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In Vitro Micropropagation of Nine Grape Cultivars

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ABSTRACT

Grape, *Vitis vinifera*, cultivars are susceptible to a broad spectrum of pests and diseases, including Pierce's disease. *Vitis rotundifolia* cultivars that are resistant or tolerant to many pests and diseases affecting *V. vinifera*, are the closest relatives that could contribute to tolerance in *V. vinifera*. However, conventional crosses between *V. vinifera* and *V. rotundifolia* are complicated due to chromosome number differences and genetic incompatibility when the latter is used as the female parent. The use of *V. rotundifolia* as a rootstock is also problematic due to poor rooting and graft incompatibility with *V. vinifera*. Hence, an efficient *in vitro* propagation protocol is needed for clonal multiplication and genetic manipulation. The *in vitro* cultivation capability of the *V. vinifera* cultivars 'Cabernet sauvignon' and 'Syrah', the French × American hybrid SV 12-375, the *Vitis bourquiniana* cultivars 'Black Spanish' and Herbemont and the *Vitis rotundifolia* cultivars 'Black Beauty', 'Higgens, 'Scuppernong' and 'Magnolia' were evaluated. Once organogenesis was induced, tissues were easily converted to callus by manipulation of cytokinin levels. Such calli were induced to form organs and regenerated into whole plants, which could again be reverted to callus and so on. Shoots of 'Black Beauty', 'Higgens', 'Magnolia', 'Scuppernong', 'Cabernet Sauvignon' and 'Syrah' rooted easily *in vitro* and were successfully transferred to the greenhouse. These characteristics are highly desirable for genetic manipulation.

Additional Index Words: *Vitis*, tissue culture, protoplast

Grapes are among the most economically important fruit crops worldwide with large-scale production in France, Italy, Spain, United States and other countries (Carimi et al., 2005; FAOSTAT data, 2006.). The genus *Vitis* L. contains the subgenus *Euvitis* Planch. in which *Vitis vinifera*, the major grape species cultivated in the world is placed, and the subgenus *Muscadinia* Planch., containing the *Vitis rotundifolia* species (Lu et al., 2000). *V. vinifera* cultivars possess excellent fruit quality characteristics and are used for table, wine and raisin production (Ramming et al., 2000). However, they are susceptible to many pests and diseases which interfere with the quality of fruit and wine produced and increase both production costs and the risk of undesirable effects caused by excessive use of pesticides, nematicides and fungicides. In contrast, *V. rotundifolia* cultivars are resistant or tolerant to a number of pests and diseases including Phylloxera [*Dactulosphaira vitifoliae* (Fitch)], the nematode *Xiphinena index* which transmits the grapevine fanleaf virus (Bouquet et al., 2000; Grzegorczyk and Walker, 1998; Heeswijck et al., 2003; Schmid and Ruhl, 2003), Pierce's disease caused by the bacterium *Xyllela fastidiosa* (Krivanek and Walker, 2003; Robacker and Chang, 1992; Van Sluyter et al., 2005), downy mildew caused by *Plasmopora viticola* (Barker et al., 2005; Dai et al., 1995; Van Sluyter et al., 2005) and to root-knot nematodes, *Meloidogyne* spp. (Walker et al., 1994). They are also heat tolerant (Ramming et al., 2000). However, *V. rotundifolia* cultivars root poorly in dormant cuttings, display graft-incompatibility with *V. vinifera* cultivars and are highly susceptible to lime-induced chlorosis (Bouquet et al., 2000; Torregrossa and Bouquet, 1995; Walker et al., 1994), which is a drawback for their use as rootstocks. *V. rotundifolia* fruit present loose clusters with few large thick skinned berries with large seeds and are considered of low quality compared to *V. vinifera* fruit (Krivanek and Walter, 2003; Lu et al., 2000; Van Sluyter et al., 2005). French × American hybrids and American cultivars are usually more resistant to pests and diseases, but they are less appreciated than European cultivars, which are more famous and preferred by consumers mostly because of cultural aspects (G.R. McEachern, personal communication).

Hybridizations between *V. vinifera* and *V. rotundifolia* have been successfully performed (Bouquet et al., 2000; Firoozabady and Olmo, 1982; Lu at al., 2000; Olmo, 1971; Ramming et al., 2000) mainly when *V. rotundifolia* is used as the male parent. However, production of hybrids is usually low and exhibit poor fertility (Bouquet et al., 2000; Dai et al., 1995). Such results are probably due to the difference in chromosome number, (2n = 40) in *V. rotundifolia* and (2n = 38) in *V. vinifera* (Schmid and Ruhl, 2003; Xu and Lu, 2004; Wang et al., 1999). Hybridization using *V. rotundifolia* as female parent is problematic because germinating *V. vinifera* pollen usually fails to reach the *V. rotundifolia* embryo sac. As the male parent, *V. rotundifolia* pollen cannot be used fresh and must be stored for one year because *V. vinifera* blooms earlier than *V. rotundifolia* (Lu et al., 2000). Such characteristics are, unfortunately, drawbacks for using them in conventional breeding programs.

Interest in production of the muscadines has increased substantially in recent years due to their high resistance and tolerance to pests and diseases in addition of being an important source of essential vitamins, minerals, fiber, antioxidants and phenolic compounds (Striegler et al., 2005). Use of modern breeding methods may offer promise to overcome hybridization hindrances and facilitate the use of *V. rotundifolia* cultivars as potential sources of genes for both molecular cloning and transfer to *V. vinifera* cultivars. Improved cultivars could be developed via genetic transformation or somatic hybridization once proper *in vitro* culture procedures are enhanced. Furthermore, tissue cultured materials are useful as explant sources for virus-free germplasm, clonal propagation, protoplast isolation, and genetic transformation. The application of tissue culture techniques to muscadines has great potential for grape cultivar improvement (Wetzstein and Myers, 1994) since tissue culture allows rapid propagation of healthy plants. Furthermore, it could facilitate the use of muscadines for studying graft-incompatibility problems and for rapid multiplication of hybrids in conventional breeding. Tissue culture of the *V. rotundifolia* cultivars 'Summit', 'Jumbo', 'Carlos', 'Dixie', AD3-42, 'Magnolia', 'Sterling', 'Regale' and 'Fry', has been attempted with some success, however, for *Euvitis* grapes it has been more difficult (Gray and Fisher, 1985; Lee and Wetzstein, 1990; Meyerson et al., 1994; Robacker, 1993; Sudarsono and Goldy, 1991; Thies and Graves, 1992).

In the present study, cultivars were chosen based on cultural importance, quality, and resistance to pests and diseases. The objective was to develop tissue culture procedures for the highly acclaimed *V vinifera* cultivars 'Cabernet Sauvignon' and 'Syrah', the French × American hybrid SV 12-375 ('Villard Blanc'), which are somewhat tolerant to Pierce's disease, the *V. bourquiniana* cultivars 'Black Spanish' (LeNoir) and 'Herbemont', considered tolerant to Pierce's disease (G.R. McEachern, unpublished, 2005) and the *V. rotundifolia* cultivars 'Black Beauty', 'Higgens', 'Scuppernong', and 'Magnolia' considered resistant or tolerant to several pests and diseases (Barker et al., 2005; Firoozabady and Olmo, 1982; Heeswijck et al., 2003; Krivanek and Walker, 2003; Van Sluyter et al., 2005; Xu and Lu, 2004; Walker et al., 1994).

MATERIALS AND METHODS

Plant material. Dormant stem pieces from the *V. vinifera* cultivars 'Cabernet Sauvignon' and 'Syrah', the French × American hybrid SV 12-375, the *V. bourquiniana* cultivars 'Herbemont' and 'Black Spanish', were collected from mature healthy plants at the Texas A&M University Grape Orchard (College Station, TX, USA). 'Scuppernong' and 'Magnolia' cuttings were kindly provided by Bluefford Hancock (College Station, TX, USA). 'Scuppernong' cuttings were collected from mature plants, while 'Magnolia' cuttings were obtained from a very young plant. The *V. rotundifolia* cultivars 'Higgens' and 'Black Beauty' were purchased from a commercial nursery.

Material preparation, sanitation and storage. Canes were cut into pieces of approximately 20 cm long, treated with 0.3% Captan®, dried or rolled in wet paper, placed in plastic bags and refrigerated at 4 °C (Dalal et al., 1992). Other cuttings were either placed directly in commercial soil mix to observe rooting potential or dipped in 1 g l^{-1} naphthalene acetic acid (NAA) before planting. 'Higgens' and 'Black Beauty' were planted in pots containing commercial soil mix (Scotts® Metro mix No. 200 growing medium) and maintained in a greenhouse.

Explants and surface sterilization. The explants used for *in vitro* propagation were: green stem pieces of 0.5-1 cm with one bud; shoot apices of 0.2-0.4 mm containing the apical meristem and leaf primordia, excised in the presence of 1% ascorbic acid; or apical and lateral buds (less than 0.5 cm) from refrigerated cuttings that had the ends soaked in water, with water changed every two days until bud swelling, or were directly from greenhouse plants. Explants were surface disinfested by washing once in sterile distilled water followed by immersion in 70% EtOH for 1 min, transferred to 10% commercial bleach, containing one drop of commercial detergent, for 10 min, and rinsed four times in sterile distilled water.

In vitro culture initiation. The initiation

medium (IM) used was a half strength MS medium (Murashige and Skoog, 1962; Catalog No. M 5519, Sigma, St. Louis, MO) containing full-strength vitamins and inositol, 30 g l^{-1} of sucrose, 10 µM BA and 7 g l^{-1} of agar (Sigma, St. Louis, MO) at pH 7.0 (Torregrossa and Bouquet, 1995). Explants presenting oxidation but still alive, as well as new explants were placed in IM plus 7 g l^{-1} of silver nitrate ($AgNO_3$), an ethylene inhibitor (Meskaoui and Tremblay, 2001). Explants that did not adapt to this medium were transferred to 75% strength WPM medium (Lloyd and McCown's Woody Plant Medium (Lloyd and McCown, 1980); Catalog No. M 6774, Sigma, St. Louis, MO), containing 2% sucrose, 555 µM myo-inositol, 0.45 µM BA, 0.5 g.L^{-1} agar (Sigma, St. Louis, MO), and 1 g l^{-1} gelrite (Gelrite@ gellan gum; Sigma, St. Louis, MO) at pH 5.7 (Thies and Graves, 1992). This step was necessary to select the best and most homogeneous materials for later experiments. After autoclaving, Clavamox® (amoxicillin trihydrate/ clavulanate potassium, Pfizer Animal Health, New York, N.Y.) at 250 mg l^{-1} was added to cooled medium to eliminate microbial infections in cultures.

A test for optimal cytokinin concentration was conducted using randomly chosen homogeneous *in vitro* material, cultured on IM plus $AgNO_3$ or WPM, and containing benzyladenine (BA) in the concentrations of 0, 0.3, 1, 3 and 10 µM. Cultivars tested were: *V. vinifera* cultivars 'Cabernet Sauvignon' and 'Syrah', the French × American hybrid SV 12-375; the *Vitis bourquiniana* cultivar 'Herbemont', and V. *rotundifolia* cultivars 'Higgens' and 'Black beauty'. A completely randomized design was used. Each treatment consisted of three replicates containing three explants each. Data on the number of shoots per explant and the percentage of explant mortality were recorded after 30 days of culture and subjected to a two-way Analysis of Variance with variety and concentration as factors using the Proc GLM of SAS (SAS, 2001). Treatment means were separated with the Student Newman- Keuls (SNK) test (Zar, 1999).

RESULTS AND DISCUSSION

Initial plant material manipulation. Dormant cuttings failed to root in the commercial mix, even with the application of NAA, indicating that external auxin-like application is not always effective. However, cuttings of 'Cabernet Sauvignon', 'Syrah', 'Black Spanish' and SV 12-375 soaked in water did root and were planted in pots with commercial soil mix. 'Herbemont' and all *V. rotundifolia* cultivars did not root. Viability of cuttings stored at 4 ºC varied among cultivars, from approximately two months for 'Black Spanish' and 'Herbemont', which putrefied after this time, to more than six months for the other cultivars. The muscadine cultivars, with exception of 'Magnolia', were healthy and dormant for the whole period, indicative of a good storage capacity. 'Magnolia' cuttings sprouted during incubation at 4 ºC. Dormancy in this cultivar could not be maintained by cold temperature; however, the source for these cuttings was young and probably juvenile while the others were collected from mature plants, which may explain the different behavior.

Establishment of *in vitro* culture. *In vitro* culture initiation was essential for the preparation of the explants, to homogenize the plant tissue populations. There were no differences in using apical or lateral buds as explant sources for *in vitro* culture initiation, which is in agreement with the results obtained by Meyersen et al. (1994) using both axillary buds and apical meristems for shoot micropropagation of bunch grapes and muscadine cultivars. Thies and Graves (1992) suggested that meristem tips (0.2 to 0.4 mm), herein called microshoot tips, may be superior to shoot tips (4 to 6 mm) in terms of avoidance of systemic pathogens in micropropagation of *V. rotundifolia*. However, attempts to use microshoot tips in the present study failed due to oxidation even when using ascorbic acid during explant preparation. Qiu et al. (2004) were also unable to prevent browning of *V. aestivalis* cv. 'Norton' microshoot tips using a mix of ascorbic acid and citric acid, cysteine or heat treatments, but were able to increase its viability with dithiothreitol (DTT). Such behavior explains why shoot tip culture is used in grapes rather than microshoot tip culture (Lee and Wetzstein, 1990; Robacker and Chang, 1992; Sudarsono and Goldy, 1991, Wetzstein and Myers, 1994). Microcuttings containing one bud and adjacent stem tissues were also tested for *in vitro* culture initiation. However, as also observed by Bianch et al. (2003), they presented high levels of contamination due to a larger physical area and close proximity to mature vascular tissue which usually harbors a variety of microorganisms.

In vitro culture of grapes and other woody species is frequently hampered by browning and necrosis of tissue, especially when culture is initiated and use of phenol binding agents and antioxidants have provided limited success (Dalal et al., 1992). In our study, buds became severely oxidized in MS medium; however, the addition of silver nitrate ($AgNO_3$) at 7 mg.l^{-1} enabled shoot growth for most cultivars tested. Vigorous growth was observed after transferring buds from MS to WPM. After the initial phases of cultivation, *V. vinifera* cultivars, Herbemont and the hybrid SV 12-375 showed a slight preference for the 50% MS medium containing $AgNO_3$ but also grew well in WPM without $AgNO_3$. Culture initiation was

better in WPM for all cultivars tested, with no oxidation observed, and it was visibly preferred by 'Black Spanish' and the *V. rotundifolia* tissues for all cultivation phases. Dzazio et al. (2004) suggested full strength MS medium during the initial cultivation and half strength MS for elongation and multiplication of *V. berlandieri* × *V. riparia* rootstock '420-A', but noticed no significant difference when using Nitsch and Nitsch (NN) medium or WPM. Lee and Wetzstein (1990) observed a slow shoot proliferation during culture establishment of muscadine cultivars using MS medium, while Thies and Graves (1992) reported efficient micropropagation using meristems of the muscadine cultivars 'Carlos', 'Doreen', 'Jumbo', 'Magnolia' and 'Sterling', using WPM 75% strength with 0.45 µM of BA for culture initiation and 8.92 µM BA for shoot proliferation.

During in vitro cultivation, eventual bacterial contamination occurred; however, this was promptly solved by transferring the infected material to fresh WPM medium containing Clavamox® (amoxicillin trihydrate/clavulanate potassium, Pfizer Animal Health).

Shoot induction. Healthy explants were transferred from the initiation culture medium to either half strength MS plus $AgNO_3$ or 75% strength WPM containing BA at 0, 0.3, 1, 3 and 10 µM. Evaluation was performed after 30 days of culture. Two experiments were performed and both presented similar response of the explants to BA. The number of shoots per explant was significantly affected by the concentration of BA (F= 14.03; df= 4, 217; P < 0.0001) and the variety (F= 2.88; df= 5, 217; P= 0.0154); likewise, the percentage of mortality of explant was significantly affected by the concentration of BA (F= 3.87; df= 4, 51; P= 0.008), and by the variety (F=13.56; df= 5, 51; P <0.001). Because of the significant effect of the interaction between concentration and variety for the numbers of shoots per explant (F= 3.88; df= 20, 217; P< 0.0001) and for the percentage of mortality of explant (F= 2.42; df= 18, 51; P= 0.007), the mean comparison for the different concentrations was conducted for each variety.

The 3 µM BA treatment provided the best shoot induction with well expanded leaves for 'Black Beauty', 'Higgens' and 'Cabernet Sauvignon' (Fig. 1A, B, C). There was no significant difference in shoot induction at 10 µM BA compared to 3 µM BA for 'Black Beauty' and 'Higgens', however, the shoots at 10 µM BA were not well developed. Lee and Wetzstein (1990) also observed strong inhibition of shoot elongation with almost no elongated shoots using higher than 10 µM BA concentrations in the muscadine 'Summit'. There was no mortality for 'Black Beauty'. Mortality was low for 'Higgens', 'Cabernet Sauvignon' and 'Syrah' at 3 and 10 µM BA (Fig. 1 G, H, J)). 'Cabernet Sauvignon' showed high mortality at 0 and 0.3 µM BA (Fig. 1 J) suggesting a need for cytokinin in the medium for *in vitro* cultivation. Explant root formation occurred in 0 and 0.3 µM of BA in 'Black Beauty', 'Cabernet Sauvignon' and 'Higgens' (data not shown). The spontaneous nature of root formation in these cultivars may have occurred due to elevated internal auxin levels. In studies performed by Barlass and Skene (1980a, b), 'Cabernet Sauvignon' shoots also rooted easily on an auxin-free medium.

Shoot induction for 'Syrah', 'Herbemont' and SV 12-375 did not show significant differences in the BA concentrations tested (Fig. 1 D, E, F). 'Herbemont' explants produced great masses of calli, in 1 µM BA, which increased as the concentration of cytokinin increased (Fig. 2). Leaf primordia and underdeveloped shoots were observed with 3 µM BA, however no further development occurred.

For SV 12-375, concentrations from 0.3 to 3 µM BA was the best option for calli formation. There was an increase in the percentage of mortality and a decrease in shoot formation at 10 µM of BA, which appeared to be toxic to the cultivar (Fig. 1 D, I). Undeveloped chunky leaves, which were thick and deformed, were observed with 3 µM BA, but showed no further development.

'Magnolia', 'Scuppernong' and 'Black Spanish' were not part of the BA experiment; however, concentrations of 0.3, 1, 3, 4 and 10 µM BA were tested on these cultivars. 'Magnolia' and 'Scuppernong' produced a good amount of shoots and leaves with 3 and 4 µM BA and Black Spanish with 1 µM BA (data not shown).

In summary, 'Black Beauty', 'Higgens', 'Cabernet Sauvignon', 'Magnolia', 'Scuppernong' and 'Syrah' shoot tissues were successfully maintained *in vitro,* produced callus in 10 µM BA, and well developed leaves and shoots in 3 µM BA, with the exception of 'Syrah', which produced well developed leaves and shoots in 1 µM BA. All of these cultivars were able to go from shoots or leaves to callus and vice-versa by manipulating the cytokinin concentration. This is important because callus and *in vitro* leaves can be good sources of explants for protoplast isolation and transformation. Regeneration protocols that enable differentiated tissues to become dedifferentiated, and differentiate again can be important in the development of genetic transformation systems for grapes (Perl et al., 1995).

Four of the five muscadine grapes studied herein were as easily propagated as the *V. vinifera* cultivars 'Cabernet Sauvignon', and 'Syrah', even though

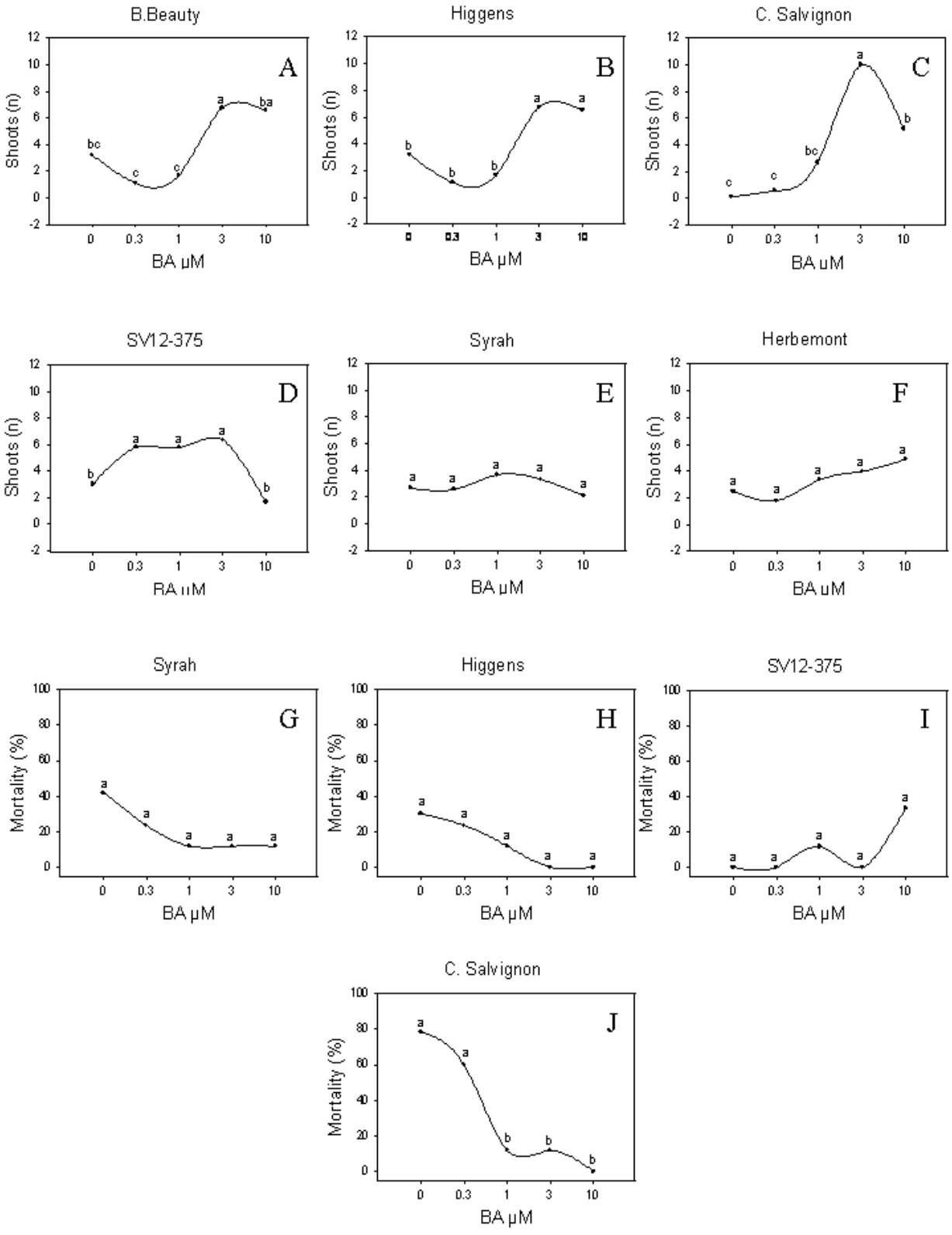

Fig. 1. Number of shoots and percentage of mortality with different concentrations of BA: (A) 'Black Beauty' shoot production; (B) 'Higgens' shoot production; (C) 'Cabernet Sauvignon' shoot production; (D) SV 12-375 shoot production; (E) 'Syrah' shoot production; (F) 'Herbemont' shoot production; (G) 'Syrah' mortality; (H) 'Higgens' mortality; (I) SV 12-375 mortality; (J) 'Cabernet Sauvignon' mortality.

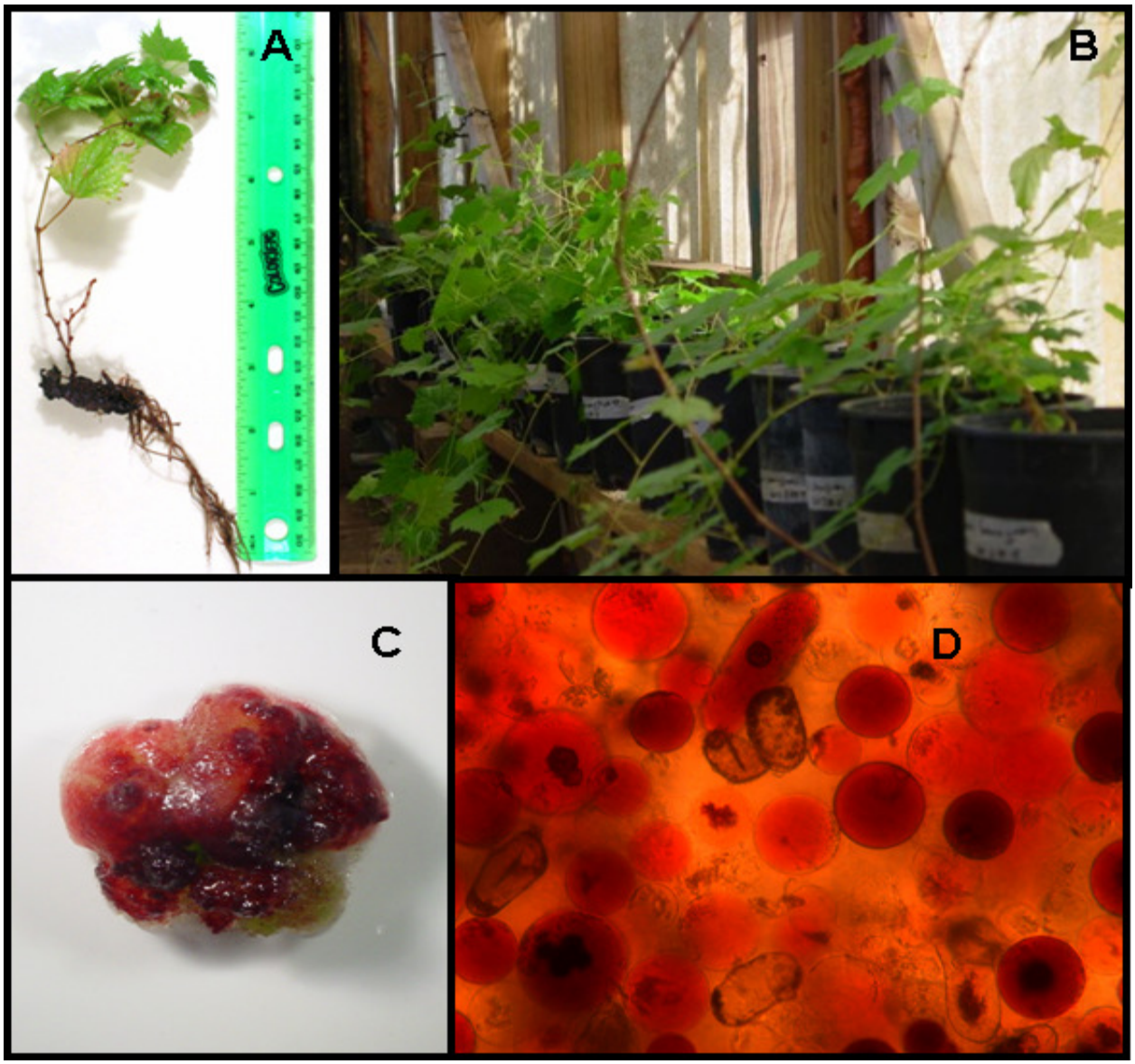

Fig. 2. *In vitro* culture of grape cultivars. (A) *V. rotundifolia* plantlet produced *in vitro*; (B) 'Black Beauty', 'Higgens', 'Magnolia', 'Scuppernong', 'Cabernet Sauvignon and 'Syrah' plants produced *in vitro*; © 'Herbemont' callus; (D) 'Herbemont' protoplasts.

Muscadinia and *Muscadinia* × *Vitis* hybrids are generally considered to be more difficult to propagate in vitro than *V. vinifera* (Olmo, 1986; Sudarsono and Goldy, 1991).

Rooting occurred spontaneously *in vitro* for the cultivars 'Black Beauty', 'Higgins', 'Magnolia', 'Scuppernong', 'Cabernet Sauvignon' and 'Syrah' (Fig. 2A) without addition of auxin to the medium, which is a very important trait especially for muscadines, which are considered to be a difficult-to-root species. Conventional propagation of muscadines is usually done by layering or with softwood cuttings under constant mist, however, this method requires succulent, immature tip cuttings, and the percentage of

loss can be high (Lee and Wetzstein, 1990). Moreover, earlier attempts at rooting by low temperature, external auxin application and air layering failed (Castro et al., 1994; Pacheco et al., 1998).

Rooted plants of 'Black Beauty', 'Higgens', 'Magnolia', 'Scuppernong', 'Cabernet Sauvignon' and 'Syrah' were successfully acclimatized and have been growing for more than one year in the greenhouse, showing very healthy and prolific development (Fig. 2B). However, no plants from 'Herbemont', SV 12-375 and 'Black Spanish' were recovered from the cytokinin concentrations tested. Callus of 'Herbemont', SV 12-375, 'Black Spanish', 'Cabernet Sauvignon', 'Syrah' and 'Magnolia' have been successfully maintained in vitro for more than two and one half years.

In the present study it was possible to isolate clean protoplasts (Fig. 2D) and to produce microcalli (i.e. cells growing in aggregates) from 'Cabernet Sauvignon' tissue cultured leaves and from 'Herbemont' callus (Fig. 2C).

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LITERATURE CITED

Barlass, M. and K.G. M. Skene. 1980a. Studies on the fragmented shoot apex of grapevine. I. The regenerative capacity of leaf primordial fragments *in vitro*. J. Exp. Botany, **31,** 483-488.

Barlass, M. and K.G. M. Skene. 1980b. Studies on the fragmented shoot apex of grapevine: II. Factors affecting growth and differentiation *in vitro*. J. Exp. Botany, **31,** 489-495.

Barker, C. L., T. Donald, J. Pauquet, M. B. Ratnaparkhe, A. Bouquet, A. F. Adam-Blondon, M. R. Thomas, and I. Dry. 2005. Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*, using a bacterial artificial chromosome library. Theor. Appl. Genet., **11**, 370-377.

Bianchi, V. J., A. C. Chaves, M. W. Schuch, and J. C. Fachinello. 2003. *In vitro* establishment of quince: effect of the explant type and time of immersion in sodium hypochlorite. Rev. Brasileira Agroc., **9**, 177-179.

Bouquet, A., Y. Danglot, M. Bongiovannii, P. Castagnone-Sereno, and L. Torregrossa. 2000. Breeding rootstocks resistant to grape fanleaf virus spread, using *Vitis* × *Muscadinia* hybridization. Acta Hort., **528**, 517-526.

Carimi, F., E. Barizza, M. Gardiman, and F. Lo Schiavo. 2005. Somatic embryogenesis from stigmas and styles of grapevine. In Vitro Cell. Devel. Biology- Plant **41**, 249-252.

Castro, P.R.C., E. Melotto, F. C. Soares, I. R. S. Passos, and C. V. Pommer. 1994. Rooting stimulation in muscadine grape cuttings. Scientia Agric., **51**, 436-440.

Dai, G.H., C. Andary, L. Mondolot-Cosson, and D. Boubals. 1995. Histochemical studies on the interaction between three species of grapevine, *Vitis vinifera*, *V. rupestris* and *V. rotundifolia* and the downy mildew fungus, *Plasmopora viticola*. Physiol. Molec. Plant Pathol. **46**, 177-188.

Dalal, M. A., B. B. Sharma, and M. S. Rao. 1992. Studies on stock plant treatment and initiation culture mode in control of oxidative browning *in vitro* cultures of grapevine. Scientia Hort., **51,** 35-41.

Dzazio, P. M., L. A. Biasi, and F. Zanette. 2004. Micropropagation of '420-A' grapevine rootstock. Rev. Brasileira Frutic., **24**, 759-764.

FAOSTAT. 2006. http://www.fao.org (last accessed in May, 2006).

Firoozabady, E. and H. P. Olmo. 1982. The heritability of resistance to root-knot nematode (*Meloidogyne incognita acrita* CHIT.) in *Vitis vinifera* × *V. rotundifolia* hybrid derivatives. Vitis, 21:136-144

Gray, D.J. and L. C. Fisher. 1985. In vitro propagation of grape species, hybrids and cultivars. Proc. Florida State Hort. Soc., **98**,172-174.

Grzegorczyk, W. and M. A. Walker. 1998. Evaluating resistance to grape *Phylloxera* in *Vitis* species with an *in vitro* dual culture assay. Amer. J. Enol. Vitic., **49,** 17-22.

Heeswijck, R.V., A. Bondar, L. Croser, and T. Franks (2003). Molecular and cellular events during the interaction of *Phyloxera* with grapevine roots. Acta Hort. **617,** 13-16.

Krivanek, A.F. and M. A. Walker. 2003. Progress in understanding the genetics of resistance from the grape *Muscadinia rotundifolia* to the bacterial pathogen *Xyllela fastidiosa*. Acta Hort., **603**, 429-432.

Lee, N. and H.Y. Wetzstein. 1990. *In vitro* propagation of muscadine grape by axillary shoot proliferation. J. Amer. Soc. Hort. Sci., **115**, 324-329.

Lloyd, G and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Proc. Intern. Plant Prop. Soc., **30**, 421-427.

Lu, J., Schell, L., and D. W. Ramming. 2000. Interspecific hybridization between *Vitis rotundifolia* and *Vitis vinifera* and evaluation of the hybrids. Acta Hort., **528,** 479-486.

Meskaoui, A.E. and F. M. Tremblay. 2001. Involvement of ethylene in the maturation of black spruce embryogenic cell lines with different maturation capacities. J. Exp. Bot., **52**, 761-769.

Meyerson, M.E., C. M. Benton, and D. J. Gray. 1994. A comparison of shoot micropropagation among bunch and muscadine grape species and cultivars. Proc Florida State Hort. Soc., **107**, 311-312.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plantarum, **15**, 473-479.

Olmo, H.P. 1986. The potential role of (*vinifera* × *rotundifolia*) hybrids in grape variety improvement. Cell. Mol. Life Sci., **42**, 921-926.

Olmo, H.P. 1971. *Vinifera rotundifolia* hybrids as wine grapes. Amer. J. Enol. Vitic., **22**, 87-91.

Pacheco, A.C., P. R. C. Castro, and B. Apezzato-da-Gloria. 1998. Aspectos anatomicos do enraizamento da videira muscadinia (*Vitis rotundifolia* Michx.) atraves da alporquia. Scientia Agric., **55**, 210-217.

Perl, A., S. Saad, N. Sahar, and D. Holland. 1995. Establishment of long-term embryogenic cultures of seedless *Vitis vinifera* cultivars – a synergistic effect of auxins and the role of abscisic acid. Plant Sci., **104**, 193-200.

Qiu, W., S. Fekete, T. Todd, and L. Kovacs. 2004. Facilitation of microshoot tip propagation of *Vitis aestivalis* var. Norton by combined application of an antioxidant and cytokinins. Amer. J. Enol. Vitic., **55**, 112-114.

Ramming, D.W., R. L. Emershad, and R. Tarailo. 2000. A stenospermocarpic, seedless *Vitis vinifera* × *Vitis rotundifolia* hybrid developed by embryo rescue. HortScience, **35,** 732-734.

Robacker, C. 1993. Somatic embryogenesis and plant regeneration from muscadine grape leaf explants. HortScience, **28**, 53-55.

Robacker, C.D. and C.J. Chang. 1992 Shoot-tip culture of muscadine grape to eliminate Pierce's disease bacteria. HortScience, **27,** 449-450.

SAS Institute Inc. 1999. SAS/STAT® User's Guide, Version 8, Cary, NC, USA.

Schmid, J. and E. H. Ruhl. 2003 Performance of *Vitis cinerea* hybrids in motherblock and nursery preliminary results. Acta Hort., **617**, 141-145.

Striegler, R.K., P. M. Carter, J. R. Morris, J. R. Clark, R. T. Threlfall, and L. R. Howard. 2005. Yield, quality, and nutraceutical potential of selected muscadine cultivars grown in southwestern Arkansas. HortTechnology, **15**, 276-284.

Sudarsono A. and R. G. Goldy. 1991. Growth regulator and axillary bud position effects on *in vitro* establishment of *Vitis rotundifolia*. HortScience, **26**, 304-307.

Thies, K. and C. H. Graves Jr. 1992. Meristem micropropagation protocols for *Vitis rotundifolia* Michx. HortScience, **27**, 447-449.

Torregrossa, L. and A. Bouquet. 1995. *In vitro* propagation of *Vitis* × *Muscadinia* hybrids by microcuttings or axillary budding. Vitis. **34**, 237-238.

Van Sluyter, S., M. J. Durako, and C. J. Halkides. 2005. Comparison of grape chitinase activities in chardonnay and Cabernet Sauvignon with *Vitis rotundifolia* cv. Fry. Amer. J. Enol. Vitic., **56,** 81-85.

Walker, M.A., H. Ferris, and M. Eyre. 1994. Resistance in *Vitis* and *Muscadinia* species to *Meloidogyne incognita*. Plant Disease, **78,** 1055-1058.

Wang, Y., J. Chen, J. Lu, and O. Lamikanra. 1999. Randomly amplified polymorphic DNA analysis of *Vitis* species and Florida bunch grapes. Scientia Hort., **82**, 85-94.

Wetzstein, H.Y. and S. C. Myers. 1994. Vegetative and yield component characteristics of micropropagated musdadine grape (*Vitis rotundifolia* Michx.). J. Hort. Sci., **69**, 747-753.

Xu, X and J. Lu. 2004. Cytogenetic study of interspecific hybrids between *Vitis rotundifolia* and *Vitis vinifera*. Acta Hort., **640,** 269-273.

Zar, J.H. 1999 Biostatistic Analysis. 4th Edition. Prentice Hall, Inc., Upper Saddle River, NJ, USA.