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Full Length Research Paper

The effect of inoculation with plant growth rhizobacteria (PGPR) on root formation of mint (*Mentha piperita* L.) cuttings

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This study was conducted both in field and greenhouse conditions at Atatürk University, College of Agriculture, Erzurum, Turkey, during 2004 and 2006. The objective of this study was to determine the effect of some bacteria isolates on root formation, root length and dry matter content of roots of mint (*Mentha piperita* L.). Mint and *Agrebacterium rubi* (strain A16), *Burkholderia gladii* (strain BA7), *Peseudomonas putidea* (strain BA8), *Bacillus subtilus* (strain OSU142) *Bacillus megatorium* (strain M3) were used as rooting agent, respectively. The highest rooting percentage was obtained by application of A16 (88.70; 89.85%), M3 (86.12; 91.15%) and BA8 (87.27; 87.77%). Overall, the lowest was observed in controls (79.31 and 76.96). Root length was greater when cuttings were treated with BA7, A16 and M3 compared to the other treatments. Mint cuttings inoculated with M3 had more dry matter content than control and the other treatments in both experiments. Rooting performance, root length and dry matter content of root, cuttings of mint treated with bacterial solutions showed better performance than control cuttings in both experiments. In addition, when evaluated bacteria strains were compared, M3 was found more effective than the others on the examined properties.

Key words: Bacteria strains, cutting, PGPR, *Mentha piperita* L.

INTRODUCTION

Mint (*Mentha piperita* L.) belonging to Labiatae family is accepted as a kind of vegetable which is produced both in greenhouse and field economically. The plant is a cool-season vegetable that does not grow well on hot and dry weather. Although, mint is generally considered hardy in cold temperatures, its optimum growth temperature is 12 - 15°C (Decoteau, 2000). It can be grown well in sandy-loamy soils, and can be multiplied by different methods such as cuttings and seeds. However, Turkish growers prefer cutting method. Commercial production areas are Mediterranean, Aegean and Marmara agricultural regions. Wild and cultivated mint plants has been spreading throughout Turkey with 3000 tons annual production (Anonymous, 1998).

The general term for cultivated mint in Turkey is 'Nane'; it is consumed both fresh and dried, in particular mixed as aroma source with salads (Vural et al., 2000). Mint is also used in medicine and food industry in Turkey (Ceylan, 1995).

There are many physiological and environmental factors that influence root formation, with exogenous treatments on cuttings being particularly important (Couvillon, 1998). Growers have attempted to stimulate rooting by applying growth regulators, various chemical substances, etc. However, the use of chemicals can produce environmental problems and increased proportion costs. Environmental problems have raised interest in environmental friendly sustainable agricultural practices

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(Salantur et al., 2005).

Recent studies showed that bacteria in several genera (*Agrebacterium, Bacillus, Streptomyces, Peseudomonas* and *Alcaligenes*) induce root formation in stem cuttings (Bassil et al., 1991; Hatta et al., 1996; Rinallo et al., 1999; Ercisli et al., 2004). Plant growth promoting rhizobacteria (PGPR) are able to exert a beneficial effect upon plant growth such as increases root growth and root weight (Nelson, 2004). In addition, it is known that growth promotion and increase in root formation in response to PGPR inoculation may involve various mechanisms. Most PGPR strains may work through multiple mechanisms, which accounts for the observed beneficial effects on plant growth. Many researchers are of the view that a very important mechanism of direct growth promotion may be the production of plant growth regulators by PGPR (Lifshitz et al., 1987; Arshad and Frankenberger, 1993, 1998; Frankenberger and Arshad, 1995).

In recent years, PGPR were used for various fruit species such as sour cherry (Esitken et al., 2003), rose (Ercisli et al., 2004) and kiwifruit (Ercisli et al., 2003). However, very little is known about the effects of bacteria genera on root formation of vegetable cuttings. We have not found any report on the effect of bacteria genera on rooting of mint cuttings. Therefore, the objective of this work was to determine the effect of some bacteria isolates on rooting, root length and dry matter content of roots of mint (*Mentha piperita* L.) cuttings.

MATERIALS AND METHODS

This study was conducted in both field and greenhouse conditions at Atatürk University, College of Agriculture, Erzurum, Turkey, during the years 2004 to 2006. Mint (*Mentha piperita* L.) semi-hardwood cuttings were used as plant material.

The mother plant material was collected from Coruh Valley in September in 2004. Then, these mother plants were preserved in medium from September 2004 to May 2005. The mother plants were dug out from perlite and moved to field in May 2005. They were cultivated in the field from May 2005 - July 2006. Mint cuttings with root were planted on inter row spacing of 20 cm and intra row spacing of 10 cm (Vural et al., 2000) in field in May in 2005. The plants were irrigated with mini springs. During the development phases, all of the plant care practices have been irrespectively applied on the plants. The semi-hardwood cuttings were collected from these plants and prepared as 10, 15 and 20 cm length in 2006.

To determine the effect of bacteria inoculation on root formation on mint cuttings, bacterial treatments were performed by dipping the cuttings into *Agrebacterium rubi* (strain A 16), *Burkholderia gladii* (strain BA7), *Peseudomonas putidea* (strain BA8), *Bacillus subtilus* (strain OSU142) and *Bacillus megatorium* (strain M3) bacterial suspensions which were prepared in sterile water at a concentration of 10^9 cfu/mL^{-1}. Mint cuttings were held in prepared solutions for 45 min (Ercisli et al., 2004). Cuttings not exposed to bacterial suspensions served as controls.

Following bacterial treatments, inoculated cuttings were placed a depth of 8 - 9 cm in trays (35 x 70 cm) filled with sterile perlite and irrigated by using mini springs as mist in a greenhouse maintained at 25 ± 2°C in July to October in 2006.

At the end of the study, rooting percentage, root length and dry matter content of root were determined. Main effects were cutting length (3 levels) and bacteria inoculation (5 levels). The experiment was replicated twice as Experiment 1 and 2. Twenty semi-hardwood cuttings prepared as 10, 15 and 20 cm length cuttings were used in each replicate in Experiment 1 and 2.

The experimental design was a completely randomized block design with 3 replications. ANOVA was applied on the data obtained in this study and the differences between means were compared using Duncan's multiple range test. Data was analyzed using SPSS 13.0 statistical program.

RESULTS AND DISCUSSION

Overall, there were significant differences among plant growth rhizobacteria (PGPR) on rooting percentage in mint cuttings ($P < 0.01$). However, the effect of cutting length on root percentage was non significant. The highest rooting percentage was observed in A16 (88.70 and 89.85%), followed by M3 (86.12 and 91.15%) and BA8 (87.27 and 87.77%), while the lowest were observed in controls (79.31 and 76.96) in both experiments. From these results, it can be said that A16, BA8 and M3 were found to be more effective to improve rooting percentage than control cuttings in the two experiments (Table 1). It has been previously shown that inoculation with *Agrobacterium* increased rooting percentage both in rose and hazelnut stem cuttings (Bassil et al., 1991; Ercisli et al., 2004). In addition, Esitken et al. (2003) demonstrated that A16 has the best performance on rooting of wild sour cherry cuttings.

As seen on the Table 2, the effects of bacteria inoculation and cutting length on root length of mint cuttings were found statistically significant at 0.01 probability level in both experiments. Root length was greater when cuttings were treated with BA7, A16 and M3 compared the other treatments. The lowest root length was obtained from control, followed by BA8 and OSU142 (Table 2). The root length changed from 11.3 cm (control) in 15 root length in experiment 1 to 22.9 cm (M3) in 20 cm root length in experiment 2. In addition, the effects of cutting length on root length on mint cuttings were different; length of 20 cm in first experiment; 15 and 20 cm cuttings in the second experiment showed better performance than the other treatments. When the bacteria activity was evaluated, it was observed that BA7, A16 and M3 were more effective than the others. Ribaudo et al. (2006) reported that inoculation with *A. brasilense* FT 326 increased root length of tomato plants and Hall et al. (1996) mentioned that when the canola, lettuce, tomato, and wheat seeds were treated with *P. putida* GR12-2 and/or AVG root length increased.

Dry matter content of roots were affected by bacterial treatments ($P< 0.05$ and $P < 0.01$) and mint cuttings inoculated with M3 had more dry matter content than control and the other treatments in both experiments. In the first experiment, length of 20 cm cuttings showed bet-

Table 1. Effect of bacteria inoculation and cutting length on rooting percentage (%) of mint cuttings.

Bacteria Strains	Cutting length (cm)			
	10 cm	15 cm	20 cm	Mean
Experiment 1				
Control	84.43 ^{NS}	69.85 b*	83.64 ^{NS}	79.31 B*
A 16	83.20	90.30 a	92.59	88.70 A
BA 7	76.10	90.46 a	83.20	83.25 AB
BA 8	84.45	90.00 a	87.37	87.27 A
OSU 142	77.88	79.63 ab	89.63	82.38 AB
M 3	81.00	90.91 a	86.44	86.12 A
Mean	81.18 ^{NS}	85.19	87.15	
Experiment 2				
Control	71.82 ^{NS}	75.56 bc*	83.50 ^{NS}	76.96 C*
A 16	93.64	85.93 b	90.00	89.85 A
BA 7	73.50	86.60 b	83.76	81.29 BC
BA 8	84.01	100.00 a	79.29	87.77 AB
OSU 142	86.44	70.00 c	79.87	78.77 C
M 3	84.55	100.00 a	88.89	91.15 A
Mean	82.35 ^{NS}	86.35	84.22	

*, **Significant differences among bacteria strains and cutting length at 0.05 and 0.01 probability levels, respectively. NS, not significant

Table 2. Effect of bacteria inoculation and cutting length on root length (cm) of mint cuttings.

Bacteria Strains	Cutting length (cm)			
	10 cm	15 cm	20 cm	Mean
Experiment 1				
Control	11.9 b**	8.1 c**	14.9 b**	11.7 C**
A 16	14.1 ab	17.9 a	21.3 a	17.6 A
BA 7	13.4 b	12.9 b	20.7 a	14.0 B
BA 8	11.5 b	14.1 b	15.7 b	13.4 BC
OSU 142	16.5 a	19.4 a	14.5 b	19.0 A
M 3	14.0 ab	21.0 a	21.6 a	18.9 A
Mean	13.6 C**	15.6 B	18.1 A	
Experiment 2				
Control	11.0 b*	11.2 c**	15.5 c*	12.6 C**
A 16	17.7 a	22.6 a	22.6 a	21.0 A
BA 7	15.3 a	16.1 ab	17.3 bc	16.2 B
BA 8	12.3 b	15.8 ab	14.1 c	14.1 BC
OSU 142	17.9 a	22.6 a	21.7 ab	20.5 A
M 3	15.5 a	19.9 a	22.9 a	19.4 A
Mean	14.9 B	18.0 A	18.9 A	

*, **Significant differences among bacteria strains and cutting length at 0.05 and 0.01 probability levels, respectively.

ter performance with respect to dry matter content of root, while it was not significant in the second experiment (Table 3). It was reported that PGPR inoculation enhanced plant dry weight (Xia et al., 1990). Similarly, Ribaudo et al. (2006) reported that inoculation with *A. brasilense* FT 326 increased root fresh weight of tomato plants.

The analysis of variance (Table 4) showed significant

Table 3. The effect of bacteria inoculation and cutting length on dry matter content of root of mint cuttings (%).

Bacteria Strains	Cutting length (cm)			
	10 cm	15 cm	20 cm	Mean
Experiment 1				
Control	13.4 ^{NS}	15.1 ab**	15.3 ab**	14.6 B*
A 16	12.4	13.1 b	17.4 ab	14.3 B
BA 7	13.1	13.8 ab	16.2 ab	14.4 B
BA 8	12.5	14.3 ab	16.9 ab	14.5 B
OSU 142	14.7	12.3 b	13.9 b	13.7 B
M 3	14.5	16.8 a	18.6 a	16.7 A
Mean	13.4 B*	14.2 B	16.4 A	
Experiment 2				
Control	15.0 ab**	8.5 b*	12.4 b*	11.9 C*
A 16	13.6 ab	16.1 a	17.1 a	15.6 AB
BA 7	15.5 a	15.2 a	15.5 ab	15.4 AB
BA 8	11.1 b	16.8 a	14.4 ab	14.1 B
OSU 142	13.4 ab	14.1 a	15.9 a	14.5 B
M 3	16.6 a	15.5 a	16.7 a	16.3 A
Mean	14.2 ^{NS}	14.4	15.3	

*, **Significant differences among bacteria strains and cutting length at 0.05 and 0.01 probability levels, respectively. NS, not significant.

Table 4. Interactions between cutting length and bacteria strains.

Source of variation	F-values		
	Rooting percentage	Root length	DW
Cutting Length (CL)	3.267*	34.436**	11.541**
Bacteria Strains (BS)	6.816**	41.356**	5.869**
CL x BS	3.285**	3.528**	2.341*

*Significant at $P = 0.05\%$ level, **Significant at $P = 0.01\%$ level.

differences among the cutting length (CL) and bacteria strains (BS) for rooting percentage, root length and dry matter content of root (DW). The CL x BS interaction was significant for rooting percentage, root length and dry matter content of root. Significant CL x BS interaction for studied characters demonstrated that the effect of bacteria strains varied considerably. In other words, this interaction also indicated that performance of cuttings under different length was affected positively by bacteria strains for rooting percentage, root length and dry matter content of root

From the standpoint of rooting percentage, root length and dry matter content of root, cuttings of mints treated with bacterial solutions *A. rubi* (strain A16), *B. gladii* (strain BA7), *P. putidea* (strain BA8), *B. subtilus* (strain BA142) *B. megatorium* (strain M3) had higher values than control cuttings in both experiments. However, when bacteria strains were compared, it was observed that M3 was more effective than the others on the examined properties.

Growers have attempted to stimulate rooting by applying growth regulators, various chemical substances, etc. Among the plant growth regulators, it is well known that auxin play a major role in the initiation and development of roots. However, the intensive use of exogenous plant growth regulators could result in environmental problems. Recently, environmental problems have raised interest in environmental friendly sustainable agricultural practices (Salantur et al., 2005). Therefore, the use of growth promoting bacteria (PGPR) can overcome environmental problems.

In conclusion, our study shows that isolate M3 was clearly more consistent in improving different root parameters of mint cuttings. Work is in progress to test

M3 and other strains on different parameters such as optimal concentration range (10^x cfu/mL^{-1}). Additionally, our study presents the first data on the effect of bacteria (PGPR) inoculation on rooting of mint semi-hardwood cuttings.

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