26. Neumann, P. M. and Z. Stein. 1983. Xylem transport and the regulation of leaf metabolism. What's New in Plant Physiol. 14:33-36.
27. Nobel, P. S., L. J. Zaragoza, and W. K. Smith. 1975. Relation between mesophyll surface area, photosynthetic rate and illumination level during development for leaves of *Plectranthus parviflorus* Henckel. Plant Physiol. 55:1067-1070.
28. Palmer, J. W. 1988. Annual dry matter production and partitioning over the first 5 years of a bed system of Crispin/M.27 apple trees at four spacings. J. Applied Ecol. 25:569-578.
29. Porpiglia, P. J. and J. A. Barden. 1980. Seasonal trends in net photosynthetic potential, dark respiration, and specific leaf weight of apple leaves as affected by canopy position. J. Amer. Soc. Hort. Sci. 105:920-923.
30. Sage, R. F. and R. W. Pearcy. 1987. The nitrogen use efficiency of C3 and C4 plants. II. Leaf Nitrogen effects on the gas exchange characteristics of *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). Plant Physiol. 84:959-963.
31. Sage, R. F., R. W. Pearcy, and J. R. Seemann. 1987. The nitrogen use efficiency of C3 and C4 plants III Leaf nitrogen effects on the activity of carboxylating enzymes in *Chenopodium album* (L.) and *Amaranthus retroflexus* (L.). Plant Physiol. 85:355-359.
32. Seemann, J. R., T. D. Sharkey, J. Wang, and C. B. Osmond. 1987. Environmental effects on photosynthesis, nitrogen-use efficiency, and metabolite pools in leaves of sun and shade plants. Plant Physiol. 84:796-802.
33. Sinclair, T. R. and T. Horie. 1989. Leaf nitrogen, photosynthesis and crop radiation use efficiency: A review. Crop Sci. 29:90-98.
34. Skene, D. S. 1974. Chloroplast structure in mature apple leaves grown under different levels of illumination and their response to changed illumination. Proc. R. Soc. Lond. B. 186:75-78.
35. Stephenson, A. G. 1981. Flower and fruit abortion: Proximate causes and ultimate functions. Annu. Rev. Ecol. Syst. 12:253-279.
36. Syvertsen, J. P. 1984. Light acclimation in citrus leaves. II CO_2 assimilation and light, water, and nitrogen use efficiency. J. Amer. Soc. Hort. Sci. 109:812-817.
37. Walters, M. B. and C. B. Field. 1987. Photosynthetic light acclimation in two rainforest Piper species with different ecological amplitudes. Oecologia 72:449-456.
38. Weinbaum, S. A., S. M. Southwick, K. A. Shackel, T. T. Muraoka, W. Krueger, and J. T. Yeager. 1989. Photosynthetic photon flux influences macroelement weight and leaf dry weight per unit of leaf area in prune tree canopies. J. Amer. Soc. Hort. Sci. 114:720-723.

Fruit Varieties Journal 48(3):170-173 1994

Improved Rooting of Ottawa 3 Apple Rootstock by Soft Wood Cuttings Using Micropropagated Plants as a Cutting Source

H. A. QUAMME AND E. J. HOGUE[1]

Abstract

The rooting of Ottawa 3 apple rootstock by softwood cuttings under intermittent mist was improved by using a source of micropropagated plants (63%) in comparison to two conventionally propagated sources (47 and 42%). Softwood cuttings from the micropropagated plants also developed larger root masses than softwood cuttings from the conventional sources. Basal cuttings rooted better than tip cuttings (71 *vs.* 23%).

Ottawa 3 (O.3) is a precocious apple rootstock between M.9 EMLA and M.26 in size-controlling potential (6, 9, 13). The main advantage of O.3 is its resistance to winter injury (2, 12, 15). However, it has the disadvantage of being difficult to propagate by stool-bed-layering (2, 13), the preferred commercial method of apple rootstock propagation. It has been successfully propagated by root cuttings (4), but results with softwood cuttings have been variable. Spangelo et al. (13) reported that rooting of softwood cuttings taken from conventional beds ranged from 34 to 62%, whereas Nelson (8) reported that rooting ranged from 89 to 100%. A 94% success rate was

[1]Agriculture Canada, Research Station, Summerland, British Columbia V0H 1Z0. Contribution No.854.

found in rooting of O.3 derived from tissue culture (8).

Recently, improved rooting of cuttings of M.9 and O.3 derived from tissue cultured plants was reported by Webster and Jones (1992). They obtained 91% rooting by softwood cuttings from micropropagated plants, whereas 41% rooting was obtained from conventional ones. In this paper, we confirm that softwood cuttings of O.3 taken from tissue cultured plants root more readily and develop larger root masses than those taken from plants propagated conventionally.

Three sources of Ottawa 3 were established in stoolbeds at Summerland Research Station for rooting trials: tissue cultured plants from Agri-Forest Industries, Kelowna, B.C.; plants from Agriculture Canada Plant Quarantine Station, Sidney, B.C., propagated on their own roots by nurse grafts (Sidney source); and plants from the University of Saskatchewan, Saskatoon, Saskatchewan, propagated by softwood cuttings (Saskatoon source). The plants used by Agri-Forest Industries, Kelowna, as a source for tissue culture material, were obtained from the Agriculture Canada Plant Quarantine Station, the same source as the Sidney plants. These plants were originally obtained from the Ottawa Central Experimental Farm, Agriculture Canada, Ottawa, Ontario. The number of subcultures that were performed on the tissue cultured stock plants is unknown. The Saskatoon source was originally derived directly from the stoolbeds at the Ottawa Central Experimental Farm and maintained in stoolbeds at Saskatoon with heavy cutting before transfer to Summerland. All sources of O.3 were virus-free and were grown in adjacent stoolbeds for at least three years before harvesting cuttings.

Table 1. Rooting of softwood cuttings under intermittent mist harvested from three sources.

Cutting block source	Percentage rooting	Average root score (0-3)[x]
Tissue cultured	63 a[y]	1.7 a
Saskatoon	47 ab	1.4 ab
Sidney	42 b	1.2 b

[y]Mean separation within columns by Duncan's Multiple Range Test, P = 0.05 (combined for years 1988, 1989, and 1991, n = 24 plots of 5 to 10 cuttings.)

[x]0 = no roots; no callus; 1 = no roots; callus; 2 = 1-3 rooting points; and 3 = > 3 rooting points.

Water was applied to the stoolbeds with overhead microsprinkler irrigation. Nitrogen was applied (200 g N per m) and foliar applications of 20-20-20 (5 kg/ha) was applied 2 or 3 times per season. The stoolbeds were cut back to the crown each year.

Shoots approximately 40 cm in length were taken from stoolbed plants in the fourth week of June each of the three years of the trial. The top several cm of succulent growth was removed and shoots were sectioned into two cuttings, tip and base, about 15 cm long. All the leaves were removed from the cutting except the top two and the bases of the cuttings were dipped for 10 sec in a 2000 ppm indolebutyric acid solution in 50% ethanol. When dried, the cuttings were inserted into moist perlite, placed in a greenhouse room with intermittent mist for eight weeks, and then examined for rooting and decay. The percentage of rooting was recorded and the amount of root mass scored.

The cuttings were placed in the mist bed in a complete randomized block design with eight replicates each containing 5 to 10 cuttings. The trial was carried out for three years, 1988, 1989, and 1991. The data was analyzed as a factorial design using the SAS General Linear Model (12). An arcsin transformation was performed on percent data prior to analysis and mean separation by Duncan's multiple range test. Data in Table 1 are non-transformed means. In 1989 the difference in percentage rooting between cuttings made from tips and bases of shoots was recorded and analyzed by SAS-GLM.

Since no significant differences were found between years, the data was

combined and means of all years are presented. The cuttings from the tissue cultured source had significantly higher percentage rooting and root scores than the cuttings from the Sidney source, but not significantly higher than that of the Saskatoon source (Table 1). The Sidney source was not significantly different in percentage rooting and root scores from the Saskatoon source. The Saskatoon source originated from the source used by Nelson to achieve high percentage rooting (89 to 100%), but this source did not yield as high a percentage rooting in our trials. The relative differences in root score was similar to relative differences in percentage rooting. In 1989 when basal cuttings were compared to tip cuttings, basal cuttings had a significantly higher mean rooting percentage than tip cuttings (71% vs. 23%, significantly different at $P = 0.05$). The percentage rooting of cuttings from the tissue cultured source observed in this study (63%) is relatively low compared to that reported by Hogue and Neilsen (5) (94%) but this may be explained by the fact that succulent tips were included in our study and not in Hogue and Neilsen's study. Webster and Jones (16) achieved higher rooting with softwood and hardwood cuttings from micropropagated plants than from conventional ones. Although rooting by softwood cuttings may differ depending on rooting conditions and cutting type, the use of tissue cultured plants as a cutting source may be used to improve rooting of 0.3.

Nothing is known about the difference in growth and cropping of scions grown on O.3 cuttings propagated from micropropagated plants. A difference in size-controlling potential was found between tissue cultured and stool-layered M.9 rootstocks (7). However, no difference in size-controlling potential was reported between tissue cultured and stool-layered M.26 (7, 11). Budded trees on rooted cuttings of the different sources of O.3 would have to be grown in orchard trials to determine if there is any difference in growth response.

A possible explanation for the improved rooting is rejuvenation of the clone by micropropagation. The juvenile phase of apple has been shown to have greater rooting capacity and to differ in leaf shape, anthocyanin content, and morphology compared to the mature phase (1, 3, 14). Juvenile buds may have been selected during the course of tissue culture or may have arisen from reversions. However, plants grown from cuttings of the three sources in the greenhouse did not show any obvious difference in leaf shape, or shoot color and morphology characteristic of the two phases of growth. A similar observation on M.9 and O.3 was made by Webster and Jones (16).

References

1. Blair, D. S., M. MacArthur, and S. H. Nelson. 1961. Observation in the growth phases of fruit trees. Proc. Amer. Soc. Hort. Sci. 67:75-79.
2. Domoto, P. A. 1991. Low temperature tolerance of 'Starkspur Supreme Delicious' on nine rootstocks in Iowa 1980-1981 NC-140 Cooperative Planting. Fruit Var. J. 45:224-229.
3. Fritzsche, R. 1948. Untersuchungen über die Jugendformen des Apfel-und Birnbaumes und ihre Kansequenzen für du Unterlagen-und Sorten züchtung. Ber. Schwiez Bot. Ges. 58:206-207.
4. Granger, R. L., and E. J. Hogue. 1974. Propagating apple rootstocks by root cuttings. Agric. Can. Tech. Bull. J550.
5. Hogue, E. J., and D. Neilsen. 1991. Rapid production methods for Ottawa-3 rootstock and branched apple nursery stock. HortScience 26:1416-1419.
6. NC-140. 1991. Performance of 'Starkspur Supreme Delicious' on 9 rootstocks at 27 sites over 10 years. Fruit Var. J. 45:192-199.
7. Navatel, J. C., M. Nio, P. Vaysse, and M. Edin. 1988. Pommier. Comportement en marcottières et aux vergers des porte-greffes micropropagés de type *Malus*. Infos, Centre Technique Interprofessionnel des Fruits et Légumes, France 44:25-28.
8. Nelson, S. H. 1976. Propagation of Ottawa apple rootstocks by softwood cuttings. Can. J. Plant Sci. 56:511-515.

9. Quamme, H. A., and R. T. Brownlee. 1990a. Rootstocks for high density apple plantings in British Columbia. Agr. Can. Summerland, B.C. Tech. Rpt. 90-02.
10. Quamme, H. A., and R. T. Brownlee. 1990b. Stool layering ability of thirty-one apple rootstock cultivars. Fruit Var. J. 44:165-169.
11. Quamme, H. A., and R. T. Brownlee. 1993. Early performance of micropropagated trees of several *Malus* and *Prunus* cultivars on their own roots. Can. J. Plant Sci. 73:847-855.
12. SAS Institute. 1990. SAS/STAT user's guide. Version 6, 4th edition. SAS Institute, Cary N.C.
13. Spangelo, L. P. S., S. O. Fejer, S. J. Leuty, and R. L. Granger. 1974. Ottawa 3 clonal apple rootstock. Can. J. Plant Sci. 54:601-603.
14. Stoutmeyer, V. T. 1932. Regeneration in various types of wood. Iowa State College. Research Bulletin 220:311-353.
15. Warmund, M. R., D. C. Ferree, P. Domoto, J. A. Barden, C. A. Mullins, and R. L. Granger. 1991. Blackheart injury in 'Starkspur Supreme Delicious' on rootstocks in the 1980-1981 NC-140 cooperative planting. Fruit Var. J. 45:219-223.
16. Webster, C. A., and O. P. Jones. 1992. Performance of field hedge and stoolbed plants of micropropagated dwarfing apple rootstock clones with different degrees of apparent rejuvenation. J. Hort. Sci. 67:521-528.

Fruit Varieties Journal 48(3):173-175 1994

Culture Date and Germination Procedure Affects Success of Nectarine Ovule and Embryo Culture

JOSE X. CHAPARRO AND W. B. SHERMAN

Abstract

Immature 'Sunlite' nectarine ovules were grown on Knop's ovule growth medium for 30 days and germinated following stratification either in vitro on a modified Knop's germination medium or in moist perlite. The minimum age at which any embryos were successfully ovule cultured and germinated was 53 days after full bloom. Germination percentage increased significantly at 60 and 67 days after full bloom. Germination percentage in perlite was not greatly different from that of ovules cultured in vitro on modified Knop's germination medium, but the 53 and 60 days after full bloom embryos germinated in perlite were visibly weaker.

Embryo culture is an important component of many classical breeding programs and has been used to rescue immature embryos from early ripening fruits, in fruits that exhibit embryo abortion, and in embryos from interspecific hybrids where embryo abortion occurs during development. Embryo culture is now being used by grape (*Vitis vinifera* L.) breeders to develop seedless table grape cultivars (2, 3, 8). Embryo (without seed coat) and ovule (with seed coat) culture techniques are also commonly used by *Prunus* breeders in the development of early ripening cultivars (7).

Embryo rescue is of particular importance in the peach (*Prunus persica* (L.) Batsch) breeding program at the University of Florida. The window for peach and nectarine production in Florida is early and narrow. Fruit must ripen within 100 days after flowering, ie. fruit development must occur between spring frost and the summer rainy season in early June. The emphasis on early-ripening enhances the need for embryo rescue so that both parents contribute to a short fruit development period in the hybrid progeny, thereby increasing the percentage of early ripening individuals from which selections can be made (5).

Embryo survival in vitro depends on the medium used and the stage of embryo development (7). The younger the embryo, the more complex the media required to insure embryo survival growth (6). These experiments

Florida Agricultural Experiment Station Journal Series No. R03343.