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In vitro plant regeneration in seedless grapes (*Vitis vinifera* L.)

by

ZORA SINGH and S.J.S. BRAR

Department of Horticulture, Punjab Agricultural University, Ludhiana, India

Summary: Young leaves, shoot apices, nodal and internodal segments from mature field-grown grapevines (*Vitis vinifera* L.) cvs Thompson Seedless and Perlette were cultured on MS medium containing different concentrations and combinations of NAA, 2,4-D, Kin and BAP. The genotype, nature of explant and chemical composition of medium affected the callusing frequency and type of callus. The media containing auxins produced friable, soft and creamish white to green calli. Such calli turned brown and died within 4–6 weeks. The medium containing BAP or Kin produced green compact and nodular calli. The subculturing of green nodular calli on MS basal medium induced 20.0 and 12.5 % rooting in cvs Perlette and Thompson Seedless respectively. Subculturing of calli on MS medium containing BAP (2 mg/l) resulted in 12.1 % complete plant regeneration in Thompson Seedless, cv. Perlette failed to regenerate. Adventitious shoots developed in 65.4 and 50.0 % of the cases from the *in vitro* derived leaves of Thompson Seedless and Perlette when cultured on MS medium containing BAP (2 mg/l). More than 85 % of the adventitious shoots of both the cultivars rooted successfully on MS medium containing IBA (1 mg/l).

Key words: adventitious shoot, explant, grapevine, regeneration, Vitis vinifera.

Abbreviations: NAA (α-naphthalene acetic acid); 2,4-D (2,4 dichloro phenoxy acetic acid); IBA (indole butyric acid); BAP (benzyl amino purine); Kin (6-furfuryl amino purine); MS medium (MURASHIGE and SKOOG's medium 1962).

Introduction

Grape is usually consumed as table fruit in India. Perlette and Thompson Seedless cultivars are commercially grown for seedless table grapes in northern plains of India. Since embryo abortion from the developing seeds is a major bottle-neck in breeding seedless grapes (for literature see: SINGH and BRAR 1992), somatic cell culture offers an attractive alternative for the incremental improvement of the existing commercial seedless cultivars of grapes (discussed in SINGH *et al.* 1992). Since the first report on successful grape culture *in vitro* (MOREL 1944), callus cultures have been initiated from various explants viz. stem, tendrils, petioles, flowers and berries of *Vitis* (for literature see: KRUL and WORLEY 1977). Later on, adventitious shoot organogenesis and plant regeneration have been achieved from fragmented shoot apices (BARLASS and SKENE 1980), internodal segments (FAVRE 1977, RAJASEKARAN and MULLINS 1981) and leaves (CLOG *et al.* 1990; STAMP *et al.* 1990). The present investigations deal with callus culture and plant regeneration from various explants of seedless grapes.

Materials and methods

The present investigations were initiated on 15-year-old grapevine (*Vitis vinifera* L.) cvs Thompson Seedless and Perlette at Ludhiana (32 °N and 76 °E) in Northern India. The experimental vines were kept under similar cultural practices for intensity and time of pruning, fertilizers, irrigation and plant protection (ANONYMOUS 1989).

Correspondence to: Dr. ZORA SINGH, C. Biol. M.I.Biol. (London), Department of Horticulture, Punjab Agricultural University, Ludhiana 141 004, India

Young leaves, shoot apices, nodal and internodal segments (5 top nodes from 1-month-old shoots) were excised from field-grown mature vines of both cultivars. After washing with running tap water, they were surface-sterilized with mercuric chloride solution (0.1 %) containing sodium lauryl sulphate (0.1 %) for 2-15 min depending upon the type of explant. The different explants were aseptically cultured on MS medium supplemented with different concentrations and combinations of NAA, 2,4-D, BAP and Kin. The cultures were incubated at 25 ± 2°C under 16 h fluorescent light and subcultured at 3-4 weeks intervals. The observations on callusing were recorded 6 weeks post culturing. The nodular green calli derived from nodal segments were transferred to basal MS medium and rhizogenesis was recorded 5 weeks post culturing. Green nodular calli derived from nodal segments were transferred to MS media containing varying levels of BAP. Plant regeneration was recorded 6 weeks post culturing.

In another experiment, *in vitro* developed leaves were cultured on MS medium containing BAP (0-2.0 mg/l). The cultural conditions including temperature and light were similar as mentioned above. The observations on the adventitious shoot were excised and transferred to MS medium containing different concentrations of IBA and rooting was recorded 6 weeks post culturing.

Results and discussion

C a l l u s i n g : The type of callus and frequency of callusing varied among different explants, culture media and cultivars (Table). In cv. Perlette maximum callus induction (91.6 %) was from young leaves cultured on MS medium containing NAA (10 mg/l) + BAP (0.5 mg/l), whereas in cv. Thompson Seedless the maximum callus induction was 86.3 % from young leaves cultured on MS supplemented with NAA (5 mg/l) + BAP (0.5 mg/l). The shoot apices of Perlette and Thompson Seedless cultured on MS containing NAA (10 mg/l) + BAP (0.5 mg/l) and MS supplemented with BAP (5 mg/l) included highest callusing (86.8 and 76.4 %, respectively; Table). The nodal segments of Perlette and Thompson Seedless cultured on MS containing Kin (2 mg/l) + BAP (2 mg/l) and MS supplemented with NAA (10 mg/l) + BAP (0.5 mg/l) produced maximum callusing (89.4 and 85.7 %, respectively). The internodal segments resulted in 83.3 % callusing in Perlette and 73.0 % in Thompson Seedless when cultured on MS medium containing BAP (2 mg/l).

The medium containing 2,4-D failed to induce callusing in all the explants except in young leaves of Perlette. In general, the genotype, nature of explant and chemical composition of the culture media affected callogenesis. Likewise, the determining effects of various explants viz. leaf, stem or petiole segments have been reported earlier (KRUL and WORLEY 1977). The media containing 2,4-D failed to induce callusing, possibly due to its phytotoxicity on dicots. In general, calli were produced from the cut ends of the explants. The media containing auxins produced friable, soft and creamish white to green calli which turned brown and died within 4-6 weeks post subculturing. The results of the present investigations are in conformity with those reported by KRUL and WORLEY (1977): they stated that the callus culture of grapes began to turn brown after two subdivisions at 30-day intervals. The long term maintenance of callus in *Vitis* in presence of auxins is not always possible. Similar observations have also been reported by SRINIVASAN and MULLINS (1980). The media containing cytokinins produced green nodular and compact calli. The green nodular calli were maintained for 1.5 years by subculturing on medium containing BAP (5 mg/l).

R h i z o g e n e s i s a n d p l a n t r e g e n e r a t i o n : From a total number of 80 and 96 calli derived from nodal segments 20.0 and 12.5 % showed rhizogenesis in Perlette and Thompson Seedless, respectively, when cultured on MS hormone free basal medium. When non-nodular or friable calli, derived from any explant of Perlette and Thompson Seedless, were cultured on media containing various levels of BAP (0.1, 0.5, 1.0 and 2.0 mg/l), they failed to

Table

Percent of explants producing callus and type of callus obtained from different explants of *Vitis vinifera* cvs Perlette and Thompson Seedless, 4-6 weeks post culturing on MS (1962) medium containing different concentrations and combinations NAA, 2,4-D, Kin and BAP.

Medium	Young leaves		Shoot apices		Nodal segments		Internodal segments	
	Percent callus	Type of callus	Percent callus	Type of callus	Percent callus	Type of callus	Percent callus	Type of callus
				Perlette				
M1	40.0	-	-	-	-	-	43.7	LGNH
M2	85.7	FCr	64.3	NCG	-	-	83.3	CrCN
M3	66.6	FCr	65.0	NLG	-	-	58.8	CrCN
M4	50.0	-	83.3	NLG	89.4	NLGH	52.6	CrCN
M5	33.0	-	41.3	-	-	-	0.0	-
M6	0.0	-	0.0	-	-	-	0.0	-
M7	80.0	FCr	55.5	NLGS	-	-	-	-
M8	91.6	FCr	86.6	NLGS	-	-	-	-
				Thompson Seedless				
M1	23.5	NWG	-	-	42.8	NCCrG	40.0	-
M2	67.5	NCrGS	52.0	CrGSN	76.9	NCCrG	73.0	CrCN
M3	78.1	NCrGS	76.0	CrGSN	83.3	NCCrG	68.0	CrCN
M4	50.0	LGSN	71.4	WGSN	40.0	NCCrG	50.0	CrCN
M5	0.0	-	0.0	-	0.0	-	0.0	-
M6	0.0	-	0.0	-	0.0	-	0.0	-
M7	86.3	CrGNS	70.0	LGNS	-	-	-	-
M8	82.5	CrGNS	68.7	LGNS	85.7	WGHN	71.4	LGN

-: callus died; F: friable; Cr: creamish; C: compact; N: nodular; G: green; S: soft; H: hard; L: light; W: white; M1: MS (1962) basal; M2: MS + BAP (2 mg/l); M3: MS + BAP (5 mg/l); M4: MS + BAP (2 mg/l) + Kin (2 mg/l); M5: MS + 2,4-D (2 mg/l) + BAP (0.5 mg/l); M6: MS + 2,4-D (5 mg/l) + BAP (0.5 mg/l); M7: MS + NAA (5 mg/l) + BAP (0.5 mg/l); M8: MS + NAA (10 mg/l) + BAP (0.5 mg/l).
Data based on 50-70 effective cultures on each medium. Each culture containing a single explant per test tube.

regenerate in most cases. Only cv. Thompson Seedless on MS medium containing BAP (2 mg/l) regenerated 12.1 % complete plants (including roots and adventitious shoots) whereas the cv. Perlette failed to regenerate. Rhizogenesis and plant regeneration seemed greatly influenced by the type of callus and genotype. Generally, nodular calli produced roots and/or complete plantlets (depending upon the composition of culture media) whereas friable callus failed to produce roots and plantlets when cultured on the same medium. Successful

organogenesis from internodal explants of grapevines has also been reported by RAJASEKARAN and MULLINS (1981) and by CLOG *et al.* (1990).

Organogenesis from *in vitro* leaves: Leaves excised from micropropagated grapes failed to develop adventitious structures when cultured on MS medium without or with 0.5 mg/l BAP. Adventitious buds were observed within 2 weeks of culture on media containing 1.0 or 2.0 mg/l of BAP: Thompson Seedless: 4.5 ± 3.4 % and 65.4 ± 4.8 % resp.; Perlette 40.1 ± 3.6 and 50.0 ± 4.1, resp. After 5 weeks the regenerating explants exhibited a mass of adventitious buds (10-25) without callus formation. The shoot elongation occurred upon transfer to fresh medium. The response of explants forming shoots was dependent on cytokinin concentration and genotype. The differences in the response of cultured leaves obtained from field-grown vines and *in vitro*-derived leaves may be ascribed to the differences in the physiological status of the leaves at the time of culturing, particulary the endogeneous levels of phytohormones Excised shoots were transferred to MS medium containing IBA (1 mg/l) and developed roots within 4-5 weeks. The rooting was recorded on more than 85% adventitious shoots. Direct shoot organogenesis and plant regeneration from leaves of *Vitis* species has also been reported by FAVRE (1977) and STAMP *et al.* (1990). Although the adventitious shoot formation and subsequent plant regeneration may not exhibit somaclonal variation because an intermediary callus stage was lacking the method may have good potentialities in gene transfer.

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