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In vitro Propagation of Bitter Gourd (*Momordica charantia* L.) from Nodal and Root Segments

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Abstract

Explants of nodal and root segments of bitter gourd were cultured on MS supplemented with various concentrations of BAP in combination with either 2,4-D or NAA. Nodal segments produced the highest percentage (93.75) of callus in MS supplemented with 1.0 mg/l 2,4-D and 1.0 mg/l BAP whereas, root segments produced the highest (85.00%) callus in 0.6 mg/l NAA and 2.5 mg/l BAP combination. A combination of 1.0 mg/l 2,4-D and 1.0 mg/l BAP exhibited 75.00% shoot regeneration from nodal segments. The highest shoot length (5.15 cm) was recorded with 2.5 mg/l BAP and 0.2 mg/l IAA from nodal segments. No sign of regeneration of shoot was found from root segments in any of the combinations. However, some combinations produced only roots.

Introduction

Bitter gourd (*Momordica charantia* L.) belongs to the family Cucurbiteceae. It is an economically important, expensive summer vegetable in Bangladesh. The fruits of bitter gourd contain nutritionally useful essential minerals and amino acids. It has hypoglycemic activity which reduces the blood glucose in diabetic rats (Perl 1988, Khan 1999), anti-tumor activity (Xue et al. 1998) and antispermatogenic and androgenic activities (Naseem et al. 1998). Bitter gourd contains bright red seeds due to high lycopene, a pigment that can be used as an artificial food colorant (Yen and Hwang 1985). Bitter gourd protein (MRK29) has been reported to have HIV inhibitor properties (Jiratchariyakul et al. 2001). Bitter gourd is tolerant to a range of limiting factors of the environments (Lim 1998) and can be grown in tropical and subtropical climates (Reyes et al. 1994).

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Traditional breeding for varietal improvement of any crop is time consuming. Improvement of this crop and development of new varieties are obviously necessary which could be done through the applications of modern techniques of biotechnology. A reproducible protocol for *in vitro* regeneration is a prerequisite for varietal improvement through genetic engineering. However, very limited efforts have been made for the improvement of this crop using biotechnological technique.

A balance between auxin and cytokinin determines the *in vitro* regeneration of plants grown in artificial medium. Type of explants, media composition, growth conditions, genotypes and physiological condition of the explants affect callus induction and plant regeneration. Therefore, the best growth condition, suitable explants and genotypes are needed to identify large-scale utilization in biotechnology. There are some reports on tissue culture of bitter gourd in Bangladesh or its neighboring countries where bitter gourd is grown as an economic crop. Therefore, the present study was carried out to select the most suitable explants and growth regulators for *in vitro* regeneration and to develop an efficient protocol for *in vitro* plant regeneration.

Materials and Methods

The experiment was carried out during February to November, 2006 in the laboratory of the Department of Biotechnology, Bangladesh Agricultural University, Mymensingh. Seeds of a hybrid variety of bitter gourd (*Momordica charantia* L.) marketed by the East West Seed (Bangladesh) Ltd. were purchased from the local market. For better germination, collected seeds were treated with hot water (55 - 57°C) for 15 min by using "Vegetable Seed Treating device" developed by IPM Lab., Department of Plant Pathology, Bangladesh Agricultural University. Then they were dried overnight under shade. Surface sterilization of seeds was done with 0.1% (w/v) $HgCl_2$ solution for five minutes. The seeds were then rinsed for three - four times by sterile distilled water to remove traces of the $HgCl_2$. Then the seeds were placed on hormone free half strength MS medium. Seeds started germination within four days of inoculation and subsequently developed into rooted plantlets within ten to 12 days. Nodal and root segments were dissected from the plantlets and were used as explants. Explants were cultured on MS supplemented with various concentrations and combinations of 2,4-D (0.0, 0.25, 0.50, 1.0 and 5.0 mg/l), BAP (0.2, 0.5, 1.0, 2.0, 2.5 and 3.0 mg/l) and NAA (0.1, 0.3 and 0.6 mg/l) for callus induction. For shoot regeneration, calli derived from both types of explants were cultured on MS fortified with various concentrations and combinations of 2,4-D, BAP, NAA, IAA (0.1, 0.2, 0.6 mg/l), IBA and GA_3 (0.1 and 0.2 mg/l). The pH of the medium was adjusted to 5.8 ± 0.1 before addition of 0.8% (w/v) agar for solidification. All the cultures were maintained in 16 hr

photoperiod, with light intensity of 2000 - 3000 lux at 25 ± 1°C. Subcultures were carried out at regular intervals. After sufficient development of shoot and root systems, the small plantlets were taken out from the culture vessels and washed to remove excess of agar around the roots. Then it was placed in normal environment for acclimatization. Then they were transplanted to small pots. The experiments were arranged in CRD with four replications. Data recorded for different parameters under study were statistically analyzed. The significance of difference between the pair of means was evaluated at 5% level of significance by DMRT (Gomez and Gomez 1984).

Results and Discussion

A combination of 2.5 mg/l BAP and 0.6 mg/l NAA produced the highest percentage of callus (86.25) within the minimum number of days (7.75) in nodal segments (Table 1). In root segments, it was found that 2.5 mg/l BAP combined with 0.6 mg/l NAA produced the highest percentage of callus (85) and 26 days were required for callus induction (Table 1). Shafiullah et al. (2003) found optimum friable light green callus in cotyledon at 1.0 BAP with 1.0 mg/l IAA. The contradiction in levels of BAP and IAA might be due to dissimilarity of explants and genotype.

In general, 2,4-D alone has been reported to induce callus efficiently in many plant species. However, a combination of an auxin and a cytokinin has been found to improve the callus induction ability of some species. In nodal segments, 1.0 mg/l 2,4-D combined with 1.0 mg/l BAP had produced the highest percentage of callus (93.75) within a minimum number (8.25) of days (Table 2). This combination produced plenty of calli and the calli were light green in colour and compact in nature. (Fig. 1a, b). A combination of auxin and cytokinins has been reported to obtain callus from nodal explants of *Coccinia indica* on medium supplemented with 1.5 mg/l BA + 0.50 mg/l Kn + 0.50 mg/l NAA (Josekutty et al. 1993). In root segments, the same combination had produced the highest percentage (75) of callus (Table 2).

Different concentrations of growth regulators were applied in MS to achieve the shoot regeneration from nodal segments. Among the treatments, 1.0 mg/l BAP with 1.0 mg/l 2,4-D had produced the highest percentage (75.0) of shoot regeneration and the lowest percentage (50) was found in 2.0 mg/l BAP and 1.0 mg/l 2,4-D (Table 3, Fig. 2). Sultana and Miah (2003) observed best response towards shoot regeneration obtained from the nodal segments of *Momordica charantia* L. on MS supplemented with 2.0 mg/l BA and 0.2 mg/l NAA. Mondal et al. (2003) observed maximum green plant with 2 mg/l BAP and 0.5 mg/l NAA in case of indica rice. Rapid regeneration through organogenesis from callus cultures of *Coccinia indica*

Table 1. Effect of different concentrations of BAP and NAA on callus induction from different segments.

Concentration (mg/l)		Nodal segments		Root segments	
BAP	NAA	% callus induction	Days to callus induction	% callus induction	Days to callus induction
	0.1	30.00 fg	12.00a	0.00 g	0.00 g
1.5	0.3	23.75 g	11.50 a	25.00 f	28.25 b
	0.6	48.75 e	10.00 b	40.00 e	28.25 b
	0.1	67.50 cd	10.00 b	53.75 d	27.50 bc
2.0	0.3	62.50 d	10.00 b	65.00 c	26.00 d-f
	0.6	75.00 bc	10.00 b	67.50 c	25.75 d-f
	0.1	65.00 d	10.25 b	75.00 b	24.75 f
2.5	0.3	82.50 ab	10.25 b	70.00 bc	25.50 ef
	0.6	86.25 a	7.75 c	85.00 a	26.00 c-f
	0.1	33.75 f	9.50 b	68.75 c	27.25 b-d
3.0	0.3	23.75 g	10.50 b	48.75 d	26.75 b-e
	0.6	30.00 fg	11.50 a	63.75 c	30.00 a

In a column, the figures with the same letter(s) do not differ significantly. *Twenty explants were cultured per treatment and each treatment was replicate 4 times.

Table 2. Effect of different concentrations of 2,4-D and BAP on callus induction from nodal segments.

Concentrations (mg/l)		Nodal segments		Root segments	
2,4-D	BAP	% callus induction	Days to callus induction	% callus induction	Days to callus induction
0	0.2	25.00 k	14.00 a	0.00 i	0.00 j
	0.5	37.50 i	13.25 a	0.00 i	0.00 j
	1.0	42.50 h	11.75 bc	0.00 i	0.00 j
	2.0	37.50 i	12.25 b	0.00 i	0.00 j
0.25	0.2	61.25 f	11.25 c-e	0.00 i	0.00 j
	0.5	72.50 e	10.25 f-i	0.00 i	0.00 j
	1.0	77.50 d	11.00 c-f	0.00 i	0.00 j
	2.0	78.75 d	11.50 b-d	0.00 i	0.00 j
0.50	0.2	85.00 bc	10.25 f-h	31.25 h	37.75 a
	0.5	62.50 f	10.00 g-j	41.25 g	35.75 b
	1.0	87.50 bc	9.75 h-j	47.50 f	34.75 c
	2.0	75.00 de	10.75 d-g	43.75 g	33.25 de
1.0	0.2	83.75 c	9.50 ij	51.25 e	34.00 cd
	0.5	88.75 b	9.25 j	61.25 c	32.00 fg
	1.0	93.75 a	8.25 k	76.25 a	30.25 i
	2.0	65.00 f	9.25 j	75.00 a	31.00 hi
5.0	0.2	52.50 g	10.25 f-i	58.75 c	34.25 ce
	0.5	41.25 hi	12.25 b	55.00 d	31.50 gh
	1.0	37.50 i	10.75 d-g	71.25 b	32.50 ef
	2.0	32.50 j	10.50 e-h	68.75 b	34.25 c

In a column, the figures with the same letter(s) do not differ significantly.

was achieved on MS supplement with 1.5 mg/l BA, 0.5 mg/l Kn and 0.1 mg/l IBA (Josekutty et al. 1993). The findings agree well with the findings of the present study.

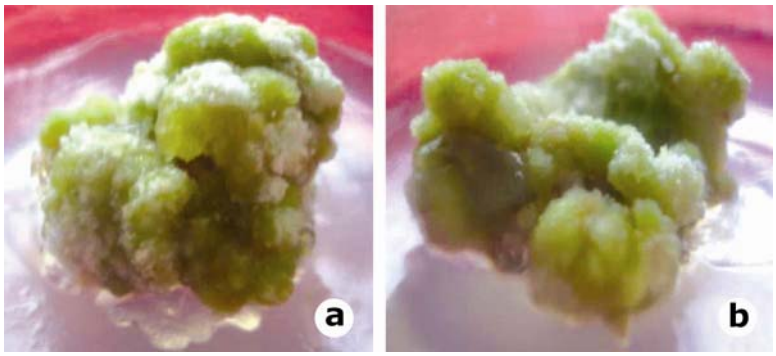

Fig. 1. Callus induced from different explants of bitter gourd cultured on media containing different concentrations of growth regulators: a. Nodal segments at 1.0 mg/l 2,4-D and 1.0 mg/l BAP (16 DAC). b. Root segments at 1.0 mg/l 2,4-D and 0.50 mg/l BAP (38 DAC).

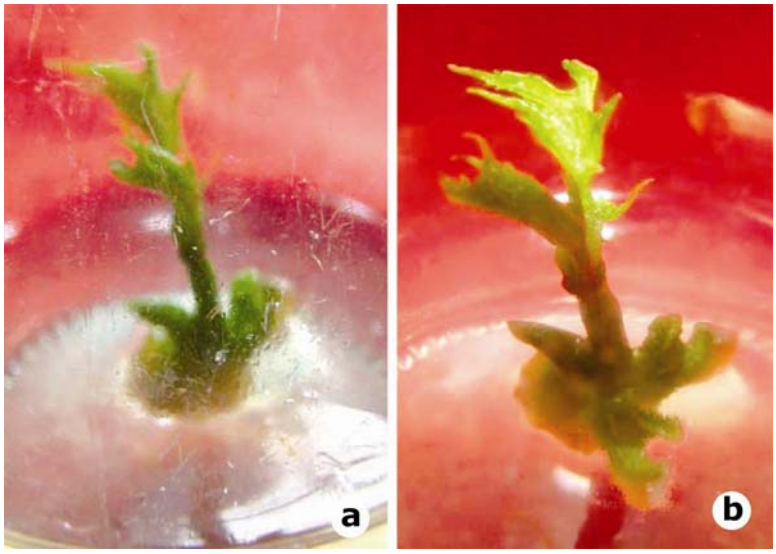

Fig. 2. Shoot regeneration from nodal segments callus media containing at 1.0 mg/l BAP (a) and 1.0 mg/l 2,4-D (b) after 45 days of culture.

The highest percentage (75.0) of root was produced by 2.0 mg/l BAP, 0.5 mg/l IBA and 0.2 GA_3 and the lowest percentage (45.0) was found in 1.0 mg/l 2,4-D and 2.0 mg/l BAP and 2.5 mg/l BAP and 0.2 mg/l IAA (Table 3). The highest shoot length (5.15 cm) was recorded in 2.5 BAP and 0.2 mg/l IAA which was statistically similar (Table 3) to 2.0 mg/l BAP, 0.5 mg/l IBA and 0.2 mg/l GA_3 (5.08 cm). Shafiullah et al. (2003) reported that the highest length of shoot (5.20)

was observed in 2.5 mg/l BAP in combination with 0.2 mg/l IAA which was similar to the present findings.

Table 3. Effect of different concentrations and combinations of growth regulators for different parameters of organogenesis from nodal segments.

Growth regulators (mg/l)			Per cent of shoot	% root induction*	Shoot length (cm)	Number of root	Root length (cm)
BAP	2,4-D						
0.50	0.25		52.50 f	52.50 d-g	3.10 gh	5.25 d	2.17
0.50	0.50		55.00 e	55.00 d-f	2.85 i	4.00 e	2.22
1.0	1.0		75.00 a	57.50 c-e	3.68 b	3.50 e	2.35
1.5	1.0		67.50 c	55.00 d-f	3.43 cd	5.75 cd	2.25
2.0	1.0		50.00 g	45.00 g	3.03 h	5.75 cd	2.28
	IAA						
2.0	0.2		60.0 d	52.50 d-g	3.13 gh	5.00 d	2.13
2.0	0.6		62.50 d	50.00e-g	3.45 cd	5.25 d	2.13
2.5	0.2		62.50 d	45.00 g	5.15 a	5.75 cd	2.28
2.5	0.6		52.50 f	47.50 fg	3.55 bc	5.50 cd	2.30
3.0	0.2		52.50 f	55.00 d-f	3.40 c-e	4.50 d	2.15
3.0	0.6		50.00 g	47.50 fg	3.13 gh	5.00 d	2.17
	IBA	GA_3					
1.0	0.5	0.2	65.00 c	52.50 d-g	3.30 d-f	5.75 cd	2.08
2.0	0.5	0.2	70.00 b	75.00 a	5.08 a	5.25 d	2.25
3.5	0.5	0.2	55.00 e	67.50 ab	3.20 fg	5.50 cd	2.03
	NAA						
2.0	0.6		0.00 h	52.50 d-g	0.00 j	5.00 d	2.20
2.0	0.3		0.00 h	52.50 d-g	0.00 j	5.00 d	2.05
2.5	0.1		0.00 h	67.50 ab	0.00 j	6.25 bc	2.25
2.5	0.6		0.00 h	65.00 a-c	0.00 j	6.75 ab	2.20
3.0	0.1		0.00 h	60.00 b-d	0.00 j	7.50 a	2.10
3.0	0.3		0.00 h	52.50 d-g	0.00 j	5.25 d	2.17

In a column, the figures with the same letter(s) do not differ significantly. * Roots formed either regenerated shoot or callus.

The highest number of root (7.50) was found in 3.0 mg/l BAP and 0.1 mg/l NAA and the lowest (3.5) was found in 1.0 mg/l 2,4-D and 1.0 mg/l BAP. The root length was statistically not significantly different (Table 3). However, 2.5 and 0.6 mg/l IAA was better.

Different concentrations of growth regulators were applied in MS for morphogenesis from root calli. Among the concentrations, none showed shoot regeneration. However, some treatment combinations produced only root (Fig. 3a). The highest percentage (75) of callus showing root was obtained in 2.5 mg/l BAP and 0.1 mg/l NAA whereas the lowest percentage (52.5) was obtained in 3.0 mg/l BAP and 0.3 mg/l NAA (Table 4).

The highest number of roots per callus (7.55) was found in 3.0 mg/l BAP and 0.1 mg/l NAA and lowest 4.25 was found in 3.0 mg/l BAP and 0.30 mg/l NAA. The highest root length (2.75 cm) was found in 3.0 mg/l BAP and 0.10 mg/l NAA and lowest was found in 2.0 mg/l BAP and 0.3 mg/l NAA (Table 4). Similar result was reported by Sultana and Miah (2003). They observed that the highest number (8.25) of root per callus and the highest length of root (2.75 cm) were obtained from 0.5 mg/l NAA.

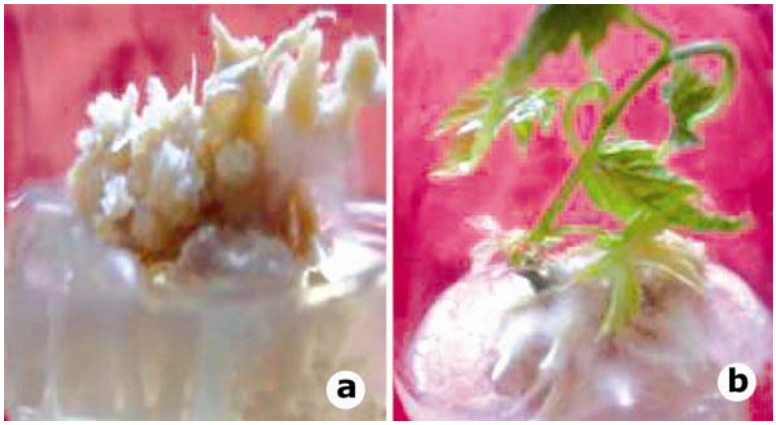

Fig. 3: (a) Only root induction from root segments derived callus in medium containing 1.0 mg/l 2,4-D and 1.0 mg/l BAP. (b) Rooted plantlets from nodal callus on 2.0 mg/l BAP and 0.20 mg/l IAA 80 days after culture.

Table 4. Effect of different concentrations and combinations of growth regulators for different parameters of organogenesis from root segments.

Growth regulator (mg/l)		% callus showing root induction	Number of root per callus	Root length (cm)
BAP	NAA			
2.0	0.6	52.50 b	5.45 c	2.20 b
2.0	0.3	55.00 b	5.50 c	2.15 b
2.5	0.1	75.00 a	6.45 b	2.65 a
2.5	0.6	67.50 b	5.15 c	2.25 b
3.0	0.1	70.00 a	7.55 a	2.75 a
3.0	0.3	52.50 b	4.25 d	2.22 b

In a column, the figures with the same letter(s) do not differ significantly.

After sufficient development of shoot and root, the small plantlets were transplanted in small pots containing soil. When the plantlets grew to a height of 5 - 6 cm, they were transferred to plastic pots. The growth of plantlets was satisfactory. The rate of success of plantlet survival was recorded to be above 75 per cent. The established plants in pots were kept in natural condition to grow them with proper care.

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References

Gomez AC and **Gomez AA** (1984) Statistical Procedures for Agricultural Research (2nd Edn.). John Wiley and Sons, New York. pp. 207-215.

Jiratchariyakul W, Wiwat C, Vongsakul M, Somanabandhu A, Leelamanit W, Fujii I, Suwannaroj N, Ebizuka Y, Weena J, Chanpen W, Molvibha V, Somanabandhu A, Leelamanit W and **Suwannaro1 N** (2001) HIV inhibitor from Thai bitter gourd. Planta Medica **67**(4): 350-353.

Josekutty PC, Shah S, Prathapasenan G and **Shah S** (1993) Direct and indirect organogenesis in *Coccinia indica.* J. Hort. Sci. **68**(1): 31-35.

Khan RA (1999) A study of Karela (*Momordica charantia* Linn.) on blood glucose. Hamdard Medicus **42**(2): 56-61.

Lim TK (1998) Loofahs gourds, melons and snake beans. The New Rural Industries. (Ed.: K W Hyde.) Canberra, Rural Industries Res. and Dev. Corp. pp. 212-218.

Mondal AB, Maiti T and **Bsiwas A** (2003) Somatic embryogenesis in root derived callus in indica rice. Plant Tissue Cult. **13**(2): 125-133.

Naseem MZ, Patil SR, Patil SR, Ravindra and **Patil SB** (1998) Antispennatogenic and androgenic activities of *Momordica charantia* (Karela) in albino rats. J. Ethnopharmacol **61**(1): 9-16.

Perl M (1988) The biochemical basis of the hypoglycemic effect of some plant extract. *In:* Craker L.E. and Simon J.E. (eds.), Herbs, Species and Medicinal plants: Recent advantaces in botany, horticulture and pharmacology. Orys press, phoenix AZ **3**: 49-70.

Reyes MEC, Gildemacher BH and **Jansen GJ** (1994) *Momordica L. In:* Plant Resources of South-East Asia: Vegetables. (Ed. Siemonsma, J S and K Piluek). Pudoc. Sci. Pub. pp. 206-210.

Shafiullah M, Sikdar B and **Joarder I** (2003) Embryogenesis and organogenesis from cotyledon-derived callus in bitter gourd. Mol. Biol. Biotech. J. **l**(l&2): 17-19.

Sultana RS and **Miah MAB** (2003) *In vitro* Propagation of Karalla (*Momordica charantea* Linn.) from nodal segment and shoot tip. J. Biol. Sci. **3**(12): 1134-1139.

Xue Y, Song S, Chen H, Xue Y, Song SH, Chen H and **Peron JY** (1998) Possible anti-tumor promoting properties of bitter gourd and some Chinese vegetables. Third international symposium on diversification of vegetable crops. Belling, China, 24-27 September 1996. Acta Hort. **467**: 55-64.

Yen GC, and **Hwang LS** (1985) Lycopene from the seeds of ripe bitter melon *(Momordica charantia)* as a potential red food colorant. II. Storage stability, preparation of powdered lycopene and food application. J. Chin. Agr. Chem. Soc. **23**: 151-161.