Indian J. Plant Physiol., Vol. 9, No. 2, (N.S.) pp. 145-157 (April-June, 2004)

IN VITRO MULTIPLICATION OF GRAPE (*VITIS VINIFERA* L.) CV. TAS-A-GANESH FROM NODAL SECTOR AND AXILLARY BUD EXPLANTS

A.R. NALWADE AND M.G. SHITOLE*

Department of Botany, University of Pune, Pune – 411007

Received on 7 May, 2003, Revised on 15 May, 2004

SUMMARY

Nodal sector (3 cm) and axillary bud (1 cm diameter) explants of field grown grape (*Vitis vinifera* L.) cv. Tas-A-Ganesh aseptically cultured on Murashinge and Skoog's (MS) basal medium containing 0.0 to 24.2 µM BAP, 0.0 to 34.2 µM IAA, 0.0 to 29.54 µM IBA, 0.0 to 22.6 µM 2, 4-D and 0.0 to 26.8 µM NAA separately and in various combinations. Eighty and eighty five per cent of cultures showed callusing at 19.72 µM NAA and 9.04 µM 2, 4-D, respectively. The maximum growth of callus of weight 1.987 g and 1.825 g per explant was produced at 10.2 µM NAA and at 9.04 µM 2,4-D, respectively, indicating these concentrations to be optimum for the growth of the callus. All cultures produced callus at very high rate using a combination of 2.22 µM of BAP + 10.72 µM NAA, indicating that combination of BAP and NAA is best for induction of callus and callus growth where 2.15 g of callus produced per explant. Multiple shoots induced with 2.2 to 22.0 µM BAP from both the explants. Maximum number of shoots (3.7±0.48 per explant) were obtained at 13.2 µM BAP alone. However, combination of 11.0 µM BAP + 2.85 µM IAA induced healthy and stout shoots (4.1±0.31 per explant), indicating it is the best hormone combination than supplementing BAP alone. *In vitro* grown shoots (2.0 to 8.0 cm height and 30 days old) when transferred to MS medium supplemented with increasing concentrations (0.0 to 34.2 µM) of IAA and (0.0 to 29.52 µM) IBA separately, 93 and 99% cultures induced rooting at 5.7 µM IAA and 4.92 µM IBA, respectively. Rooting at all concentrations of IBA was superior than IAA in this experiment. IBA (4.92 µM) was the best for rooting of *in vitro* plantlets. Fifty one per cent of *in vitro* grown plantlets were hardened at 80 to 85% humidity and transferred to field successfully.

***Key words*: Callus, grape, multiple shoots, rooting.**

INTRODUCTION

Vitis vinifera L. is an important commercial species of family Vitaceae. It is important for the production of high-quality wine and table fruit. *Vitis* is a temperate plant. With good management, grapes are cultivated in tropical climates and with careful pruning may even produce two crops per year.

Three potential methods for rapid clonal multiplication of grapevines were developed during 1970s. Regeneration of plantlets of cvs. *V. rupestris, V. riparia, V. vinifera* from axillary bud explants on Galzy medium were observed by Galzy (1961, 1963), while Jona and Webb (1978) produced plantlets of *V. vinifera* L. cv. Sylvaner Riesling on Campbell and Durzan (1975) medium from axillary bud. Chee and Pool (1985) obtained multiple shoots in *Vitis* hybrid Remaily Seedless with BAP.

* Correspondings author, E-mail: mgs@unipune.ernet.in

Some of the requirements for propagation using internode cuttings (Pool and Powell 1975, Ecevit 1979), nodal explants bearing a single axillary bud (Mhatre *et al*. 2000) and the rapid multiplication of shoot tips and virus elimination (Jona and Webb 1978, Harris and Stevenson (1979) were reported. A novel method for rapid multiplication involving the induction of adventive buds from fragmented apical meristem explants was also described (Barlass and Skene 1978) in eleven genotypes of *V. vinifera*.

In vitro plantlets from various explants such as axillary buds, stem, fruit stalk and tendrils have been obtained in various cultivars of grapes by using BAP, IBA, NAA, and zeatin alone and in combination. (Novak and Juvova 1982, Skene and Barlass 1983, Wang *et al*. 1985, Diaz *et al*. 1995).

However, the work on micro-propagation of Indian cultivar (Tas-A-Ganesh of *V. vinifera* L.) is meager. Recently, Mhatre and coworkers (2000) succeeded in the micro-propagation of Indian cultivars, viz. Sonaka, Tas-A-Ganesh and Thompson Seedless by using G16, GM2, MS2 and GR1 media where nodal explants bearing a single axillary bud had to culture successively on these above mentioned media. They did not try MS medium and also the long time (about 6 months) was required for raising *in vitro* plants. Looking at its commercial importance, there is an urgent need to develop quick, simple, easy and low cost protocol for this recent promising cultivar Tas-A-Ganesh. An attempt was therefore made to develop a protocol for micropropagation of *V. vinifera* L. cv. Tas-A-Ganesh which is widely cultivated in the state of Maharashtra for table fruit. In this paper we report the optimization of micropropagation protocol in grape cv Tas-A-Ganesh.

MATERIALS AND METHODS

Young shoot (45 days old) of field grown vines of cv. Tas-A-Ganesh were procured from National Centre for Grapes, Manjari Farm, Pune, Maharashtra. The nodal sectors were trimmed to about 3 cm and axillary buds were cut to remove the excess bark up to about 1 cm diameter around the axillary buds; washed with detergent and water and then surface sterilized in $HgCl_2$ (0.1% w/v) for 15 min and rinsed four times with sterile distilled water. Nodal sectors and axillary buds between seventh and eleventh buds from the apex were selected for the investigation. Initially 1st to 16th (from the apex) buds were cultured on MS medium supplemented with 0.0 to 24.2 µM BAP. It was found that 7th to 11th buds from the apex responded to culture conditions. It was hence, for further experiments 7th to 11th buds were used.

The basal medium consisted of full-strength and half strength (for rooting) of Murashige and Skoog (1962) supplemented with sucrose (30 g/l) and Difco-Bacto agar (6 g/l). The growth regulators with their increasing concentrations (0.0 to 24.2 µM) of BAP, (0.0 to 34.2 µM) IAA, (0.0 to 29.52 µM) IBA, (0.0 to 26.8 µM) NAA and (0.0 to 22.6 µM) 2, 4-D were added alone or in combinations in the medium before autoclaving. All chemicals, vitamins and growth regulators were obtained from Sigma Chem., USA. The pH of medium was adjusted to 5.8 with 0.1 N NaOH before autoclaving. The medium was digested on the water bath for 30 min. Nutrient medium (20 ml) was poured into 150 x 25 mm corning glass tubes which were plugged with non-absorbent cotton. Tubes containing medium were autoclaved for 20 min. Explants were transferred in the culture medium under aseptic conditions. *In vitro* cultures were incubated at 25 ± 2ºC in a temperature-controlled growth room under 16 h photoperiod (cool white fluorescent Philips tubes 19.75 µmol $m^{-2}s^{-1}$).

Axillary bud explants were transferred on MS basal medium supplemented with 0.0, 2.68, 5.87, 10.72, 16.08, 21.44, 26.8 µM NAA; 0.0, 2.26, 4.52, 9.04, 13.56, 18.08, 22.6 2, 4-D; 0.0, 2.2, 4.4, 6.6, 8.88, 11.0, 13.2, 15.4, 17.6, 19.8, 22.0, 24.2 µM BAP separately and 0.0 to 24.2 µM BAP and 0.0 to 17.1 µM IAA in combination and 0.0 to 24.2 µM BAP in combination with 0.0 to 26.8 µM NAA. Nodal sector and axillary bud explants were transferred on MS basal medium supplemented with 0.0 to 24.2 µM BAP.

In vitro grown shoots (2-8 cm) of 30 days old were transferred to rooting in half strength MS basal medium supplemented with 0.0 to 34.2 µM and 0.0 to 29.52 µM concentrations of IAA and IBA respectively.

Rooted plantlets were removed carefully from the culture medium and washed with sterile distilled water.

Plantlets were transplanted in small plastic pots with drainage holes containing 1 : 1 soilrite and washed sand (with pebble size of 1.0 – 1.5 mm) and nourished with half strength of MS basal medium. The plastic pots were covered with polythene bags to maintain high (70 to 80%) humidity levels. Pots were kept in shade at 650 lux light intensity and temp 25 ± 2ºC. This condition was maintained for first two weeks. Afterwards polythene bags were removed and pots were kept in the shade (650 lux) for one week. Plantlets were exposed to full sunlight (99,000 lux) for 4 h in the 4th week, 6 h in the 5th week, and 8 h in the 6th week. In the 7th week plantlets were taken to the filed conditions.

RESULTS AND DISCUSSION

Effect of NAA on axillary bud explants

The data presented in Table 1 clearly indicate that callusing was induced at 2.68 µM NAA. The number of cultures showing callusing and growth of callus per explant increased with increasing level of NAA to 10.72 µM, where highest (80%) number of cultures showed callus growth and growth of callus per explant (1.987 g fw) was highest (Table 1, Fig. 1). At higher concentrations (16.8 µM) number of cultures producing callus as well as average growth of callus per explant decreased. Callus induction was inhibited at very high (16.8 µM) concentration of NAA (Table 1), thereby indicating that 10.72 µM is the optimum concentration for callus induction and callus growth in axillary bud explants of this cultivar of grape. The calli initiation delayed at every increasing level of NAA (Table 1). At higher levels of NAA, calli were white and fragile and when subcultured on the same medium, turned brown and died within 10 days. Singh *et al.* (1993) reported

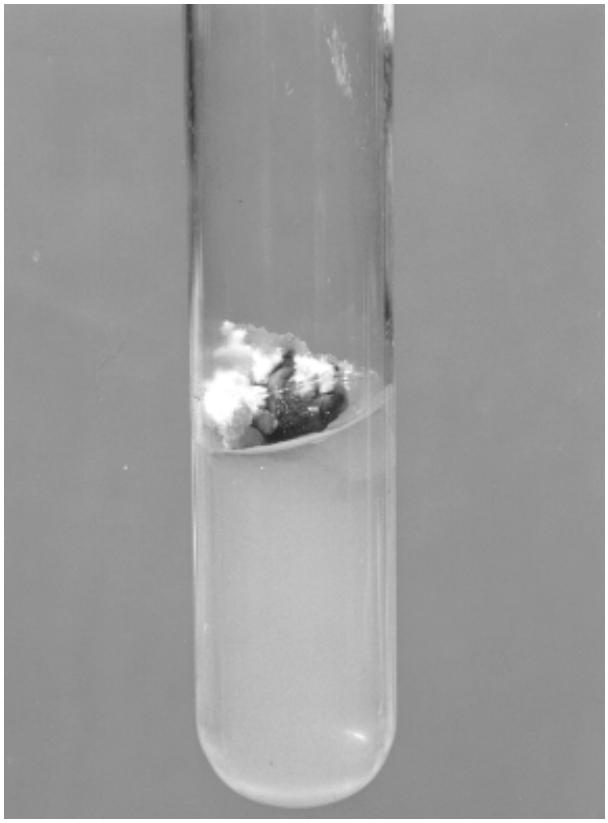


Fig. 1. Callus growth from axillary bud explants during 30 days at 10.72 µM NAA

Table 1. Effect of increasing concentration of NAA on axillary bud explants of grape (*V. vinifera* L.) cv. Tas-A-Ganesh.

NAA µM	Callus initiation: Days after inoculation	Percentage of cultures producing callus	Fresh weight of callus per explant after 30 days of inoculation (g)
0.0	--	0.0	--
2.68	10	20.33	0.621 ± 0.003
5.37	10	55.00	1.225 ± 0.021
10.72	12	80.00	1.987 ± 0.052
16.08	14	50.00	1.023 ± 0.075
21.44	17	25.00	0.726 ± 0.139
26.8	--	--	--

* Results are mean of three replicates (20 x 3) ± SE

-- Explant turned brown after 4 days of inoculation and died within 15 days.

that when young leaves, shoot apices and internodal segments of grapevine (*V. vinifera* L.) cv. Thompson seedless and Perlette cultured on MS medium containing different concentrations of NAA. NAA produced friable, soft and creamish white to green calli, such calli turned brown and died within 4-6 weeks. NAA promoted callus formation in the axillary bud cultures of grapevine (Novak and Juvova 1982). NAA produced a larger basal callus at 0.3 mg/l or higher to the *in vitro* grown shoots of grape (Harris and Stevenson 1982).

When explants of Cabernet Souvignon and Chardonny and of the rootstock Rupestris du Lot cultured on modified MS medium with different concentrations of NAA (over 0.05 mg/l), inhibited regeneration and induced callus on the base of cultured microcuttings (Dimitrova 1995). Results of the present investigation (Table 1, Fig. 1) suggested that optimum concentration for callus formation was 10.72 µM NAA which is in accordance with the previous reports (Novak and Juvova 1982, Singh *et al.* 1993 and Dimitrova 1995).

Fig. 2. Callus growth from axillary bud explants during 30 days at 9.04 µM 2, 4-D

Effect of 2, 4-D on axillary bud explants

Results depicted in Table 2 showed that number of cultures showing callus and callus growth per axillary bud explant increased with increasing concentration of 2, 4-D up to 9.04 µM where 85% of the cultures showed callus growth and callus growth per axillary bud explant (1.825 g fw) was highest (Table 2, Fig. 2). This indicated that the optimum concentration of 2, 4-D for callus induction and its growth in grape cv. Tas-A-Ganesh is 9.04 µM. At all higher concentration of 2, 4-D (13.56 to 18.08 µM), both the parameters decreased with increasing concentration. At 22.6 µM 2, 4-D there was no induction of callus suggesting that high concentration is inhibitory for callus induction. The callus induction delayed at every increasing level of 2, 4-D (Table 2). Calli were cream

Table 2. Effect of increasing concentrations of 2, 4-D on axillary bud explants of grape (*V. vinifera* L.) cv. Tas-A-Ganesh.

2, 4-D µM	Callus initiation: Days after inoculation	Percentage of cultures producing callus	Fresh weight of callus per explant after 30 days of inoculation (g)
0.0	*	0.0	*
2.26	7	45.00	0.575 ± 0.340
4.52	8	66.60	1.030 ± 0.082
9.04	10	85.00	1.825 ± 1.939
13.56	12	51.60	0.932 ± 0.023
18.08	17	31.33	0.625 ± 0.040
22.60	--	--	--

Results are mean of three replicates (20 x 3) ± SE.
* No callus initiaion
-- Explants turned brown after 4 days of inoculation and died within 12 days.

coloured and fragile in all the treatments (Fig. 2). These calli when subcultured (after one month) on the same medium and at respective levels of 2, 4-D, turned brown and died within 12 days of subculture.

Leaf explants of grape hybrids showed strong callogenesis during the first month of culture on MS medium supplemented with 5 µM 2, 4-D (Torregrosa and Bouquet 1995). El-Din and coworkers (1997) obtained callus from muscadine grapes cv. Triumph, Fry, Regale, Golden and Nespitt apical meristems on MS medium containing 2, 4-D but no organogenesis was observed. Singh and coworkers (1993) when cultured young leaves, shoot apices, nodal and internodal segments of *V. vinifera* L. cv. Thompson seedless and Perlette on MS medium, found that the media containing 2,4-D produced friable, soft, creamish white to green calli. Such calli turned brown and died within 4-6 weeks. Similar results were obtained by Singh and cowokers (1993) and Torregrosa and Bouquet (1995) in grape. Results of the present investigation (Table 2, Fig. 2) on Tas-A-Ganesh also revealed that callus produced from axillary bud, leaf, shoot apices, nodal and internodal sectors on MS medium was cream colored, fragile, soft and died within 12 days after subculture.

Effect of increasing concentrations of BAP on nodal sector and axillary bud explants

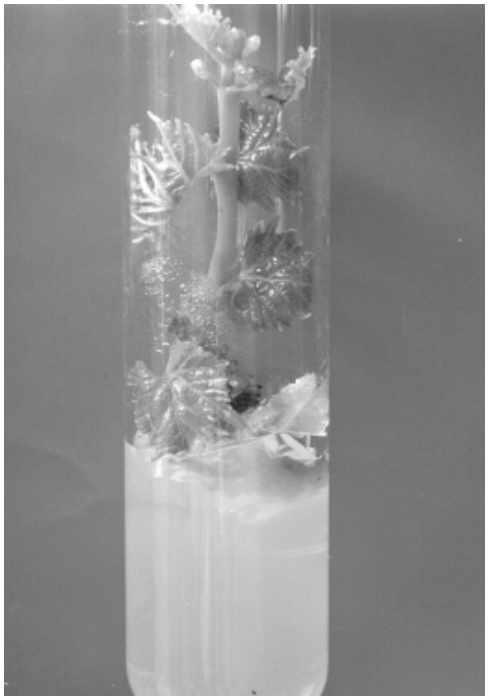
Nodal sector and axillary bud explants which were cultured on MS basal medium containing increasing concentrations (4.4 to 22 µM) of BAP showed that number of shoots per explant increased with increasing concentrations of BAP up to 13.2 µM BAP and decreased at higher levels (Table 3, Fig. 3). Maximum number of shoots (3.7 ± 0.48) per explant was observed at 13.2 µM BAP (Table 3, Fig. 3) in both the explants indicating optimum concentration for shoot initiation. However, it was found that average shoot height was decreased linearly with increasing concentrations of BAP (Table 3, Fig. 3a, b, c, d). Chee and Pool (1985) reported multiple shoot production in *Vitis* hybrid Remaily Seedless with 5.0 µM BAP. Similar observations were recorded with cv. Albarina of *Vitis vinifera* L. with 1.0 mg/l BAP on MS medium (Diaz *et al.* 1995). Recently, Mhatre and coworkers (2000) succeeded in the micropropagation of Indian cultivars, viz. Sonaka, Tas-e-Ganesh and Thompson Seedless by using G16, GM2, MS2 and GR1 media using 2.25 mg/l BAP, where nodal explants bearing a single axillary bud had to culture successively on above media which required 6 months (from initial explant to rooted plantlets in soil).

Peixoto and Pasqual (1996) cultured nodal segments from apical, middle and basal sections of two *in vitro* grown grape vine rootstocks. The segments were cultured on C2D (modified MS) medium supplemented with 0.0, 2.2, 4.4 or 8.8 µM BAP and 0.0, 0.22 or 0.44µM NAA. They observed that concentrations of 4.4 and 8.8 µM BAP gave the best rates of proliferation and growth in rootstock R-99. Results of present

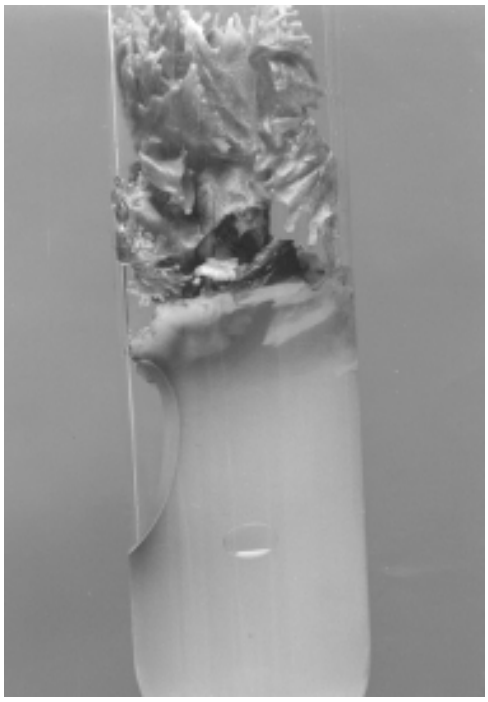
Table 3. Effect of increasing concentrations of BAP on nodal sector and axillary bud explants of grape (*Vitis vinifera* L.) cv. Tas-A-Ganesh.

BAP (µM)	Nodal sector explant		Axillary bud expalnt	
	Number of shoots per culture (per explant)	Average height of shoots after 30 days (cm.)	Number of shoots per culture (per explant)	Average height of shoots after 30 days (cm.)
0.0	--	--	--	--
4.4	1.3 ± 0.67	8.2 ± 0.42	1.1 ± 0.31	8.1 ± 0.32
8.8	2.8 ± 0.42	6.8 ± 0.42	2.8 ± 0.42	6.7 ± 0.48
13.2	3.7 ± 0.48	4.9 ± 0.56	3.7 ± 0.48	4.2 ± 0.42
17.6	1.2 ± 0.42	2.2 ± 0.42	1.2 ± 0.42	2.1 ± 0.31
22.0	0.5 ± 0.42	0.2 ± 0.42	0.1 ± 0.31	0.5 ± 0.31
24.2	--	--	--	--

* Results are mean of three replicates (30 X 3) ± SE.
-- Shoot not induced.

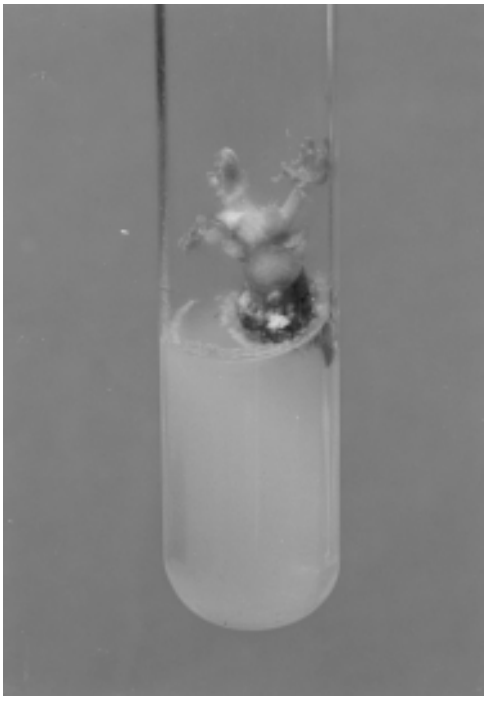


3 (a)



3 (b)

3 (c)



3 (d)

Fig. 3. (a) Multiple shoots (1.1±0.31) from axillary bud explants during 30 days at 4.4 µM BAP, (b) Multiple shoots (2.8±0.42) from axillary bud explants during 30 days at 8.8 µM BAP, (c) Multiple shoots (3.7±0.48) from axillary bud explants during 30 days at 13.2 µM BAP, (d) Multiple shoots (1.2±0.42) from axillary bud explants during 30 days at 17.6 µM BAP

investigation (Table 3, Fig. 3) indicated that BAP is suitable for induction of shoot in grape cv. Tas-A-Ganesh which is in line with the work of Chee and Pool (1985), Diaz and coworkers (1995), Peixoto and Pasqual (1996) and Mhatre and associates (2000).

Effect of increasing concentrations of BAP and IAA in combination on axillary bud explants :

IAA (5.7, 8.55, 11.4 µM) induced callus and roots within 20 days after inoculation while at all higher concentration of it , explant turned brown after 7 days of inoculation and died within 20 days (Table 4). Addition of 2.85 to 17.1 µM of IAA to 2.2 to 22.0 µM BAP, reduced number of cultures showing shoots and increased number of cultures inducing shoots and callus (Table 4). Number of shoots per explant decreased at all levels of BAP by addition of all concentrations of IAA except at 11.0 µM BAP plus 2.85 µM IAA where number of shoots per explants (4.1±0.31) was highest (Table 4, Fig. 4). The shoots produced at this concentration of BAP and IAA were shorter than those produced at 13.2 µM BAP alone (Table 3, Fig. 3c). However, shoots produced at 11.0 µM BAP plus 2.85 µM IAA were more thicker and healthy. This revealed that BAP and IAA in combination is the best for obtaining healthy, thicker and more shoots per explant in this cultivar of grape (Table 4, Fig. 4). The regeneration of shoot reduced with the addition of 0.1 to 0.25 mg/l IAA and it was completely inhibited at 0.5 to 10 mg/l IAA which could be attributed to inhibitory effect of high concentration of IAA on shoot morphogenesis (Pierik 1987). In this cultivar, BAP alone induced shoots while IAA induced roots via callus (Table 4, Fig. 4) while, BAP and IAA in combination induced shoots via callus but not pure callus, suggesting that effect of BAP and IAA in combination is different in different cultivars of grape.

Jako and Nitsch (1980) reported that marked tissue and organ differentiation induced in *V. vinifera* L. shoot tips cultured on MS-Nitsch media with addition of 1.0 mg/l IAA and 2.0 mg/l BAP. According to Choi and coworkers (1992) when meristems were cultured on MS medium, shoot proliferation was the best at 1.0 mg/l BAP + 0.1 mg/l IAA for cv. Khyoho of grape, while, Jiang and coworkers (1995) observed axillary bud propagation and shoot elongation with combined application of BAP and IAA (both at 1.0 mg/l). Feucht and associates (1998) reported growth promotion of calli from internodes of vigorously growing grape cvs. Spatburgunder and Rhmulus on MS medium containing BAP alone and BAP combined with 1.0 or 5.0 mg/1 IAA. The growth was lower on media containing BAP alone but it was more on media with BAP and IAA. Results of the present investigation are in line with these reported by others (Jako *et al.* 1990, Choi *et al.* 1992, Jiang *et al.* 1995, Feucht *et al.* 1998). Present results, therefore, suggested that BAP in combination with IAA is most suitable for multiple shoot induction and growth from axillary bud explant of grape cv. Tas-A-Ganesh (Table 4, Fig. 4).

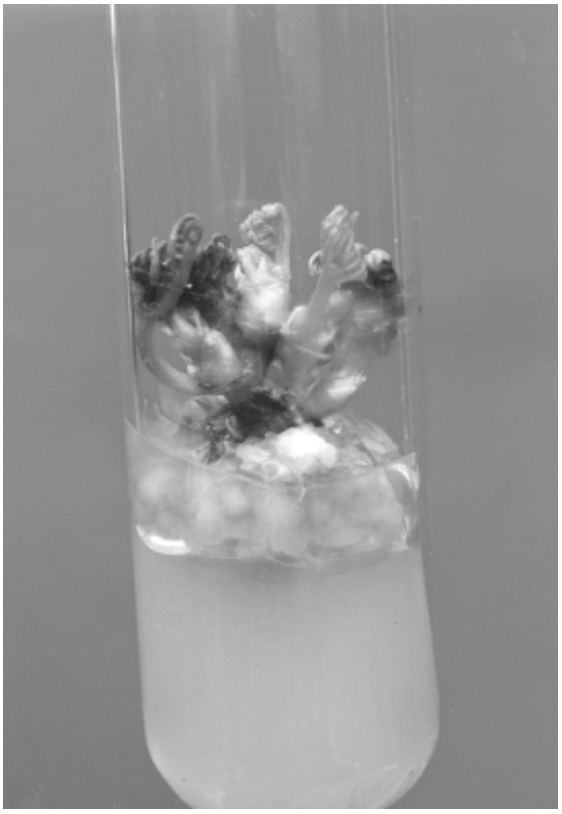


Fig. 4. Multiple shoots (4.1±0.31) from axillary bud explants during 30 days at 11.00 µM BAP + 2.56 µM IAA

Effect of increasing concentration of BAP and NAA on axillary bud explants

The results revealed that addition of 2.68 to 21.44 µM of NAA to 2.22 to 22.0 µM BAP inhibited induction of shoots at all concentrations of BAP by all levels of NAA and in all combinations callus growth was observed (Table 5, Fig. 5). At 2.22 µM BAP + 10.72 µM NAA, 100% cultures showed callus growth and highest growth

Table 4. Effect of increasing concentrations of BAP and IAA in combination on axillary bud expants of grape (*V. vinifera* L.) cv. Tas-A-Ganesh.

Hormones	IAA µM 0.0			2.85			5.7			8.55			11.4			14.25			17.1	
BAP µM	Culture with shoot*	No. of shoots X	Shoot height cm.	Callus and shoot*	No. of shoots X	Shoot height cm.	Callus and shoot*	No. of shoots X	Shoot height cm.	Callus and shoot*	No. of shoots X	Shoot height cm.	Callus and shoot*	No. of shoots X	Shoot height cm.	Callus and shoot*	No. of shoots X	Shoot height cm.	Callus and shoot*	No. of shoots X	Shoot height cm.
0.0	—	—	—	—	—	—	18.00 **	—	—	18.00 **	—	—	19.00 **	—	—	—	—	—	—		
2.2	66.00	1.1±0 31	8.4	57.00	1.2±0 42	4.5	49.00	1.1±0 31	3.5	49.00	1.2±0 63	2.9	47.00	1.3±0 48	2.7	40.00	1.1±0 42	2.6	23.67	0.9±0 42	2.6
4.4	96.00	1.3±0 67	8.1	65.00	1.4±0 51	5.0	54.00	1.6±0 51	6.5	52.00	1.5±0 70	4.5	49.33	1.5±0 52	3.9	45.00	1.6±0 51	3.8	40.00	1.6±0 69	5.5
6.66	93.00	1.4±0 42	7.2	67.33	1.7±0 42	7.5	44.33	1.8±0 42	7.1	40.00	1.8±7 8	6.2	33.00	1.7±0 48	5.8	31.33	1.8±0 63	5.7	31.33	1.9±0 73	5.7
8.88	90.00	2.8±0 42	6.7	49.33	2.5±0 52	6.0	38.00	2.1±0 31	5.5	35.00	1.9±0 56	5.4	30.00	1.8±0 78	5.4	23.66	1.9±0 56	5.9	21.67	2.0±0 81	6.8
11.0	89.00	2.9±0 31	5.3	43.00	4.1±0 31	4.1	23.66	2.4±0 42	4.5	23.66	2.1±0 56	4.1	16.33	2.0±0 47	3.6	15.00	1.9±0 73	3.5	15.00	1.9±0 87	4.6
13.2	87.00	3.7±0 48	4.2	21.00	2.4±0 51	3.7	17.66	1.8±0 42	3.4	15.00	1.8±0 78	3.2	12.00	1.8±0 63	2.7	11.00	1.6±0 69	2.9	11.00	1.0±0 52	3.2
15.4	67.33	1.2±0 52	3.6	12.00	2.3±0 48	2.5	9.00	1.6±0 51	2.4	8.00	1.6±0 70	2.1	8.00	1.6±0 51	1.6	6.66	1.4±0 69	1.6	6.66	1.1±0 53	2.8
17.6	44.33	1.2±0 42	2.1	9.00	2.1±0 31	1.6	7.00	1.2±0 42	1.3	7.00	1.1±0 56	0.9	6.00	1.0±0 47	0.6	5.00	0.8±0 42	0.6	4.33	0.7±0 46	0.5
19.8	17.66	0.7±0 46	1.2	6.00	1.6±0 51	0.9	3.00	0.9±0 30	0.7	2.00	0.8±0 42	0.6	—	—	—	—	—	—	—	—	—
22.0	9.00	0.1±0 31	0.5	3.00	0.8±0 42	0.6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24.2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

*Values are expressed as percentage of cultures.

**Root formation

X No. of shoots per explant.

Table 5. Effect of increasing concentrations of BAP and NAA in combination on axillary bud explants of grape (*V. vinifera* L.) cv. Tas-A-Ganesh.

Hormones	NAA µM																				
BAP	0.0			2.68			5.37			10.72			16.08			21.44			26.8		
µM	Shoot	Callus		Shoot	Callus	W	Shoot	Callus	W	Shoot	Callus	W	Shoot	Callus	W	Shoot	Callus	W	Shoot	Callus	W
0.0	—	—		—	20	0.62	—	56	1.22	—	80	1.99	—	60	1.12	—	28	0.73	—	—	—
2.2	66	—		—	80	1.12	—	84	1.28	—	100	2.15	—	90	1.66	—	48	0.85	—	—	—
4.44	96	—		—	88	1.25	—	86	1.35	—	96	1.86	—	85	1.48	—	37	0.69	—	—	—
6.66	93	—		—	94	1.32	—	90	1.41	—	96	1.77	—	80	1.25	—	31	0.52	—	—	—
8.88	90	—		—	96	1.48	—	94	1.53	—	80	1.58	—	69	1.10	—	27	0.47	—	—	—
11.00	89	—		—	84	1.27	—	78	1.39	—	70	1.46	—	57	0.74	—	21	0.35	—	—	—
13.2	87	—		—	65	0.88	—	60	0.92	—	51	1.10	—	30	0.53	—	13	0.30	—	—	—
15.4	67	—		—	40	0.67	—	38	0.70	—	29	0.80	—	12	0.36	—	—	—	—	—	—
17.6	44	—		—	25	0.41	—	15	0.45	—	11	0.53	—	—	—	—	—	—	—	—	—
19.8	17	—		—	10	0.35	—	5	0.37	—	—	—	—	—	—	—	—	—	—	—	—
22.0	9	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
24.2	—	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

Values are expressed as percentage of cultures

W = Fresh weight in g per explant

— = Shoot or callus not induced

of callus (2.15 g fw per explant) during 30 days (Table 5, Fig. 5), indicating that this is the optimum hormone combination for callus induction and growth in grape cv. Tas-A-Ganesh.

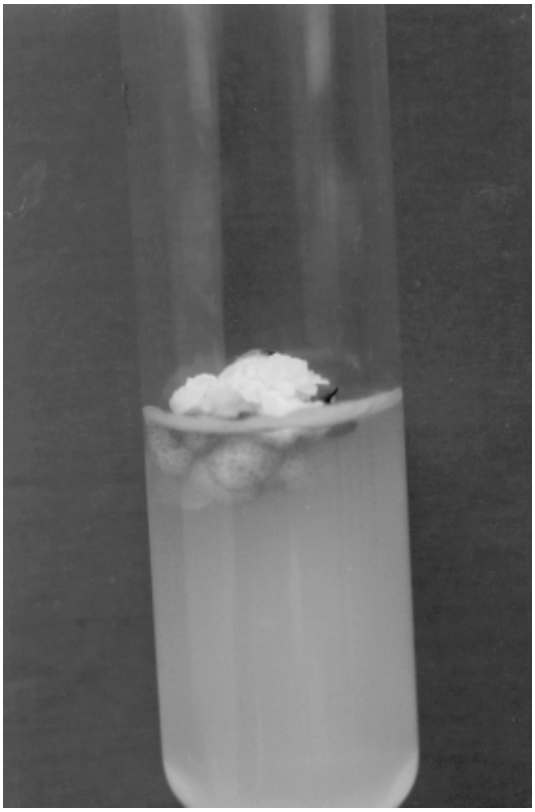


Fig. 5. Growth of callus from axillary bud explants during 30 days at 2.2 µM BAP + 10.72 µM NAA

Tapia and Read (1998) cultured axillary buds of three hybrids on MS medium containing 0.0, 0.1, 0.5, 1.0 or 2.0 mg BAP/l combined with 0.0 or 0.01 mg NAA/l. They reported that BAP at 0.5 or 1.0 mg/l in combination with 0.01 mg NAA/l proved to be the best medium for explant establishment and shoot production. High concentrations of BAP in the absence of NAA were detrimental to plantlets quality. Results of the present investigation (Table 5, Fig. 5) are contradictory to the results of Tapia and Read (1998). The work of Matsuta (1992) revealed that when shoot tips of Perlette vines cultured on MS medium supplemented with 5, 10 or 15 µM BAP and 0, 0.5, 2.0 or 2.5 µM NAA, all treatments influenced the growth and development of shoots but a combination of 0.5 µM NAA and 10.0 µM BAP resulted in the highest survival rate (85%). This indicated that there is cultivar difference in grape vines for requirement of hormones to induce shoots as well as callus. It may be due to difference in requirement of hormone combinations for different explants in grapes. This could be attributed to the difference in endogenous hormones level of various cultivars of grape.

Effect of IAA and IBA on rooting of in vitro grown shoots

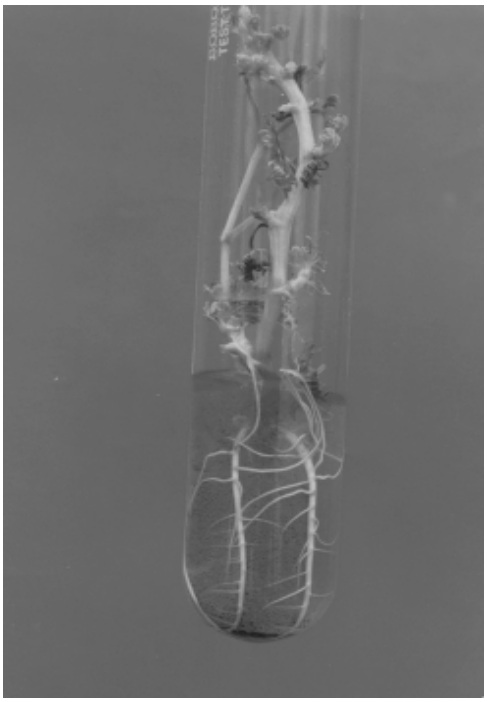
In vitro grown shoots from axillary bud and nodal sector explants produced with 13.2 µM BAP alone and from axillary bud explants obtained with 11.4 µM BAP + 2.85 µM IAA were transferred to full and half strength MS medium supplemented with 0.0, 5.7, 11.4, 17.1, 22.8, 28.5, 34.2 µM IAA and 0.0, 4.92, 9.84, 14.76, 19.68, 24.62, 29.54 µM IBA separately. With full strength MS medium alone and with all concentrations of plant growth regulators IAA and IBA used in the experiment, rooting of shoots was very poor. Hence, half strength MS medium was used as suggested by Novak and Juvova (1982) and Li and Eaton (1984).

The maximum number of shoots (93%) rooted at 5.7 µM IAA and rooting decreased with increasing concentrations (11.4 to 22.4 µM) of IAA (Table 6). IBA (4.92 µM) showed highest number (99%) of rooted plantlets. However, at all higher concentrations (9.84 to 24.62 µM), rooting decreased (Table 6). The number of roots per explant was more at all concentrations of IBA than IAA used in the experiment. Within IAA treatments,

Table 6. Effect of increasing concentrations of IAA and IBA on rooting of *in vitro* shoots of grape (*V. vinifera* L.) cv. Tas-A-Ganesh.

IAA µM	Per cent plantlets showing rooting	IBA µM	Per cent plantlets showing rooting
0.0	--	0.0	--
5.7	93.00	4.92	99.00
11.4	80.00	9.84	87.00
17.1	56.67	14.76	69.67
22.8	32.33	19.68	44.67
28.5	11.67	24.62	12.33
34.2	--	29.54	--

-- = Roots not induced.



6 (a)

6 (b)

Fig. 6 . (a) Rooting of *in vitro* shoots at 5.7 µM IAA during 30 days, (b) Rooting of *in vitro* shoots at 4.92 µM IBA during 30 days

5.7 µM IAA induced highest rooting (Fig. 6a). However, 4.92 µM IBA was the best for rooting in this cultivar (Fig. 6b) where number of roots was more and they were healthy. IAA at 34.2 and IBA at 29.54 µM could not induce roots (Table 6).

Thus, for rooting, IBA is superior to IAA for *in vitro* shoots of cv. Tas-A-Ganesh and these results are similar to the results of Novak and Juvova (1982) and Morini and coworkers (1985). Li and Eaton (1984) reported an increased rooting response of cultivars Marechal Foch and Cascade on MS medium when *in vitro* shoots were pretreated for 15 min in solution of IBA before being placed in growth regulator-free medium. They observed maximum rooting at 2.4 and 3.9 µM IBA. However, Harries and Stevenson (1982) reported 100% rooting with IAA (0.1 mg/l) in eleven cultivars on MS medium and they have not used IBA. This may be due to cultivar differences in endogenous level of plant growth regulators.

Fifty one per cent plantlets survived in the field by the method described in material and methods section (Fig. 7a, c). Thus grape cv. Tas-A-Ganesh is very sensitive and difficult for hardening. Further work is needed to improve per cent survival of plantlets when transferred to the natural conditions.

7 (a)

7 (b)

7 (c)

Fig. 7 . (a) *In vitro* rooted and hardened plantlets in pots, (b) Growth of *in vitro* plantlets (7 weeks), (c) Growth of *in vitro* plantlets in pots (6 months)

REFERENCES

Barlass, M. and Skene, K.G.M. (1978). *In vitro* propagation of grapevine (*Vitis vinifera* L.) from fragmented shoot apices. *Vitis* **17**: 335-340.

Chee, R. and Pool, R.M. (1985). In *vitro* propagation of *Vitis. Vitis* **24**: 106-118.

Choi, S.Y., Oh, J.Y., Kim, J.S., Pak, D.M., Lee, S.B. and Choi, D.U. (1992). Research report of the Rural Development Administration Biotechnology **34**: 1-9.

Diaz, T., Mosquera, M.V., San Jose, M.C. and Gonzalez, E. (1995). Effect of culture media and explant type on propagation of *Vitis vinifera* L. cv. Albrano. *Exp. Bot.* **60:** 11-15.

Dimitrova, V. (1995). Morphogenetic effect of the growth regulator alpha-NAA in *in vitro* propagation of grapevine. *Rasteniev* dni-Nauki **32:** 190-193.

Ecevit, F.M. (1979). Asamin steril ortamada (*in vitro*) mineral beslenmesi. *Ege University Zirat Fak. Derg.* **16:** 95-108.

El-Din, T.N., Rizk, I.A. and Madkour, M. (1997). *In vitro* propagation of muscadine grapes *(Vitis rotundifolia*). Bulletin of Faculty of Agriculture, University of Cairo. **48:** 129-142.

Feucht, W., Treutter, D., Keukenkamp, I. (1998). Growth enhancement of grapevine callus by catechin on auxin-free media. *Vitis* **37:** 67-71.

Galzy, R. (1961). Confirmation de la nature virale courtnou'e de la vigne par des essais de thermotherapie sur des culture *in vitro*. *CR Acad. Sci. Paris* **253:** 706-708.

Galzy, R. (1963). Thermotherapie de quelque varietes de vigne. *Prog. Agric. Vitic.* **160:** 255-261 and 292-297.

Harris, R.E. and Stevenson, J.H. (1982). *In vitro* propagation of *Vitis. Vitis* **21:** 22-32.

Jako, N. and Nitsch, C. (1980). Changing hormonal requirement of tissue cultures from shoot tip meristem with *Vitis vinifera* L. cv. Sultana depending on the stage of development. *Mitt. Klosterneuburg* **30:** 231-237.

Jiang-Aili, Jin-Peifang, Jiang A.L. and Jin, P.E. (1995). Effect of some plant growth regulators on the growth of plantlets of several wild grape species in test tubes. *Acta-Agriculturae-Shanghai* **11:** 87-89.

Jona, R. and Webb, K.J. (1978). Callus and Axillary bud culture of *Vitis vinifera* 'Sylvaner Riesling'. *Sci. Hortic.* **9:** 55-60.

Li, J.R. and Eaton, G.W. (1984). Growth and rooting of grape apices *in vitro*. *Hort. Sci. 1***9:** 64-66.

Matsuta, N. (1992). Effect of auxin on somatic embryogenesis from leaf callus in grape (*Vitis* spp.). *Japanese J. Breed.* **42:** 879-883.

Mhatre, M., Salunkhe, C.K. and Rao, P.S. (2000). Micropropagation of *Vitis vinifera* L. Towards an improved protocol. *Sci. Hortic.* **84:** 357-363.

Morini, S., Marzialetti, P. and Barbieri, C. (1985). *In vitro* propagation of grapevine. *Riv Ortoflorofrutt It.* **69:** 385-396.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* **15:** 473-497.

Novak, F.J. and Juvova, Z. (1982). Clonal propagation of grapevine through *in vitro* axillary bud culture. *Sci. Hortic.* **18:** 231-240.

Peixoto, P.H.P. and Pasqual, M. (1996). Effect of explant origin on *in vitro* multiplication and rooting of grapevine rootstocks. *Ciencia-e-Agrotecnologia* **20:** 301-306.

Pool, R.M. and Powell, L.E. (1975). Influence of cytokinins on *in vitro* shoot development of Concord grape. *J. Am. Hortic. Soc.* **100:** 200-202.

Singh, Z., Brar, S.J.S. and Singh, Z. (1993). *In vitro* plant generation in seedless grapes (*Vitis vinifera* L.). *Vitis* **34:** 229-232.

Skene, K.G.M. and Barlass, M. (1983). Studies on the fragmented shoot apex of grapevine. IV. Separation of phenotypes in a periclinal chimera *in vitro. J. Exp. Bot.* **34:** 1271-1280.

Tapia, M.I. and Read, P.E. (1998). Propagation of grape hybrids by *in vitro* culture of axillary buds. *Agro-Cinencia* **14:** 35-41.

Torregrosa, L. and Bouquet, A. (1995). *In vitro* propagation of *Vitis* X Muscadina hybrids by microcuttings or axillary budding. *Vitis* **34:** 237-238.

Wang, Y., Ge, K., Zou, G., Yang, J. and Ye, M. (1985). Callus induction and plantlets regeneration in grapevines. *Acta. Bot. Sin.* **27:** 661-664.