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Effects of Culture Medium on in Vitro Rooting of Antonovka 313 Apple

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Abstract. **In vitro propagated shoots of the apple rootstock, Antonovka 313 (*Malus pumila* Mill.), were rooted successfully in vitro. Roots became visible in 6–8 days, and 100% rooting after 2 weeks was achieved consistently in shoots cultured on modified Murashige and Skoog (MS) salt medium supplemented with 0.25 μM indole-3-butyric acid (IBA). Sucrose was the most influential medium component for rhizogenesis. Inorganic nutrients, IBA and vitamins did not influence rooting. Omitting activated charcoal caused only a slight decrease. A 1.5% sucrose solution added to a peat-vermiculite growing medium in vitro resulted in higher rooting than in treatments without sucrose.**

Scion and rootstock cultivars of *Malus* can be propagated through in vitro techniques (1, 5, 6, 12, 16). Direct rooting tissue culture-produced shoots (microshoots) in plant growing medium reduces plant cost and shortens the time required to produce acclimated plantlets. In addition to economic advantages, direct rooting may circumvent anatomical and physiological complications which often occur with in vitro rooting (2, 3, 4, 13).

The apple rootstock Antonovka 313 is propagated commercially through in vitro techniques. Acclimated plantlets can be produced within 4 weeks after placement of shoots onto rooting medium. However, this cultivar is especially difficult to direct root. The objectives of this research were to identify the critical medium component(s) for rooting in vitro, and to determine the potential for direct rooting.

Aseptic cultures of Antonovka 313 were purchased from Microplant Nurseries. The Stage II medium was composed of modified MS salts (8) supplemented with (per liter) 1.4 mM myo-inositol 0.2 H_2O, 7.4 μM thiamine HCl, 1.1 μM 6-benzylaminopurine (BA), 4.9×10^{-2} μM IBA, 3% sucrose and 0.6% TC agar. The pH was adjusted to 5.7 prior to adding agar. Medium was dispensed in 25-ml aliquots into 25 × 150-mm test tubes and autoclaved at 1.1 kg/cm^2 and 121°C for 15 min.

Stage II cultures were grown at 24° ± 3°C and 30–40 μmol $s^{-1}m^{-2}$/16-hr day from cool-white fluorescent lights. After 3–4 weeks a 4- to 7-fold increase in shoot number occurred. Shoot clumps were subcultured every 4 weeks, with 2- to 4-cm long shoots being used for rooting experiments.

For rooting in vitro, the macronutrients were reduced to one-fourth strength, micronutrients reduced to one-half strength, BA omitted, and sucrose and vitamins reduced to one-half strength of the Stage II medium (Suttle, personal communication). Acid-washed activated charcoal (1 g/liter) and 0.25 μM IBA were added to the medium, which was solidified with 0.7% Gibco Phytagar after adjusting the pH to 5.7. Stage III cultures were incubated at 24° ± 3°C under continuous light at 50–60 μmol $s^{-1}m^{-2}$.

The individual classes of medium components were tested for their influence on rhizogenesis of Antonovka 313 shoots. Shoots were placed on rooting media lacking only one of the following components: sucrose, macronutrients, micronutrients, IBA, vitamins, activated charcoal, or agar. The control consisted of the complete stage III medium. To evaluate rooting without agar, explants were supported on filter paper bridges in liquid medium. Rooting percentage was determined every 2nd day, beginning 6 days after placement of microshoots on rooting media.

Roots became visible by day 6 (Table 1). Rooting of Antonovka 313 microshoots without IBA was similar to the shoots rooted on the complete medium on day 14. This response contrasts with results reported elsewhere indicating a strict auxin requirement for rooting other apple cultivars (7, 12, 14, 15, 16). Shoots grown on media lacking macronutrients or micronutrients became slightly discolored and pale, but exhibited good rooting. Rooting percentage for shoots on media lacking vitamins or activated charcoal was slightly lower than for the shoots rooted on the complete medium. Excessive callus formation occurred at the shoot bases of explants cultured on medium lacking activated charcoal. Shoots rooted poorly on the liquid medium. However, many of the shoot bases were lifted from the filter paper bridges by leaf contact with the test tube during the course of the experiment. About 45% of the shoots that maintained good contact with the filter paper bridges rooted.

Shoots placed on medium without sucrose exhibited significantly lower rooting than all other treatments, except the minus agar treatment. However, some rooting did occur in the absence of sucrose, indicating that an exogeneous carbohydrate is not absolutely essential for root formation of Antonovka 313 in vitro; sucrose ensured consistently high rooting percentages. Shoots cultured on medium lacking sucrose appeared healthy and showed no lack of vigor or chlorosis. The lack of a carbohydrate requirement for rooting by Antonovka 313 is unlike other tissue cultures (11, 12, 16). 'Granny Smith' apple shoots died within one week without sucrose (12) and *Rhododendron* stem explants turned black and died within 2 weeks without sugar in the medium (9).

The poor rooting response for direct rooted Antonovka 313 shoots in preliminary experiments may have been caused by an insufficient carbohydrate supply. A peat-vermiculite growing medium (Redi-Earth) was saturated with various nutrient media: 1) water (control); 2) 1.5% sucrose; 3) rooting medium without sucrose; 4) complete rooting medium with 1.5% sucrose. The liquid media were adjusted to pH 5.7 prior to dispensing 15-ml aliquots into 20 cc volumes of Redi-Earth in 25 × 150-mm test tubes. The tubes were autoclaved and cultures were grown under the conditions described for rooting in vitro. Forty tubes were used per treatment. Rooting percentage was determined after 14 days.

Table 1. Rooting of 'Antonovka 313' shoots on apple rooting medium lacking various medium components.

Apple rooting medium (without)	% Rooting on day				
	6	8	10	12	14
Complete	10 ab[z]	60 ab	88 ab	95 a	100 a
Micronutrients	8 ab	60 ab	93 a	93 a	93 ab
Macronutrients	0 b	40 b	68 b	93 a	98 ab
Activated charcoal	13 a	68 a	80 ab	85 a	85 b
IBA	8 ab	43 b	80 ab	85 a	98 ab
Vitamins	3 ab	58 ab	70 b	85 a	90 ab
Sucrose	0 b	0 c	3 c	5 b	23 c
Agar	0 b	10 c	18 c	23 b	30 c

[z]Mean separation by 2-tailed Fisher's Exact Test for differences in proportions (n = 40).

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Table 2. Rooting in vitro of Antonovka 313 shoots on peat-vermiculite growing medium saturated with various nutrient solutions.

Sucrose	Salts –	Salts +	Mean x̄
–	16 b[z]	16 b	16
+	52 a	43 a	48
x̄	34	30	

[z]Mean separation by 2-tailed Fisher's Exact Test for difference in proportions (n = 40).

Antonovka 313 shoots rooted poorly on the peat-vermiculite medium, even when sucrose was supplied (Table 2). Maximum rooting occurred when sucrose was added to the growing medium and salts did not influence rooting.

Our experiments revealed that Antonovka 313 shoots require a carbohydrate source for achieving consistent and rapid root formation. Low rooting percentages for shoots rooted on peat-vermiculite medium saturated with sucrose may indicate that some other factor besides carbohydrate supply limits root development in vivo. Research has indicated that agar medium may enhance diffusion of carbohydrates, nutrients, and hormones throughout the medium (10), as well as aid in the diffusion of substances inhibitory to rhizogenesis away from explants (9).

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Specificity and Interaction among Auxins, Light, and pH in Rooting of Australian Woody Species in Vitro

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Abstract. **A number of Australian woody species are shown to differ widely in adventitious root production in vitro in response to the type and combination of hormones applied, although the most effective combinations usually included IBA. Shoots of *Eremophila lanii* F. Muell produced roots on a medium containing cytokinins and no auxin. Two species, *Prostanthera striatiflora* F.v.M and *Correa decumbens* F.v.M., required reduced pH and a period of darkness for root induction, whereas these conditions inhibited *Grevillea biternata* Meissner. There also is an interaction among hormone treatment, media pH, and the effect of continuous light vs. a period of darkness. Chemical names used: 1*H*-indole-3-butanoic acid (IBA); naphthhalene acetic acid (NAA); indole-3-acetic acid (IAA); B-naphthoxyacetic acid (NOA); 6-furfurylaminopurine (KIN); *N*-(phenylmethyl)-1*H*-purin-6-amine (BA).**

There is considerable interest in the in vitro propagation of Australian native woody plant species for horticultural, forestry, and conservation purposes, and a number of successes have been reported, e.g., *Eucalyptus* (1, 3), *Araucaria* (6), *Prostanthera* and *Dampiera* (9), and *Santalum* (2). Difficulty is still commonly encountered with the induction of roots, however, particularly on cultures derived from mature woody explants, e.g., with *Eucalyptus* (3, 4) and *Santalum* (2).

A limiting factor in the general application of reported culture techniques is the wide variation in responses obtained. Most attempts to induce roots on shoots of woody plants produced in vitro involve the application of exogenous auxin. IBA and NAA are most commonly used, but the responses vary between species and laboratories. Effects of medium pH (2) and light (8) also have been reported. We have examined the interaction of these factors in rooting a range of Australian native species with potential for use as ornamental shrubs.

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All media used included Murashige and Skoog (7) minerals plus vitamins and amino acids, as modified by de Fossard (5), with 1% sucrose and 0.8% Difco Bacto agar. The pH was adjusted to 5.5 unless otherwise indicated, and all media were autoclaved at 121°C and 1.1 kg/cm^{-2} for 20 min after the addition of hormones as required. Ten ml of medium was used in each 8 × 2.5 cm screw-capped, polycarbonate tube.

Rooting experiments were carried out on 10 species of woody plants (Table 1) ranging from prostrate ground cover species to small shrubs. Adventitious and axillary shoots 3–4 cm long were excised from shoot proliferating cultures established from mature plants growing under cultivation or in the wild, using the general methods reported previously (9). Each treatment was applied to at least 5 shoots, and each experiment was duplicated in time. Mean percentages are presented in the tables along with the standard error.

The first group of experiments involved the testing of a wide range of hormone treatments to induce rooting on shoots produced in vitro. Once substantial rooting was achieved for a species, no further treatments were tested. Therefore, not all treatments were applied to each species. Rooting was assessed 6 weeks after shoots were placed on the rooting medium. The following hormones were added to the medium either singly or in combinations according to the treatment re-