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EFFECT OF DN1 BACTERIAL STRAIN APPLIED BY DIFFERENT METHODS ON SOME MORPHOLOGICAL CHARACTERISTICS OF STRAWBERRY CV. SAN ANDREAS (*Fragaria X ananassa* Duch.)

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ABSTRACT

There have been quite intensive studies on the use of Plant Growth-Promoting Rhizobacteria (PGPR) in agriculture. *Acidovorax facilis* strain DN1 is one of the PGPR commonly used. The effect of DN1 bacterial strain on some morphological characteristics of strawberry cv. San Andreas was investigated. The DN1 bacterial strain was applied via soil, leaf, and soil + leaf, for 3 months (once a month) to strawberry plants. The DN1 spores were prepared with 0.2% boron, 10% corn starch and distilled water. The bacterial solution was applied to plants at the following day with a hand pump (to leaves; 50 cc) and graduated cylinder (250 cc each 5-liter pot). After 3 treatments, plants removed from pots and data collected.
According to the results, DN1 bacterial strain often had a positive effect on the morphological and fruit characteristics. Spraying treatment was the most effective way for the stem and root traits we evaluated (crown diameter: 36.87 mm; stem fresh weight: 63.64 g; leaf number: 38.69; root fresh weight: 34.89 g). In addition, soil + leaf treatment had a positive effect on mean fruit weight (23.57 g) and fruit diameter (27.64 mm). The effect on other properties was also positive, but the root length (26.34 cm) was reduced in leaf treatment compared to the control (29.69 cm). It is expected that the most effective treatment is the combined (leaf + soil) treatment, while the leaf treatment may be the most effective method on soils with boron toxicity.

Keywords: *PGPR, DN1 (Acidovorax facilis) strawberry, and boron (B*)

INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) is an herbaceous and perennial fruit which has an important place among the berries (Ağaoğlu, 1986). Strawberry is a species belonging to the genus *Fragaria*, the family *Rosaceae*, and the order *Rosales*. Commercially produced species *Fragaria x ananassa* Duch. has a history of 250 years (Hancock, 1999). Strawberry plants can grow in many parts of the world thanks to short, neutral and long day cultivars (Yılmaz, 2009).

Recently, researchers have been searching for various ways to reduce the use of chemical fertilizers and pesticides in agricultural production. These searches are particularly focused on the use of microorganisms (Adesemoye et al., 2008; Ekici et al., 2015). Plant Growth-Promoting Rhizobacteria (PGPR) can be used as a biocontrol agent and/or biofertilizer to stimulate plant growth. Most of these microorganisms belong to genus *Alcaligenes, Azotobacter, Bacillus, Pseudomonas,* and *Rhizobium* (Glick, 1995; Burdman et al., 2000; Romerio, 2000; Somers et al., 2004; Tuzlacı, 2014). The use of PGPRs to replace chemical fertilizers which causes soil and water pollution is increasing year by year (Çakmakçı, 2005; Ahemad and Kibret, 2014; Bashan et al., 2014). PGPRs increase plant growth and productivity by reducing harmful effects of the phytopathological microorganisms or give substances to the plant environment (rhizosphere and phyllosphere) which they produce **(**Altın and Bora, 2005; Saleem et al., 2007; Glick, 2012; Bashan et al., 2014). PGPRs are used as biological fertilizers, phytostimulators, rhizoremidators, phytoremediators and biopesticides (Lucy et al., 2004; Somers et al., 2004; Aontoun et al., 1998; Siddiqui, 2006). It has been reported by many researchers that PGPRs can be treated to plants by root inoculation and leaf spraying (Kokalis-Burelle, 2003; Eşitken et al., 2006; Malusa et al., 2006; Aslantaş et al., 2009; Eşitken et al., 2009; Eşitken et al., 2010; Pırlak and Köse, 2010; Ertürk et al., 2012). In strawberry studies, it has been suggested that bacterial treatments increase seedling number and quality (Aslantaş et al., 2010), fruit yield and quality (Pırlak and Köse, 2009; Ertürk et al., 2012), as well as plant growth (Kokalis-Burelle, 2003; Eşitken et al., 2010).

DN1 is a strain of *Acidovorax facilis* (AY581467) and isolated from bermudagrass roots by Wang and Skipper (2004). In this study, cv. San Andreas plants were treated 3 times (once a month) with DN1 bacterial strains through soil inoculation, spraying, and spraying + soil inoculation. The effects of DN1 bacterial strain on some morphological and fruit characteristics were investigated.

MATERIAL AND METHODS

This study was carried out in the greenhouse of the Department of Horticulture on the Faculty of Agriculture of Selcuk University. The plants were planted with a mixture of peat-perlite (2: 1) in 5-liter pots (February 5, 2015). Bacterial solutions (*Acidovorax facilis* strain DN1) were prepared by adding 1 g DN1 spore, 0.2 g boron, and 10 g of cornstarch with 1-liter of distilled water. After 24 h of incubation at room temperature, the solution was treated 3 times (once a month) with a hand pump (spraying treatment: 50 cc to each replicate) and graduated cylinder (soil inoculation: 250 cc to each plant).

The plants were removed in November, and data for crown diameter (CD), plant height (PH), stem fresh weight (SFW), root fresh weight (RFW), crown number (CN), leaf number (LN), root length (RL), chlorophyll content (CC), mean fruit weight (MFW), fruit length (FL), and fruit diameter (FD) were collected. The data were analyzed with One Way Analysis of Variance (ANOVA) and the Duncan Multiple Comparison Test ($p \leq 0.05$) with the IBM SPSS v.20 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY) statistical software package.

RESULTS AND DISCUSSION

The effect of *Acidovorax facilis* strain DN1 on fruit and morphological characteristics was found statistically significant ($p \leq 0.05$). While the spraying DN1 treatment (36.87 mm) maximizes the crown diameter (CD), soil inoculation (26.92 mm) is the treatment that minimizes plant height (PH) (Table 1). However, spraying + soil inoculation (31.88 mm) reduced the CD compared to the control (33.18 mm). In addition, while spraying + soil inoculation (23.10 cm) has been the most beneficial treatment on PH, the shortest plants were obtained from the control (21.76 cm). On the other hand, the most effective treatment for stem fresh weight (SFW) was spraying (63.64 g), while soil inoculation (61.36 g) was the second. Spraying + soil inoculation (53.85 g) was not as effective on SFW as other DN1 treatments, but it is more effective than control (51.17 g). The most effective treatment on the leaf number (LN) associated with SFW was spraying (38.89), but minimal LN was obtained from the control (26.00). However, unlike SFW, soil inoculation (34.90) was less effective than spraying + soil inoculation (34.61).
Soil inoculation and spraying + soil inoculation treatments may be more ineffective than spraying treatment because of the toxic effect of boron used when preparing the bacterial solution. Bacterial treatments reported having a positive effect on the above-mentioned properties on previous studies (Tahmatsidou et al., 2006; Pırlak et al., 2007; Ertürk et al., 2010; Karlıdağ et al., 2013). In a study conducted by Ekici et al. (2015) on broccoli (*Brassica oleracea L. var. italica*) seedlings, it was reported that bacterial treatments increased PH, CD, and SFW compared to control. In another study, it was reported that bacterial treatments increased the length of shoots and shoot diameters in apple (Pırlak et al., 2007). The use of *Bacillus subtilis* strain FZB24-WG in strawberry increased SFW (Tahmatsidou et al., 2006), and bacterial treatments increased the root diameter of the Hayward kiwifruit seedling cuttings (Ertürk et al., 2010) to a considerable extent. In another study, Karlıdağ et al. (2013) reported that the treatment of bacteria to strawberry plants under salt stress increased the SFW.

Table 1. Effects of *Acidovorax facilis* strain DN1 on the means of crown diameter (CD), plant height (PH), stem fresh weight (SFW), and leaf number.

	Crown Diameter (mm)	Plant Height (cm)	Stem Fresh Weight (g)	Leaf Number
Control	33.18 ± 0.37[b*]	21.76 ± 0.15[c*]	51.17 ± 0.50[d*]	26.00 ± 0.25[d*]
Soil inoculation	26.92 ± 0.57[d]	23.10 ± 0.08[a]	61.36 ± 0.39[b]	34.90 ± 0.36[c]
Spraying	36.87 ± 0.34[a]	22.34 ± 0.29[b]	63.64 ± 0.26[a]	38.89 ± 0.34[a]
Spray. + s. inoc.	31.88 ± 0.52[c]	22.73 ± 0.28[ab]	53.85 ± 0.19[c]	34.61 ± 0.35[b]
p	0.000	0.000	0.000	0.000

*Significant at p≤0.05 and the value after ± is the standard deviation.

DN1 bacterial treatments used in this study positively affected crown number (CN), root length (RL), root fresh weight (RFW), and chlorophyll content (CC). The most favorable effect on CN and RL was observed in soil inoculation treatment, while the spraying treatment had the best results in terms of RFW (Table 2). On the other hand spraying + soil, inoculation treatment had the best results on CC (Table 2). In addition, all treatments increased CN, RFW, and CC values compared to the control. However, spraying and spraying + soil inoculation on RL were not as effective as a control (Table 2). This might be due to the stronger effect of mineral and water search of roots than the effect of DN1 treatments. According to previous observations, bacterial treatments have a positive effect on above mentioned traits. In some studies, bacterial treatments increased the CN **(**Aslantaş et al., 2010), RL (Ertürk et al., 2010; Ekici et al., 2015), RFW (Tahmatsidou et al., 2006; Karlıdağ et al., 2013; Ekici et al., 2015), and CC (Karlıdağ et al., 2013; Ekici et al., 2015) compared to control. When considering the effects of bacteria on traits except for RL, it is understood that there are similarities between the previous studies and the present study. On the other hand, the boron used while preparing the solution in spraying + soil inoculation treatment may have been toxic to the strawberry plants. As a result of this, the root length may have been shorter than the control. But, it cannot be clearly understood why the roots obtained from spraying treatment were shorter than the control.

Table 2. Effects of *Acidovorax facilis* strain DN1 on the crown number (CN), root length (RL), root fresh weight (RFW), and chlorophyll content (CC).

	Crown Number	Root Length (cm)	Root Fresh Weight (g)	Chlorophyll Content (μg/cm2)
Control	4.85 ± 0.13[b*]	29.69 ± 0.62[a*]	27.57 ± 0.43[d*]	42.67 ± 0.39[c*]
Soil inoculation	5.25 ± 0.05[a]	29.70 ± 0.34[a]	31.41 ± 0.32[b]	45.50 ± 0.41[b]
Spraying	4.93 ± 0.12[b]	26.34 ± 0.41[b]	34.89 ± 0.39[a]	46.16 ± 0.45[ab]
Spray. + s. inoc.	4.87 ± 0.12[b]	26.72 ± 0.27[b]	29.36 ± 0.27[c]	46.57 ± 0.13[a]
p	0.006	0.000	0.000	0.000

* Significant at p≤0.05 and the value after ± is the standard deviation.

It is understood that the effect of DN1 strain on other traits as well as on the fruit characteristics is positive (Table 3). However, the most effective treatments differ for mean fruit weight (MFW), fruit length (FL), and fruit diameter (FD). While spraying + soil inoculation was the most effective treatment on MFW (13.57 g) and FD (27.64 mm), the most effective treatment for the FL was spraying (35.53 mm). On the other hand, the lowest values for all three fruit characteristics were obtained from the control (MFW: 11.51 g, FL: 33.98 mm, and FD: 26.05 mm). It can be said that spraying and spraying + soil inoculations were effective treatments on fruit characteristics in general.

Table 3. Effects of *Acidovorax facilis* strain DN1 on mean fruit weight (MFW), fruit length (FL), and fruit diameter (FD).

	Mean Fruit Weight (g)	Fruit Length (mm)	Fruit Diameter (mm)
Control	11.51 ± 0.07^{d*}	33.98 ± 0.06^{c*}	26.05 ± 0.09^{c*}
Soil inoculation	12.29 ± 0.16^{c}	35.19 ± 0.07^{b}	26.94 ± 0.,12^{b}
Spraying	12.48 ± 0.06^{b}	35.53 ± 0.15^{a}	26.82 ± 0.12^{b}
Spray. + s. inoc.	13.57 ± 0.11^{a}	34.99 ± 0.14^{b}	27,.64 ± 0.09^{a}
p	0.000		0.000

*Significant at p≤0.05 and the value after ± is the standard deviation.

In previous studies, bacterial treatments had positive effects on fruit characteristics. Ipek et al (2014) reported that bacterial treatments have a positive effect on MFW. Some researchers reported that the efficacy is mixed (Tuzlacı, 2014; Ağgün, 2018), while others reported statistically insignificant effects (Tahmatsidou et al., 2006; Eşitken et al., 2010; Pesakoviç et al., 2013). The results obtained from the present study were in agreement with the study conducted by Ipek et al (2014). According to Pesakoviç et al (2013), bacterial treatments (depending on the bacterial species) have different effects on the FL. The effect on FD was insignificant according to the same study. In the present study, the DN1 strain was positively affected both FL and FD as opposed to the study mentioned above.

CONCLUSION

Given the results obtained from this study, the *Acidovorax facilis* strain DN1 positively affected the morphological and fruit characteristics of San Andreas strawberry cultivar. Particularly spraying has been the treatment that increases most of the features. However, the ineffectiveness of soil inoculation and spraying + soil inoculation treatments may be due to boron toxicity. Consequently, because of the possible boron toxicity, an optimum DN1 treatment was not identified. The DN1 strain dose, number of treatments and the amount of boron in the DN1 solution should be determined in future studies.

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