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EFFECT OF GROWTH HORMONES ON MICROPROPAGATION OF *VITIS VINIFERA* L. CV. PERLETTE

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Abstract

In vitro propagation of *Vitis* offers opportunities for increasing plant material for cultivation. Cultures were established and maintained *in vitro* on MS medium supplemented with BA (0, 5 and 10 µM) for shoot and NAA (0, 1, 5 and 10 µM) for callus induction. The best explant sterilization was achieved with 10% chlorox treatment either for 10 or 15 minutes. Oxidative browning was effectively controlled by three subcultures of explants on fresh media. Better shooting was observed on MS medium with 5 µM BA. Shoot proliferation (80%) was obtained by subculturing the micro cuttings on same media as used for shoot formation. Maximum rooting (80%) occurred on medium with 10 µM IBA. The highest callus induction was 80% from stem segments on 5 µM BA followed by leaf disk explants on NAA (1 µM). All callus cultures initiated aerial roots. Leaf disk explants (60%) developed embryoids on MS medium supplemented with NAA (2 mg/l).

Introduction

In Pakistan, grape is a minor fruit used as fresh and dry. It ranks on 10th position among fruits. Total area of grapes in Pakistan is 13.1 thousand hectare with annual production of 48.8 thousand tones (Anon., 2005-06). In conventional method of propagation nearly all grape varieties (scion or rootstock) are propagated through stem cutting, layering and grafting. Conventional method of propagation is sometimes hampered by seedling heterozygosity, space and time consideration, seed and cutting dormancy and limited yield. Many serious diseases of fungi like powdery mildew, gray rot; viral diseases like fan leaf roll fleck, stem pitting, corkey bark and bacterial diseases like pierces, necrosis are accountable for drop in production and shortened life span of plants. These are usually spread by infected source of propagation material. The improvement in production and quality of grapes can be achieved by practicing genetic and sanitary clonal selection through incorporation of unconventional propagation method like tissue culture which is adopted an established method for the commercial propagation of herbaceous and woody plant species (Lewandowski, 1991).

The use of apices and axillary buds for the *in vitro* propagation of various species and cultivars of *Vitis* is documented (Gray & Fischer, 1985) and micropropagation protocols are reported for muscadine grapes (Thies & Graves, 1992; Torregrossa & Bouquet, 1995), but studies with cultivars of *V. vinifera* L., have met with limited success (Chee & Pool, 1983; Zatiko & Molnar, 1985). Salunkhe *et al*., (1997) while working on tendril explants of cultivars of grape reported somatic embryogenesis and low frequency conversion of embryos to plants. *In vitro* micropropagation of woody plant species is an increasingly applied practice. Its feasibility depends on the shoot multiplication rate from subculture shoot and percentage rooting of the shoots. Rapid micropropagation of grape vine can be achieved not only by combining shoot proliferation and rooting of new shoots, but also by sequential rooting of one node shoots segments from *in vitro* or

greenhouse grown vines. However, the use of cytokinin may be avoided which may result in higher incidence of undesired somaclonal variation (Beauchesne, 1982).

The present report describes a procedure for the micropropagation of grape cv. Perlette, a potential cultivar under Punjab agro-climatic conditions, using shoot tip, nodes and leaf disks from field- and *in vitro*-grown vines as an initial explant.

Materials and Methods

Different explants from grape variety Perlette were selected. Nodal segments (0.5-0.8 cm), shoot tips (0.3-0.5 cm) and leaf disk (0.5 cm^2) were dissected and sterilized. Explant sterilization was accomplished firstly by rinsing in tap water for 5 minutes. Then the explants were treated with 70% ethanol plus 2 drops of Tween 20 for 5 minutes followed by 3 washings with autoclaved distilled water. Finally the explants were treated with NaOCl (10%) for 5, 10 and 15 minutes.

Callus induction in different explants was examined on MS medium (Murashige & Skoog, 1962) supplemented with NAA (1, 5 and 10 µM). For shoot proliferation and rooting in nodal explants, BA (0, 5 and 10 µM) and IBA (0, 5 and 10 µM) were used, respectively. The problem of phenolic exudation from explants was tackled by repeated sub-culturing on fresh media. The pH of the media was adjusted at 5.7 and autoclaved at 121°C with 15 psi pressure for 15 minutes. Callus cultures were maintained in dark for 2 weeks before shifting to light (16 h day). Shoot proliferation cultures were subjected to light with photoperiod of 16 h day. Plantlets were acclimated to *ex vitro* conditions with *in vitro* rooting. The experiment was designed as completely randomized design in factorial arrangements with four replications. Means were compared according to Duncan's multiple range test (Steel & Torrie, 1984).

Results and Discussion

Explant sterilization and oxidative browning: The problem of contamination was tried to solve with 10% chlorox treatment for 5, 10 and 15 minutes. Both higher treatment for 10 and 15 minutes showed non-significant differences and successfully controlled contamination (Table 1). Chlorox (10%) treatment for 5 minutes resulted in only 41.3% clean cultures.

Like other woody plants the oxidative browning was also a problem in grapes. It was tackled by continuous subculture on fresh media up to 3 subcultures (Table 2). Similarly Dalal *et al*., (1991) reported oxidative browning control but with modification in MS macro salts.

Shoot and root formation: Nodal segments from dormant shoots were cultured on MS media having BA (0, 5 or 10 µM). None of the explants showed shoot formation. Thereafter nodal explants from new growth of shoots were cultured on MS medium containing BA (0, 5 or 10 µM). Nodal segments responded the best for shoot formation at BA (5 µM) (Table 2). Further the nodal segments from these *in vitro* grown shoots were cultured on the same media for shoot proliferation. MS medium having BA (5 µM) significantly affected on shoot proliferation and average number of shoots were 8 per culture. Similar rate of shoot proliferation was reported by Barlass & Skene (1978) who produced 800 plantlets in 3-4 months from single shoot tips by proliferation whereas Yae *et al*., (1990) obtained shoot proliferation on MS medium plus BA with combination of different hormones.

Table 1. Effect of chlorox (10%) on asepsis of cultures with different treatment times.

Treatments	Contamination (%)	Clean culture (%)
5 minutes	58.7 a	41.3 b
10 minutes	19.3 b	80.7 a
15 minutes	3.7 b	96.3 a

Table 2. Effect of subculture on browning and BA on callus and shoot formation from nodal explants of grapes.

Treatments	Browning (%)	Callus (%)	Shoot formation (%)
MS	0.3	0 c	3.7 b
MS + 5 µM BA	0.5	80 a	93.7 a
MS + 10 µM BA	0.2	50 b	83.3 a

Micro shoots were transferred on MS medium supplemented with IBA (0, 5 and 10 µM) for root induction. Media having 10 µM IBA proved the best for root formation in micro shoots (Table 3). MS medium without IBA showed complete failure in root formation. Hicks & Dorey (1988) also reported roots at high frequency on MS plus IBA but level of IBA was different than our treatment which may be due to different varietal response. Similarly Lewandowski (1991) also obtained 95% rooting of micro-cuttings on MS having a combination of IBA and NAA.

Callus induction and embryogenesis: Nodal segments induced 80% callus on media having 5 µM BA. Only the 50% stem segments induced callus on media with 10 µM BA whereas the cultures on MS media without BA did not respond (Table 2). *In vitro* grown nodal segments did not show any callus production on any of the BA treatment. However, Wang *et al*., (1985) reported callus development on different concentrations of BA and 2,4-D.

Another set of explants was cultured on MS medium having NAA (0, 1, 5 and 10 µM) for callus formation. Data presented in Table 4 indicates that maximum nodal segments induced callus at 10 µM NAA (80%) followed by 5 µM NAA. In contrast to nodal segments, maximum leaf disk explants (80%) stimulated to develop callus on media having 1 µM NAA while higher concentrations of NAA (5 or 10 µM) were statistically similar for callus production. Shoot tip explants cultured on different levels of NAA showed non-significant and low callus production than other explants. These results are supported by Clog *et al*., (1990) and Robacker (1993) who obtained callus in grapes using different levels of NAA from leaf disk and stem segments. Aerial root formation was also observed in case of induced callus. Nodal segments cultures at NAA (10 µM) produced more aerial roots as compared with other treatments (Table 4). Number and length of roots in these cultures increased with passage of time.

Callus multiplication and embryogenesis on MS media supplemented with 5 µM BA or NAA showed maximum callus multiplication (80% and 70%, respectively). IBA (10 µM) did not depict any increase in callus mass. Callus after removal of aerial roots was transferred on MS plus 2 mg/l NAA. Callus cultures induced from leaf disk explants developed maximum percentage of somatic embryos (60%) as compared with callus from nodal segments. However, Rajasekaron & Mullins (1979) reported somatic embryo from grapes anthers on Nitsch media containing 2,4-D and BA.

Table 3. Effect of different concentrations of IBA on rooting of grapes shoots *in vitro*.

Treatments	Rooting (%)	No. of roots/culture
MS + 0 µM IBA	0 c	0
MS + 5 µM IBA	30 b	2.3
MS + 10 µM IBA	80 a	3.7

Table 4. Effect of different concentrations of NAA on callus induction from different explants of grapes.

Treatments	Node		Leaf disk		Shoot tip	
	Callus (%)	Aerial rooting (%)	Callus (%)	Aerial rooting (%)	Callus (%)	Aerial rooting (%)
MS	0 c	0 c	0 c	0 c	0 c	0 c
MS + 1 µM NAA	40 b	40 b	80 a	60 a	10 b	10 b
MS + 5 µM NAA	70 a	30 b	40 b	20 b	20 b	20 b
MS + 10 µM NAA	80 a	50 a	50 b	30 b	30 a	30 a

Table 5. Comparison of morphological characteristics of plantlets developed from *in vivo*- and *in vitro*-grown shoots of grapes.

Types of explants	No. of branches	Length of branches (cm)	No. of roots	Length of roots (cm)	No. of leaves
In vivo shoots	3.5	4.4	3	6.5	5.6
In vitro shoots	7.5	3.8	7	2.9	11.3

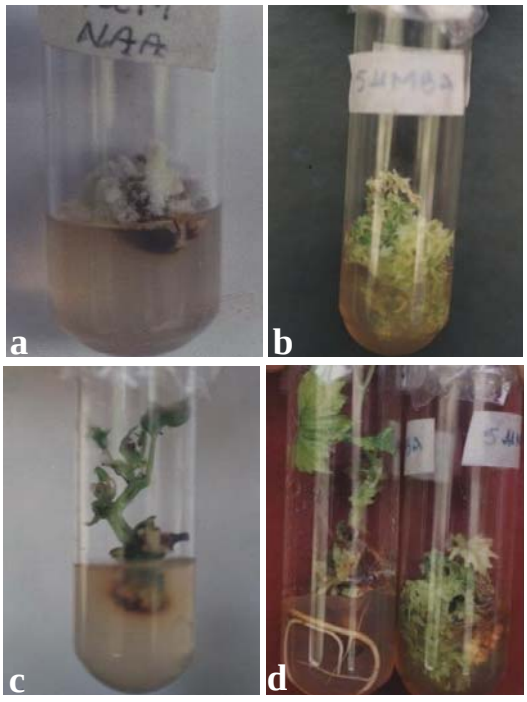

Fig. 1. a) callus induction on 10 µM NAA media; b) somatic embryos developed on 5 µM BA; c) shoot induction from nodal explants on 5 µM BA ; d) root development on 10 µM IBA.

Comparison of plantlets grown from different explant source: Comparison between morphological characteristics of plantlets micropropagated from nodal segments of field grown shoots and *in vitro* proliferated plantlets was also made (Table 5). Higher number of roots, shoots and leaves but smaller length of roots, shoots and size of leaves were recorded in *in vitro* proliferated plantlets than micropropagted from nodal segments.

The present study describes a method for table grape (*Vitis vinifera*) cv. Perlette propagation. Both shoot proliferation and callus formation can be accomplished by the use of BA or NAA. This method of clonal propagation is characterized by higher regeneration efficiency, achieved through simple *in vitro* manipulations.

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