns}	0.189^{ns}	0.003^{ns}	0.0015^{ns}	0.001^{ns}
Irrigation (I)	3	10.95^{**}	1.01^{**}	2.79^{**}	587.67^{**}	5.74^{**}	0.031^{**}	1.97^{**}
Biofertilizer (F)	7	0.98^{**}	0.004^{**}	0.01^{**}	82.67^{**}	3.52^{**}	0.035^{**}	0.53^{**}
I × F	21	0.10^{**}	0.004^{**}	0.03^{**}	5.97^{**}	0.03^{**}	0.0002^{**}	0.02^{**}
Error	62	0.001	0.0001	0.0001	0.37	0.002	0.0001	0.0001
CV (%)	-	1.98	1.82	1.82	3.79	1.27	1.50	1.17

ns, **: Not significant and significant at $p \leq 0.01$, respectively; CAT: Catalase, POX, Peroxidase.

Table 2. Proline content for the combination of biofertilizers with water-deficit stress conditions in green peas.

Biological fertilizers	I_1	I_2	I_3	I_4
F_1	1.09m	1.31k	2.44d	2.70b
F_2	1.03mn	1.30k	2.42d	2.67b
F_3	0.82p	1.28k	2.27f	2.58c
F_4	0.94o	1.25k	1.81g	2.06f
F_5	0.79p	0.96o	1.62i	2.04f
F_6	0.96o	1.18l	1.85g	2.21e
F_7	0.69q	0.97no	1.30k	1.72h
F_8	1.160l	1.42j	2.56c	2.97a

Different letters in each column indicate a significant difference at $p \leq 0.01$ (Duncan's multiple range test); I_1, I_2, I_3, I_4: regular irrigation, water shortage at the grain filling stage, water shortage at the flowering stage and no irrigation, respectively; F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8: *Azotobacter coroccum*, *Azospirillum lipoferum*, *Mycorrhiza arbuscular*, *Azotobacter coroccum* + *Azospirillum lipoferum*, *Azotobacter coroccum* + *Mycorrhiza arbuscular*, *Azospirillum lipoferum* + *Maycorrhiza arbuscular*, *Azotobacter coroccum* + *Azospirillum lipoferum* + *Mycorrhiza arbuscular*, control.

Table 3. The catalase activity for the combination of biofertilizers with water-deficit stress conditions in green peas.

Biological fertilizers	I_1	I_2	I_3	I_4
F_1	0.17op	0.21n	0.45g	0.58d
F_2	0.20n1	0.29i	0.46g	0.61c
F_3	0.15pq	0.23mn	0.45h	0.58d
F_4	0.18o	0.24klm	0.52ef	0.64b
F_5	0.15opq	0.26jk	0.53ef	0.65b
F_6	0.16opq	0.25kl	0.50f	0.64b
F_7	0.14q	0.23lmn	0.53e	0.70a
F_8	0.22mn	0.28ij	0.43h	0.56d

Different letters in each column indicate a significant difference at $p \leq 0.01$ (Duncan's multiple range test); I_1, I_2, I_3, I_4: regular irrigation, water shortage at the grain filling stage, water shortage at the flowering stage and no irrigation, respectively; F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8: *Azotobacter coroccum*, *Azospirillum lipoferum*, *Mycorrhiza arbuscular*, *Azotobacter coroccum* + *Azospirillum lipoferum*, *Azotobacter coroccum* + *Mycorrhiza arbuscular*, *Azospirillum lipoferum* + *Maycorrhiza arbuscular*, *Azotobacter coroccum* + *Azospirillum lipoferum* + *Mycorrhiza arbuscular*, control.

Peroxidase activity

Due to the interaction between the drought stress and biofertilizer, the highest amount of peroxidase (1.38 nmol min^{-1} mg^{-1} protein) was achieved in a combination of three biofertilizers at the non-irrigated conditions, and the lowest amount (0.31 nmol min^{-1} mg^{-1} protein) which was related to the combination of three fertilizers and normal irrigation (Table 4).

Peroxidase plays a role in some cellular processes in higher plants under stress, such as defense mechanisms, glycoprotein cross-linking rich in hydroxyl proline monomers in the cell wall, and cross-linking of pectic polysaccharides with phenolic acids (Das and Roychoudhury 2014). Hinojosa *et al* (2018) evaluated the effect of drought stress on quinoa. They reported that peroxidase has an essential role in protecting against environmental stresses and detoxifying H_2O_2. Although chemical fertilizers have a positive effect on quantity and quality of quinoa under drought stress, it has been reported that an adequate supply of nutrients through inoculation with biofertilizers can balance nutrients required by plants and produce sufficient energy to escape oxidative and osmotic stress (Basra e*t al*. 2014). Applying Mycorrhiza and PGPRs together increases antioxidant production, reduces ROS, and protects cells against oxidative stress (Fouad *et al*. 2014).

Effect of biofertilizer and water deficit stress on the chlorophyll index

The highest chlorophyll index (30.01) was observed in the mixture of three biofertilizers at the normal irrigation conditions, and the lowest chlorophyll index (8.300) was related to the treatment with no biofertilizer at non-irrigated conditions (Table 5). No significant difference was observed between *Azospirillium* and *Mycorrhiza* treatments.

Drought stress significantly reduces the chlorophyll content in soybean leaves (Sairam *et al*. 2011). The formation of chloroplasts during leaf growth depends on nitrogen supply which increases leaf chlorophyll (Singh *et al*. 2016). The increase in the chlorophyll content in the plant,

Table 4. The peroxidase activity for the combination of biofertilizers with water-deficit stress conditions in green peas.

Biological fertilizers	I1	I2	I3	I4
F_1	0.42q	0.53m	0.83jk	1.02f
F_2	0.43q	0.52n	0.81k	1.06e
F_3	0.38r	0.43q	0.85j	0.98g
F_4	0.43q	0.50o	0.91i	1.12d
F_5	0.35s	0.42q	0.93hi	1.21b
F_6	0.36s	0.43q	0.93hi	1.16c
F_7	0.31t	0.37rs	1.13d	1.38a
F_8	0.45p	0.53mn	0.71l	0.93h

Different letters in each column indicate a significant difference at $p \leq 0.01$ (Duncan's multiple range test); I_1, I_2, I_3, I_4: regular irrigation, water shortage at the grain filling stage, water shortage at the flowering stage and no irrigation, respectively; F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8: *Azotobacter coroccum*, *Azospirillum lipoferum*, *Mycorrhiza arbuscular*, *Azotobacter coroccum* + *Azospirillum lipoferum*, *Azotobacter coroccum* + *Mycorrhiza arbuscular*, *Azospirillum lipoferum* + *Maycorrhiza arbuscular*, *Azotobacter coroccum* + *Azospirillum lipoferum* + *Mycorrhiza arbuscular*, control.

Table 5. The chlorophyll index for the combination of biofertilizers with water-deficit stress conditions in green peas.

Biological fertilizers	I_1	I_2	I_3	I_4
F_1	21.37d	17.89ef	14.27l	12.44jk
F_2	19.29e	17.82ef	13.22ijk	10.77im
F_3	22.63cd	17.28fg	12.09kl	10.30m
F_4	21.26d	18.41ef	13.29ijk	9.92m
F_5	24.40b	18.87e	13.45ijk	12.36jk
F_6	23.06bc	18.85e	13.81ij	12.35jk
F_7	30.01a	24.05b	16.16gh	13.64ij
F_8	15.80h	12.28jk	9.58mn	8.30n

Different letters in each column indicate a significant difference at $p \leq 0.01$ (Duncan's multiple range test); I_1, I_2, I_3, I_4: regular irrigation, water shortage at the grain filling stage, water shortage at the flowering stage and no irrigation, respectively; F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8: *Azotobacter coroccum*, *Azospirillum lipoferum*, *Mycorrhiza arbuscular*, *Azotobacter coroccum* + *Azospirillum lipoferum*, *Azotobacter coroccum* + *Mycorrhiza arbuscular*, *Azospirillum lipoferum* + *Maycorrhiza arbuscular*, *Azotobacter coroccum* + *Azospirillum lipoferum* + *Mycorrhiza arbuscular*, control.

subsequently enhances the sunlight absorbance and produce more assimilates, finally increasing plant yield (Salehi *et al*. 2016). Chlorophyll concentration is an indicator of source strength, so photosynthesis and yield reduction under drought stress can be due to the decrease in chlorophyll concentration. Therefore, the protection of the chlorophyll under stress conditions may help the stability of the plant's performance. Fungal hyphae penetrate the internal tissues of the roots and form a complementary adsorption system, increasing root expansion in the soil. As a result, the roots gain access to more room in the soil (Allen *et al*. 1982).

Effect of biofertilizer and water-deficit stress on the nitrogen content

The combined treatment of three biofertilizers had a higher amount of nitrogen (4.96%) compared to the control treatment with no biofertilizer at non-irrigated conditions (2.09%). The highest

percentage of nitrogen was obtained at normal irrigation + a combination of three types of biofertilizers (4.96%). The lowest percentage of nitrogen (2.09%) was observed in the treatment without biofertilizer at the non-irrigated conditions (Table 6). Behl *et al.* (2006) reported that the application of *Aztobacter* significantly increased grain yield, number of tillers, dry matter yield, nitrogen, phosphorus, and potassium uptake in the wheat. PGPRs can activate some enzymes involved in nitrogen metabolism, such as nitrate reductase, and improves nitrogen level under water-deficit stress conditions (Ansari and Ahmad 2019). The higher the soil moisture content, the more nitrogen is absorbed by the plant (Jones 1980).

Effect of biofertilizer and water-deficit stress on the phosphorus content

The highest amount of phosphorus (0.37%) was observed in the combined treatment of three biofertilizers at normal irrigation conditions (Table 7). Drought stress reduced phosphorus uptake compared to non-irrigated conditions in all biofertilizer types.

Under a drought environment, the diffusion rate of phosphorus from soil to roots is slower than other nutrients owing to adhering of phosphate ions to the clay particles and becoming less available to the roots (Marschner 1995). The ability of soybean roots to absorb phosphorus is weak due to the reduced movement of phosphorus in soils with low water potential (Marschner 1995).

The combined application of three types of biofertilizers increased the amount of phosphorus by 85% at normal irrigation conditions and by 133% when the plants were not irrigated compared to the related controls. The use of a combination of nitrogen-fixing and phosphate-solubilizing bacteria combined with the help of 50% of the regular nitrogen, phosphorus, and potassium fertilizers in the sour tea herb, led to an increase in the percentage of nitrogen and phosphorus in the leaves and the amount of anthocyanin, vitamin C, and pH in the sepals (Abo-Baker and Mostafa

Table 6. The N content (%) for the combination of biofertilizers with water-deficit stress conditions in green peas.

Biological fertilizers	I_1	I_2	I_3	I_4
F_1	4.62b	4.41d	3.83hi	3.31k
F_2	4.25e	4.03g	3.61j	3.05l
F_3	3.72i	3.29k	2.71m	2.45n
F_4	4.22ef	4.13fg	3.81hi	3.33k
F_5	4.45cd	4.28g	3.89h	3.56j
F_6	4.28e	4.12fg	3.71i	3.39k
F_7	4.96a	4.55bc	4.19ef	3.81hi
F_8	3.31k	3.14l	2.54n	2.09o

Different letters in each column indicate a significant difference at $p \leq 0.01$ (Duncan's multiple range test); I_1, I_2, I_3, I_4: regular irrigation, water shortage at the grain filling stage, water shortage at the flowering stage and no irrigation, respectively; F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8: *Azotobacter coroccum*, *Azospirillum lipoferum*, *Mycorrhiza arbuscular*, *Azotobacter coroccum + Azospirillum lipoferum*, *Azotobacter coroccum + Mycorrhiza arbuscular*, *Azospirillum lipoferum + Maycorrhiza arbuscular*, *Azotobacter coroccum + Azospirillum lipoferum + Mycorrhiza arbuscular*, control.

Table 7. The P content for the combination of biofertilizers with water-deficit stress conditions in green peas.

Biological fertilizers	I_1	I_2	I_3	I_4
F_1	0.27hij	0.25jklmn	0.23nop	0.20q
F_2	0.26ijk	0.25jklmn	0.22opq	0.21pq
F_3	0.34cde	0.30fg	0.26ijkl	0.23mnop
F_4	0.28hi	0.26jklm	0.24mno	0.21pq
F_5	0.36ab	0.33def	0.31fg	0.26ijk
F_6	0.35abc	0.31f	0.29gh	0.24klmno
F_7	0.37a	0.34bcd	0.32ef	0.28hi
F_8	0.20qr	0.18r	0.15s	0.12t

Different letters in each column indicate a significant difference at $p \leq 0.01$ (Duncan's multiple range test); I_1, I_2, I_3, I_4: regular irrigation, water shortage at the grain filling stage, water shortage at the flowering stage and no irrigation, respectively; F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8: *Azotobacter coroccum*, *Azospirillum lipoferum*, *Mycorrhiza arbuscular*, *Azotobacter coroccum + Azospirillum lipoferum*, *Azotobacter coroccum + Mycorrhiza arbuscular*, *Azospirillum lipoferum + Maycorrhiza arbuscular*, *Azotobacter coroccum + Azospirillum lipoferum + Mycorrhiza arbuscular*, control.

2011).

Effect of biofertilizer and water-deficit stress on potassium content

The highest amount of potassium (2.75%) was obtained when three biofertilizers were combined at the non-irrigated conditions, and the lowest amount of potassium (1.35%) was observed in the full irrigation conditions when no biofertilizer was used (Table 8). The increase in the potassium content following drought stress in our experiment was not consistent with the results of some researchers, which reported a decrease in the potassium content under drought conditions. They attributed this decrease to the reduction of soil water, which leads to the decline in the flow of elements from the soil to the plant (Wu and Xia 2006). However, biofertilizers increased the amount of potassium at all irrigation conditions compared to the related controls.

Table 8. The K content for the combination of biofertilizers with water-deficit stress conditions in green peas.

Biological fertilizers	I1	I2	I3	I4
F_1	1.49r	1.78lm	1.96i	2.15f
F_2	1.46s	1.59q	1.76m	1.96i
F_3	1.58q	1.90j	2.00h	2.38d
F_4	1.62p	1.79l	1.97i	2.09g
F_5	1.64p	1.88k	1.96i	2.56b
F_6	1.73n	1.96i	2.16f	2.53c
F_7	1.88jk	2.02h	2.19e	2.75a
F_8	1.35t	1.46s	1.67o	1.76m

Different letters in each column indicate a significant difference at $p \leq 0.01$ (Duncan's multiple range test); I_1, I_2, I_3, I_4: regular irrigation, water shortage at the grain filling stage, water shortage at the flowering stage and no irrigation, respectively; F_1, F_2, F_3, F_4, F_5, F_6, F_7, F_8: *Azotobacter coroccum*, *Azospirillum lipoferum*, *Mycorrhiza arbuscular*, *Azotobacter coroccum + Azospirillum lipoferum*, *Azotobacter coroccum + Mycorrhiza arbuscular*, *Azospirillum lipoferum + Maycorrhiza arbuscular*, *Azotobacter coroccum + Azospirillum lipoferum + Mycorrhiza arbuscular*, control.

Conclusions

The present study showed that water deficit has a negative effect on chlorophyll content and nutrient uptake (P and N), and biofertilizer has a positive impact on chlorophyll content and element uptake. These results show that using PGPR is an effective technique for overcoming the negative effects of drought stress on plants.

Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

References

Abo-Baker AA and Mostafa GG, 2011. Effect of bio-and chemical fertilizers on growth, sepals yield and chemical composition of *Hibiscus abdariffa at* new reclaimed soil of south valley area. Asian Journal of Crop Science 3:16-25.

Agarwal S and Pandey V, 2004. Antioxidant enzyme responses to NaCl stress in *Cassia angustifolia.* Biologia Plantarum 48: 555-560.

Allen M, Moore JTS, and Christensen M, 1982. Phytohormone changes in *Boutelou agracilis* infected by vesicular-arbuscular mycorrhizae: II. Altered levels of gibberellin-like substances and abscisic acid in the host plant. Canadian Journal of Botany 60: 468-471.

Al-Naggar AMM, Abd El-Salam RM, Badran AEE, and El-Moghazi MA, 2017. Drought tolerance of five quinoa (*Chenopodium quinoa Willd*.) genotypes and its association with other traits under moderate and severe drought stress. Asian Journal of Advances in Agricultural Research 3: 2456-2468.

Anonymous, 1980. Official methods of analysis. 13th ed. The Association of Official Analytical Chemists. Washington DC, USA.

Ansari FA and Ahmad I, 2019. Alleviating drought stress of crops through PGPR: mechanism and application. In: Singh D, Gupta V, and Prabha R (eds). Microbial Interventions in Agriculture and Environment. Springer, Singapore.

Atouei MT, Pourbabaee AA, and Shorafa M, 2019. Alleviation of salinity stress on some growth parameters of wheat by exopolysaccharide-producing bacteria. Iran. Journal of Science and Technology 43: 2725-2733.

Barnawal D, Bharti N, Pandey SS, Pandey A, Chanotiya CS, and Kalra A, 2017. Plant growth-promoting rhizobacteria enhance wheat salt and drought stress tolerance by altering endogenous phytohormone levels and TaCTR1/TaDREB2 expression. Physiologia Plantarum 161: 502–514.

Basra SMA, Iqbal S, and Afzal I, 2014. Evaluating the response of nitrogen application on growth, development, and yield of quinoa genotypes, International Journal of Agriculture and Biology 16: 886-892.

Behl RK, Narula N, Vasudeva M, Sato A, Shinano T, and Osaki M, 2006. Harnessing wheat genotype x*Azotobacter* strain interactions for sustainable wheat production in semi-arid tropics. Tropics 15: 123-133.

Chance B and Maehly SK, 1955. Assay of catalase and peroxidase. Methods in Enzymology 2: 764-775.

Das K and Roychoudhury A, 2014. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Frontiers in Environmental Science 2: 53-66.

Enebe MC and Babalola OO, 2018. The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. Applied Microbiology and Biotechnology 102: 7821-7835.

Fang Y and Xiong L, 2015. General mechanisms of drought response and their application in drought resistance improvement in plants. Cellular and Molecular Life Sciences 72: 673-689.

Farooq M, Hussain M, and Siddique KHM, 2014. Drought stress in wheat during flowering and grain-filling periods. Critical Reviews in Plant Sciences 33: 331-349.

Forni C, Duca D, and Glick BR, 2017. Mechanisms of plant response to salt and drought stress and their alteration by rhizobacteria. Plant and Soil 410: 335-356.

Fouad MO, Essahibi A, Benhiba A, and Qaddoury A, 2014. Effectiveness of arbuscular mycorrhizal fungi in the protection of olive plants against oxidative stress induced by drought. Spanish Journal of Agricultural Research 12:763-771.

Gilik BR, Penrose D, and Wenbo M, 2001. Bacterial promotion of plant growth. Biotechnology Advances 19: 135-138.

Hinojosa L, González J, Barrios-Masias F, Fuentes F, and Murphy K, 2018. Quinoa abiotic stress responses: a review. Plants **7**: 106-138.

Jackson ML, 1973. Soil Chemical Analysis. Prentice-Hall, New Delhi.

Jones HG, 1980. Interaction and integration of adaptive response to water stress. Royal Science Society of London 273: 193-205.

Kamara AY, Ekeleme F, Chikoye D, and Omoigui LO, 2009. Planting date and cultivar effects on grain yield in dryland corn production. Agronomy Journal 101: 91-98.

Kang SM, Radhakrishnam R, Khan AL, Kim MJ, Park JM, Kim BR, Shin DH, and Lee IJ, 2014. Gibberellin secreting rhizobacterium, *Pseudomonas putida* H-2-3 modulates the hormonal and stress physiology of soybean to improve the plant growth under saline and drought conditions. Plant Physiology and Biochemistry 84: 115-124.

Marschner H, 1995. Mineral Nutrition of Higher plants. Academic Press, USA, 889pp.

Meher MP, Ashok RK, and Manohar RD, 2018. Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. Saudi Journal of Biological Science 25:285-289.

Mir RR, Zaman-Allah M, Sreenivasulu N, Trethowan R, and Varshney RK, 2012. Integrated genomics, physiology, and breeding approaches for improving drought tolerance in crops. Theoretical and Applied Genetics 125: 625-645.

Nemeskeri E, Molnar K, Racz C, Dobos AC, and Helyes L, 2019. Effect of water supply on spectral traits and their relationship with the productivity of sweet corns. Agronomy 9: 63.

Ngumbi E and Kloepper J, 2016. Bacterial-mediated drought tolerance: current and future prospects. Applied Soil Ecology 105: 109-125.

Parray JA, Jan S, Kamili AN, Qadri RA, Egamberdieva D, and Ahmad P, 2016. Current perspectives on plant growth-promoting rhizobacteria. Journal of Plant Growth Regulation 35: 877–902.

Ray P, 2015. Hi-tech horticulture climate change. In: Choudhary ML, Patel VB, Siddiqui MW, and Mahdi SS (eds). Climate Dynamics in Horticultural Science, Principles and Applications. Apple Academic Press, NY, USA, pp. 1-22.

Ruiz-Lozano JM, 2003. Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress, new perspectives for molecular studies. Mycorrhiza 13: 309-317.

Sairam RK, Deshmukh PS, and Saxna DC, 2011. Role of antioxidant systems in Anethum genotype tolerance to water stress. Journal of Applied Environmental and Biological Sciences 41: 387-394.

Salehi A, Tasdighi H, and Gholamhoseini M, 2016. Evaluation of proline, chlorophyll, soluble sugar content, and uptake of nutrients in the German chamomile (*Matricaria chamomilla* L.) under drought stress and organic fertilizer treatments. Asian Pacific Journal of Tropical Biomedicine 6: 886-891.

Schutz M and Fangmeir E, 2001 Growth and yield responses of spring wheat (*Triticumaestivum* L. cv. Minaret) to elevated CO_2 and water limitation. Environmental Pollution 114: 187-194.

Sharma KD and Kuhad MS, 2006. Influence of potassium level and soil moisture regime on biochemical metabolites of *Brassica* Species. Brassica Journal 8: 71-74.

Shirinbayan S, Khosravi H, and Malakouti MJ, 2019. Alleviation of drought stress in maize (Zea mays) by inoculation with Azotobacter strains isolated from semi-arid regions. Applied Soil Ecology 133:138-145.

Singh M, Khan MMA, and Naeem M, 2016. Effect of nitrogen on growth, nutrient assimilation, essential oil content, yield, and quality attributes in *Zingiber officinale* Rosch. Journal of the Saudi Society of Agricultural Sciences 15: 171-178.

Sinha AK, 1972. Colorimetric assay of catalase. Analytical Biochemistry 47: 389-394.

Vurukonda SS, Vardharajula S, Shrivastava M, and Sk ZA, 2016. Enhancement of drought stress tolerance in crops by plant growth-promoting rhizobacteria. Microbiological Research 184: 13-24.

Wu Q and Xia R, 2006. Arbuscular mycorrhizal fungi influence growth, osmotic adjustment, and water stress conditions. Journal of Plant Physiology 163: 417-425.

You J and Chan Z, 2015. ROS regulation during abiotic stress responses in crop plants. Frontiers in Plant Science 6: 1092.

تاثیر باکتری‌های تثبیت کننده نیتروژن و مایکوریزا بر خواص بیوشیمیایی و جذب عناصر ضروری در نخود فرنگی تحت تاثیر سطوح مختلف کم آبی

مهدی قاسم بگلو*، محمد صدقی، رئوف سید شریفی و سلیم فرزانه

گروه مهندسی تولید و ژنتیک گیاهی، دانشکده کشاورزی و منابع طبیعی، دانشگاه محقق اردبیلی، اردبیل
*مسئول مکاتبه؛ Email: gasembagloomehdi@yahoo.com

چکیده

خشکی یکی از تنش‌های بحرانی غیربیولوژیکی است که به دلیل کاهش عملکرد، بر بهره‌وری گیاه تأثیر منفی می‌گذارد. ریزوباکترهای محرک رشد گیاهی (PGPR) مانند ازتوباکتر و آزوسپیریلیوم بر فیزیولوژی گیاه، به ویژه در شرایط تنش خشکی، تأثیر مثبت دارند. این مطالعه با هدف بررسی اثرات قارچ میکوریزا و PGPR بر فعالیت آنزیم‌های آنتی اکسیدانی و میزان جذب مواد مغذی در شرایط کمبود آب انجام شد. آزمایش به صورت فاکتوریل در قالب طرح بلوک‌های کامل تصادفی در سه تکرار انجام شد. فاکتورها شامل آبیاری (آبیاری معمولی، کمبود آب در مرحله پر شدن دانه، کمبود آب در مرحله گلدهی، عدم آبیاری) و قارچ میکوریزا و PGPR (کود آزتوباکتر کروکوکوم، آزوسپریلیوم لیپوفرم، مایکوریزا آربوسکولار، تلفیق آزوسپریلیوم لیپوفرم و ازتوباکتر، تلفیق مایکوریزا آربوسکولار و ازتوباکتر کروکوکوم، تلفیق مایکوریزا آربوسکولار و آزوسپریلیوملیپوفرم، تلفیق ازتوباکتر کروکوکوم، آزوسپریلیوم لیپوفرم و مایکوریزا آربوسکولار، عدم تلقیح) بودند. تنش خشکی باعث کاهش میزان کلروفیل و جذب عناصر و افزایش فعالیت پرولین، کاتالاز و پراکسیداز شد. همچنین کودهای زیستی باعث کاهش میزان پرولین، کاتالاز و پراکسیداز و افزایش میزان کلروفیل و جذب عناصر شدند.

واژه‌های کلیدی: آنزیم آنتی اکسیدان؛ خشکی؛ کود زیستی؛ مواد مغذی