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In vitro micropropagation of pointed gourd (*Trichosanthes dioica* Roxb.) from shoot tip and nodal segment

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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 91.66%, when nodal explants were cultured on MS+1.0 mg/1 BAP. The maximum number of shoots (4.8) per explant was observed in MS + 1.0 mg/1 BAP + 0.2 mg/1 NAA from nodal segment. Among the two explants, nodal segment was found better for shoot regeneration and multiplication. The best response towards root induction was achieved on half MS medium supplemented with 0.4 mg/1 NAA. The regenerated healthy rooted plantlets were transferred to small plastic pot containing garden soil and compost in a ratio of 2:1. Immediately after transplantation the plantlets were covered with polythene bag to prevent desiccation. After acclimatization the plantlets were successfully transferred to the garden soil and the percentage of survivability in such condition was 90%.

Key Words: *In vitro* multiplication, Shoot regeneration, Pointed gourd, Transplantation, Acclimatization.

Introduction

Pointed gourd (*Trichosanthes dioica* Roxb.) locally known as 'patal' is an important 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 combination with other vegetables or meats. The pointed gourd is one of the most nutritive cucurbit vegetables that holds a coveted position in the vegetable market during summer and rainy season (Singh *et al.,* 1992). Patal is a good source of carbohydrates, protein, vitamin A and C. Pointed gourd proteins showing peroxidase activity (Satar and Husain, 2009). It also contains a variety of trace elements considered beneficial for the human physiology, such as magnesium, potassium, copper, sulphur and chlorine (Singh, 1989). From the ayurvedic point of view, it is a tridoshic vegetable and is excellent for the balancing of all five fundamental elements. It is extremely ojas enhancing, easy to digest and assimilate into the physiology, does not create any ama and is nurturing for all seven layers of the skin. The fruits are easily digestible and diuretic in nature. It is febrifuge, laxative and antibilious (Vashista, 1974). Play important role in circulatory system, especially in lowering total cholesterol and blood sugar (Chandrasekar *et al.,* 1988; Sharma *et al.*, 1988). It is also known to have antiulcerous effects (Som *et al.,* 1993). The aqueous extract of *Trichosanthes dioica* leaves has good hypoglycemic potential along with a high anti-diabetic profile. It has also high industrial value as different types of jam, jelly and pickles can be made from this vegetable. Fresh juice of unripe fruit is used as cooling and laxative. The fruit is also used in spermatorrhoea (Sharma, 2004).

Pointed gourd is multiplied through stem cuttings and root suckers usually by the farmers. But stem and root cuttings are labor intensive and also require bulk amount of vines and roots, which restrict their multiplication at commercial level and ultimately increase its cost. Propagation using seed is not feasible primarily due to poor germination (Kumar *et al.,* 2007). Seed based populations have a tendency to give more male than female plants and in some cases the ratio goes up to 85:15 (Som *et al.,* 1993). In such a case, *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 micro-propagation protocol for plant regeneration of pointed gourd through *in-vitro* culture of shoot tip and nodal segment.

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Materials and Methods

The experiment was conducted at Plant Tissue Culture Section, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhanmondi, Dhaka. Healthy and profusely growing vines of pointed gourd were collected from various places of Bangladesh such as Magura, Kushtia and Manikgang and used as sources of explants for this experiment. Shoot tips and stem nodes with a single axillary bud were used for this experiment. The explants were surface sterilized with soft detergent for three times followed by washing with a few drops of Tween 20 and thoroughly washed in running tap water for 20-25 minutes. Then the explants were transferred in autoclaved plastic pot and treated with 0.1% mercuric chloride ($HgCl_2$) for 5 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 supplemented with alone or combination of BAP (0.5, 1.0, 1.5, 2.0, 2.5 & 3.0 mg/l), Kn (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l), NAA (0.1, 0.2, 0.3, 0.4, 0.5 mg/l), IAA (0.1, 0.3, 0.5, 1.0 mg/l) and IBA (0.1, 0.3, 0.5, 1.0 mg/l) for shoot regeneration and multiplication. The pH of the medium was adjusted to 5.7 ± 0.1 using 0.1N sodium hydroxide (NaOH) or 0.1N HCI. In order to solidify the media, laboratory grade agar of 5.5g (0.55%) 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^{o}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 ± 1^{o}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 and number of shoots per explant have been recorded after four weeks of culture. *In vitro* shoots of pointed gourd were cultured in ½ MS medium supplemented with different concentrations of NAA (0.1, 0.2, 0.3, 0.4, 0.5 and 1.0 mg/1), IAA (0.1, 0.3, 0.5, 1.0 mg/l) and IBA (0.1, 0.3, 0.5, 1.0 mg/l) for root initiation. The well rooted plantlets were then kept in room temperature for 2-3 days and transferred to plastic pot containing garden soil and compost in ratio of 2:1 and moist them adequately for proper hardening.

Results and Discussion

The regeneration response from shoot tips and nodal segments of pointed gourd in different concentrations of BAP and Kn is presented in Table I. The regeneration of shoots

Table I: Effect of different concentrations of BAP and Kn on shoot induction and proliferation from nodal segment and shoot tip explants of pointed gourd

Growth regulators (mg/l)	Shoot tip			Nodal segment		
	Shoot induction (%)	Days to shoot initiation	Shoot number/ explant	Shoot induction (%)	Days to shoot initiation	Shoot number/ explant
BAP						
0.5	13.33	10	1.50	8.33	10	2.30
1.0	80.00	8	3.25	91.66	7	4.00
1.5	73.33	7	3.00	83.33	7	3.50
2.0	53.33	8	2.25	58.33	7	2.70
2.5	20.00	9	2.00	8.33	8	2.25
3.0	6.66	10	1.90	-	-	-
Kn						
0.5	8.33	10	1.75	11.11	10	1.80
1.0	41.66	8	2.00	44.44	9	2.20
1.5	58.33	8	2.25	66.66	9	2.30
2.5	8.33	10	2.00	11.00	9	1.90
3.0	-	-	-	-	-	-

varied both with the type of explants and kind of supplements used. In MS + 1.0 mg/1 BAP and MS + 1.5 mg/1 BAP, it took the shortest time (7 days) for shoot induction. Among different concentrations, MS + 1.0 mg/1 BAP showed higher percentage of shoot induction (91.66%) and maximum number of shoots per explant (4.00) from nodal segments. Shoot initiation and proliferation from shoot tip explant on MS medium supplemented with 1.0 mg/1 BAP are shown in Fig. A. In case of Kn, MS + 1.5 mg/1 Kn higher percentage of shoot induction (66.66%) and maximum number of shoots per explant (2.30) from nodal segments were found and it was 58.33% and 2.25 respectively, for shoot tip explant. The ability of BAP to induce auxillary branching is well documented (George, 1993). In general, herbaceous plants are highly responsible to BAP treatments and most of the cultures produce robust well formed shoots suitable for further shoot proliferation (Debergh and Zimmerman, 1991).

The results on different concentrations and combinations of BAP with Kn, NAA, IAA, and IBA from shoot tip and nodal segments shown in Table II indicates that MS medium with 1.0 mg/1 BAP + 0.2 mg/1 NAA regenerated higher percentage of shoot induction (88.88%) and maximum number of shoots per explant (4.80) from nodal segment than any other combinations in both explants. The greater respons of nodal explants over shoot apices can be attributed to the absence of apical dominance and the presence of axillary buds in 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 (Hutchiunson, 1981) and Thom less blackberry (Zimmerman and Broome, 1980) nodal segments proved to be good explants for micropropagation. 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).

For acclimatization of micropropagated shoots, it is necessary to develop sufficient root. Different auxins NAA, IAA and IBA were used in different concentrations on half strength of MS basal medium for root induction (Table III). Among the three auxins NAA showed best response of root induction. The effectiveness of half-strength MS medium

Table II: Effect of different concentrations and combinations of BAP with Kn, NAA, IAA and IBA on shoot induction and proliferation from nodal segment and shoot tip explants of pointed gourd

Growth regulators (mg/l)	Shoot tip			Nodal segment		
	Shoot induction (%)	Days to shoot initiation	Shoot number/ explant	Shoot induction (%)	Days to shoot initiation	Shoot number/ explant
BAP+Kn						
1.0+0.5	58.33	8	3.20	62.50	10	3.40
1.0+1.0	66.66	8	3.60	75.00	8	3.80
1.0+1.5	33.33	9	3.40	50.00	9	3.25
1.0+2.0	16.66	10	2.90	25.00	10	3.00
BAP+NAA						
1.0+0.1	58.33	8	3.25	77.77	8	4.10
1.0+0.2	75.00	8	4.25	88.88	7	4.80
1.0+0.3	50.00	8	3.80	55.55	8	4.20
1.0+0.5	33.33	9	3.20	44.44	9	3.80
BAP+IAA						
1.0+0.1	60.00	9	3.20	62.50	9	3.35
1.0+0.5	40.00	10	3.10	50.00	9	3.45
1.0+1.0	30.00	11	2.90	25.00	11	3.10
BAP+IBA						
1.0+0.1	60.00	9	3.25	70.00	9	3.45
1.0+0.3	50.00	10	3.10	60.00	9	3.20
1.0+1.0	30.00	11	2.70	30.00	11	2.80

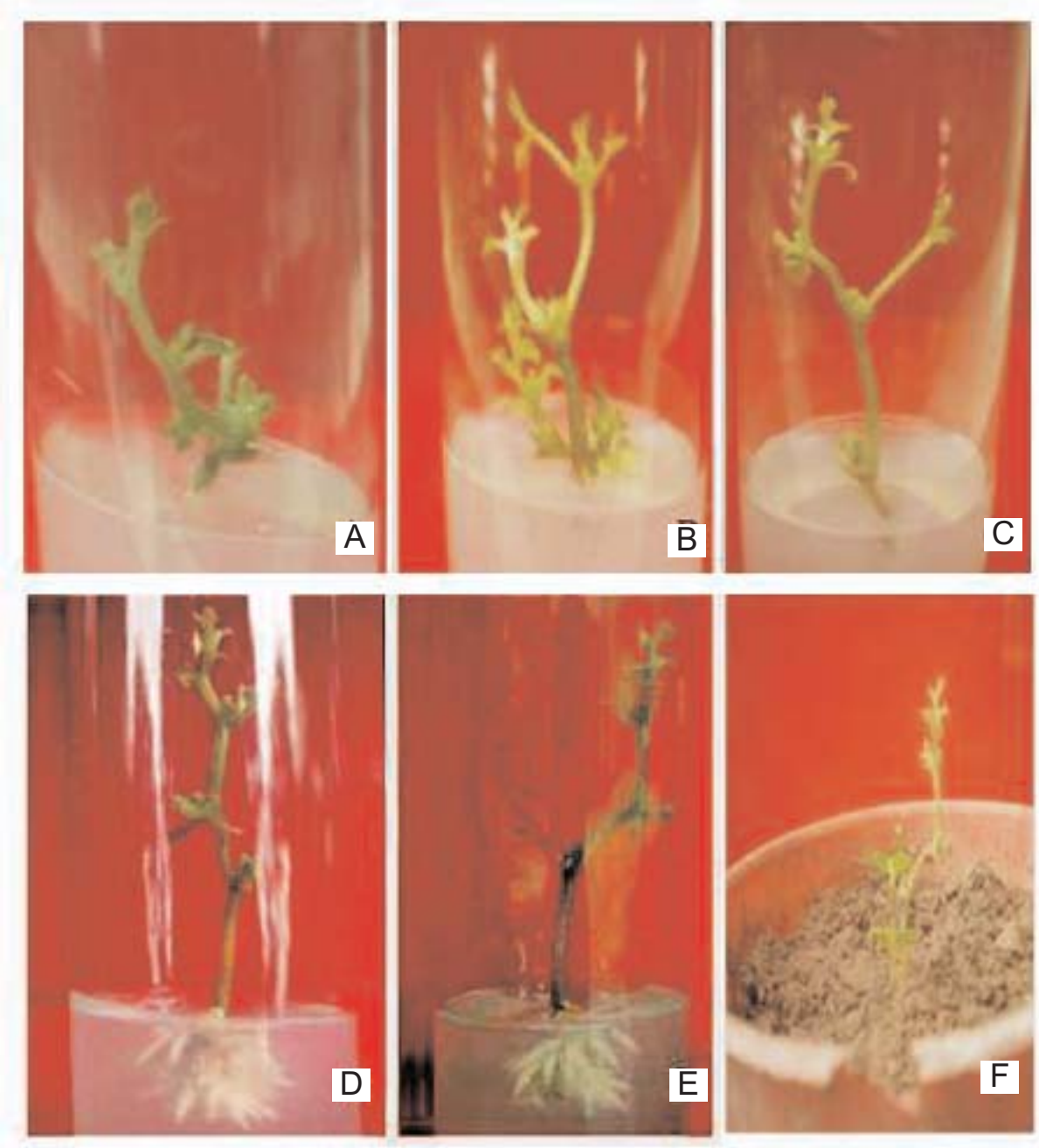

Fig. : ***In-vitro*** **micropropagation of** ***Trichonsanthes dioica*****.**
A. Multiple shoots regeneration from Shoot tip explant on MS+mg/1BAP + 0.2 mg/1 NAA.
B. Multiple shoots of nodal segment explant on MS+1mg/1BAP+0.2 mg/1 NAA.
C. Multiple shoots of Shoot tip explant on MS+1 mg/1BAP.
D. Root formation on 1/2 MS+0.5 mg/1BAP.
E. Root formation on 1/2 MS+0.4 mg/1 BAP.
F. Transplantation in earthen pot.

supplemented with auxin on root induction has been reported in many plants (Ahamed *et al.,* 2005; Huda *et al.,* 2003). Response of different concentrations of NAA, IAA and IBA on half strength MS medium on *in vitro* adventitious root formation is presented in Table III. The highest percent of root formation (87.50%) and number of roots per explant (11) were found in ½ MS + 0.4 mg/l NAA (Fig. D) which was significantly different from other treatments. Use of auxins singly or in combination for rooting was also reported by different authors (Sahoo and Chand, 1998; Ajithkumar and Seeni, 1998; Rai, 2002; Sivakumar and Krishnamurthy, 2000; Hassan and Roy, 2004; Baksha *et al.,* 2007; Hassan, 2008).

Table III: Effect of different concentrations of NAA, IAA and IBA in ½ MS medium on root induction from *in vitro* grown shoots of pointed gourd

Growth regulators (mg/l)	Root induction (%)	Days to root induction	Root number of explant
NAA			
0.1	-	-	-
0.3	50.00	15	7
0.4	87.50	14	11
0.5	62.00	14	10
1.0	-	-	-
IAA			
0.1	-	-	-
0.3	14.29	19	5
0.5	28.57	17	7
1.0	-	-	-
IBA			
0.1	14.29	21	6
0.3	42.86	18	8
0.5	14.29	20	7
1.0	-	-	-

The regenerated healthy rooted plantlets were carefully removed from the culture vessels. After thoroughly washing the roots in tap water to remove the traces of nutrients, the rooted plantlets were successfully transferred to plastic pot containing garden soil and compost in ratio of 2:1. The pots were immediately covered with polythene bag to prevent desiccation. About 88% plants were successfully grown. Established plantlets were transferred to the garden soil and it has been also observed that the *in vitro* raised plantlets were 90% reestablished.

Conclusion

The best result was observed in MS medium containing 1.0 mg/l BAP + 0.2 mg/l NAA for shoot initiation and multiplication when nodal segment used as explant and in case of root formation, the best result was observed in ½ MS medium containing 0.4 mg/l NAA. These results may be great helpful for the *in vitro* multiplication of pointed gourd.

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