

Full Length Research Paper

# Effects of some humidity and IBA hormone dose applications on rooting of M9 apple clonal rootstock Softwood top cuttings

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Accepted 2 November, 2009

In this study, softwood cuttings were taken from M9 dwarf apple rootstocks in early June. Different indole-3-butyric acid (IBA) concentrations [0 (control), 500, 1500, 2500 and 3500 ppm], different air humidity levels (85 - 90%, 95-100%) and rooting media of perlite effects on rooting capability and root formation of M9 apple rootstock cuttings were examined. All the cuttings remained alive. The highest ratio of cutting callus formation was found in control group (58%) in 95 - 100% humidity level and 2500 ppm IBA hormone dose application in 85 - 90% humidity level. The highest rooting ratio was obtained from control group (46%) in 95 - 100% humidity level; the lowest one was 3500 ppm IBA dose application (17%) in 85 - 90% relative humidity level. The highest rooting surface length was found in 1500 ppm hormone dose (0.53 cm) in 95 - 100% and 2500 ppm IBA dose application (0.42 cm) in 85 - 90% humidity level. With respect to root numbers, the highest value was from 1500 ppm IBA application (1.29 number/cutting) in 95 - 100% relative humidity, and 500 and 2500 ppm IBA doses (1.04 number/cutting) in 85 - 90% relative humidity level. The longest root was obtained from control group (2.03 cm) in 95 - 100% humidity level and 500 ppm IBA hormone dose (1.80 cm) in 85 - 90% humidity level. The shortest root was obtained from 2500 ppm IBA hormone dose application (0.09 cm) in 85-90% humidity level. The highest root branching value was obtained from 1500 ppm hormone dose application (0.88 number/cutting) in 95 - 100% humidity level.

**Key words:** M9, softwood top cutting, misting system, humidity, hormone, rooting.

## INTRODUCTION

Anatolia is the origin of apple, like many other fruits. Apple is the pomaceous fruit of the apple tree; species of *Malus domestica*, belonging to the rose family, *Rosaceae*. It is one of the most widely cultivated tree fruits. Apple is the most significant species in the temperate climate fruits and has been grown since ancient times. In one of the fundamental studies on the origin of cultivated plants, "L'origin des plantes cultivees", De Candolle (1883) revealed that apple has been cultivated for over 4000 years (Özbek, 1978).

The tree originates from Central Asia, where its wild ancestor is still found today. There are more than 7,500 known cultivars of apples resulting in range of desired

characteristics (Anonymous, 2009). Apples can be canned, juiced and optionally fermented to produce apple juice, cider, ciderkin, vinegar and pectin. Distilled apple cider produces the spirits applejack and Calvados. Apple wine can also be made. Apples are an important ingredient in many desserts, such as apple pie, apple crumble, apple crisp and apple cake. They are often eaten baked or stewed and they can also be dried and eaten or re-constituted (soaked in water, alcohol or some other liquid) for later use (Anonymous, 2009). The chemical structure of the fruit is affected by rootstock, variety, climate, soil, growing conditions and tree growth itself (Holland et al., 1992). Rootstock utilization has gained value for solving the limiting factors of production (soil, climate and pest etc) and conditioning of market demands (fresh or processed) on productivity, short juvenility period and high fruit quality (Demirkeser et al., 2009).

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Rootstocks are used to propagate the fruiting scion onto a rooting system in order to gain uniformity and precocity in fruiting portion, compared to seedlings. They are also done in order to control tree size (vigor), to enable the tree to adapt to adverse soil conditions (pH, drought, texture, drainage), to tolerate soil pests (nematodes, insects, diseases) and to increase hardiness of the tree to low temperatures. (Dolgov and Hanke, 2006). The most used vegetative methods in the production of rootstocks in recent years are cutting, layering and tissue culture (Ağaoğlu et al., 2001). Both manufacturers and researchers have been busy on cutting propagation of fruit trees for many years. From the beginning onwards, except several fruit species that are easy to root, cutting could not be propagated successfully. However, success of cutting propagation researchs increased with the discovery of the plant hormones in 1930s (Kankaya and Özyiğit, 1998). Today, M9 clonal apple root-stock is propagated with different layering methods, but these methods have some disadvantages, as they are slow, labor-intensive and cumbersome (Ağaoğlu et al., 2001).

Apple can be grown in almost every part of the South and West coast of Turkey and Eastern Anatolia, except the high lands. Apple cultivation is widespread in plants grafted on by seedling rootstock (Şen et al., 2000). However, in recent years, especially in modern fruit cultivation, the use of clonal apple rootstocks is widespread (Dolgun et al., 2009). With the use of clonal root-stocks, more trees can be planted in the unit area and more fruit can be harvested. Roots are more superficial, so cultural processes, especially irrigation and fertilization need to be done more carefully (Öz et al., 1993; Hartmann et al., 1990; Barrit et al., 1995). Therefore, new apple types should be grafted on the dwarf M27, M9, M26 and M7 apple rootstocks. Moreover, new apple gardens with these plants should be established (Barrit, 1992; Özkan, 2004).

Turkey produced about 6 million pieces of apple saplings in 2007 (Anonymous, 2008). This production accounts for approximately 1.73 million M9, 1.08 million MM 106, 1.04 million MM 111 and 1.03 million for seedling rootstock. In the country, with new varieties, the gardens are established based on modern system and apple cultivation in the coming years is thought to be a profitable investment (Küden and Kaşka, 1995). By means of conducted experiments with the cloning rootstocks, the highest yield was obtained from the M9 and MM 106 root-stocks and are recommended for the country (Burak et al., 1997; Pamir and Öz, 1997).

In 1950s, the use of the mist propagation system for plant production was realized and this plant propagation method was considered to be revolutionary (Hudson, 1997). The system, which maintains the atmosphere with low evaporative demand, reduces water loss through transpiration (Hartmann et al., 1997; Osterc and Spethmann, 1998). "Mist Propagation System" is effec-

tive in increasing the amount of rooted cuttings and is an economic system that is widely used for cutting propagation in the nursery.

The aim of the present study is to determine the effects of "Mist Propagation System" high air relative humidity levels, plant growth regulator (IBA) dose applications and perlite media on rooting of M9 clonal rootstock cuttings.

## MATERIALS AND METHODS

### Materials

In this research, the softwood top cuttings of M9 apple rootstock taken from Nursery of Selcuk University Sarayönü Vocational High School were used. Different humidity levels (85 - 90% and 95 - 100%), perlite media (0.0 - 5.0 mm) and indole-3-Butirik Asit (IBA) in different doses [0 (control), 500, 1500, 2500 and 3000 ppm] were utilized.

### Methods

The research was carried out in the "Mist Propagation Unit" of Research and Practice plastic greenhouse at Selcuk University Sarayönü Vocational High School. Softwood top cuttings were taken to be 20 - 25 cm length, 3 - 4 mm diameter; and the pinched leaves are below the point where one or two leaves are attached to the stem and then planted into the rooting media (Kalyoncu, 1996).

In the research, 0 ppm (control), 1000, 2000, 3000, 4000 ppm indole-3-Butirik Asit (IBA) doses were applied. In practice, in the form of sheaves of cutting, 1 - 2 cm of the bottom parts were dipped in IBA solution for a period of five seconds and we waited for a short period of time for the alcohol to release. Then cuttings were planted in a rooting media (buried about 2/3 their height) by 10 x 10 row and plant spacing (Kalyoncu, 1996). The relative humidity levels, rooting area temperature and ambient temperature in the mist propagation unit were 85 - 90%; 95 - 100%; 18 - 20°C; 29 - 31°C, respectively. This experiment was carried out in a randomized block factorial designs, with 3 replicates. Each replicate consisted of 8 cuttings.

Later, M9 apple rootstock cuttings, for a period of 47 days, were subjected to root mist propagation system; they were investigated in the following areas: cutting vitality (number), cutting length (cm), callus status (%), rooting ratio (%), cutting diameter (cm), rooting surface area length (cm), root number (number/cutting), the longest root length (cm), the shortest root length (cm), root branching (number/cutting), root diameter (cm). Being reviewed in terms of these properties, measurement and counting were done according to Kalyoncu (1996) method. "MINITAB" computer package program was used in the statistical analysis. The differences between averages were controlled by Duncan test (Düzgüneş et al., 1987).

## RESULTS AND DISCUSSION

Some studies related to M9 clonal apple rootstock have been carried out these days, but the ones with softwood cutting works on rooting are rare. In the study, the highest rooting percentage was found to be 46% in the softwood top cuttings of M9. Some properties of the cuttings: cutting vitality (number), cutting length (cm), callus status (%), rooting ratio (%), cutting diameter (cm), rooting surface area length (cm), root number (number/cutting),

**Table 1.** Effects of moisture level and hormone dose applications in the softwood top cuttings of M9 clonal apple rootstock cuttings.

Cutting Properties	Humidity Ratio (%)	IBA Hormone Doses (ppm)					Average Humidity
		0 (control)	500	1500	2500	3500	
Cutting Length (cm)	95-100	21.23(1.173)	20.21(0.560)	21.04(2.189)	20.65(0.546)	20.91(1.367)	20.81(1.170)
	85-90	21.15(0.977)	21.50(0.817)	20.29(1.512)	21.21(0.469)	21.81(0.325)	21.20(0.940)
<b>Average Hormone</b>		21.19(0.967)	20.85(0.945)	20.66(1.733)	20.93(0.550)	21.36(1.018)	21.00(1.061)
Callus Formation (%)	95-100	58a* (19.00)	25bc(12.500)	17c (14.400)	13c (0.000)	46ab(10.100)	32(21.200)
	85-90	49a (7.200)	46a (31.400)	43a (14.200)	58a (7.200)	33a (19.000)	45(17.400)
<b>Average Hormone</b>		52 (14.600)	35 (24.200)	30 (19.200)	35 (25.500)	40 (15.200)	38(20.300)
Rooting ratio (%)	95-100	46 (31.400)	25 (0.000)	25 (21.600)	25 (25.000)	37 (17.600)	32(20.400)
	85-90	38 (21.600)	29 (19.000)	33 (21.800)	29 (28.800)	17 (19.000)	29(20.200)
<b>Average Hormone</b>		42 (24.500)	27 (12.200)	29 (19.900)	27 (24.200)	27 (19.800)	30 (20.000)
Rooting surface area (cm)	95-100	0.33 (0.241)	0.15 (0.033)	0.53 (0.808)	0.28 (0.245)	0.31 (0.082)	0.32 (0.356)
	85-90	0.25 (0.187)	0.19 (0.160)	0.19 (0.092)	0.42 (0.308)	0.08 (0.095)	0.23(0.193)
<b>Average Hormone</b>		0.29 (0.197)	0.17 (0.106)	0.36 (0.546)	0.35 (0.260)	0.19 (0.145)	0.27 (0.286)
Root number (unit/cutting)	95-100	1.33 (1.092)	0.92 (0.072)	1.29 (1.808)	0.79 (0.753)	1.62 (0.359)	1.19 (0.913)
	85-90	0.75 (0.125)	1.04 (1.371)	0.62 (0.165)	1.04 (1.063)	0.54 (0.732)	0.80 (0.748)
<b>Average Hormone</b>		1.04 (0.765)	0.98 (0.871)	0.96 (1.206)	0.92 (0.835)	1.08 (0.783)	0.99 (0.844)
The longest root (cm)	95-100	2.03 (1.515)	1.01 (0.229)	1.98 (1.566)	1.22 (1.225)	1.28 (0.676)	1.50 (1.074)
	85-90	1.09 (0.461)	1.80 (0.746)	0.72 (0.043)	0.71 (1.107)	0.52 (0.789)	0.84 (0.664)
<b>Average Hormone</b>		1.56 (1.125)	1.09 (0.502)	1.35 (1.207)	0.96 (1.081)	0.80 (0.778)	1.17 (0.939)
The shortest root (cm)	95-100	0.42 (0.130)	0.48 (0.344)	0.79 (0.665)	0.20 (0.189)	0.75 (1.117)	0.53 (0.563)
	85-90	0.88 (0.593)	0.36 (0.219)	0.47 (0.316)	0.09 (0.086)	0.11 (0.101)	0.38 (0.407)
<b>Average Hormone</b>		0.65 (0.459)	0.42 (0.266)	0.63 (0.498)	0.13 (0.153)	0.43 (0.792)	0.45 (0.489)
Root Branching (unit/cutting)	95-100	0.50 (0.330)	0.29 (0.190)	0.88 (1.192)	0.21 (0.260)	0.51 (0.443)	0.48 (0.564)
	85-90	0.63 (0.642)	0.63 (0.866)	0.19 (0.082)	0.13 (0.216)	0.13 (0.216)	0.34 (0.490)
<b>Average Hormone</b>		0.57 (0.462)	0.46 (0.589)	0.53 (0.843)	0.17 (0.218)	0.32 (0.375)	0.41 (0.524)
Root diameter (mm)	95-100	0.60 (0.494)	0.37 (0.088)	0.33 (0.295)	0.39 (0.338)	0.61 (0.414)	0.46 (0.323)
	85-90	0.54 (0.262)	0.37 (0.367)	0.47 (0.291)	0.40 (0.426)	0.23 (0.264)	0.40 (0.298)
<b>Average Hormone</b>		0.57 (0.355)	0.37 (0.238)	0.40 (0.273)	0.40 (0.344)	0.42 (0.374)	0.43 (0.307)
Cutting diameter (cm)	95-100	3.63 (0.143)	3.43 (0.099)	3.37 (0.407)	3.50 (0.186)	3.64 (0.319)	3.52 (0.243)
	85-90	3.84 (0.331)	3.63 (0.070)	3.68 (0.246)	3.27 (0.167)	3.62 (0.202)	3.61 (0.267)
<b>Average Hormone</b>		3.73 (0.256)	3.53 (0.131)	3.53 (0.344)	3.39 (0.202)	3.63 (0.239)	3.56 (0.255)

\*Data are the means of three replications  $\pm$  standard error. Values within column followed by the same letter are not significantly different at  $P = 0.05$  (Duncan's Multiple Range test).

the longest root length (cm), the shortest root length (cm), root branching (number/cutting), root diameter (cm) were determined according to Kalyoncu method (1996), and the results of Duncan test were given in Table 1.

In the experiment all of the cuttings (100%) remained alive. Babaoğlu (2007) has investigated the effects of different humidity levels and indole Butirik Acid (IBA) doses in some apple rootstock softwood cuttings. As a result, M9 softwood cuttings showed the highest cutting vitality at 8000 and 10000 ppm IBA dose applications (100%) and the lowest in the control group (90.47%) in the 95 - 100% relative humidity level. Küçükbaşmacı (2002) indicated that the highest cutting vitality was in control group (31.11%) in M9 apple rootstock cutting. She also indicated that the auxin applications did not affect the cutting vitality ratio too. Baykal (2001) determined that

IBA applications had lower vitality than control group.

The results of the study by Babaoğlu (2007) are similar to ours, whereas those of the other researchers have remained contradictory, since their levels were very low. In terms of callus formation, the interaction of moisture x IBA doses in M9 softwood cuttings was found statistically significant ( $P < 0.05$ ). The highest callus formation was obtained in the control group (58%) in 95 - 100% humidity level. The lowest callus formation was obtained from 2500 ppm IBA dose application (13%) in 95 - 100% humidity level. As for the results by Babaoğlu (2007), the highest callus formation was obtained from 8000 ppm IBA dose application (70.83%) in 95 - 100% humidity level, while the lowest value was obtained from 2000 ppm IBA dose application (4.74%) in the 85 - 90% humidity level. Eccher and Annoni (1985) revealed that out of the

different varieties of apple wood cuttings in their work, 50% of cuttings formed callus formation. Results in our study were in parallel with their results.

Examining the roots of cutting between the types and applications, including the control group, statistical differences could not be found. In 95 - 100% humidity level, the maximum rooting ratio in control group (46%) and the lowest rooting ratio in the 500, 2500 and 3500 ppm IBA dose applications (25%) were obtained. 85 - 90% moisture level in the environment, the highest value (38%) in the control group, the lowest value in the 3500 ppm IBA dose application (17%) were obtained. In Babaoğlu's (2007) study, the highest rooting ratio (4000 ppm) IBA dose application (77.78%), the lowest in the control group (39.68%) were obtained in the 95 - 100% humidity level. The highest rooting ratio was found to be: 13% IBA dose application, being 2500 ppm according to Kankaya and Özyiğit (1998); 29.63% IBA dose application, being 8000 ppm according to Ülger and Bakır (1995); 30% IBA application, being 20 mg/l according to Lilov et al. (1988); 13.3% IBA application at 24-28°C according to Eccher and Annoni (1985); 13% IBA application, being 4000 ppm according to Baykal (2001); 22.22% IBA application, being 1000 ppm according to Küçükbaşmacı (2002). The ratios obtained in our research were lower than those of Babaoğlu (2007), but higher than the ones obtained by other researchers.

M9 softwood cutting is examined in terms of the length of root surface and moistures and IBA doses were not found statistically significant. Root length surface was examined in terms of the moisture and thus the highest value was obtained from 1500 ppm IBA dose application (0.53 cm) in the 95 -100% humidity level, while the lowest one (0.15 cm) was obtained by applying a dose of 3500 ppm (0.08 cm) in the 85 - 90% humidity level. Babaoğlu (2007) revealed that, the highest root surface length was obtained from 2000 ppm IBA dose application (2.17 cm) in the 95 -100% humidity level, while the lowest value was seen in 10000 ppm IBA dose application (0.28 cm). Values in terms of root surface length in this experiment were lower than those of Babaoğlu (2007). Effects of two different moisture levels (85-90% and 95-100%), different IBA dose applications, and perlite medium on rooting surface length of gilaburu softwood cuttings taken in early June were examined by Kalyoncu and Özer (2000). The root surface length values in both of the moisture levels were increased as IBA doses increased. Reported values in 85-90% humidity level was higher than 95-100% moisture level. The values obtained from different types by Babaoğlu were higher than those of ours (Kalyoncu and Ecevit, 1995; Kalyoncu, 1996; Özer and Kalyoncu, 2007; Kalyoncu et al., 2007).

When applications were examined in terms of the number of roots, it was observed they were not statistically significant. The maximum number of root was obtained from 3500 ppm dose application (1.62 units/cutting) in the 95-100% humidity level, while the lowest

one was from 3500 ppm dose application (0.54 units/cutting) in the 85-90% humidity level. Babaoğlu (2007) cited that the maximum root number was from 4000 ppm IBA dose application (16.05 units/cutting) in 95-100% humidity level, while the lowest was from 10000 and 8000 ppm dose applications (1.92 units/cutting).

Küçükbaşmacı (2002) found that M9 (2.57 units) were lower than MM106 (2.63 units) in terms of root numbers. The values of Babaoğlu (2007) were higher than that of ours.

In our study when averages were examined in terms of the longest root values, statistical differences were not found among applications. The longest root length value (2.03 cm) was from the control group in the 95 - 100% humidity level, while the lowest value (1.00 cm) was obtained by applying a dose of 3500 ppm (0.52 cm) in the 85 - 90% humidity level. For Babaoğlu (2007), the longest root status in the M9 softwood cuttings was observed in 8000 ppm IBA dose application (3.80 cm), while the lowest one was in the control group (0.46 cm) in the 95 - 100% humidity level.

In terms of the shortest root length, the highest value was in 10000 ppm dose application (0.75) at 95 - 100% humidity level, while the lowest value was in the control group (0.22 cm) in the 85 - 90% moisture level. Küçükbaşmacı (2002) determined that the lowest root length (1.74 mm) was obtained from M9 cutting. The results in this study in terms of root length are in parallel with the results of Babaoğlu (2007).

When the averages are examined in terms of the root branches in the cuttings, they were not found to be significant. As regards the root branching, the highest value was in 1500 ppm dose application (0.88 unit / cutting), while the lowest one was in 2500 ppm dose application (0.13 unit / cutting) at 95 - 100% moisture level. Babaoğlu (2007) found that the highest value in the root branching was determined from 4000 ppm dose treatment (1.28 units/cutting) in the 95 - 100% humidity level, while the lowest one was from the control group (0.00 units/cutting) in the 85 - 90% humidity level. Babaoğlu 's (2007) research results are similar to our experiment results. The results obtained by other researchers support our findings, but branching is higher in their studies.

Kalyoncu and Özer (2000) cited that, root branching of softwood gilaburu cuttings was obtained in the control group in the highest moisture level and that in terms of the root branching, control groups demonstrated the higher branching than IBA dose applications. They reported that when cuttings stay less in the rooting media, they become easy to root and root branches occur less. Other researchers also support this conclusion (Kalyoncu and Ecevit, 1995; Kalyoncu, 1996; Özer and Kalyoncu, 2007; Kalyoncu et al., 2007).

When cuttings are examined in terms of the average root diameter, the difference among applications was insignificant statistically. At 95 - 100% humidity level, the

highest root diameter (0.61 mm) was obtained from 3500 ppm dose level, while the lowest one was from the 3500 ppm dose (0.23 mm) at moisture level of 85 - 90%. Babaoğlu (2007) determined that, the highest root diameter was in 8000 ppm dose application (0.88 mm), while the lowest one was in the control group (0.45 mm) in 95-100% humidity level. Babaoğlu's (2007) research results are similar to our results.

As a result of different applications in M9 softwood cuttings, and in contrast to none, highly successful results have been obtained through this propagation method. It is proposed because it furnishes us with several advantages such as a wider unit area, the fight against unemployment, evaluation of small-scale land, ease, applicability, positive results, improving socio-economic structure of the farmers, contribution to the country's economy and agriculture.

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