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EFFECTS OF SOME RHIZOBACTERIA AND INDOLE-3-BUTYRIC ACID ON ROOTING OF BLACK AND WHITE MULBERRY HARDWOOD CUTTINGS

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ABSTRACT

This research was conducted to determine the effects of treatments with indole-3-butric acid (0, 2, 4 and 6 g l^{-1} IBA) and plant growth promoting rhizobacteria (PGPR) (*Burkholderia gladii*-BA7,*Bacillussubtilus*-OSU142 and *Bacillusmegatorium*-M3) strains have on rooting and root growth of hardwood stem cuttings of black and white mulberry both alone and in combination with each bacterial strain. Cuttings in the control group were treated with 50% ethanol + 50% distilled water. The hardwood stem cuttings (middle parts of one-year-old shoots) for rooting were selected from 15-year-old healthy donor black and white mulberries trees on February 1, 2015 and 2016 in Bolu, Turkey. The treated cuttings were placed in perlite medium in unheated trays of a greenhouse with automated misting system for 90 days. The rooting rate, root number, root length and diameter were evaluated. The rooting rate varied from 12.0 to 85.0%. The number of root varied from 2.05 to 10.19. The root length varied from 2.75 to 8.72 cm. The root diameter varied from 0.81 to 2.49 mm. The results indicated that treatment with 4 g l^{-1} IBA plus *B. megatorium*-M3 solution had a profound effect in increasing rooting capacity and quality in comparison to the control, and all other PGPR and IBA treatments. Overall, rooting rate and root quality of black mulberry hardwood cuttings was found to be lower than those of white mulberry. Moreover, 4 g l^{-1} IBA was the most appropriate dose and *B. megatorium*-M3 bacterial strain was the most appropriate rhizobacteria for rooting of mulberry cuttings.

Keywords: Mulberry, cutting, rooting, auxin, bacteria, propagation.

INTRODUCTION

Propagation for the mulberry can be accomplished by seeds, cuttings, grafting, layering and tissue culture (Lu, 2002; Anis *et al*., 2003). It can most easily be propagated via seeds (Güneş and Çekiç, 2004; 2011). However, propagation through seeds is undesirable because of enormous heterozygosity in the plants resulting from cross pollination (Anis *et al*., 2003).If cultivated for its fruit, mulberry should be clonally propagated. Grafting or budding propagation is not practical as it calls for specialized labour-power and nursery practice is expensive. Furthermore, grafting success is dependent on internal factors like as 'milk' exudation, compatibility, activity of cambium; and it can be affected by external conditions such as temperature, humidity and soil characteristics (Ünal *et al*., 1992; Koyuncu and Şenel, 2003).Although there are no reported guaranteed protocols to date, tissue culture techniques can be used for mulberry propagation (Anis *et al*., 2003). Methods of in vitro propagation require specialist staff and expensive facilities.

Cutting regeneration is still one of the most economical methods of clonal propagation of plants. The rooting abilities of the cuttings vary greatly depending on the species and cultivars. However, the age of donor plants, collection time, environmental conditions and treatments with plant growth regulators also affect the rooting of the cuttings (Ünal *et al*., 1992; Koyuncu and Şenel, 2003; Hartmann *et al*., 2011). Mulberry is limited due to the difficulties in cutting. The cutting cannot display rooting success due to milk secretion emerging under cuttings and the space that emerges under the bud tissue on the mulberry (Ünal *et al*., 1992). Auxin induce formation of callus and new vascular tissue. Various auxins, which affected xylem and phloem differentiation, had significant effects on cutting, as well as on the process of lignification, which is regarded as very important factors in rooting (Kako, 2012). Authors indicate that IBA is critical for hardwood cutting which is well documented and is appropriately cited in this manuscript (Koyuncu and Şenel, 2003; Kalyoncu *et al*., 2009; Çekiç *et al*., 2013; Husen *et al*., 2015). Authors introduce the idea that bacteria can induce root formation in different plant cutting (Bassil *et al*., 1991;Eşitken *et al*., 2003; Ercişli *et al*., 2003; Kaymak *et al*., 2008; Ertürk *et al*.,2008; Ertürk *et al*.,2010). It has been reported that these bacteria produce indole-3-acetic acid (IAA) (Goto, 1990). Authors indicate that cutting treated with both bacteria and IBA can have accelerated rooting (Bassil *et al*., 1991; Falasca *et al*., 2000; Ercişli *et al*., 2004).

Cuttings are the most widely used method for propagation of mulberry saplings around the world. Black

mulberry fruits, with their high fresh weight, black to purple colour and amazing taste, are increasingly attracting consumers and thus, the need for these plants has also surged in recent years. This research was conducted to determine the effects of treatments with IBA (0, 2, 4 and 6 g l^{-1}) and some plant growth promoting rhizobacteria (PGPR) (*Burkholderia gladii*-BA7,*Bacillus subtilus*-OSU142 and *Bacillus megatorium*-M3) strains alone and combination with each bacterial strains on rooting and root growth in black and white mulberry hardwood cuttings.

MATERIALS AND METHODS

Study Site: This study was conducted at the Abant İzzet Baysal University, Vocational Community College of Bolu research greenhouse located in Bolu Center, Turkey (North: 40° 43', East: 31° 33', Altitude: 768 m) during 2015 and 2016.

Plant Materials: Plants of black (*Morus nigra* L.)and white mulberry (*Morus alba* L.) were used for cutting collection. The hardwood stem cuttings (middle parts of one-year-old shoots) for rooting were selected from 15-year-old healthy donor black and white mulberries trees on February 1, 2015 and 2016in Bolu, Turkey.

Preparation of cuttings: Dormant hardwood cuttings (15-20 cm length) containing 3-4 buds were prepared. The cuttings were disinfected by a fungicide (0.2% Benlate for 10 minutes) against fungus infections. After disinfection they were quickly washed three times with distilled water.

Bacterial strains and IBA treatment: Cuttings were put through 1 of 16 treatments. In IBA treatments, the basal portion of cuttings (2 cm) were dipped in a 2, 4 or 6 g l^{-1} aqueous solution of IBA, dissolved in 50% ethanol, for 5 min and were then left air dry (for 30 min). In order to perform bacterial treatments, the 2 cm basal portion of cuttings were dipped into the bacterial suspension (prepared in distilled water with a concentration of 10^9cfu ml^{-1} from *Bacillus subtilus* (strain OSU142), *Burkholderia gladii* (strain BA7), and *Bacillus megatorium* (strain M3) strains for 30 min. IBA+ bacteria combined treatments, IBA treated cuttings were dipped into the bacterial suspension. Cuttings in the control group were drenched in 50% ethanol + 50% distilled water.

Rooting media and growth conditions: Following treatments cuttings were put in perlite-filled trays (sterile agri-perlite) incubated under mist (15 s/ 6 min) in a green house kept at temperatures of 22±2°Cand were placed to a depth of 10 cm. The automatic and time-dependent mist-propagation system was set at 70-80% air humidity for the rooting process. The data were recorded after 90 days.

Rooting parameters followed: When the rooting period concluded; rooting rate (%), root number (per cutting), root length (cm) and root diameter (mm) (Zenginbal and Özcan, 2014) were determined.

Statistical analysis: The experimental design for this study was a randomized complete block design with four replications. In each replication there were 25 cuttings, spaced 5 cm apart. The percentage data (rooting rate) were modified using arc-sin$\sqrt{x}$ transformation. The data was analysed using SPSS 13.0 statistical software. Before the ANOVA tests, homogeneity of variances were examined with Leven's variance homogeneity test. Variances of all traits were found homogeneous (p>0.05). Duncan's test was used for multiple comparisons. There were no statistical differences between the years, therefore the data were pooled.

RESULTS AND DISCUSSION

The effect of trio interaction (IBA x PGPR strains x cultivar) on rooting rate, root number, root length and diameter in black and white mulberry hardwood cuttings are summarized in Table 1 and Table 2. In research, trio interaction (IBA x PGPR strains x cultivar) effect was found statistically significant (P<0.05) for all parameters. Among cuttings from both mulberry cultivars, those that were treated with IBA and PGPR strains rooted better than the control cuttings. Nevertheless, the response of the two mulberry cultivars to the imposed treatments varied. The results indicated that treatment of white mulberry cuttings with 4 g l^{-1} IBA plus *B. megatorium*-M3 solution was highly effective in increasing rooting capacity and quality when compared to control, and all other PGPR and IBA treatments.

Main effects of treatments with IBA on rooting rate, root number, root length and diameter of two mulberry cv. cuttings are summarized in Table 3. Assessment in terms of rooting rate, root number, root length and diameter showed statistically significant differences (P<0.001). The treatments with IBA increased the rooting and root quality. The results showed that mulberry was able to root at low rate (16.75%) without any additional treatments (control). Rooting rate of cuttings treated with IBA resulted in 50.63-70.0%. Maximum rooting rate (70.0%) was obtained after 4 g l^{-1} IBA treatments. The number of roots varied from 2.76 to 9.64. Root number was greater in cases where cuttings were treated with 4 g l^{-1} IBA (9.46) rather than other treatments. Root number was lowest at control variant (2.76). Based on these results, it could be claimed that treatments of cuttings with IBA significantly improved the root number as compared to non-treated cuttings. As seen on the Table 3, treatments with IBA significantly

increased the root length. The root length of cuttings treated with IBA the values were 5.62-8.23 cm. The highest root length of cuttings (8.23 cm) was observed after treatment with 4 g l^{-1} IBA, followed by 6 g l^{-1} IBA (7.03 cm) and 2 g l^{-1} IBA (5.62 cm).The lowest root number was obtained at control variant (3.40). Treatments with IBA also increased the root diameter and diameters of root varied from 1.06 mm to 2.31 mm. Root diameter was greater in cases where cuttings were treated with 4 g l^{-1} IBA (2.31 mm) rather than other treatments. At control variant (1.06 mm), root number was the lowest.

Main effects of treatments with bacterial strains on root rate, root number, root length and diameter of two mulberry cv. cuttings are summarized in Table 4. As seen on the Table 4, effects of treatments with PGPR strains on rooting rateof mulberry cuttings were found statistically significant ($P < 0.001$). The treatments with PGPR strains increased the rooting rate. The results showed that mulberry was able to root at low rate (46.13%) without any additional treatments (control). Maximum rooting rate (53.63%) was obtained after *B. megatorium* (M3) strain treatments. Statistically significant differences ($P<0.001$) in terms of the root number was found. The number of roots varied from 5.92 to 6.15. For cuttings that were treated with *B. megatorium* (M3) strain, root number was greater compared to the other treatments. Based on these results, it could be claimed that treatments of cuttings with PGPR strains significantly improved the root number as compared to non-treated cuttings (control). As seen on the Table 4, statistically significant differences ($P<0.001$) were observed in terms of root length. Treatments with PGPR strains significantly increased the root length. The highest root length of cuttings (6.44 cm) was observed after treatment with *B. megatorium* (M3) strain. The lowest root number was obtained at control variant (5.76 cm). Treatments with PGPR strains had effects on root diameter of mulberry cuttings that were noted to be statistically significant ($P < 0.001$). For cuttings that were treated with *B. megatorium* (M3) strain, root diameter was greater compared to the other treatments.

Main effects of mulberry cultivars on rooting rate, root number, root length and diameter of mulberry cuttings are summarized in Table 5. Statistically significant differences ($P<0.001$) were found in terms of the rooting rate and diameter. Rooting rate and root diameter of black mulberry hardwood cuttings were lower than those of white mulberry. Mulberry cultivars effect was found statistically insignificant ($P>0.05$) for root number and root length parameters. Overall, rooting of black mulberry hardwood cuttings was lower than those of white mulberry.

Both IBA and PGPR treated black and white mulberry cuttings rooted significantly better than those from the control variant. Positive effects of IBA and PGPR applications on mulberry cuttings may be explained by auxin produced by IBA and bacterial strains. It is known that auxin is deeply involved in the process by which callus and adventitious roots from in cuttings (Weaver, 1972). Among our PGPR treatments the best rooting ratio and root quality was observed after treatment with M3 bacterial strain. It has previously been demonstrated that with *Agrobacterium* strains could induce adventitious rooting in recalcitrant woody genotypes. Bassil *et al*. (1991) showed that rooting percentage of hazelnut stem cuttings was improved by treatment with *Agrobacterium* strains. Eşitken *et al*. (2003) and Ercişli *et al*. (2003) found that *Agrobacterium rubi* A16 was the most efficient from among the three bacterial strains tested for rooting of wild sour cherry and kiwifruit cuttings. Kaymak *et al*. (2008) reported that rooting percentage for mint increased when cuttings were treated with M3 bacterial strains. Furthermore, Ercişli *et al*. (2004), Ertürk *et al*. (2008) and Ertürk *et al*. (2010) tested PGPR for rooting in rosehip, tea and kiwifruit cuttings and found that PGPR was efficient in obtaining high rooting percentage and root quality. In the present study, control cuttings rooted poorly, but the cuttings responded to the best of treatments with IBA 4 g l^{-1}. Studies by Weaver (1972) and Hartmann *et al*. (2011) showed that growth regulators (auxins) altered the number and the strong fringe root was produced by the type of root. Also, several researchers (Kalyoncu *et al*., 2009; Kako,2012; Çekiç *et al*., 2013; Husen *et al*., 2015) reported that treating cuttings with IBA (between 3 and 6 g l^{-1}) increased the percentage of rooting and as well as root quality in mulberry. In generally, our results are parallel with these formerly reported data. For recalcitrant woody genotypes, inoculation with rhizobacteria strains (especially *B. megatorium*-M3) might induce adventitious rooting and in most cases, this could also require exogenous auxin. Falasca *et al*. (2000) and Ercişli *et al*. (2004) showed that exogenous IBA treatments enhanced rooting on walnut and rosehip cuttings which were bacteria inoculated. Results from our study were general in line with previously reported data (Bassil *et al*., 1991; Esitken *et al*., 2003; Ercişli *et al*., 2004), revealing that IBA-bacteria combined treatments showed greater capacity than IBA or bacteria alone treatments in enhancing rooting of cuttings. The significant difference between mulberry cultivars in terms of rooting rate and rooting quality success may potentially be attributed to genetic difference. Likewise, Hartmann *et al*. (2011) reported that genetic factors had a significant effect on rooting rate. Similar results were reported by Ünal *et al*. (1992), Kalyoncu *et al*. (2009) and Çekiç *et al*. (2013) in mulberry cultivars.

Table 1. Effects of IBA and PGPR strains on the rooting (%) and root number of hardwood stem cuttings of black and white mulberry cultivars (average of 2015 and 2016).

IBA ($g\ l^{-1}$)	Bacterial strains	Rooting rate (%)		Average root number (per cutting)	
		Morus nigra	Morus alba	Morus nigra	Morus alba
0	None	12.0 ± 1.63 r	18.0 ± 2.58 pr	2.05 ± 0.08 f	2.15 ± 0.17 e-f
	OSU 142	14.0 ± 2.58 pr	20.0 ± 1.63 pr	2.83 ± 0.18 ef	2.93 ± 0.44 ef
	M 3	16.0 ± 1.63 pr	23.0 ± 3.00 p	3.13 ± 0.36 e	3.60 ± 0.21 e
	BA 7	12.0 ± 1.63 r	19.0 ± 3.42 pr	3.03 ± 0.17 ef	2.83 ± 0.22 e-f
2	None	41.0 ± 1.91 o	55.0 ± 3.42 h-m	5.69 ± 0.23 cd	5.77 ± 0.61 cd
	OSU 142	44.0 ± 3.65 no	58.0 ± 1.15 f-k	5.69 ± 0.33 cd	5.85 ± 0.50 b-d
	M 3	49.0 ± 3.42 k-o	60.0 ± 2.83 f-j	6.19 ± 0.15 b-d	6.07 ± 0.51 b-d
	BA 7	45.0 ± 4.43 m-o	53.0 ± 3.42 i-n	5.80 ± 0.11 cd	5.55 ± 0.47 d
4	None	57.0 ± 4.43 g-l	75.0 ± 3.42 b-d	9.46 ± 0.40 a	9.60 ± 0.35 a
	OSU 142	61. 0± 3.42 f-i	79.0 ± 3.42 ab	9.51 ± 0.22 a	9.62 ± 0.29 a
	M 3	66.0 ± 2.58 d-g	85.0 ± 2.52 a	9.79 ± 0.19 a	10.19 ± 0.39 a
	BA 7	60.0 ± 2.83 f-j	77.0 ± 2.52 a-c	9.37 ± 0.40 a	9.62 ± 0.21 a
6	None	47.0 ± 1.91 l-o	64.0 ± 3.65 e-h	6.08 ± 0.46 b-d	6.55 ± 0.24 b-d
	OSU 142	55.0 ± 4.43 h-m	68.0 ± 3.65 c-f	6.33 ± 0.52 b-d	6.75 ± 0.16 bc
	M 3	57.0 ± 5.00 g-l	73.0 ± 4.43 b-e	6.44 ± 0.30 b-d	6.93 ± 0.15 b
	BA 7	50. 0± 2.58 j-o	63.0 ± 3.42 f-i	6.28 ± 0.13 b-d	6.75 ± 0.30 bc
P value		<0.05		<0.05	

Means ± standard errors within a column followed by the same letter are not significantly different.

Table 2. Effects of IBA and bacterial strains on the root length and diameter of hardwood stem cuttings of black and white mulberry cultivars (average of 2015 and 2016).

IBA ($g\ l^{-1}$)	Bacterial strains	Average root length (cm)		Average root diameter (mm)	
		Morus nigra	Morus alba	Morus nigra	Morus alba
0	None	2.75 ± 0.19 m	3.03 ± 0.20 lm	0.81 ± 0.15 k	0.88 ± 0.10 k
	OSU 142	3.69 ±0.27 lm	3.17 ±0.16 lm	0.95 ± 0.05 jk	1.13 ± 0.09 i-k
	M 3	4.17 ± 0.33 j-l	3.47 ± 0.17 lm	1.17 ± 0.09 h-k	1.49 ± 0.21 f-j
	BA 7	3.88 ± 0.18 k-m	3.09 ± 0.27 lm	0.91 ± 0.08 k	1.20 ± 0.14 h-k
2	None	5.01 ± 0.14 i-k	5.80 ± 0.27 g-i	1.24 ± 0.06 g-k	1.72 ± 0.15 d-h
	OSU 142	5.16 ± 0.30 h-j	5.94 ± 0.20 f-i	1.31 ± 0.04 f-k	1.69 ± 0.16 d-i
	M 3	5.44 ± 0.58 hi	6.35 ± 0.10 e-h	1.34 ± 0.01 f-k	1.83 ± 0.19 b-f
	BA 7	5.28 ± 0.38 h-j	5.99 ± 0.11 f-i	1.28 ± 0.04 f-k	1.76 ± 0.07 d-h
4	None	7.93 ± 0.33 a-d	8.36 ± 0.23 a-c	2.08 ± 0.10 a-e	2.36 ± 0.09 ab
	OSU 142	8.15 ± 0.21 a-d	8.07 ± 0.57 a-d	2.19 ± 0.10 a-d	2.31 ± 0.15 a-c
	M 3	8.57 ± 0.47 ab	8.72 ± 0.44 a	2.37 ± 0.27 ab	2.49 ± 0.14 a
	BA 7	7.90 ± 0.17 a-d	8.17 ± 0.58 a-d	2.32 ± 0.20 ab	2.34 ± 0.06 ab
6	None	6.37 ± 0.21 e-h	6.87 ± 0.36 d-g	1.25 ± 0.10 g-k	2.32 ± 0.28 ab
	OSU 142	7.13 ± 0.77 c-f	7.20 ± 0.67 c-f	1.26 ± 0.07 g-k	2.39 ± 0.22 ab
	M 3	7.33 ± 0.54 b-e	7.45 ± 0.63 a-e	2.26 ± 0.34 a-c	2.60 ± 0.34 a
	BA 7	6.82 ± 0.44 d-g	7.08 ± 0.78 c-g	1.57 ± 0.31 e-i	2.26 ± 020 a-c
P value		<0.05		<0.05	

Means ± standard errors within a column followed by the same letter are not significantly different.

Table 3. Effect of IBA on the rooting, root number, root length and diameter of hardwood stem cuttings of black and white mulberry cultivars (average of 2015 and 2016).

IBA ($g\ l^{-1}$)	Rooting rate (%)	Average root number (per cutting)	Average root length (cm)	Average root diameter (mm)
0	16.75 ± 0.99d	2.76 ± 0.11d	3.40 ± 0.11d	1.06 ± 0.05d
2	50.63 ± 1.53c	5.83 ± 0.13c	5.62 ± 0.12c	1.52 ± 0.05c
4	70.00 ± 2.00 a	9.64 ± 0.11a	8.23 ± 0.14a	2.31 ± 0.05a
6	59.63 ± 1.90 b	6.51 ± 0.11b	7.03 ± 0.19b	1.99 ± 0.12b
P value	<0.001	<0.001	<0.001	<0.001

Means ± standard errors within a column followed by the same letter are not significantly different.

Table 4. Effect of bacterial strains on the rooting, root number, root length and diameter of hardwood stem cuttings of black and white mulberry cultivars (average of 2015 and 2016).

Bacterial strains	Rooting rate (%)	Average root number (per cutting)	Average root length (cm)	Average root diameter (mm)
None	46.13 ± 3.78 c	5.92 ± 0.49 b	5.76 ± 0.36 b	1.58 ± 0.11 b
OSU 142	49.88 ± 3.94 b	6.19 ± 0.44 ab	6.06 ± 0.35 ab	1.65 ± 0.10 b
M 3	53.63 ± 4.12 a	6.49 ± 0.45 a	6.44 ± 0.36 a	1.94 ± 0.12 a
BA 7	47.38 ± 3.82 bc	6.15 ± 0.43 ab	6.02 ± 0.34 ab	1.70 ± 0.11 b
P value	<0.001	<0.001	<0.001	<0.001

Means ± standard errors within a column followed by the same letter are not significantly different.

Table 5. Effect of mulberry cultivars on the rooting, root number, root length and diameter of hardwood stem cuttings of black and white mulberry cultivars (average of 2015 and 2016).

Cultivar	Rooting rate (%)	Average root number (per cutting)	Average root length (cm)	Average root diameter (mm)
Morus nigra	42.88 ± 2.39b	6.10 ± 0.31	5.97 ± 0.24	1.52 ± 0.07b
Morus alba	55.63 ± 2.89a	6.27 ± 0.32	6.17 ± 0.26	1.92 ± 0.08a
P value	<0.001	>0.05	>0.05	<0.001

Means ± standard errors within a column followed by the same letter are not significantly different.

Conclusion: The results in research indicated that treatment with 4 $g\ l^{-1}$ IBA plus *B. megatorium*-M3 solution was highly effective in increasing rooting capacity and quality when compared to control, and all other PGPR and IBA treatments. It has been reported by the present study that IBA increased the rooting and root quality in mulberry cuttings. The PGPR application may be of benefit in rooting cuttings of mulberry cultivars, particularly for organic farming. In addition, 4 $g\ l^{-1}$ IBA was the most appropriate dose and *B. megatorium*-M3 bacterial strain was the most appropriate rhizobacteriafor rooting of mulberry cuttings. Overall, rooting rate and root quality of black mulberry hardwood cuttings was lower than those of white mulberry. Moreover, our study presented primary data on the effect of bacterial (PGPR) treatment has on rooting and root growth of black and white mulberry hardwood cuttings.

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