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RESEARCH ARTICLE

Effects of Rhizobacteria on Plant Development, Quality of Flowering and Bulb Mineral Contents in *Hyacinthus orientalis* L.

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ABSTRACT

Size of bulbs is directly proportional to the quality of the flower, the commercial value of the bulb and getting more bulblet. The research was carried out to evaluate the effects of PGPR on plant growth parameters, flowering, bulb quality and bulb mineral contents in hyacinth (*Hyacinthus orientalis* L. cv. Aiolos) under greenhouse condition. In the study, there were 5 applications: (T_1) *Pseudomonas putida strain* RCK-42A, (T_2) *Kluyvera cryocrescens strain* RCK-113C, (T_3) *Paenibacillus polymyxa strain* RCK-12E, (T_4) *Bacillus subtilis strain* RCK-17C, and (T_5) Control (uninoculated bacteria). The surface-sterilized bulbs were incubated separately by shaking at 80 rpm for two hours at 28 ºC to coat the bulbs with the bacteria. The chlorophyll content (50.02), leaf length (26.03 cm), leaf area (268.38cm^2), flower fresh and dry weight (15.54 g and 0.88 g) in T_2 (*Kluyvera cryocrescens* strain RCK-113C) was found as the maximum according to other applications. The highest leaf width (6.37 cm) and the highest floret number were observed in T_4. It was shown that the maximum bulb diameter (42.57 mm), bulb length (40.01 mm) and bulb weight (12.01 g) were determined in T_2. The maximum N (2.90%), P (1.98%) and Ca (1.74%) were found in T_3. Maximum Fe (0.48 mg kg^{-1}), Mn (151.20 mg kg^{-1}) and Zn (35.28 mg kg^{-1}) were found in T_1. Use of especially *Kluyvera cryocrescens* strain RCK-113C and *Pseudomonas putida* strain RCK-42A bacterial isolates may be effective in maintaining the sustainability of the environment and growing medium in the cultivation of hyacinth and also the development of bio fertilizer.

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Introduction

Hyacinth (*Hyacinthus orientalis L.)* belongs to *Hyacinthaceae* Batsch ex Borkh family and *Hyacinthus* genus. Hyacinths are used in the landscape studies (Xie and Wu, 2017) and cultivated mainly for indoor, outdoor and balcony decorations (Ekim et al., 2000). The plant has commercial importance for cut flower and as well as in garden designs, and duplicating bulbs are also sold on the market in order to contribute to the economy (Xie and Wu, 2017). The plant is also used industries related to perfumery for obtaining essential oil extracts (Kizil et al., 2016).

Seeds using for development of new cultivars are not preferred for commercial multiplication in hyacinth. Their natural propagation rates are very slow and take 4–6 years to develop a bulb size capable of flowering and seed set under optimum conditions (Kizil et al., 2016). In general, the amounts of stored reserves present in corm, bulb or rhizome have certain effects on the performance of vegetative propagated plants. Size of bulbs is directly proportional to the quality of the flower, the commercial value of the bulb and getting more bulblet (Rees, 1969; Padhye and Cameron, 2007; Parlakova, 2014). In the direction of this information, plant nutrition is important for the best development of *hyacinth*, bulb growth and number of bulbs.

The production and profit increase in agriculture brought along the intensive use of inputs. In this case, different microorganisms selected from rhizosphere are used for nutrition in order to increase the plant growth. Plant growth

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promoting rhizobacteria (PGPR) that promote plant growth are used as organic fertilizer because of the useful effects on plant growth. PGPR have several important bacterial characteristics that have been generally attributed to their ability to fix atmospheric nitrogen, secretion of certain organic compounds, solubilize soil phosphate, produce antibiotics, phytohormones and siderophores, or suppress deleterious rhizobacteria (Glick, 1995; Pérez-Montaño et al., 2014). There are many reports showing that PGPR have promoted the reproductive and growth parameters of ornamental plants (Parlakova, 2014; Arab et al., 2015; Parlakova Karagöz and Dursun 2019a,b), vegetable crops (Botta et al., 2013; Pahari et al., 2017), fruits (Arikan and Pirlak, 2016; Pii et al., 2017.) field crops (Mirshekari et al., 2012; Di Benedetto et al., 2016; Nosheen et al., 2018). There is no study using PGPR as plant growth promoting agent in hyacinth cultivation around the world.

The aim of this study was to examine the effects of PGPR (*Paenibacillus polymyxa, Pseudomonas putida, Kluyvera cryocrescens* and *Bacillus subtilis*) on growth parameters, flowering, bulb quality and bulb mineral contents of hyacinth. This study also aimed at producing big sized quality bulbs in a maximum number and good quality by using PGPR during the cultivation of hyacinth.

Materials and Methods

Materials and Plant Set-up

Bulbs of *Hyacinthus orientalis* L. (cv. Aiolos) used in the experiments were purchased from Asya Lale Company in Turkey (Konya). Bulbs free of rotten and wounded were at 16-17 cm circumference length of bulbs. The study was carried out under the natural light in greenhouse at the Department of Horticulture of Agricultural Faculty, Atatürk University between on December 8 in 2016 and on June 10 in 2017.

The daytime temperatures were recorded as 26 ± 2 ^{0}C and night temperatures were recorded as 10 ± 2 ^{0}C in the greenhouse. Surfaces of bulbs were disinfected in 3% sodium hypochlorite by dipping the bulb for 3 min and washing three times in distilled water.

All of the bacterial strains (*Pseudomonas putida strain* RCK-42A*, Kluyvera cryocrescens strain* RCK-113C*, Paenibacillus polymyxa strain* RCK-12E and *Bacillus subtilis strain* RCK-17C*)* were acquired from the culture collection unit in the Department of Plant Protection, Faculty of Agriculture at Atatürk University (Table 1).

Table 1. Nitrogen fixation (N), phosphate-solubilising activity (P) of the tested bacterial strains.

Code of application	Bacterial strains	Isolated from	N	P
T_1	*Pseudomonas putida* RCK-42A	*Poaceae* sp.	W+	W+
T_2	*Kluyvera cryocrescens* RCK-113C	*Allium* sp.	+	+
T_3	*Paenibacillus polymyxa* RCK-12E	*Poaceae* sp.	S+	+
T_4	*Bacillus subtilis* RCK-17C	*Rubus sp.*	S+	W+

+: Positive; S+: Strong positive; W+: Weak positive; -: Negative
Kotan et al., 2005; Kotan et al., 1999; Erman et al., 2010

The best nitrogen fixing and best phosphorus solubilizing of the bacterial strains given in Table 1 were selected by considering the results obtained in previous studies and some biochemical test results of each strain (Erman et al., 2010). Absorbance of the bacterial suspensions was measured spectrophotometrically at 600 nm. The bacterial suspensions were properly diluted to 1x10^8 CFU ml^{-1} in distilled water. Approximately, 0.2g of sucrose (10 mg mL^{-1}) was put in each Erlenmeyer flasks. The surface-sterilized bulbs were soaked separately in these suspensions and incubated by shaking at 80 rpm for two hours at 28 ºC in *the Erlenmeyer flasks* to coat the bulbs with the bacteria. The bulbs untreated by bacteria were used as the control. The experimental growing medium includes 1:1 ratio of farm soil in field condition and sand for ensuring drainage. Control and treated bulbs were planted in black polyethylene bag having 3-liter volume, 14.5 cm length and 20.5 cm^2 diameter on December 8 in 2016 and harvested on June 10 in 2017.

Experimental Design and Greenhouse Studies

In the study, there were 5 applications: (**T_1**) *Pseudomonas putida strain* RCK-42A, (**T_2**) *Kluyvera cryocrescens strain* RCK-113C, (**T_3**) *Paenibacillus polymyxa strain* RCK-12E, (**T_4**) *Bacillus subtilis strain* RCK-17C, and (**T_5**) Control (uninoculated bacteria). Research was established in a completely randomized design with 3 replications having 5 plants in each replication. For each application, total 15 hyacinth bulbs were planted, one bulb per pot. Total 75 bulbs (at 16-17 cm circumference length) were used. During the experiment period, irrigation was performed according to the irrigation needs of the hyacinth plant.

Quantitative and Qualitative Parameters and Measurements

Vegetative growth of hyacinth plant [diameter of stems (mm), chlorophyll content (SPAD), leaf area (cm^2), leaf width (cm), leaf length (cm)], some morphological parameters of hyacinth, bulbs [average bulb diameter (mm), bulb length (mm) and bulb weight (g)] and some morphological parameters of hyacinth flower [flower stem diameter (mm), number of floret per flower, flower diameter (mm), flower length (mm), flower fresh and dry weight (g)] were determined. Total leaf width and length were recorded as the sum of all the individual leaf widths and lengths for one particular plant (Addai, 2010). The leaf area was measured using CI 202 Portable digital brand leaf area meter. Bulb tissue samples were oven dried at 68 °C

for 48 h, ground, and passed through a 1-mm sieve. The Vapodest 10 Rapid Kjeldahl Distillation Unit (Gerhardt, Germany) and the Kjeldahl method and were used to determine total N (Bremner, 1996). Macro (K, P, Ca and Mg) and micro (Fe, Na, Mn, Cu, Zn) nutrients bulbs were also determined according to the Mertens (2005) method. The contents of K^{+}, Mg^{+2} and Ca^{+2} were determined after wet digestion of dried and ground sub-samples in a H_2SO_4-Se-Salisylic acid preparation. Phosphorus (P) was determined spectrophotometrically by the vanadomolybdophosphoric method (Lott et al., 1956) after reaction with ascorbic acid. K^{+} and Ca^{+2} were determined by flame photometry, and Mg^{+2}, Fe, Cu, Na, Mn and Zn were determined by atomic absorption spectrometry using the AOAC (1990) method.

Statistical Analysis

Data have been evaluated by analysis of variance, which was performed using the SPSS version 20.0 statistical software package (SPSS Inc., Chicago, IL, USA). Duncan's multiple range was used to compare significant difference. $p<0.05$ has been set to be the maximum acceptable limit and to be considered a significant result.

Results

According to the results, applications exerted a significant effect on diameter of stem (mm), chlorophyll content (SPAD), leaf area (cm^2), leaf width (cm) and leaf length from some morphological values in studied hyacinth cultivar (Table 2).

The highest diameter of stem was obtained from the T_3 (17.76 mm). There were significant ($p>0.05$) differences in terms of chlorophyll content (SPAD) and leaf area (cm^2) in all bacterial applications compared to the control application (T_5). However, chlorophyll content (50.02) and leaf area (268.38cm^2) in T_2 application was found as the maximum according to other applications. The highest leaf width was obtained from T_4 (6.37 cm). The highest leaf length (26.03 cm) was determined in T_2. The T_3 in terms of the leaf length parameter is in the same statistical group with the control (Table 2).

Table 2. The effects of the applications on some morphological values of hyacinth plant.

Applications	Diameter of stems (mm)	Chlorophyll content (SPAD)	Leaf area (cm^2)	Leaf width (cm)	Leaf length (cm)
T_1	16.13±0.81 b	38.29±0.96 c	252.17±0.95 b	4.20±0.10 c	23.10±0.53 b
T_2	15.27±0.44 c	50.02±1.51 a	268.38±0.47 a	5.63±0.21 b	26.03±0.93 a
T_3	17.76±0.85 a	45.81±0.76 d	228.04±9.76 c	5.83±0.06 b	15.83±0.81 c
T_4	14.76±0.63 c	44.37±0.86 e	257.01±5.64 b	6.37±0.38 a	21.83±1.68 b
T_5	12.78±0.54 d	45.08±1.73 b	182.98±2.85 d	3.43±0.25 d	17.23±1.05 c
Mean	15.34±1.79	44.71±4.03	237.72±31.74	5.09±1.15	20.80±4.01
F	22.25*	35.34*	126.85*	85.65*	46.64*

ns: non-significant at $p>0.05$, * Significant at $P<0.05$. Data (means±SD). There is no difference between the means shown with the same letter at $p<0.05$ significance level. T_1: *Pseudomonas putida strain* RCK-42A; T_2: *Kluyvera cryocrescens strain* RCK-113C; T_3: *Paenibacillus polymyxa strain* RCK-12E; T_4: *Bacillus subtilis strain* RCK-17C; T_5: Control (uninoculated bacteria).

The applications had significant ($p<0.05$) effects on the floret number (number $flower^{-1}$), and the average floret number was 13.25 number $flower^{-1}$ (Table 3). The highest floret number was obtained from T_4.

Flower stem diameter and flower diameter was not significantly affected by bacterial applications (Table 3). The T_1 produced the highest flower length (20.15 mm), while the flower length was 15.74 mm in the case of control application (T_5).

Table 3. The effects of applications on some morphological values of hyacinth flowers.

Applications	Flower stem Diameter (mm)	Floret number (number/flower)	Flower diameter (mm)	Flower length (mm)	Flower fresh weight (g)	Flower dry weight (g)
T_1	7.36±0.53	13.00±0.60 b	8.17±0.47	20.15±.73 a	9.48±0.74 c	0.66±0.06 b
T_2	7.50±0.17	13.98±0.73 ab	7.57±0.13	18.73±0.47 b	14.54±0.69 a	0.88±0.05 a
T_3	7.60±0.43	13.97±0.31 ab	7.53±0.31	17.08±0.90 c	12.46±1.31 b	0.72±0.06 b
T_4	7.90±0.60	14.17±0.17 a	7.90±0.60	15.06±0.63 d	12.12±0.81 b	0.88±0.09 a
T_5	7.38±0.37	11.12±0.74 c	7.38±0.36	15.74±0.77 d	8.84±0.24 c	0.67±0.04 b
Mean	7.55±0.43	13.25±1.27	7.71±0.45	17.35±2.04	11.49±2.27	0.76±0.11
F	0.74 ns	15.56*	1.89 ns	26.00*	23.59*	8.74*

The highest flower fresh weight (14.54 g) and flower dry weight (0.88 g) were found in T_2 application. In terms of flower dry weight, T_2 was in the same group with T_4 application.

The effects of the applications on bulb diameter, bulb length and bulb weight of hyacinth plant were presented in Table 4. It was determined that the highest bulb diameter was in T_2 bacteria application. The bulb length was found significant ($p<0.05$) in applications. T_1 and T_2 applications were in the same group with bulb length compared to the control. The effect of bacteria applications on bulb weight (g plant $^{-1}$) was determined significant ($p<0.05$). The highest bulb weight was in T_2 application.

Table 4. The effects of the applications on some morphological values of hyacinth bulbs.

Applications	Bulb diameter (mm)	Bulb length (mm)	Bulb weight (g plant^{-1})
T_1	41.28±1.27 b	39.66±0.62 a	11.18±1.44 a
T_2	42.57±0.43 a	40.01±0.58 a	12.01±0.77 a
T_3	40.87±.0.76 b	37.93±0.11 b	11.27±0.79 a
T_4	38.28±0.19 c	36.55±0.76 c	8.81±0.38 b
T_5	35.98±0.33 d	34.88±0.95 d	7.91±0.29 b
Mean	39.80±2.052	37.81±2.06	10.23±.79
F	41.67*	31.50*	13.43*

Table 5. Findings of macro (%) and micro (mg kg^{-1}) nutrient analysis of hyacinth bulbs.

Applications	N	P	K	Ca	Mg
T_1	2.80±0.20 a	1.76±0.20 a	0.29±0.04 a	1.44±0.19 b	210.40±1.90 a
T_2	2.40±0.01 b	1.88±0.13 a	0.33±0.06 a	1.66±0.02 ab	187.00±12.00 c
T_3	2.90±0.05 a	1.98±0.10 a	0.25±0.03 b	1.74±0.07 a	203.82±1.93 ab
T_4	2.77±0.17 a	1.98±0.39 a	0.29±0.05 a	1.50±0.20 ab	199.12±1.27 b
T_5	1.70±0.32 c	1.33±0.02 b	0.18±0.01 b	1.18±0.13 c	184.26±0.98 c
Mean	2.51±0.48	1.78±0.31	0.27±0.06	1.50±0.23	196.92±11.30
F	20.70*	5.06*	5.55*	7.22*	12.01*
Applications	***Na***	***Fe***	***Mn***	***Zn***	***Cu***
T_1	0.50±0.08	0.48±0.06 a	151.20±0.40 a	35.28±1.01 a	52.00±1.00 a
T_2	0.44±0.03	0.39±0.02 b	142.00±3.00 b	27.00±2.00 c	52.00±1.89 a
T_3	0.49±0.03	0.34±0.02 b	147.73±2.73 b	31.64±.064 b	51.35±.1.40 a
T_4	0.45±0.06	0.39±0.01 b	135.28±2.00 c	33.00±1.00 b	53.33±1.53 a
T_5	0.37±0.20	0.28±0.02 c	90.28±1.14 d	15.80±0.74	30.74±.0.09 b
Mean	0.45±.0.63	0.38±0.07	133.30±23.02	28.54±7.23	47.89±8.97
F	3.26 ^{ns}	16.62*	420.82*	128.78*	156.11*

The applications had significant effects on N, Ca, Mg, P and K (at $p<0.05$) from macro-nutrient elements. The maximum N (2.90%), P (1.98%) and Ca (1.74%) were found in T_3 application while the maximum (0.33%) K was found in T_2 application. It was found that N macro-nutrient element was the same group in T_1, T_3 and T_4 bacteria applications. All of the bacteria applications significantly increased P content of plant compared to the control (T_5). All the applications were in the same group for K macro-nutrient element when compared to the control (T_5) and T_3 applications (Table 5). Applications had significant (at $p<0.05$) effects on Mg. According to control application, maximum Mg (210.40 mg kg^{-1}) was found in T_1 application (Table 5). There were no significant ($p>0.05$) differences between the applications in terms of Na. Applications had significant effects on Fe, Zn, Mn and Cu micro nutrient elements at $p<0.05$. Maximum Fe (0.48 mg kg^{-1}), Mn (151.20 mg kg^{-1}) and Zn (35.28 mg kg^{-1}) were found in T_1 bacteria application, while maximum Cu (53.33 mg kg^{-1}) was found in T_4 (Table 5).

Discussion

The present study showed that the effects of PGPR supply on growth and development of the hyacinth plants and bulbs are important. This is the first report on the growth promoting effect of bacterial application on hyacinth. However, similar reports were obtained for different plant species. Researchers reported that bacterial applications including *Bacillus* and *Pseudomonas* strains can stimulate the growth and increase the yield in (Nelson, 2004; Sahu et al., 2018) [tomato (Mena-Violante and Olalde-Portugal, 2007; Le et al., 2018), sugar beet (Çakmakçı et al., 2006), chickpea (Elkoca et al., 2008),

apricot (Altindag et al., 2006), strawberry (Pii et al., 2017) eg.].

It is clear that the rate of growth of a plant depends on a thick stem. The results indicate that diameter of stem hyacinth was high with T_3 application (17.76 mm). Asghar et al. (2002) stated that inoculation with isolate S84 increased (33.3%) stem diameter in *Brassica juncea* L. Likewise, Esitken et al. (2006) reported that applying *Bacillus* OSU-142 increased the stem diameter and leaf area of sweet cherry trees. The positive effects of *Paenibacillus polymxa* RCK-12 E on the diameter of stem hyacinth was explained by N_2 fixation ability and production of antimicrobial substance (Glick 1995; Pérez-Montaño et al., 2014). The chlorophyll content is intimately related to plant dry matter production (Buttery and Buzzell, 1977). Therefore, any increase in leaf chlorophyll content would rise net photosynthesis and thus rise total plant growth and development. The chlorophyll content of hyacinth leaves ranged between 38.29 and 50.02 in the present study. Alam et al. (2001) illustrated that bacterial inoculation of rice plants led to the increase in chlorophyll content. Bailey (1963) stated that the length of hyacinth leaves is 20-30 cm and leaf width is 1.25-3.75 cm. Smigielska et al. (2014) reported that leaf lengths ranged between 24.30 and 28.40 cm. The average leaf length was the same with the finding. However, the average width of leaf was different from the finding and the applications had enhancing effect in terms of the parameter. In addition, the highest leaf width (6.37 cm) was determined in T_4 bacteria application.

De Silva et al. (2000) stated that the treatment of *Pseudomonas fluorescens* Pf 5 increased in the stem diameter and leaf area of high bush blueberry. These findings, in which the maximum leaf area was obtained by T_2 bacteria application, were supported by the findings of De Silva et al. (2000). Also, Sharaf-Eldin et al. (2008) stated that inoculation of *Bacillus subtilis* FZB24 increased the flowers per corm and leaf length.

In flower bulbs, inflorescence is an important sink organ (Van Die et al., 1970). The reason for this is that the flowering is dependent on the existing photosynthesis or reserves stored in the bulb scales (Wassink, 1965). In this study, the decrease in the parameters of some leaves (Chlorophyll content) may be interpreted because the possibility that more reserves may be transferred into the flowers for the development of inflorescence instead of leaf and bulb growth. It was obtained in the present study that the bacterial applications had promoting effects on floret number, flower length and flower stem diameter. Before flower initiation, the quality of the inflorescence and offsets can thus be influenced by the nutritional status of the bulbs (Roodbol et al., 2002). As a result, inflorescence will be able to develop and grow at the expense of bulb growth with a good plant nutrition.

In active growth period, bulbs with root and leaves need nutrients and water; basic needs of bulbs are phosphate and superphosphate or ammoniumphosphate are reported to be beneficial (Addai, 2011). Deficiencies in nitrogen are cause the development of small plants and bulbs by reason of early maturity (Scott, 2008; D'Haene et al., 2018). The number of florets per inflorescence is influenced in nutrition in the previous season and season when the plant blooms. The nutritional requirements of the bulb change according to cultivar types (Roodbol et al., 2002). They observed that the large bulbs required higher nutrient levels than small bulbs (Roodbol et al., 2002).

The concentrations of macro and micro plant nutrient content in hyacinth bulbs were significantly affected by PGPR applications. The reasons of the increases in plant growth may be due to the increasing nutrient uptake, providing plant growth hormones, improving chlorophyll content and organic acids with bacterial applications. These findings in the present study were found to be consistent with the findings of previous studies (Shen et al., 2004; Zare et al., 2011; Parewa et al., 2014; Parlakova Karagöz et al., 2016). In the PGPR applications, the highest contents of N, P and Ca were observed in the T_3 bacteria application. In the PGPR applications, the highest contents of P, K, Mg Ca were observed in the *Pseudomonas+Azotobacter* application that had differed significantly from other applications (Zare et al., 2011). The highest contents of Mg, Fe, Mn and Zn were determined in T_1, *Pseudomonas putida strain* RCK-42A, application. Gravel et al. (2007) reported that *Pseudomonas putida* B strain 1 increased Mg content of leaves of tomato plants. PGPR inoculation could compensate for nutrient deficiency, improve a plant development by microorganisms in the root zone, stimulate root development of plants and result in better absorption of nutrients and water from the grown medium (Egamberdiyeva, 2007; Soussi et al., 2016).

In conclusion, in hyacinth cultivation, the PGPR applications (especially *Kluyvera cryocrescens strain* RCK-113C and *Pseudomonas putida strain* RCK-42A) may have a potential for the production of biofertilizer required in organic agriculture because of rendering insoluble phosphates into soluble form and, biological N_2 fixation encouraged directly to improve the plant growth by means of the bacteria. The PGPR applications could be ideal in the cultivation of hyacinth as cut flowers, landscaping plants, potted plants. So, sustainability in the landscapes may be achieving. We expect that this demand can be met by this study.

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