

Original Research

Some Phenological and Morphological Properties of *Hyacinthus Orientalis* cv. “Delft Blue” with Treated Bacterial Inoculations

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Abstract

This study was conducted to determine the effect of N:P:K fertilization and some bacterial species on the flowering and development of *Hyacinthus orientalis* cv. ‘Delft Blue’ plant. In the study carried out under laboratory conditions according to randomized plot experiment design, full and half (1/2) dose of 20:20:20 N:P:K commercial fertilizer was applied to hyacinth bulbs as inorganic fertilizer. Nitrogen-fixing bacteria: *Cellulomonas turbata* (TV54A), phosphate solubilizing bacteria: *Bacillus*-GC Group (TV119E) and nitrogen-fixing and phosphate solubilizing bacteria: *Kluyvera cryocrescens* (TV113C) and TV54A and TV119E bacteria types were used as biological fertilizers. In the study in which some phenological and morphological observations were made, the earliest time of first flowering, full flowering and harvesting were determined as 52.07, 53.70 and 54.66 days in 1/2 N:P:K application and the difference between the applications was found to be statistically significant ($P<0.001$). Bacterial applications caused a ten-day delay. The highest leaf width average (24.43 mm) was obtained in TV119E application ($P<0.01$). The maximum number of leaves was 7.13 (1/2 N:P:K), leaf length was 245.57 mm (TV113C) and plant height was 364.24 mm (TV119E). It was found that bacteria prolong the flowering start times, that is, N:P:K fertilization makes flowers bloom in a shorter time. In addition, leaf growth and bacterial inoculation of the plant were determined to be effective.

Keywords: flowering, *Hyacinthus orientalis*, nitrogen-fixing bacteria, ornamental plant, phosphate-solubilizing bacteria

Introduction

Natural vegetation is one of the important components which increases visual landscape quality and is highly appreciated by the user [1]. However,

the use and trade of plants in various ways increases the threats on plant species in nature. Medicinal plants and bulbous-tuberous plants are the most adversely affected. The usage and trade of geophytes are increasing day by day due to their being visually attractive in the environment in which they are located as potted flowers and cut flowers. Hyacinth (*Hyacinthus orientalis*) is another group that draws attention among

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these plants. Fertilization is not necessary for the development of hyacinth plants [2], but it is stated that bulbs will be much stronger in case they are fertilized [3]. Fertilization with 2.5 N: 1 P₂O₅: 3.5 K₂O: 2 CaO in hyacinth has been reported to be necessary. And more importantly, it is reported that high doses of nitrogen-containing fertilization should be done in two phases before planting, especially in spring [4]. It is recommended that only nitrogenous fertilization should not be applied in bulbous plants and phosphorus and potassium fertilization should also be performed together with nitrogen [5]. Chemical fertilizers play an important role in the increase of production. However, excessive use of chemical fertilizers causes a decrease in soil fertility and environmental degradation. Theoretically, it is not possible to achieve a higher increase in yield with chemical fertilizers reaching the highest level of use. The cost of chemical fertilizers and environmental damages have brought to the agenda the research, advancement and adaptation of biological alternatives that reduce the environmental impact of chemical fertilizers to acceptable levels. It also brought the importance and adoption of biological fertilization into the discussion. Research is being carried out in order to create biological fertilizer formulations for clean living area and healthy productions in the world [6]. According to [7], there is an increasing interest in the idea of reducing the use of chemicals in agricultural areas to protect plant health and reduce production costs. Therefore, the use of bacteria (not in excess) in

the root rhizosphere of horticultural and ornamental plants has recently gained importance. It has been demonstrated on cultivated plants that plant growth regulator bacteria (PGPR) can be applied successfully in search of alternative solutions to chemical fertilizers, thus reducing the dose of chemical fertilizer used [8].

There are very few studies on the use of rhizobacteria that promote plant growth in the cultivation of ornamental plants [7, 9-12].

The aim of this study is to determine the effect of bacteria on the development of an ornamental plant, hyacinth, in order to prevent the use of inorganic fertilizers so as to obtain more yields in agricultural products, especially in recent years and to draw attention to the alternative biological fertilization.

Experimental

Material

Hyacinthus orientalis cv. “Delft Blue” commercial bulbs were used as plant material in the experiment (Fig. 1). Bacteria contaminated with bulbs were selected from nitrogen fixing and phosphate solubilizing species and were obtained as stock from Siirt University, Faculty of Agriculture, Department of Field Crops. Nitrogen-fixing *Cellulomonas turbata* (TV54A), phosphate solubilizing *Bacillus*-GC Group (TV119E)



Fig. 1. Flowers of *Hyacinthus orientalis* cv. ‘Delft Blue’.

and nitrogen-fixing and phosphate solubilizing *Kluyvera cryocrescens* (TV113C) bacteria as well as N:P:K chemical fertilizer were used.

Method

Experimental Design

The experiment was conducted under laboratory conditions with three replications and five replicates per repetition according to the randomized plot design. A total of seven treatments were applied to the plants which were treated with N:P:K and bacteria, including the control group.

Fertilizer Application

N:P:K 20:20:20 half and full doses of chemical fertilizer were applied to the pots one week after planting bulbs, and only once in the form of irrigation water.

Bacterial Application

Before planting, all the bulbs were rinsed by surface sterilization in tap water and detergent water, respectively. After that, they were left with 5% (v/v) sodium hypochlorite for 20 minutes and washed 3 times with distilled water. In the last stage, bacteria were inoculated for 5 hours on surface sterilized bulbs [13].

The bacteria used in the experiment were obtained from Siirt University, Faculty of Agriculture, and Department of Field Crops. Bacterial isolates were isolated from the Van Lake Basin with the TOVAG 108O147 TÜBİTAK project and their PGPB activity was detected [14]. These bacteria were diagnosed with the microbial identification system (MIS) and identified as Plant Growth Promoting Bacteria (PGPB) activity under greenhouse and field conditions.

These bacteria were selected as nitrogen-fixing bacteria which have nitrogen fixing and phosphate solubilizing properties, were applied alone; an application was also made using a combination of the bacteria *Cellulomonas turbata* (TV54A) and *Bacillus-GC* Group (TV119E).

For bacterial solution, 20 g of nutrient agar was added to one liter of distilled water, adjusted to pH 7.0 and the mixture was sterilized by autoclave for 15 minutes at 121°C. After sterilization, it was cooled to 50°C, then transferred to petri dishes and allowed to solidify. The stock cultures of the bacteria were planted in nutrient agar medium with the help of the needle and incubated at 26±2°C for 24 hours [15]. The nutrient broth (Merck-VM775843711) was used as the liquid nutrient. 8 g of nutrient broth was added to one liter of distilled water and pH was adjusted to 7.0. The mixture was sterilized by autoclave for 15 minutes at 121°C and then allowed to cool. A single colony was taken from the bacteria developed in nutrient agar and was

transferred to nutrient broth in aseptic conditions. The bacteria transferred to the liquid nutrient were incubated at 26±2°C for 24 hours and at 120 rpm in the horizontal shaker. After incubation, the bacteria concentrations were turbidimetrically adjusted to ~10⁸cfu / ml. Then the bulbs were spread on blotting papers and allowed to dry. These dried bulbs were planted one day later.

Parameters Investigated

Since the hyacinth bulbs grown in the study were planted, the first flowering times (opening of the first floret), full flowering times (opening of 50% of the florets) and harvesting times (the last few florets were closed) were calculated as the number of days.

While the plants are found by counting the number of leaves (leaf / plant), the plant height, leaf width and length were determined by measuring with digital caliper (mm).

Statistical Evaluations

The analysis of the data was done in the SAS 9.1 statistical package program according to randomized plot design. Duncan multiple comparison test was used for comparing the averages. Tests were performed at $\alpha = 0.05$ significance level [16]. Descriptive statistics in terms of the traits were given.

Results and Discussion

The effect of N:P:K, nitrogen fixing and phosphate solubilizing bacteria on the growth and flowering criteria of hyacinth plant is given in Table 1 and Fig. 2.

The effects of N:P:K and bacterial applications on the first flowering, full flowering and harvesting period were found to be statistically significant in hyacinth ($P < 0.001$). In plants with half (1/2) and full dose N:P:K application and control plants that were not subjected to any application, the first florets were opened at the earliest time, while plants inoculated with bacteria were found to have a delay of about ten days. The first floret opening was determined in 52.07 days in ½ N:P:K application and the latest first floret opening in 63.07 days in TV54A application. As in the first flowering time, the control and N:P:K fertilizer applications brought full flowering time back ten days compared to bacterial applications. The lowest average of full flower duration was determined in ½ N:P:K as 53.70 days and the highest average was determined in TV54A application as 64.16 days. N:P:K fertilizer applications had brought back the harvesting time an average of ten days compared to bacterial applications. The earliest harvest was obtained from ½ N:P:K fertilizer application (54.66 days), the latest harvest (65.67 days) was obtained from TV113C and TV119E applications. Bacterial applications were statistically in the same group and allowed them to reach later

Table 1. The effect of N:P:K and bacterial applications on plant growth and flowering in hyacinths.

Properties/Applications	Control	N:P:K	½ N:P:K	TV54A	TV119E	TV54A + TV119E	TV113C	Significant degree
First flowering time*** (day)	53.85 B	54.15 B	52.07 B	63.07 A	62.07 A	61.13 A	62.33 A	P<0.001
Full flowering time *** (day)	55.05 B	56.10 B	53.70 B	64.16 A	63.80 A	63.20 A	64.14 A	P<0.001
Harvest time *** (day)	58.23 B	58.10 B	54.66 B	65.58 A	65.67 A	65.53 A	65.67 A	P<0.001
Leaf number	7.12	7.00	7.13	6.37	6.73	6.53	6.40	NS
Leaf length (mm)	230.53	230.70	221.00	240.17	238.80	239.55	245.57	NS
Leaf width** (mm)	19.95 B	20.23 AB	20.29 AB	23.82 AB	24.43 A	24.14 AB	22.95 AB	P<0.01
Plant height (mm)	307.97	300.98	313.66	341.26	364.24	353.22	338.34	NS

NS: Not significant, **: P<0.01, ***: P<0.001; Means followed by the same letter indicate no statistical difference

TV54A: Nitrogen fixing bacteria-*Cellulomonas turbata*; TV119E: Phosphate solubilizing bacteria-*Bacillus*-GC Group; TV113C: Nitrogen-fixing and phosphate-solubilizing bacteria -*Kluyvera cryocrescens*

harvest time than N:P:K, ½ N:P:K and control groups. The application of the bacteria with two features as a result of the application consisting of nitrogen fixing and phosphate solubilizing bacteria alone or together, resulted the plants to bloom later than N:P:K treated plants in first flowering, full flower and harvest time.

Although the effects of N:P:K and bacterial treatments on leaf number and length of hyacinth were statistically insignificant, the effect on leaf width on (P<0.01) level was found to be significant. The highest average value of leaves (7.13) was obtained from ½ N:P:K application and the lowest average value (6.37) was obtained from TV54A bacteria application. The application of bacteria having both properties by single or combined applications of nitrogen fixing and phosphate solubilizing bacteria resulted in fewer number of leaves than N:P:K treated plants in the control group. The longest leaf length average (245.57 mm) was achieved with TV113C bacteria application and the shortest leaf length average (221.00 mm) was reached with ½ N:P:K application. Although the difference between leaf length averages was statistically insignificant, bacterial applications increased leaf length by approximately 15 mm. The largest leaf average value (24.43 mm) was obtained from TV119E bacterial application and the lowest leaf width (19.95 mm) was obtained from the control group.

In terms of plant height, the difference between the applications was found to be statistically insignificant. The longest plant length (364.24 mm) was obtained by application of TV119E bacteria, while the shortest plant length average (300.98 mm) was obtained in N:P:K fertilizer application. Although the differences between the applications were statistically insignificant, bacterial applications were found to be more effective on plant height than fertilizer applications and control group.

In one of the different studies on bacterial applications in ornamental plants, *Burkholderia phytofirmans* (PsJN), T2*Bacillus* sp. (MN-54), T3*Enterobacter* sp. (MN-17) and *Caulobacter* sp. (FA-13) isolates were used as foliar fertilizer in tulip (*Tulipa gesneriana* L. cv. 'Clear Water') plant. The results showed that tulip reacted well to bacterial strains and showed significant improvement in morphological characteristics, bulb characteristics and other quality parameters. In flowering times, the bacteria caused a delay of 4-6 days compared to the control application [17]. PGPR formulations were applied to two different varieties (Christmas Feelings and Christmas Eve) of poinsettia (*Euphorbia pulcherrima* Willd.ex Klotzsch.) grown in greenhouse conditions: Formulation 1 (*Paenibacillus polymyxa* TV-12E + *Pseudomonas putida* TV-42A + *Pantoea agglomerans* RK-79), Formulation 2 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Bacillus subtilis* TV-17C), Formulation 3 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-92 + *Kluyvera cryocrescens* TV-113C), Formulation 4 (*Bacillus megaterium* TV-91C + *Pantoea agglomerans* RK-79 + *Bacillus megaterium* TV-6D) and 100% to 50% chemical fertilizer was applied [18]. It is aimed to reduce the use of chemical fertilizers by giving these fertilizers in singles and in combination. In addition to the recommended amount of chemical fertilizer application (100%), it has been found that formulation applications in BI and BIII bacteria have positive effects on the shortening of the period until flowering and early flowering on poinsettia growing. Poinsettia plants grew in the shortest marketable time compared to control when supplied with BIII + chemical fertilizer application. In this study on poinsettia, the shortest time required for flowering was done with bacteria + chemical fertilizer. In our study, while bacteria delayed flowering in hyacinth, N:P:K fertilization caused early blooming. Hyacinths and tulips are bulbous ornamental

plants. Similar results were obtained in both studies and it was seen from both studies that N:P:K fertilizer was effective in bringing forward the time required for flowering.

In a study of *P. fluorescens* and *B. pumilus* rhizobacteria applied to wild jasmine, leaf area and

stem diameter were increased [19]. *Pseudomonas putida* bacteria were effective in increasing the number of leaves of poinsettia plant [20]. In a study, PGPRs grafted to *Cistus ladanifer* flower seedlings increased the number of leaves [21]. The reasons for the differences determined in terms of the total number of

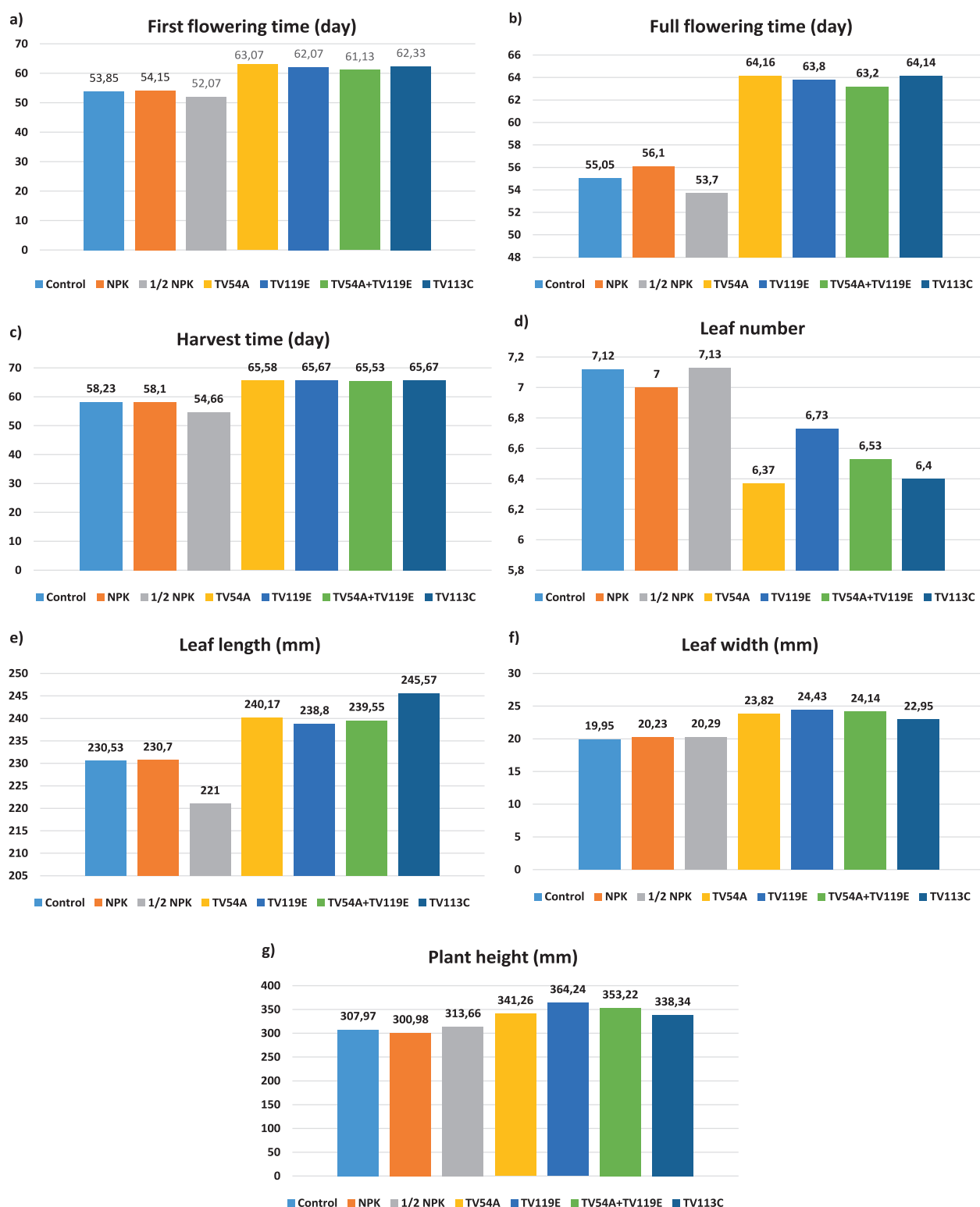


Fig. 2. Effects of bacterial inoculation and fertilizer applications on plant a) First flowering time, b) Full flowering time, c) Harvest time, d) Leaf number, e) Leaf length, f) Leaf width, g) Plant height.

leaves between the applications is thought to be related to the species and amounts of microorganisms in the growing environment in previous studies [22] and in the study of poinsettia flower. It is also estimated that the plant is transformed into a form in which it can receive nutrients [18]. As seen from the studies, the number of leaves varying according to plant species and applications were obtained. Hyacinth leaf length and width values obtained from our study are within the range of 20-30 cm and 1.25-3.75 specified by [23].

It has been determined by many researchers that plant height increases with bacterial applications. Foliar fertilization was carried out on tulip (*Tulipa gesneriana* L. cv. 'Clear Water') plant with many bacterial treatments. Among them, *Burkholderia pyrofirman* (PsJN) gave the maximum stalk length value, whereas control application gave the minimum value compared to other applications [17]. In a study using rooted cuttings of poinsettia (*Euphorbia pulcherrima* Willd. Ex Klotzsch cv. Christmas Feelings), different PGPR formulations, chemical fertilizers (KG) and combinations were applied [24]. Four types of formulations were made with bacteria and 50% to 100% of chemical fertilizer was used. According to chemical fertilizer and control applications, BIV + KG application increased plant height between 2.87% and 5.27%. Bacterial strains used in our study were used in similar studies and had a positive effect on plant growth parameters [14, 15, 25, 26]. With the inoculation of PGPRs on *Hyacinthus orientalis* cv. 'Aiolos', there had been increases in leaf width and length, number of florets, flower diameter and length, stalk thickness, bulb diameter, length and weight in greenhouse conditions [27]. It has been concluded that the bacterial formulation applications increased in the number of bulbs and quality of the tulip cultivars in the field conditions [28]. In greenhouse, the application of autoclaved vermicompost + PGPRs on *Gladiolus grandiflorus* L. cv. 'Red Beauty' had increased the diameter of flowers for number of leaves per plant, number of florets per spike, stem diameter, spike length, fresh and dry weight of flowers, the number and diameter of corm [29]. In the greenhouse, bacterial applications have increased rooting quality (root ball width, root length and number of main roots) without rooting percentage in rooting rosehip (*Rosa canina*) shoots [30]. But according to [31] the control plants demonstrated the highest value (with no bacterial application and fertilization) of the weight, length and width of *Hyacinthus orientalis* cv. 'Delft Blue' bulb (both laboratory and field conditions), however bacterial applications had negative effects on these values. There are a lot of studies on the effects of hormones and plant nutrients on ornamental plants on plant development, and the number of studies on the effects of PGPR applications, which are frequently emphasized and which can be used as bio fertilizers in agriculture, on ornamental plants is not high [32]. As can be seen in our study, bacterial applications had a positive effect on plant height. It

is thought that it can be used together with inorganic fertilizer as in some studies to increase plant height which is estimated to vary according to plant and bacterial species.

The use of PGPR bacteria will reduce the use of chemical inputs and increase environmental pollution and vegetative growth and yield. However, the biggest obstacle in the widespread use of these bacteria is high concentration of salt and heavy metal, high pH, temperature and other highly competitive microorganisms. PGPR shows positive effects in case of choosing suitable environment and plants [33].

Conclusions

In the study carried out with the hyacinth plant, the first flowering period was approximately delayed ten days (63.07 days) according to the control group and fertilizer applications. TV54A bacterial application revealed the opening of the florets. In the same way, it was seen that bacterial applications prolonged the full flower period (64.16 days with TV54A) and harvest periods (65.67 days with TV113C). The highest number of leaves (7.13) was found with $\frac{1}{2}$ N:P:K fertilizer application, the highest leaf length (245.57 mm) was found with TV113C bacteria application, the largest leaf width (24.43 mm) was found with TV119E application and the longest plant height (364.24 mm) was found with TV119E bacteria application. When leaf growth is taken into consideration, the bacteria TV113C in length and TV119E in width were effective. The positive effect of bacteria on leaf growth is remarkable.

Bacterial inoculations at all stages of flowering seem to cause a delay of about ten days. N:P:K fertilization and early flowering of the control group plants becoming ready for harvest are notable. In terms of earliness, it can be said that bacterial inoculation on hyacinths has a negative effect. Phosphate solubilizing bacteria in plant height were more effective than other applications. The effect of plant height indicates that it increases stalk growth. It is thought that bacteria can be applied to increase plant height. If the period when hyacinth flowers will be used is important, it may be recommended to have a bacteria application with calculations on ten days late flowering and harvest time in order to continue after all the flowering has ended.

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Conflict of Interest

The authors declare no conflict of interest.

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