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IN VITRO MICROPROPAGATION AND PLANT ESTABLISHMENT OF 'BLANC DU BOIS' GRAPE

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Abstract. **Methods to micropropagate 'Blanc du Bois', a Florida hybrid bunch grape (*Vitis* spp.), were developed. Fifty percent of shoot apex explants obtained from field-grown plants produced viable cultures. Of four medium salt formulations tested, Murashige and Skoog (MS) and C_2D (a modification of MS) produced the most shoots per cultured apex. Comparison of the effect of the cytokinins benzyladenine and thidiazuron on shoot production showed that all concentrations of each that were tested produced significantly more shoots per apex than no-cytokinin controls (4.0 and 4.3, respectively vs. 0.7) whereas the best level of kinetin (10μM) produced only 0.6. Although similar in overall response, shoots produced with benzyladenine were larger and more normal in appearance than those from thidiazuron, which were small and stunted. Both in vitro and in vivo rooting methods were examined. For in vitro rooting, shoots placed on medium containing 1μM naphthaleneacetic acid produced longer roots than those on unsupplemented medium, although the total number of shoots that rooted and the number of roots per shoot were statistically similar. In vivo rooting was accomplished by placing shoots directly into moist potting mix, with or without a commercial rooting powder pretreatment. Rooting powder significantly increased root number but not length. Overall, in vivo rooting percentage was greater than that obtained from in vitro. In vivo rooting was more efficient since vigorous, acclimated plants were produced in less time. Furthermore, a major in vitro manipulation was eliminated by in vivo rooting.**

'Blanc du Bois' is a new Florida hybrid bunch grape cultivar released by the University of Florida in 1987 (5). It produces a premium white wine that sets a new quality standard for this state. The wine potential shown by this grape has resulted in an increase in acreage and a shortage of plants. In vitro micropropagation can be used to produce plants at rates in excess of conventional propagation methods (1, 3). Micropropagation has successfully been demonstrated for many grape species, hybrids and cultivars, including several Florida bunch grape hybrids (2, 3). In this report, we adapt micropropagation technology to 'Blanc du Bois'.

Materials and Methods

Twenty shoot tips approximately 4 cm in length were excised from rapidly growing plants at the CFREC Leesburg experimental vineyard and placed between layers of moist paper towels. In the laboratory, shoot tips were further dissected to approximately 6 mm in length and all leaves and tendrils were removed, except for those very small appendages enclosing the shoot apical meristem. The shoot tips were surface sterilized for 2.5-3 min by agitation in 25% commercial bleach containing a drop of Triton X surfactant. The shoot tips were rinsed twice and stored in sterile distilled water. The apex of each shoot (approximately 1 mm in diameter) was micro-dissected and placed, cut surface down, on autoclaved C_2D medium (1), containing 5 μM benzyladenine (BA) as previously described (2, 3). Cultures were incubated at 25C with an 18 hr cool white fluorescent light/6 hr dark cycle. The percentage of shoot tips that remained contaminant-free and proliferated as adventitious bud cultures was determined after 6 weeks.

Apical meristems from these initial cultures were used as explants for experiments to evaluate the effects of various medium salt formulations on micropropagation. Medium salt formulae tested were: Murashige and Skoog (MS) (6), 1/2 MS, C_2D (1) and woody plant medium (WPM) (4). Each medium contained 5 μM BA, 0.7% agar and 3% sucrose. Twenty apices were placed, five to a plate, on each medium and incubated as described above. After 6 weeks, the resulting number of shoots produced per apex was determined and an additional sample of 20 apices was recultured on the same respective medium. This cycle was repeated 3 times. Shoot proliferation data for the 3 cycles was pooled in determining average proliferation rates.

Effect of various cytokinins at different concentrations was determined using MS medium. Cytokinins and concentrations tested were: 5, 10 and 20 μM BA; 10, 20 and 40 μM kinetin (Kin); and 0.5, 1 and 5 μM thidiazuron (TD). These differential activity ranges for each cytokinin were determined in previous experiments. A no-cytokinin control treatment was also included. Twenty apices obtained on MS medium with 5 μM BA were plated on each cytokinin-concentration treatment and the number of shoots produced per apex was determined after 6 weeks. The experiment was repeated 3 times as above.

In vivo rooting of shoots was compared with in vitro rooting. For in vivo rooting, shoots with four-to-six nodes were excised and placed either directly in Pro-mix com-

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mercial potting mix in planting flats or first dipped into Rootone-F commercial rooting powder. The flats were covered with clear domes and placed in a greenhouse mist chamber. For in vitro rooting, shoots were placed in MS medium with or without 1 μM naphthaleneacetic acid (NAA). The number of: rooted shoots, roots/shoot as well as the average root length were determined after 6 weeks.

All data were subjected to an analysis of variance and mean separation was by the Duncan's multiple range method.

Results and Discussion

Fifty percent of 'Blanc du Bois' shoot apices plated on C_2D medium with 5 μM BA became dark green and began to enlarge within 2 weeks. The other apices either became contaminated with bacteria and/or fungi or became necrotic and did not grow. By 4 weeks, the apices had grown to approximately 5 mm in diameter, producing leaf-like structures and buds. The proliferating apical material was composed of green, hard and contorted tissue, that resembled abnormal stem material, from which buds formed and subsequently elongated into shoots. At week 6, some of the proliferating apices had shoots with over 6 nodes that were up to 3 cm in length. In older cultures, the shoots grew in clumps from the basal mass of apex-derived tissue.

Comparison of the effect of medium formulation on shoot production showed that MS and C_2D were significantly better than 1/2 MS or WPM, producing an average of 3.6 and 3.4 shoots per apex vs. 2.6 and 2.8, respectively (Table 1). Since the cultures were maintained over 3 successive cycles, these results are an indication of long-term responses. Our previous studies of 'Orlando Seedless' (3), another Florida hybrid bunch grape, similarly showed that use of MS and C_2D, a modification of MS formulated for northeastern bunch grape cultivars (hybrids of *Vitis labrusca* L.) (1), resulted in similar shoot proliferation rates. Given the equivalent responses obtained from these media, MS is preferable for commercial production since it is in common usage and can be purchased as a preformulated mix. Therefore, MS was utilized in all subsequent experiments.

Cytokinin type and concentration had a dramatic effect on shoot proliferation (Table 2). Both BA and TD produced equivalently high numbers of shoots per apex (4.0 and 4.3) at optimum concentrations of 10 and 5 μM, respectively, when compared to the no-cytokinin control treatment (0.7). Kinetin was ineffective at all concentrations tested, since it (nonsignificantly) produced fewer shoots than the control. Average shoot production obtained from the control and the three test cytokinins, at their respective optimum concentrations, are compared in Figure 1. Although BA and TD were equivalent in shoot production, shoots formed on TD were smaller and stunted. In contrast, shoots formed on BA-containing medium were larger and more vigorous. Therefore, BA is preferable to TD. Our previous studies (2, 3) have utilized only 5 μM BA as a cytokinin since this type and amount was previously shown to be adequate by others (1). Results with 'Blanc du Bois' suggest that optimum cytokinin concentration is cultivar dependent and should be determined on a case-by-case basis.

For in vitro rooting, there was no statistical difference between basal medium and medium with NAA when considering the percentage of shoots that rooted or the number of roots per shoot (Table 3). However, shoots placed on NAA-containing medium produced longer roots than those on control medium. In vivo, a rooting powder pretreatment resulted in more roots per shoot but no difference in length (Table 4). NAA is an auxin and Rootone-F rooting powder contains an array of the auxins NAA, 1-naphthaleneacetamide, 2-methyl-naphthaleneacetamide

Table 1. Effect of medium formulation on shoot micropropagation of 'Blanc du Bois' grape.

Medium	Apices cultured (no.)	Shoots/apex[z] (no.)
Murashige and Skoog (MS)	60	3.6a
1/2 MS	59	2.6b
C_2D	60	3.4a
Woody plant (WPM)	55	2.8b

[z]Mean separation by Duncan's multiple range test, 5% level.

Table 2. Effect of cytokinins on shoot micropropagation of 'Blanc du Bois' grape.

Cytokinin Conc. (μM)	Apices cultured (no.)	Shoots/apex[z] (no.)
Kinetin (Kin)		
0	32	0.7a
10	28	0.6a
20	28	0.5a
40	23	0.5a
Benzyladenine (BA)		
0	32	0.7a
5	50	3.3b
10	59	4.0c
20	50	3.8bc
Thidiazuron (TD)		
0	32	0.7a
0.5	55	3.6b
1	55	3.7b
5	50	4.3b

[z]Mean separation by Duncan's multiple range test, 5% level.

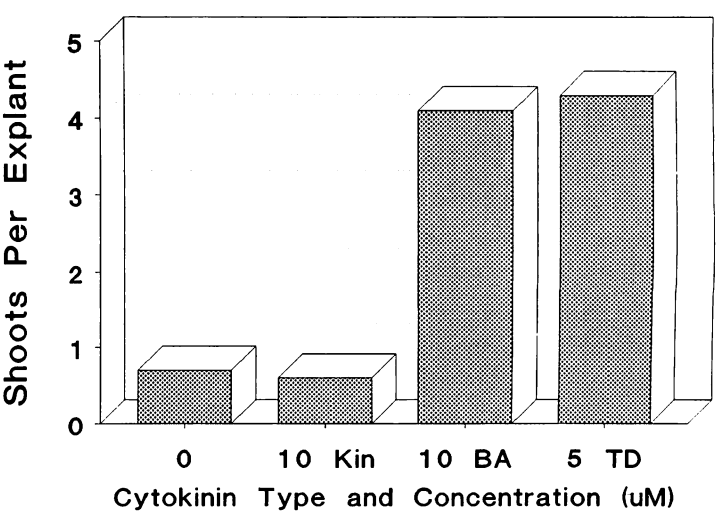

Fig. 1. Effect of cytokinins at optimum concentrations on shoot production from apices of 'Blanc du Bois' cultured on MS medium.

Table 3. In vitro rooting of micropropagated 'Blanc du Bois' shoots.

Rooting treatment	Rooted shoots[z] (%)	Roots per shoot (no.)	Root length (mm)
0	57a	1.7a	6.7a
1μM NAA[y]	70a	2.2a	23.0b

[z]Mean separation within columns by Duncan's multiple range test, 5% level.
[y]Naphthaleneacetic acid.

Table 4. In vivo rooting of micropropagated 'Blanc du Bois' shoots.

Rooting treatment	Rooted shoots[z] (%)	Roots per shoot (no.)	Root length (mm)
0	71a	4.1a	14.1a
Rootone dip	68a	9.1b	17.8a

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

and indole-3-butyric acid. The stimulative effect of auxins on rooting is vividly demonstrated by these data. It appears that such exogenously supplied auxins stimulated root primordia to form during in vivo treatments because more roots developed from auxin-treated shoots. Similarly, auxin probably accelerated the in vitro rooting response since auxin-treated roots were longer (Table 3). In comparing the two, the in vivo method was clearly more efficient because more roots per shoot were formed (9.1 vs. 2.2) and a major tissue culture step was eliminated. Vigorous plants were produced with less time and effort. Elimination of a culture step also reduces possible errors that could spell disaster in commercial production.

This study demonstrated that 'Blanc du Bois' could be readily micropropagated. Because this cultivar does not require grafting to a rootstock (5), micropropagated plants can be planted directly in the field once adequate size has been obtained. The proliferation rate of 4 shoots per apex per 6 weeks in the best treatment is adequate for commercial production since, in practice, apices and nodes of proliferating cultures can be used to establish new cultures and, thus increase culture mass. For example, considering an initial plating of 20 apices that produced 4 shoots with at least 3 nodes every three weeks, over 1,300,000 shoots could be produced in 6 months since each shoot would contain 4 explants (the apex and 3 nodes). These shoots could be rooted and established in liners within an additional 2 months. Thus, successful implementation of micropropagation technology for 'Blanc du Bois' would circumvent shortages in plant availability due to rapid increases in acreage.

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YIELDS AND OTHER CHARACTERISTICS OF MUSCADINE GRAPE CULTIVARS AT LEESBURG

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Abstract. **Muscadine grape (*Vitis rotundifolia* Michx.) cultivars and selections planted in 1974 were evaluated in a 6-replicate trial on Blanton fine sand with single vine replicates. Vines were spaced 15.5 ft apart in rows 12 ft apart and trained to a modified Geneva Double Curtain system. Fruit was harvested once when most berries on a vine were ripe by shaking into catch frames. Yields, date of harvest, evenness of ripening, soluble solids, percentage dry scar (at pedicel attachment to berry), and ease of harvesting were measured each year from 1979 through 1986. The most productive among 30 cultivars were 'Regale', 'Redgate', 'Tarheel', 'Noble', 'Doreen', 'Carlos', and 'Welder' cultivars, Ga. 3-9-2, and N.C. selections 77-21, 80-74, 154-2, and 184-4. Yields from these cultivars averaged 6.0 to 8.9 t/a. Yields of other cultivars ranged from 0.5 t/a for 'Sugargate' to 5.8 t/ha for 'Dixie'. Mean fruit ripening dates occurred between August 17 and September 13, depending on cultivar. Decline in yields in 1986 was attributed to heavy grape root borer (*Vitacea polistiformis* Harris) infestations. Characteristics are discussed and recommendations are made of Cowart, Dixie, Fry, and Southland as cultivars for fresh market; Carlos, Doreen, and Welder for white wine; Noble for red wine.**

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Muscadine grape growing in Florida dooryards dates back many decades. More recently, with newer cultivars from Georgia (3), Mississippi, and North Carolina (4), commercial production of muscadine grapes is feasible in Florida (5). Several cultivars suitable for fresh fruit (7) and processing (2) are available. Muscadine yield trials were reported at Leesburg (6), Monticello (1, 6), and Fort Pierce (8). The purpose of this paper is to report yields and other