Short communication

Micropropagation of *Vitis vinifera* L: towards an improved protocol

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Abstract

Nodal explants bearing a single axillary bud, from three cultivars of cultivated grape, viz., ‘Thompson seedless’, ‘Sonaka’ and ‘Tas-e-Ganesh’, were used to initiate shoot cultures on G16 medium containing adenine sulphate, monobasic sodium phosphate, BAP and NAA. Each shoot, developed from an axillary bud, produced a tuft of multiple shoots on a medium containing BAP, calcium pantothenate, monobasic sodium phosphate and IBA. Subculture of the tuft of multiple shoots to an ‘elongation medium’ resulted in distinct individual shoots. Rooting of shoots and plantlet formation was achieved on IAA-containing liquid medium. A culture procedure for enhanced multiple shoot production and the recovery of complete plants is described. The use of this protocol for the commercial exploitation of cultivated grape is discussed. © 2000 Published by Elsevier Science B.V. All rights reserved.

Keywords: *Vitis*; Axillary buds; In vitro multiple shoots; Micropropagation

Abbreviations: B\(_5\), Gamborg et al. (1968) medium; BAP, 6-benzyl amino purine; IAA, 3-indoleacetic acid; IBA, 3-indolebutyric acid; LS, Linsmaier and Skoog (1965) medium; MS, Murashige and Skoog (1962) medium; NN, Nitsch and Nitsch (1969) medium; NAA, l-naphthaleneacetic acid; WPM, woody plant medium

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1. Introduction

Tissue culture is an established method for the commercial clonal propagation of herbaceous and woody plant species (Lewandowski, 1991). Although the use of apices and axillary buds for the in vitro propagation of various species and cultivars of *Vitis* is documented (Gray and Fischer, 1985) and micropropagation protocols are reported for muscadine grapes (Thies and Graves, 1992; Torregrossa and Bouquet, 1995), studies with cultivars of *V. vinifera* have met with limited success (Chee and Pool, 1983; Zatiko and Molnar, 1985). In an earlier study, on tendril explants of cultivars of grape, we reported somatic embryogenesis and low frequency conversion of embryos to plants (Salunkhe et al., 1997). This communication documents a procedure for the micropropagation of three commercially important cultivars of *V. vinifera* viz., ‘Thompson seedless’, ‘Sonaka’ and ‘Tas-e-Ganesh’, using axillary buds from greenhouse-grown vines as initial explants.

2. Material and methods

Axillary buds, isolated from three-months-old greenhouse-grown vines of *V. vinifera* viz., ‘Thompson seedless’, ‘Sonaka’ and ‘Tas-e-Ganesh’, were used as source material for culture. Nodal explants (0.5–1 cm) each containing a single axillary bud, were washed thoroughly with detergent and water and subsequently sterilised with HgCl₂ (0.1% w/v, 10 min) and used as initial explants for experiments.

Pilot experiments were carried out to determine the effect of growth regulators added individually or in combinations and varying concentrations to media. The following media combinations were chosen for the micropropagation protocol:

- **G16**, *initiation medium*, comprised of NN major and minor salts, LS vitamins, Fe EDTA, 2% (w/v) sucrose, 10 mg l⁻¹ thiamine HCl, 40.53 mg l⁻¹ adenine sulphate, 218.4 mg l⁻¹ monobasic sodium phosphate, 2.25 mg l⁻¹ BAP and 0.09 mg l⁻¹ NAA.
- **GM2**, *multiplication medium*, comprised of WPM (Sigma catalogue, 1994) major and minor salts, B5 vitamins, Fe EDTA, 3% (w/v) sucrose, 2 mg l⁻¹ calcium pantothenate, 168 mg l⁻¹ monobasic sodium phosphate, 0.5 mg l⁻¹ IBA and 2.2 mg l⁻¹ BAP.
- **MS2**, *shoot elongation medium*, comprised of MS major and minor salts, MS vitamins, Fe EDTA, 2% (w/v) sucrose, 0.5 mg l⁻¹ BAP and 0.2 mg l⁻¹ IAA.
- **GR1**, *rooting medium* (liquid), comprised of half strength MS major and minor salts, full strength MS vitamins, Fe EDTA, 1% (w/v) sucrose and 0.1 mg l⁻¹ IAA.
All the media contained 100 mg l\(^{-1}\) myo-inositol and unless otherwise specified, were solidified with 0.8% agar (Hi-Media Labs., Mumbai). The pH was adjusted to 5.8 prior to autoclaving (121°C, 1 kg cm\(^{-2}\) for 15 min). The cultures were maintained at 25 ± 2°C in 16 h/8 h dark/illumination (Philips TL34, 25 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)) cycle. There were 48 replicates per treatment and the experiments were repeated thrice.

Axillary buds were cultured on G16 medium. Each bud sprouted and produced three shoots after four weeks. One shoot was cultured on liquid GR1 medium on filter paper bridge for rooting. The remaining two shoots were cultured individually on GM2 medium for multiple shoot production. Tufts of multiple shoots from GM2 medium were subcultured on MS2 medium for shoot elongation. Elongated shoots were cultured on liquid GR1 medium on filter paper bridges to obtain complete plants. In vitro produced plants were transferred initially to plastic cups containing ‘Soilrite’ (mixture of 75% Irish peatmoss and 25% expanded Perlite; M/s. Chougule Industries, Mumbai) and eventually to soil in the greenhouse.

3. Results and discussion

Each axillary bud, borne on nodal segments (initial explants), produced three shoots in a span of three to four weeks on G16 medium (Fig. 2). One of the shoots was excised and cultured on GR1 medium to obtain an individual rooted plantlet and this served as a stock culture. Some of these plantlets were established in the greenhouse and were used as a source of axillary buds. The remaining two shoots were cultured independently on GM2 medium (Fig. 2) which produced tufts of multiple shoots within six weeks of culture (Fig. 1a). Both G16 and GM2 media contained monobasic sodium phosphate (218.4 and 1.68 mg l\(^{-1}\), respectively) as one of the constituents, in addition to 40.53 mg l\(^{-1}\) adenine sulphate in G16. Our findings are in agreement with those of Harris and Stevenson (1979) on enhanced shoot formation in grapevine cultures following the addition of adenine sulphate and monobasic sodium phosphate.

BAP has been reported to elicit optimum shoot multiplication rate at a concentration of 1.125–2.25 mg l\(^{-1}\) (Harris and Stevenson, 1982; Chee and Pool, 1985). In our studies too, both initiation (G16) and multiplication (GM2) media required BAP (2.25 and 2 mg l\(^{-1}\), respectively) for the induction of shoots. Hyperhydricity (vitrification) has been noted in grapevine cultures mainly during proliferation (Morini et al., 1985). Though the mechanism for this remains to be elucidated, it has been suggested that high concentration of cytokinin, NH\(_4\) ions or agar, may influence hyperhydricity (Ziv, 1991). Helior et al. (1997) reported that, maintenance of multiplication rate and reduction of hyperhydricity are possible only when the BAP concentration is reduced from 2 to 1 mg l\(^{-1}\) in the
medium. Our results indicate that BAP at 2.25 mg l\(^{-1}\) is necessary for multiplication and does not result in hyperhydricity. This may be so, because both G16 and GM2 media contain monobasic sodium phosphate in addition to BAP and this could be responsible for a synergistic effect of cytokinin and NH\(_4^+\) ions. Chee and Pool (1982) have reported that 0.09 mg l\(^{-1}\) NAA is required for optimum bud formation in cultures of *Vitis* sp. cv. Rougeon. Our studies stress the requirement of not only 0.09 mg l\(^{-1}\) NAA but also 0.5 mg l\(^{-1}\) IBA in the initiation and multiplication media, respectively.

The multiple shoots grew as tufts (Fig. 1a; Fig. 2) and it became necessary to define a medium to promote shoot elongation. Although BAP alone at 0.5 mg l\(^{-1}\) did produce a few elongated shoots, addition of 0.2 mg l\(^{-1}\) IAA in the elongation medium (MS2) enhanced this effect. A similar observation has been made for bud formation in cultures of *Vitis* spp. cv. Rougeon (Chee and Pool, 1982). On MS2 medium, the shoots grew as distinct entities and could easily be separated for rooting (Fig. 2). Rooting of isolated shoots was achieved on liquid GR1 medium.

Fig. 1. Multiple shoots and plantlets in grape: (a) tuft of multiple shoots obtained on GM2 medium, (b) rooting of in vitro shoots on filter paper bridges in liquid GR1 medium, and (c) in vitro produced rooted plantlets established in plastic cups with soilrite.
on filter paper bridges (Fig. 1b; Fig. 2). Elimination of cytokinin and monobasic sodium phosphate, reduction of basal salts and sucrose to half strength and the addition of 0.1 mg l\(^{-1}\) IAA in GR1 medium induced rooting in more than 80% of cultures in all three varieties. Helior et al. (1997) have reported that IBA serves as a suitable auxin for in vitro rooting of \textit{V. vinifera} cv. ‘Pinot noir’ and addition of NAA does not give rise to more roots, but leads to callus formation. In our studies, use of 0.1 mg l\(^{-1}\) IAA in the rooting medium not only induced roots but also eliminated callus formation. Of the cultivars, ‘Sonaka’ gave rise to a greater number of elongated shoots (\(\sim 11–12\)) on MS1 medium as compared with four to six shoots for ‘Thompson seedless’ and ‘Tas-e-Ganesh’ (Fig. 3). The rooting percentage was marginally better in ‘Tas-e-Ganesh’ (83.3%) and ‘Sonaka’ (81–82%) than in ‘Thompson seedless’ (77%) on GR1 medium. The number of plantlets generated by the present micropropagation protocol is higher than that obtained from somatic embryogenesis of tendril callus of ‘Sonaka’ and ‘Tas-e-Ganesh’ (Salunkhe et al., 1997).

Acclimatisation of in vitro developed plants was a crucial step in the present study and was accomplished by initial growth in ‘Soilrite’ in plastic cups for four weeks (Fig. 1c). Subsequently plantlets were transferred to soil in the greenhouse. The entire micropropagation process from the initial explant to rooted plantlets in
soil requires six months. Earlier studies on in vitro propagation of Vitis have indicated that the degree of success at each stage of culture is genotype-dependent and varies under a given set of culture conditions (Monette, 1988). But it should be possible to obtain a higher number of vines by a reproducible micropropagation procedure and our study demonstrates the feasibility of a culture protocol yielding multiple shoot induction and predictable recovery of grapevine plantlets for commercial exploitation.

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References


