Effects of indole-3-butyric acid levels and activated charcoal on rooting of in vitro shoots of apple rootstocks

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Summary: Rooting responses of rootstocks cvs. JTE-H, M. 26 and MM. 106 were studied to different concentration of IBA in root induction media and to presence of activated charcoal in root elongation media. High rooting rate (>90%) could be achieved in cvs. JTE-H and M. 26, while cv. MM. 106 showed weak rooting ability at each IBA level tested. Increasing IBA content depressed the rooting only in cv. M. 26. Presence of activated charcoal decreased considerable the rooting rate in cv. M. 26 and decreased the number of roots in cvs. JTE-H and M. 26. These cultivars developed longer roots on media containing activated charcoal, while cv. MM. 106 did not showed any reaction for it.

Key words: adventitious rooting, M. 26, MM. 106, JTE-H, IBA, activated charcoal

Abbreviations: IBA-indole-3-butyric acid, BA-6-benzylaminopurine, BAR-6-benzylaminopurine riboside, NAA-naphthaleneacetic acid

Introduction

Successful rooting and acclimatisation of apple shoots are very important steps of *in vitro* clonal propagation. The rooting methods of *in vitro* apple shoots often include an auxin treatment followed by the transfer of the shoots to achieve root elongation *(Druart* 1997, *Bolar* et al. 1998, *Harbage & Stimart* 1996). Although several factors can affect the rooting capacity of shoots, auxin plays primary role in root induction of *in vitro* apple shoots *(Sriskandarajah* et al. 1990, *Harbage* et al. 1993, *De Klerk* et al. 1995). Comparing different auxins *Zimmermann & Fordham* (1985) found IBA to be the most efficient to induce rooting. *Jones* (1979) studied the micropropagation possibilities of several apple cultivars and reported that rootstocks need higher level of auxin for induction of adventitious roots than scions, but the optimal concentration of auxin for rooting is genotype-dependent *(Yepes & Aldwinckle* 1994, *Alvarez* et al. 1988, *Machnik & Lisek* 1996). After the auxin-sensitive phase auxins inhibit the growth of roots *(Lane* 1978, *James & Thurbon* 1979, *Druart* 1997). During post-induction phase presence of activated charcoal in root elongation media increased the rooting capacity of *in vitro* apple shoots *(Modgil* et al. 1999). Using of activated charcoal to enhance rooting is common during micropropagation of other species such as *Philodendron erubescens* and *P. tuxtlanum (Jámborné Benczúr E.* et al. 1998, *Jámbor-Benczúr & Márta-Riffer* 1990) and *Hosta fortunei (Szafián* et al., 1996). The aim of this work was to study the responses of rootstocks to different auxin levels and to describe the influence of the activated charcoal on rooting characteristics.

Material and method

Plant material

In vitro cultures of cvs. M. 26, MM. 106 and JTE-H rootstocks were established as reported earlier *(Dobránszki* et al., 2000). Media for shoot proliferation contained *Murashige-Skoog* (1962) salts and vitamins, supplemented with 100 mg l^{-1} myo-inozitol and 3% saccharose. The media was solidified by 0.7% agar-agar and pH was adjusted to 5.7 before autoclaving. Previously different hormone combinations were tested and shoots were collected from the best proliferation media for rooting experiments. Accordingly, the proliferation media contained 0.3 mg l^{-1} IBA + 1.0 mg l^{-1} BAR for cv. JTE-H, 0.1 mg l^{-1} IBA + 0.5 mg l^{-1} BA for cv. M. 26 and 0.1 mg l^{-1} IBA + 1.0 mg l^{-1} BAR for cv. MM. 106. All media were supplemented with 0.2 mg l^{-1} GA_3.

Rooting experiments

Four-week-old shoots (15–25 mm in length) were used for rooting after removing bottom leaves. Five shoots per baby jar were placed vertically on 30 ml root induction media (RIM), which contained MS salts at half strength, 100

mg l^{-1} myo-inozitol, 0.5 mg l^{-1} vitamin B_1, 2% saccharose, and 0.7% agar-agar. Effects of three IBA levels were tested: 1.0, 2.0 and 3.0 mg l^{-1}. The pH was adjusted to 5.5 prior to autoclaving. Cultures were incubated at 26 °C in total darkness for a week before transferring to hormone-free root elongation medium (REM). REM contained MS salts at half strength supplemented with 50 mg l^{-1} myo-inozitol, 3% saccharose, 2.0 ml l^{-1} Wuxal and 0.7% agar-agar. Half of shoots were cultured on REM containing 2.5 g l^{-1} activated charcoal (E1), the other half were cultured on REM without activated charcoal (E2).

These cultures were incubated at 22±2 °C with 16–h photoperiod provided by warm-white lamps (Tungsram) at PPF of 105 µmol s^{-1} m^{-2}. Rooting percentage, number of roots per rooted shoot and the length of roots were observed after two weeks and rooted shoots were planted in Jiffy-7 pellets after removing the medium. Previously, Jiffy-7s were soaked in a sterile solution containing MS salts in 0.1 strength and 0.15% Previcur to prevent fungal contamination. Acclimatisation was made according to *Bolar* et al. (1998). Each treatment consisted of 30 plantlets.

The statistical analysis was made by analysis of variance followed by Tukey's test, by using of SPSS 7.5 for Windows program.

Results and discussion

Responses of rootstocks to IBA levels were genotype dependent. Cv. JTE-H showed very high rooting capacity on each IBA level (91–100%), while very low rooting rate could be obtained in cv. MM. 106 in each treatment (33–46%) *(Table 1)*. High rooting ability of cv. JTE-H maybe due to higher endogenous free auxin level in the shoot base *(Alvarez* et al., 1989) or differences maybe present in endogenous metabolism of exogenous auxin between genotypes *(James,* 1983). The highest rooting percentage (94%) was achieved by the lowest IBA concentration in the case of cv. M. 26 and the rate of rooted shoots decreased as IBA levels increased *(Table 1)*. *Jámborné Benczúr* (1993) induced rooting in high rate (90%) by use of very low IBA concentration (0.2 mg l^{-1}) for rooting of cv. M. 26. *Similarly, Alvarez* et al. (1988) found very low (0.4 mg l^{-1}) IBA concentration to be optimal for rooting of cv. M. 26 microcuttings.

Modgil et al. (1999) found that activated charcoal decreased the callus formation and increased the rooting capacity of *in vitro* shoots of cv. Tydeman Early Worcester. In contrast we found, that presence of activated charcoal in hormone-free root elongation media decreased the rooting rate of shoots depending on genotype and IBA content of RIM. When root induction media contained 1.0 or 2.0 mg l^{-1} IBA, the rooting percentage of cv. JTE-H was slightly decreased by activated charcoal. Strong negative effect of activated charcoal could be observed in the case of cv. M. 26 after each IBA level *(Table 1)*. The root developing media contained IBA in experiments published by *Modgil* et al. (1999) thus enhancing effect of activated charcoal maybe due to adsorption of the IBA from media. Aromatic compounds such as auxins could have great adsorption affinity for activated charcoal *(Pan & Staden,* 1998) and in our preliminary experiments we found that the favourable effect of activated charcoal mainly due to the adsorption of NAA. In contrast, presence of activated charcoal can decrease the rooting percentage on media containing auxin in *Prunus x davidopersica* 'Piroska' *(Jámbor-Benczúr* et al., 2001). Applying hormone-free root developing media *Snir & Erez* (1980) found that activated charcoal influenced only the length of roots. Similarly to their findings, we observed that activated charcoal increased significantly the length of roots in the case of cvs. JTE-H and M. 26 *(Figure 1–2)*. Raising of IBA content in RIM can decrease the length of roots, although the differences were proved statistically significant only in cv. JTE-H, when REM applied without activated charcoal. Even though the longest roots (9.0 mm) were obtained after the lowest IBA level on media without activated charcoal, any significant differences could not be detected in the case of cv. MM. 106. The length of roots varied only between 6.0 and 7.0 mm in other treatments (data not shown).

Harbage & Stimart (1996) found that the number of roots increased with an increase in IBA content up to 2.0 mg l^{-1}. In cvs. JTE-H and M. 26 we observed the same phenomena only in the presence of activated charcoal in REM *(Figure 3–4)*.

Moreover, presence of activated charcoal decreased significantly the number of roots for these rootstocks, especially when lower IBA levels were applied in RIM *(Figure 3–4)*. *Szafián* et al. (1996) observed the same effect

Table 1 Effect of IBA levels and activated charcoal on rooting rate of three apple rootstock

Rate of rooted shoots (%)	IBA content in root induction media (mg l^{-1})					
	1.0	2.0	3.0	1.0	2.0	3.0
	E1			E2		
cv. JTE-H	96.7	91.4	100	100	100	100
cv. M. 26	65.7	57.1	40	94.3	74.3	62.9
cv. MM.106	40	33	46	40	46	37

E1: root elongation media with activated charcoal
E2: root elongation media without activated charcoal

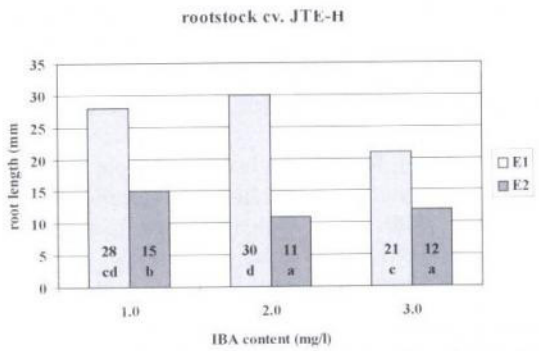

Figure 1 Effects of IBA levels and activated charcoal on the root length in cv. JTE-H. E1: root elongation media with activated charcoal, E2: root elongation media without activated charcoal. The small letters mean the homogenous groups according to Tukey's test.

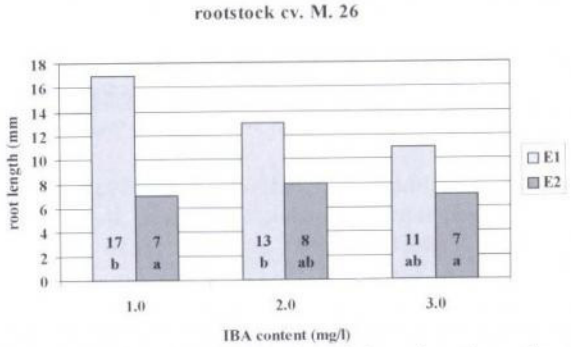

Figure 2 Effects of IBA levels and activated charcoal on the root length in cv. M. 26. E1: root elongation media with activated charcoal, E2: root elongation media without activated charcoal. The small letters mean the homogenous groups according to Tukey's test.

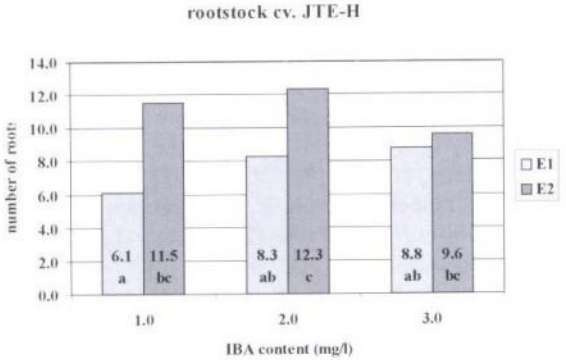

Figure 3 Effects of IBA levels and activated charcoal on the number of roots in cv. JTE- H. E1: root elongation media with activated charcoal, E2: root elongation media without activated charcoal. The small letters mean the homogenous groups according to Tukey's test

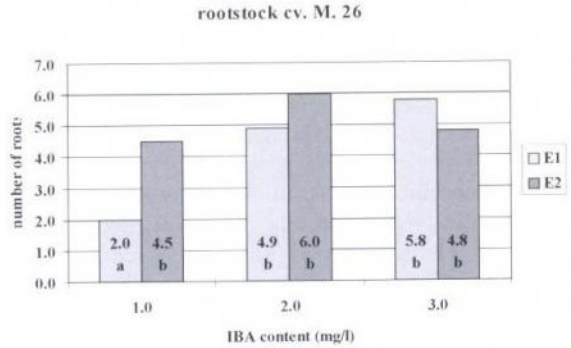

Figure 4 Effects of IBA levels and activated charcoal on the number of roots in cv. M. 26. E1: root elongation media with activated charcoal, E2: root elongation media without activated charcoal.The small letters mean the homogenous groups according to Tukey's test

of activated charcoal during *in vitro* propagation of *Hosta fortunei*.

The number of roots was not affected by any treatments in the case of cv. MM. 106. The root number ranged between 4,4–5,1 per shoots (data not shown).

Rooted shoots were planted in Jiffy-7 pellets after cutting back of too long roots to 10 mm *(Thomas & Ravindra,* 1997). High rate of plants survived (92–100%) in the first phase of acclimatisation maybe due to the presence of Previcur and MS salts applied similarly to the method of *Bolar* et al. (1998). In this first phase of acclimatisation almost each plantlet survived except for one shoot of cv. JTE-H, which originated from media with 2.0 mg l^{-1} IBA and one shoot of cv. MM. 106 originated from media with 1.0 mg l^{-1} IBA, both plantlets rooted without activated charcoal.

Although in this study we could not find any favourable effect of activated charcoal on rooting characteristics, the plants originated from media contained activated charcoal grew more vigorously during rooting and acclimatisation. Shorter roots developed on REM without activated charcoal should be more advantageous for acclimatisation *(Jámbor-Benczúr* et al., 2001) but in our experiments the long roots were stumped so the length of roots did not affected the survival.

IBA content of root induction media had no carry-over effect on acclimatisation. When roots became visible on the surface of Jiffy-7 pellets, the plantlets were planted into pots (80-mm diameter). All plantlets survived in pots and continued vigorous growth.

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