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REGENERATION OF PLANTLETS FROM GRAPE (*Vitis vinifera* L.) THROUGH DIFFERENT EXPLANTS

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ABSTRACT

Chowdhury MMH, Ashrafuzzaman M, Begum SN, Islam MM, Dhar P (2012) Regeneration of plantlets from grape (*Vitis vinifera* L.) through different explants. *Int. J. Sustain. Crop Prod.* 7(2), 12-18.

The experiment was conducted to investigate the effect of different plant growth regulators on growth and plantlet regeneration of grape 'Zakkao'cultivar by tissue culture technique. Among the different concentration of BAP at 2.5 mg/l supplemented to LS medium was the best response for callus induction and moderate shoot initiation and BAP 2.0 mg/l + 0.5 mg/l NAA for plantlet regeneration, shoot development from shoot tip, node and leaf explants. Maximum shoot height and shoot fresh weight was obtained from the BAP 2.0 mg/l+0.5 mg/l of NAA. For rooting, half strength MS medium with 3.0 mg/l IBA showed best performance in the respect of root length and root fresh weight from node, shoot tip and leaf explants derived callus. An efficient observation found that for *in vitro* grapevine regeneration, only half strength MS medium without hormonal combination was effective for root and shoot induction from node and shoot tip explants.

Key words: *growth regulators, BAP, NAA, IBA, LS, MS medium*

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crops grown in the world today in terms of both total acreage and dollar value (Galletta & Himerlic, 1989). Grapes belong to the Vitaceae family, which comprises of about 60 species. There are over 100 species reported in the literature, 65 of which are thought to be genuine and another 44 are questionable, probably they are inter specific hybrids. Preliminary observation trail conducted at BARI concluded that grape can be grown in Bangladesh as a fruit crop (Biswas and Nazrul, 1997). Recently farmers also want to make vineyard at commercial scale. But the major problem is non availability of good varieties and virus free planting materials. Bangladesh Agricultural Research Institute (BARI) has introduced about 48 germplasm of grape. In preliminary selection 12 germplasms produced fruit of acceptable quality and quantity. Tissue culture is a newly emerging, highly rewarding technology with large potential application in crop improvements which is highly appropriate for developing countries like Bangladesh. Tissue culture technique has several advantages over traditional propagation methods. Cultures have started with small plant parts (Node, shoot, leaf etc.) therefore, only a small amount of space is required to multiply large no. of plants. The use of *in vitro* techniques for propagation of various *V. vinifera* cultivars has been well documented (Chee and Pool, 1982; Singh *et al.* 2000; Mhatre *et al*. 2000; Singh *et al.* 2004). Protocols have also been reported for muscadine grape (Lee and Wetzstein, 1990; Gray and Benton, 1991; Sudarsono and Goldy, 1991; Thies and Graves, 1992; Torregrosa and Bouquet, 1995) and some wild grapes (Poudel *et al*. 2005). It is evident that there is little empirical data on the *in vitro* cloning and performance of several new grape rootstocks. The first report of *in vitro* culture of grapevines was by Morel (1944). Since that early study, culture of callus, production of protoplasts, development of somatic embryos, regeneration via organogenesis with or without a callus phase, and multiplication through axillary bud or nodal culture have all been attempted with varying results and achievements. Several reviews of the topic have been published (Krul *et al*. 1984; Read *et al.* 2004; Torregrosa *et al*. 2001). Recently, Zakkao cultivar of grape was found to be produced successfully in our country (Nuruzzaman 1994). The present investigation was undertaken to identify the best hormonal combination for callusing of *V. vinefera* on different media and investigate the shoot regeneration and root induction ability of different explants of grape.

MATERIALS AND METHODS

The experiment was conducted at the Plant Tissue Culture Laboratory of Plant Breeding Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh during the period from March to June 2011. In the present study, node, shoot-tip and leaf segments of a recently introduced grape cultivar named 'Zakkao' were used, the variety 'Zakkao' was collected from Horticulture Development Centre of BADC, Kasimpur. This is an introduced and locally acclimatized variety and now spread all over the country for commercial cultivation. In the present experiment, node, shoot tip and leaf segments of recently introduced grape cultivar named 'Zakkao' were used.

Before implanting onto culture media these were cut into small pieces for explants. Shoot tip, leaf and nodal segment explants were cultured on ½MS media, LS media and supplemented different concentration and combinations of BAP alone or in combination with NAA or IBA for callus formation, shoot initiation & root regeneration. The first step in the preparation of the medium was the preparation of stock solutions. The various

constituents of the medium were prepared into stock solutions for ready use to expedite the preparation of' the medium. Separate stock solutions for macronutrients, micro-nutrients, iron, vitamin, growth regulators etc. were prepared and used. To ensure aseptic condition all instruments, glassware and culture media were sterilized by autoclaving. For the shoot, root and plantlets regeneration the methods are employed that explants prepared by washing and surface sterilization then inoculation, incubation and sub cultured are completed. To investigate the effect of different treatments of the experiment, data were collected on percent of callus induction, Percentage of callus developed Shoots and Percentage of callus developed roots.

RESULTS AND DISCUSSION

The present study was carried out to determine an efficient and reproducible method for *in vitro* regeneration of Grape from different explants of Zakkao cultiver (*V. vinifera).* Different concentrations of BAP alone and in combination with NAA or IBA were used in MS and LS medium to observe their effect on callus initiation and shoot, root regeneration. The results obtained from the experiment are described as follows (Table 1; Plate A, B). In case of plant regeneration, the first experiment conducted that there were no need of plant growth regulators for regenerating grape from two types of explants namely, shoot tip and nodal explants on half MS medium. Basinger and Durhane (2000) have compared rooting ability of many species of native grape vine by culturing them on half strength MS media and noticed a relatively high rooting and shooting ability except in one of the species used. A significant observation was found for regeneration of shoot (68%), root (75%) from node, and shoot tip culture without hormonal combination. The Average length of shoot, root was found 5.5 cm, 7.7 cm from node and on the other hand, 4.16 cm, 5.19 cm from shoot tip. Zhanga *et al.* (2010) reported that ½ MS was the most suitable medium for rooting, shoot proliferation, and plantlet production of *V. piasezkii* var. pagnucii and Gansu (GS) medium was the second most suitable one.

The second experiment were conducted that, LS medium supplemented with of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l BAP were used for callus formation, shoot induction, from node, shoot tip and leaf explants. Gradual increase of BAP concentration in to LS medium up to 2.5 mg/l enhanced the percentage of callus formation (100%) and produces average shoot initiation from nodal explants. Nodal segments responded the best for shoot formation at BA 5µM (Muhammad *et al*. 2008). When LS medium supplemented with 2.0 mg/l, 2.5 mg/l of BAP, 100% leaf explants produced callus and 2.5 mg/l BAP produced maximum shoot. Das *et al.* (2002) reported that in MS basal medium supplemented with 1 mg BAP and 0.1 mg 2,4-D, leaf disc cultured for two weeks under dark conditions produced callus in over 80% of the cultures. The shoot tip explants showed best callus response (100%) on LS medium supplemented with 0.5 mg/l, 1.0 mg/l, 2.0mg/l and 2.5mg/l BAP and produce maximum shoot in 2.5 mg/l BAP. Sajid *et al.* (2006) observed that shoot number and shoot mass increased when the concentration of BAP was increased in a linear fashion. The combination of LS+2.5 mg/l BAP was found most effective for callus formation and moderately shoot regeneration (Table 2; Plate C, D & E).

The third experiment were conducted that, LS medium supplemented with of 0.5, 1.0, 1.5, 2.0 and 2.5 mg/l BAP with 0.5 mg/l NAA were used for callus formation, shoot induction, from node, shoot tip and leaf explants. There was statistically significant difference between the concentration of BAP+0.5 mg/l NAA in the callus formation from nodal segment. 100% callus formation was the highest on BAP 2.0 mg/l+0.5 mg/l NAA and the same concentration of BAP with 0.5 mg NAA produced the 33.33% regenerated shoot from callus. Singh, *et al*. (2010) reported that using nodal segments the treatment, 2.0 mgl-1 BAP + 0.2 mgl-1 NAA (Naphthalene acetic acid) was most effective with regard to enhancement in culture establishment and reduction in time to bud sprouting. The highest length of shoot (2.17 cm) and the maximum fresh weight of shoot (0.13gm) were found in the medium containing 2.0 mg/l of BAP with 0.5 mg/l NAA. Gradual increase of BAP concentration with 0.5 mg/l NAA into LS medium up to 2.0 mg/l was enhanced the per cent of callus formation and shoot regeneration from leaf explants. The highest percentage of callus from leaf explants (100%) was observed on BAP at 1.5, 2.0, 2.5 mg/l with 0.5 mg/l NAA. The highest shoot regeneration percentage (40%) was found at 2.0 mg/l BAP with 0.5 mg/l NAA. The highest length of shoot (1.89 cm) and the maximum fresh weight of shoot (0.129 gm) were found in the medium containing 2.0 mg/l of BAP with 0.5 mg/l NAA. In the case of leaf these values were significantly higher than that of other treatments. Richard *et al*. (2010) reported that abundant embryogenic callus was obtained from leaf and floral explants supplemented with 9 µM 2, 4-D+17 µM IASP + either 1 µM BA or 1 µM TDZ (ECIM) in darkness. Shoot tip was another explants of grape and affected by different concentration of BAP with 0.5 mg/l NAA. When LS medium supplemented with 0.0, 0.5, 1.0, 2.0 mg/l BAP and 0.5 mg/l NAA, (100%) explants produce callus and this value was significantly higher than that of other treatment. The highest percentage of shoot regeneration (40%) from callus, highest length of shoot (2.8 cm) and the maximum Fresh weights of shoot (0.16gm) were observed at 2.0 mg/l BAP with 0.5 mg/l NAA. The culture media used (1.5 mgL-1 BA), and (1 mgL-1 IBA + 1.5 mgL-1 BA) for highest average number shoots per cultured apex from shoot tip (Aazami 2010). Finally, LS supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA was found most effective for plantlet regeneration (Table 3; Plate F, G & H). There were no sufficient responses found in control treatment (Table 3).

The fourth experiment were conducted that, half MS medium supplemented with of 1.0, 2.0 and 3.0 mg/l IBA were used for callus formation, root induction, from node, shoot tip and leaf explants. Well root initiation is very important for *in vitro* regenerated plantlets In these experiment we observed that, root is directly initiated from the different explants not the rooting of *in vitro* grown shoots of grape. Gradual increase of IBA concentration into medium up to 3.0 mg/l was enhanced root initiation percentage (100%) and callus formation (100%) from nodal explants. IBA (10µM) did not depict any increase in callus mass (Muhammad *et al*. 2008). Concentration of IBA also affected the length of root and fresh weight. The highest length of root (2.7 cm) and fresh weight of root (0.9 gm) was found at 3.0 mg/l of IBA. A significant observation was found for regeneration of shoot, root from node without IBA combination that is control treatment. The Average length of root was found 6.6 cm; fresh weight (0.5 gm). When half MS medium supplemented with 3.0 mg/l of IBA, 85.6% leaf explants were callus formation and 85.6% root initiated. Skene *et al.* (1980) reported that root initiation was possible by using hormones free half strength MS medium containing 0.1 mg/l NAA. The highest length of root (1.7 cm) and fresh weight of root (0.721 gm) was found at 3.0 mg/l of IBA. Without IBA combination, callus and root is not initiated from the leaf explants that are control treatment. The callus formation, root initiation, root height and root fresh weight from shoot tip explants were also affected by different concentration of IBA with half MS medium. 3.0 mg/l of IBA, 100% explants were rooted and callus formed. Root length (1.5 cm), root fresh weight (0.016 gm) observed when shoot tip explants were cultured in half MS medium containing 3.0 mg/l IBA. A significant observation was found for regeneration of shoot, root from shoot tip without IBA combination. The Average length of root was found 7.2 cm; fresh weight (0.7 gm). Novak and Juvova (1983) reported that half strength medium with the addition of 0.1 µM IBA was effective for root formation in most of the shoot tip clones. As a result the best response of callus formation, root initiation, root length, root fresh weight was observed on half MS medium supplemented with 3.0 mg/l of IBA (Table 4; Plate I, J, K & L).

Table 1. Regeneration ability of plant from nodal segment, shoot tip explants without hormonal combination on half MS medium. Data were recorded at 45 DAI

Explant Name	No.of explants in conical flask	No.of explant regenerated	Regeneration of shoot (%)	Regeneration of root (%)	Shoot length (Average) cm	Root length (Average) cm
Node	25	17	68	68	5.5	7.7
Shoot tip	4	3	75	75	4.16	5.9

Table 2. Different concentration of BAP in LS medium on shoot regeneration for three types explants of grape. Data were recorded at 30 DAI

Concentration of BAP(mg/l)	Explants	No. of explants cultured	% of callus formation	% of shoot regeneration
LS (0)	Node	5	4 (80%)	+
	Leaf	5	4 (80%)	-
	Shoot tip	2	1 (50%)	-
0.5	Node	6	5 (83.33%)	+
	Leaf	5	5 (100%)	+
	Shoot tip	2	2 (100%)	+
1.0	Node	5	5 (100%)	+
	Leaf	6	5 (83.33%)	-
	Shoot tip	1	1 (100%)	+
1.5	Node	7	7 (100%)	+
	Leaf	5	5 (100%)	+
	Shoot tip	2	1 (50%)	-
2.0	Node	5	4 (80%)	++
	Leaf	6	6 (100%)	+
	Shoot tip	1	1 (100%)	++
2.5	Node	6	6 (100%)	++
	Leaf	4	4 (100%)	++
	Shoot tip	2	2 (100%)	++
Lsd (5%)			3.37	

No regeneration= -, Poor= +, Good= ++, BAP=6-Benzyl Amino Purine. DAI= Days After Inoculation. Lsd=least significant difference. The Lsd value is Significant at 5% level (*)

Table 3. Different concentration of BAP+NAA in LS medium on shoot regeneration for three types explants of grape. Data were recorded at 30 DAI

Concentration of(BAP+0.5 NAA) mgL^{-1}	Explants	% of callus formation (average)	% of shoot regeneration (average)	Shoot length(cm)	Shoot fresh weight(gm)
LS(0+0)	Node	70%	20%	1.00	0.056
	Leaf	85%	-	-	-
	Shoot tip	100%	-	-	-
0.5+0.5	Node	94%	-	-	-
	Leaf	80%	-	-	-
	Shoot tip	100%	33.33%	2.3	0.129
1.0+0.5	Node	90%	-	-	-
	Leaf	83.33%	20%	1.5	0.09
	Shoot tip	100%	-	-	-
1.5+0.5	Node	83.33%	20%	1.8	0.12
	Leaf	100%	16.66%	1.6	0.11
	Shoot tip	66.6%	-	-	-
2.0+0.5	Node	100%	33.33%	2.17	0.13
	Leaf	100%	40%	1.89	0.126
	Shoot tip	100%	50%	2.8	0.16
2.5+0.5	Node	87.5%	12.5%	2.5	0.14
	Leaf	100%	20%	1.5	0.084
	Shoot tip	50%	50%	1.5	0.078
Lsd (5%)		4.512	1.797	0.218	0.0172

No regeneration = -, NAA= Naphthalene Acetic Acid. The Lsd values are Significent at 5% level of probability (*)

Table 4. Different concentration of IBA on root formation (without *in vitro* Regenerated Shoot) from different explants of grape on half MS medium. Data were recorded at 35 (DAI)

Concentration of IBA (mg/l)	Explants	No.of explants cultured	% of callus formation	%of explants rooted	Root length, cm (average)	Root fresh weight, gm (average)
½MS	Node	7	-	5(71.42%)	6.6	0.5
	Leaf	3	-	-	-	-
	Shoot tip	2		2(100%)	7.2	0.7
½MS + 1.00	Node	5	4(80%)	80%	2.4	0.04
	Leaf	5	3(60%)	60%	1.3	0.6
	Shoot tip	2	1(50%)	50%	1.4	0.014
½MS + 2.00	Node	5	2(40%)	40%	2.3	0.038
	Leaf	8	4(50%)	50%	1.22	0.05
	Shoot tip	1	-	-	-	-
½MS + 3.00	Node	5	5(100%)	100%	2.7	0.9
	Leaf	7	6(85.6%)	85.6%	1.6	0.721
	Shoot tip	2	2(100%)	100%	1.5	0.016
Lsd (5%)			3.76	2.678	0.336	0.0568

No regeneration = -, The Lsd value are Significent at 5% level (*), IBA= Indole Butyric Acid.

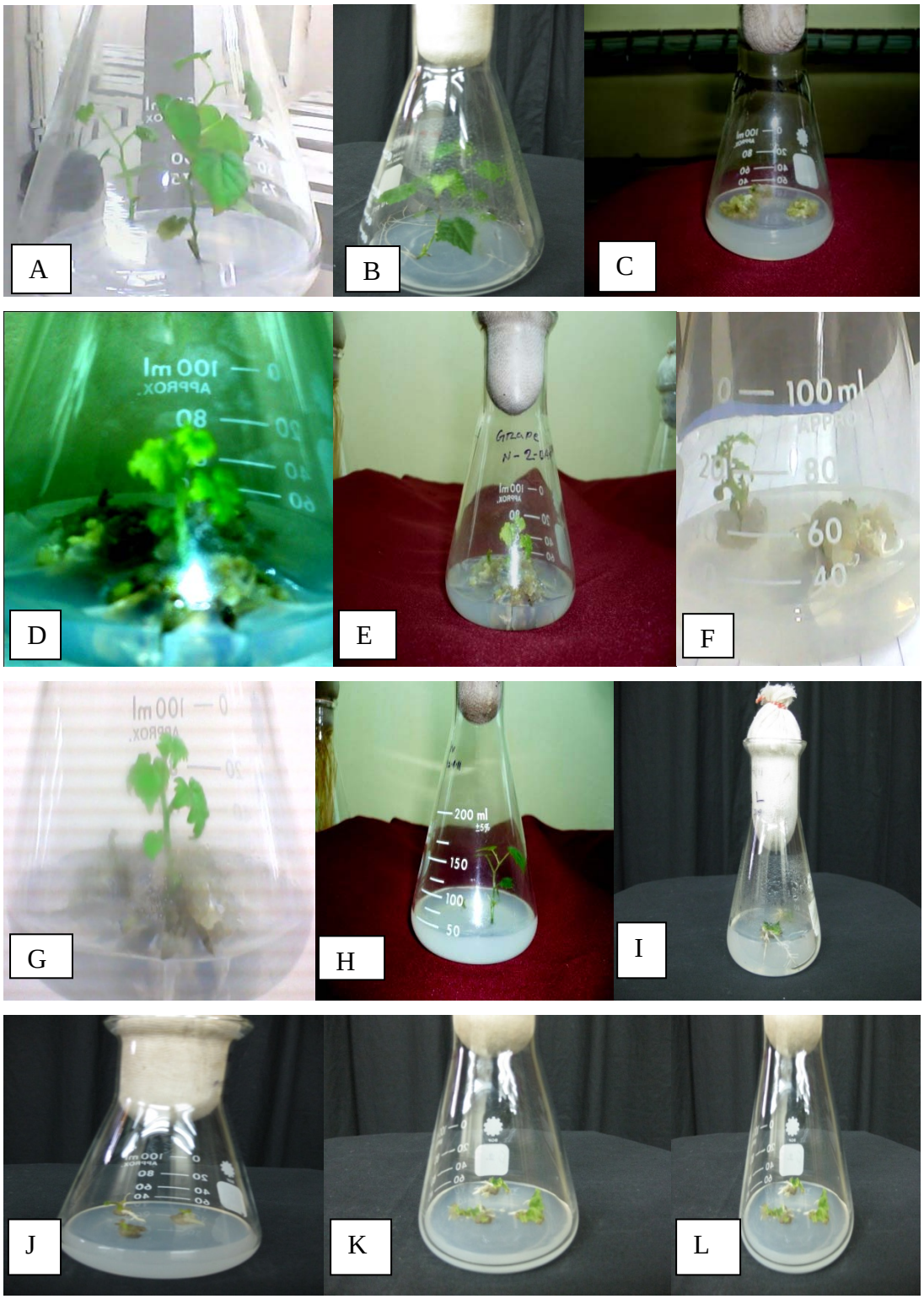

Plate A. Regeneration of Shoot & Root from Node on half MS medium. Plate B. Regeneration of Shoot & Root from Shoot tip on half MS medium. Plate C. Callus formation from Leaf explants of grape On LS medium supplemented with 2.5 mg/l BAP. Plate D. Callus formation from shoot tip explants of grape On LS medium supplemented with 2.5 mg/l BAP. Plate E. Callus formation & shoot initiation from nodal explants of grape supplemented with 2.5 mg/l BAP. Plate F. Shoot regeneration from leaf explants of Grape on LS medium supplemented with 2.0mg/l BAP + 0.5 mg/l NAA. Plate G. Shoot regeneration from node explants of Grape on LS medium supplemented with 2.0mg/l BAP + 0.5 mg/l NAA. Plate H. Shoot regeneration from shoot tip explants of Grape on LS medium supplemented with 2.0mg/l BAP + 0.5 mg/l NAA. Plate I. Rooting of *in vitro* grown explants of grape on half strength MS medium without hormonal combination. Plate J. Callus formation and Root initiation from node explants of Grape on half MS medium supplemented with 3.0mg/l IBA. Plate K. Callus formation and Root initiation from shoot tip explants of Grape on half MS medium supplemented with 3.0mg/l IBA. Plate L. Callus formation and Root initiation from leaf explants of Grape on half MS medium supplemented with 3.0mg/l IBA.

CONCLUSIONS

From the above experiments, the following findings could be drawn that regeneration ability of grape without hormonal combination was effective for shoot, root induction, proliferation and elongation. Benzylaminopurine (BAP) at 2.5 mg/l in LS medium was effective for callus formation and partially shoots induction but 2.0 mg/l BAP + 0.5 mg/l NAA was most effective for callus formation and shoot regeneration. IBA at 3.0 mg/l in half MS medium was the best for direct callus formation and rooting from explants.

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