Journal of Agricultural Technology 2011 Vol. 7(3): 721-737
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Plant regeneration of *Vitis vinifera* (L) *via* direct and indirect organogenesis from cultured nodal segments

Kurmi, U.S.[1], Sharma, D.K.[1], Tripathi, M.K.[1*], Tiwari, R.[2], Baghel, B.S.[3] and Tiwari, S.[4]

[1]Horticultural Biotechnology Laboratory, Jawaharlal Nehru Agricultural University, Jabalpur 482 004, India.
[2]Department of Fruit Science, Jawaharlal Nehru Agricultural University, Jabalpur 482 004, India.
[3]Office of Dean, KNK-College of Horticulture, Mandsaur, Jawaharlal Nehru Agricultural University, Jabalpur 482 004, India.
[4]Division of Transgenic & Tissue Culture, Biotechnology Centre, Jawaharlal Nehru Agricultural University, Jabalpur 482 004, India.

Kurmi, U.S., Sharma, D.K., Tripathi, M.K., Tiwari, R., Baghel, B.S. and Tiwari, S. (2011). Plant regeneration of *Vitis vinifera* (L) *via* direct and indirect organogenesis from cultured nodal segments. Journal of Agricultural Technology 7(3): 721-737.

A protocol was standardized to regenerate three cultivars *namely*: Thompson seedless, Karnet and Local of *Vitis vinifera* (L.) through indirect and direct *in vitro* organogenesis from nodal segment explants excised from *in vitro* grown regenerants as well as one-year-old plants. During present study, induction medium MS2D (MS + 2.0 mg.l^{-1} 2, 4 -D + 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) induced callusing in higher frequencies (84.22%). Culture medium MS3B (MS+3.0 mg.l^{-1} BA + 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) was found to be more responsive for proliferation of shoots in higher frequencies (80.67%). Shoot (s) per explant in higher numbers (14.08) was attained with culture medium MS.2Td (MS+ 0.2 mg.l^{-1} TDZ + 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar), while nutrient medium MS.2Td.5N (MS+ 0.2 mg. l^{-1} TDZ + 0.5 mg.l^{-1}NAA + 30.0 g.l^{-1} sucrose + 7.5 g.l^{-1} agar) produced shoot of higher length (8.78cm). Higher *in vitro* rooting response *i.e.* root proliferation ability (89.94%), number (s) of roots (5.78) and root length (7.75 cm) was exhibited by MS rooting medium fortified with 1.0 mg.l^{-1} IBA, 10.0 g.l^{-1} sucrose and 7.5 g.l^{-1} agar. Genotype Thompson Seedless responded better than Karnet and Local for the most of the attributes investigated. The *in vitro* raised plantlets were acclimatized and established successfully in the field.

Key words: *Vitis vinifera*, nodal segment culture, morphogenesis, indirect organogenesis, direct organogenesis, plantlet regeneration

[*]Corresponding Auther: M.K.Tripathi; e-mail: ms_1tripathi@yahoo.com

Introduction

Grape (*Vitis vinifera* L.) is one of the most important crops worldwide. Genetic improvement of grapevine is exacerbated by polyploidy, high level of heterozygosity and inbreeding depression; it is also a time-consuming process due to 2-3 years generation cycle (Gray and Meredith, 1992; Jayasankar *et al*., 1999). Plant biotechnology could be an attractive means for improving grape (Kuksova *et al*., 1997); however, this requires, as a first step, an efficient and reproducible regeneration system from somatic tissues of mature plants that permits both transformation and regeneration into plantlets. *In vitro* techniques could be used for vine breeding to overcome the difficulties in conventional breeding studies and obtaining new cultivars (Maitz *et al*., 2000).

In grape, plant regeneration can be achieved from several explant types by both shoot organogenesis and somatic embryogenesis. In *Vitis vinfera*, somatic embryogenesis and regeneration of complete plants was first described by Mullins and Srinivasan (1976), subsequently, regeneration of *Vitis vinfera via* somatic embryogenesis and/or organogenesis has been reported from applying diverse explants such as nodal segment (Lee and Wetzstein, 1990; Heloir *et al*.,1997; Mhatre *et al.*, 2000; Sajid *et al*., 2006; Muhammad *et al.*, 2008; Alizadeh *et al*., 2010), leaf discs (James *et al.*, 1990; Robacker, 1993; Das *et al*., 2002; Muhammad *et al.,* 2008; Sadanand *et al.*, 2009), anthers (Lopez-Perez *et al.*, 2005; Cutanda *et al*., 2008), immature ovaries (Nakano *et al*., 1997), style and stigma (Morgana *et al*., 2004; Carimi *et al*., 2005), pistals and stamens (Cutanda *et al*., 2008), as well as immature zygotic embryos (Stamp and Meredith, 1988; Tangolar *et al*., 2008) with varying degree of success.

In vitro morphogenesis, however, appears to be dependent on the interaction between genotype, explant source and culture medium, and thus, it is necessary to develop specific regeneration protocols for each *Vitis* cultivar (Lpez-Perez *et al*., 2005). In this paper, we describe the use of nodal segments as valuable explants excised from mature somatic tissue and available throughout the year for the induction of morphogenesis in higher frequencies by searching appropriate culture medium combinations (especially appropriate concentration and combinations of plant growth regulators) ensuing an effectual protocol for regeneration of three cultivars of grape already under cultivation in Madhya Pradesh and adjoining areas of India.

Materials and methods

Experiments were conducted at the Horticultural Biotechnology Laboratory, KNK College of Horticulture, Mandsaur, Jawaharlal Nehru Agricultural University, Jabalpur (M. P.) with three grape cultivars *namely*:

Thompson seedless, Karnet and Local for nodal segment cultures. Experimental materials were obtained from *in vitro* grown regenerants as well as one-year-old plants propagated in orchard. For *in vitro* grown regenerants, no surface sterilization method was adopted; while for sticks collected from orchard an intensive surface sterilization protocol was standardized prior to culture nodal segments. For this purpose, the indeterminate shoots from top or mid portion of canopy were collected from one-year-old plant in distilled water. Top 1-10 nodal segments were rejected being very soft whereas the nodal segments from 10-20 th nodes were trimmed to 1-2 cm length and determinate shoots attached with nodes were removed. These nodal segments were kept under running tap water for 30 minutes to remove adhering dirt particles. Then nodal segments were placed into double distilled water containing 2% Tween 20 (v/v) for 10 minutes. The cleaned segments were then treated with 70% (v/v) ethanol for 1 minute followed by a treatment with diverse concentrations of Bavistin® (BASF, Germany) for varying durations. Segments subsequently dipped into an aqueous solutions of two different surface sterilizing agents *i.e*. Ca $(OCl)_2$ and $HgCl_2$ in varying concentrations and combinations for diverse durations with initial vacuum of 100 psi. Finally the nodal segments were washed 4-5 times with sterile double distilled water.

To start with a preliminary experiment, two different fortifications of basal media *viz*: MS (Murashige and Skoog, 1962) and WP (Llyod and McCown, 1980) were venerated to search out better *in vitro* response. The basal MS and WP media supplemented with different concentrations of plant growth regulators in various concentrations (Tables 1-4). All the initial culture media were amended with 30.0 g l^{-1} sucrose and 7.5 g. l^{-1} agar. Warm culture media, still in liquid state was poured into baby food bottles (50-60 ml / bottle) and culture tubes (15-20 ml/tube) followed by autoclaving at 121^{o}C under 15 psi pressure for 20 minutes after adjusting the pH to 5.6 ± 0.1 with 1 N KOH. Readymade MS basal medium, plant growth regulators and other ingredients were procured from Hi-media Laboratories, Mumbai, India.

In baby food bottles, 1-2 pieces of nodal segments were cultured and sealed with Lab film (Parafilm®) and were incubated under complete darkness at 25 ± 2°C for one week. Later *in vitro* cultured nodal segments were incubated for 16 hours photoperiod regime with 1200 lux light intensity provided by PAR (Photosynthetically Active Radiation) lamps at 25 ± 2 ^{o} C temperatures and 70% relative humidity.

After 4-5 weeks of initial culturing, nodal segments followed either direct plant regeneration (auxiliary bud proliferation) or indirect organogenesis (*via* callus formation). Shootlets obtained from direct proliferation were transferred to MS elongation medium supplemented with 1.0 mg.l^{-1} GA_3, 15.0 g.l^{-1} sucrose

and 7.5 $g.l^{-1}$ agar. However, calli obtained from indirect organogenesis were subcultured again on the initial medium after four weeks of culture. Cultures were subjected to 25±2°C temperature and photoperiod regimes of 60 μ $mol.m^{-2}.s^{-1}$ luminance provided by cool fluorescent tubes for 16 hr. At times root formation was not attained on regeneration medium, plantlets were subsequently transferred to MS rooting medium fortified with varying strength of IBA, NAA, BA and Kn (alone as well as in combinations), 10 $g.l^{-1}$ sucrose and 7.5 $g.l^{-1}$ agar. Plants uprooted from cultures and thoroughly washed with running tap water to remove the adhering agar were planted in 2.5 cm root trainers filled with 1:1:1 sand, soil and FYM sterilized mixture. Root trainers with transplanted plants were placed under 30±2°C and 70 ± 5% RH for 20-30 days in Environmental Growth Cabinet for acclimatization. Acclimatized plants then were transferred to Net House for 30 days for hardening before transplanting to the field.

Factorial Completely Randomized Design (CRD-factorial) was used to find out the significance of genotype, culture media combinations and their interactive effect. Each treatment was consisted of two replications. Per replication approximately 100-120 nodal segments of each genotype were randomized and cultured on each media. Since all the data were recorded in percentage the arc-sine transformation was made before analysis. The data were analyzed as per method suggested by Snedecor and Cochran (1967).

Results

There were highly significant ($p<0.01$) differences among the response of different surface sterilizing and antifungal agents. The highest percentage of survived nodal segment (72.30%) with aseptic culture (79.05%) was achieved with 1% Bavistin in combination with 0.1% $HgCl_2$ for 10 minutes (data not presented). Therefore, this surface sterilization combination was used throughout the experiment.

During present investigation, three different sets of plant growth regulators were added to fortify MS basal media for culturing nodal segment. Depending upon the nature of different culture media combinations, cultured nodal segments followed either direct or indirect pathway of plant regeneration. In direct approach, plantlets were regenerated on explant surface directly without callus formation (*via* auxiliary bud proliferation); and in indirect mode plantlets were originated *via* callus formation (indirect organogenesis). In direct mode of regeneration, shootlets were developed directly from the meristematic zones of cultured nodal segments (Fig. 1A-D). Shoot formation from cultured nodal segments started approximately 7-15 days from initial culturing and proliferated 1-15 shoots (Fig. 1A-D).

Plantlet also regenerated *via* organogenesis from the callus surface (Fig. 1E-H).The first response of cultured explants was similar after 4 -7 days and mostly independent from explant and culture media. All explants became swollen and no callus proliferation was evident during first few days. Callus proliferation usually started from the portion in contact with the medium and spread upward after 2 weeks of culture (Fig. 1E). Initiated callus tissue developed distinct phenotypes. These pheno-variants were rough, hard, dense and glossy reflecting different developmental potentials. Plantlet regeneration occurred routinely (Fig. 1 F-H) after sub culturing of these organogenic calli. Shootlets obtained from direct as well as indirect modes subsequently transferred on MS elongation medium containing 1.0 $mg.l^{-1}GA_3$, where length of shoots increased subsequently (Fig. 1 I-J). Initiation of root started after 10-15 days of transferring of shootlets in rooting medium (Fig. 1 K). Regenerated plantlets (Fig. 1 L) were subjected to phenotypic evaluation. Although the traits were not scored quantitatively, regenerated plants appeared phenotypically normal and true to the type.

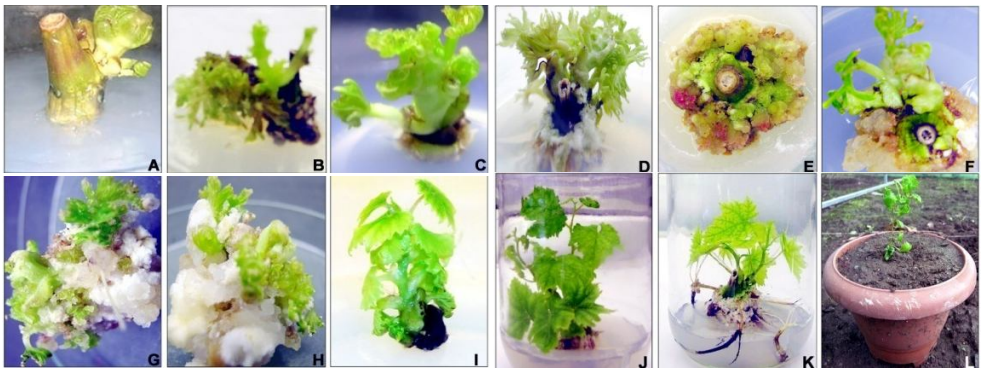

Fig. 1. Plant regeneration from nodal segments of *Vitis vinifera*: A. Initiation of single shoot after 7-10 days in culture; B. initiation of multiple shoots after 10-15 days in culture; C. Formation of multiple shoots after 15-20 days in culture; D. Formation of multiple shoots after 25-30 days in culture; E. Proliferation of callus after 10-15 days in culture; F-H. Indirect organogenesis *in vitro*; I. Elongated shoot after 30-35 days in culture; J. Elongated shoot after 35-40 days in culture; K. *In vitro* rooting of regenerant; L. Hardening of regenerant in Net House.

Nodal segments of three cultivars of grape were cultured on different fortifications of MS medium. Three auxins (NAA, 2, 4-D and 2, 4, 5-T) and three cytokinins (BAP, Kinetin and TDZ) were used as sole (Table 1 and 2) as

well as in a number of combinations and concentrations (Table 3) for culture establishment. The analysis of variance (Table 1-4) revealed highly significant ($P<0.01$) differences among the response of different genotypes and culture media combinations for overall callus induction, shoot proliferating efficiency, number of shoot(s) per responding explant, mean shoot length, root proliferating efficiency, number of root(s) and mean root length. It indicates the presence of considerable amount of variability amongst the different genotypes and culture media combinations. However, non-significant differences were observed for interaction of genotypes with culture media for the most of the attributes investigated.

Effects of different auxins in varying concentrations on *in vitro* are presented in Table 1. Higher callus initiation with culture media fortified with 2,4-D MS2D (84.22%) and MSD (80.56%) and low callus initiation on media devoid of 2,4-D such as MS5T (40.01%) and MS5N (44.40%) reveal that 1.0 - 2.0 $mg.l^{-1}$ 2,4-D induces callus in higher frequencies ($\geq$ 80%). The size of callus also enlarged with the increased level of 2,4-D however, levels above 2.0 $mg.\ l^{-1}$ reduced the response. At the concentration of 4.0 $mg.l^{-1}$ most of the calli turned black with apparent cell mortality. Culture medium altered with 2, 4, 5-T produced calli in lower to moderate frequencies depending upon the concentrations. On the other hand, NAA in the range of 1.0-2.0 $mg.l^{-1}$ proved to be remarkably superior for shoot proliferating efficiency, since culture media MSN (54.48%) and MS2N (51.83%) performed higher for this attribute. However, beyond this concentration, ratio of non-morphogenic calli increased subsequently. Auxins 2,4-D MS5D (31.18%) and 2,4,5-T MS.1T (30.93%) responded poorly for this attribute.

In vitro response of different cytokinins is presented in the Table 2. Varying concentrations of BAP, Kinetin and TDZ supplemented in the medium exhibited very poor callus induction. Maximum callus induction frequency (~35-45%) was recorded with induction media fortified with lower concentration of cytokinins (in range of 0.1-1.0 $mg.l^{-1}$). For shoot proliferating ability, proliferation media MS3B (80.67%) and MS.2Td (78.41%) followed by two *statistically* similar performing nutrient media MS4B (77.72%) and M.1Td (76.51%) proved remarkably superior as compared to other media combinations. Culture media MS.2Td (14.08) and MS.1Td (11.39) followed by MS.3Td (10.31) proved significantly superior for producing shootlets in higher numbers. Shoot with higher length were attained on culture media MS.3Td (4.41), MS.2Td (4.29) and MSB (4.27cm) numerically, however, *statistically* non-significant differences was observed for this attribute.

Combined effects of different added auxins and cytokinins in varying concentrations on various culture phases are presented in Table 3. Maximum

callus induction was documented by induction media MS3N.5B (78.87%) followed by two *statistically* similar responding media *viz*: MS4N.5B (74.82%) and MS2N.5B (74.46%). Inoculation media MS2B.5N (76.42%) and MS3B.5N (75.26%) followed by MS.2Td.5N (74.19%) proved remarkably superior for shoot proliferating efficiency. Culture media MS.2Td.5N (10.13), MS.1Td.5N (9.56), MS.5Td.5N (8.97) and MS3B.5N (7.74) with *at par* performance proved significantly superior for producing shootlets in higher numbers, whereas, shoot of higher length were recovered from culture media MS.2Td.5N (8.78 cm) and MS2NB (7.19 cm).

In terms of genotypic response, genotype Thompson Seedless was proved remarkably superior to others for the most of cultures phases. Maximum callus induction frequency was exhibited by cultivar Thompson Seedless (65.28%) followed by Karnet (64.32%) and Local (62.96%). Shoots in higher numbers also achieved in cultivar Thompson Seedless (7.29) with the medium fortified with combination of an auxin and a cytokinin, however, higher shoot proliferating ability was shown by cultivar Thompson Seedless (68.52%) followed by Karnet (67.20%) and Local (64.13%) on medium amended with cytokinin as alone. Cultivar Karnet (4.69 cm) followed by Local (4.56 cm) and Thompson Seedless (4.50) produced shoots of higher length numerically.

Shootlets of grape were transferred into twenty-one different fortifications of basal MS media for induction of *in vitro* rooting (Table 4). Maximum *in vitro* root proliferating ability was demonstrated by rooting media MSI (89.94%), MS2I (89.65%) and MS I.5Kn (88.57%) with statistically *at par* performance. For higher number (s) of root initiation per explant, culture medium MSI (5.78) was found to be numerically superior. The roots of higher length were developed by two similar responding nutrient media *i.e.*: MSI (7.75 cm) and MS2I (7.20cm).

Discussion

The purpose of the present investigation was to develop reliable protocol for plant regeneration in higher frequencies from nodal segments derived cultures in order to use them for mass *in vitro* propagation and genetic transformation experiments. It is well known fact that *in vitro* regeneration of an explant is influenced by several internal and external factors such as nature and hormonal composition of culture medium, species, genotype, explants and various other culture conditions.

Table 1. Effect of different auxins (alone) in varying concentrations on *in vitro* response of cultured nodal segments.

Culture media ▼ Genotypes ►	Auxins	Conc. mg.l^{-1}	*In vitro* response															
			Callus proliferating nodal segments (%)				Shoot proliferating nodal segments (%)				Number of shoot (s) per responding nodal segments				Mean shoot length (in cm)			
			Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean
MS.1N	NAA	0.1	55.08	52.31	53.74	**53.71^{i}**	40.09	38.50	36.09	**38.22^{f}**	1.22	1.20	1.18	**1.20^{a}**	3.82	3.80	3.78	**3.80^{b}**
MS.5N		0.5	65.61	64.80	64.20	**64.87^{g}**	50.15	45.09	44.08	**46.44^{d}**	1.48	1.38	1.30	**1.38^{a}**	4.63	3.61	3.59	**3.94^{b}**
MSN		1.0	70.22	70.09	69.09	**69.80^{e}**	55.15	54.49	53.80	**54.48^{a}**	1.96	1.82	1.74	**1.82^{a}**	5.65	4.59	4.62	**4.92^{ab}**
MS2N		2.0	71.58	70.39	70.05	**70.00^{e}**	54.39	51.09	50.02	**51.83^{ab}**	2.28	2.12	1.98	**2.12^{a}**	7.32	6.28	6.25	**6.62^{a}**
MS3N		3.0	75.22	74.11	74.88	**70.67^{c}**	51.25	48.60	49.02	**49.62^{c}**	2.10	2.01	1.82	**1.97^{a}**	6.84	5.74	5.82	**6.13^{a}**
MS4N		4.0	50.11	48.20	49.15	**49.15^{j}**	45.60	45.08	44.72	**45.13^{e}**	1.90	1.82	1.66	**1.79^{a}**	4.69	4.61	4.58	**4.62^{b}**
MS5N		5.0	45.30	44.25	43.65	**44.40^{k}**	48.20	41.02	40.20	**43.58^{e}**	1.85	1.78	1.69	**1.77^{a}**	3.48	2.50	2.49	**2.82^{c}**
MS.1D	2,4-D	0.1	70.28	69.60	70.12	**70.0^{de}**	41.01	39.20	39.01	**39.74^{f}**	1.64	1.60	1.48	**1.57^{a}**	2.72	2.69	2.66	**2.54^{c}**
MS.5D		0.5	75.61	74.32	72.60	**74.17^{c}**	48.02	46.02	45.90	**46.64^{d}**	1.80	1.75	1.62	**1.72^{a}**	2.82	2.78	2.80	**2.80^{c}**
MSD		1.0	80.60	81.02	80.08	**80.56^{b}**	50.75	50.40	49.82	**50.32^{bc}**	1.89	1.72	1.69	**1.76^{a}**	3.40	3.38	3.36	**3.38^{c}**
MS2D		2.0	85.32	84.15	83.21	**84.22^{a}**	46.32	45.15	45.01	**45.49^{de}**	1.81	1.72	1.61	**1.71^{a}**	3.89	2.76	2.78	**3.14^{c}**
MS3D		3.0	70.32	68.12	70.02	**69.48^{e}**	45.66	43.08	42.01	**43.38^{e}**	1.71	1.65	1.55	**1.63^{a}**	2.32	2.30	2.28	**2.30^{c}**
MS4D		4.0	60.18	60.09	59.82	**60.03^{h}**	40.66	38.52	40.21	**39.79^{f}**	1.42	1.33	1.22	**1.32^{a}**	2.44	1.92	1.90	**2.08^{c}**
MS5D		5.0	52.09	99.82	48.22	**66.71^{i}**	32.22	30.02	31.30	**31.18^{h}**	1.32	1.20	1.09	**1.20^{a}**	1.28	1.26	1.23	**1.26^{c}**
MS.1T	2,4,5-T	0.1	54.60	52.30	50.44	**52.44^{i}**	31.20	31.01	30.60	**30.93^{h}**	1.76	1.65	1.38	**1.59^{a}**	2.69	2.56	2.49	**2.58^{c}**
MS.5T		0.5	65.80	64.25	64.09	**64.71^{g}**	39.60	38.10	38.01	**38.57^{f}**	1.82	1.76	1.52	**1.70^{a}**	3.52	3.49	3.46	**3.49^{bc}**
MST		1.0	70.15	66.26	65.39	**67.26^{f}**	48.70	48.20	47.09	**47.99^{cd}**	1.91	1.89	1.73	**1.84^{a}**	3.66	3.59	3.62	**3.62^{b}**
MS2T		2.0	74.40	71.09	70.09	**71.86^{d}**	45.10	44.80	44.08	**44.66^{e}**	1.90	1.86	1.70	**1.82^{a}**	2.52	2.48	2.49	**2.49^{c}**
MS3T		3.0	70.60	70.32	65.82	**68.91^{ef}**	44.10	44.01	43.02	**43.71^{e}**	1.89	1.78	162	**1.76^{a}**	2.46	2.41	2.38	**2.42^{c}**
MS4T		4.0	45.30	44.05	42.30	**43.88^{k}**	33.25	35.02	34.15	**34.80^{g}**	1.62	1.50	1.52	**1.54^{a}**	1.46	1.38	1.36	**1.40^{c}**
MS5T		5.0	40.31	39.72	40.01	**40.01^{l}**	34.09	33.80	33.16	**33.68^{gh}**	1.25	1.09	1.15	**1.14^{a}**	1.29	1.25	1.23	**1.26^{c}**
Mean			**64.22^{a}**	**62.82^{b}**	**62.23^{b}**		**44.18**	**42.44**	**41.96**		**1.73^{a}**	**1.64^{a}**	**1.53^{a}**		**3.47**	**3.11**	**3.26**	
CD 0.05																		
Genotypes						**0.79**				**NS**				**0.52**				**NS**
Media						**2.09**				**2.97**				**1.38**				**2.29**
G x M						**NS**				**NS**				**NS**				**NS**

Values within column followed by different letters are significantly different at 5% probability level.

Table 2. Effect of different cytokinins (alone) in varying concentrations on *in vitro* response of cultured nodal segments.

Culture media ▼ Genotypes ►	Cyto-kinins	Conc. mg.l^{-1}	*In vitro* response															
			Callus proliferating nodal segments (%)				Shoot proliferating nodal segments (%)				Number of shoot (s) per responding nodal segments				Average shoot length (in cm)			
			Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean
MS.1B	BA	0.1	32.01	30.20	30.01	**30.74^{d}**	68.09	66.42	66.02	**66.84^{f}**	3.52	4.50	2.48	**2.50^{e}**	2.39	2.35	2.32	**2.35^{a}**
MS.5B		0.5	45.30	43.31	40.20	**42.93^{a}**	68.42	68.30	68.25	**68.32^{ef}**	2.98	2.90	2.69	**2.85^{e}**	2.66	2.61	2.60	**2.62^{a}**
MSB		1.0	40.22	38.62	35.38	**38.67^{b}**	73.40	73.20	72.15	**72.91^{cd}**	3.62	3.51	3.50	**3.54^{e}**	4.33	4.28	4.21	**4.27^{a}**
MS2B		2.0	30.18	30.22	30.08	**30.16^{d}**	78.30	76.20	73.60	**76.03^{bc}**	4.41	11.07	3.96	**6.48^{cd}**	3.29	3.26	3.20	**3.25^{a}**
MS3B		3.0	33.60	31.02	25.08	**29.90^{d}**	83.51	80.22	78.30	**80.67^{a}**	3.96	3.20	3.16	**3.44^{e}**	2.18	2.09	2.15	**2.14^{a}**
MS4B		4.0	25.80	22.32	20.29	**22.80^{f}**	78.35	78.02	76.80	**77.72^{b}**	2.89	3.09	2.96	**2.98^{e}**	2.18	2.12	2.09	**2.13^{a}**
MS5B		5.0	20.16	18.10	15.08	**17.78^{h}**	54.51	54.30	54.16	**54.32^{h}**	2.28	2.03	1.96	**2.09^{e}**	1.06	0.99	0.92	**0.99^{a}**
MS.1Kn	Kn	0.1	35.48	34.90	33.65	**34.67^{c}**	58.16	58.12	58.01	**58.09^{g}**	2.78	1.98	1.77	**2.17^{e}**	2.28	2.21	2.15	**2.21^{a}**
MS.5Kn		0.5	30.58	30.23	30.22	**30.34^{d}**	58.18	58.09	58.02	**58.09^{g}**	2.82	2.11	2.02	**2.31^{e}**	2.39	2.32	2.29)	**2.33^{a}**
MSKn		1.0	25.38	22.61	21.80	**23.26^{e}**	65.09	64.38	65.66	**65.04^{f}**	2.76	2.19	1.95	**2.30^{e}**	2.32	3.38	3.35	**3.01^{a}**
MS2Kn		2.0	25.44	22.61	20.32	**22.79^{f}**	68.30	68.10	67.50	**67.96^{f}**	3.36	2.71	2.48	**2.69^{e}**	2.58	2.48	2.51	**2.52^{a}**
MS3Kn		3.0	20.18	21.32	20.15	**20.55^{fg}**	70.80	68.39	66.30	**68.49^{e}**	2.89	2.41	2.17	**2.49^{e}**	3.40	2.29	2.25	**2.64^{a}**
MS4Kn		4.0	20.10	20.08	18.09	**19.42^{gh}**	73.18	73.01	68.22	**71.47^{de}**	2.16	2.09	2.08	**2.11^{e}**	2.30	2.26	2.22	**2.26^{a}**
MS5Kn		5.0	15.20	14.22	12.18	**13.86^{i}**	43.25	43.05	43.01	**43.10^{j}**	2.43	1.10	1.08	**1.53^{e}**	1.92	1.68	1.18	**1.59^{a}**
MS.1Td	TDZ	0.1	25.62	24.60	25.23	**25.15^{e}**	78.40	78.13	73.59	**76.51^{b}**	12.08	11.09	11.02	**11.39^{ab}**	2.61	2.56	2.42	**2.53^{a}**
MS.2Td		0.2	20.19	20.09	15.58	**18.62^{h}**	83.35	78.30	73.60	**78.41^{ab}**	14.46	14.22	13.56	**14.08^{a}**	4.48	4.42	4.35	**4.41^{a}**
MS.3Td		0.3	15.69	15.38	14.39	**15.15^{i}**	79.20	74.35	74.15	**75.90^{c}**	10.94	10.87	9.12	**10.31^{b}**	4.39	4.26	4.22	**4.29^{a}**
MS.4Td		0.4	15.09	14.08	12.48	**13.88^{i}**	74.22	74.08	69.45	**72.78^{d}**	9.35	8.92	8.46	**8.91^{bc}**	3.98	3.89	2.98	**3.61^{a}**
MS.5Td		0.5	10.59	10.45	10.15	**10.39^{j}**	73.38	68.15	68.20	**69.91^{e}**	8.62	8.32	7.88	**8.27^{c}**	2.98	2.75	2.70	**2.81^{a}**
MSTd		1.0	9.92	08.62	08.40	**8.98^{jk}**	59.33	59.25	54.10	**57.56^{gh}**	5.74	5.17	4.98	**5.29^{d}**	2.88	2.72	2.75	**2.78^{a}**
MS2Td		2.0	9.10	08.05	06.05	**7.73^{k}**	49.52	49.22	49.12	**49.28^{j}**	4.90	3.98	3.48	**4.12^{de}**	2.54	2.49	2.38	**2.47^{a}**
Mean			**24.08^{a}**	**22.90^{b}**	**21.19^{c}**		**68.52^{a}**	**67.20^{b}**	**55.60^{c}**		**5.18**	**4.79**	**4.71**		**2.81**	**2.73**	**2.41**	
CD 0.05																		
Genotypes						**0.70**				**1.25**				**NS**				**NS**
Media						**1.39**				**3.33**				**3.26**				**3.61**
G x M						**2.41**				**NS**				**NS**				**NS**

Values within column followed by different letters are significantly different at 5% probability level.

Table 3. Combined effect of different added auxins and cytokinins in varying concentrations and combinations on *in vitro* response of cultured nodal segments.

Culture media ▼ Genotypes ►	Plant Growth regulators		*In vitro* response															
	Auxin NAA $mg.l^{-1}$	Cyto-kinins $mg.l^{-1}$	Callus proliferating nodal segments (%)				Shoot proliferating nodal segments (%)				Number of shoot (s) per responding nodal segments				Average shoot length (in cm)			
			Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean
MS.5B.5N	0.5	BA, 0.5	65.28	65.20	65.02	**65.16^{e}**	70.08	70.35	69.80	**70.07^{c}**	5.62	5.02	4.94	**5.19^{bc}**	4.25	4.11	3.95	**4.10^{c}**
MSB.5N	0.5	BA, 1.0	70.61	68.50	69.79	**69.63^{cd}**	72.41	71.22	70.06	**71.23^{c}**	6.75	6.29	5.82	**6.28^{b}**	4.35	4.21	4.10	**4.22^{c}**
MS2B.5N	0.5	BA, 2.0	64.39	62.32	61.31	**62.67^{f}**	70.18	80.08	79.02	**76.42^{a}**	8.12	7.82	7.16	**7.70^{b}**	3.92	3.88	3.96	**3.92^{c}**
MS3B.5N	0.5	BA, 3.0	58.42	57.39	56.82	**57.54^{h}**	76.33	75.20	74.26	**75.26^{b}**	8.46	7.72	7.06	**7.74^{ab}**	3.08	2.96	2.80	**2.94^{d}**
MS4B.5N	0.5	BA, 4.0	50.34	50.23	49.79	**50.12^{j}**	80.92	70.08	70.02	**73.67^{b}**	5.79	4.88	4.08	**4.91^{c}**	2.95	2.89	2.79	**2.87^{d}**
MS5B.5N	0.5	BA, 5.0	45.25	45.19	45.06	**45.16^{k}**	75.32	73.18	70.55	**73.01^{bc}**	3.82	1.49	3.21	**2.84^{c}**	2.77	2.67	2.72	**2.72^{d}**
MS.5Kn.5N	0.5	Kn, 0.5	74.39	70.30	69.39	**71.36^{c}**	65.32	65.30	60.05	**63.55^{de}**	3.95	3.79	3.22	**3.65^{c}**	5.54	4.39	4.26	**4.73^{bc}**
MSKn.5N	0.5	Kn 1.0	72.52	71.16	70.62	**71.43^{c}**	66.62	64.08	65.21	**65.30^{d}**	4.38	3.99	3.12	**3.83^{c}**	4.26	4.16	4.11	**4.17^{c}**
MS2Kn.5N	0.5	Kn, 2.0	65.10	65.09	60.18	**63.45^{f}**	65.08	65.05	65.01	**65.04^{e}**	4.58	4.04	3.83	**4.15^{c}**	4.18	4.06	3.94)	**4.06^{c}**
MS3Kn.5N	0.5	Kn, 3.0	60.51	59.09	58.28	**59.29^{gh}**	60.88	60.20	60.03	**60.37^{ef}**	4.36	4.12	4.01	**4.16^{c}**	3.10	2.98	2.83	**2.97^{cd}**
MS4Kn.5N	0.5	Kn, 4.0	70.80	70.20	65.34	**68.78^{d}**	62.40	60.32	55.02	**59.24^{f}**	4.89	3.98	3.28	**4.05^{c}**	3.82	3.72	3.65	**3.73^{c}**
MS5Kn.5N	0.5	Kn, 5.0	50.18	56.42	55.22	**53.94^{i}**	56.22	55.08	55.02	**55.44^{gh}**	2.55	2.35	2.16	**2.35^{c}**	2.78	2.58	2.50	**2.62^{d}**
MSN.5B	1.0	BA, 0.5	72.36	71.29	70.39	**71.34^{c}**	68.32	66.23	65.08	**66.54^{d}**	4.27	4.09	3.86	**4.07^{c}**	6.08	6.02	5.98	**6.02^{b}**
MS2N.5B	2.0	BA, 0.5	75.62	74.39	73.38	**74.46^{b}**	68.21	63.32	60.07	**63.85^{d}**	4.62	3.92	3.69	**4.07^{c}**	7.49	7.18	6.92	**7.12^{ab}**
MS3N.5B	3.0	BA, 0.5	80.25	78.29	78.09	**78.87^{a}**	65.22	64.18	60.15	**63.18^{e}**	3.94	3.36	3.10	**3.46^{c}**	6.89	6.70	6.53	**6.70^{b}**
MS4N.5B	4.0	BA, 0.5	75.48	75.69	73.29	**74.82^{b}**	60.51	60.35	58.44	**59.76^{f}**	2.86	2.39	1.98	**2.41^{c}**	5.58	5.42	5.32	**5.44^{b}**
MS5N.5B	5.0	BA, 0.5	70.28	70.09	68.09	**69.48^{d}**	58.30	55.60	50.02	**54.60^{h}**	2.15	1.91	1.56	**1.87^{c}**	4.66	4.36	3.91	**4.31^{cd}**
MS.1Td.5N	0.5	TDZ, 0.1	65.38	65.21	64.30	**64.96^{e}**	70.40	69.65	70.12	**70.05^{c}**	16.74	6.17	5.77	**9.56^{a}**	6.48	6.39	5.92	**6.26^{bc}**
MS.2Td.5N	0.5	TDZ, 0.2	65.36	65.16	60.08	**63.53^{ef}**	75.19	74.22	73.01	**74.14^{b}**	17.19	6.90	6.32	**10.13^{a}**	8.92	8.78	8.64	**8.78^{a}**
MS.5Td.5N	0.5	TDZ, 0.5	60.25	60.18	60.02	**60.15^{g}**	65.82	64.66	65.02	**65.16^{d}**	16.09	5.68	5.14	**8.97^{a}**	6.38	6.03	5.96	**6.12^{b}**
MSTd.5N	0.5	TDZ, 1.0	60.14	58.46	55.59	**58.06^{h}**	63.22	62.61	60.28	**62.03^{e}**	14.82	4.19	3.83	**7.61^{b}**	5.96	5.82	5.76	**5.84^{b}**
MS2Td.5N	0.5	TDZ, 2.0	55.40	55.30	54.44	**55.04^{i}**	55.53	65.23	54.72	**58.49^{fg}**	9.94	3.26	3.05	**5.41^{b}**	4.23	3.98	3.88	**4.03^{c}**
Mean			**65.28^{a}**	**64.32^{b}**	**62.96^{c}**		**66.93^{a}**	**66.21^{a}**	**64.13^{b}**		**7.29^{a}**	**4.79^{b}**	**4.50^{b}**		**4.50**	**4.69**	**4.56**	
CD 0.05																		
Genotypes						**0.78**				**1.18**				**1.27**				**NS**
Media						**2.11**				**3.18**				**3.46**				**2.08**
G x M						**NS**				**NS**				**NS**				**NS**

Values within column followed by different letters are significantly different at 5% probability level.

Table 4. Effects of different plant growth regulators on *in vitro* rooting of shootlets obtained from nodal segment culture.

Culture media ▼ Genotypes ►	Plant growth regulators mg.l^{-1}				*In vitro* rooting response											
					Root proliferating shootlets (%)				Number of root (s)				Root length (in cm)			
	IB.	NAA	BAl	K	Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean	Thompson Seedless	Karnet	Local	Mean
MS.1I	0.1	-	-	-	80.62	79.92	78.89	**79.81^{d}**	3.89	3.82	3.76	**3.82**	5.82	5.79	4.62	**5.41**
MS.5I	0.5	-	-	-	85.80	84.39	84.29	**84.82^{bc}**	4.79	4.70	4.59	**4.69**	6.78	6.62	5.49	**6.29**
MSI	1.0	-	-	-	90.62	89.69	89.52	**89.94^{a}**	5.86	4.56	4.41	**4.94**	8.65	8.39	6.23	**7.75**
MS2I	2.0	-	-	-	90.32	89.84	88.81	**89.65^{a}**	5.76	5.84	5.75	**5.78**	7.86	6.92	6.82	**7.20**
MS3I	3.0	-	-	-	82.39	81.89	81.28	**81.85^{cd}**	4.25	4.23	4.18	**4.22**	6.70	5.82	5.39	**5.97**
MS.1N	-	0.1	-	-	70.42	70.39	70.18	**70.33^{gh}**	3.12	3.02	2.98	**3.04**	4.14	3.88	3.70	**3.90**
MS.5N	-	0.5	-	-	76.28	75.33	74.34	**75.31^{ef}**	3.29	3.19	3.04	**3.17**	4.64	3.76	3.82	**4.07**
MSN	-	1.0	-	-	71.84	70.69	70.48	**71.00^{fg}**	3.59	3.40	3.36	**3.45**	5.28	4.32	4.20	**4.60**
MS2N	-	2.0	-	-	66.74	65.85	65.35	**65.98^{hi}**	3.58	3.50	3.32	**3.46**	5.05	4.96	4.82	**4.94**
MS3N	-	3.0	-	-	60.39	60.30	59.82	**60.17^{ijk}**	2.82	2.64	2.46	**2.64**	4.29	4.92	4.79	**4.67**
MS.1Kn	-	-	-	0.1	58.79	57.62	56.82	**57.74^{kl}**	3.52	3.19	3.11	**3.27**	3.69	3.73	3.56	**3.66**
MS.5Kn	-	-	-	0.5	62.55	62.40	61.82	**62.25^{ij}**	3.92	3.72	3.71	**3.78**	4.39	3.59	3.49	**3.82**
MSKn	-	-	-	1.0	65.08	64.54	64.32	**64.64^{i}**	4.65	4.56	4.50	**4.57**	3.98	3.89	3.78	**3.88**
MS2Kn	-	-	-	2.0	55.69	54.38	54.18	**54.75^{lm}**	4.78	4.42	4.32	**4.50**	4.78	3.89	4.79	**4.48**
MS3Kn	-	-	-	3.0	50.58	50.32	49.94	**50.28^{l}**	4.49	4.39	4.22	**4.36**	3.64	4.34	4.32	**4.10**
MS.5I.5B	0.5		0.5	-	80.82	79.81	79.12	**79.91^{d}**	3.56	3.38	3.28	**3.40**	3.69	3.29	3.09	**3.35**
MSI.5B	1.0		0.5	-	80.74	79.61	79.22	**79.85^{d}**	3.92	3.84	3.72	**3.82**	4.19	3.59	3.28	**3.68**
MS2I.5B	2.0		0.5	-	78.39	78.29	78.12	**78.26^{de}**	3.98	3.62	3.61	**3.73**	5.96	4.09	3.97	**4.67**
MS.5I.5kn	0.5	-	-	0.5	87.39	86.42	86.28	**86.69^{b}**	4.04	3.96	3.75	**3.91**	5.62	3.96	3.92	**4.50**
MS I.5Kn	1.0	-	-	0.5	89.72	88.61	87.39	**88.57^{ab}**	5.10	4.82	4.92	**4.94**	4.14	5.68	5.62	**5.14**
MS2I.5Kn	2.0	-	-	0.5	85.32	84.34	83.62	**84.42^{c}**	4.95	4.89	4.76	**4.86**	6.22	6.52	6.46	**6.40**
Mean					**75.45**	**74.30**	**74.30**		**4.18**	**3.98**	**3.89**		**5.21**	**4.90**	**4.56**	
CD (0.05)																
Genotypes								**NS**				**NS**				**NS**
Media								**4.41**				**NS**				**NS**
G x M								**NS**				**NS**				**NS**

Values within column followed by different letters are significantly different at 5% probability level.

Nodal segment excised from top 1-10 nodes could not induce higher bud break, which may be due to tender nature of buds. The exposure of explants with sterilants/antioxidants may exterminate these soft buds. Maximum bud break was observed from 10-20 nodes, probably due to juvenile status and competence to resist the toxic effect of surface sterilizing agents. These results are in accordance to the findings of Tran Than Van (1973) and Alizadeh *et al*. (2010) who found higher morphogenic comeback followed by plantlet regeneration that appears to be highly dependent on position of explant. Studies have revealed that lower or mid portion of the branch are easier to establish *in vitro* than upper part of the branch (Alizadeh *et al*., 2010).

In grape, although, plants from tissue cultures have been regenerated on an array of basal medium such as MS medium (Lee and Wetzstein, 1990; Torregrosa and Bouquet, 1996; Heloir *et al*., 1997; Mhatre *et al.*, 2000; Muhammad *et al.,* 2008), Nitsch medium (James *et al*., 1990; Robacker, 1993) and MT medium (Xu *et al*., 2005), in our experiments, MS basal medium was used throughout the experiments, as it was found more amenable in preliminary experimentations than others (data not presented). As per studies conducted so far, composition of culture media does not seem to play leading role in *in vitro* response as much as the type and concentration of plant growth regulators.

Some of the most intriguing questions faced by us concern the conditions that reprogramme hitherto differentiated or otherwise committed cells into forming morphogenic structures in culture and that determine the path taken, direct or indirect, shoot or callus. By varying growth regulator levels and types, one can determine the route of *in vitro* morphogenesis. During present investigation, presence of an auxin alone in culture medium supported profuse callus growth. Such type calli after transfer to the medium without growth regulators did not exhibit morphogenesis at full potential. Nutrient medium containing higher concentration of auxins in combination with a comparatively lower concentration of cytokinins preferred indirect shoot organogenesis with low morphogenic competence. Culture medium amended with comparatively higher concentrations of cytokinins in association with lower concentrations of auxins also preferred indirect organogenesis mode of regeneration but the frequency of shoot proliferation was extremely higher. Cytokinins as sole preferred direct auxiliary bud proliferation (direct shoot organogenesis) and regenerated multiple shoots.p

Culture medium fortified with higher concentrations of auxins in alone (2, 4-D, NAA or 2, 4, 5-T) initiated calli in higher frequencies. Higher degree of callus initiation was observed on culture media MS2D and MS3N as compared to culture media MS3N.5B and MS4N.5B containing higher concentration of an auxin in combination with lower concentration of cytokinins, MS5B.5N and

MS5Kn.5N supplemented with relatively higher concentration of cytokinins in combination with lower concentration of an auxin as well as MS5B, MS5Kn, MS2Td with cytokinins at higher concentration suggested that an auxin alone is adequate for higher degree of callus initiation from cultured nodal segments. A similar result was also reported by Torregrosa and Bouquet (1996) in grape. Regeneration of multiple shoots from nodal segment explants directly as well as from nodal segment-derived callus of grape has been reported earlier in response to cytokinins BA, Kn and TDZ (Barreto and Nookaraju, 2007; Torregrosa and Bouquet, 1996). Both adenine (BA and Kn) and phenyl urea derivatives (TDZ) of cytokinins were used in the present study for shoot regeneration. It was observed that BA and TDZ in alone as well as in combination with a lower concentration of NAA (0.5mg.l^{-1})was better for shoot proliferating ability when compared with Kn. Growth regulator BAP promotes adventitious buds in excised organs and tissues *in vitro* (Bhojwani and Johri 1971). In the earlier studies BA was found also superior for shoot proliferation in grape (Thies and Graves, 1992; Torregrosa and Bouquet, 1996; Mhatre *et al*., 2000; Das *et al*., 2002; Singh *et al*., 2004; Alizadeh *et al*., 2010) which was in accordance with present findings. During present study, it was also observed that TDZ was equally effective for shoot proliferating competence to BA but the numbers of shoots formed per explant were extremely higher in the presence of TDZ. Recovery of shoots in higher numbers on medium supplemented with cytokinin TDZ as compared to supplementation of BA as well as Kn perhaps due to occurrence of direct organogenic mode of regeneration. The presence of TDZ in culture medium prevents callus initiation and forced to nodal segments to regenerate plantlets from meristemtic zones directly. Furthermore, TDZ was found more effective at lower concentrations (0.1-0.5 mg.l^{-1}) as compared to BA and Kn (they were found more effective in the range of 2.0 -4.0 mg.l^{-1}). Effectiveness of TDZ at lower level perhaps, was due to induced accumulation of endogenous cytokinins, as reported earlier by Murthy *et al*. (1995). TDZ also affected auxin transport in hypocotyl tissues of *Pelargonium* and others (Murch and Saxena, 2001) and promoted regeneration frequency by altering the levels of abscisic acid, ethylene and perhaps proline (Murch and Saxena, 2001). In the present study, TDZ was found to be unresponsive when supplemented into media at the concentration beyond to 1.0 mg.l^{-1}. This finding is in accordance with the findings of Huetteman and Preece (1993) who also reported that TDZ at higher concentrations inhibit shoot elongation in many species. However, the exact mechanism of TDZ induced shoot proliferation in plants is not all that clear.

Cytokinin Kn performed moderately in all phases of culture. Kinetin preferred rhizogenesis alone *in vitro* instead of gammogenesis. In all cultivars

BAP treatments either singly or in combination with 0.5 mgl^{-1} NAA showed better response than single or combined kinetin treatments. This finding is in accordance with the Alizadeh *et al*. (2010) for nodal segment culture of grape. In contrary to our results, Poudel *et al*. (2005) reported the effectiveness of kinetin on culture establishment of two wild grape cultivars. This may be due to difference in genotypes utilized; because different plant growth regulators at varying concentrations show considerable variability for *in vitro* regeneration of different species or cultivars. Plant growth regulator (s) effective for one species may not be equally effective for another cultivar or species (Poudel *et al*., 2005; Singh *et al*., 2004).

Shoot of higher length was attained on culture media MS.2Td.5N and MS2NB with higher quantity of an auxin in combination with lower concentration of a cytokinin as compared to nutrient media MS5B.5N with relatively higher cytokinin quantity in combination of lower proportion of an auxin suggested that culture medium was more effective when higher concentration of an auxin with lower concentration of a cytokinin were added in culture medium. A comparatively lower response was recorded when auxins was used separately in the medium. Much lower results were documented with supplementation of alone cytokinins into medium. A review of literatures indicates that addition of either 2,4-D or NAA in the culture medium improved the response in a number of species in terms of overall shoot length. We observed that 2.0 $mg.l^{-1}$ NAA and 0.2 $mg.l^{-1}$ TDZ solely in MS medium proliferated shoot of higher length 6.62cm and 4.29cm respectively. On the other hand, addition of 0.5 mg. l^{-1} BA with NAA and 0.5 mg. l^{-1} NAA with TDZ elevated the shoot length considerably. Shoot of maximum length 7.19cm and 8.78 cm respectively have been achieved with the combined application of auxin and cytokinin.

During present investigation, rhizogenesis frequencies were found higher after transferring of shootlets into the rooting medium. In grape, auxins like IBA (Barreto and Nookaraju, 2007), IAA (Alizadeh *et al*., 2010) as well as NAA (Sajid *et al*., 2006) was found effective for inducing *in vitro* rooting. In present study, higher root proliferation ability, number (s) of roots as well as root of higher length were attained on full strength MS rooting medium amended with IBA alone at the concentration ranging from 1.0-2.0 $mg.l^{-1}$. The results clearly indicated that rooting of *in vitro* shoots of grape required lower to moderate concentrations of IBA. Furthermore, IBA responded better as compared to NAA, BA or Kn. Medium supplemented with higher auxins augmented the intensity of callus induction, while, the number of roots produced per shoot and root growth reduced considerably. Culture media supplemented with IBA alone responded well as compared to media fortified

with other synthetic auxin (NAA) even in higher concentrations suggested that IBA promotes *in vitro* rooting of shootlets in grape. Auxins promoted adventitious root development on intact plants as well as excised stems. Of these, IBA was the most effective than any other synthetic auxins in most of the cases, apparently because it is not destroyed by IAA oxidase or other enzymes and therefore persists longer.

Significant differences in shoot induction efficiency were also detected among three *Vitas* cultivars. Under the same culture cues, genotype Thompson seedless was found more responsive that Karnet and Local for the most of culture phases indicating the presence of genotype/cultivar-specific reactions. Cultivar-dependent variation in shoot regeneration capacity has been previously reported in various explants cultures of *Vitas* by Stamp and Meredith (1988); Torregrosa and Bouquet (1996); Carimi *et al*. (2005); Xu *et al*. (2005); Barreto and Nookaraju (2007) and Sadanand *et al.*(2009). Previous work and ours demonstrated that regeneration potential of the cultured nodal segments was tightly correlated with genetic background of the stock plants, which may be due to different levels of endogenous metabolites and/or hormones that are involved in regeneration process (Norstog, 1970).

In conclusion, the present results substantiate the fact that nodal segments is an excellent explant source and are available throughout the year especially in grape where choice of explants is limited due to non-availability of seeds. The present study shows that nodal segment explants of grape carry a high potential for rapid multiple shoot regeneration and subsequent micropropagation. Utilizing our regeneration system and existing transformation technologies in grape breeding program could provide an additional strategy for the enhancement of elite cultivar.

References

Alizadeh, M., Singh, S.K. and Patel, V.B. (2010). Comparative performance of *in vitro* multiplication in four grape (*Vitis* spp.) rootstock genotypes. International Journal of Plant Production 4: 41-50.

Barreto, M. S. and Nookaraju, A. (2007). Effect of auxin types on *in vitro* rooting and acclimatization of grapevine as influenced by substrates. Ind. J. Hort. 64: 5-11.

Bhojwani, S.S. and Johri, B.M. (1971). Morphogenetic studies on cultured mature endosperm of *Croton bonplandianum*. New Phytol. 70: 761-766.

Carimi, F., Barizza, E. Cardiman, M. and Schiavo, F. L. (2005). Somatic embryogenesis from stigmas and styles of grapevine. *In vitro* cell Dev. Biol. plant. 41: 249-252.

Cutanda, M.C., Bouquet, A., Chatelet, P., Lopez, G., Botella, O., Montero, F. J. and Torregros, L. (2008). Somatic embryogenesis and plant regeneration of *Vitis Vinifera* cultivars 'macabeo' and 'tempranillo'. Vitis. 47: 159-162.

Das, D., Reddy, M., Upadhyaya, S. and Sopory, S. (2002). An efficient leaf disc culture method for the regeneration *via* somatic embryogenesis and transformation of grape (*Vitis vinifera* L.). Plant Cell Rep.20: 999-1005.

Gray, D. J. and Meredith, C. P. (1992). Grape. In: F. A. Hammerschlag, R. E. Litz (Eds): Biotechnology in Perennial Fruit Crops, 229-262. CAB. Intern.

Heloir, M.C., Fournioux, J.C., Oziol, L. and Bessis, R. (1997). An improved procedure for the propagation *in vitro* of grapevine (*Vitis vinifera cv*. Pinot noir) using auxiliary-bud microcuttings. Plant Cell Tiss. & Org. Cult. 49: 223-225.

Huetteman, C. A. and Preece, J. E. (1993). Thidizuron: A potent cytokinin for woody plant tissue culture. Plant Cell Tissue Org. Cult. 33:105-119.

James, A. S., Sheila, M.C., and Carole, P. M. (1990). Improved shoot organogenesis from leaves of grape. J. Amer. Soc. Horti. Sci. 115: 1038-1042.

Jayashankar, S., Gray, D. J. and Litz, R. E. (1999). High-efficiency somatic embryogenesis and plant regeneration from suspension cultures of grapevine. Plant Cell Reports 18: 533-537.

Kuksova, V. B., Piven, N. M. and Gleba, Y. Yu. (1997). Somaclonal variation and *in vitro* induced mutagenesis in grapevine. Plant Cell Tiss. & Org. Cult. 49: 17-27.

Lee, Ni and Wetzstein, Y. H. (1990). *In vitro* propagation of Muscadine grape by auxiliary shoot proliferation. J. Amer. Soc. Hort. Sci. 115: 324-329.

Lloyd, G. and McCown, B. (1980). Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot tip culture. Proceeding Int. Plant Prop. Soc. 30: 421-427.

Lopez-Perez, A.J., Carreno J., Martinez, C. A. and Dabauza, M. (2005). High embryogenic ability and plant regeneration of table grapevine cultivars (*Vitis vinifera* L.) induced by activated charcoal. Vitis. 44: 79-85.

Maitz, M., Trampler, F., Groschl, M., Camara Mchada, A. Da. and Laimer Da. Camara Mchada, M. (2000). Use of ultrasound cell retention system for the size fraction of somatic embryos of woody species. Plant Cell Reports.19: 1057-1063.

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

Morgana, C., Dilorenzo, R. and Carmi, F. (2004). Somatic embryogenesis of *Vitis vinifera* L. (cv.Sugarcane) from stigma and style culture. Vitis. 43: 169-173.

Muhammad, J., Jaskani, H. A., Sultana, R., Khan, M.M., Qasim, M. and Khan, I. A. (2008). Effect of growth hormones on micropropagation of *Vitis vinifera* L cv. Perlette. Pak. J. Bot. 40: 105-109.

Mullins, M.G. and Srinivasan, C. (1976). Somatic embryos and plantlets from an ancient clone of the grapevine (cv'Cabernet-Sauvignon') by apomixes *in vitro*. J.Exp.Bot. 27:1022-1030.

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

Murch, S. J.and Saxena, P.K. (2001). Molecular fate of thidizuron and its effects on auxin transport in hypocotyls tissues of Pelargonium x hortorum Bailey. Plant Growth Regul. 35:269-275.

Murthy, B.N.S., Murch, S. and Saxena, P.K. (1995).TDZ-induced somatic embryogenesis in intact seedlings of peanut (*Arachis hypogaea*).Endogenous growth regulator levels and significance of cotyledons. Physiol Plant. 94:268-276.

Nakano, M., Sakakibara, T., Watanabe, Y. and Mii, M. (1997). Establishment of embryogenic culture in several cultivars of *Vitis vinifera* x *Vitis labruscana*. Vitis. 36: 141-145.

Norstog, K. (1970). Induction of embryo like structures by kinetin in cultured barley embryos. Dev. Biol. 23: 665-670.

Poudel, P.R., Kataoka, I. and Mochioka, R. (2005). Effect of plant growth regulators on *in vitro* propagation of *Vitis ficifolia* var. *ganebu* and its interspecific hybrid grape. Asian J. Plant Sci. 4: 466-71.

Robacker, C. (1993). Somatic embryogenesis and plant regeneration from muscadine grape leaf explants. Hort Sci. 28: 53-55.

Sadanand, A. D. and Zhijian, T. Li. (2009). Optimizing initiation and maintenance of *Vitis* embryogenic cultures. Horti. Sci. 44: 1400-1406.

Sajid, G.M., Ilyas, M.K. and Anwer, R. (2006). Effect of diverse hormonal regimes on *in vitro* growth germplam. Pak. J. Bot. 38: 385-391.

Singh, S.K., Khawale, R.N. and Singh, S.P. (2004). Techniques for rapid *in vitro* multiplication of *Vitis vinifera* L.cultivars. J. Hort. Sci. Biotech. 19: 267-272.

Snedecor, G.W. and Cochran, W. G. (1967). Statistical methods. VI Ed. Oxford IBH Pub. Co. Delhi.

Stamp, J.A. and Meredith, C.P. (1988). Somatic embryogenesis from leaves and anthers of grapevine. Sci. Horti. 35: 235-250.

Tangolar, S.G., Buyukalaca, S. and Ergenoglu, F. (2008). High efficiency somatic embryogenesis from immature zygotic embryos of grapevine: the effect of genotype, media, 2,4-D and incubation conditions. Turk J. Agric. 32: 311-317.

Thies, K.L. and Graves, C.H. (1992). Meristem micropropagation protocols for *Vitis rotundifolia* Michx. Hort. Sci. 27: 447-449.

Torregrosa, L. and Bouquet, A. (1996). Adventitious bud formation and shoot development from *in vitro* leaves of *Vitis x Muscadinia* hybrids. Plant Cell Tiss. & Org. Cult. 45: 245-252.

Tran Than Van, K. (1973). Direct flower neoformantion from superficial tissue of small explant of *Nicortiana tabacum*. Planta115:87-92.

Xu, X., Lu, J., Ren, Z., Wang, H. and Leong, S. (2005). Callus induction and somatic embryogenesis in muscadine and seedless bunch grapes (*Vitis*) from immature ovule culture. Proc.Fla.State.Hort.Soc. 118:260-262.

(Received 4 November 2010; accepted 27 March 2011)