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Assessment of the Effects of Some Bacterial Isolates and Hormones on Corm Formation and Some Plant Properties in Saffron (*Crocus sativus* L.)

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ABSTRACT

The saffron, from the Iridaceae family and an autumn-flowering geophytes, is one of cormous plants. The biggest obstacle in the development of this plant, production having the most economic value as one of the medicinal and aromatic plants, is the insufficient bulbous used for propagation. Bacterial isolates showing capacity to grow in nitrogen-free conditions, for hormones production (IAA, GA_3) and to solubilise phosphate as microbial fertilizer were used to reproduce the corms of saffron plants. Thus, the disappearance of saffron from the species that are under threat of extinction can be prevented and the continuation of the species can be provided by its widespread propagation as an ornamental plant. In this study, a total of ten treatments; (1) *Achromobacter xylosoxidans* strain TV-42A, (2) *Brevibacillus choshinensis* strain TV-53D, (3) *Myroides odoratus* strain TV-85C, (4) *Bacillus megaterium* strain TV-87A, (5) *Colwellia psycrerytreae* strain TV-108G, (6) *Kluyvera cryocrescens* strain TV-113C and (7) *Bacillus* GC group B strain TV119E, (8) Control (untreated bacteria or hormones) (9) Control 2 [100 mg L^{-1} *IBA* (indole-3 butyric acid)] and (10) Control 3 [100 mg L^{-1} GA_3 (gibberellic acid)] were tested to see their effects on the plant growth and development parameters of saffron. The number of cormlet, average cormlet diameter (mm), cormlet length (mm), cormlet weight (g), macro and micro plant nutrients (N, K, P, Mg, S, Ca, Na, Fe, Mn, Zn, Cu, Pb, B and Cd) contents of corms were determined in greenhouse assays. Some of the bacterial applications gave growth and yields of saffron equal to or higher than the hormones applied. Bio-fertilizers used in organic farming, increase in plant growth and development of saffron were concluded to have positive effect.

Keywords: PGPR; GA_3; IBA; Saffron; Cormlet

Bazı Bakteri İzolatları ve Hormon Uygulamalarının Safran (*Crocus sativus* L.) Bitkisinde Korm Oluşumu ve Kimi Bitki Özelliklerine Etkisi

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ÖZET

Safran, süsengiller (*Iridaceae*) familyasından ve sonbaharda çiçek açan, soğanlı bitkilerden biridir. Ekonomik değeri yüksek, tıbbı ve aromatik bitkilerden biri olan bu bitkinin üretiminin geliştirilmesinde en büyük engel tohumluk olarak kullanılan soğanların yetersizliğidir. Safran bitkisinin kormlarının çoğalmasında mikrobiyal gübre olarak azot fiksasyonu yapabilme, fosfatı çözebilme ve hormon (IAA, GA_3) üretebilme özelliğine sahip bakteri izolatlarının kullanımı hedeflenmiştir. Böylece, nesli tehlike altında olan türlerden olan safranın yok olması önlenebilir ve bir süs bitkisi olarak üretiminin yaygınlaştırılmasıyla türlerin devamı sağlanabilir. Bu çalışmada, toplam on uygulama (1) *Achromobacter xylosoxidans* strain TV-42A, (2) *Brevibacillus choshinensis* strain TV-53D, (3) *Myroides odoratus* strain TV-85C, (4) *Bacillus megaterium* strain TV-87A, (5) *Colwellia psycrerytreae* strain TV-108G, (6) *Kluyvera cryocrescens* strain TV-113C and (7) *Bacillus* GC group B strain TV119E, (8) Kontrol (bakteri ve hormon uygulamasız) (9) Kontrol 2 [100 mg L^{-1} *IBA* (indole-3 butyric acid)] ve (10) Kontrol 3 [100 mg L^{-1} GA_3 (gibberellic acid)] safranın bitki büyüme ve gelişim parametreleri üzerindeki etkilerini belirlemek için test edilmiştir. Sera koşullarında, yavru korm sayısı, yavru korm çapı (mm), yavru korm uzunluğu (mm), yavru korm ağırlığı (g) ve kormların makro ve mikro (N, K, P, Mg, S, Ca, Na, Fe, Mn, Zn, Cu, Pb, B ve Cd) besin içerikleri belirlenmiştir. Safranın büyüme ve verim değerleri, bakteri uygulamalarının bazılarında hormon uygulamalarından daha yüksek veya eşit şekilde elde edilmiştir. Organik tarımda kullanılan biyo-gübrelerin, safranın bitki büyüme ve gelişimini artırması üzerine olumlu bir etkiye sahip olduğu sonucuna varılmıştır.

Anahtar Kelimeler: PGPR; GA_3; IBA; Safran; Yavru korm

1. Introduction

Crocus sativus L. (saffron) belonging to Iridaceae family show spread in tropical and subtropical regions of the northern hemisphere of the world. This species has been cultivated for 4300 years (Escribano et al 2000). It is a perennial herbaceous plant cultured corm in many countries bordering the Mediterranean Sea especially in Italy, Spain, Greece and Turkey as well as in Japan, China, Iran and Azerbaijan (Vurdu et al 1997).

Saffron name is generally used both for the plant and spice. Saffron is known to be economically very important. The plant has been used by humans for centuries due to its smell, colour and curative effects (Tarantilis & Polissiou 1997; Lozano et al 1999; Carmona et al 2006). In the year of 1000 BC, saffron was rumoured to be used to paint the mummies that were stored in coffin-shaped crates or mummification in Egypt. Firstly, saffron was used as a hair dye in Romans and as a perfume later on (Basker & Negbi 1983).

C. sativus is to be grown in semi-arid climate having hot and dry winds, which are similar to habitat of the Mediterranean maquis and North America chaparral vegetation (similar to the Mediterranean maquis vegetation). Plants can survive cold winters and they may remain under snow until -10 °C in a short period of time (Chichiricco 1984; Amirghasemi 2001).

The *Crocus* genus includes approximately 80 species worldwide. There are about 32 species of *Crocus* genus in Turkey (Vurdu & Güney 2004). Some species also include subspecies between 2 and 10. A part of *Crocus* species bloom in autumn and some of them bloom in spring period. About 30 of these species are grown as ornamental plants (İpek et al 2009). Saffron cultivation area is limited in Turkey. Saffron has been cultured in an area of 1.5 ha in Karabük in Turkey (Kara 2010). The product obtained cannot even meet the domestic consumption. Therefore, Turkey needs the saffron corm. Some *Crocus* species are produced from both corm and seeds. But, *Crocus sativus* L. can only be produced from corm (Chichiricco 1984). Saffron flowers are sterile; i.e., the plant is not able to set viable seed. The pollen of Saffron sterility is auto triploid (2n= 24) (Chichiricco 1984). The propagation of corms is necessary because of the triploid nature of *Crocus sativus* L. (Warburg 1957).

Corms with 2-3 cm diameter have the best production of flowers and corms. Corm measurements

are important in terms of the relationship between flower number and weight of stigmas.

Modern agriculture is faced with increasing difficulty of growing worldwide, the decline in soil productivity and product quality, and due to rising consumer demand. Therefore, there is need for new and highly effective fertilizer to protect the ecological balance of nature. In this regard, plant growth-promoting bacteria (PGPR) could have a very important role.

Benefits of PGPRs on plants are the inclusion biocontrol, biological N_2 fixation, phosphorus solubilisation, production of siderophore and/or production of phytohormone encouraged directly to improve plant growth by means of bacteria (Mia et al 2012; Turan et al 2014). Bacteria are also encouraging to improve plant growth such as to suppress plant pathogens by indirect means (Kotan & Şahin 2002; Dobbelaere et al 2003; Şahin et al 2004; Çakmakçı et al 2006). Therefore, PGPR application is stated to increase the plant growth and yield as well as on improving the soil quality.

PGPRs produce phytohormones plant growth-promoting compounds- as auxins, cytokinins and gibberellins (Saikia et al 2006). Auxin causes cell expansion and growth, encourages cell elongation, tissue growth and root formation (Grunewald et al 2009). Auxin is an effective substance in cell volume and fraction (meristem formation). Therefore, it is effective in growth and development. In addition, it is used to end the dormancy in the some plants (Budak et al 1994; Kaynak & Ersoy 1997; Kaynak & Memiş 1997). Various development processes, such as stem elongation, control various aspects of seed germination, including dormancy break and mobilization of endosperm reserves, are influenced by endogenous gibberellins. Moreover, gibberellins influence the transition from juvenile stage to mature stage, induction of flowering, sex determination and fruit set establishment in the reproductive development (Taiz & Zeiger 2004).

Saffron cultivation area is limited in Turkey. Turkey has lost importance in saffron trade. The product obtained cannot even meet the domestic consumption. Therefore, Turkey needs the saffron corm. Saffron has several beautiful flowers and carries the perennial property due to corm. Because of this feature, the use of saffron as an ornamental plant in flower beds, among the grass, balconies and terraces, in the regulation of the roof garden will be of great importance in terms of providing the continuation of the species. There are few studies using PGPR as plant growth promoting agent in the cultivation of saffron (Sharaf-Eldin et al 2008; Parray et al 2013) around world and there is no study in Turkey. The aim of this study was to evaluate the plant growth parameters and corm formation on saffron by using PGPRs (*Achromobacter xylosoxidans* strain TV-42A, *Brevibacillus choshinensis* strain TV-53D, *Myroides odoratus* strain TV-85C*, Bacillus megaterium* strain TV-87A*, Colwellia psycrerytreae* strain TV-108G, *Kluyvera cryocrescens* strain TV-113C and *Bacillus* GC group B strain TV119E) and hormones (indole-3 butyric acid and gibberellic acid) treatments in saffron in greenhouse assays.

2. Material and Methods

2.1. Materials

2.1.1. Plant material

A total of 150 corms of saffron used in the experiments were obtained from the Safranbolu Directorate of District Food, Agriculture and Livestock, Karabük, Turkey. The corms were selected free of wounds and rots, and as homogeneous as possible in size (2.0 to 2.5 cm). They were stored at 1-4 ^{o}C until using. The study was conducted under the natural light in greenhouse condition at the Faculty of Agriculture at Atatürk University. Temperatures inside the greenhouse were determined as 15±2 ^{o}C at night and daytime temperatures were determined as 27±2 ^{o}C. Treated corms were planted on July 28, 2012 then the harvest was made on 08 May 2013.

2.1.2. PGPR bacteria and hormones

All of the bacterial strains (*Achromobacter xylosoxidans* strain TV-42A, *Brevibacillus choshinensis* strain TV-53D, *Myroides odoratus*

strain TV-85C, *Bacillus megaterium* strain TV-87A, *Colwellia psycrerytreae* strain TV-108G, *Kluyvera cryocrescens* strain TV-113C and *Bacillus* GC group B strain TV119E) were obtained from the culture collection unit in the Department of Plant Protection, Faculty of Agriculture at Atatürk University (Table 1). These non-pathogenic bacterial strains had been isolated from the rhizosphere and phyllosphere of wild and traditionally cultivated plants growing in Erzurum and Van Cities located in the Eastern Anatolia Region of Turkey (Kotan et al 2005; Erman et al 2010). The identity of all bacterial strains used in this study was confirmed according to fatty acid methyl esters (FAME) analysis by using Sherlock Microbial Identification System (Microbial ID, Newark, DE, USA) (Miller 1982). Bacterial cultures were grown on nutrient agar (NA; Difco™) for routine use, and maintained in Luria Broth (LB; Difco™) with 15% glycerol at -80 ºC for long-term storage. In the previous studies, all strains used in this study were determined that they showed capacity to grow in N-free conditions, for hormones production (IAA, GA_3) and to solubilise phosphate (Table 1) (Ekinci et al 2014; Kotan et al 2014; Turan et al 2014). In addition, the effectiveness of the commercially available indole-3 butyric acid (IBA; Indole-3 butyric acid/SIGMA/anhydrous, molecular weight 203.2 g) and gibberellic acids (GA_3 molecular weight 346.38 g) of hormones with the effectiveness of selected bacteria was used to compare and was aimed to determine the degree of activity.

2.2. Method

2.2.1. Corms surface disinfection with sodium hypochlorite

Corms were surface disinfected to avoid the presence of any saprophytic and/or pathogenic microorganisms on the corm surface. Corms disinfection was performed by dipping the corm for 3 min. in 3% sodium hypochlorite and washing four times in sterilized and distilled water (sdH_2O). The corms were left to dry on sterile filter paper sheets overnight in the laminar flow hood to be used in further studies.

2.2.2. Media and growth condition

Tryptic Soy Agar (TSA, Oxoid) and Tryptic Soy Broth (TSB, Oxoid) medium were used in the experiments. All bacterial isolates were incubated in TSA at 27 °C for 24 h. After the incubation period, a single colony was transferred to 500 mL flasks containing TSB, and grown aerobically in the flasks on a rotating shaker (150 rpm) for at 27 °C for 48 h (Merck KGaA, Germany). Bacterial suspension was then diluted in sdH_2O to a final concentration of $1x10^8$ cfu mL^{-1} with a turbidimeter.

Table 1- Bacterial strains, their host, nitrogen fixation (N) and phosphate-solubilising activity (P) properties and hormones (IAA, GA_3) production (µg mL^{-1}) (Ekinci et al 2014; Turan et al 2014)

Çizelge 1- Kullanılan bakteri izolatlarının izole edildikleri bitki, azot fiksasyonu ve fosfat çözebilme özellikleri ve hormon (IAA, GA_3) üretimi (µg mL^{-1})

Bacterial strains	*Isolated from*	*N*	*P*	*GA_3*	*IAA*
Achromobacter xylosoxidans TV-42A	*Poaceae* sp.	S+	W+	299.532	1.402
Brevibacillus choshinensis TV-53D	*Taraxacum* sp.	S+	S+	362.206	1.140
Myroides odoratus TV-85C	Sugar beet	-	-	243.893	0.324
Bacillus megaterium TV-87A	Sugar beet	+	-	262.163	0.608
Colwellia psychrerytreae TV-108G	*Poaceae* sp.	+	-	166.856	0.000
Kluyvera cryocrescens TV-113C	*Allium* sp.	+	+	171.620	10.325
Bacillus GC group A TV-119E	*Poaceae* sp.	W+	+	290.349	0.509

+, positive; S+, strong positive; W+, weak positive; -, negative

2.2.3. Coating procedure of the bacteria on the corms

The bacteria were grown in TSB as described above. Absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm and properly diluted to $1x10^8$ CFU mL^{-1} in sdH_2O. Approximately, 0.2 g of sucrose (10 mg mL^{-1}) was added to each Erlenmeyer flasks, and the surface-sterilized corms were soaked separately in this suspension. The corms were incubated in the flasks by shaking at 80 rpm for two hours at 28 ºC to coat the corms with the bacteria. As controls applications, untreated bacteria and hormones (100 mg L^{-1} IBA and 100 mg L^{-1} GA_3) were used in this study. Concentration of the hormone was prepared at the rate of 100 mg L^{-1} with drinking water. After then, the corms were soaked in these solutions for two hours to coat the corms with the hormones and untreated bacteria. After shaking, the corms were taken out and air-dried on sterile Whatman filter paper sheets overnight in the laminar flow hood.

2.2.4. Greenhouse studies

In the study, there were 10 treatments: (1) *Achromobacter xylosoxidans* strain TV-42A, (2) *Brevibacillus choshinensis* strain TV-53D, (3) *Myroides odoratus* strain TV-85C, (4) *Bacillus megaterium* strain TV-87A, (5) *Colwellia psycrerytreae* strain TV-108G, (6) *Kluyvera cryocrescens* strain TV-113C, (7) *Bacillus* GC group B strain TV119E, (8) Control 1 (untreated bacteria or hormones), (9) Control 2 (only IBA treatment) and (10) Control 3 (only GA_3 treatment). Research was established in a complete randomized design with 3 replications and each replication have 5 plants. For each application, 5 saffron corms were planted. A total of 150 corms were used, and the study was conducted under greenhouse conditions. Experimental soil composed of 1:1:1 ratio of soil, sand and farmyard manure. Treated corms were planted in black pouch having 3 litres volume. During the study period, considering the humidity and temperature values of greenhouse, irrigation was performed according to the irrigation requirement of the saffron plant.

2.2.5. Evaluation of the results

Vegetative growth of saffron plant such as the number of days between planting and emergence, emergence ratio (%), number of stems (number $plant^{-1}$), number of leaf (number $plant^{-1}$), the thickness of root collar (mm), number of cormlet, average cormlet diameter (mm), cormlet length (mm) and cormlet weight (g) of saffron plant were determined. Macro and micro nutrient (N, K, P, Mg, S, Ca, Na, Fe, Mn, Zn, Cu, Pb, B and Cd) contents of corms were also determined. Plant samples were oven-dried at 68 °C for 48 h and were then ground. Potassium (K), Ca and Mg were determined after wet digestion of dried and ground sub-samples in a H_2SO_4-Se-Salicylic acid mixture. Phosphorus (P) was determined spectrophotometrically by the vanadomolybdophosphoric-yellow method (Lott et al 1956). Potassium (K) and Ca were determined by flame photometry, and Mg, Cu, Fe, Mn, Na, Zn, Pb, B and Cd were determined by atomic absorption spectrometry using the methods of AOAC (1990). Boron was determined, after dry-ashing of plant samples, spectrophotometrically at 550 nm by the curcumin method (Odom 1992).

2.3. Statistical analysis

Data treated by the analysis of variance by using the SPSS version 17.0 statistical software package (SPSS Inc., Chicago, IL, USA). For the significance level, 5% has been set to be the maximum acceptable limit to be considered as a significant result.

3. Results

The emergence dates of saffron subjected to different treatments were given in Table 2. The treatments affected the emergence dates of saffron. The earliest emergence date was obtained from *M. odoratus* TV-85C and *C. psycrerytrea* TV-108G bacterial strains as 48-53 days (Table 2). Emergence of plant continued from the 1st week of September to the 2nd week of October.

The resulting effects of the treatments on emergence ratio and some morphological values of saffron plant were given in Table 3. According

Table 2- The effect of different treatments on saffron-emergence dates

Çizelge 2- Farklı uygulamaların safran çıkış sürelerine etkisi

Treatments	*Number of days between planting and emergence*
A. xylosoxidans TV-42A	50-54
B. choshinensis TV-53D	48-60
M. odoratus TV-85C	48-53
B. megaterium TV-87A	49-60
C. psychrerytreae TV-108G	48-53
K. cryocrescens TV-113C	50-54
Bacillus GC group A TV-119E	51-65
Control 1 (untreated)	50-58
Control 2 (100 mg L^{-1} IBA)	51-58
Control 3 (100 mg L^{-1} GA_3)	52-65

to these results, there were no significant (P>0.05) differences in terms of IBA, GA_3 and all bacterial treatments when compared to the control treatment on emergence ratio (%). However, emergence ratio in GA_3 treatment (86.67%) was found low according to the other treatments. It was found that emergence ratio was approximately the same to the bacteria treatments and IBA treatment.

The effects of the all bacterial treatments on the number of saffron stems was significant (at P<0.001) according to the control 3 (100 mg L^{-1} GA_3). The average number of stem of all applications was 2.65 number $plant^{-1}$. Minimum number of stem (1.70 number $plant^{-1}$) was obtained from the GA_3 treatment. The maximum number of stems wre obtained from *A. xylosoxidans* TV-42A. The increase in *A. xylosoxidans* TV-42A application as the control 1, 2 and 3 were 73.9%, 60.0% and 135.3%, respectively. *B. choshinensis* TV-53D, *M. odoratus* TV-85C, *B. megaterium* TV-87A, *Bacillus* GC group A TV-119E, control 1 (untreated) and control 2 (100 mg L^{-1} IBA) were located in the same group. There were no significant (P>0.001) differences in terms of IBA, GA_3 and bacterial treatments when compared to the untreated control treatment on the number of saffron leaf. The highest number of leaf were obtained from *A. xylosoxidans* TV-42A (17.20 number $plant^{-1}$), *C. psychrerytreae* TV-108G (16.63 number $plant^{-1}$) and *K. cryocrescens* TV-113C (15.60 number $plant^{-1}$). Average number of leaf was 14.39 number $plant^{-1}$. There were no significant (at P>0.05) effects of treatments on the thickness of root collar. According to the control treatment, the maximum thickness of root collar (2.75 mm) was obtained from *C. psycrerytreae* TV-108G bacteria treatment.

Table 3- The effects of the bacteria and hormones treatments on the emergence ratio and some morphological values of saffron plant

Çizelge 3- Bakteri ve hormon uygulamalarının safran bitkisinin çıkış oranı ve kimi morfolojik özelliklerine etkisi

Treatments	*Emergence ratio (%)*	*Number of stems (number $plant^{-1}$)*	*Number of leaf (number $plant^{-1}$)*	*The thickness of root collar (mm)*
A. xylosoxidans TV-42A	100.00±0.00	4.00±0.20 a	17.20±0.72 a	2.32±0.36
B. choshinensis TV-53D	93.33±11.54	2.47±0.12 c	14.80±0.20 cd	2.57±0.19
M. odoratus TV-85C	93.33±11.55	2.40±0.00 c	12.40±0.60 f	2.42±0.10
B. megaterium TV-87A	93.33±11.55	2.33±0.08 c	13.80±0.00 def	2.61±0.13
C. psychrerytreae TV-108G	100.00±0.00	3.00±0.00 b	16.63±1.38 ab	2.75±0.22
K. cryocrescens TV-113C	93.33±11.55	3.20±0.00 b	15.60±0.80 bc	2.44±0.01
Bacillus GC group A TV-119E	100.00±0.00	2.60±0.20 c	13.00±0.60 ef	2.49±0.18
Control 1 (untreated)	100.00±0.00	2.30±0.30 c	12.40±1.00 f	2.62±0.07
Control 2 (100 mg L^{-1} IBA)	100.00±0.00	2.50±0.30 c	14.00±1.20 def	2.59±0.30
Control 3 (100 mg L^{-1} GA_3)	86.67±23.09	1.70±0.30 d	14.10±1.10 cde	2.52±0.33
Average	96.00±9.68	2.65±0.62	14.39±1.76	2.53±0.22
F-values	0.61ns	31.60***	11.16***	0.95ns

ns, non-significant; ***, significant at P<0.001; difference between the means with same letter in a column is not significant

The effects of the treatments on the number of cormlet, cormlet diameter, cormlet length and cormlet weight of saffron plant were given in Table 4. As shown, the bacteria except *B. megaterium* TV-87A and hormones treatments significantly ($P<0.001$) increased the number of saffron cormlets according to control 1 (untreated). When the values obtained in the yield are compared with the value before planting, a reduction is observed. The maximum number of cormlet was obtained from *A. xylosoxidans* TV-42A (2.8 number plant^{-1}) and *K. cryocrescens* TV-113C (2.4 number plant^{-1}) application. Cormlet diameter (mm) and cormlet length (mm) were found significant ($P<0.001$) in bacteria and hormones treatments when compared to the control 1. Also, effect of bacteria and hormones treatments on cormlet weight (g plant^{-1}) was found significant ($P<0.05$). The maximum cormlet diameter, cormlet length and cormlet weight were obtained from *B. megaterium* TV-87A. However, this application was in the same group with control 1 (untreated) treatment.

The treatments had significant effects on P, Ca, S, K, Mg (at $P<0.001$) and N (at $P<0.01$) (Table 5). The maximum P (1.98%) and K (1.85%) were found in *B. megaterium* TV-87A treatment while the maximum (1.80%) total N was found in *K. cryocrescens* TV-113C bacteria treatment. The maximum Mg (0.21%) was determined in *Bacillus* GC group TV-119E treatment. *K. cryocrescens* TV-113C, *Bacillus GC group* TV-119E bacteria treatments and GA_3 treatments were in the same group and it was concluded that there were no significant differences among these treatments (Table 5).

The treatments had significant effects on Fe, Na, Mn, Zn, Cu, Pb, Cd and B nutrient elements at $P<0.001$. The maximum total Na (225.25 mg kg^{-1}) was found in *B. choshinensis* TV-53D bacteria treatment while the maximum Fe (157.08 mg kg^{-1}) and Zn (54.60 mg kg^{-1}) were found in *B. megaterium* TV-87A bacteria treatment. The maximum (35.20 mg kg^{-1}) total Mn was obtained from *B. megaterium* TV-87A bacteria treatment, but there was no significant difference between it and the control. According to control 1 treatment, the maximum (52.80 mg kg^{-1}) total Cu and the maximum (8.80 mg kg^{-1}) total Pb were obtained from control 2 (100 mg L^{-1} IBA) treatment. According to the control treatment, the maximum (21.32 mg kg^{-1}) total B was determined in

Table 4- The effects of the bacteria and hormones treatments on some morphological values of saffron corms

Çizelge 4- Bakteri ve hormon uygulamalarının safran kormlarının kimi morfolojik özelliklerine etkisi

Treatments	*Number of cormlet (number plant^{-1})*	*Cormlet diameter (mm)*	*Cormlet length (mm)*	*Cormlet weight (g plant^{-1})*
A. xylosoxidans TV-42A	2.80±0.40 a	10.05±0.61 de	7.51±1.01 d	0.77±0.10 b
B. choshinensis TV-53D	2.10±0.10 bc	11.03±0.79 cd	8.42±0.85 cd	1.37±0.20 a
M. odoratus TV-85C	2.30±0.30 bc	11.23±0.28 bcd	8.28±0.45 cd	1.28±0.13 a
B. megaterium TV-87A	1.40±0.00 e	12.80±0.99 a	10.00±0.03 a	1.50±0.20 a
C. psychrerytreae TV-108G	2.20±0.20 bc	12.44±1.53 ab	9.69±0.33 ab	1.38±0.34 a
K. cryocrescens TV-113C	2.40±0.35 ab	11.21±0.65 bcd	8.38±0.61 cd	1.18±0.02 a
Bacillus GC group A TV-119E	1.60±0.00 de	8.87±0.13 e	8.69±0.48 bc	1.33±0.30 a
Control 1 (untreated)	2.30±0.30 bc	12.42±0.47 ab	9.98±0.81 a	1.28±0.14 a
Control 2 (100 mg L^{-1} IBA)	1.90±0.10 cd	11.67±0.49 abc	9.89±0.08 a	1.21±0.14 a
Control 3 (100 mg L^{-1} GA_3)	1.60±0.20 de	12.32±0.08 abc	9.98±0.71 a	1.44±0.28 a
Average	2.05±0.46	11.40±1.32	9.08±1.03	1.27±0.26
F-values	10.02***	8.44***	6.74***	2.97*

*, significant at $P<0.05$; ***, significant at $P<0.001$; difference between the means with same letter in a column is not significant

Table 5- Macronutrient concentrations of saffron corm (%)

Çizelge 5- Safran kormlarına makro besin elementi konsantrasyonları (%)

Treatments	*N*	*P*	*K*	*Ca*	*S*	*Mg*
A. xylosoxidans TV-42A	1.60±0.10 ab	0.24±0.02 e	1.56±0.04 c	0.62±0.03 f	0.51±0.02 d	0.33±0.02 b
B. choshinensis TV-53D	1.70±0.10 ab	0.24±0.01 e	1.44±0.08 d	0.62±0.02 f	0.42±0.05 e	0.37±0.02 a
M. odoratus TV-85C	1.60±0.20 ab	0.25±0.02 e	1.66±0.03 b	0.68±0.03 e	0.43±0.02 e	0.36±0.02 ab
B. megaterium TV-87A	1.60±0.10 ab	0.28±0.01 e	1.85±0.02 a	0.70±0.01 e	0.49±0.01 d	0.38±0.01 a
C. psychrerytreae TV-108G	1.30±0.10 c	0.17±0.01 f	1.12±0.02 e	0.37±0.02 g	0.35±0.01 f	0.25±0.02 cd
K. cryocrescens TV-113C	1.80±0.10 a	1.98±0.02 a	0.29±0.03 f	1.50±0.03 ab	0.67±0.02 a	0.20±0.01 d
Bacillus GC group A TV-119E	1.70±0.10 ab	1.66±0.08 d	0.24±0.02 f	1.47±0.03 b	0.63±0.02 b	0.21±0.01 d
Control 1 (untreated)	1.60±0.10 ab	1.85±0.04 b	0.24±0.01 f	1.42±0.03 c	0.62±0.01 bc	0.20±0.02 d
Control 2 (100 mg L^{-1} IBA)	1.50±0.10 b	1.76±0.03 c	0.26±0.02 f	1.52±0.02 a	0.69±0.01 a	0.21±0.02 d
Control 3 (100 mg L^{-1} GA_3)	1.60±0.10 ab	1.77±0.04 c	0.26±0.01 f	1.20±0.02 d	0.59±0.01 c	0.19±0.03 d
Average	1.60±0.16	1.02±0.80	0.89±0.67	1.01±0.44	0.54±0.11	0.27±0.08
F-values	4.10**	1725.00***	1240.68***	986.45***	90.96***	53.33***

, significant at P<0.01; *, significant at P<0.001; difference between the means with same letter in a column is not significant

A. xylosoxidans TV-42A bacteria treatment. However, *M. odoratus* TV-85C and *B. megaterium* TV-87A treatments and *A. xylosoxidans* TV-42A treatment were in the same group. The maximum (1.91 mg kg^{-1}) total Cd was determined in the control 3 (100 mg L^{-1} GA_3) treatment (Table 6).

Table 6- Micronutrient and heavy metal concentrations of saffron corm (mg kg^{-1})

Çizelge 6- Safran kormlarına mikro besin elementi ve ağır metal konsantrasyonları (mg kg^{-1})

Treatments	*Na*	*Fe*	*Mn*	*Zn*	*Cu*	*Pb*	*B*	*Cd*
A. xylosoxidans TV-42A	187.31±0.69 d	151.70±1.67 c	28.88±0.65 c	48.38±0.94 b	10.95±0.08 g	0.15±0.01 f	21.32±0.80 a	0.17±0.02 g
B. choshinensis TV-53D	225.25±3.28 a	128.70±0.33 g	30.81±0.17 bc	47.45±1.56 b	11.06±0.83 g	0.47±0.02 c	17,.26±0.28 b	0.35±0.01 e
M. odoratus TV-85C	182.78±1.20 e	139.04±0.97 d	31.08±1.01 b	48.98±0.42 b	12.64±0.43 f	0.37±0.01 e	20.54±0.55 a	0.22±0.01 fg
B. megaterium TV-87A	206.40±2.92 b	157.08±0.19 b	35.20±1.58 a	54.60±1.03 a	11.20±0.89 g	0.40±0.01 d	21.00±0.78 a	0.16±0.01 g
C. psychrerytreae TV-108G	191.73±3.59 c	185.64±1.34 a	14.40±0.96 d	27.38±0.99 e	7.20±0.39 h	0.07±0.01 g	13.34±0.81 c	0.29±0.01 ef
K. cryocrescens TV-113C	145.01±1.08 g	139.54±0.49 d	13.53±1.00 d	22.96±0.16 f	34.31±0.53 e	0.68±0.01 b	10.25±0.53 d	1.72±0.12 cd
Bacillus GC group A TV-119E	150.15±2.85 f	132.36±1.53 f	14.38±1.60 d	27.32±0.43 e	43.45±0.56 c	0.87±0.02 a	10.36±0.71 d	1.67±0.10 d
Control 1 (untreated)	138.48±1.57 h	135.00±3.01 e	12.88±0.68 d	33.18±0.60 c	39.50±0.53 d	0.67±0.02 b	10.16±0.58 d	1.85±0.04 ab
Control 2 (100 mg L^{-1} IBA)	144.00±1.64 g	139.00±2.03 d	14.08±1.5 d	31.08±1.01 d	52.80±0.24 a	0.88±0.02 a	10.34±0.25 d	1.80±0.05 bc
Control 3 (100 mg L^{-1} GA_3)	132.34±0.74 ı	121.00±0.91 h	14.28±1.30 d	26.85±1.16 e	45.20±1.15 b	0.69±0.01 b	10.34±0.50 d	1.91±0.01 a
Average	170.35±31.27	142.91±17.66	20.95±8.94	36.82±11.30	26.83±17.14	0.53±0.27	14.49±4.82	1.01±0.79
F-values	639.62***	449.43***	194.82***	483.11***	2317.43***	1072.2***	199.73***	689.959***

***, significant at P<0.001; difference between the means with same letter in a column is not significant

4. Discussion

There are several PGPR inoculants presently commercialized that seem to promote growth through at least one mechanism; improved nutrient acquisition (Biofertilizers) or phytohormone production (Biostimulants). In this study, a total of seven bacterial strains and hormone applications were tested to see their effects on plant growth promoters of saffron.

There has no information on the number of stems per plant so far. The number of stem is an important character of which there was a very close relationship between the number of stem and the number of corm per plant. As a matter of fact, İpek et al (2009) reported the same finding. Deo (2003) indicated that the large bulb had between 5-11 leaves. The average number of leaves was the same with the findings and the treatments had enhancing effect on the parameter. It is considered that there may be closely related to the number of leaves and the number of stems.

Frank (1986) stated that bulbous plants blooming in autumn are usually blooming in the second year. Soheilivand et al (2007), reported that Iranian origin saffron bulbous bloomed in the second year. Saffron bulbous of our research were not bloomed in the first year. Findings of our research were in accordance with these findings. As a matter of fact, the aim of our study was to get a large number of quality corms and to investigate the availability of the resulting quality corms as well as an ornamental plant. Based on the obtained results, we can interpret that the use of these bacterial isolates in the cultivation of ornamental plants may be one of the important tools for decreasing the maintenance cost and achieving sustainability in the landscapes. Benschop (1993) determined that *Crocus sativus* is the most important specie bloomed in autumn and Crocus species is used as a garden plant and pot. After all other flowering plants seeding in October, they bloom bright-coloured flowers with a darker purple colour from light pastel mauve (Willard 2002). Flowers are similar to lilies and they are at size of tulips (Safranbolu 2015).

Bacterial inoculations increased the plant nutrient element content. The plant nutrient elements Pb, Cd, S and Ca that obtained from GA_3 application were excluded. The highest content of K, Mg, Mn, Zn and B were obtained from *B. megaterium* TV-87A bacterial application. Furthermore, maximum cormlet diameter, cormlet length and cormlet weight values were obtained from this application. Corm diameter is the most important factor affecting the yield (Çavuşoğlu & Erkel 2005). Also, the highest content of N, P and S were obtained from *K. cryocrescens* strain TV-113C bacterial application. Increasing the mineral content may be explained by organic acids plant growth hormones and amino acids production by plant and bacterial inoculations (Table 1). In the study of Turan et al (2014), *B. megaterium* TV-87A inoculation increased the plant growth parameters such as fresh shoot weight, dry shoot weight, root diameter, root length, fresh root weight, dry root weight, plant height, stem diameter, leaf area and chlorophyll contents of cauliflower transplant. Except for indole acetic acid (IAA), the values of abscisic acid (ABA), gibberellic acid (GA) and salicylic acid (SA) increased in the applications by some ratio compared to the control.

Many publications have been previously documented on plant growth promoting bacteria (Erman et al 2010; Ekinci et al 2014; Turan et al 2014). However, the effects of the PGPR and hormones treatments on plant growth and formation of corm in saffron cultivation has not been enough studied. Sharaf-Eldin et al (2008) studied the effect of *B. subtilis* FZB24 on saffron (*Crocus sativus* L.) corms under *ex-vitro* conditions in Egypt and reported that inoculation of *B. subtilis* FZB24 significantly increased the leaf length, flowers per corm, weight of the first flower stigma, total stigma biomass and significantly decreased the time required for corms to sprout and the number of shoots. In addition, Parray et al (2013) stated that some important plant growth promoting bacteria as *B. subtilis* and *Pseudomonas* ssp., showed IAA production, phosphate solubilisation production and siderophore production can be used as biofertilizers and application of these rhizobacterial strains

may provide some benefit to saffron growers by speeding corm growth (earlier shoot emergence) and increasing stigma biomass. The present study showed that some of the bacterial application increased the plant growth parameters of saffron. These results are in agreement with the previous literature reports on bacteria. To our best knowledge, there is no investigation on the effects of PGPR on plant growth and quality of saffron in Turkey. For this reason, our study is one of the first of its kind in Turkey.

However, P, Ca, S, Mg, Mn, Cu, Pb and Cd content of the plants applied bacteria decreased whereas the uptake of K, Na, Fe, Zn and B slightly increased (Table 5 and 6). In recent years, the heavy metals pollution resulting from industrial and agricultural activities has emerged as a major problem. It was reported to be significant in the level of the metal products as Mn, Cu, Cd, Pb and Hg (Saharan & Nehra 2011). Contamination of soil with Cd can negatively affect biodiversity and the activity of soil microbial communities (Chen et al 2003). In this study, bacteria applied plants reduced Cu, Cd, P, Ca, S and Pb concentration while the Na, Fe, Zn, Mn, K, Mg and B were abundantly present as compared to control. The presence of these bacteria in the soil as biofertilizers can protect the plant from metal toxicity and stimulate plant growth. In addition, Mayak et al (2004) reported that *A. piechaudii* having ACC deaminase activity significantly increased the fresh and dry weights of tomato seedlings grown in the presence of NaCl. The bacterium reduced the production of ethylene in tomato seedlings, which was otherwise stimulated when seedlings were challenged with an increase in salt concentrations.

In conclusion, the main objective was to achieve the most and best quality corm by using some hormones and PGPR improving the quality, growth and the yield of saffron product. The results were statistically evaluated. From these results, it could be concluded that the tested bacterial strains have some important plant growth promoting traits that can be used as biofertilizers and application of these bacterial strains may provide some benefit to saffron growers by speeding corm growth (earlier shoot emergence), and increasing the stem and leaf biomass and number of cormlet and cormlet weight. In conclusion, the bacteria treatments used in research were determined as significant in terms of plant development and corm of saffron. The bacterial strain tested in this study may be a potential to be used as a biofertilizer in sustainable and organic common vetch production. The bacterial bio-formulations used in organic farming increase plant growth, and the development of saffron complete that positive affect. Saffron blooms several beautiful flowers and carries perennial feature due to the corm. Because of these properties, saffron can be used as ornamental plant in rock gardens, in flower beds, in grass, in balconies and terraces and in the regulation of the roof garden. Use of these bacterial isolates in the cultivation of ornamental plants may be one of the important tools for decreasing the maintenance cost and achieving sustainability in the landscapes. The effects of the PGPR and hormones treatments on plant growth and formation of corm in saffron cultivation has not been studied enough. Our study is one of the first of its kind in this sense. The bacteria treatments used in this study had significant effects on plant development and corm of saffron. Further, it is thought that further investigation should be made by using different bacterial breeds. Further similar study will be useful with more material. Results should be transferred into practice and converted into economic benefits by sharing with employees in the crop production sector**.**

References

Amirghasemi T (2001). Saffron, the red gold of Iran. Ayandegan publishing cultural Institution. Tehran, Iran

AOAC (1990). Official methods of Analysis of the AOAC. Volume 2 (No. Ed. 15). Association of Official Analytical Chemists Inc

Basker D & Negbi M (1983). Uses of Saffron. *Economic Botany* **37**(2): 228-236

Benschop M (1993). Crocus. The physiology of flower bulbs. pp. 257-272

Budak N, Çalışkan C F & Çaylak Ö (1994). Plant growth regulators and use of agricultural production. *Ege University Journal of Faculty of Agriculture* **31**: 289-296

Çakmakçı R, Dönmez F, Aydın A & Şahın F (2006). Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biology & Biochemistry* **38**(6): 1482-1487

Carmona M, Zalacain A, Sanchez A M, Novella J L & Alonso G L (2006). Crocetin esters, picrocrocin and its related compounds present in *Crocus sativus* stigmas and Gardenia jasminoides fruits. Tentative identification of seven new compounds by LC-ESI-MS. *Journal of Agriculture and Food Chemistry* **54**: 973-979

Çavuşoğlu A & Erkel E İ (2005). The effect of different planting areas and corm size on yield and harvest period of saffron (*Crocus sativus* L.) in Kocaeli province. *Akdeniz University Journal of the Faculty of Agriculture* **18**(2): 179-184

Chen Y, Yang Y, Zheng S & Wong M (2003). Effect of cadmium on nodulation and N2 fixation of soyabean in contaminated soils. *Chemosphere* **50**: 781-787

Chichiricco G (1984). Karyotype and meiotic behaviour of the triploit *Crocus sativus* L. *Caryologia* **37**: 233-239

Deo B (2003). Growing saffron-the world's most expensive spice. *Crop and Food Research* **20**(1): 1-4

Dobbelaere S, Vanderleyden J & Okon Y (2003). Plant growth-promoting effects of diazotrophs in the rhizosphere. *Critical Reviews in Plant Sciences* **22**(2): 107-149

Ekinci M, Turan M, Yildirim E, Güneş A, Kotan R & Dursun A (2014). Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone content of cauliflower (*Brassica oleracea* l. var. *botrytis*) transplants. *Acta Scientiarum Polonorum Hortorum Cultus* **13**(6): 71-85

Erman M, Kotan R, Çakmakçı R, Çığ F, Karagöz K & Sezen M (2010). Effect of nitrogen fixing and phosphate-solubilizing rhizobacteria isolated from Van Lake Basin on the growth and quality properties in wheat and sugar beet. *Turkey IV. Organic Farming Symposium*, 28 June-1 July, Erzurum, Turkey, pp. 325-329

Escribano J, Diaz-Guerra M J M, Riese H H, Alvarez A, Proenza R & Fernandez J A (2000). The cytolytic effect of a glycoconjugate extracted from corms of saffron plant (*Crocus sativus* L.) on human cell lines in culture. *Planta Medica* **66**: 157-162

Frank R (1986). 'Zwiebel und Knollengewächse' Eugen Ulmer Verlag, Stuttgart, DE, ISBN 3-8001-6159-1

Grunewald W, Noorden G V, Isterdael G V, Beeckman T, Gheysen G & Mathesius U (2009). Manipulation of auxin transport in plant roots during rhizobium symbiosis and nematode parasitism. *The Plant Cell* **21**: 2553-2562

İpek A, Arslan N & Sarıhan E (2009). Effects of different planting depth and bulb sizes on yield and yield components of saffron (*Crocus sativus* L.). *Tarım Bilimleri Dergisi-Journal of Agricultural Science* **15**(1): 38-46

Kara I (2010). Saffron Cultivation in Eskisehir. *Eskişehir Trade Exchange Journal* **1**(1): 29-31

Kaynak L & Ersoy N (1997). General properties of plant growth regulator and uses. *Akdeniz University Faculty Agricultural Journal* **10**: 223-236

Kaynak L & Memiş K (1997). Effect of plant growth blocker and delay mechanisms. *Akdeniz University Faculty Agricultural Journal* **10**: 237-248

Kotan R & Şahin F (2002). Use of bacterial organisms in biological control of plant disease. *Atatürk University Faculty Agriculture Journal* **33**: 111-119

Kotan R, Sahin F & Ala A (2005). Identification and pathogenicity of bacteria isolated from pome fruits trees in Eastern Anatolia Region of Turkey. *Journal of Plant Diseases and Protection* **113**: 8-13

Kotan R, Çakir A, Ozer H, Kordali Ş, Çakmakci R, Dadasoglu F, Dikbaş N, Aydin T & Kazaz C (2014). Antibacterial effects of *Origanum onites* against phytopathogenic bacteria: Possible use of the extracts from protection of disease caused by some phytopathogenic bacteria. *Scientia Horticulturae* **172**: 210-220

Lott W L, Nery J P, Gallo J R & Medcalf J C (1956). Leaf analysis technique in coffee research. *IBEC Research Institute* **9**: 21-24

Lozano P, Castellar M R, Simancas M J & Iborra J L (1999). Quantitative highperformance liquid chromatographic method to analyse commercial saffron (*Crocus sativus* L) products. *Journal of Chromatography A* **830**: 477-483

Mayak S, Tirosh T & Glick B R (2004). Plant growth-promoting bacteria confer resistance in tomato plants

to salt stress. *Plant Physiology and Biochemistry* **42**: 565-572

Mia M A B, Hossain M M, Shamsuddin Z H & Islam M T (2012). Plant-associated bacteria in nitrogen nutrition in crops with special reference to rice and banana. In: D. K. Maheshwari (ed.), *Bacteria in Agrobiology: Plant Nutrition.* pp. 97-126

Miller L T (1982). Single derivatization method for routine analysis of bacterial whole-cell fatty acid methyl esters, including hydroxy acids. *Journal Clinical Microbiology* **16**: 584-586

Odom J W (1992). Determination of Total Boron in Plants by the Curcumin Method. Plant Analysis Reference Procedures for the Southern Region of the United States. Southern Cooperative Series Bulletin No. 368, The University of Georgia Crops and Soil Science Dept. Athens. GA

Parray J A, Kamili A N, Reshi Z A, Hamid R & Qadri R A (2013). Screening of beneficial properties of rhizobacteria isolated from Saffron (*Crocus sativus* L.) rhizosphere. *African Journal of Microbiology Research* **7**(23): 2905-2910

Safranbolu (2015). Safranbolu Kaymakamlığı. http://www.safranbolu.gov.tr/default_B0.aspx?content=86. (Erişim tarihi: 26.01.2015)

Saharan B S & Nehra V (2011). Plant growth promoting rhizobacteria: A critical review. *Life Sciences and Medicine Research* **2011**: LSMR-21

Saikia R, Kumar R, Arora D K, Gogoi D K & Azad P (2006). *Pseudomonas aeruginosa* inducing rice resistance against *Rhizoctonia solani*: Production of salicylic acid and peroxidases. *Folia Microbiologica* **51**: 375-380

Sharaf-Eldin M, Shereen E, Jose-Antonio F, Helmut J, Ronald C, Jose G & Pamela W (2008). *Bacillus subtilis* FZB24 affects flower quantity and quality of Saffron (*Crocus sativus*). *Planta Medica* **74**: 732-734

Soheilivand S, Agayev Y M, Shakib A M, Fathi M & Rezvani E (2007). Comparsion of diversity in flowering rate of two saffron (*Crocus sativus L.*) populations of Iran. *Acta Horticulturae* (ISHS) **739**: 303-306

Şahin F, Çakmakçı R & Kantar F (2004). Sugar beet and barley yields in relation to inoculation with N_2-fixing and phosphate solubilizing bacteria. *Plant and Soil* **265**: 123-129

Taiz L & Zeiger E (2004). Fisiologia Vegetal. 3.ed. *Porto Alegre: Artmed*, 719 pp

Tarantilis P A & Polissiou M G (1997). Isolation and identification of the aroma components from saffron (*Crocus sativus* L.). *Journal of Agricultural and Food Chemistry* **45**: 459-462

Turan M, Ekinci M, Yildirim E, Güneş A, Karagöz K, Kotan R & Dursun A (2014). Plant growth-promoting rhizobacteria improved growth, nutrient, and hormone content of cabbage (*Brassica oleracea*) seedlings. *Turkish Journal of Agriculture and Forestry* **38**: 327-333

Vurdu H & Güney K (2004). Kırmızı Altın. ISBN:975-92006-0-0, 108 s, Bizim Büro Basımevi, Ankara

Vurdu N, Allahverdiev S & Vurdu H (1997). The effects of hormones on the growth of the saffron crocus (*Crocus sativus* L.). *Kastamonu Education Journal* **4**: 85-89

Warburg E F (1957). Crocuses. *Endeavour* **16**: 209-216

Willard P (2002). Secrets of Saffron: The Vagabond Life of the World's Most Seductive Spice. Beacon Press