ISSN 0258-7122
Bangladesh J. Agril. Res. 35(3) : 465-473, September 2010

IN VITRO REGENERATION THROUGH CALLUS IN POINTED GOURD *(Trichosanthes dioica* Roxb.)

M. A. MALEK[1], M. A. MANNAN[2], D. KHANAM[3]
M. H. MOLLA[4] AND M. KHATUN[5]

Abstract

An efficient protocol was developed for *in vitro* plant regeneration and multiplication through callus culture in pointed gourd. Among the explants, highest percentage of cotyledon explants (92.00%) produced callus when this explant cultured in MS medium supplemented with NAA (0.1, 0.5, 1.0, 1.5, and 2.0 mg/l) and 2, 4-D (0.1, 0.5, 1.0, 1.5, and 2.0 mg/l). The highest number of shoots per explant was observed in MS + 0.5 mg/l BAP + 0.5 mg/l NAA followed by 1.0 mg/l BAP + 0.5 mg/l NAA when inter-node derived callus cultured in MS medium. Among the explants derived calli from leaf, inter-node and cotyledon in *in vitro* regeneration study, inter-node appeared as the most suitable explant for callusing and plant regeneration. The best response towards root induction was achieved on half MS medium supplemented with 0.5 mg/l NAA. The regenerated plantlets were successfully established in prepared earthen soil pot.

Keywords: *In vitro* regeneration, pointed gourd.

Introduction

Pointed gourd *(Trichosanthes dioica* Roxb.) is an under exploited important summer vegetable in Bangladesh. It is one of the most nutritive cucurbit vegetables that hold a coveted position in the vegetable market during summer and rainy season (Singh *et al.,* 1992). Being very rich in protein and vitamin A, it has certain medicinal properties and many reports are available regarding its role in circulatory system, especially in lowering blood sugar and serum triglycerides (Sheshadri, 1990). The success of a crop improvement programme, depends on selection of desirable plants, which is possible if wide variation is present in the base population. But there is less variability in pointed gourd (Uddin, 2000). Variability can be created by somaclonal variation or by *in vitro* polyploidization (Hoque *et al.,* 1998). So, it is necessary to develop *in vitro* plant regeneration protocol for pointed gourd for getting somaclonal variation. The technique of *in vitro* culture aims to keep the culture free from microbes and to ensure desird development in the cells and organs in suitable nutrient media and environmental conditions (Raghuvanshi, 2001). But less attention has been given to tissue

[1]Plant Genetic Resources Centre, Bangladesh Agricultural Research Institute (BARI), Gazipur, [2]Farm Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, [3,4&5]Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Gazipur, Bangladesh.

culture of pointed gourd than its closely related taxa, such as cucumber and melon (Dong and Jia, 1991). Plantlet formation has already been reported in *Cucurbita pepo* (Jelaska, 1974), water melon (Dong and Jia, 1991), cucumber (Chee, 1990; Gambley and Dodd, 1990) and *Momordica charantia* (Islam *et al.*, 1994). Many reports are available on callus initiation from herbaceous and vegetable crop plant species (Yamada *et al.*, 1967; Matsuoka and Hinata, 1983; Antonioli *et al.*, 1983, and Misra *et al.*, 1983). But there have not been many studies on *in vitro* regeneration through callus culture of pointed gourd in Bangladesh or in neighbouring countries. Therefore, the present investigation was conducted with a view to developing a protocol for plant regeneration through *in vitro* callus culture of pointed gourd.

Materials and Method

Leaf, inter-node and root from *in vitro* grown plantiets and cotyledon generated from mature seed of pointed gourd were used as the explants for callus formation and plant regeneration. The experiments were conducted in the Plant Biotechnology Laboratory, Institute of Biological Sciences (IBSc.), University of Rajshahi, Rajshahi. The experiments were conducted following Completely Randomized Design (CRD). The data were analyzed for mean value and standard error (S. E).

Inoculation

The excised leaf, inter-node, root, and cotyledons (obtained from mature seed of pointed gourd) were inoculated in each culture test tubes containing MS (Murashige and Skoog, 1962) media with various concentrations of 2,4-D (0.1, 0.5, 1.0, 1.5, and 2.0 mg/l) and NAA (0.1, 0.5, 1.0, 1.5, and 2.0 mg/l) and combinations of BAP (0.5 and 1.0 mg/l) with NAA (0.1, 0.5, 1.0, 1.5, and 2.0 mg/l) for callus formation. The pH of the medium was adjusted to 5.7 ± 0.1 using 0.1 N sodium hydroxide (NaOH) or 0.1 N HCI. In order to solidify the media, laboratory grade agar of 8.0 g (0.8%) was added to the solution. The culture tubes were plugged with aluminum foil and marked with glass marker pen to indicate specific hormonal supplement. The culture tubes were sterilized at 1.09 kg/cm^2 pressure at 121°C for 15 minutes in an autoclave. After auto claving, the culture media were taken out and allowed to cool and solidify. For growth and development of cultures, the temperature was set at 25±1°C and a light intensity of 2000-3000 lux from fluorescent tubular lamps and the photoperiod was maintained generally at 16 hours light and 8 hours dark (16 L/8 D) cycle and relative humidity was 60-70%. The well rooted plantlets were then kept in room temperature for 2-3 days and transferred to polyethylene bags containing a mixture of soil, sand, and rice bran ash (1:1:1) and moist them adequately for proper hardening.

Results and Discussion

Callus induction and shoot regeneration

Response of different explants of *in vitro* plantlets of pointed gourd, such as leaf, internode, cotyledon, and root for induction of callus at different concentrations of growth regulators viz. 2, 4-D, NAA and combinations of BAP with NAA are presented in Table 1. These explants when cultured in MS medium without hormonal supplements (control) remained fresh for long time without showing any sign of callus formation. All the explants except root produced callus when the explants were cultured in MS medium supplemented with different concentrations of 2, 4-D (0.1, 0.5, 1.0, 1.5, and 2.0 mg/l) and NAA (0.1, 0.5, 1.0, 1.5, and 2.0 mg/l) and combinations of BAP with NAA. However, the roots remained fresh in culture media for long time. Eighty percent of leaf, 88% of internode, and 92% of cotyledon explants produced callus in MS medium supplemented with different concentrations of NAA. Similarly, 88%, 84% and 92% of leaf, inter-node, and cotyledon explants, respectively, produced callus when cultured in the media supplemented with 2, 4-D and 68%, 80%, and 60% of the same explants produced callus when cultured in MS medium with different concentrations and combinations of BAP and NAA. However, inter-node produced roots in MS medium supplemented with different concentrations of NAA and 2, 4-D.

Table 1. Effect of different explants of pointed gourd in MS medium supplemented with 2, 4-D, NAA and combinations of BAP and NAA on callus induction.

Explants	No. of explants cultured	No. of for responsive explants for callus induction			% of explants forming callus		
		NAA	2, 4-D	BAP + NAA	NAA	2, 4-D	BAP + NAA
Leaf	25	20	22	17	80	88	68
Inter-node	25	22	21	20	88	84	80
Cotyledon	25	23	23	15	92	92	60
Root	25	NR	NR	NR	NR	NR	NR

NR: No response

Effect of different hormonal concentrations on morphogenic response of callus is presented in Table 2. The calli derived from different explants were morphologically different. The calli induced from leaf were soft, creamy, and friable; inter-node calli were white and loose and cotyledon calli were creamy and soft. The auxin 2, 4-D at 0.1-2.0 mg/l concentrations showed callus growth after 8-11 days of culture. The optimum level of callus induction was found when leaf explants were cultured in MS medium supplemented with 0.5 mg/l of 2, 4-D.

Average callus formation was observed in 0.1 mg/l and 1.0 mg/l of 2, 4-D. But 2, 4-D at the highest concentrations *(*1.5 mg/l and 2.0 mg/l) produced minimum amount of callus. Optimum level of callus induction was produced by inter-node explants when cultured in 0.5 mg/l of 2, 4-D followed by average level of callus produced in 1.0 mg/l and 1.5 mg/l of 2, 4-D. Cotyledon also produced different levels of callus in different concentrations of 2,4-D. Optimum level of callus was found in 1.0 mg/l of 2, 4-D and average amount of callus observed in 0.1 mg/l, 0.5 mg/l and 1.5 mg/l of 2, 4-D. Masum (1999) investigated different concentrations of 2, 4-D for callus production in ginger *(Zingiber officinale* Rose.) and reported that MS medium supplemented with 3.0 mg/l of 2, 4-D was the most suitable treatment for callus induction. Nanda Lal and Pramvir Singh Ahuja (1996) reported that MS medium supplemented with 0.5-2.0 mg/l of 2, 4-D induced callus from shoot cuttings and leaf explants of *Picrorhiza heerroa* Royle ex Benth. within two weeks.

Table 2. Effect of different hormonal concentrations for callus and shoot induction on different explants of *in vitro* grown plantlets of pointed gourd.

Concentrations of growth regulator (mg/l)	Morphogenic responses of explants			
	Leaf	Inter-node	Cotyledon	Root
2, 4-D				
0.1	++C,SS Creamy, Friable	+C Loose, white	++C, Creamy, Soft	NR
0.5	+++ C, Creamy, Friable	+++ C Loose, white	++ C, Creamy, Soft	NR
1.0	++C, Creamy, Friable	+++C Loose, white	+++C, Creamy, Soft, MS	NR
1.5	+C, Creamy, Friable	++C Loose, white	++C, Creamy, Soft	NR
2.0	+C, Creamy, Friable	+C, Loose, white	+C, Creamy, Soft	NR
NAA				
0.1	+ C, Creamy	+++C, MS Loose, white	++ C, Creamy	NR
0.5	++ C, Creamy	++ C, MS Loose, white	++ C, Creamy	NR
1.0	++ C, Creamy	++ C, MS Loose, white	++ C, Creamy	NR
1.5	++ C, Creamy	+ C, Profuse root	++ C, Creamy	NR
2.0	NR	+ C, Profuse root	+ C, Creamy	NR

+ C=Minimum callus, ++ C=Average callus, +++ C=Optimum callus, SS=Single shoot, MS=Multiple shoot, NR=No response.

Effect of NAA at various levels of concentrations (0.1- 2.0 mg/l) for callus formation is also presented in Table 2. Callus formation was completed within 10 days of culture. The average level of callus induction was observed when the leaf explants was cultured in MS medium supplemented with 0.5 mg/l NAA, 1.0 mg/l NAA, and 1.5 mg/l NAA. Lowest amount of callus was observed in 0.1 mg/l NAA. Inter-node explants produced optimum level of callus at the lowest concentrations of NAA (0.1 mg/l) and average level of callus was observed in 0.5 mg/l NAA and 1.0 mg/l NAA. The average level of callus was found when cotyledon explant was cultured in MS medium supplemented with 0.1 mg/l NAA, 0.5 mg/l NAA, 1.0 mg/l NAA, and 1.5 mg/l NAA, and minimum amount of callus was observed at the highest concentrations of NAA (2.0 mg/l). No callus was observed at different concentrations of NAA when the root explant was cultured in MS medium.

Table 3. Combined effects of growth regulators for callus formation and shoot regeneration from different explants of *in vitro* grown plantlets of pointed gourd.

Concentrations of growth regulator (mg/l)		Morphogenic responses of explants			
		Leaf	Inter-node	Cotyledon	Root
BAP	NAA				
0.5	0.1	+ C, Creamy	+ C, White, Root	+ C, Creamy	NR
0.5	0.5	+ + C, Creamy	+ + + C, White, Root and MS	+ C, Creamy	NR
0.5	1.0	+ C, Creamy	+ C, White Root	+ + C, Creamy	NR
0.5	1.5	+ C, Creamy	+ C, White Root	+ C, Creamy	NR
0.5	2.0	+ C, Creamy	Profuse root only	+ C, Creamy	NR
1.0	0.1	+ C, Creamy	+ C, White	+ + C, Creamy	NR
1.0	0.5	+ + C, Creamy	+ + + C, White Root and MS	+ + C, Creamy	NR
1.0	1.0	+ C, Creamy	+ C, White	+ C, Creamy	NR
1.0	1.5	+ C, Creamy	+ C, White, Root	+ C, Creamy	MR
1.0	2.0	+ C, Creamy	Profuse root only	+ C, Creamy	NR

+ C= Minimum callus, ++ C = Average callus, +++ C = Optimum callus, MS = Multiple shoot, NR= No response.

The results of combinations of BAP and NAA on callus formation are presented in Table 3. The average amount of creamy callus was observed in combination of 0.5 mg/l BAP + 0.5 mg/l NAA and 1.0 mg/l BAP + 0.5 mg/l NAA when leaf explant was cultured in the medium. The optimum amount of white callus was observed in 0.5 mg/l BAP + 0.5 mg/l NAA and 1.0 mg/l BAP +

0.5 mg/l NAA along with root and multiple shoot when inter-node explant was used in the culture media. The inter-node explant produced profuse roots only without formation of callus in 0.5 mg/l BAP + 2.0 mg/l NAA and 1.0 mg/l BAP + 2.0 mg/l NAA. Cotyledon explants produced average amount of callus by the combination treatments of 0.5 mg/l BAP + 1.0 mg/l NAA, 1.0 mg/l BAP + 0.1 mg/l NAA and 1.0 mg/l BAP + 0.5 mg/l NAA. No root was observed in case of leaf and cotyledon explants.

Table 4. Effect of different concentrations of 2, 4-D and NAA and combination of BAP with NAA on shoot regeneration and proliferation from leaf and inter-node derived callus.

Treatment (mg/l)		Days to shoot induction		Shoot number per culture		Shoot length (cm)		Leaf number per shoot	
		Leaf	Internode	Leaf	Internode	Leaf	Internode	Leaf	Internode
2, 4-D									
0.1		28	-	2.0	-	5.80	-	9.00	-
0.5		-	-	-	-	-	-	-	-
1.0		-	-	-	-	-	-	-	-
1.5		-	-	-	-	-	-	-	-
2.0		-	-	-	-	-	-	-	-
NAA									
0.1		-	-	-	-	-	-	-	-
0.5		-	-	-	-	-	-	-	-
1.0		28	-	3.00	-	6.50	-	10.00	-
1.5		-	-	-	-	-	-	-	-
2.0		-	-	-	-	-	-	-	-
BAP	NAA								
0.5	0.1	-	-	-	-	-	-	-	-
0.5	0.5	-	35	-	7.00	-	6.25	-	8.10
0.5	1.0	-	-	-	-	-	-	-	-
0.5	1.5	-	-	-	-	-	-	-	-
0.5	2.0	-	-	-	-	-	-	-	-
1.0	0.1	-	-	-	-	-	-	-	-
1.0	0.5	-	30	-	3.00	-	5.70	-	7.20
1.0	1.0	-	-	-	-	-	-	-	-
1.0	1.5	-	-	-	-	-	-	-	-
1.0	2.0	-	-	-	-	-	-	-	-

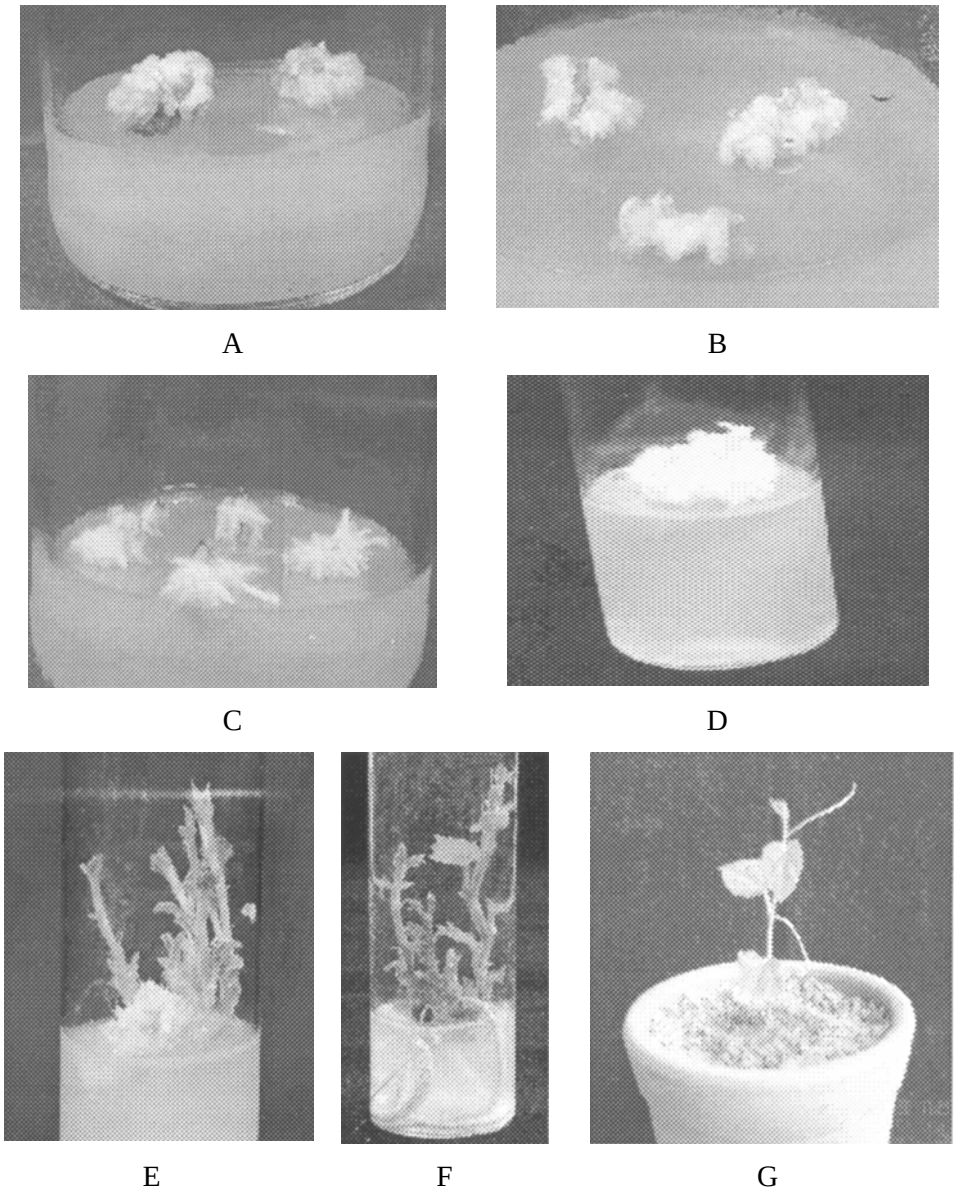

Fig. 1. Different stages of regeneration of pointed gourd.

A. Callus of leaf
B. Callus of inter-node
C. Callus with root
D. Callus of cotyledon
E. Shoot derived from callus
F. Multiple shoots with root
G. Established plant in earthen pot

In another experiment, 2, 4-D and NAA were used singly and in combination of BAP with NAA on shoot regeneration from leaf and inter-node derived callus. The effect of different concentrations of 2, 4-D and NAA alone and combination of BAP and NAA are presented in Table 4. Leaf explant produced good callus growth with multiple shoot formation when cultured in the medium containing 0.1 mg/l of 2, 4-D. Other concentrations of 2, 4-D produced only callus without showing shoot when the *in vitro* leaf explant was cultured. The inter-node explant produced callus as well as multiple shoot in treatment 1.0 mg/l NAA. The other concentrations of NAA produced only callus and root without any shoot.

The combined effects of BAP and NAA responded differently for shoot regeneration from leaf and inter-node derived callus of pointed gourd (Table 4). Internode explant derived callus showed multiple shoots when cultured on MS medium supplemented with 0.5 mg/l of BAP + 0.5 mg/l of NAA and 1.0 mg/l BAP + 0.5 mg/l NAA. Highest number of shoots (7.00) was observed in 0.5 mg/l of BAP + 0.5 mg/l of NAA followed by 1.0 mg/l of BAP + 0.5 mg/l of NAA when inter-node derived callus cultured in the medium.

The well rooted plantlets were kept in room temperature for 2-3 days and transferred to polyethylene bags containing a mixture of soil, sand, and rice bran ash (1:1:1) and moist them adequately for proper hardening. The well developed plants were then transferred to earthen pot. The survival rate was 60%.

Under the present study, efforts have been made to establish the protocol for regeneration of pointed gourd through callus under the treatment of different hormonal formulations. Multiple shoot propagation in pointed gourd *(Trichosanthes dioica* Roxb) was successfully developed to facilitate further advance research on genetic improvement through application of biotechnological approaches.

References

Antonioli C, L, Martinelli C Solanum and R Alliccho. 1983. Miglioramento genetoco sanitario della melanzana: Selezione *in vitro* dilence cellulari resistenti regenerazione di plante. *Notiziario Tecnico* (Italy). **13:** 20-23.

Chee, P.P. 1990. High frequency of somatic embryogenesis and recovery of fertile cucumber plants. *Hort. Sci.* **25:** 792-793.

Dong, J and S. Jia. 1991. High frequency plant regeneration from cotyledons of water melon *(Citrullus vulgaris* Schred). *Plant Cell Rep*. **9:** 559-562.

Gambley, R. I. and W. A. Dodd. 1990. An *in vitro* technique for the production of de novo multiple shoots on cotyledon explants of cucumber *(Cucurbits sativus* L.). *Plant Cell Tissue and Org. Cult.* **20:** 177-183.

Hoque, E. M., A. Bhowmik and M. Khalequzzaman. 1998. *In vitro* culture of Pointed gourd *(Trichosanthes dioica* Roxb.). *Thai. J. Agric. Sci.* **31**(3): 369-374.

Islam, R., K. P. Sarkar, A. T. M. Naderuzzaman and 0. I. Joarder. 1994. *In vitro* regeneration of plant from cotyledons of *Momordica charantia* L. *Plant Tissue Cult.* **4** (2): 105-109.

Jelaska, S. 1974. Embryogenesis and organogenesis in pumpkin explants. *Physiol. Plant.* **31:** 157-161.

Masum A. S. M. H. 1999. *In vitro* plants regeneration in ginger *(Zingiber officinale* Rosc.) An M.S. Thesis submitted for the Department of Genetics and Plant Breeding, BSMRAU. p. 1-47.

Matsuoka H. and H. Hinata. 1983. Factors affecting embryoid formation in hypocotyl callus *of Solanum melongena* L. *Japan J. Breed.* **33:** 303-309.

Misra N. R., Verghese, T. M. Mahaerchandani and N. R. K. Jam. 1983. Studies on induction and differentiation of androgenic callus of *Solanum melongena* L. *In:* Plant Cell Culture in crop improvement. *Basic Life Sciences* (USA) Vol. 22 Plinem Press, New York. pp. 465-468.

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

Nanda Lal and Pramvir Sing Ahuja 1996. Plantlet regeneration from callus in *Picrorhiza kurroa* Royle ex Benth. -An Endangered Medicinal Plant. *Plant Tissue Cult.* **6** (2): 127-134.

Raghuvanshi H. K. 2001. Plant tissue culture genetic in human welfare. *Perspective in Cytology and Genetics.* **10:** 271-283.

Rashid M. M. 1993. Vegetable Science (in Bengali) 2nd ed. Rashid Publication, Dhaka, Bangladesh. pp. 494-496.

Sheshadri, V. S. 1990. Cucurbits. *In:* Vegetable Crops in India, edited by Bose T. K. and Som M. G., *Naya Prokash*, Calcutta, India.

Singh A. K., R. D. Singh and K. Singh. 1992. Genetic variability, heritability and genetic advance for some traits in pointed gourd *(Trichosanthes dioica* Roxb.). *Hatyana J. Hort. Sc.* **21** (3 & 4): 236-240.

Uddin, S. 2000. *In vitro* propagation of pointed gourd *(Trichosanthes dioica* Roxb.). M S thesis, BSMRAU, Salna, Gazipur 1703. p. 2

Yamada Y, H. Nakagawa and Y. Sinnoto. 1967. Studies on the differentiation in cultured cells. Embryogenesis in three strains of *Solanum* callus. *Bot.Ag.* Tokyo, **80:** 63-74.