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Effect of Indole-3-Butyric Acid and Different Strains of *Agrobacterium rubi* on Adventive Root Formation from Softwood and Semi-Hardwood Wild Sour Cherry Cuttings

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Abstract: This study was conducted to evaluate the effects of a range of Indole-3-butyric acid (IBA) concentrations (250, 500 and 750 mg l^{-1}) alone and in combination with three strains of *Agrobacterium rubi* (A1, A16 and A18) on the rooting capacity of wild sour cherry (*Prunus cerasus* L.) softwood and semi-hardwood cuttings. The bacterial strains used in the present study were isolated from the foliage of pome fruits (from apple and pear orchards) growing in the eastern Anatolia region of Turkey. No rooting was observed on the cuttings of wild sour cherry with control treatment (no IBA or bacterial treatment) in both types of cuttings, whereas different rooting percentages were observed on the cuttings treated with IBA and bacteria. The highest rooting percentages were 65% for softwood and 70% for semi-hardwood cuttings when they were treated with 250 mg l^{-1} IBA + A16 treatments. In softwood cutting treatments, the bacteria strains A16 (43.8%) and A1 (42.5%) were found to be more effective than the strain A18 (18.8%) and the control (13.1%). Among the hormone doses, the best rooting percentage was found at the treatment of 250 mg l^{-1} IBA (39.4%). In semi-hardwood cuttings the highest rooting percentage among the bacterial strains and hormone doses was obtained with the treatments of A16 (49.4%) and 750 mg l^{-1} IBA (46.9%). The results indicate that the combination of IBA + bacteria is highly effective in increasing rooting capacity when compared to the control, or bacteria and IBA treatments alone.

Key Words: Sour cherry, softwood and semi-hardwood cutting, *Agrobacterium rubi*, IBA, adventive rooting

IBA ve Bakteri (*Agrobacterium rubi*) Uygulamalarının Yeşil ve Yarı Odunsu Yabani Vişne Çeliklerinde Adventif Kök Oluşumu Üzerine Etkileri

Özet: Yabani vişne (*Prunus cerasus* L.)'nin yeşil ve yarı odunsu çeliklerinde köklenme üzerine IBA ve *Agrobacterium rubi*'nin etkisinin incelendiği araştırmada, IBA'nın 0, 250, 500 ve 750 mg l^{-1} dozları tek başına veya *Agrobacterium rubi*'nin üç şuşu (A1, A16 ve A18) ile kombine edilerek uygulanmıştır. Araştırma sonunda, yabani vişne çeliklerinde her iki çelik tipinin kontrol uygulamalarında köklenme elde edilememiştir. Yabani vişnenin yeşil ve yarı odunsu çeliklerine bakteri, IBA ve IBA+bakteri uygulamalarında köklenmenin teşvik edildiği saptanmıştır. En yüksek köklenme oranı yeşil çelikte 65% ve yarı odunsu çelikte 70% ile 250 mg l^{-1} IBA+A16 uygulamasından elde edilmiştir. Yeşil çeliklerde bakteri şuşları içerisinde A16 (43.8%) ve A1 (42.5%), A18 (18.8%) ve kontrolden (13.1%) daha etkili bulunmuştur. Hormon dozları arasında 250 mg l^{-1} IBA (39.4%) en yüksek köklenme oranını vermiştir. Yarı odunsu çeliklerde ise, en yüksek köklenme oranını bakteri şuşları içerisinde A16 (49.4%) ve hormon dozları içerisinde 750 mg l^{-1} IBA (46.9%) uygulamasından elde edilmiştir. Sonuç olarak, IBA+bakteri uygulamaları çeliklerde adventif kök oluşumu için tek bakteri, IBA ve kontrol uygulamalarından daha etkili bulunmuştur.

Anahtar Sözcükler: Vişne, çelik, *Agrobacterium rubi*, IBA, köklenme

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Introduction

Wild sour cherry (*Prunus cerasus* L.) is one of the most important rootstocks for cultivated cherries in Turkey. This rootstock is more resistant to soil wetness and cold climates than others used as rootstocks such as mazzard (*Prunus avium* L.) and mahaleb (*Prunus mahaleb* L.). Hence, this species is the most preferred one where extreme environmental conditions are present. Dwarfing and early bearing qualities are some other advantages of sour cherries as rootstocks for cherries. On the other hand, most of the sour cherry rootstocks obtained from the same parents have heterogeneous horticultural characteristics due to a wide range of variation in the genetic background of the seedlings when they were germinated from seeds (Webster and Schmidt, 1996).

Thus, it is believed that the vegetative propagation of sour cherry rootstocks with superior horticultural characteristics and adaptation ability for given locations and conditions is useful and necessary for overcoming drawbacks, and providing genetically uniform and cost-effective planting material (Perry, 1987). As is commonly known, the rooting of cuttings of temperate zone fruit species is very difficult. So far, there have been many attempts to stimulate the rooting of cuttings by various treatments including treatment with plant growth regulators, carbohydrates and various other chemical substances (Doud and Carlson, 1972; Çelik and Ağaoğlu, 1983; Couvillon, 1988). It is well known that auxins have an important role in the rooting of hardwood cuttings on many temperate species (Sims and Lawes, 1981; Yıldız and Eti, 1995; Ercişli and Güleryüz, 1999).

Recent studies showed that some bacteria in the genera of *Agrobacterium, Bacillus, Streptomyces, Pseudomonas* and *Alcaligenes* might induce root formation in stem cuttings (Bassil et al., 1991; Jacob et al., 1991; Jacob and Hamdan, 1992; Hatta et al., 1996; Rinallo et al., 1999). It has been reported that these bacteria produce indole-3-acetic acid (IAA) (Goto, 1990), and several studies have also shown that the rooting of bacteria-inoculated cuttings can be further accelerated by exogenous Indole 3-butyric acid (IBA) application (Bassil et al., 1991; Falasca et al., 2000). The bacterial strains of *Agrobacterium rubi* (A1, A16 and A18) used in this study were selected on the basis of their IAA production abilities, which were determined in our previous studies (Ercişli et al., 2000 a; Ercişli et al., 2000 b).

The objective of the present study was to determine the effect of *Agrobacterium rubi* strains (A1, A16 and A18) alone and in combination with exogenous auxin treatment on the root formation of softwood and semi-hardwood cuttings of the wild sour cherry.

Materials and Methods

The softwood and semi-hardwood cuttings used in this study were taken from wild cherry selection type (32-Is-4), which is the most preferred rootstock for wet soils and is commonly grown in the provinces of Afyon and Isparta in Turkey. Various concentrations of IBA and/or different strains of *Agrobacterium rubi* (A1, A16 and A18) were applied to the cuttings. These bacterial strains were isolated from the foliage of pome fruits (from apple and pear orchards) growing in the eastern Anatolia region of Turkey (Kotan, 2002).

For IBA treatments, the basal portions of the cuttings were dipped in aqueous solutions of 250, 500, or 750 mg l^{-1} IBA (50% ethanol) for 5 min and allowed to air dry. Bacterial treatments were performed by dipping the cuttings into the bacterial suspension prepared in sterile water at a concentration of 10^9 cfu ml^{-1} from *Agrobacterium rubi* (A1, A16 and A18) for 30 min and then dried.

Combined IBA + bacteria treatments were applied by dipping IBA-treated cuttings into the bacterial suspension. Cuttings in the control group were dipped in 50% ethanol. Following treatment, the cuttings were placed in perlite media to a depth of 10 cm under mist (15 s/6 min) in a greenhouse maintained at 21 ± 2 °C. The experimental design was completely randomized with four replications. Each replication contained 10 cuttings spaced 50 mm apart. Data were collected on the percentage of rooting, number of roots and root length (cm). Data were subjected to analysis of variance (ANOVA) and were separated using Duncan's multiple range test.

Results

Softwood cuttings

The effects of the treatment (IBA, *Agrobacterium rubi* strains and their combinations) on the rooting of sour cherry softwood cuttings are summarized in Table 1. Rooting was significantly affected by the interaction of

Table 1. The effects of IBA, *Agrobacterium rubi* strains and their combinations on the rooting (%), root number and root length (cm) of softwood cuttings.

IBA Doses (mg l^{-1})	Bacteria strains	Rooting (%)	Root Number	Root Length (cm)
0	None	0.0 f	0.0 d	0.00 f
	A1	10.0 f	3.0 cd	1.13 ef
	A16	10.0 f	2.5 cd	2.47 e
	A18	0.0 f	0.0 d	0.00 f
Means		5.0	1.4	0.90
250	None	10.0 f	6.0 bc	8.60 abc
	A1	60.0 ab	8.4 ab	9.70 a
	A16	65.0 a	8.8 ab	9.95 a
	A18	22.5 e	3.5 cd	6.20 cd
Means		39.4	6.7	8.61
500	None	10.0 f	9.0 ab	4.40 de
	A1	50.0 bc	11.0 a	10.05 a
	A16	60.0 ab	12.0 a	9.10 a
	A18	30.0 de	5.5 bc	6.10 cd
Means		37.5	9.4	7.41
750	None	32.5 de	5.5 bc	4.50 de
	A1	50.0 bc	6.2 bc	6.60 bc
	A16	40.0 cd	4.0 cd	6.20 cd
	A18	22.5 e	3.1 cd	3.75 e
Means		36.3	4.7	5.26
LSD		0.01: 10.08	0.01: 4.02	0.01: 2.06
Bacteria strains		***	***	***
IBA doses		***	***	***
Bacteria x IBA		***	***	***

bacteria and IBA treatments in the 32-Is-4 wild sour cherry genotype ($p \leq 0.001$).

The softwood cuttings of 32-Is-4 could not be rooted in the control treatment. All the other combinations of treatments including bacteria, IBA or their combinations increased the rooting percentage.

There were statistical differences between the bacterial treatments on the rooting percentage of softwood cuttings obtained from 32-Is-4. The rooting percentages of bacterial treatments varied between 0.0 and 10.0% (Table 1).

An analysis of rooting percentage data showed that there were significant differences between the treatments. No rooting was observed on the control cuttings, whereas variable rooting success (0-32.5%) was obtained with the IBA treatments (Table 1).

Treatments with bacteria + IBA also resulted in greater rooting than for the controls in this genotype. The difference in the rooting percentage between the treatments with IBA + bacteria and the water control was significant for this genotype ($p \leq 0.001$). The rooting percentages were highest in 250 mg l^{-1} IBA combined with the A16 treatment at 65.0% in softwood cuttings (Table 1).

The differences in average root number of cuttings with different treatments were also found to be statistically significant ($p \leq 0.01$). Except for the control and one bacterial treatment (A18) that did not trigger root production, all the other applications induced root numbers ranging from 2.5 to 11.0 per sour cherry cutting (Table 1).

Better root development was observed in the cuttings treated with IBA + bacteria combinations, in which the average root length ranged from 3.75 (750 mg l^{-1} IBA + A18) to 10.05 cm (500 mg l^{-1} IBA + A1). The average root lengths in the other applications were as follows: 4.40-8.60 cm in single concentrations of IBA; 1.13-2.47 cm in bacterial strain alone; and 0.0 in the control.

The highest rooting percentage (39.4%) and root length (8.61 cm) were obtained from 250 mg l^{-1} IBA treatments while the greatest root number (9.4) was observed for 500 mg l^{-1} IBA doses in softwood cuttings (Table 1).

Semi-Hardwood Cuttings

Table 2 shows the percentage of rooting, root number and root length obtained with 32-Is-4 semi-hardwood cuttings. The differences in the average percentage of rooting, root length and root number with different treatments were statistically significant ($p \leq 0.001$). Rooting was significantly affected by the interaction of bacteria and IBA treatments ($p \leq 0.001$). As shown in Table 2, the control group of semi-hardwood cuttings did not yield rooting. The application of *Agrobacterium rubi* strains alone also gave poor rooting results, which ranged from 0.0 to 7.50%.

Cuttings treated with IBA yielded moderate rooting results, ranging from 20.0 to 50.0%. However, cuttings treated with IBA + *Agrobacterium rubi* showed significant improvement in terms of rooting percentage (35.0-70.0%) (Table 2).

The differences in the average number of roots and root length of semi-hardwood cuttings with different treatments were also found to be statistically significant ($p \leq 0.001$). Except for the control, two bacterial treatments, A1 and A18, did not trigger root production; all the other applications induced root numbers ranging from 1.3 to 12.3 per sour cherry cutting (Table 2). Better root development was observed on the cuttings treated with the IBA + bacteria combination in which the average root length ranged from 2.37 to 5.57 cm. The average root lengths of the other applications were as follows: 0.73-4.07 cm in single concentrations of IBA, 0.0-1.68 cm in bacterial strain alone, and 0.0 in the control.

In semi-hardwood cuttings, although the highest root number was for 250 mg l^{-1} IBA (8.0) treatments, the

Table 2. The effects of IBA, *Agrobacterium rubi* strains and their combinations on the rooting (%), root number and root length (cm) of semi-hardwood cuttings.

IBA Doses (mg l^{-1})	Bacteria strains	Rooting (%)	Root Number	Root Length (cm)
0	None	0.0 f	0.0 f	0.00 f
	A1	0.0 f	0.0 f	0.00 f
	A16	7.5 f	1.5 f	1.68 e
	A18	0.0 f	0.0 f	0.00 f
Means		1.9	0.4	0.42
250	None	20.0 e	5.5 cd	0.73 f
	A1	35.0 d	8.5 b	2.37 de
	A16	70.0 a	12.3 a	5.43 a
	A18	35.0 d	5.7 cd	3.37 cd
Means		40.0	8.0	2.98
500	None	22.5 e	4.2 de	2.20 e
	A1	37.5 d	6.5 c	3.53 c
	A16	57.5 bc	5.7 cd	4.60 ab
	A18	35.0 d	5.3 cde	4.17 bc
Means		38.1	5.4	3.63
750	None	35.0 d	3.9 e	4.07 bc
	A1	50.0 c	4.7 de	3.17 cd
	A16	62.5 ab	5.7 cd	5.57 a
	A18	40.0 d	1.3 f	3.77 bc
Means		46.9	3.9	4.15
LSD		0.01: 9.39	0.01: 1.52	0.01: 1.08
Bacteria strains		***	***	***
IBA doses		***	***	***
Bacteria x IBA		***	***	***

highest rooting percentage (46.9%) and root length (4.15 cm) were determined for 750 mg l^{-1} IBA applications (Table 2).

Discussion

The results here showed no rooting in the control treatment in either cutting type. In addition, except for the A1 treatment of softwood cuttings and the A16 treatment of semi-hardwood cuttings, the treatments using only the bacterial strain were not capable of inducing the rooting of sour cherry cuttings. However, the use of bacterial strains in combination with IBA applications significantly increased the rooting of cuttings. This confirm the evidence that there are differences in the capacity of potential bacterial strains used for promoting the rooting of hardwood plant cuttings like sour cherry, hazelnut, jujube, elm and mulberry species (Nagarajan et al., 1989; Bassil et al., 1991; Hatta et al., 1996; Rinallo et al., 1999; Ercişli et al., 2000).

Even though there were some differences between the treatments at different IBA concentrations (250, 500 and 750 mg l^{-1}), all doses of IBA increased rooting percentage, root number and root length of sour cherry cuttings tested (Tables 1 and 2). These results are in good agreement with several previous studies. For example, Prizhmontas (1994) found a variation in the rooting ratio (52.2-58.9%) of sour cherry cuttings depending on the differences in cultivars and IBA doses used. Likewise, Burak and Öz (1987) obtained a variable rooting percentage (0.0-53.0) on cuttings of F 12/1 rootstocks by applying different IBA treatments.

The results of the present study provide additional evidence for IBA treatments stimulating the rooting of cuttings. However, the best concentration of IBA needs to be determined for each plant species.

Our results are also in general agreement with previously reported data (Bassil et al., 1991; Benavides, 1998; Ercişli et al., 2000), showing that IBA + bacteria combined treatments have greater capacity for enhancing rooting, root number and root length of sour cherry cuttings (Tables 1 and 2). The major question that should be discussed is the mechanism of IBA action and bacterial strains, which have additive effects on the rooting of plant cuttings. The clarification of these mechanisms could greatly contribute to better improving the rooting capacity of trees. It has been shown that auxins stimulate the initiation of lateral and adventitious roots because of their effect on cell division. Therefore, it is expected that the exogenous application of auxin-like plant growth regulators, such as IBA, will induce root formation in the cuttings used for plant propagation.

In other studies, the rooting enhancing capacity of bacterial strains is believed to be related to IAA production (Goto, 1990; Bassil et al., 1991). Since the bacterial strains used in our study were not examined for IAA production, the future direction of this research is to determine the role of bacterial strains in cutting rooting.

References

Bassil, N.V., W.M. Proebsting, L.W. Moore and D.A. Lightfoot. 1991. Propagation of hazelnut stem cuttings using *Agrobacterium rhizogenes*. HortScience 26(8): 1058-1060.

Benavides, M.P. and S. Radice. 1998. Root induction in *Simmondsia chiensis* (Link) Schneid. using *Agrobacterium rhizogenes*. Biocell 22(2): 109-114.

Burak, M. and F. Öz. 1987. Mazzard F 12/1(Kiraz-Vişne Anacı) anacının yeşil çelikle çoğaltılması. Bahçe 16(1-2): 39-43.

Couvillon, G.A. 1988. Rooting responses to different treatments. Acta Hort. 227: 187-197.

Çelik, H. and Y.S. Ağaoğlu. 1983. Effects of different treatments on the rooting of American grape rootstock cuttings. U.Ü. Ziraat Fak. Dergisi, 2(1): 49-54.

Doud, S.L. and R.F. Carlson. 1972. Propagation methods of fruit tree cultivars from hardwood cuttings. Hort. Abst. 43(7): 4178.

Ercişli, S. and M. Güleryüz. 1999. A study of the propagation of the hardwood cuttings of some rose hips. Tr. J. of Agriculture and Forestry. 23 (Supplement 2): 305-310.

Ercişli, S., A. Eşitken and F. Şahin. 2000 a. The effect of IBA and bacteria (*Agrobacterium rubi*) on the rooting of cutting of sour cherry cv. Kütahya. Bahçe 29(1-2): 75-80.

Ercişli, S., A. Eşitken and F. Şahin. 2000 b. IBA ve bakteri (*Agrobacterium rubi*) uygulamalarının kuşburnu çeliklerinin köklenmesi üzerine etkisi" II. Ulusal Fidancılık Simpozyumu, www.agr.ege.edu.tr/~fitekno, İzmir.

Falasca, G., M. Reverberi, P. Lauri, E. Caboni, A. De Stradis and M.M. Altamura. 2000. How *Agrobacterium rhizogenes* triggers de novo root formation in a recalcitrant woody plant: An integrated histological, ultrastructural and molecular analysis. New Phytologist 145 (1): 77-93.

Goto, M. 1990. Fundamentals of bacterial plant pathology. Academic Press, New York.

Hatta, M., C.A. Beyl, S. Garton and A.M. Diner. 1996. Induction of roots on jujube softwood cuttings using *Agrobacterium rhizogenes*. J. Hort. Sci. 71(6): 881-886.

Jacob, M., A. Plietzsch and K. Schulze. 1991. Results of model trials on the rooting of ornamental trees and shrubs. Gartenbau magazin 38(3): 44-46.

Jacob, M. and I. Hamdam. 1992. Use of beneficial bacteria for *Pelargonium zonale*. Gartenbau magazin 1(3): 105-107.

Kotan, R. 2002. Doğu Anadolu Bölgesinde yetiştirilen yumuşak çekirdekli meyve ağaçlarından izole edilen patojenik ve saprofitik bakteriyel organizmaların klasik ve moleküler metodlar ile tanısı ve biyolojik mücadele imkanlarının araştırılması. Doktora Tezi, Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, p 217.

Nagarajan, P., N.V. Ratha, D. Kandasamye, G. Oblisami and S. Jayaraj. 1989. Effect of combined inoculation of *Azospirillum brasilense* and *Glomus fasciculatum* on mulberry. Madras Agric. J. 76(11): 601-605.

Perry, R.L. 1987. Cherry rootstocks (in Rootstocks for Fruit Crops, Edited by Rom, R.C. and R.F. Carlson). A Wiley-Interscience Publication, New York, USA, pp. 217-264.

Prizhmontas, T. 1994. Action of auxins on rooting of sour cherry softwood cuttings. Hort. Abst. 64 (3): 1670.

Rinallo, C., L. Mittempergher, G. Frugis and D. Mariotti. 1999. Clonal propagation in the genus *Ulmus*: Improvement of rooting ability by *Agrobacterium rhizogenes* T-DNA genes. J. Hort. Sci. Biotech. 74 (4): 502-506.

Sim, B.L. and G.S. Lawes. 1981. Propagation of kiwifruit from stem cuttings. Gartenbauwissenschaft, 46(2): 65-68.

Webster, A.D. and H. Schmidt. 1996. Rootstocks for sweet and sour cherries. In: Cherries: Crop physiology, production and uses (Eds: A.D. Webster and N.E. Looney) CAB International, Cambridge, UK, pp. 127-166.

Yıldız, A. and S. Eti. 1995. Keçiboynuzu çeliklerinin köklenmesi üzerine farklı IBA dozlarının etkisi üzerinde bir araştırma. II. Ulusal Bahçe Bitkileri Kongresi, Cilt 1 (Meyve): 746-751.