MICROPROPAGATION OF APPLE SCION CULTIVARS

S. SRISKANDARAJAH and M.G. MULLINS

Department of Agronomy and Horticultural Science
University of Sydney, Sydney, New South Wales, 2006

Abstract Shoots produced by isolated buds of the normally difficult-to-root cultivars, Granny Smith, Jonathan, and Delicious, were induced to form adventitious roots *in vitro* Isolated buds from adult trees were cultured in MS medium containing BA (10 μM) to produce proliferating shoot cultures These shoots were made into microcuttings for induction of roots The highest rooting percentage (80%) was obtained in 'Granny Smith' when microcuttings were grown in continuously agitated liquid culture (half-strength MS) with IBA (10 μM) The most effective culture method for 'Jonathan' was growing the microcuttings on filter paper bridges with either NAA (10 μM) or IAA (100 μM) Up to 80% of microcuttings of 'Delicious' produced roots when the bases of cuttings were dipped in IBA (750 μM) and grown in liquid medium Exogenous auxin was needed to stimulate root formation in all cultivars There was a progressive improvement in the rooting of microcuttings with increasing numbers of subcultures Newly established cultures had a low rooting capacity After 9 subcultures, 95% of 'Jonathan' microcuttings formed roots With 'Delicious' the percent rooting increased from 21% after 4 subcultures to 79% after 31 subcultures

INTRODUCTION

A wide range of plant species can now be propagated *in vitro* and the uses and limitations of tissue culture in horticulture have ben discussed in several reviews (2,13,18,15). The most extensive commercial use of plant tissue culture has been in clonal propagation of ornamentals, especially ferns and orchids. The application of aseptic methods to species that are easy-to-propagate by conventional methods is warranted only in special situations, for example, for the rapid multiplication of scarce material. In woody perennials, tissue culture could have an important role in the propagation of species which are difficult-to-root by conventional methods.

Although many species of woody plants have been successfully propagated by aseptic methods, there are still several crop and forest trees which cannot, as yet, be regenerated *in vitro*. In many recent reports on micropropagation, difficulties have been experienced in root induction in cultures derived from mature adult trees. It was also observed that tissue from juvenile seedlings and rootstocks had high regenerative capacity (7,8,10). These results are in accord with experience in conventional plant propagation. It is well established that there is a progressive loss of regenerative capacity during seedling ontogeny and that cuttings from adult flowering trees are usually very difficult to root (8). This lack of ability to form adventitious root primordia is a persistent character. When adult material is multiplied by grafting onto rootstocks the regrowth from the scion remains difficult-to-propagate. For

example, most commercially-important scion cultivars are difficult-to-root from cuttings and, with a few exceptions (1,9,16), apple cultivars have proved to be difficult to regenerate by micropropagation (3,4,14,17).

This paper summarises our work on the production *in vitro* of rooted plants of some normally difficult-to-root scion cultivars of apple.

MATERIALS AND METHODS

Establishment of cultures. Dormant one-year-old scions from virus-indexed mother trees of 'Granny Smith', 'Jonathan' and 'Delicious' were grafted onto apple seedlings and were grown either in controlled environment chambers (16 hr photoperiod, radiant flux denisty 350 μE $m^{-2}s^{-1}$) or in the glasshouse with natural illumination Regrowth from these scions was used as a source of explants The segments were surface sterilized and cut into single node segments and then planted into test tubes containing Murashige and Skoog medium (MS) (12) and 6-benzyladenine (10 μM, BA). The sucrose concentration was 30g l^{-1} The medium was solidifed with agar (0.8% w/v) Cultures were grown at 26 ± 2°C with continuous illumination provided by Osram MCFE 40W cool white fluorescent tubes

Production of microcuttings. Each nodal segment produced a single shoot 50 mm in length within 5 weeks of incubation. The shoots were cut into leafy segments and subcultured in larger culture vessels containing cytokinin medium as before. There were 3 to 4 segments per flask and within 5 weeks each segment had produced up to a dozen shoots by outgrowth of axillary meristems. These shoots were then harvested for use as microcuttings and the remains of the culture were cut into fragments and subcultured with cytokinin medium to produce a new generation of shoots

RESULTS AND DISCUSSION

Shoots were harvested from 30-day-old cultures and made into microcuttings, 20 to 30 mm in length. All experiments concerned with induction of roots were made at constant temperature 26° ± 2°C) with continuous illumination (Osram MCFE cool white fluorescent tubes, 90 $\mu E^{-2}sec^{-1}$). The microcuttings were grown by different methods, i.e., on agar-based media, on filter paper bridges with liquid media, in stationary liquid media, or in agitated liquid culture (shaken continuously on a reciprocating platform shaker — 70 strokes min^{-1}, displacement. 20 mm) Agar-based media were found to be unsuitable for induction of rooting. There were marked differ-

ences among cultivars in the most effective culture method for induction of adventitious roots. Agitated liquid culture was effective for 'Granny Smith' but not for 'Jonathan' or 'Delicious.' Up to 80% of microcuttings of 'Granny Smith' formed roots when grown with half-strength MS medium containing γ-(indole-3)-butyric acid (IBA, 10 μM) (16). The mechanism by which continuous shaking or agitation leads to the formation of adventitious roots is not yet clear.

A very high frequency of rooting (95%) was obtained with 'Jonathan' when microcuttings were grown on filter paper bridges in test tubes containing half-strength MS medium with either α-naphthalene acetic acid (NAA, 10 μM) or indolylacetic acid (IAA, 100 μM). With 'Delicious,' the bases of microcuttings were dipped in IBA (750 μM) and then grown with basal medium. About 80% of the cuttings of long-established cultures of 'Delicious' produced roots.

Exogenous auxins were needed to stimulate root formation in all cultivars. IBA (10 μM) and β-naphthoxyacetic acid (NOA, 10 μM) both promoted root formation in 'Granny Smith'. Higher concentrations of auxins produced fewer roots and soft white callus at the bases of cuttings IAA (100 μM) and NAA (10 μM) were both effective in promoting root formation. IBA (750 μM) was found to be the most effective auxin for rooting 'Delicious', particularly when given as a basal dip.

It was found that newly established cultures of 'Jonathan' and 'Delicious' had a low regenerative capacity. The initial explants, and microcuttings from the first few subcultures, produced roots at very low frequency (Table 1). A marked increase in root formation was observed in later subcultures. In 'Jonathan', for example, the rooting percentage at the 4th subculture (62%) was almost 8 times greater than the rooting percentage at the initial culture (8%).

Table 1 Adventitious root formation *in vitro* in apple cultivars effects of subculture

Jonathan				Delicious			
Culture No	No micro-cuttings	No rooted	Percent rooted	Culture No	No micro-cuttings	No rooted	Percent rooted
†0	100	0	0	0	34	0	0
*1	62	5	8	1	21	1	5
**1	105	34	32	1	85	13	15
2	117	51	44	2	86	17	20
3	106	55	52	3	90	-	-***
4	166	102	62	4	86	18	21
5	149	105	70				
9	105	100	95	31	24	19	79
25	112	108	96	32	20	14	70
28	92	87	95				

† Nodal cuttings from whole plant tested in vitro, * Initial culture, ** First subculture, *** Culture lost

Our results show that the normally difficult-to-root cultivars, 'Jonathan' and 'Delicious' can be transformed *in vitro* into easily rooted plants. The possibility that the transformation is associated with genetic change cannot be discounted until orchard testing for trueness-to-type has been completed. The occurrence of genetic change in standard apple cultivars would be of great interest from the viewpoint of clonal selection but it would render the subculture method useless for routine plant propagation.

The physiological changes in subcultured shoots which favour adventitious root formation are still being investigated. There seem to be parallels between the subculture technique and procedures such as hedging which are used in horticulture and forestry to produce cuttings with an enhanced capacity for root formation (11). In all cases the stock plants are subjected to repeated pruning and these are indications that this treatment leads to rejuvenation (5), the restoration of morphological and/or physiological characteristics of the juvenile growth phase. In subcultures of 'Jonathan' and 'Delicious' the improved root formation of microcuttings was associated with the appearance of morphological characters in shoots which are reminiscent of apple seedlings, for example, highly serrated leaf margins, a paucity of primary phloem fibres and production of anthocyanin pigment by the stem epidermis (S. Sriskandarajah, unpublished results).

Finally, it is possible that cultivation of explants in agitated media or use of the repeated subculture technique, could be applied to many other difficult-to-propagate species.

Acknowledgment. This research is supported by the Rural Credits Development Fund, Reserve Bank of Australia

LITERATURE CITED

1 Abbott, A J and E Whiteley, 1976 Culture of *Malus* tissues *in vitro* 1 Multiplication of apple plants from isolated shoot apices *Scientia Hort* 4 183-189

2 Abbott, A J 1978 Practice and promise of micropropagation of woody species *Acta Hort* 79 113-127

3 Dutcher, R D and L E Powell 1972 Culture of apple shoots from buds *in vitro* *J Am Soc Hort Sci* 97 511-514

4 Elliott, R F 1972 Axenic culture of shoot apices of apple *N Z J Bot* 10 254-258

5. Garner, R J and E S T Hatcher 1962 Regeneration in relation to vegetative vigour and flowering *16th Int Hort Congr Brussels*

6 Hartmann, H T and D E Kester 1975 Plant Propagation Principles and Practices, 3rd ed , Prentice-Hall, Englewood Cliffs, New Jersey

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

8 Jones, O P and S G S Hatfield 1976 Root initiation in apple shoots cultured *in vitro* with auxin and phenolic compounds *J Hort Sci* 51 495-499

9 Jones, O P , C A Pontikis and M E Hopgood 1979 Propagation *in vitro* of five apple scion cultivars *J Hort Sci* 54 155-158

10 Lane, D W 1978 Regeneration of apple plants from stock meristem-tips *Plant Sci Lett* 13 281-285

11 Libby, W J and J V Hood 1976 Juvenility in hedged Radiata pine *Acta Hort* 56 91

12 Murashige, T and F Skoog 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures *Physiol Plant* 15 473-497

13 Murashige, T 1974 Plant propagation through tissue cultures *Ann Rev Plant Physiology* 25 135-166

14 Quoirin, M 1974 Premiers r´esultats obenus dans la culture in vitro du m´erist´eme apical de sujets po greffe de pommier Bull Rech Agron Gemblous 9 182-192

15 Rowe, R N 1981 Tissue culture — its advantages and limitations for the multiplication of horticultural crop plants *Seed and Nursery Trader*, April pp 9-16

16 Sriskandarajah, S and M G Mullins 1981 Micropropagation of Granny Smith apple Factors affecting root formation *in vitro* *J Hort Sci* 56 71-76

17 Walkey, D G 1972 Production of apple plantlets from axillary-bud meristems *Can J Pl Sci* 52 1085-1087

18 Winton, L L 1978 Morphogenesis in clonal propagation of woody plants In Frontiers of Plant Tissue Culture, ed Thorpe, T A pp 419-426

REGENERATION OF GRAPEVINES BY ASEPTIC METHODS

K. RAJASEKARAN and M.G. MULLINS

Department of Agronomy and Horticultural Science
University of Sydney, Sydney, New South Wales 2006

Abstract. Techniques are described for high-frequency somatic embryo and plantlet formation from (i) cell suspensions derived from nucellar callus of unfertilized ovules, and (ii) somatic cells of cultured anthers Plantlet regeneration by organogenesis, induction of adventitious buds and adventitious roots, has been achieved in a few genotypes of *Vitis* and in the Muscadine grape Factors affecting the regenerative competence *in vitro* of grape tissues include genotype (species, cultivar), growth phase (juvenile or adult), and origin of explants Competence is a heritable character Evidence is accumulating that grapevines produced *in vitro* are variable and that tissue culture *per se* leads to genetic variation It is concluded that the

Abbreviations.
BA — benzyladenine (syn 6-benzylaminopurine)
2,4-D — 2,4-dichlorophenoxy acetic acid
NOA — β-naphthoxy acetic acid
PBA — 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine