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Organogenesis in Teasle Gourd (*Momordica dioica* Roxb.)

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Abstract

Of the four types of explants of teasle gourd (*Momordica dioica* Roxb.) viz. node, shoot tip, leaf and the cotyledon, the cotyledon showed the best performance. The combination 1.0 mg/l BAP and 0.1 mg/l NAA was found most suitable in callus induction followed by 0.2 mg/l NAA. The highest number of multiple and tallest shoots were obtained on MS medium fortified with 1.0 mg/l BAP and 0.1 mg/l NAA. For rooting, half strengh MS supplemented with IBA proved to be better than IAA, although on half strnegth MS supplemented with IAA tallest shoots were observed.

Introduction

Teasle gourd (*Momordica dioica* Roxb.), commonly known as Kakrol, a cucurbitaceous crop originated in the Indo-Malayan region (Rashid 1976 and Singh 1990) and has been cultivated in India, Bangladesh and neighbouring countries for a long time. It is rich in carotene, protein, carbohydrate (Rashid 1976) and vitamin C (Bhuiya et al. 1977). Kakrol as an important summer vegetable is widely grown in Comilla, Brahmanbaria, Rangpur, Norshingdi and Sylhet districts of Bangladesh and has high economic value with export potential. Improvement of this crop has not been attempted adequately, because of its dioecious nature and its vegetative mode of propagation. Presently its propagation entirely depends on underground tuberous roots, which occupy the valuable cultivable land for a long period i.e. until next planting season. Maintaining tuber quality in field condition as well as to conserve it in storage is difficult. Micropropagation may help overcome these problems to a great extent. An attempt of *in vitro* propagation of teasle gourd was demonstrated by Hoque et al. (1995). There have not been many studies on micropropagation of teasle gourd in Bangladesh or in neighbouring countries.

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So, adequate information on this aspect is not available. Therefore, the present experiment has been designed to develop an efficient protocol for *in vitro* plant regeneration of teasle gourd and to select suitable explants for *in vtiro* propagation.

Materials and Methods

As explants shoot tip, leaves and nodes were taken from field grown mature plants. MS medium and different concentrations of growth regulators such as BAP, NAA, IAA and IBA were used.

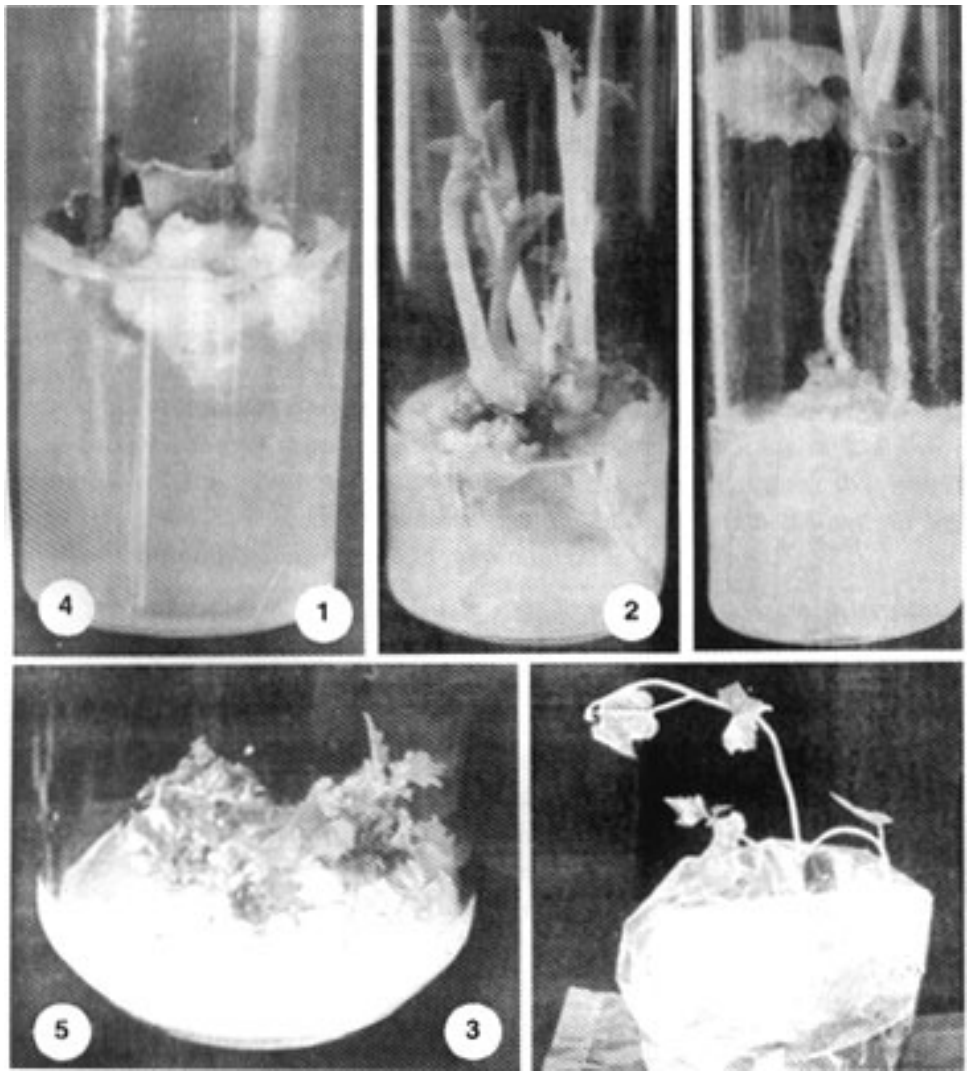
Explants were washed thoroughly under running tap water in plastic pot for 10 to 15 min. Later plants were cut into pieces and washed with detergent powder (Wheel brand) and 1 - 2 drops of Tween-20 for 10 min and then sterilized with 0.1 % $HgCl_2$ solution along with 1 - 2 drops of Tween-20 for 5 min followed by three - four rinses in autoclaved distilled water to remove traces of $HgCl_2$ under a laminar airflow cabinet.

Small segments (0.5 - 1.0 cm) were cultured on MS supplemented with specific concentration of growth regulators (BAP and NAA) in combination adding 3 % high quality sugar (SOS brand, Singapore) and 0.7 % agar, pH was adjusted and maintained at 5.8. Subcultures were done every 30 days interval. Cultures were kept for callus induction and maintenance; shoot initiation, proliferation and elongation. Each proliferated and adventitious shoot was cut from the basal end and subcultued again for further multiple shoot induction. Regenerated multiple shoots were cut and individual shoots were placed in half strength MS containing different concentrations of IBA and IAA for root induction. All cultures were kept at a temperature of 25 ± 1_C under 16 h photoperiod at 2000 - 3000 lux from fluorescent tubular lamps. The experiment was conducted under controlled conditions and followed complete randomized design (CRD) with three replications. Duncan's Multiple Range Test (DMRT) was used with the help of MSTAT software.

Results and Discussion

Various concentrations and combinations of BAP and NAA were used in MS medium and observed on the morphogenic responses of different explants of teasle gourd. Of the four explants such as shoot tip, leaf, node and cotyledons maximum callus was obtained from cotyledons (Fig. 1). Swelling of the leaf was observed in MS supplemented with 2.0 mg/l BAP + 0.1 mg/l NAA (Table 1) and high callus growth in 2.0 mg/l BAP + 0.2 mg/l NAA. Shoot regeneration was observed from shoot tip and node explants fortified with 1.0 mg/l BAP and 0.3 mg/l NAA. A high rate of callus growth and shoot regeneration were also

obtained in the same medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA (Organogenic callus). A combination of 1.0 mg/l BAP and 0.1 mg/l NAA resulted in the maximum percentage of callus growth (79.16) after 12 days of culture followed by 50 % in the medium fortified with 0.1 mg/l NAA (Table 1). MS fortified with 1.0 mg/l BAP and 0.2 mg/l NAA produced shoots and roots (Organogenic callus). Others had no response to shoot initiation (non-organogenic).



Figs. 1 - 5 : 1. *In vitro* callus formation in teasle gourd from cotyledon explants. 2. Multiple shoot proliferation in test tube. 3. Multiple shoot induction in a big jar. 4. Profuse rooting *in vitro.* 5. *Ex vitro* establishment of *in vitro* teasle gourd seedlings in polybag.

Table 1. Effect of different concentrations and combinations of BAP and NAA on MS in morphogenic responses of explants of teasle gound.

Concentration (mg/l)		Morphogenic response of explants				Production of callus		Type of callus	Morphogenic response of callus derived from cotyledons
BAP	NAA	Shoot tip	Node	Leaf	Cotyledon	Amount of callus	Percentage		
0	0	-	-	-	+ C	+	8.33	Non-organogenic	No callus growth
1	0	-	-	-	+ C	+	20.83	Non-organogenic	No callus growth
2	0	-	-	-	++ C	++	20.83	Organogenic	Good callus growth with shoot bud primodia
3	0	-	-	-	–C	0	0	Non-organogenic	No callus growth
0	0.1	-	-	-	+ C, S	+	16.66	Organogenic	No callus but shooting
1	0.1	-	-	-	+++ C, S	+++	79.16	Organogenic	Good callus growth with shoot bud primordia
2	0.1	-	-	-	++ C, S	++	16.66	Organogenic	Good callus growth with shoot bud primordia
3	0.1	-	-	-	++ C, S	++	20.83	Organogenic	Good callus growth with shoot bud primordia
0	0.2	-	-	-	++ C, S	++	50.00	Organogenic	Slow callus growth
1	0.2	-	-	-	+++ C, S, R	+++	29.16	Organogenic	Good callus growth with shoot bud primordia
2	0.2	-	-	+++ C	+++ C	+++	41.66	Organogenic	Good callus growth with shoot bud primordia
3	0.2	++, S	++, S	+++ C	–C	0	0	Non-organogenic	No callus growth
0	0.3	-	-	-	–C	0	0	Non-organogenic	No callus growth but shooting
1	0.3	+++, S	+++, S	-	–C	0	0	Non-organogenic	No callus growth
2	0.3	-	-	-	+ C	+	16.66	Non-organogenic	No callus growth but shoot initiation
3	0.3	-	-	-	+ C	+	20.83	Non-organogenic	Slow callus growth but shoot initiation

- = No growth, + = low growth, ++ = medium growth, +++ = High growth, –C = no callus, + C = low callus, ++ C = medium callus, +++ C = high callus, S = shoot and R = root.

The results of the present investigation agree with the findings of Hoque et al. (1995). They found that a combination of 1.5 mg/l BA and 0.1 mg/l NAA was more suitable for adventitious multiple shoot formation in teasle gourd whereas in this experiment 1.0 mg/l BAP + 0.1 mg/l NAA was observed to be best for the production of multiple shoots.

Islam et al. (1994) obtained the highest frequency of shoot formation (78 %) with 7.9 shoots per explants in MS supplemented with 2.0 mg/l BA. There are some reports on several related species. Multiple shoot regeneration of *Cucumis melo* using shoot tips as explant in 2.5 mg/l NAA and 1.0 mg/l BAP was obtained by Moreno et al. (1985). Halder and Gadgril (1982) was able to produce callus from cotyledons and embryo axis in squash (*Cucumis melo*) in MS supplemented with 2.0 mg/l NAA, and 15 % coconut milk. He obtained adventitious shoots and roots from 1.0 mg/l NAA. Wehner and Locky (1981) achieved adventitious shoot formation from the callus of cotyledon culture of *Cucumis sativas.* Lee and Thomas (1985) succeeded in obtaining multiple shoot proliferation and rooted plantlets from shoot tips and stem nodes of *Cucurbita foctidissima* in MS supplemented with 1.0 mg/l BAP + 0.1 mg/l NAA. Halder and Gadgil (1982) obtained callus and shoot bud development when cotyledons were cultured in MS supplemented with 0.1 - 1.0 mg/l NAA and 33.8 mg/l adenine. Rao et al. (1982) were also able to regenerate adventitious shoots and roots from dissected seed cotyledons and the embryo axis of *C. melo* in MS supplemented with 5.0 mg/l Kn + 15 % coconut milk.

The differentiation of plantlets from callus in teasle gourd using cotyledons as explants on MS supplemented with BAP and NAA is shown in Table 1. Significant differences were observed among the treatments on multiple shoot proliferation. Among the concentrations and combinations of BAP and NAA, the highest number of adventitious multiple shoots (25.33) was produced (Fig. 2) in MS supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA followed by the MS fortified with 2.0 mg/l BAP (21.33). The regenerated shoots were comparatively weak in medium supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA than others. The lowest number of multiple shoots was observed in MS fortified with 1.0 mg/l BAP and 0.2 mg/l NAA (8.66).

The highest shoot regeneration rate (88%) of teasle gourd was achieved by Hoque et al. (1995). They also obtained maximum number of shoots per regenerating explants (8.8) when 1.5 mg/l BA and 0.1 mg/l NAA was added on MS medium. In the present study, the highest number of regenerated shoots was 25.33 (Fig. 3). The highest number of shoot regeneration as reported by Hoque et al. (1998) was found close to the lowest number of shoots obtained in the present

study. This variation might be due to the treatment concentration and combination of BAP and NAA and variation due to different genotypes used by the two investigators.

The longest shoot (4.55 cm) was observed in MS supplemented with 1.0 mg/l BAP and 0.1 mg/l NAA (Table 2) followed by 2.0 mg/l BAP and 0.2 mg/l NAA (4.10 cm). The shortest shoot (2.22) was found in the medium containing 2.0 mg/l BAP.

Table 2. Effect of BAP and NAA on shoot proliferation (Number and length of shoot) in teasle gourd using cotyledon as explants.

Concentration (mg/l)		Number of shoots	Length of shoots (cm)
BAP	NAA		
2.0	0.0	21.33^{b}	2.22^{e}
1.0	0.1	25.33^{a}	4.55^{a}
2.0	0.1	10.66^{d}	2.61^{d}
3.0	0.1	10.00^{d}	2.27^{de}
1.0	0.2	8.66^{d}	3.38^{c}
2.0	0.2	15.66^{c}	4.10^{b}

In column means followed by uncommon letter varied significantly from each other at 5 % level of significance following DMRT.

Hoque et al. (1998) failed to elongate the shoot buds induced on the medium when BAP and Kn alone were used. There are some reports on shoot elongation in closely related species. The best shoot elongation was observed by Hossain et al. (1997) on pointed gourd (*Trichosanthus diocia* Roxb.) in MS supplemented with 1.0 mg/l BAP, 0.1 mg/l NAA and 10 mg/l adenine sulphate. This inference was near to the findings of the present investigation.

The data presented in Table 3 showed significant result on the root proliferation and length of the roots. *In vitro* grown multiple shoots were excised and cultured on half strength of MS supplemented with IBA and IAA. Most of the shoots produced roots within 12 to 20 days.

Highest number of root (2.80) was produced (Fig. 4) in MS supplemented with 0.2 mg/l IBA followed by 0.2 mg/l (2.66) and 0.2 mg/l IAA (2.60). These were statistically similar.

Hoque et al. (1998) cultured excised shoots of *in vitro* grown kakrol and transferred them to rooting medium and obtained 7 - 11 roots per plant on half strength of MS supplemented with 1.0 mg/l IBA within 15 to 20 days.

The present investigation reveals that auxin; IBA is better than IAA in terms of rooting ability. On the other hand, the number of roots per plants was lower than that of the findings of Hoque et al. (1998). This might be due to the genotypic variation of the explants and along with the cultural and environmental conditions.

Table 3. Effect of different concentration of IAA and IBA in half strength MS for root induction and elongation of teasel gourd using cotyledon as explants.

Concentration of growth regulator (mg/l)		Number of roots/ plantlet	Length of roots (cm)
IAA	IBA		
	Control	1.93^{b}	3.51^{ab}
0.2	-	1.13^{c}	4.14^{a}
0.3	-	2.60^{a}	3.48^{ab}
-	0.2	2.66^{a}	2.77^{b}
-	0.3	2.80^{a}	1.89^{c}

In column means followed by uncommon letter varied significantly from each other at 5 % level of significance following DMRT.

For the establishment of plant, regenerated healthy rooted plantlets were placed at room temperature for one - two weeks. Then the plantlets were removed from the culture bottle and carefully cleaned the plantlets to remove adhering agar. Plantlets were sprayed with fungicide and planted to normal and sterilized soil to observe the accomplishment of the planted media in polyethylene bag. The polyethylene bags (Fig. 5) were kept in the net-house.

It was observed that sterilized soil showed relatively cent per cent survivability than normal soil (83.33 %). Nevertheless, sterilization is a costly process. Thus, normal soil is more preferable for the practical purpose. Before going to transplant seedling in polyethylene bag, hardening is essential. To preserve moisture, watering is allowed as and when necessary. After 25 days of hardening, seedlings were ready for planting in the field.

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