controllers of plant responses instead of looking at all of the factors. A ten-fold increase in auxin or ethylene can have dramatic effects on a plant's growth response, but so too can a ten-fold change in water supply or carbon dioxide.

LITERATURE CITED

1 Crossett, R D and D J. Cambell 1975 The effects of ethylene in the root environment upon the development of barley. *Plant and Soil* 42,2 453-464

2 Fabijan, D., J S Taylor and D M Reid 1981 Adventitious rooting in hypocotyls of sunflower (*Helianthus annuus*) seedlings, II Action of gibberellins, cytokinins, auxins, and ethylene *Physiol Plant.* 53,4 589-597

3 Geneve, R L and C W. Heuser 1983 The relationship between ethephon and auxin on adventitious root initiation in cuttings of *Vigna radiata* (L). R. Wilcz *Jour Amer Soc. Hort Sci* 108(2):330-333.

4 Kays, S J , C W Nicklow and D H Simons 1974. Ethylene in relation to the response of roots to physical impedance. *Plant and Soil.* 40.565-571

5 Maleike, R 1978 Ethylene and adventitious root formation *Proc Inter Plant Prop Soc.* 28·519-525

6 Nakayama, M and Y. Ota 1980 Physiological action of ethylene in crop plants 6 Effect of hydrocarbons, especially ethylene, on the root growth of soybean and rice seedlings *Jap Jour. Crop Sci* 49(2):366-372.

7 Robbins, J A , J.A. Dirr, and S J Kays. 1983 Enhanced rooting of wounded mung bean cuttings by wounding and ethephon *Jour Amer Soc Hort. Sci.* 108(2).325-329

8 Swanson, B T , Jr 1974 Ethrel as an aid in rooting *Proc. Inter. Plant Prop Soc.* 24 351-361

9 Thimann, K.V. and F W Went 1934. On the chemical nature of the root-forming hormone. *Proc K Ned Akad. Wet., Amsterdam* 37.456-459

10 Zimmermann, P.W and A E Hitchcock 1933. Initiation and stimulation of adventitious roots caused by unsaturated hydrocarbon gases *Contr. Boyce Thompson Inst.* 5 351-369

MICROPROPAGATION OF 'NORTHERN SPY' APPLE ROOTSTOCK

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Abstract. The literature on the micropropagation of apple rootstocks is briefly reviewed. Detailed results are presented on factors affecting the establishment of cultures, shoot proliferation, adventitious root initiation, and growth and establishment in potting medium of the apple rootstock 'Northern Spy.'

REVIEW OF LITERATURE

Apple trees are normally propagated by budding or grafting the cultivar required (scion) onto a rootstock which is, itself, propagated by seed, cuttings, or from stool beds. The maintenance of stool beds is labour intensive and the production of rootstocks can be expensive.

A less expensive and more efficient method of producing clonal apple rootstocks is by micropropagation. Over the last 5 years there has been considerable progress in the micropropagation of apple rootstocks and scions. A number of commercial laboratories have been established that now specialize in the micropropagation of fruit trees (4,5,27,47). The success of micropropagation for apples is based largely on the finding that the cytokinin, BAP, can stimulate the growth of shoot-tips (18) and induce shoot proliferation (31), and that phloroglucinol can synergise auxins during rooting (21). Further advances have identified that the cytokinin type (28) and concentration (23,25), light levels (12), explant type (12), and agar concentration (34) are important for optimum shoot proliferation.

Recently, considerable attention has been given to studies on root initiation and growth. The stimulatory effect of phloroglucinol was first shown on shoot proliferation with 'M-7' and 'M-26' (19) and on root initiation with 'M-26' (21). Others found that phloroglucinol had no advantage (36), resulting in growth inhibition (45), or variable response, depending on cultivar (50) and concentration and the growth phase of the stock plant (42). Phloroglucinol was included routinely with 'M-9' cultures (16) and, although it had no effect on proliferation, it enhanced subsequent root initiation. The mode of action of phloroglucinol is not known and it has been suggested that it increases root initiation by increasing auxin uptake (15), or as an auxin protector by acting as an alternative substrate for IAA oxidase and/or peroxidase (17).

Investigations of the effect of auxin levels on rooting has led some workers to use a high auxin medium for root initiation followed by a low or auxin-free medium for root elongation (14,16,22,36). Lowering the salt concentration may also be

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Abbreviations:
LS, Linsmaier and Skoog;
2ip, isopentenylaminopurine,
BAP, 6-benzylaminopurine,
IAA, indole-3-acetic acid,
IBA, indole-3-butyric acid,
NAA, αnaphthalenacetic acid;
NOA, β-naphthoxyacetic acid,
PAA, phenylacetic acid,
GA_3, gibberellic acid

necessary for adequate rooting (23,30,36,43). Other factors affecting rooting include agar concentration (43), wounding the base of the stem (36,37), temperature (23), the time shoots are on proliferation medium (17) and the number of time shoots have been sub-cultured (38,40).

The benefits of micropropagation depend on rapid establishment and high survival rates once plantlets are transferred to an external environment in a potting medium. Few detailed studies have been made on this transfer procedure, but the problem areas primarily associated with poor water relations have been identified. There are four major factors. *Firstly*, there is a reduction or absence of epicuticular wax on the leaves of *in vitro* plants compared to glasshouse-grown plants (8,39). *Secondly*, the adequate functioning of stomata is delayed. Leaves of *in vitro* 'Pixy' plum lost more water than glasshouse-grown plants (3), the water loss occurring entirely from the underside of the leaves (6). For the first 3 days after transferring cultured 'Mac 9' apple to potting medium stomatal closure was low and water loss high, and only after 4 days did the stomatal closure mechanism develop (1). Treatments known to induce rapid stomatal closure were not effective with cultured leaves (2). *Thirdly*, high water loss has been attributed to reduced epicuticular wax and incomplete vascular development between the roots and the stem in cauliflower (9). *Finally*, leaf anatomical studies have revealed large air spaces between palisade and spongy mesophyll cells of cultured plants which may result in excess water loss (3,44).

Other factors found important for successful transfer of cultured plants include the use of antitranspirants (41, Hutchinson unpublished), the use of chilling or sprays with GA_3 when post-transfer growth is poor (10), and the incorporation or inoculation with appropriate mycorrhizal fungi (7,29,32,46).

With regard to successful transfer procedures, there are many problems still to be solved and many questions remain unanswered. In particular, the optimum potting medium and transfer environment need to be defined. The interaction of the prior cultural environment on the success of establishment is another area requiring further study. One recent advance is the initiation of roots and establishment of 'M-26' rootstocks combining the features of micropropagation and establishment in a single process (33).

Attention is drawn to more extensive reviews on fruit tree tissue culture (20,35,48), the micropropagation of deciduous fruit and nut species (13,24), and apple tissue culture (49).

The purpose of this paper is to briefly describe some factors which influence the establishment, shoot proliferation,

and root initiation and growth of the apple cultivar 'Northern Spy.' This cultivar was selected because certified virus-tested material is in limited supply, yet it is extensively used as a rootstock in Australia because it has semi-dwarfing characteristics and is resistant to woolly aphid (*Eriosoma lanigerum* Hausm.). Aspects of this work have been published previously (11,12).

MATERIALS AND METHODS

General: The basal medium of Linsmaier and Skoog (26), supplemented with 5 μM l^{-1} BAP and 1 μM l^{-1} IBA was used. Media were gelled with Difco Bacto agar at 0.8%. The pH of all media was adjusted to 5.8 prior to autoclaving at 100 kPa for 15 minutes. Cultures were incubated at 25°C with a 16:8 hour (light:dark) photoperiod under cool-white fluorescent tubes providing 75 μM m^{-2} sec^{-1}. Variations are indicated in the text. Shoot-tips 3 to 5 cm long were collected from the nursery at the Horticultural Research Institute, Knoxfield. They were brought to the laboratory and kept in running water for 1 hour before being sterilised for 1 minute in 70% ethanol containing 0.1% Tween 20 followed by 15 minutes in freshly prepared and filtered 5% calcium hypochlorite (w/v) containing 0.1% Tween 20 after which they were rinsed three times in sterile water. All subsequent manipulations were done in a laminar flow cabinet. After surface sterilization, the apical 3 to 5 mm was dissected out and placed vertically on the medium.

RESULTS AND DISCUSSION

Culture Establishment

a) *Time of year.* The effect of time of the year was studied by collecting shoot-tips in mid-spring, mid-summer, mid-autumn, and mid-winter.

Cultures could be established at any time during the year although contamination was least, tissue browning less of a problem, and growth more rapid if explants were collected in mid-spring or mid-summer. Explant browning was a significant problem with material collected in mid-autumn or mid-winter (Table 1).

Table 1. Effect of time of year on establishment, explant browning, and contamination

Time of year	Established	Explant browning	Contaminated
mid-spring	80%	15%	5%
mid-summer	90	10	0
mid-autumn	45	30	25
mid-winter	20	60	20

Medium LS with 5 μM l^{-1} BAP, 1 μM l^{-1} IBA and 1 mM l^{-1} phloroglucinol
n = 20

b) *Role of phloroglucinol.* The role of 1 mM l^{-1} phloroglucinol was studied on the establishment and following 5 sub-cultures using explants collected in mid-spring. The control medium had phloroglucinol omitted.

Phloroglucinol was effective in establishing proliferating cultures and more than doubled the number of shoots produced in each of the first two sub-cultures; however, by the fourth sub-culture phloroglucinol did not result in increased shoot number (Table 2). With these experiments phloroglucinol may be aiding in the uptake of cytokinins resulting in increased shoot proliferation.

Table 2. Effect of incorporating 1 mM l^{-1} phloroglucinol on shoot number for five sub-cultures

Sub-culture number	Plus phloroglucinol	Minus phloroglucinol
1	4.0	1.9
2	8.1	2.4
3	9.4	5.9
4	8 9	7 9
5	10.0	9.6

Medium: LS with 5 μM l^{-1} BAP and 1 μM l^{-1} IBA n = 20

Shoot proliferation

a) *Type of explant.* Five explant types from aseptic cultures were compared. Proliferation of the commonly used shoot-tips was compared with other explant types: i) single nodes placed vertically, ii) two nodes placed horizontally, iii) two or three shoot-tips resulting from stem fasciation and, iv) the basal mass material remaining after the removal of tissue in i) to iii).

All explant types were suitable for shoot proliferation. Vertical nodes and basal mass explants were better than single shoot-tips, clusters of two to three shoot-tips, and horizontal nodes (Table 3). The majority (63%) of single shoot-tips produced up to 6 shoots. The spread of shoot numbers was less and the nodal number was greater from clusters of two to three shoot-tips, greater again from single nodes, and even greater from basal mass explants. With nodes placed horizontally, 28% of the cultures produced single shoots and about 30% produced 10 to 16 shoots. The proliferation obtained with other explant types other than shoot-tips is interesting although not unexpected considering they contain dormant buds. What is unexpected is that shoot-tips are not the best type of explant to use to maintain cultures as the majority produce relatively few shoots. This allows other explant types to be used for routine proliferation and shoot-tips to be used immediately for root initiation, reducing further the time to obtain plantlets.

Table 3. Effect of explant type on shoot proliferation.

Explant type	Shoot number	Number of cultures examined
Single shoot tips	9 5 ± 0.5*	234
Clusters of 2-3 shoot tips	9.5 ± 0 4	146
Single nodes (vertical)	12 4 ± 0.4	129
Two nodes (horizontal)	8.3 ± 0 7	72
Basal mass	12 4 ± 0 5	32

Medium LS with 5 μM l^{-1} BAP and 1 μM l^{-1} IBA
*Standard error of mean

b) *Cytokinins.* Three concentrations (1, 5, and 10 μM l^{-1}) of each of four cytokinins (BAP, 2iP, kinetin, and zeatin) in factorial combination with light at five levels (25, 50, 75, 100, and 125 μM m^{-2} sec^{-1}) were evaluated. Single nodes and single shoot-tips were both tested as explant types.

Of the cytokinins tested BAP was by far the most effective for inducing shoot proliferation from both nodal buds and shoot-tips. For both explant types there was an increase in shoot number with increasing BAP concentration up to a light level of 75 μM m^{-2} sec^{-1}, after which it becomes supra-optimal, reducing proliferation. While proliferation was greatest at 10 μM l^{-1} BAP, the shoots were usually less than 1 cm tall and difficult to sub-culture, whereas with 1 or 5 μM l^{-1} shoots were between 1.5 and 2.5 cm tall and able to be readily sub-cultured. Kinetin, zeatin, and 2iP at, any concentration or light level, failed to induce any growth with nodal explants, whereas kinetin and zeatin were slightly promotive at 10 μM l^{-1} with shoot-tips (Tables 4 and 5). Single shoots with about 6 nodes developed with 2iP from shoot-tips. Since it was shown that BAP was suitable for inducing shoot proliferation in apple (31) it has been the most widely used cytokinin. These experiments confirm the results of Lundergan and Janick (28) which showed that BAP was superior to either kinetin or 2iP. With nodal explants BAP also has the advantage of breaking dormancy, whereas zeatin, kinetin, and 2iP are not.

Table 4. Effect of light level and BAP on shoot number using nodal explants

	Light level (μM m^{-2} sec^{-1}				
BAP concentration (μM l^{-1})	25	50	75	100	125
1 0	1.0	3 5	5 2	4.6	2.6
5.0	2 5	6 5	10.8	8.9	6 5
10 0	5 0	7 0	19 8	10.7	10.1

Basal medium LS with 1 μM l^{-1} IBA

Table 5. Effect of light level, and cytokinin type and concentration on shoot number, using shoot-tip explants

Cytokinin concentration (μM l^{-1})		Light level (μM m^{-2} sec^{-1}				
		25	50	75	100	125
Kinetin	1 0	2.1	1.0	1 0	1 0	1 0
	5 0	2.2	0.9	1.1	1.0	1 0
	10 0	4.0	3.5	3.5	3.5	1 2
Zeatin	1.0	1 5	1.1	1.8	1.4	1.5
	5 0	1 6	2 4	2 6	1 8	1 5
	10 0	6.1	4 8	4 6	4 5	1 5
BAP	1 0	4.0	4 2	4 4	4 3	4 3
	5 0	4.7	10.0	10.1	9 6	6 0
	10.0	4 9	16 0	17.1	16.8	9.8

Basal medium LS with 1 μM l^{-1} IBA

Root initiation and growth

a) *Auxins.* Three concentrations (1, 5, and 10 μM l^{-1}) of each of five auxins (IAA, IBA, NAA, NOA, and PAA) were tested in factorial combination with light at levels as for shoot proliferation.

Of the auxins tested, only IAA and IBA at 1 or 5 μM l^{-1} were suitable for root initiation. Both NAA and NOA tended to produce callus at the base of the stem with roots emerging from the callus but with no vascular connection between the roots and the stem. Phenylacetic acid was unsatisfactory as an auxin, with only about 20% root initiation at 10 μM l^{-1}.

b) *Salt concentration and physical support.* The effect of half and full strength LS salts (with organic addenda at normal concentration), and 1 and 5 μM l^{-1} IBA were tested in factorial combination with nine physical supports: agar, perlite, vermiculite, perlite:vermiculite (1:1), peat moss, coarse sand, peat moss:coarse sand (1:3), filter paper bridge, and liquid rotated medium (1 rpm).

Half-strength salts with 1 μM l^{-1} IBA was generally better than other chemical combinations, with high percentage root initiation in agar, perlite, coarse sand, and liquid rotated medium; however root number and length were low in agar and in perlite, compared to coarse sand and liquid rotated medium. This increase with coarse sand may be due to better aeration of the medium. Better aeration coupled with a greater absorptive area may be why a liquid rotated medium is so good; however, the resulting plantlets are fragile and translucent, making them difficult to establish in a glasshouse environment. The physical properties of vermiculite and peat moss are satisfactory for root initiation *in vivo* but may be unsuitable in this situation because of pH. These results support the finding that low salt concentrations are beneficial (23,36,43)

but only if used with low IBA concentrations and suggest that, depending on the choice of physical support, a one-step procedure may be suitable for both root initiation and elongation.

Establishment in potting medium

The influence of potting media was tested by evaluating mixes of peat moss:coarse sand (1:3), or perlite:vermiculite (1:1), with 5 sec. misting each 15 min. for 14 days. In addition, the effect of the same potting media were tested but with two applications of the anti-transpirant, Folicote® at 5% (v/v), one at the time of transfer and another 5 days later.

It is possible to transfer and establish rooted plants in potting media. Of those tested, a mixture of vermiculite:perlite was better than peat moss:coarse sand, if misting was used. Poor survival with peat moss:coarse sand may have been due to poor drainage. If anti-transpirants were used there was no difference between potting media (Table 6). Regenerated plants have shown no morphological differences to conventionally propagated plants.

Table 6. Effect of potting medium, misting, and anti-transpirant on survival percentage

Potting medium	Mist	Anti-transpirant	Control
Vermiculite perlite (1:1)	80	80	0
Peatmoss:coarse sand (1:3)	50	85	0

CONCLUSIONS

Aseptic cultures of 'Northern Spy' can be established at any time during the year although mid-spring and mid-summer are best. Shoot proliferation is maximum using nodal explants from aseptic cultures with 10 μM l^{-1} BAP and a light level of 75 μM m^{-2} s^{-1}, but the shoots are dwarfed and difficult to sub-culture. Better quality shoots, suitable for root initiation, were obtained when BAP concentration was reduced to 5 μM l^{-1}. Half-strength salts and 1 μM l^{-1} IBA result in maximum percentage root initiation and, if used in combination with a number of different physical supports, allowed control over the number and length of roots formed. Plantlets can be established in potting medium with a mixture of vermiculite-perlite, and either intermittent misting or sprays with an anti-transpirant.

LITERATURE CITED

1 Brainerd, K E. and L.H. Fuchigami 1981 Acclimatization of aseptically cultured apple plants to low relative humidity. *Jour. Amer. Soc. Hort. Sci* 106 515-518

2. Brainerd, K.E and L.H. Fuchigami 1982 Stomatal functioning of in *vitro* and greenhouse apple leaves in darkness, mannitol, ABA and CO_2. *Journ. Exp. Bot.* 33 388-392

3 Brainerd, K E , L H Fuchigami, S Kwiatkowski and C.S Clarke 1981 Leaf anatomy and water stress of aseptically cultured 'Pixy' plum grown under different environments. *HortScience* 16.173-175

4. Dunstan, D I 1981 Transplantation and post-transplantation of micropropagated tree-fruit rootstocks *Proc. Inter. Plant Prop Soc* 31 39-45

5 Frecon, J 1980 New tissue culture nursery opens in Oregon. *Compact Fruit Tree* 13 145.

6. Fuchigami, L H , T Y Cheng and A Soeldner 1981. Abaxial transpiration and water loss in aseptically cultured plum *Jour Amer. Soc. Hort. Sci* 106 519-522

7 Granger, R L., C Plenchette and J A Fortin. 1983. Effect of a vesicular arbuscular (VA) endomycorrhizal fungus (*Glomus epigaeum*) on the growth and leaf mineral content of two apple clones propagated *in vitro*. *Can Jour Plant Sci.* 63 551-555

8 Grout, B W W 1975 Wax development on leaf surfaces of *Brassica oleracea* var Currawong regenerated from meristem culture. *Plant Sci Lett* 5 401-405

9 Grout, B W W and M.J Ashton 1977 Transplanting of cauliflower plants regenerated from meristem culture I Water loss and water transfer related to changes in leaf wax and to xylem regeneration *Hort Res* 17 1-7

10. Howard, B H and V H Oehl. 1981 Improved establishment of *in vitro* propagated plum micropropagules following treatment with GA_3 or prior chilling. *Jour Hort Sci* 56 1-7

11. Hutchinson, J.F. 1982 *In vitro* propagation of apple using organ culture In A Fujiwara (Ed.), *Plant Tissue Culture 1982*. Proc 5th Intl Cong Plant Tissue and Cell Culture, Japan. pp 729-730

12. Hutchinson, J F 1984 Factors affecting shoot proliferation and root initiation in organ cultures of the apple 'Northern Spy' *Scientia Hortic* 22 347-358.

13 Hutchinson, J.F. and R H Zimmerman. Micropropagation of fruit and nut trees (In preparation.)

14 James, D.J. and I J Thurbon 1979. Rapid *in vitro* rooting of the apple rootstock M.9. *Jour Hort Sci* 54 309-311.

15 James, D J and I J Thurbon. 1980 *Report of East Malling Research Station for 1979*. p. 189

16 James, D.J. and I J Thurbon 1981 Shoot and root initiation *in vitro* in the apple rootstock M 9 and the promotive effects of phloroglucinol. *Jour Hort Sci* 15 15-20.

17 James, D.J ,and I J Thurbon. 1981 Phenolic compounds and other factors controlling rhizogenesis *in vitro* in the apple rootstocks M.9 and M 26 *Z Pflanzenphysiol* 105 1-10

18 Jones, O P 1967. Effect of benzyladenine on isolated apple shoots *Nature*. 215 1514-1515

19. Jones, O P 1976 Effect of phloridzin and phloroglucinol on apple shoots *Nature* 262·392-393 Erratum, *ibid* 262 274.

20 Jones, O P. 1983. *In vitro* propagation of tree crops In· S H Mantell and H. Smith (Eds.), *Plant Biotechnology*. Cambridge University Press pp. 139-159

21 Jones, O P and S.G S Hatfield. 1976 Root initiation in apple shoots cultured *in vitro* with auxins and phenolic compounds *Jour. Hort Sci.* 52 235-238

22. Jones, O P , C.A. Pontikis and M E Hopgood 1979 Propagation in vitro of five apple scion cultivars. *Jour Hort. Sci* 54.155-158.

23 Lane, W D 1978 Regeneration of apple plants from shoot meristem-tips *Plant Sci. Lett* 13 281-287

24 Lane, W D 1982. Tissue culture and *in vitro* propagation of deciduous *fruit and nut species In D.T Tomes, B.E Ellis, P.M. Harney, K J. Kasha,* and R L Peterson (Eds.), *Application of Plant Cell and Tissue Culture to Agriculture and Industry.* University of Guelph. pp 163-186.

25. Lane, W D. and J M McDougald 1982 Shoot tissue culture of apple. comparative response of five cultivars to cytokinin and auxin *Can. Jour. Plant Sci* 62 689-694

26 Linsmaier, E M and F Skoog. 1965 Organic growth factor requirements for tobacco tissue culture *Physiol. Plant* 18 100-127

27 Loreti, F and S Morini 1982 Mass propagation of fruit trees in Italy by tissue culture present status and perspectives *Proc. Inter Plant Prop Soc* 32 283-291

28 Lundergan, C A and J Janick 1980 Regulation of apple shoot proliferation and growth *in vitro Hort Res* 20 19-24

29 Morandi, D , S Gianinazzi and V Gianinazzi-Pearson 1979. Intérêt de léndomycorhization dans la reprise et al crossance du Frumboisier issu de multiplication végétative *in vitro. Ann Amélior Plantes* 29 623-630.

30 Nemeth, G 1981 Adventitious root induction by substituted 2-chloro-3-phenyl propionitriles in apple rootstocks cultured *in vitro Scientia Hortic* 14 253-259

31. Pieniazek, J 1968 The growth *in vitro* of isolated apple-shoot tips from young seedlings on media containing growth regulators. *Bull Acad. Polon. Sci , Cl.V.* 16 179-183.

32 Pons, F , V Gianinazzi-Pearson, S. Gianinazzi and J.C Navatel 1983 Studies of VA mycorrhizae in *vitro* mycorrhizal synthesis of axenically propagated wild cherry (*Prunus avium* L) plants *Plant and Soil* 71 217-221

33 Simmonds, J 1983 Direct rooting of micropropagated M26 apple rootstocks. *Scientia Hortic* 21 233-241

34 Singha, S 1982 Influence of agar concentration on in vitro shoot proliferation of Malus sp 'Almey' and Pyrus communis 'Seckel' Jour. Amer. *Soc Hort Sci* 107 657-660

35 Skirvin, R M. 1981 Fruit crops. In B V. Conger (Ed) *Cloning Agricultural Plants via In Vitro Techniques* CRC Press. pp 51-139

36 Snir, I and A Erez 1980 *In vitro* propagation of Malling-Merton apple rootstocks. *HortScience* 15 597-598

37. Sriskandarajah, S. and M.G. Mullins. 1981 Micropropagation of Granny Smith apple factors affecting root formation *in vitro. Jour Hort. Sci.* 56 71-76

38 Sriskandarajah, S , M G Mullins and Y Nair. 1982. Induction of adventitious rooting *in vitro* in difficult-to-propagate cultivars of apple. *Plant Sci. Lett* 24:1-9

39 Sutter, E and R W Langhans 1979. Epicuticular wax formation on carnation plantlets regenerated from shoot-tip culture *Jour Amer. Soc. Hort. Sci* 104 493-496

40 Takeno, K , J S Taylor, S Sriskandarajah, R P Pharis and M G Mullins 1982/83 Endogenous gibberellin- and cytokinin-like substances in cultured shoot tissues of apple *Malus pumila* cv. Jonathan, in relation to adventitious root formation. *Plant Growth Regulation* 1.261-268

41 Wardle, K , A Quinlan and L. Simpkins 1979 Abscisic acid and the regulation of water loss in plantlets of *Brassica oleracea* L var *botrytis* regenerated through apical meristem culture. *Ann Bot* 43 745-752

42 Welander, M and I. Huntrieser 1981 The rooting ability of shoots raised *in vitro* from the apple rootstock A2 in juvenile and in adult growth phase *Physiol Plant.* 53.301-306

43. Werner, E.M. and A.A. Boe 1980. *In vitro* propagation of Malling 7 apple rootstock *HortScience* 15.509-510

44. Wetzstein, H.V and H.E. Sommer. 1982 Leaf anatomy of tissue-cultured *Liquidambar styraciflua* (Hamamelidaceae) during acclimitization *Amer Jour. Bot* 69 1579-1586

45 Whiteley, E and A.J Abbott 1977 *Report Long Ashton Research Station for 1976* pp 62-63

46 Wooi, K C , C Y. Wong and R.H V Corley. 1982 Tissue culture of palms — a review In A N. Rao (Editor), *Tissue Culture of Economically Important Plants* GOSTED and ANBS, pp 138-144

47. Zimmerman, R H 1979 The laboratory of micropropagation at Cesna, Italy *Proc Inter Plant Prop Soc.* 29.398-400

48. Zimmerman, R H (Ed.). 1980 *Proceedings of the Conference on Nursery Production of Fruit Plants through Tissue Culture — Applications and Feasibility.* U S Dept. Agric , Sci. and Educ Adm , Agric Res Results ARR-NE-11, Beltsville, M.D. pp 119.

49 Zimmerman, R H 1984 Apple tissue culture. In. W R Sharp, D Evans, P Ammirato, and Y. Yamada (Eds), *Handbook of Plant Cell Culture Volume 2. Crop Species.* Macmillan, New York. pp 369-395.

50 Zimmerman, R.H. and O C. Broome. 1981 Phloroglucinol and *in vitro* rooting of apple cultivar cuttings *Jour Amer. Soc Hort. Sci* 106.648-652

PROPAGATION OF *CAMELLIA JAPONICA* USING HORTICULTURAL ROCKWOOL

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Our nursery has been experimenting for the past 5 years with various rooting media for camellia propagation. In the spring of 1982 it was suggested to us that we try Rockwool as a medium. The nurseryman making the suggestion had experienced great success in its use for the propagation of miniature roses. We purchased from the manufacturer of Rockwool, approximately 10,000 blocks measuring 38 × 38 × 40 mm. These blocks came in sheets of 28 units measuring 266 × 152 × 40 mm.