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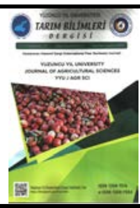

Research Article (Araştırma Makalesi)

Effects of Endophytic Bacteria on Some Physiological Traits and Nutrient Contents in Pepper Seedlings under Drought Stress**

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Drought,
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Abstract: The present study was conducted to determine the effects of the endophytic bacteria (EB) on some physiological traits and nutrient contents in pepper (*Capsicum annuum* L.) seedlings grown under drought stress. The pepper cv. Mostar F_1 and two EB isolates [*Bacillus thurigiensis* (CA41/1) and *Ochrobactrum* sp. (CB36/1)] were employed under drought stress condition. The first EB application was at a density of 10^8 CFU mL^{-1} as 10 mL $plant^{-1}$ at the first cotyledon leaf stage and the second one was two weeks later. The seedlings were irrigated by gravimetric method on a regular basis every two days. Twenty days after EB application, irrigation was terminated completely in half of the applications in order to form drought stress for 7 days. Among the studied traits, membrane damage index, leaf relative water content, amount of malondialdehyde, catalase enzyme activity, ascorbate peroxidase enzyme activity, and the contents of some mineral elements (K, Ca and Mg) were significantly different in drought stressed seedlings compared the control (regularly irrigated) seedlings. EB (Especially CA41/1) had generally positive effects on most studied traits, whereas drought stress had generally negative effects on the mentioned traits. There might be a high potential of EB fighting against drought stress in pepper; however, one keeps in mind that there is variation in the performance of EB; therefore, the best EB combinations have to be determined even for cultivars in each plant species in future studies.

Endofitik Bakterilerin Kuraklık Stresi Altındaki Biber Fidelerinin Bazı Fizyolojik Özellikleri ve Besin İçerikleri Üzerine Etkileri

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Anahtar kelimeler

Biber,
EB'ler,
Enzim aktivitesi,
Büyüme,

Öz: Bu çalışma, kuraklık stresi altında yetiştirilen biber (*Capsicum annuum* L.) fidelerinde endofitik bakterilerin (EB) bazı fizyolojik özellikler ve besin içerikleri üzerine etkilerini belirlemek amacıyla yapılmıştır. Mostar F_1 Biber çeşidi, iki EB izolatı [*Bacillus thurigiensis* (CA41 / 1) ve *Ochrobactrum* sp. (CB36 / 1)] kuraklık stresi altında kullanılmıştır. İlk EB uygulaması, ilk kotiledon yaprağı aşamasında 10 mL/bitki olarak 10^8 CFU/mL yoğunlukta ve ikincisi ise iki hafta sonra yapılmıştır. Fideler, iki günde bir düzenli olarak gravimetrik yöntemle sulanmıştır. EB uygulamasından yirmi gün sonra, 7 günlük kuraklık stresi oluşturmak için uygulamaların yarısında sulamaya tamamen son verilmiştir. Çalışılan özellikler arasında, kuraklık stresi altındaki fidelerde kontrol (düzenli olarak sulanan) fideler ile karşılaştırıldığında, membran zararlanma indeksi, yaprak oransal su içeriği, malondialdehit miktarı, katalaz enzim aktivitesi,

Kuraklık,
Mineral maddeler.

askorbat peroksidaz enzim aktivitesi ve bazı mineral elementlerin içerikleri (K, Ca ve Mg) önemli ölçüde farklı bulunmuştur. EB'ler (Özellikle CA41 / 1), incelenen özelliklerin çoğunda genel olarak olumlu etkilere sahipken, kuraklık stresi belirtilen özellikler üzerinde genellikle olumsuz etkilere yol açmıştır. Biberde kuraklık stresine karşı savaşan yüksek bir EB potansiyeli olabilir; ancak, EB'lerin performansında farklılıklar olduğu unutulmamalıdır; bu nedenle, en iyi EB kombinasyonları, gelecekteki çalışmalarda her bitki türündeki çeşitler için belirlenmelidir.

**This article extracted from M.Sc. thesis of Aynur Sadak.

1. Introduction

The need for water in agriculture is indispensable and vegetables need high amount of irrigation water (Sensoy et al., 2007). The world is threatened by global warming and climate change. This climate change causes several stress factors in agricultural areas. These stress conditions also constitute drought stress (Turkes et al., 2000; Ozturk, 2002). Plants experience different biotic or abiotic stress conditions for the duration of their life cycle. Drought stress is a common abiotic stresses disturbing plant growth and yield (Kabay and Sensoy, 2016, 2017; Kusvuran and Dasgan, 2017; Cakmakci et al., 2017; Ekinci and Başbağ, 2019; Söylemez et al., 2020). Drought stress occurs by irregular rainfalls accompanied by extreme wind and extreme temperatures (Ozturk, 2002). As a result of the increase in the world population, it is seen that water consumption increases and climate change such as global warming cause the water to decrease gradually (Çakmakcı et al., 2016; Sahin et al., 2016).

It is known that some biostimulant applications give positive results in abiotic stress conditions (Turkmen et al., 2005). Plant growth promoting rhizobacteria and a recent focus within this bacteria group of endophytic bacteria, have also significant potential. Endophytic bacteria (EB) have plant growth promoting effect that is EB have a vegetative and generative growth-enhancing effect in varying proportions in plants (Akkopru et al., 2018). EB reside in the internal plant tissues and do not have deleterious effects on the plant (Rosenblueth and Martínez-Romero, 2006; Hardoim et al., 2008).

EB might affect plant growth directly or indirectly**.** EB might also produce the plant growth hormones (Van Loon, 2007). Moreover, EB cause reduction in ethylene synthesis, convert mineral matters into much useful forms and stimulate plant defense mechanisms (Saharan and Nehra, 2011). EB might also play a role as a biocontrol agent reducing the efficacy and quantity of pathogens, encouraging beneficial symbiotic relationships or decomposing xenobiotics found in soil (Saharan and Nehra, 2011). EB colonization is not restricted to a certain region of the plant and the effects of EB might be ameliorated by plant transport system (Rosenblueth and Martínez- Romero, 2006; Hardoim *et al*., 2008). The interior tissues of the plant might be protected by EB from biotic and abiotic stress factors (Rosenblueth and Martínez-Romero, 2006).

The pepper which is an important vegetable crop worldwide and contains a wide genetic variability (Aktas et al., 2009), is affected severely by biotic and abiotic stress conditions. Pepper is also very susceptible to drought stress (Yildirim et al., 1994; Dagdelen et al., 2004; Ertek et al., 2007). EB might help pepper seedlings to lessen drought stress. The study was carried out to determine the effects of the endophytic bacteria (EB) on the growth of the pepper (*Capsicum annuum* L.) seedlings developed under drought stress.

2. Material and Methods

The pepper cv. Mostar F1 was employed as plant material and two bacteria isolates [*Bacillus thurigiensis* (CA41/1) and *Ochrobactrum* sp. (CB36/1)] whose PGPR activities previously determined (Ozaktan et al., 2015) were used as biological control agents. The study was carried out with randomized plots with three replications. There were 6 applications of EB and drought applications (1: control, 2: drought, 3: CA41/1, 4: CA41/1+drought, 5: CB36/1 and 6: CB36/1+drougt applications) with 3 replications each having 5 pots and 1 plant per pot (2 L pots filled with 1 peat: 1 perlite). The pepper seeds were sown in a growth chamber at a 70 % relative humidity and a temperature of 24 ± 1°C with 14 h fluorescent illumination (approximately 8000 lx light intensity).

2.1. PGPR applications

EB applied to seedlings as: 48-hour EB cultures developed on King's B medium (Pepton 20 g L^{-1}, K_2HPO_4 1.5 g L^{-1}, $MgSO_47H_2O$ 1.5 g L^{-1}, Glycerol 10 ml L^{-1}, Agar 15 g L^{-1}) were prepared in suspension with 10^8 colony-forming units (CFU) mL^{-1} density (Ozaktan et al., 2015). The suspensions were applied twice to the seedlings by drenching method with 10 ml $plant^{-1}$, the first EB application was performed when the first true leaves began to open, and the second one was two weeks later.

2.2. Drought stress

During the experiment period, the seedlings were irrigated by gravimetric method on a regular basis every two days. The experiment was terminated after 8 weeks. The field capacity was determined by taking the weight of the pots in the first seed sowing. With an equal amount of tap water regularly was applied every two days to all the pots. All seedlings were irrigated with equal amount of water for 7 weeks after seed sowing. For the last week, control plants was irrigated to field capacity and for drought stress irrigation was completely stopped (Kusvuran, 2010; Kabay, 2014).

2.3. Determination of leaf water content

To determine the proportional water content of leaf samples taken from plants at the 8^{th} week, fresh leaf samples were weighed (FW) and held in distilled water for 4 h to determine the turgor weights (TW); then, the samples were held in an oven (65 °C) for 48 h and weighted (DW). Leaf water content was determined as (Kusvuran, 2010; Kabay et al., 2017):

LWC = (FW-DW) / (TW-DW) x100 FW

2.4. Membrane damage index

After the application of EB and drought, the disc-shaped leaf samples in 17 mm diameter were obtained from the 3^{rd} leaves of pepper seedlings, incubated for 5 h in 10 mL distilled water, and the EC values were determined. The same disc samples were held at 100 °C for 10 min, and their EC values were determined again. Membrane Damage Index (MDI) was determined as (Dlugokecka and Kacperska-Palacz, 1978; Fan and Blake, 1994; Kusvuran, 2010).

2.5. Malondialdehyde (MDA) product of lipid peroxidation in plants

0.5 g of leaf samples were homogenized with 10 ml of 0.1% (w/v) tri-chloro-acetic acid and the homogenate was centrifuged at 15 000 rpm for 5 min. 1 mL of the supernatant was added with 0.5 % of thio-barbituric-acid dissolved in 4 mL 20% tri-chloro-acetic acid. The mixture was incubated at 95 °C for 30 min and immediately cooled in ice bath; then centrifuged at 10 000 rpm for 10 min and absorbance readings were accomplished for the supernatant at 532 and 600 nm wavelengths. The content of MDA was determined according to the following equation (Heath and Packer, 1968; Sairam and Saxena, 2000).

2.6. Antioxidative enzyme analyses

The frozen 1 g leaf sample (third leaf from the bottom of the plants) was homogenized (4 °C for 30 minutes at 18 000 rpm) with a mixture of 5 ml of cold 0.1 M Na-phosphate, 0.5 mM Na-EDTA and 1 mM ascorbic acid (pH: 7.5). The ascorbate peroxidase (APX) activity was determined immediately in the homogenate with this preparation. The frozen 1 g leaf sample (third leaf from the bottom of the plants) was homogenized (4 °C for 30 min at 18 000 rpm) with 5 ml of cold 0.1 M Na-phosphate, 0.5 mM Na-EDTA mixture (pH: 7.5). Catalase activity (CAT) activity was detected in the homogenate (Jebara et al., 2005; Guneri Bagci, 2010; Kabay, 2014; Guzel et al. 2018). CAT activity was detected by monitoring the disappearance of H_2O_2 at a wavelength of 240 nm. 0.05 M phosphate buffer (KH_2PO_4), 1.5 mM H_2O_2 mixture (pH: 7.0) was employed as the reaction solution. 2.5 mL of reaction solution and 0.2 ml of plant extract were mixed. In spectrophotometer, 0^{th} and 60^{th} seconds readings were observed at 240 nm wavelength. The reaction was initiated by the addition of 0.1 mL enzyme extract. The evaluation was completed based on the change in absorbance within 1 min (Jebara et al., 2005; Guneri Bagci, 2010; Kabay, 2014). Ascorbate peroxidase (APX) activity was detected at 290 nm depending on the ascorbic acid reducing H_2O_2. As the reaction solution, 50 mM phosphate buffer (KH_2PO_4), 0.5 mM ascorbic acid, 0.1 mM EDTA, 1.5 mM H_2O_2 mixture (pH: 7.0) were employed. 3 mL of reaction solution and 0.1 mL of plant extract were mixed. The 0^{th} and 60^{th} seconds readings were taken at 290 nm wavelength in the spectrophotometer. The reaction was started by the addition of 0.1 ml enzyme extract. The evaluation was completed based on the change in absorbance within 1 min (Jebara et al., 2005; Guneri Bagci, 2010; Kabay, 2014).

2.7. Mineral element analysis

The mineral matter contents of the shoot samples from the applications were determined in an atomic absorption device (Thermo no: ice3000) (Kacar, 1994; Kacar and Inal, 2008). The mineral matter contents having significant results such as Mg, K, and Ca were presented in the results.

2.8. Statistical analysis

To determine the effect of stress in drought and control plants according to the experimental design of randomized plots, the data statistically analyzed by using package program IBM SPSS Statistics 21.0 were used. The t test was used to compare the drought application means, and the Duncan test was used to compare the application means of EB and interactions.

3. Results and Discussion

The effect of EB on growth of pepper seedlings grown under drought stress was studied and the obtained results were presented below (Tables 1-3). Drought stress negatively affected pepper seedlings growth. In general, EB application positively influenced drought-stressed pepper seedlings (Table 1-3). For the membrane damage index (MDI), it was determined that pepper seedlings inoculated with EB had lower membrane damage index values compared to the control application (Table 1). EB application CB36/1 (*Ochrobactrum* sp.) had 65.74 % (10.07) lower MDI value compared to control application (29.39). Moreover, the other EB application [*Bacillus thurigiensis* (CA41/1)] had also 47.09 % (15.55) lower MDI value compared to control application (29.39). It is known that the free radical structures formed by stress in the plants damage the membrane structure and harms the membrane permeability (Shewfelt and Purvis, 1995). For leaf water content (LWC), although there were no significant differences among the applications, there was up to 2.87 % increase in LWC at EB applications (from 86.46 to 88.95). It has been reported that PGPR applications decrease the membrane damage in plants in drought stress conditions by increasing the synthesis of proline and by protecting the water status in the cell (Ansary et al., 2012; Chakraborty et al., 2013; Sarma and Saikia, 2014). Pepper is an important vegetables species in the world and Turkey. Water stress conditions adversely affect pepper seedlings sensitive to drought stress or water restriction (Yildirim et al., 1994; Ozturk, 2002; Dagdelen et al., 2004). Drought stress also negatively affects cv. Mostar F1 pepper. It has been reported that some endophyte bacteria on the seedlings of cv. Mostar F1 on post-stress recovery are relatively promising in seedlings exposed to drought stress (Sadak et al., 2019).

Table 1. Membrane damage index and leaf water content and of drought stressed EB applications

	Leaf Water Content (%)	Membrane Damage Index (%)
EB	Drought application	Drought application
Control	86.46±17.10 ^{ns}	29.39±6.82 A**
CB36/1	88.95±11.42	10.07±3.11 C
CA41/1	88.10±16.23	15.55±4.25 B
Mean	87.70±14.91	18.34±4.72

^{ns}: non-significant.
$^{A-C}$: Means in the same column not sharing the same superscript are significantly different (**p<0.01).

The response of MDA content and antioxidant enzyme activities (CAT and APX) have been presented in Table 2. MDA content of the drought stressed pepper seedlings increased 248.77 % (13.16) compared the control seedlings (5.19). There were insignificant increases in MDA contents of EB inoculated seedlings. There were also increases in CAT and APX contents of EB inoculated seedlings. However, only CA41/1 (*Bacillus thurigiensis*) caused significant increases in CAT and APX contents compared to control application (0.110 and 3.03, respectively) by 218.18 % (0.240) and 131.68 % (3.99), respectively. In a study dealing with drought stress in common bean, it was reported that there were increases in MDA level and CAT, SOD and APX activities (Kabay and Sensoy, 2017). In another study conducted in beans, MDA content is reported to increase in plants with drought stress (Kusvuran and Dasgan, 2017). It is obvious that antioxidant enzyme activity plays an important role in increasing tolerance to drought stress in plants. The antioxidant enzyme activity could reduce the negative effects of free radicals, especially in stress conditions. In recent studies, it is stated that with the increasing use of PGPRs in arid conditions, tolerance can be achieved by increasing antioxidant enzyme activity against the negative effects of stress (Sarma and Saikia, 2014). Kohler et al. (2008) reported that the antioxidant enzyme level was increased, and therefore, the tolerance to stress was increased in drought stressed lettuce inoculated with *Pseudomonas mendocina*. It was also stated that mung bean inoculated with *Pseudomonas aeruginosa* improved the activity of SOD and CAT; this might contribute to increase the tolerance to stress (Sarma and Saikia, 2014). Kumari et al. (2018) reported three isolates expect one strain showed positive response for CAT and PGPR with CAT activity have been stated to defend plants against biotic stress agents by maintaining plant ROS levels.

Table 2. CAT, APX and MDA values obtained in drought and EB applications

CAT (nmol g^{-1} FW)			
EB	Control	Drought	Mean
Control	0.063±0.011 ^{ns}	0.157±0.082	0.110±0.046 B **
CB36 / 1	0.157±0.025	0.125±0.036	0.141±0.030 B
CA41 / 1	0.250±0.096	0.230±0.016	0.240±0.056A
Average	0.156±0.068 ^{ns}	0.170±0.123	
APX (nmol g^{-1} FW)			
EB	Control	Drought	Mean
Control	2.51±0.65 c *	3.55±0.72 bc	3.03±0.68 B **
CB36 / 1	2.99±0.82 bc	3.89±0.83 b	3.44±0.82 B
CA41 / 1	2.95±0.76 bc	5.03±0.96 a	3.99±0.86 A
Average	2.82±0.74 B**	4.15±0.83A	
MDA (nmol / g TA)			
EB	Control	Drought	Mean
Control	4.45±1.23 ^{ns}	11.29±2.96	7.87±2.09 ^{ns}
CB36 / 1	6.62±2.06	13.74±3.01	9.47±2.53
CA41 / 1	4.52±1.26	14.45±2.49	8.49±1.87
Average	5.19±1.51 B**	13.16±2.82 A	

^{ns}: non-significant.
$^{A\text{-}B}$: Means in the same column or line not sharing the same superscript are significantly different (**p<0.01).
$^{a–c}$: Means in the interactions not sharing the same superscript are significantly different (*p<0.05).

There were also significant differences of the applications on Mg, K, and Ca contents of pepper seedlings (Table 3). Potassium (K) content of the drought stressed pepper seedlings decreased approximately 18 % (2992 ppm) compared the control seedlings (3641 ppm). There were increases in Ca content of EB inoculated seedlings. However, only CA41/1 (*Bacillus thurigiensis*) inoculated applications caused significant increases in Ca content compared to control application and CB36/1 (*Ochrobactrum* sp.) by 49 % (6891 ppm) and 41.6 % (5941 ppm) respectively. These reactions at drought condition, the pepper plants inoculated with CA41/1 were more effective in the Mg content when compared to the plants that were un-inoculated. Kabay and Sensoy, (2016 and 2017) and Kabay et al. (2017) also reported reduction in K, Ca, and Mg contents due to abiotic stress conditions.

Mechanisms for the closure of stomata in plants under water stress conditions are related with the amount of K^+ ion, the accumulation of ABA hormone and turgor pressures of closure cells. In the stress of water, the amount of ABA increases in the stoma cells of the plants and thus the water-insoluble starch is formed and the K^+ ion decreases (Okturen and Sonmez, 2005). Singh et al. (2018) stated that promising EB isolates, for use as inoculants to improve micronutrient uptake and accumulation in grains. Win et al. (2018) reported that one *Pseudomonas* strains (OFT5) decreased salt induced ethylene production in tomato seedlings, and although it did not diminish shoot uptake of Na, it encouraged shoot uptake of some macronutrients and micronutrients. They proposed that the mentioned nutrients might trigger processes that lessen the effects of salt, suggesting that OFT5 can be utilized to improve nutrient uptake and plant growth under moderate salt stress condition.

Table 3. Ca, K, and Mg values obtained in drought and EB applications

Ca ($mg\ kg^{-1}$)			
EB	Control	Drought	Mean
Control	6217.4±1124.1 ab**	5102.8±1032.8 b	5660.1±1078.4 B**
CB36 / 1	6512.5±1101.3 ab	5369.4±1097.2 b	5941.0±1099.9 B
CA41 / 1	6180.5±1201.6 ab	7603.1±1162.8 a	6891.8±1181.5 A
Average	6328.3±1142.5 ^{ns}	5709.4±1097.6	
K ($mg\ kg^{-1}$)			
EB	Control	Drought	Mean
Control	3518±1023 ^{ns}	2996±936	3257±979 ^{ns}
CB36 / 1	3666±1501	2698±969	3182±1235
CA41 / 1	3835±1432	3572±1006	3703±1219
Average	3641±1325 A**	2992± 970 B	
Mg ($mg\ kg^{-1}$)			
EB	Control	Drought	Mean
Control	2823.0±856.9 bc**	2603.0±932.5 b	2713.0±894.5 ^{ns}
CB36 / 1	3099.3±896.6 bc	2542.7±965.7 b	2821.0±930.9
CA41 / 1	2598.2±762.1 c	3285.4±1003.5 a	2941.8±882.6
Average	2888.6±838.5 ^{ns}	2715.4±966.8	

^{ns}: non-significant.
$^{A\text{-}B}$: Means in the same column or line not sharing the same superscript are significantly different (**p<0.01).
$^{a\text{–}c}$: Means in the interactions not sharing the same superscript are significantly different (**p<0.01).

5. Conclusions

In general, drought stress affected negatively membrane damage index and leaf water content in pepper seedlings. On the other hand, EB application in the present study had some ameliorative effects on drought stressed pepper seedlings. Both EB [*Bacillus thurigiensis* (CA41/1) and *Ochrobactrum* sp. (CB36/1)] lessened substantially the membrane damage index (MDI). The MDA content of the drought stressed pepper seedlings increased significantly, but there were insignificant increases in MDA contents of EB inoculated seedlings. There were also increases in CAT and APX contents of EB inoculated seedlings. However, only CA41/1 (*Bacillus thurigiensis*) caused significant decreases in the mentioned enzymes in drought stressed pepper seedlings. Drought stress decreased K contents of pepper seedlings significantly. CA41/1 (*Bacillus thurigiensis*) inoculations caused significant increases in Ca content.

In conclusion, the effect of two endophytic bacteria varied in drought stressed pepper seedlings. Drought stress beside several abiotic stress factors is an important problem worldwide. Microbial organisms that exist in natural environments might help to increase tolerance level of plants to biotic and abiotic stress agents. Endophytic bacteria (EB) colonizing the roots and having plant growth promoting effect ameliorate plants tolerance level to the a/biotic stresses. There is a high potential of EB fighting against various stress factors in sustainable agriculture practices; however, one keeps in mind that there is variation in the performance of EB; therefore, the best EB combinations have to be determined even for cultivars in each plant species in future studies.

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