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Full Length Research Paper

Effects of indol–3-butyric acid (IBA), plant growth promoting rhizobacteria (PGPR) and carbohydrates on rooting of hardwood cutting of MM106 Apple rootstock

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This investigation was conducted to evaluate the effects of a range of indole-3-butyric acid (IBA) concentrations (1000, 2000 and 4000 ppm), two strains of *Agrobacterium rubi* (A-18) and *Bacillus subtilis* (OSU-142) and four carbohydrates (Glucose, Sucrose, Sorbitol and Mannitol) alone, in combination with two and three treatments on the rooting capacity of the hardwood cuttings of MM106 apple rootstock in greenhouse conditions. No rooting was obtained from control treatments but only low callus formation (10%). Single treatments did not induce rooting, except IBA-1000 ppm, OSU-142 and A-18, but induced better callus formation (20%) compared to control. Double and the three combinations were more successful in terms of rooting and callus formation. A-18+sorbitol, OSU-142+sorbitol+IBA-2000, A-18+sorbitol+IBA-2000 and A-18+sorbitol+IBA-4000 treatments were obtained from the highest rooting formation (30%) and OSU-142+sorbitol treatment had the highest callus rate (70%), that may be a precursor of adventitious root formation. IBA-1000 ppm treatment had the highest adventitious root number (16.5). A-18 treatment had the highest average adventitious root thickness (1.61 mm). The results indicate that double and triple combination of IBA, bacteria and carbohydrates are more effective in increasing rooting capacity and more quality rooting when compared to control, or carbohydrate, IBA and, bacteria alone.

Key words: Apple rootstock, hardwood cutting, IBA, bacteria, carbohydrate.

INTRODUCTİON

Rootstock production by vegetative propagation ensures uniform trees with similar cropping charact-eristics, and for apple in particular provides a range of size-controlling clonal rootstocks. Trees on MM106 are well anchored, do not sucker, are semidwarfing (60 - 75% the size of trees on apple seedlings), and very pro-ductive (Wiley, 1987).

Apple is an economically important fruit tree around the world (Karakurt, 2006; Aslantas and Karakurt, 2007); it is vital that the propagation problems are solved (Uosukainen, 1992). Various vegetative methods have been used to propagate fruit saplings. One of the these methods is rooting of the cutting. This method has been commonly used for propagation of some fruit species and clonal rootstocks. But, there are some endogenous and ex-ogenous factors affecting rooting of cuttings. Endoge-nous factors such as growth substances, anatomical structure of cutting and carbohydrate level (Hartmann et al., 2002) and exogenous factors such as humidity, air and light condition in rooting environ-ment, taking date of cutting are always required to obtain satisfactory propa-gation (Ercisli et al., 2003).

The use of hardwood cuttings is one of the least ex-pensive and easiest methods of vegetative propagation. Cutting propagation is the most important means of clo-nal regeneration of many horticultural crops such as fruit, nuts, ornamentals, and vegetables (Hartmann et al., 2002).

Some treatments have been used to enhance success rate on the cutting propagation. Exogenous plant growth regulators are one of the most commonly used methods (Polat and Kamiloğlu, 2007). İn recent time, PGR treat-ments promoting root formation of cutting (Ercisli et al., 2003), and carbohydrate and vitamin treatments and con-centration heve been used in micropropagation methods (Uosukainen, 1992).

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The using goal of these substances is to accelerate root formation of cutting in especially hard-to-root spe-cies, and increase root number and quality per cutting (Zengibal et al., 2006).

The most successful results have been obtained from IBA treatments including auxin hormone group. IBA has been found to be critical for both softwood and hardwood cuttings (Delargy and Wright, 1979; Christov and Koleva, 1995; Ofori et al., 1999; Polat et al., 2000; Güneş and Şen, 2001; Yıldız, 2001; Ercisli et al., 2003; Koyuncu and Senel, 2003; Nawrocka-Grzeskowiak, 2003; Sebastiani and Tognetti, 2004; Erdoğan and Aygün, 2006; Tworkoski and Takeda, 2007).

Recent studies have confirmed the findings that some bacteria in the genera of Agrobacterium, Bacillus, Streptomeyces, Pseudomonas and Alcaligenes may induce root formation in stem cuttings. These bacteria have been reported to produce indol-3-acetic acid and several studies have also shown that rooting of cuttings inoculated with bacteria can be accelerated by exogenous IBA applications (Falasca et al., 2000; Ercisli et al., 2003; Esitken et al., 2003).

The relationship between carbohydrates and adventitious root formation remains controversial. The carbohydrate pools of sugars (soluble carbohydrates) and storage carbohydrates (starches or insoluble carbohydrates) are important to rooting as building block of complex macromolecules, structural elements, and energy sources. A positive correlation between carbohydrate content and rooting may reveal that the supply of current photosynthate is insufficient for supporting optimal rooting. High C/N ratios in tissue of cutting promote rooting but do not accurately predict the degree of rooting response. İn some species, callus formation is a precursor of adventitious root formation. Actually, the formation of callus and the formation of roots are independent of each other. Hovewer, both involve cell division and roots frequently emerge through the callus (Hartmann et al., 2002). The type of carbohydrate utilized during the rooting stage together with auxin treatment can be essential to the rooting success (Uosukainen, 1992; Pawlicki and Welander, 1995). There are reasons to believe that sucrose enhances the sensitivity to auxin (Caboni et al., 1992; Calamar and Klerk, 2002; Anonymous, 2008). As a result of poor nutrition uptake or because of a possible hormonal inbalance, young apple plants had a tendency to lapse into arrest of growth (Uosukainen, 1992).

The aim of this investigation was carried to increase rooting of cuttings by treating different treatments on MM106 semi-dwarf apple rootstock, which is hard-to-root. ppm) were investigated to determine the effects on rooting hardwood cuttings of kiwi and found that IBA-

MATERİAL AND METHODS

The hardwood cuttings of MM106 semi-dwarf apple rootstock were collected from the apple rootstock field at Research and Application Orchard of Department of Horticulture at Agriculture Faculty of Atatürk University in Erzurum province (Altitude, 1850 m) in the first week of April. These cuttings had uniform length (25 cm) and diameter and was paid attention each of them to have equal bud number on itself. The cuttings in present investigation were held in rooting media almost three months after treatments and then the obtained results were evaluated.

Two strains of *Agrobacterium rubi* (A-18) and *Bacillus subtilis* (OSU-142), four carbohydrates (glucose, sucrose, sorbitol, and mannitol) and three doses of indol-3-butyric acid (IBA 1000, 2000, 4000 ppm) alone, in combination with two and three treatments in turn in order were treated to investigate the effects on rooting of MM106 hardwood cuttings. Two bacteria strains from Department of Plant Protection at Agriculture Faculty of Atatürk University and IBA and carbohydrates from Horticulture Laboratuar in our department were used in this investigation.

Carbohydrate solutions, glucose (115 mM), sucrose (55 mM), sorbitol (55 mM), and mannitol (25 mM) were prepared and each treatment group of cuttings were kept by dipping into each of these solution for equal duration (6 h). Bacterial suspension prepared was diluted in distilled water to a final concentration of 10^9 cfu ml^{-1} ((Karakurt, 2006; Aslantas et al., 2007) and cuttings were dipped in this suspension for 45 min. For IBA treat-ments, the basal portion of cuttings was dipped in an aqueous solution of 1000, 2000 and 4000 ppm (50% ethanol) for 15 s and allowed to air dry (Ercisli et al., 2003). Cuttings in the control group were treated with sterile water and before treatments, all cuttings were sterilized with 25% $NaHCO_3$ for 5 min (Aslantas et al., 2007). The combination treatments with double and the three were performed as followed below.

Glucose (6 h) + A-18 (45 min) Sucrose (6 h) + A-18 (45 min)
Glucose (6 h) + OSU-142 (45 min) Sucrose (6 h) + OSU-142 (45 min)
Sorbitol (6 h) + A-18 (45 min) Mannitol (6 h) + A-18 (45 min)
Sorbitol (6 h) + OSU-142 (45 min) Mannitol (6 h) + OSU-142 (45 min)
Sorbitol (6 h) + A-18 (45 min) + IBA 1000 ppm (15 s)
Sorbitol (6 h) + A-18 (45 min) + IBA 2000 ppm (15 s)
Sorbitol (6 h) + A-18 (45 min) + IBA 4000 ppm (15 s)
Sorbitol (6 h) + OSU-142 (45 min) + IBA 2000 ppm (15 s)
Sorbitol (6 h) + OSU-142 (45 min) + IBA 2000 ppm (15 s)
Sorbitol (6 h) + OSU-142 (45 min) + IBA 4000 ppm (15 s)

Following treatments, cuttings were placed in trays filled with perlite mgfedia to a depth of 10 cm in a greenhouse maintained at 25 ± 2°C. The experimental design used was a randomised complete block with 2 replications. Each replication contained 10 cuttings spaced 50 mm apart. Data were analysed by using SPSS software program and differences between means were seperated to Duncan Multiple Range Test (Düzgüneş et al., 1993).

Some preliminary investigations were performed to determine the most suitable concentration and type of carbohydrates for hardwood cuttings of MM106, based on treatments usually used to root microcuttings in *in-vitro* conditions. As a result of these, the suitable treatments inducing callus formation on basal portion of cuttings were chosen to investigate in present study. Similarly, the suitable concentration and duration of IBA and bacteria treatments were applied by examining the investigations previously carried out in various fruit species and periods

RESULTS

There were statitically significant differences ($P < 0.01$) in terms of rooting rate, callus rate, adventitious root number, root length and root thickness in this investigation. A-18+sorbitol, OSU-142+sorbitol+IBA-2000, A-18+sorbitol + IBA-2000 and A-18+sorbitol+IBA-4000 treatments pre-

sented the highest rooting rate (30%) compared to control (0%). IBA-1000 ppm, OSU-142, A-18, A-18+glucose, A-18+mannitol, OSU-142+sorbitol, OSU-142+sorbitol + IBA-1000 ppm, A-18+sorbitol+IBA-1000 ppm followed by 20% rooting. The rest of treatments had no rooting.

OSU-142+sorbitol treatment had the highest callus rate (70%) and sucrose, OSU-142+sucrose and OSU-142 + sorbitol + IBA-2000 ppm treatments followed by (50%) compared to control (10%).

IBA-1000 ppm treatment had the highest adventitious root number (16.5). A-18+glucose, A-18+sorbitol + 2000 ppm-IBA, A-18, A-18 + sorbitol + IBA-1000 ppm followed by 15.0; 11.5; 8.0; 7.5, respectively.

A-18+Glucose treatment had the highest adventitious root length (15.5 cm). IBA-1000 ppm tretament by 15.0 cm, OSU-142 by 9.75 cm, A + 18 + sorbitol + IBA-2000 ppm by 9.35 cm tretaments followed that, respectively.

The difference between A-18 and A-18 + Mannitol treatments had statistically the same. A-18 treatment occured the highest avarage adventitious root thickness (1.61 mm). A-18+Mannitol by 1.57, OSU-142 by 1.43 followed that, respectively (Table 1).

DISCUSSION

This experiment was designed to determine the effects of single, double and the three treatments of IBA, bacteria, carbohydrates, which may affect the rooting performance of hardwood stem cuttings of hardwood cuttings of MM106 semi-dwarf apple rootstock.

Three different concentrations (1000, 2000, 4000 ppm) of IBA were applied singly and combined bacteria plus sorbitol.

In single IBA treatments, IBA-1000 ppm had the best by rooting rate (20%), callus rate (30%), avarage root number (16.5), root length (15.0 cm) and root thickness (1.11 mm). Single treatments of IBA-2000 ppm and 4000 ppm did not occured rooting, but induced more callus formation compared to control.

Many treatments have been usually performed to determine the best suitable IBA concentrations for rooting of cuttings taken from different periods of a year in lots of fruit species (Ercisli et al., 2003). İndeed, various concentration of IBA (0, 50, 100, 150, 2000,4000, 6000 6000 ppm had the best (Zengibal and Özcan, 2006).

Similarly, IBA-8000 ppm occured rooting rate (50%) in the hardwood cuttings of kiwi (Spriovska, 1982). IBA-2000 ppm had the highest rooting rate (36.22%) in the hardwood cuttings of rosehip (Güneş and Şen, 2001).

In the three treatments, IBA-2000 ppm combined OSU-142 plus sorbitol had a noteworthy rooting rate (30%) and callus rate (50%).

It may be explained that IBA-1000 ppm in single treatments, IBA-2000 ppm in the three treatments had the succesful results in terms of the rooting of MM106 and IBA-4000 ppm did not occured rooting in both single and the three treatments. This concentration of IBA may affect undesirably to the rooting of the hardwood cuttings of MM106 rootstock.

OSU-142 and A-18 bacteria treatments provided the equal rooting rate in single treatments. Single A-18 occured more callus formation (20%), adventitious root number (8) and adventitious root thickness (1.61 mm) than those of OSU-142.

In double treatments, combined A-18 plus sorbitol had the highest rooting rate (30%) and OSU-142 plus sorbitol had the highest callus rate (70%). It was observed that the callus formation had been more distinctive and adventitious root traces dispersed densely around the portion of cuttings especially for these two treatments.

It may be stated that more successful rooting rate would be obtained from these two treatments (Combined A-18 plus sorbitol and OSU-142 plus sorbitol), in case of the experiment were carried on more 10 - 15 days. These will be promising for the rooting of the hardwood cuttings of MM106 rootstock.

OSU-142+sorbitol+IBA-2000 ppm and A-18+sorbitol+ IBA-2000 ppm treatments were more considerable due to high rooting and callus formation. In addition, we may state that A-18 was better in all single, double and the three treatments compared to OSU-142.

Esitken et al. (2003) in wild sour cherry cuttings and Ercisli et al. (2003) in kiwi cuttings were determined that *Agrobacterium rubi* strains (A-1, A-16, and A-18) provided considerable rooting as treated single, together with IBA. It has been reported that these bacteria produce indole-3-acetic acid (IAA) (Goto, 1990) and Aslantas et al. (2007) were determined that OSU-142 had the capacity of IAA production. This situation shows these bacteria with IAA-production can be important for the rooting investigations. There have been few studies on the effect of carbohydrates on adventitious root formation (Pawlicki and Welander, 1995; Calamar and Klerk, 2002; Correa and Paim, 2005). The carbohydrates have usually been used for rooting of microcuttings of hard-to-root species in in-vitro studies. However, they were used directly single, combined bacteria and IBA for rooting of the hardwood cuttings of MM106 in greenhouse conditions. These did not incur rooting by single treatments but induced more callus formation compared to control. More successful results were obtained from double and the three treatments. It has been reported that sucrose increses e-thylene production in plant tissues. In apple, ethylene increases rootting when added briefly after taking the cutting probably acting as an elicitor making cells sensitive to respond to auxin. Thus, during the initial days of the rooting treatment sucrose may enhance rooting by increasing ethylene synthesis (Calamar and Klerk, 2002). This situation may be explained that the carbohydrates increases the response of cells in the basal portion of cutting to IBA and bacteria in this experiment. There have been many attempts to stimulate the rooting of cuttings by various treatments including treatments with plant growth regulators, carbohydrates and various other chemical substances (Esitken

Table 1. The effects of some single and different combination treatments on rooting of the hardwood cuttings of MM106 apple rootstock.

Treatments	Rooting rate (%)	Callus rate (%)	Adventitious root number	Adventitious root length (cm)	Adventitious root thickness (mm)
Control	0 b	10c (13.28)	0 e	0 e	0 e
IBA-2000 ppm	0 b	30b (32.89)	0 e	0 e	0 e
IBA-4000 ppm	0 b	40ab (39.23)	0 e	0 e	0 e
IBA-1000 ppm	20a (26.56)	30b (32.89)	16.5a	15.0a	1.11bcd
OSU-142	20a (26.56)	10c (13.28)	6.0c	9.75b	1.43ab
A-18	20a (26.56)	20bc (26.56)	8.0c	1.72cde	1.61a
Sorbitol	0 b	30b (32.89)	0 e	0 e	0 e
Glucose	0 b	30b (32.89)	0 e	0 e	0 e
Mannitol	0 b	20bc (26.56)	0 e	0 e	0 e
Sucrose	0 b	50ab (45.0)	0 e	0 e	0 e
OSU-142+Sucrose	0 b	50ab (45.0)	0 e	0 e	0 e
A-18+Sucrose	0 b	30b (32.89)	0 e	0 e	0 e
OSU-142+Glucose	0 b	30b (32.89)	0 e	0 e	0 e
A-18+Glucose	20a (26.56)	30b (32.89)	15.0a	15.5a	1.10bcd
OSU-142+Mannitol	0 b	30b (32.89)	0 e	0 e	0 e
A-18+Mannitol	20a (26.56)	30b (32.89)	6.0c	4.0c	1.57a
*OSU-142+Sorbitol	20a (26.56)	70a (57.10)	1.5de	1.6cde	0.89cd
*A-18+Sorbitol	30a (32.89)	40ab (39.23)	7.0c	1.05de	0.83d
OSU-142+Sorbitol+IBA-1000 ppm	20a (26.56)	20bc (26.56)	2.5de	0.35e	1.14bcd
A-18+Sorbitol+IBA-1000 ppm	20a (26.56)	40ab (39.23)	7.5c	0.75e	0.93cd
OSU-142+Sorbitol+IBA-2000 ppm	30a (32.89)	50ab (45.0)	3.0d	3.5cd	1.08bcd
A-18+Sorbitol+IBA-2000 ppm	30a (32.89)	40ab (39.23)	11.5b	9.35b	0.97cd
OSU-142+Sorbitol+IBA-4000 ppm	0 b	40ab (39.23)	0 e	0 e	0 e
A-18+Sorbitol+IBA-4000 ppm	30a (32.89)	40ab (39.23)	5.5c	8.30b	1.30abc
Significant level	**	**	**	**	**

Means followed with the same letter within each column are not significant different, (**: $P < 0.01$).
The statistical analyse of percentage values was performed by using transformed values (in parantesis).

et al., 2003). İt was reported that high C/N ratios in tissue of cutting promote rooting (Hartmann et al., 2002).

In present study, more successful results were obtained from double and the three treatments of IBA, bacteria and carbohydrates, although single IBA and bacteria treatments had the considerable results. As a result of these, we can recommend that these treatments can be used for rooting of hard-to-root rootstocks, because the propagation method by cuttings is less expensive and more practical than other vegetative propagation methods. In future, studies should be carried on acquiring more comprehensive and usable results by shifting the sampling date of cuttings and the concentration of chemical substance.

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