

IN VITRO REGENERATION IN POINTED GOURD

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Abstract

An efficient protocol was developed for plant regeneration, multiplication and rooting under *in vitro* condition in pointed gourd. Highest percent of shoot regeneration was 93.86 when nodal explants were cultured on MS+2.0 mg/l BAP. The maximum number of shoots (4.00) per explant was observed in MS + 2.0 mg/l BAP + 0.3 mg/l NAA from nodal segment. Among the two explants, nodal segment was found better for shoot regeneration. Female genotypes responded better than the male genotypes for shoot induction and proliferation. Lower nodal segment performed the best shoot 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.

Key Words: *In vitro* regeneration, pointed gourd

Introduction

Pointed gourd (*Trichosanthes dioica* Roxb.) locally known as *patal* is an under exploited important summer cucurbit vegetable in Bangladesh. The Bengal and Assam region of India is the primary centre of its origin (Singh *et al.*, 1992). It is grown almost in every districts of Bangladesh, especially in Rajshahi, Bogra, Pabna, Jessore and Kushtia (Rashid, 1993). It is a perennial crop and available in the market to the end of October when there is a scarcity of vegetables. The fruit is the edible part of the plant, which is cooked in various ways either alone or in combinations with other vegetables or meats. The fruits are easily digestible and diuretic in nature. Pointed gourd is rich in vitamin and minerals and contains 9.0 mg Mg, 2.6 mg Na, 83.0 mg K, 1.1 mg Cu and 17 mg S per 100 g edible part. Being 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 total cholesterol and blood sugar (Chandrasekar *et al.*, 1988; Sharma

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and Plant, 1988; Sharma *et al.*, 1988). It has also high industrial value as different types of jam, jelly and pickles can be made from this vegetable.

Traditionally pointed gourd is multiplied through stem cuttings and root suckers. Propagation through seed is not desirable due to dioecy and resulting cross-pollination the maintenance of true to type plant is another major problem. Stem and root cuttings are labour intensive and also require bulk amount of vines and roots, which restrict their multiplication at commercial level. *In vitro* multiplication of elite clones will be an attractive approach in order to meet the requirement of quality propagules at large scale for commercial cultivation. The present study was, therefore, undertaken to develop a rapid micro-propagation protocol for plant regeneration of pointed gourd through *in vitro* culture of shoot-tip and nodal segment.

Materials and Method

Shoot-tips and nodal segments were collected from tender and actively growing vines of the experimental field of pointed gourd at Plant Genetic Resources Centre, BARI, Joydebpur, Gazipur. Prepared explants were thoroughly washed in running tap water for 10-15 minutes. The explants were surface sterilized with detergent for 15 minutes in a beaker containing tap water. The explants were transferred in autoclaved plastic pot and treated with 0.1% mercuric chloride (HgCl_2) with a few drops of Tween 80 for 7 minutes for surface sterilization. The sterilized explants were then rinsed 4-5 times with sterile distilled water inside the clean bench to remove all traces of HgCl_2 . The sterilized explants were excised in the laminar airflow cabinet aseptically using a fine sterile forceps and scalpel. The excised explants were then inoculated in MS (Murashige and Skoog, 1962) medium with different levels of BAP (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l), Kn (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) and TDZ (0.1, 0.2, 0.3, 0.4 and 0.5 mg/l) and combinations of BAP (2.0 mg/l) with IBA (0.1, 0.2, 0.3, 0.4 and 0.5 mg/l), NAA (0.1, 0.2, 0.3, 0.4 and 0.5 mg/l, IAA 0.1, 0.2, 0.3, 0.4 and 0.5 mg/l) and Kn (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) for shoot regeneration. The pH of the medium was adjusted to 5.7 ± 0.1 using 0.1N sodium hydroxide (NaOH) or 0.1 N HCl. 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/cm² pressure at 121°C for 15 minutes in an autoclave. After autoclaving, the culture media were taken out and allowed to cool and solidify. The cultures were maintained in the temperature set on $26 \pm$

1°C with a light intensity of 2000-3000 lux from fluorescent tubular lamps. The maintained photoperiod was 16 hours light and 8 hours dark (16 L/8 D) and relative humidity of 60-70%.

Successful shoot formations become evident when small green fresh leaves began to emerge. Subcultures carried out regularly at an interval of 4-5 weeks. The percentage of explant induced shoot, days to shoot initiation, number of shoots per explant and length of the longest shoot have been recorded after four weeks of culture. *In vitro* shoot of pointed gourd were cultured in ½ MS medium supplemented with different concentrations of NAA (0.1, 0.2, 0.3, 0.4, and 0.5 mg/l) for root initiation. 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

Shoot regeneration

The response of shoot tips and nodal segments of pointed gourd to different concentrations of BAP, Kn and TDZ is presented in Table 1. The regeneration of shoots varied both with the type of explants and kind of supplements used. Higher concentrations took shorter duration for shoot regeneration. The shortest time (8 days) was taken by MS + 2.0 mg/l BAP and MS + 2.5 mg/l BAP. Among the various concentrations, MS + 2.0 mg/l BAP showed higher percentage of shoot induction (93.86%), maximum number of shoots per explant (3.25) and length of longest shoots per explant (4.55 cm) from nodal segments and it was 84.71%, 2.50 and 4.18 cm, respectively, for shoot induction percentage, shoot number per explant and shoot length per explant in shoot tips in medium with the same concentration of BAP. Shoot initiation and proliferation from shoot tip and nodal segment on MS medium supplemented with 2.0 mg/l BAP are shown in Fig. 1 and 2, respectively.

Table 1. Effect of different concentrations (mg/l) of BAP, Kn and TDZ on shoot induction and proliferation from nodal segment and shoot tip explants of pointed gourd.

Treatments (mg/l)	Shoot tip				Nodal segment			
	Shoot induction (%)	Days to shoot initiation	Shoot number/explant	Shoot length (cm)	Shoot induction (%)	Days to shoot initiation	Shoot number/explant	Shoot length (cm)
Control	0	0	0	0	0	0	0	0
BAP 0.5	64.29	10	2.00	3.20	75.00	10	1.75	2.73
1.0	71.43	10	2.25	3.19	80.91	10	2.75	3.73
1.5	75.00	8	2.50**	3.57	82.31	10	3.00*	3.89
2.0	84.71**	8	2.50**	4.18**	93.86**	8	3.25**	4.55**
2.5	62.50	8	2.00	3.55	57.14	8	2.00	3.59
Mean	71.58	8.80	2.25	3.53	77.84	9.20	2.55	3.69
S. E. (±)	3.576	0.438	0.100	0.161	5.376	0.438	0.258	0.261
Kn 0.5	45.26	8	1.80	2.54	55.14	9	2.00	2.94
1.0	50.33	8	2.00	2.15	57.43	9	2.25	3.00
1.5	52.60	8	2.00	2.55	68.00*	9	2.20	3.00
2.0	60.15**	8	2.37**	2.97**	75.15**	9	2.50**	3.46**
2.5	32.11	8	1.50	2.63	40.00	9	1.75	2.91
Mean	48.09	8.00	1.93	2.56	59.14	9.00	2.14	3.06
S. E. (±)	4.167	0.000	0.127	0.117	5.367	0.000	0.112	0.090
TDZ 0.1	21.00	16	1.20	0.75	25.86	16	1.00	0.87
0.2	23.57	16	1.28	0.68	28.88	16	1.00	0.66
0.3	30.32**	16	1.00	0.77	23.81	16	1.50*	0.89
0.4	33.00**	16	1.59**	1.41**	40.00**	15	1.75**	1.38**
0.5	20.16	16	0.72	0.64	22.41	15	0.75	0.65
Mean	25.61	16.00	1.15	0.85	28.19	15.60	1.20	0.89
S. E. (±)	2.297	0.000	0.129	0.126	2.814	0.219	0.164	0.118

*,** Significant at 5% and 1% levels, respectively.

Table 2. Effect of different concentration and combinations (mg/l) of BAP with IBA, NAA, IAA and Kn on shoot induction and proliferation from nodal segment and shoot tip.

Treatment (mg/l)	Shoot tip				Nodal segment			
	Shoot induction (%)	Days to shoot initiation	Shoot number/explant	Shoot length (cm)	Shoot induction (%)	Days to shoot initiation	Shoot number/explant	Shoot length (cm)
Control	0	0	0	0	0	0	0	0
BAP+IBA								
2.0+0.1	59.24	10	2.13	3.00	62.00	10	2.50	3.08
2.0+0.2	70.32**	10	2.58**	3.40**	80.00**	10	2.75**	3.70**
2.0+0.3	57.10	10	2.23	3.14*	60.00	10	2.25	3.10
2.0+0.4	53.44	10	2.20	2.70	55.56	10	2.70**	2.93
2.0+0.5	48.00	10	2.00	2.46	50.00	10	2.50	3.10
Mean	57.62	10.00	2.22	2.94	61.51	10.00	2.54	3.18
S.E (±)	3.311	0.000	0.086	0.147	4.527	0.000	0.079	0.119
BAP+NAA								
2.0+0.1	56.10	8	2.00	2.90	71.43	10	2.25	3.25
2.0+0.2	71.40*	8	2.55	3.00	81.82*	10	3.32*	4.30*
2.0+0.3	78.19**	8	3.80**	3.60**	88.78**	10	4.00	4.88**
2.0+0.4	70.24*	8	1.42	3.15	72.50	10	3.20	3.85
2.0+0.5	51.00	8	2.51	2.65	53.33	10	1.75	3.26
Mean	65.38	8.00	2.45	3.06	73.57	10.00	2.90	3.90
S.E (±)	4.733	0.000	0.35 2	0.141	5.347	0.000	0.358	0.279
BAP+IAA								
2.0+0.1	57.30	8	2.54	3.10	63.64	8	2.75	3.73
2.0+0.2	55.60	8	2.47	3.25	62.50	8	2.25	3.68
2.0+0.3	62.40	8	2.65	3.35	66.67	8	3.00*	3.80
2.0+0.4	72.00**	8	3.60**	4.10**	81.33**	7	3.25**	4.65**
2.0+0.5	58.50	8	2.31	3.30	60.00	7	2.50	3.80
Mean	61.16	8.00	2.71	3.42	66.82	7.80	2.75	3.93
S.E (±)	2.622	0.000	0.204	0.156	3.381	0.335	0.158	0.162
BAP+Kn								
2.0+0.5	54.83	8	2.60	3.42**	61.34	8	3.00*	3.56*
2.0+1.0	66.00**	8	3.40**	3.44**	72.00**	8	3.50**	3.85**
2.0+1.5	60.87*	8	2.15	3.17	60.00	8	2.45	3.24
2.0+2.0	53.40	8	2.00	2.32	56.00	8	2.00	2.65
2.0+2.5	40.00	8	2.00	2.00	42.35	8	2.00	2.82
Mean	55.02	8.00	2.43	2.87	58.33	8.00	2.59	3.22
S.E (±)	3.914	0.000	0.238	0.266	4.287	0.00	0.262	0.199

*,** Significant at 5% and 1% levels, respectively.

Table 3. Effect of different concentrations (mg/l) of BAP, Kn on shoot induction of male and female genotypes from nodal segment of pointed gourd.

Treatments (mg/l)	Female genotype (AM-8)			Male plant genotype (AM-21)		
	No. of explant cultured	Shoot induction (%)	Shoot number/ explant	No. of explant cultured	Shoot induction (%)	Shoot number/ explant
Control	0	0	0	0	0	0
BAP						
0.5	20	75.00	2.75	20	65.47	1.50
1.0	20	80.91	1.75	20	68.75	2.00
1.5	20	82.31	3.00*	20	75.00*	2.10
2.0	20	93.8**	3.25	20	80.00**	2.30**
2.5	20	57.14	2.00	20	50.00	2.20*
Mean		77.84	2.55		67.84	2.02
S. E (±)		5.376	0.258		4.578	0.120
Kn						
0.5	20	55.14	2.00	20	44.23	1.10
1.0	20	57.43	2.20	20	56.65	1.00
1.5	20	68.00*	2.50**	20	63.00**	1.90**
2.0	20	75.15**	2.50**	20	63.00**	1.90**
2.5	20	40.00	1.75	20	48.00	1.00
Mean		59.14	2.19		53.97	1.28
S. E (±)		5.367	0.129		3.069	0.153

*,** Significant at 5% and 1% level, respectively.

Table 4. Effect of different concentrations (mg/l) of BAP and Kn on shoot induction (%) and shoot number from upper and lower nodal segment of pointed gourd.

Treatment (mg/l)	Upper nodal segment				Lower nodal segment			
	Shoot induction (%)	Days to shoot initiation	Shoot number /explant	Shoot length (cm)	Shoot induction (%)	Days to shoot initiation	Shoot number /explant	Shoot length (cm)
Control	0	0	0	0	0	0	0	0
BAP 0.5	50.00	12	1.30	2.12	60.00	10	2.71	3.45
1.0	53.00	12	1.70	2.23	66.66	10	2.75	3.81*
1.5	56.00*	12	2.00*	2.28	85.71**	9	3.00	3.61
2.0	62.00**	12	2.15**	2.55**	91.66**	9	3.50**	3.97**
2.5	44.00	12	1.52	2.14	50.00	9	2.50	3.10
Mean	53.00	12.00	1.73	2.26	70.80	9.40	2.89	3.71
S.E (±)	2.683	0.000	0.138	0.069	6.996	0.219	0.153	0.079
Kn 0.5	43.00	12	1.12	2.00	50.27	9	2.00	2.50
1.0	49.00	12	1.50	1.92	55.43	9	2.50	3.00
1.5	50.00	12	1.47	2.00	61.20	9	2.15	3.00
2.0	54.00**	12	2.18**	2.38**	69.38**	9	3.00**	3.15**
2.5	32.00	12	1.40	2.00	46.00	9	1.80	2.42
Mean	47.00	12.00	1.53	2.06	56.45	9.00	2.29	2.81
S.E (±)	2.011	0.000	0.156	0.072	3.677	0.000	0.189	0.132

*,** Significant at 5% and 1% level, respectively.

Table 5. Effect of NAA in half strength of MS medium on rooting of induced shoots in pointed gourd.

Treatments (mg/l)	Days to root initiation	Root induction (%)	Root number of explant	Root length (cm)
Control	0	0	0	0
NAA 0.1	18	20	6	6.10
0.2	18	34	8	7.00*
0.3	18	64	8	6.40**
0.4	15	78*	9	7.25**
0.5	15	90*	11**	7.14*
Mean	16.80	57.20	8.40	6.77
S.E (±)	0.657	11.792	0.726	0.201

*,** Significant at 5% and 1% level, respectively.

The results on different concentrations and combinations of BAP with IBA, NAA, IAA, and Kn from shoot tip and nodal segments shown in Table 2

indicates that MS medium with 2.0 mg/l BAP + 0.3 mg/l NAA regenerated higher percentage of shoot induction (88.78%), maximum number of shoots per explant (4.00) and length of longest shoot per explant (4.88cm) from nodal segment than any other combinations in both explants. The results indicated that the nodal explants were more capable of producing multiple shoots compared to those of the shoot tip explants. The greater responsiveness of nodal explants over shoot apices can be attributed to the absence of apical dominance and the presence of axillary buds at a more advanced stage of development. It may be mentioned here that the shoot apex displays apical dominance, which might result from auxin produced at the terminal bud. Due to apical dominance, the lateral bud formation is suppressed. In apple (Hutchinson, 1981) and thornless blackberry (Zimmerman and Broome, 1980) nodal segments proved to be good explants for micro propagation. The results reported here indicate that nodal segment was more suitable for shoot regeneration and multiplication and also maximum shoot elongation. These results were in agreement with the findings of Debnath *et al.* (2000) and Uddin (2000) in pointed gourd. Zaman *et al.* (1992) demonstrated similar effects of BAP on shoot elongation in nodal segments culture of *Verbena* spp. (Hosoki and Katahira, 1994).

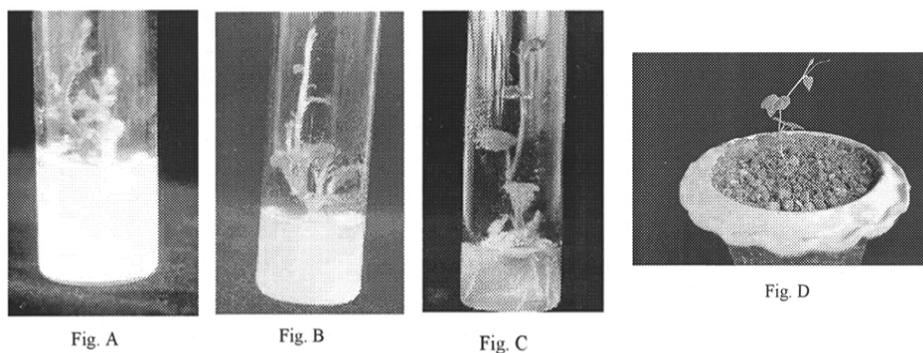


Fig. A. Multiple shoots of shoot tip explant on MS + 2.0 mg/l BAP

Fig. B. Multiple shoots of nodal segment explant on MS + 2.0 mg/l BAP + 0.3 mg/l NAA

Fig. C. Rooting of *in vitro* grown shoots on half strength MS medium supplemented with 0.5 mg/l NAA

Fig. D. Established plant in earthen pot

The regeneration of multiple shoots varied both with the type of explants and kind of supplements used. Performance of the nodal explants for multiple shoot regeneration was better than shoot tips culture. Sikdar *et al.* (2003) also observed similar results and they reported that performance of the nodal explants for multiple shoot regeneration in bitter melon was better than shoot tip. It has also been observed that with the increasing of BAP upto 2.0 mg/l, the number of shoots increased and then decreased at very high BAP concentration (2.5 mg/l). The ability of BAP to induce auxillary branching is well documented (George, 1993). In general, herbaceous plants are highly responsive to BAP treatments and most of the cultures produce robust well formed shoots suitable for further shoot proliferation (Debergh and Zimmerman, 1991).

***In vitro* response of male and female plant**

In a comparative study of *in vitro* response of male and female genotypes, only nodal segments were cultured on to MS media supplemented with different concentrations of BAP and Kn (Table 3). Morphogenic *in vitro* responses of the explants were found to vary with different hormonal formulations present in the culture media. Shoot proliferation was observed in all the treatment formulations. The female plant showed the best results on shoot induction percentage and shoot number per explant in MS + 2.0 mg/l BAP. In case of shoot induction percentage, female genotype produced highest shoot induction (93.86%) that of male genotype (80.00%) when the nodal explants cultured in MS media containing 2.0 mg/l BAP (Fig. 1). For shoot number per explant, the female genotype showed also better results (3.25) than the male genotype (2.30) in MS + 2.0 mg/l BAP. The MS basal media along with 1.5 mg/l Kn and 2.0 mg/l Kn had also better effect on female genotype than male one for shoot number per explant. In this case, female genotype produced higher shoot number per explant (2.50) than male genotype (1.90) in MS + 2.0 mg/l Kn. Moreover, shoot induction percentage (75.15%) also found better in female genotype than male genotype (63.00%) when the explant cultured on MS basal media supplemented with 2.0 mg/l Kn. These results were in agreement with the findings of Mythili and Thomas (1999) and Uddin (2000) in pointed gourd.

***In vitro* response of upper and lower nodal segment**

At the same concentrations of BAP and Kn, lower nodal segment produced maximum percent of shoot (91.66%) than the upper nodal segment. In all concentrations, lower nodal segment showed better results for percent of shoot induction, shoot number per explant and shoot length than upper nodal segments. Percent of shoot induction was decreased at MS + 2.5 mg/l BAP

(Fig. 2). Similar results were also reported by Uddin (2000) in pointed gourd. Lee *et al.* (1995) investigated *in vitro* propagation of various muskmelons (*Cucumis melo*) cultured from nodal explants. They found node from the upper part of the stem shoot well, shooting efficiency decreased with nodes taken from the lower part of the stem. These findings are reverse in our results for percent of shoot induction.

Rooting of *in vitro* grown shoot

Easy root formation is very important for establishment of *in vitro* regenerated plantlets. For successful micro propagation, healthy and strong root system is required. Response of different concentrations of NAA on half strength MS medium on *in vitro* adventitious root formations is presented in Table 5. The highest percent of root formation (90%) and number of root per explant (11) were found in MS + 0.5 mg/l NAA which was significantly different from other treatments. But longest root per explant (7.25 cm) was observed in MS + 0.4 mg/l NAA. Early root induction was obtained from the treatment of MS + 0.4 mg/l NAA and MS + 0.5 mg/l NAA. This result was in agreement with the findings of Mamun Hossain *et al.* (1997) and Uddin (2000).

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