Effect of IBA and Bacteria (Agrobacterium rubi ve Bacillus OSU 142) on the Rooting of M9 Apple Rootstock Cuttings

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Abstract: This study was carried out to investigate the effects of IBA and bacteria (*Agrobacterium rubi* ve *Bacillus* OSU 142) applications on the rooting of M9 rootstock cuttings. The cuttings treated with IBA alone at different concentrations (2000, 4000 and 6000 ppm) and in combinations of three *Agrobacterium rubi* (A1, A16, A18) and *Basillus* OSU 142 strains. The treated cuttings were placed mist propagation system including perlite medium. After three months the cuttings were uprooted and evaluated for rooting percentage (%), callusing (%) and viability rate (%).
There were determined inadequate results about rooting of cuttings. Rooting in cuttings was observed in 2000 ppm IBA (6.66 %), 4000 ppm IBA (13.33 %), and 2000 ppm IBA + *Basillus* OSU 142 combination (6.66 %), respectively. Callus were observed in all treated. Callus rate was 66.66 % in control while the rate was 84.61 % in A16 + A18 applications. Callus rate decreased depending on increasing of IBA doses. There were determined significantly differences in bacteria alone applications. According to application of bacteria combinations, the highests callus rate was A16 + A18 while the lowest was A1 + OSU 142. The best result of hormones + bacteria combinations were taken from 4000 ppm IBA + A18 application. Cutting living rate was similar to callus rating results. Many of the cuttings were live after application. Living rate was low at alone IBA application compared to that of the control.

Introduction

Apple is one of the most commonly cultivated produces around the world. Today, commercial apple (*Malus domestica* Borkh.) production is carried out through out temperate regions of both north and southern hemispheres. To date, world's apple production is 64 255 520 t (Anon. 2009). Such a widespread cultivation potential would be attributed to rich germplasm diversity of *malus* species in both cultivar and rootstock genotypes. Besides, recent improvements in utilitation of dwarf rootstocks in intensive cultivation contributed to enhance apple production.

In order to match present reguirements of modern apple orchards, rootstocks with high potential in clonal propagation apptitude are recommended to use. Dwarf and semidwarf rootstocks, particularly, provide easyness in cultivation practices, reduce production cost and increase the proportional income.

M9 is the most frequently used apple rootstock in the world to obtain dwarf plants. Malling 9 has for a long time been used as apple rootstock in Europe. Trees grafted on M9 do not exceed 2.7 m and are the 20-40% size of those grafted on seedling. Cultural processes are made without using a ladder. Thanks to excellent light exposure it provides, fruits are often well-colored and of high quality. However, it needs a backing for life because its root system is weak. Under available conditions, its roots grow up well on beds, but it is quite hard to provide root formation via woody cuttings. Production of M9 apple rootstock is usually conducted with the "Stool Bed Layering" layering method (Howard 1985; Ferree & Carlson 1987; Hartmann et al. 1990).

M9 rootstock can be produced with layering method, but can hardly be increased with cutting (Ferree & Carlson 1987). Increasing with cutting is an increasing method of great importance for all fruits as it is economical and fast. Despite its all positive features, the biggest problem that restricts its use is that cuttings can not take root because regenerating abilities of some species are very deficient (Rugini & Fedelli 1990; Webster 1997).

The cuttings belonging to the fruit species found in temperate climate group do not easily rooting. Thereby, cuttings are subject to various applications (plant growth regulators, different chemicals, bacteria

applications, carbohydrates etc.), one of which is the use of plant growth regulator, in order to remove problems about rooting in the kinds of these species (Dubeiovsky et al. 1993; Wiesman & Lavee, 1995; Grange et al. 1998; Eşitken et al. 2003). The most common application to increase the rooting ratio in cuttings is the use of auxins and especially of IBA (Hartmann et al. 1990; Howard 1985).

In a study on determining the effects of IBA application with doses of 3000 and 8000 ppm on rooting properties of M9 apple root-stocks, it was reported that the maximum rooting (29.63%) was obtained from apple cuttings treated with 8000 ppm IBA (Ülger & Baktır 1995).

In recent years, bacteria applications to cutting rootings have gained great importance. A. tumafaciens, E. milletiae, P. syringae pv. savastanoi, and P. syringae pv. myricae bacteria encourage cell divisions or gal formations in plants. These bacteria except A. tumafaciens are produced on intercell surfaces as indole-3-acetamide and indol-3-pyruvate from oxine group by IAA (Goto 1990). These bacteria produce hormones in colonized areas on plant tissues or encourage hormone production in plants. Therefore, these types of organisms cause cell divisions growth as depending on hormone production. Recently, especially some strains of A. rhizogenus (Bassil et al. 1991; Hatta et al. 1996) and A. rubi strains have been successfully been used for cutting rooting (Eşitken et al. 2003).

In a study, 2000, 4000 and 6000 ppm IBA and three different types (A1, A16, A18) of Agrobacterium rubi bacteria were applied for determining cutting rooting of Kütahya sour cherry. While no rooting was determined in the control group, the highest rooting rate (70%) was obtained with application of 2000 ppm IBA+A16 (Ercisli et al. 2000). In a similar study, Ercisli et al. (2000a) applied 2000, 4000 and 6000 ppm IBA and three different types (A1, A16, A18) of Agrobacterium rubi bacteria to rose hip and the highest rooting rate (95%) was obtained with 2000 ppm IBA + A18 application. Eşitken et al. (2003) applied IBA (250, 500, 750 ppm), Agrobacterium rubi and Bacillus OSU-142 to wild cherry, and reported that the best rooting rate (80%) was obtained with 250 ppm IBA + OSU 142.

The objective of this study was to determine the individual and combination effects of IBA, Agrobacterium rubi and Bacillus OSU 142 applications on rooting rate of M9 apple rootstock cuttings.

Material and Methods

Material

This study was carried out at Atatürk University Faculty of Agriculture Department of Horticulture to investigate the effects of different applications on the rooting of hardwood M9 cutting. By this aim, twenty six different applications were performed using various doses of IBA alone and/or distinct strains of Agrobacterium rubi and Bacillus OSU 142 bacteria. Applications are listed in Table 1. Bacteria strains were obtained from Atatürk University Faculty of Agriculture Department of Plant Protection.

1. Kontrol	8. OSU 142	15. 2000 ppm IBA + A1	22. 4000 ppm IBA + OSU 142
2. 1000 ppm IBA	9. A1 + A16	16. 2000 ppm IBA + A16	23. 6000 ppm IBA + A1
3. 2000 ppm IBA	10. A1 + A18	17. 2000 ppm IBA + A18	24. 6000 ppm IBA + A16
4. 6000 ppm IBA	11. A16 + A18	18. 2000 ppm IBA + OSU 142	25. 6000 ppm IBA + A18
5. A1	12. A1 + OSU 142	19. 4000 ppm IBA + A1	26. 6000 ppm IBA + OSU 142
6. A16	13. A16 + OSU 142	20. 4000 ppm IBA + A16	
7. A18	14. A18 + OSU 142	21. 4000 ppm IBA + A18	

Methods

Cuttings were collected from M9 clone root stocks and one-year shoots at dormancy stage. The bottom of cuttings was submitted into IBA solutions for 3-5 seconds, following evaporation then submitted into Agrobacterium rubi strains and Bacillus OSU 142 bacteria suspension with a concentration of $1x10^8$ bacteria per milliliter. The bottom of control cuttings was submitted into distilled water only (Eşitken et al. 2003). In order to smear bacteria on cutting bottoms, they were mixed for 30 minutes at 75 rounds per minute. Then cuttings were planted in fogging area in greenhouse (Figure 2.1). The growing media is automatically controlled for heating (26 °C) and relative humidity (90-95%). Perlite was used as growing media.

Rooting rates (%), callus formation rates (%) and survival rates (%) of cuttings were determined at the end of 3 month growing period.

The experimental design was completely randomized design with 3 replications (10 cuttings at each replication). Duncan's multiple comparison tests was used for mean comparisons after arcsine transformation of raw data (Düzgüneş et al. 1987).

Results

The findings on the effects of IBA and bacteria applications on rooting, callus formation and survival cuttings percentages are presented in Table 1. Root formation did not ocur in cuttings of most treatments including control. However, 2000 ppm IBA, 4000 ppm IBA and 2000 ppm IBA + OSU 142 treatments resulted in root formations, 6.66, 13.33 and 6.66 %, respectively. Differences among the treatments were statistically significant.

Applications	**Rooting (%)**	**Callus Formation (%)**	**Survival Ratio (%)**
Control	0 b *	66.66 b	60.00 c
2000 ppm IBA	6.66 ab	46.66 bcd	13.33 ef
4000 ppm IBA	13.33 a	20.00 ef	13.33 ef
6000 ppm IBA	0 b	13.33 f	13.33 ef
A1	0 b	21.42 ef	21.42 de
A16	0 b	26.66 def	40.00 cd
A18	0 b	53.33 bc	60.00 c
OSU 142	0 b	53.33 bc	66.66 bc
A1 + A16	0 b	53.33 bc	66.66 bc
A1 +A18	0 b	60.00 bc	86.66 ab
A16 +A18	0 b	84.61 a	92.30 a
A1 + OSU142	0 b	40.00 cde	66.66 bc
A16 + OSU142	0 b	46.66 bcd	53.33 cd
A18 + OSU142	0 b	46.66 bcd	53.33 cd
2000 ppm IBA + A1	0 b	20.00 ef	13.33 ef
2000 ppm A1 + A16	0 b	13.33 f	13.33 ef
2000 ppm IBA + A18	0 b	13.33 f	21.42 de
2000 ppm IBA + OSU142	6.66 ab	21.42 ef	40.00 cd
4000 ppm IBA + A1	0 b	26.66 def	40.00 cd
4000 ppm A1 + A16	0 b	46.66 bcd	73.33 bc
4000 ppm IBA + A18	0 b	60.00 bc	93.33 a
4000 ppm IBA + OSU142	0 b	40.00 cde	73.33 bc
6000 ppm IBA + A1	0 b	0 g	0 f
6000 ppm A1 + A16	0 b	0 g	0 f
6000 ppm IBA + A18	0 b	0 g	0 f
6000 ppm IBA + OSU142	0 b	0 g	0 f
LSD .01	12.85		18.29
LSD .05		4.78	

- Statistical analysis have been carried out using arc sin values.

Table 3.1. The effects of IBA and bacteria applications on the rooting, callus formation and survival ratio on M9 apple rootstock cuttings.

Callus formation occured in cuttings of most applications, except for the combinations of 6000 ppm IBA with bacteria. There were statistically significant differences between the applications for callusing. The highest callusing rate was obtained from A16 + A18 (84.61%), followed by control (66.66%).

The findings relevant to survival percentages of cuttings are generally similar to those of callus formation rates. In most cases the cuttings maintained their survives up to the end of study. However, there was no surviving cutting in combination of 6000 ppm IBA with bacteria as was seen in callusing rates. Differences in survival percentages were statistically significant. The highest percentage on this criterion was observed in 4000 ppm IBA + A18 treatment (93.33 %), while control group demonstrated considerably higher rate (60.00 %).

Discussion

Considering the overall rooting percentages, the results appear dissatisfactory. This results conform the previous statement of Parmar & Aier (1989) who clarified the general difficulties in rooting of temperate fruits as a results of their hard tissues. Besides this, rooting ağabeylity of apple cuttings as well as other temperate fruits, are solely affected by external treatments along with genotypic apptitude and environmental conditions. Auxin treatments, out of external factors, have a special interest (Goto 1990). Literature investications reveal that

auxin application do not have significant influence on rooting of M9 cuttings (Ülger & Baktır 1995).

Callus development was observed in most treatments. Cutting ratio with callus was found as 66.66% in control and increased up to the 84.61% after A16 + A18 applications. When the cuttings are placed to the suitable media for rooting, callus layer occurs at the lower part of the cuttings. Protective layer resulted from callus tissue delays the rot formation at the lower part of the cuttings. In some cases, callus layer helps water uptakes of the cuttings (Hartmann et al. 1990). On the other hand, there has been different information related to the effect of callus layer on root formation. Hartmann et al. (1990) reported that callus formation and rooting formation are independent from each other. Kantarcı & Ayfer (1989) reported similar result in one study conducted in hazelnut plant. Similarly, Tayfon (1995) obtained low rooting in hard wood cuttings of kiwi, but callus formation was high in same conditions.

In general, the results obtained from being alive ratio of cutting look like almost the callus formation ratios. Most cuttings were observed alive after treatments. For example, after pull off the cuttings, alive cutting ratios for control and 4000 ppm IBA+A18 application were 60% and 93.33%, respectively. By evaluation of the results of alive cutting ratios, it can be observed that alive cutting ratio and callus formation ratio were close to each other. The result was also supported by Hartmann et al. (1990) that the protective layer resulted from callus tissue delays the rot formation at the lower part of the cutting and in some cases, callus layer helps water uptake of cutting.

In result, the study performed to research the effects of IBA and bacterium applications on rooting ratio of cuttings in M9 apple rootstock showed that sufficient rooting ratio was not obtained. The present study was mainly focused on recent the effect of bacterium on cutting rooting that is very popular in recently. The increase or decrease in rooting may be determined properly with use of the different bacterium races or cuttings taken in different period by future studies.

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