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Research Article

In Vitro Propagation of Grape (*Vitis vinifera* L.) cv. Thompson Seedless

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ABSTRACT

In vitro propagation of Vitis offers opportunities for increasing plant material for cultivation. Explant sterilization was accomplished by washing with 0.02 per cent Active-80 for 10 minutes followed by treating for 15 minutes in a solution containing 1000 ppm Bavistin + 100 ppm Cetrimide and final surface sterilization using (0.1%) mercuric chloride for 5 minutes. Between two different explants such as nodal cuttings and petiole segments, the nodal cuttings showed the maximum response of 90 per cent towards shoot induction on half strength MS medium. While, only 10 per cent of petiole segments responded, in that only root induction was seen. Induction of multiple shoots was found to be highest (3.6) on media supplemented with 2 mg/l BAP in cv. Thompson Seedless. Rooting of microshoots was successfully achieved and was cent percent with 1 mg/l IBA. In the same treatment early root initiation, maximum number of primary roots, lengthy roots and maximum number of secondary roots were also recorded. The higher concentrations of IBA at 4 mg/l and above were found to be deleterious in its effect, in that white friable calli was observed instead of rooting. Among different hardening media, Coirpith, Sand, Vermicompost, Vermicompost: Perlite (1:1), Soil: Sand: Vermicompost (1:1:2), the highest survival percentage (90) was observed with coirpith media and none of the plantlets survived on a sand media.

Keywords: *Thompson Seedless Grape, In vitro propagation, MS medium, Nodal segment, Shoot proliferation, Root initiation.*

INTRODUCTION

Grapes (*Vitis viniefera* L.) is one of the most important, delicious and refreshing sub tropical fruit of the world having its origin in Asia Minor, the area between Caspian sea and Black sea. Grape is a crop closely associated with history of human civilization. The berries are good source of sugar, acid, minerals and vitamins. Grapes are the best table fruit. Ripe berries are tasty, juicy, attractive, nutritious and easily digestible. Grapes are used as table, wine, raisin and juice.

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Grape is one of the most widely spread out fruit crop in terms of both area (7.5 million ha) and annual production (63.3 million tonnes) in the world (Kocturk & Engindeniz, 2009). The reason for its wide distribution is its genetic diversity and broad range of environmental adaptation. Thompson Seedless is originated in Asia Minor. It is believed to be grown in every viticultural country of the world. It's bunches are medium large, long, conical to cylindrical, shouldered, and well filled to compact. The berries are yellowish green to golden yellow when fully ripe, small, seedless and have an excellent keeping quality. It is a multipurpose grape variety being used for table, wine and raisins. This variety is getting renewed attention and importance due to it is quality (Bal, 2006). Due to heterozygous nature, grape varieties are mostly propagated by vegetative means. Even though these methods are being commercially exploited, they are cumbersome, time consuming and highly season bound. By using micropropagation techniques, thousands of plants can be produced in a year from a single vine. By contrast, a nursery using hardwood cuttings might propagate about a dozen plants in a year from a single vine (Krul & Mowbray, 1984).

MATERIALS AND METHODS

The investigation was carried out at the Plant Tissue Culture Laboratory, Division of Horticulture, University of Agricultural Sciences, Bangalore – 65, India. Nodal cuttings at semi hardwood stage (2-3 cm long) and Petiole segments (1-2 cm length) of cultivar Thompson Seedless collected from the field growing vines of grapes grown at the Horticultural Research Station, Division of Horticulture, University of Agricultural Sciences, GKVK (Gandhi Krishi Vignana Kendra), Bangalore, were used as experimental material. Murashige and Skoog's (MS) media at half strength of salt concentrations were used in different experiments under present investigation. The collected explants (Nodal cuttings and petiole segments) were washed in running tap water for 30 min., followed by washing with 5-10 minutes in a surfactant solution containing 0.02 per cent Active-80 and rinsed 3 times with distilled water. They were then transferred to a solution containing 1000 ppm Bavistin + 100 ppm Cetrimide for 10-15 min followed by 3-4 times rinsing with sterile distilled water. Finally the explants were surface sterilized with 0.1 per cent mercuric chloride ($HgCl_2$) for 5 minutes and then rinsed 3-4 times with sterile DDW to remove the residues of $HgCl_2$ from the material. The cut ends of the explants were trimmed off and the explants were carefully inoculated on the prepared media in the LAF. The culture vessels were closed immediately, labeled and incubated in the growth room. The number of replications was 10 per treatment in all the experiments. The data were subjected to analysis of variance with and without transformation of data by adopting Completely Randomized Design (CRD).

RESULTS AND DISCUSSION

As the results of the experiments have shown nodal cuttings and petiole segments of Grape cultivar Thompson Seedless were used as explants and cultured on half strength MS medium without any growth regulator. Among the explants tried for initiating aseptic cultures, nodal cuttings were found to be superior. Maximum percentage of (90%) established cultures with shoot induction response were noticed with nodal cuttings. While, only 10 per cent of the petiole segments could produce roots in half strength MS medium at the end of six weeks (Table 1 and Figure 1). Similarly, the superiority of nodal segments for initiating aseptic culture is in agreement with the results reported by other workers like Kumar et al. (2008) in grape rootstock 1613C, Barreto et al. (2006) in Red Globe and Singh et al. (2004) in Pusa Urvashi and Pusa Navrang.

Table1. Effect of different explant sources on establishment of *in vitro* cultures in grapes cv. Thompson Seedless

Explant source	Percentage response	Kind of response
Nodal cutting	90	Shoot
Petiole segment	10	Root

Fig. 1: Response of different explants of grapes cv. Thompson Seedless under *in vitro* culture condition
a) Nodal cuttings 5 weeks after inoculation. b) Petiole segment 2 months after inoculation.

Explants obtained from the previous experiment were inoculated in half strength MS media with varying concentrations of BAP (0, 1, 2, 4, and 8 mg/l). The maximum shoot proliferation response (100%) was obtained with low BAP concentrations of 2 and 1 mg/l and the decreased with increase in BAP concentration, least being at 8 mg/l (68%). The microshoot response found earlier (6.1 days), the maximum number of shoots produced per explant (3.6), the highest length of shoot (2.73 cm) and the maximum number of leaves (3.6) were obtained in half strength MS medium supplemented with 1 mg/l BAP after sex weeks (Table 2 and Figure 2). Various reports are there stating the concentrations of BAP that is beneficial to the different varieties of grapes (Jaskani et al., 2008; Yae et al., 1990; Salami et al., 2005 & Alizadeh et al., 2010). In light of these findings, it can be argued that depending on the variety of grape, slightest difference in the concentration of BAP is very significant. The concentration which can induce multiple shoots in a particular variety need not do the same for another. In same cases, a particular concentration of BAP is beneficial for the induction of multiple shoots and can also inhibit the production of multiple shoots. For 'Rizamet', the optimum concentration of BA was 1 mg/l (Yae et al., 1990). The best concentration of BAP for shoot proliferation in the rootstock 'deGrasset' was also 1 mg/l (Mukherjee et al., 2010). Rapid multiplying cultures of 'Thompson Seedless' grapevine were established from isolate shoot apices and buds on modified MS supplemented with 5 ppm BA (Allam et al., 1992). A proliferation medium (MS + amendments) containing 2 mg/l BA was preferable for 'Pinot Blanc' (Ciccotti, 1982). In many *Vitis vinifera* cvs, the best concentration of BA was 2 mg/l (Stamp et al., 1990).

Table 2: Effect of BAP at different concentrations on shoot proliferation from *in vitro* microshoots of grapes cv. Thompson Seedless

Treatment	Per cent response	Days taken for response	No. of shoots/ explant	Mean length of shoot (cm)	No. of leaves/ shoot
Half MS medium (Control)	70 (57.81)	9.2	1.0	2.36	3.1
Half MS medium + BAP 1 mg/l	100 (89.43)	6.2	3.1	2.61	3.6
Half MS medium + BAP 2 mg/l	100 (89.43)	6.1	3.6	2.73	3.6
Half MS medium + BAP 4 mg/l	75 (61.28)	6.6	1.7	2.16	2.5
Half MS medium + BAP 8 mg/l	68 (55.78)	6.8	1.5	2.08	2.3
F test	**	**	**	**	**
SEm±	3.95	0.43	0.18	0.12	0.28
CD @ 1%	15.01	1.64	0.67	0.44	1.08
CD @ 5%	11.24	1.23	0.50	0.33	0.81

Note 1: Figures in parentheses represent Arcsine transformed values.
Note 2: *, ** Significant at 5% and 1% levels, respectively; NS – Non significant.

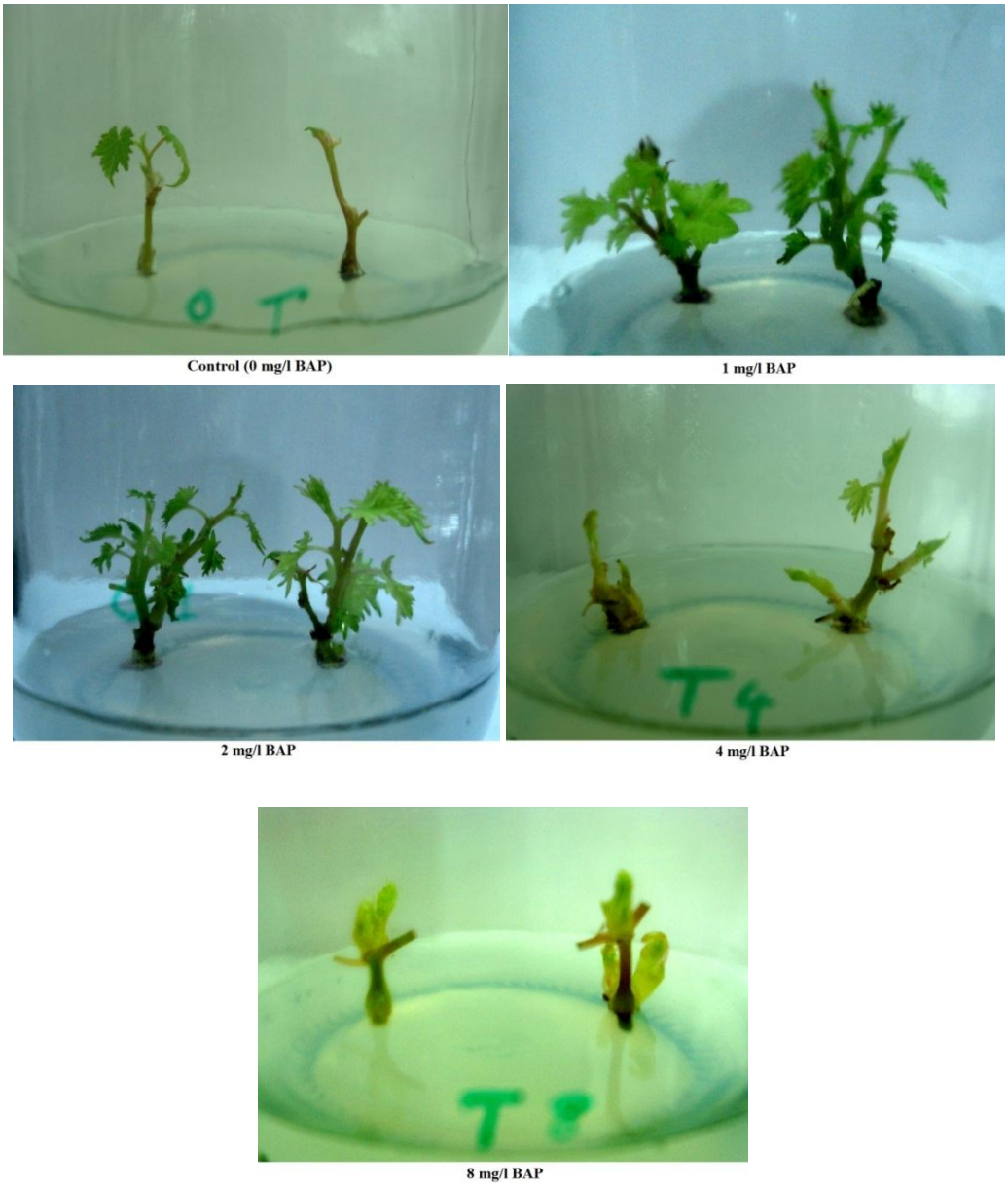


Fig. 2: *In vitro* shoot induction from microshoots of grapes cv. Thompson Seedless on half MS media containing various concentrations of BAP

Rooting of microshoots was successfully achieved and was cent percent with half strength MS medium + IBA 1 and 2 mg/l. Early root initiation (20.9 days) and the maximum number of primary roots (10.1) were noticed in half strength MS medium containing IBA 1 mg/l followed by 2 mg/l IBA (9.6). While in higher concentrations of auxin (4 and 8 mg/l IBA) white friable callus was developed at the base of microshoot which is not desirable (Figure 3). The maximum mean length of roots (2.44) and highest number of secondary roots (126.7) was recorded in the same treatment i.e., half strength MS media supplemented with IBA 1 mg/l (Table 3). The similar result, using IBA at 1 mg/l with half strength MS medium in 'Thompson Seedless' grape was reported by (Allam et al., 1992).

Table 3: Effect of different concentrations of IBA on rooting of *in vitro* microshoots of grapes cv. Thompson Seedless

Treatment	Per cent response	Days taken for response	No. of primary roots/ microshoot	Mean length of root (cm)	No. of secondary root/ microshoot
Half MS medium (Control)	65 (53.73)	26.1	2.1	2.28	23.6
Half MS medium + IBA 1 mg/l	100 (89.43)	20.9	10.1	2.44	126.7
Half MS medium + IBA 2 mg/l	100 (89.43)	21.8	9.6	2.31	120.8
Half MS medium + IBA 4 mg/l	72.5 (58.39)	24.3	3.0	1.69	19.4
Half MS medium + IBA 8 mg/l	67.5 (55.26)	25.3	2.0	1.43	8.3
F test	**	**	**	*	**
SEm±	0.53	1.29	0.77	0.28	17.44
CD @ 1%	2.01	4.91	2.93	1.06	66.33
CD @ 5%	1.50	3.68	2.19	0.79	49.66

Note 1: Figures in parentheses represent Arcsine transformed values.
Note 2: *, ** Significant at 5% and 1% levels, respectively; NS – Non significant.

Table 4: Effect of different kinds of media on plantlet survival during primary hardening of grapes plantlets cv. Thompson Seedless

Medium	Plantlet survival (%)	Length of shoot (cm)	No. of leaves
Coirpith	90 (71.57)	5.4	4.8
Sand	0.0 (0.00)	0.0	0.0
Vermicompost	20 (26.57)	4.4	3.0
Vermicompost : Perlite (1:1)	22.5 (28.28)	3.5	3.0
Soil : Sand : Vermicompost (2:1:1)	35 (36.27)	3.7	4.0
F test	**	**	**
SEm±	0.37	0.25	0.31
CD @ 1%	1.42	0.97	1.18
CD @ 5%	1.06	0.73	0.89

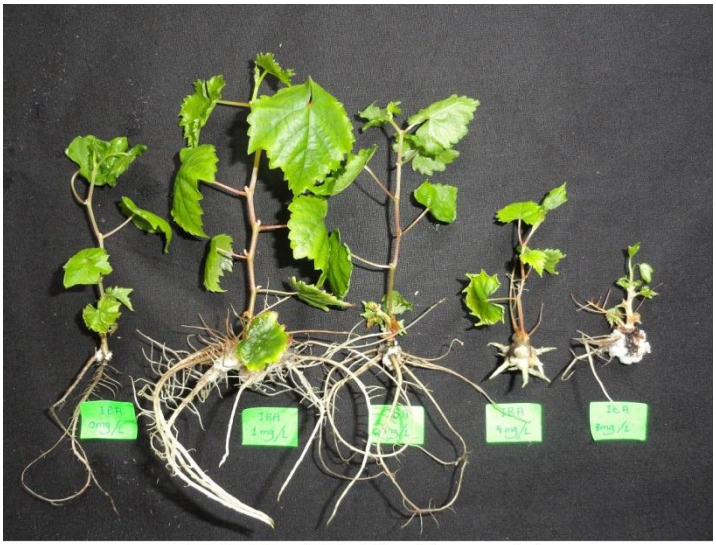
Note 1: Figures in parentheses represent Arcsine transformed values.
Note 2: *, ** Significant at 5% and 1% levels, respectively; NS – Non significant.

Table 5: Effect of different hardening treatments on plantlet survival during secondary hardening of grapes cv. Thompson Seedless

Treatment	Plantlet survival (%)	Length of shoot (cm)	No. of leaves
Soil:Sand:Coirpith (1:1:2) plus half MS basal 100 ml/week	80 (63.43)	12.6	8.0
Soil:Sand:Coirpith (1:1:2) plus half MS basal 100 ml/two weeks	60 (50.80)	6.8	5.6
{			
F test	**	**	**
SEm±	1.18	0.46	0.26
CD @ 1%	4.80	1.87	1.07
CD @ 5%	3.50	1.37	0.78

Note 1: Figures in parentheses represent Arcsine transformed values.
Note 2: *, ** Significant at 5% and 1% levels, respectively; NS – Non significant.

As it shown in Table 4, the highest survival rate (90 %) was found with coirpith media. While, none of the plantlets survived on sand media and death of 100 per cent plantlets was reported by the third week in primary hardening. The highest length of shoot (5.4) and the maximum number of leaves per plantlet (4.8) were recorded with coirpith media (Figure 4). The probable reason for good response with coirpith media may be attributed to the optimum conditions such as good water retention, richness in nutrients, aeration and good drainage provided by the substrate. These factors may have helped the plantlets to establish well. Sand on the other hand, is highly porous and has high percolation rate and thus may have resulted zero per cent survival of plantlets during hardening. During secondary hardening the higher plantlet survival rate (80%) was recorded with Soil:Sand:Coirpith (1:1:2) and half MS basal 100 ml/week/plantlet after 3 weeks. the longest length of shoot (12.1 cm) and the maximum number of leaves per plantlet (8.1) was obtained with the same treatment (Soil:Sand:Coirpith (1:1:2) with supplement of half MS basal 100 ml/week/plantlet), after a period of three weeks under shade condition (Table 5 and Figure 5). Similar findings during hardening of grape plantlets have been reported by Dzazio et al. (2002) in grape rootstock 420-A, Barreto and Nockaraju (2007) in grape cvs. 2A-Clone and Red Globe and Singh et al. (2004) in grape cvs. Pusa Urvashi and Pusa Navrang.



0, 1, 2, 4, 8 mg/l IBA, left to right, respectively

Fig. 3: Kind of roots induced on half MS media with IBA at different concentrations in grapes cv. Thompson Seedless

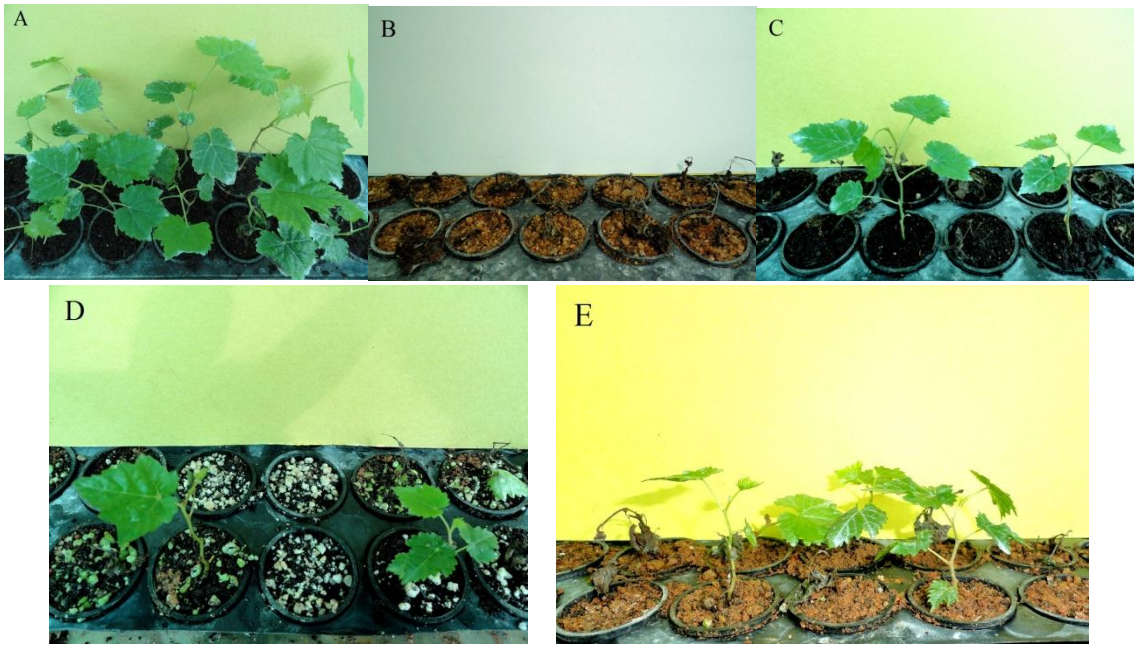


Fig. 4: Plantlets of cv. Thompson Seedless kept for acclimatization 0n different primary hardening media
a) Coirpith, b) Sand, c) Vermicompost, d) Vermicompost: Perlite (1:1), e) Soil: Sand: Vermicompost (1:1:2).

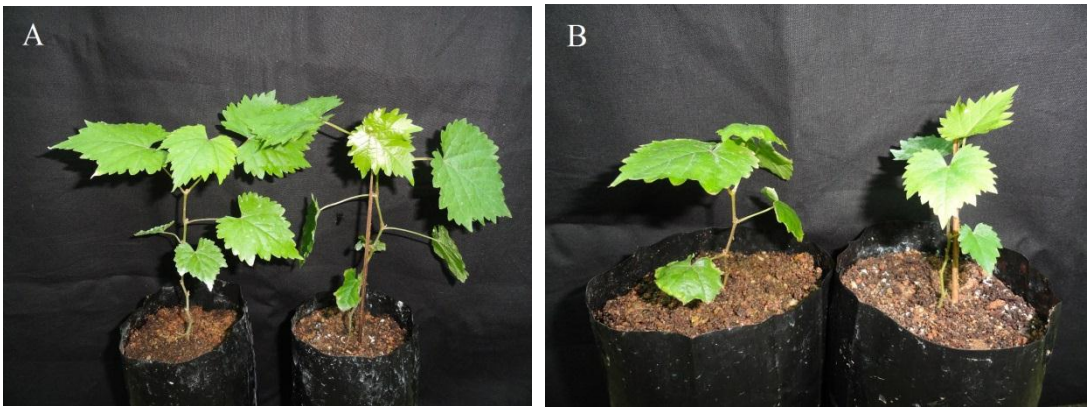


Fig. 5: Hardened plantlets of grape cv. Thompson Seedless 3 weeks after transfer to the poly bags for secondary hardening
a) Soil: Sand: Coirpith (1:1:2) plus half MS basal 100 ml/week. b) Soil: Sand: Coirpith (1:1:2) plus half MS basal 100 ml/two week.

CONCLUSION

Among two different explants i.e., nodal cuttings and petiole segments, which were tested on half MS medium, the nodal cuttings showed the maximum response of 90 per cent towards shoot induction. While, only 10 per cent of petiole segments induced root within a period of two months. Induction of multiple shoots from *in vitro* nodal explants was found to be highest on MS medium (half strength) supplemented with 2 mg/l BAP in grape cv. Thompson Seedless. For *in vitro* rooting, use of IBA at concentration of 1 mg/l recorded to be the most satisfactory result in half strength MS medium. Among different primary hardening media, the highest survival percentage of (90) was observed with coirpith media and none of the plantlets survived on a sand media. Secondary hardening was best, in a soil mix containing Soil:Sand:Coirpith at (1:1:2) plus a feeding with half MS salts at 100 ml/week/plantlet.

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