High Frequency Shoots Regeneration from Cotyledon Explants of Teasle Gourd Via Organogenesis

Mohammad Abdul Karim
Dept. of Crop Botany, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
Email:profkarim@yahoo.com, profkarim1949@gmail.com

Sheikh Shorif Uddin Ahmed and Mohammad Shahidul Haque
Dept of Crop Botany, Dept. of Biotechnology, Faculty of Agriculture, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
Email: {chisty_agric, haquems}@yahoo.com

***Abstract*—Organogenic callus induction and high frequency shoot regeneration were achieved from cotyledon explants of teasle gourd. About 94.8% of cotyledon explants derived from 18 day old cotyledon produced green, compact nodular organogenic callus in MS medium containing BA 1 mgl-1 after 51 days with 95% shoot induction. The elongated shoots rooted in MS medium supplemented with IBA 0.3 mgl-1. Rooted plants were acclimatized in growth room and subsequently established in soil with 100% survivability. This protocol produced an average of 19.8 shoots cotyledon-1 explant in 22.2 days of culturing.**

***Index Term*—Teasle gourd; cotyledon; explant; regeneration; organogenesis**

I. Introduction

Teasle gourd *(Momordica dioica* Roxb.) is an important summer vegetables in Bangladesh [10]. It is one of the most nutritive cucurbit vegetables that have important position in the vegetable market during summer and rainy season. Being very rich in protein and Vitamin A, it has certain medicinal properties. In recent years, *in vitro* procedures were used to some degree in almost every major agronomic, vegetables, and fiber crops [7]. The success of this technology requires an efficient protocol for plant regeneration from isolated organs, tissues, and cells [8]. The success of a crop improvement programmed, depends on selection of desirable plants, which is possible if wide variation is present in the base population. But there is less variability in teasle gourd [12]; Variability can be created by somaclonal variation or by *in vitro* polyploidization [7]. So, it is necessary to develop *in vitro* plant regeneration protocol for teasle gourd.

But less attention has been given to tissue culture of teasle gourd than its closely related taxa, such as cucumber and melon [3]. Therefore, the present investigation was conducted with a view to develop from a protocol for plant regeneration through *in vitro* culture of cotyledon explants teasle gourd.

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II. Materials and Methods

Cotyledon was used as explants for plant regeneration. These explants were collected from field growing 18 days old teasle gourd fruits. The cotyledon was cut into half dividing in proximal and distal parts. Subsequently each part was separated.

The excised explants were then inoculated in the culture test tubes/vials containing MS medium supplemented with various concentrations and combinations of NAA (0.0, 0.1, 0.2, 0.3 and 0.4 mg/l) with BA (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) and BA alone (0.0, 1.0, 2.0, 3.0, 4.0 and 5.0 mgl^{-1}) for *in vitro* callus induction. Growth and development of cultures were maintained at 25 ± 1 ℃ and light intensity was 2000-3000 lux... The photoperiod was maintained at 16 hours light and 8 hours dark with relative humidity 60-70%. The callus culture medium was supplemented with 3% (w/v) sucrose and adjusted to pH 5.8.

Subculture was done at 30 days after explants inoculation. Second subculture was carried out at 30 days after first subculture. After regenerated multiple shoots were carefully removed from the culture tubes/vials and each shoot was cut from the basal end and was transferred to new rooting medium which containing IBA and IAA (0.0, 0.1, 0.2, 0.3 and 0.4 mgl^{-1}) hormone. Always half strength MS medium was used for rooting media. The suitable agar concentration was 4.75 gl^{-1} (Table .I).

TABLE I. Effect of Agar Concentration on Ms Medium

Amount of agar	Comment
5.50 gl^{-1}	Very hard to remove plantlet
5.00 gl^{-1}	Hard to remove plantlet
4.75 gl^{-1}	Soft to remove plantlet
4.50 gl^{-1}	No gelling

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The data for the parameters were analyzed using SPSS statistical software. Differences among the means data were compared following Duncan's Multiple Range Test (DMRT) at 1% level of significance. Each experiment was laid out in Completely Randomized Design (CRD).

III. Results and Discussion

A. Callus Initiation

The effect of various concentrations of cytokinin individually and in combination with auxin was examined and varied results were found in all the parameters (Table II). The minimum time required for callus initiation (18.0 days) and induction (51.6 days) were observed at 1 mgl^{-1} BA. The maximum percentage (94.8%) of callus induction was found at 1 mgl^{-1} BA treatment. The maximum weight of callus at 45 and 60 days was 2.14 gm and 5.15 gm, respectively in this treatment. The colour of calli was found to be green in all the treatment and the friable calli were found at 1 mgl^{-1} BA treatment (Fig. 1 A & B). The combination 1.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA were found most suitable in callus induction from cotyledon of cultivated and wild *Momordica dioica* (Roxb.) observed by [6] and [9]. The potential of medium with only cytokinin for the induction of callus was reported in *Cucumis melo* cv. pusa madhuras [11].

TABLE II. Effect of Growth Regulators on Callus Induction From Cotyledon Explants

Concentration of hormone (mgl^{-1})		Growth of callus			Weight of callus (gm)		Callus morphology	
NAA	BA	Days to callus initiation	Days to callus induction	% of callus induction	At 45 days	At 60 days	Colour of calli	Abundance of calli
0.0, 0.1	0.0	-	-	-	-	-	-	-
0.1	0.5	26.8cd ±1.5	53.2f ± 4.5	22.6d ±2.7	0.06d ±0.01	0.12f ±0.02	green	Small & compact
0.1	1.0	30.0bc ±2.2	59.6e ±3.0	20.6d ±3.0	0.05d ±0.01	0.11f ±0.01	green	Small & compact
0.1	1.5	31.8b ±2.3	62.4de ±3.2	19.0d ±6.6	0.04d ±0.01	0.13f ±0.01	green	Small & compact
0.1	2.0	39.4a ± 3.6	65.6d ±3.9	17.4d ±3.4	0.03d ±0.01	0.10f ±0.01	green	Small & compact
1 mgl^{-1}BA		18.0f ± 3.0	51.6f ± 2.7	94.8a ±2.3	2.14a ±0.16	5.15a ±0.48	green	Plenty & friable
2 mgl^{-1}BA		21.4e ± 2.1	55.0f ± 2.3	65.4b ±3.6	2.03a ±0.25	3.98b ±0.43	green	Moderate & friable
3 mgl^{-1}BA		26.2d ±2.0	70.2c ±2.4	60.6b ±5.3	1.45b ±0.21	3.09c ±0.27	green	Moderate & friable
4 mgl^{-1}BA		26.4d ±2.0	76.2e ±3.4	44.0c ±3.6	1.34b ±0.04	2.25d ±0.15	green	Moderate & friable
5 mgl^{-1}BA		29.8bc ±3.3	81.4a ±2.3	21.8d ±5.2	0.60c ±0.18	1.05e ±0.15	green	Moderate & friable
Level of sig.		***	***	***	***	***		

Mean values ± SD for each type of hormone concentration. Mean values followed by same letter(s) do not differ significantly. *** $P < 0.01$ (0.1%).

B. Shoot Initiation

The calli derived from cotyledon explant were subcultured on different concentrations of cytokinins alone for shoot regeneration. It proliferated rapidly and became organogenic within around 3 weeks (Plate 1 E, F, G & H). It was found significantly different for all the treatments (Table III). A treatment with 1 mgl^{-1} BA took minimum time 22.2 days required for shoot initiation whereas the maximum time was required for shoot initiation (47.0 days) at 5 mgl^{-1} BA treatment (Plate 1 C & D, 2 A & B). The maximum percentage of shoot regeneration (95%), maximum length of shoot (2.0 cm) and number of shoot $callus^{-1}$ (19.8) were found at 1 mgl^{-1} BA treatment. From [9], it is observed that the highest number of multiple and tallest shoots were obtained on MS medium fortified with 1.0 mgl^{-1} BAP and 0.1 mgl^{-1} NAA in this crop. Similar observations were reported on cotyledon explant of *Momordica dioica* (Roxb.) wild [6].

TABLE III. Effect of Growth Regulators on Shoot Induction From Cotyledon Explants

Explant	Concentration of hormone (mgl-1)	Days to shoot initiation	No of explant generated shoots	% of shoot regeneration	Shoot length (cm) after 30 days	Shoot callus-1
Cotyledon	1 mgl^{-1}BA	22.2d ± 1.9	19	95	2.0a ±0.4	19.8a ± 3.2
	2 mgl^{-1}BA	25.0c ± 1.6	12	60	1.5b ±0.1	13.4b ±1.1
	3 mgl^{-1}BA	38.0b ± 2.9	9	45	0.8c ±0.1	5.6c ± 1.5
	4 mgl^{-1}BA	45.0a ± 1.5	8	40	0.5d ±0.2	5.8c ± 1.6
	5 mgl^{-1}BA	47.0a ± 1.7	5	25	0.4d ±0.1	3.4c ± 1.1
Level of significance		***			***	***

Mean values ± SD for each type of hormone concentrations. Mean values followed by same letter(s) do not differ significantly. *** $P < 0.01$ (0.1%).

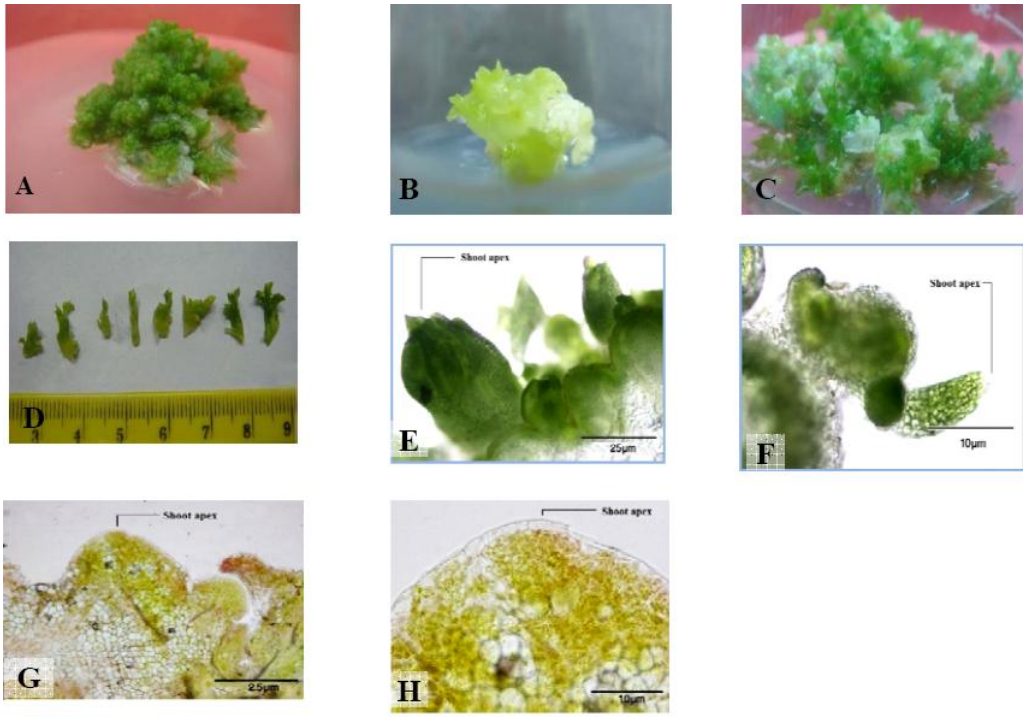

Figure 1. Callus and different stage of shoot initiation from cotyledon explant

A) Callus induction from cotyledon cultured on MS medium supplemented with 1 mgl^{-1} BA at 45 days after inoculation.
B) Callus induction from cotyledon cultured on MS medium supplemented with 3 mgl^{-1} BA at 45 days after inoculation.
C) Shoot regeneration from cotyledon callus cultured on MS medium supplemented with 1mgl^{-1} BA at 50 days after inoculation; (D) Shoots from callus for subculture.
E, F, G & H) Somatic embryogenesis from cotyledon callus cultured on MS medium supplemented with 1mgl^{-1} BA at 50 days after inoculation.

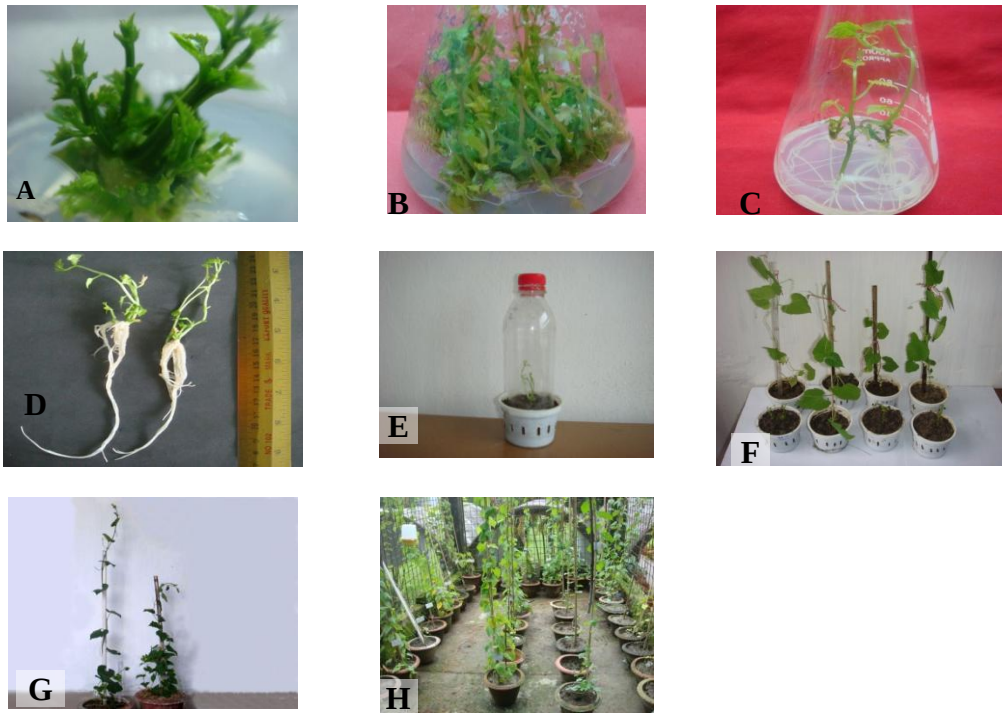

Figure 2. Shoots and roots initiation and hardened plant from cotyledon explant

A & B) Shoots induction from cotyledon explant cultured on MS medium supplemented with 1 mgl^{-1} BA at 65 days after inoculation.
C) Root induction from cotyledon explant cultured on half MS medium supplemented with 0.3 mgl^{-1} IBA at 30 days after inoculation.
D) Plantlets with healthy root system from cotyledon explants; E) Hardening by bottle.
F) Hardened plantlets from cotyledon explants; G) Natural plant (left) and regenerated plant (right); H) Regenerated plant in grill house

TABLE IV. EFFECT OF DIFFERENT CONCENTRATIONS OF IAA & IBA ON ROOTING FROM COTYLEDON

Explants	Conc. of growth regulators (mg l^{-1}) IBA	Days to root induction	Length of roots (cm)	Number of roots plantlets^{-1}
cotyledon	0.0	-	-	-
	0.1	-	-	-
	0.2	-	-	-
	0.3	17.2 ±1.5	5.6 ±1.1	21.4 ± 2.4
	0.4	-	-	-

Mean values ± SD for each type of hormone concentrations.

From [5], the authors found that hundred per cent of regenerated shoots of *M. dioica* were rooted with 5.4 mgl^{-1} IBA in full strength MS medium within two weeks. Plantlets were acclimatized successfully and later established in the grill house and also evaluation of somaclonal variation was achieved (Fig. 2 E, F, G & H).

C. Age of Cotyledon

Age of cotyledon was very important for successfully growth of callus (Table V). The minimum time required for callus initiation (17.3 days) was observed at 18 days old cotyledon whereas 14 days old cotyledon required the maximum time for callus initiation (35.0 days). Very immature cotyledon (14 DAP) produced white colour friable callus which failed to regenerate shoot (Fig. 4). The highest percentage of calli was produced by 18 days old cotyledon. Green, friable and organogenic calli were found from this cotyledon of 18 days old fruits. The authors [6] reported that green fruits (18 days after anthesis) of *M. dioica* rapid multiplication of shoot differentiation from cotyledons.

TABLE V. EFFECT OF FRUIT AGE ON CALLUS GROWTH FROM COTYLEDON

Age of fruit (DAP)	Days to callus initiation	Days to callus induction	% of callus induction	Type of callus	Type of cotyledon
12	-	-	-	-	Cotyledon is watery
14	35.0a ±2.0	105a ±4.0	18.3e ±1.5	White, friable, non-organogenic	Very soft or watery cotyledon
16	24.0c ±1.0	51.3e ±5.5	40.0d ±5.0	Light green, friable & organogenic	Medium soft
18	17.3e ±1.5	72.0bc ±5.2	92.4a ±2.5	Green, friable, organogenic	Medium hard
20	21.3d ±1.5	61.0d ±3.6	77.6b ±2.5	Green, friable, organogenic	Medium hard
22	25.4bc ±1.5	69.7c ±2.1	69.6c ±1.5	Green, friable, organogenic	hard
24	27.6b ±1.1	74.0bc ±2.6	41.3d ±3.2	Green, compact, organogenic	hard
26	17.6e ±1.5	78.3b ±1.5	37.4d ±2.5	Green, compact, organogenic	Very hard
Level of sig.	***	***	***	-	-

Mean values ± SD for each type of hormone concentration. Mean values followed by same letter(s) do not differ significantly. *** $P < 0.01$ (0.1%). DAP = Days after pollination

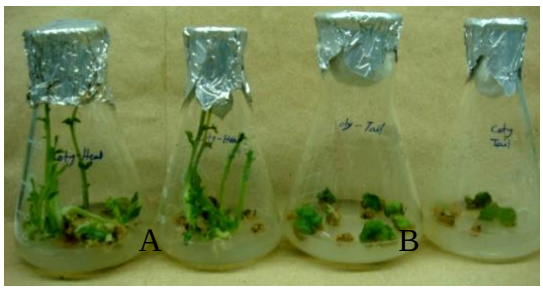

Figure 3. (A) Proximal end of cotyledon was highly regenerative. (B) Distal end of cotyledon had few regen

Figure 4. Very immature cotyledon (14 DAP) of teasle gourd couldnot produce organogenic callus.

D. Portion of Cotyledon

Portion of cotyledon was another important character for successful regeneration. It was found that proximal end of the cotyledon produced the highest number of regeneration (97%) whereas the distal end produced the lowest regeneration (19%) (Fig. 3 & 5). Proximal end produced the highest number of plants $callus^{-1}$ (26.7) and required 15 days & 51.7 days for callus initiation and callus induction respectively (Table 6). In the other hand distal end produced (3.0) plants $callus^{-1}$ and required 47.3 days and 106.3 days for callus initiation and callus induction respectively (Table IV).

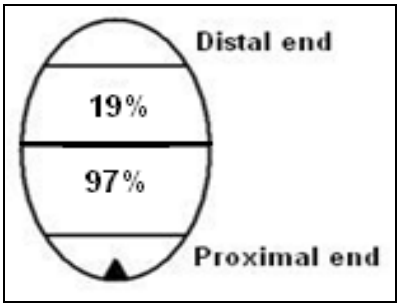

Figure 5. Proximal central section of cotyledon was highly regenerative at 1 mgl^{-1} BA of teasle gourd.

TABLE VI. REGENERATION CAPABILITY OF PROXIMAL AND DISTAL REGION OF COTYLEDON

Parameter	Days to callus initiation	Days to callus induction	Percent callus induction	Plant callus^{-1}
Central basal region	15.0b ±1.7	51.7b ±3.1	97.6a ±2.5	26.7a ±3.0
Central distal region	47.3a ±2.1	106.3a ±2.3	19.5b ±4.5	3.0b ±1.0
Sig. level	***	***	***	***

Mean values ± SD for each type of hormone concentrations. Mean values followed by same letter(s) do not differ significantly. *** $P <$ 0.01 (0.1%).

IV. CONCLUSION

Conclusion is made from the above study that proximal end of cotyledons from seeds of 18 days fruits produced highest frequency of plant regeneration.

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Mohammad A. Karim was born at Tangail, Bangladesh, on 3 December, 1949. The author has done B.Sc. in Agriculture major in Crop Botany in 1969, M.Sc in Cytogenetics in 1970 from the department of Crop Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh, and PhD in Genetics and Plant Breeding in 1983 from Indian Agricultural Research institute, New Delhi, India and post PhD in Molecular Biology in 1996 from Wye College, University of London, UK. The author's major field of study was Cytogenetics.

He started his job as a LECTURER in the department of Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh since April 9, 1973 to September 2, 1975. Later on he has served as an ASSISTANT PROFESSOR in the department of Botany, Bangladesh Agricultural University, Mymensingh, Bangladesh since September 3, 1975 to August 6, 1986. Then he worked as an ASSOCIATE PROFESSOR in the department of Crop Botany, Bangladesh Agricultural University, and Mymensingh, Bangladesh since August 7, 1986 to May 15, 1991. Later on he joined as PROFESSOR in the department of Crop Botany, Bangladesh Agricultural University, and Mymensingh, Bangladesh since May 16, 1991 to to date. 72 scientific articles have been published in different national and international journals in his credentials. The author's previous research interest was Cytogenetic and current interests in Plant tissue culture and Plant Biotechnology.

Prof. Karim is a life member of Crop Science Society of Bangladesh, Bangladesh Society of Horticultural Science, Progressive Agriculturists, Bangladesh and senior member of ICBFS and APCBEES.