High frequency regeneration from grapevine petioles: Extension to new genotypes

B. I. Reisch, M. H. Martens and Z. M. Cheng

Department of Horticultural Sciences, New York State Agricultural Experiment Station, Cornell University, Geneva, New York 14456, USA

Summary: Auxins were found to enhance shoot organogenesis when used in a high cytokinin medium at low levels. This enhancement was genotype specific. In the presence of 10.0 μM BA, Vanessa regenerated best with the inclusion of 4.0 μM IAA-dl-aspartate, while Catawba regenerated best with 2.0 μM IAA-glycine in the medium. With Ravat 51, 14-15 % of explants regenerated in the presence of either 2.0 μM IAA-glycine or 4.0 μM IAA-dl-aspartate, whereas there was no regeneration in the absence of auxin. Tests with 0.0-8.0 μM TDZ as a cytokinin source indicated that petiole explants of Vanessa regenerate best (10 %) at 4.0 μM TDZ. Our results clearly show that high cytokinin media can be easily extended to petiole explants of other genotypes to facilitate shoot organogenesis and that low levels of auxins, especially IAA conjugates can enhance the level of regeneration.

Key words: tissue culture, petiole, genotype, growth regulator, organogenesis, regeneration.

Introduction

Improved ability to produce plants from tissue cultures of *Vitis* would expedite efforts in genetic engineering, *in vitro* mutant isolation, rapid multiplication of elite stocks, and the production of haploids and then homozygous diploids.

In grapes, varying degrees of organogenesis and somatic embryogenesis have been obtained. Shoot organogenesis and embryogenesis has been obtained from vegetative tissues, such as shoot apices (Barlass and Skene 1978, 1980 a, 1980 b), leaf callus (Favre 1977; Hirabayashi 1985; Stamp and Meredith 1988), and internode callus (Krul and Worley 1977; Rajasekaran and Mullins 1981). Yet most of the available techniques are limited by low frequency of regeneration, genotype and explant specifity, and a process involving two or three steps.

The objective of our research was to extend the regeneration system developed for *Vitis x labruscana* Catawba (Cheng and Reisch 1989) to other genotypes. We also planned to examine auxin and cytokinin effects on regeneration from petiole tissues.

Materials and methods

The grape cultivars Catawba, Vanessa and Ravat 51 (Vignoles) were established *in vitro* from vines grown at the New York State Agricultural Experiment Station, Geneva, NY. All explants for regeneration experiments were derived from *in vitro* shoot cultures.

Axillary buds from the basal part of growing primary shoots were isolated and washed for 30 min in tap water with Micro detergent (International Products Corp., Trenton, NJ, USA). After woody parts and outer layers of bud scales were removed, buds were submerged in 70 % ethanol for 2-3 min and then were transferred to 1.85 % sodium hypochlorite for 15 min, followed by 3-5 washes in sterile distilled water. Apex tissues, 2-4 mm long, were isolated from buds and inoculated for shoot multiplication on MS basal medium (Murashige and Skoog 1962) supplemented with 4.0 μM BA. Conditions for further growth of shoot tip cultures were described earlier (Reisch 1986).The micropropagated shoots were later cultured in MS medium or C_2d (Chee and Pool 1987) with 2.0 or 4.0 μM BA prior to excision of explants. All media were solidified with agar (Difco bacto) at 7.0 g/l.

The basic medium for regeneration experiments was NN69 (NITSCH and NITSCH 1969). This medium was supplemented with various plant growth regulators or other components. Vitamin concentrations were those of MURASHIGE and SKOOG (1962). Myo-inositol (100 mg/l), sucrose (20 g/l), BA, IBA, TDZ and amino acid conjugates of IAA were added to the media prior to autoclaving.

Petioles from shoot cultures were isolated with great care to exclude nearby axillary buds. Each treatment included 6-12 Petri dishes (25 x 100 mm) with 10 explants each.

The cultures for regeneration were grown in the dark (in cardboard boxes) in a culture room at 24-26 °C. After 6 weeks of culture, cultures were transferred to BA containing media under a 16 : 8 light : dark photoperiod to induce further shoot growth.

Table 1: Regeneration from petioles of Vanessa and Catawba after 8 weeks on media with 10.0 µM BA plus the indicated auxin (60-120 petioles/tmt)

VARIETY	AUXIN SOURCE	% REGENERATION
Vanessa	none	3.3
Vanessa	4.0 µM IAA-dl- asp	9.0
Vanessa	2.0 µM IAA-gly	2.5
Vanessa	0.5 µM IBA	8.3
Vanessa	0.5 µM NAA	0.0
Catawba	none	5.0
Catawba	4.0 µM IAA-dl-asp	7.0
Catawba	2.0 µM IAA-gly	10.8
Catawba	0.5 µM IBA	7.5
Catawba	0.5 µM NAA	0.0

Table 2: Regeneration from Ravat 51 petioles after 6 weeks (40-90 petioles/tmt)

BA (µM)	AUXIN (µM)	% REGENERATION
10.0	0.0	0.0
10.0	4.0 IAA-dl-ala	5.9
10.0	2.0 IAA-gly	15.0
10.0	20.0 IAA-gly	2.9
10.0	4.0 IAA-dl-asp	14.4
10.0	80.0 IAA-dl-asp	5.0

Table 3: Regeneration from Vanessa petioles after 8 weeks (65-100 petioles/tmt)

TDZ (µM)	% REGENERATION
0.0	0.0
2.0	4.6
4.0	10.0
6.0	6.1
8.0	7.3

Results and discussion

Auxin effects on grapevine regeneration from petiole cultures

Earlier results with Catawba suggested that regeneration could be greatly improved with the addition of autoclaved IBA to the culture medium. In this first experiment, the effects of IBA as well as other auxin sources were examined. Petioles were excised from *in vitro* shoots of both Vanessa and Catawba. Cultures were incubated in the dark for 6 weeks, 12 plates, 10 petioles/plate, on five media: 1. 10 μM BA, 2. 10 μM BA + 4.0 μM IAA-dl-aspartate, 3. 10 μM BA + 2.0 μM IAA-glycine, 4. 10 μM BA + 0.5 μM IBA (autoclaved), and 5. 10 μM BA + 0.5 μM NAA.

Results obtained do not re-confirm the large increase seen in Catawba regeneration on IBA containing medium (Table 1). Yet they do suggest that IBA can increase the rate of regeneration. The glycine conjugate of IAA seems to be the most effective auxin source for Catawba, while the aspartate conjugate is the most effective for Vanessa regeneration. The use of NAA at the level tested prevented regeneration of both cultivars.

A second experiment concentrated on defining the optimum levels of IAA conjugates in the medium. Conjugates of IAA are reported to stabilize the levels of IAA in tissue culture media and provide for a slower, steadier release of auxin over a longer period of time. Preliminary results in our lab have shown that these conjugates are extremely active in grape tissue cultures. Using petioles from *in vitro* shoots of Ravat 51, three conjugates of IAA were tested in combination with 10 μM BA at the following levels: 10 μM BA (control), 10 μM BA + 4.0 μM IAA-dl-alanine, 10 μM BA + 2.0 μM IAA-glycine, 10 μM BA + 20.0 μM IAA-glycine, 10 μM BA + 4.0 μM IAA-dl-aspartate, and 10 μM BA + 80.0 μM IAA-dl-aspartate.

All conjugates tested effectively improved regeneration over the control (Table 2). The highest levels of regeneration with Ravat 51 petioles were achieved on media with either 2.0 μM of the glycine conjugate or 4.0 μM of the aspartate conjugate. In the latter case, the effective concentration is 2.0 μM since only the 'l' form of the conjugate can be naturally hydrolysed *in vitro*.

Cytokinin effects on grapevine regeneration from petiole cultures

Preliminary experiments with thidiazuron (TDZ) in the range of 0.0-2.0 μM showed that TDZ was an effective cytokinin-like compound when used with grape petiole cultures. We then sought to determine its optimum effective level and to test it at very high levels. Earlier results suggested that it would be effective alone or in combination with BA. This present experiment tested TDZ in the absence of other growth regulators. Petioles were derived from Vanessa shoots grown *in vitro* and cultured in the dark at the indicated concentrations of TDZ for 6 weeks followed by transfer to the light (16 h photoperiod).

Without TDZ, no callus growth or regeneration was observed (Table 3). All levels of TDZ tested (2.0-8.0 μM) produced shoots from the cut ends of the petioles. The optimum level of TDZ was determined to be 4.0 μM, a level which is twice as high as any previously tested level of TDZ in our lab.

In the present experiments, two new genotypes, Vanessa and Ravat 51, have been shown to be regenerable via the petiole regeneration system. The frequency of regeneration is lower than that reported for Catawba, yet it was comparable to the frequency for Catawba in the one experiment (Table 1) in which Catawba was run side by side with Vanessa. For unknown reasons, the level of regeneration may vary considerably from experiment to experiment. This may be due to explant physiological status on the shoot proliferation medium, node position of the petiole, and other *in vitro* factors which may affect the hormone or nutritional status of petioles prior to explanting to regeneration medium.

Acknowledgements

This research was supported by the New York State Grape Production Research Fund and Grant No. US 1181-86 from BARD - The United States - Israel Binational Agricultural Research & Development Fund. We would like to express our gratitude for the personal support provided by JOHN DYSON. Support from Hatch Project 32487 is also acknowledged.

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