Effects of activated charcoal on rooting of *in vitro* apple (*Malus domestica* Borkh.) shoots

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Summary: Rooting of *in vitro* '*Royal Gala*' shoots was studied under different conditions of root induction and root elongation phase. The rooting capacity was affected by both rooting phases. Very high rooting percentage could be reached with both liquid and solid root induction media. Raising the temperature from 22 °C to 26 °C during root induction phase increased the rooting percentage. Presence of activated charcoal in root elongation media can affect the number of roots per rooted shoots and can increase the rooting percentage, the length of roots and the rate of survival depending also on other conditions during rooting. Presence of NAA in root elongation media reduced the number and the length of roots considerably. Favourable effect of activated charcoal on rooting was mainly due to adsorption of NAA.

Abbreviations: IBA: indole-3-butyric acid, NAA: naphthalene aceticacid, BA: benzylaminopurine.

Introduction

The induction of adventitious roots and successful acclimatisation are very important steps in micropropagation. Many factors may affect the rooting capacity of *in vitro* apple shoots, including genotype (*Harbage & Stimart*, 1996, *Zhou* et al., 1992) and type of carbohydrate source (*Karhu & Ulvinen*, 1995) and auxin content of root induction media (*Harbage* et al., 1993). Ammonium nitrate content of media can inhibit the rooting (*Sriskandarajah* et al., 1990, *Druart*, 1997), while an increase in the number of subculture can enhance the rooting capacity (*Noiton* et al., 1992). Activated charcoal in medium for root elongation improved rooting of apple shoots (*Modgil* et al., 1999, *Snir & Erez*, 1980) and *Philodendron erubescens* (*Jámbor-Benczúr* et al., 1998). Activated charcoal can enhance or inhibit the *in vitro* growth of explants depending on several factors (*Pan & Staden*, 1998). The present report is concerned with factors affecting the formation of adventitious roots in '*Royal Gala*' especially with activated charcoal.

Material and methods

Plant material

In vitro culture of '*Royal Gala*' was 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% sacharose. Benzylaminopurine (BA) was added as cytokinin (0.5 mg l^{-1} in *Experiment 1.*, and 1.0 mg l^{-1} in *Experiment 2.*) and 0.3 mg l^{-1} indole-3-butyric acid (IBA) as auxin. The media was solidified by 0.7 % agar-agar and pH was adjusted to 5.7 before autoclaving. Four week old shoots (15–25 mm) were cut off and placed vertically on 30 ml root induction media (five shoots per baby jar) after removing bottom leaves.

Experiment 1

The medium for root induction was liquid or solid, containing MS salts at half strength supplemented with

100 mg l^{-1} myo-inozitol, 0.5 mg l^{-1} vitamin B_1, 20.0 g l^{-1} sucrose, 3.0 mg l^{-1} IBA and 7.0 g l^{-1} agar-agar or 20% perlite in liquid media. The pH was adjusted to 5.5 prior to autoclaving. Solid cultures were incubated at 22 or 26 °C, while liquid cultures were incubated at 26 °C with continuos shaking and all cultures were exposed to total darkness for a week. Then shoots were transferred to root elongation medium (REM), which contained MS salts at half strength supplemented with 50 mg l^{-1} myo-inozitol, 30.0 g l^{-1}sucrose, 2.0 ml l^{-1} Wuxal, 0.5 mg l^{-1} NAA, and 7 g l^{-1} 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 three weeks and rooted shoots were planted in Jiffy-7 pellets after removing 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 at least 30 explants.

Experiment 2

The solid and liquid media for root induction was the same as in previous experiment, but perlite was omitted from liquid medium. When liquid media were used the shoots were transferred to tubes in order to prevent their submerging. Cultures were incubated at 26 °C in total darkness for a week. After solid REM the shoots were passed to different root elongation media: in addition to E1 and E2 the hormone-free REM were also used containing 2.5 g l^{-1} activated charcoal but not NAA (E3) and REM without activated charcoal and NAA (E4).

Half of the shoots from liquid medium were planted to Jiffy-7 pellets immediately after root induction phase, others were passed to REM (E3).

Rooting percentage, number of roots per shoot and the length of roots were observed after two weeks and rooted shoots were planted in Jiffy-7 pellets on the same way as was described above. At least thirty shoots were evaluated per treatments.

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

Experiment 1

Temperature during root induction phase did not affect the number of roots per rooted shoot or the length of roots, although the rooting percentage was higher at higher temperature. *Similarly, Zimmerman & Fordham* (1985) found that raising of temperature during dark treatment improved rooting of several cultivars.

The rooting percentage was affected strongly by combination of treatments *(Table 1)*. Although the number of root primordia is determined in the first days of root induction treatments *(Harbage* et al., 1993), in our study the conditions during root elongation phase influenced the finally results in rooting capacity. Activated charcoal in root elongation media increased the rooting percentage when root induction was made on solid media (from 40 to 94.3% and from 66.7 to 100% when root induction was made at 22 and 26 °C, respectively), while after liquid media we found higher percentage on media without charcoal. Using of activated charcoal in root elongation media raised the number of roots, when solid medium was used during the first phase. Activated charcoal increased markedly the length of roots in each treatment. Similar effect of activated charcoal on root length were observed by *Szafián* et al., (1996) in the case of *Hosta fortunei*.

Table 1 Rooting capacity of *'Royal Gala'* shoots in different treatment combinations

RIC→	Solid media				Liquid media	
	22 °C		26 °C			
REM→	E1	E2	E1	E2	E1	E2
Rooting %	94.3	40.0	100	66.7	80	96.0
Number of roots per shoot	11.5 bc	2.9 a	13.1 c	6.1 ab	15.1 c	25.9 d
Length of roots (mm)	40.7 c	3.6 a	37.8 c	4.0 a	27.3 b	9.4 a
The rate of survival (%)	100	44.0	100	70.0	100	73.0

The same letters in rows indicate statistically homogenous groups (Tukey-groups).
RIC: root induction conditions, REM: root elongation media,
E1: activated charcoal + NAA,
E2: NAA without activated charcoal.

The liquid medium increased significantly the rooting percentage and the number of roots when the root elongation medium did not contain activated charcoal. In this case several short, thin hairy-like roots formed at the base of shoots. In contrast, the number of roots was not affected by consistence of root induction medium when the root elongation media contained activated charcoal. In this experiment the perlite was not able to prevent the submersion of the shoots in the liquid medium thus the roots differentiated on the whole surface of the shoots. Only the roots formed at the base of shoots were taken into consideration.

Ten rooted plantlets from each treatment were planted to Jiffy-7 pellets. The roots, which formed on media containing activated charcoal were too long so the planting was very difficult. The roots formed on media without activated charcoal were short and strong, callus formation could be observed. The survival rate was 100% when shoots originated from medium containing activated charcoal, while only 44–73 percent of shoots survived from medium without charcoal. After two weeks when roots emerged from Jiffy-7 pellets the plantlets were transplanted to pots and all of them survived.

Experiment 2

In this experiment the rooting percentage was not affected by any conditions, it was very high for all treatments ranging from 96% (E2 and E3) to 100% (others). Even though the conditions were similar to Experiment 1., the rooting capacities of shoots were higher. Differences in the last proliferation media or the number of subcultures may cause this phenomenon. As the number of subcultures was hardly different (two passages after 1. Experiment) the cytokinin content of media could cause the differences. *Webster & Jones* (1991) also found differences in rooting percentage between shoots originating from different content of BA in media.

The number of roots was not affected by consistence of media, but activated charcoal increased it when root elongation medium contained auxin *(Table 2)*. In contrast, activated charcoal resulted decrease in the number of roots if the medium was auxin-free. No differences could be observed for number and length of roots between media with or without auxin if they contained activated charcoal. Auxin content of root elongation media decreased the number and length of roots significantly if medium did not contain activated charcoal. De *Klerk* et al. (1995) found that high level of auxin is required only during the induction phase and *James & Thurbon* (1979) reported decreased number of roots on shoots left in continuous contact with IBA. In our study the favourable effect of activated charcoal mainly due to the adsorption of NAA. However, it showed further effects on media without auxin: it decreased significantly the number of roots and increased markedly the length of roots.

Table 2 Effect of auxin and activated charcoal on rooting of *'Royal Gala'* shoots

RIM→	Solid media				Liquid media
REM→	E1	E2	E3	E4	E3
Rooting percentage	100	96	96	100	100
Number of roots per shoot	12.1 bc	4.5 a	8.2 ab	14.5 c	7.4 a
Length of roots (mm)	23.2 c	2.5 a	25.2 c	12.7 b	14.2 b

The same letters in rows indicate statistically homogenous groups (Tukey-groups).
RIM: root induction media, REM: root elongation media, E1: activated charcoal + NAA, E2: NAA without activated charcoal, E3: activated charcoal without NAA, E4: without NAA and activated charcoal

Differences in growth vigour and physiological state of plantlets could be observed during root elongation and in the first acclimatisation phase. Plantlets on medium with auxin and activated charcoal showed the best growth vigour, they grew rapidly and formed several large dark green leaves. Growth of plantlets on auxin-free medium with activated charcoal was slightly delayed but plantlets were very healthy. Growth of plantlets on medium without auxin and activated charcoal were satisfactory, while plantlets on medium with auxin and without activated charcoal were very weak and the leaves turned yellow. Even though these differences remained visible during the first phase of acclimatisation 100 % of plantlets survived except for those originated from medium contained auxin without activated charcoal (reached only 80%).

In these experiments the liquid culture was successful, but our results showed that it was not necessary to increase the rooting capacity of *'Royal Gala'* shoots. Although *Duarte* (1997) reported successful direct rooting in vermiculite after root induction phase, in our experiments the growth of shoots planted to Jiffy-7 pellets immediately after root induction phase was less vigorous, than those that were transferred to REM before acclimatisation. However rooting percentage and the rate of survival reached 96%.

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