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IN VITRO SHOOT MICROPROPAGATION AND PLANT ESTABLISHMENT OF 'ORLANDO SEEDLESS' GRAPE AND 'TAMPA' ROOTSTOCK

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Abstract. **Factors influencing in vitro shoot production and rooting of the *Vitis* hybrid cv. Orlando Seedless grape and *Vitis* hybrid cv. Tampa rootstock were explored. 'Orlando Seedless' produced an average of 4 shoots per meristem during successive subcultures. Basal tissue masses not only had equivalent shoot proliferation rates but were easier to manipulate than meristems. Equivalent numbers of shoots were produced on Murashige and Skoog's medium and C_2D medium. Soil rooting was judged superior to in vitro rooting because a major culture manipulation was eliminated and plants were produced more rapidly. 'Tampa' rootstock produced an average of 3.5 shoots per meristem.**

Vitis hybrid cv. Orlando Seedless was released by the University of Florida in Jan. 1986 as the first seedless cultivar resistant to Pierce's disease and, thus, able to persist in Florida (3, 4). It is recommended that 'Orlando Seedless' be grafted onto *Vitis* hybrid cv. Tampa rootstock for optimum performance (3, 4). Typically, dormant wood of a new cultivar is first released to nurseries so that plants can be propagated by conventional vegetative means. However, conventional grape propagation is relatively slow and a lag time of several years exists between release and availability of commercially acceptable numbers of plants.

In vitro shoot micropropagation is an alternative to conventional propagation. Micropropagation is already commercially used for a variety of clonally propagated crops to produce plants at rates that far exceed conventional methods. Adoption by the industry of micropropagation technology for grape would alleviate the lag time between release and availability of new cultivars. We previously described simple methods to micropropagate 35 grape species, hybrids and cultivars, including 'Orlando Seedless' (2). In the present report, we study 'Orlando Seedless' in more depth in order to provide specific recommendations for micropropagating and producing rooted plants. Preliminary information for 'Tampa' rootstock is also included.

Materials and Methods

Plant material. Cuttings from vineyard plants of 'Orlando Seedless' and 'Tampa' were rooted in a mist chamber and established in 23-cm pots on greenhouse benches. Plants were pruned repeatedly to produce abundant shoot tips for explant material.

Culture initiation and maintenance. All leaves and tendrils were removed from excised shoot tips prior to surface sterilization for 2.5-3 min in 25% commercial bleach containing a drop of Triton X surfactant. Shoot tips were rinsed twice and stored in sterile distilled water. The apex of each shoot was dissected and placed, cut surface down, on autoclaved solid C_2D medium (1) containing 5 μM benzylaminopurine (BAP) and prepared as previously described (2). Cultures were incubated at 25°C with an 18 hr light/6 hr dark cycle using cool white fluorescent lights. Cultures were transferred to fresh medium every 5 weeks. The number of shoots produced by each culture was counted prior to transfer. Further details of culture methodology are provided in the results.

Results and Discussion

The first response of 'Orlando Seedless' meristems plated onto C_2D medium containing 5 μM BAP was enlargement and production of adventitious buds. Continued adventitious bud production eventually resulted in an enlarged mass of basal tissue from which shoots grew. Culture volume was increased by dissecting apical meristems from shoots and transferring them to fresh medium. Alternatively, plants were produced by rooting harvested shoots. Several experiments were conducted to evaluate overall performance of 'Orlando Seedless' tissue cultures and to determine optimum methods of propagating and producing rooted plants.

Shoot proliferation from meristems during successive subculture. In order to evaluate the reliability of micropropagation over time, meristems from established cultures were subcultured successively for three culture periods (Table 1). Although considerable variation existed, an overall average of four shoots per meristem was obtained. Considering an initial plating of 100 meristems, 25,600 shoots could be produced in four successive culture periods at this multiplication rate. This rate of increase is commercially acceptable to produce plants for grafting.

Comparison of meristem and basal tissue masses for shoot proliferation. Subculture via transfer of excised meristems is a rather precise and tedious method of culture increase. Therefore, a simpler alternate method of subculturing by transfer of basal tissue masses was tested. To utilize basal tissue, shoots from 5-week-old cultures were trimmed from the tissue mass, leaving stem remnants approximately 4 mm long. Prior to transfer to fresh C_2D medium, the bottom of the mass was trimmed to expose fresh tissue. Shoot proliferation rates from meristems and basal tissue were compared (Table 2). Although basal tissue produced more

Table 1. Shoot multiplication from meristems of 'Orlando Seedless' during successive subculture.[z]

Culture period[y]	No. observations	Shoots/meristem
1	167	3.77 ± 1.50
2	182	5.18 ± 3.15
3	227	4.19 ± 1.92

[z]Culture medium was C_2D with 5 μM benzylaminopurine.
[y]Each culture period was 5 weeks.

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Table 2. Comparison of shoot proliferation from meristem and basal tissues of 'Orlando Seedless' after 5 weeks of culture.[z]

Tissue	No. observations	Shoots/explant
Meristem	576	4.38 ± 2.19
Basal tissue	106	5.56 ± 2.49

[z]Culture medium was C_2D with 5 μM of benzylaminopurine.

shoots than meristems, the variation exhibited by each overlapped so that a statistical difference was not shown. Manipulation of basal tissue is much less tedious than meristems. Therefore, for initiation, increase and establishment of an 'Orlando Seedless' culture line, we recommend the following: 1) Culture initiation should be via meristem explants as previously described (2) in order to obtain pure cultures. 2) The increase phase should employ all possible tissue sources, including meristem and basal tissue, to produce cultures as quickly as possible. 3) Maintenance of the line should be by basal tissue due to ease of manipulation.

Comparison of C_2D with MS medium. We have used C_2D medium for all micropropagation experimentation due to its proven superiority for Eastern grape species (1). C_2D (1) is a derivative of Murashige and Skoog's (MS) medium (5), a commonly used plant tissue culture formulation. Use of MS medium would be procedurally advantageous because it can be purchased premixed whereas C_2D must be formulated in the laboratory. Therefore, shoot proliferation on C_2D was compared with MS. Approximately 4 shoots per meristem were obtained on each medium (Table 3). Consequently, MS can be substituted for C_2D medium; however, careful preliminary testing of premixed MS is recommended before large-scale incorporation into a commercial propagation system.

Comparison of rooting methods. Both in vitro and greenhouse rooting methods were tested. In vitro rooting methods were compared by placing 2-, 4- and 6-node cuttings in C_2D medium with 0.4 μM naphthaleneacetic acid (NAA) (Table 4). In greenhouse rooting tests, 4-node cuttings were placed directly in soil, either in a mist chamber or a sealed moist high humidity chamber. The best results in vitro were with 6-node cuttings where 94 percent produced roots. Fifty-six percent of 4- and 2-node cuttings rooted. For greenhouse rooting, best results were from the humidity chamber environment where 70 percent rooting was achieved compared to 40 percent for the mist chamber. Greenhouse methods not only yielded a higher rooting percentage than in vitro methods for 4-node cuttings but a significant savings in time and manpower is realized because a major tissue culture step is eliminated. In vitro rooted plants still must be transferred to soil and acclimated. In addition, the threat of contamination or other disasters during in vitro rooting is eliminated. Therefore, we favor greenhouse rooting over in vitro rooting.

Table 4. Comparison of methods for rooting in vitro shoots of 'Orlando Seedless'.

Method	*% Rooted shoots*
In Vitro[z]	
2 node cuttings	56
4 node cuttings	56
6 node cuttings	94
Greenhouse Rooting (4-node cuttings)	
Mist chamber	40
Humidity chamber	70

[z]In vitro rooting medium was C_2D with 0.4 μM naphthaleneacetic acid.

Shoot proliferation from 'Tampa' rootstock. 'Orlando Seedless' requires grafting for optimum performance. Therefore, it is also necessary to be able to rapidly increase the recommended 'Tampa' rootstock to fully utilize the efficiency of micropropagation for this table grape. 'Tampa' produces approximately 3.5 shoots per meristem when cultured on C_2D medium (Table 5). Although the shoot proliferation rate for 'Tampa' is slightly lower than that obtained for 'Orlando Seedless', it should be satisfactory to provide rootstock for commercial production.

Exploiting the full efficiency of micropropagation technology for grafted varieties such as 'Orlando Seedless' depends on use of rapid grafting techniques. Several possibilities for producing grafted micropropagated plants exist. Scion and rootstock could be grown to a suitable size and allowed to become dormant through winter before conventional grafting. However, this would impose a one year lag period before salable plants were produced. Alternatively, green wood grafting of younger plants would result in grafted plants within the first growing season. Another possibility is to produce grafted plants directly in tissue culture via micrografting. Although this technique has not been demonstrated for grape, theoretically, micrografted plants could be produced at extremely high efficiencies when compared to other grafting methods.

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Table 3. Comparison of MS and C_2D culture media[z] for shoot proliferation from meristems of 'Orlando Seedless'.

Media	No. observations	Shoots/meristem[y]
MS	73	4.03 ± 1.90
C_2D	227	4.19 ± 1.92

[z]Both media were supplemented with 5 μM benzylaminopurine.
[y]Data taken after 5 weeks of culture.

Table 5. Shoot multiplication from meristems of 'Tampa' during successive subculture.[z]

Culture period	No. observations	Shoots/explant
1	51	4.61 ± 1.99
2	66	3.09 ± 1.25
3	11	3.09 ± 1.14

[z]Culture medium was C_2D with 5 μM benzylaminopurine.