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In Vitro Regeneration of Teasle Gourd

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***Abstract*— With a view to increase commercial production of teasle gourd, an attempt was made to generate a complete reproducible protocol for large scale propagation and *in vitro* regeneration from diferent organs of teasle gourd. Internodes, nodes, leaves, shoot tips and cotyledons were used as explants. The explants were cultured on MS medium supplemented with the combination of NAA (0, 0.5, 1.0, 1.5, 2.0 mg L^{-1}) & BA (0, 0.5, 1.0, 1.5, 2.0 mg L^{-1}) but BA (0, 0.5, 1.0, 1.5, 2.0 mg L^{-1}) used as separate treatment for callus initiation. Cotyledons showed higher percentage of callus induction (98.33%) at 1.0 mg L^{-1} BA in 15.33 days whereas callus initiation was induced by 1.0 mg L^{-1} BA + 0.5 mg L^{-1} NAA from internode explant within shortest time (14.21 days). Proliferated calli were cultured on MS medium for shoot initiation. The cotyledon explants had produced highest frequency of shoot formation (89.67%). MS medium containing 1.0 mg L^{-1} BA resulted maximum number of shoots (5.33) and longest shoots (0.9 cm). The shoots were subcultured on MS medium supplemented with IBA and IAA for rooting. Sufficient roots were induced on half MS medium containing only 0.3 mg L^{-1} IBA. Thus a protocol of regeneration of teasle gourd has been developed via callus formation using cotyledon explants.**

Keywords- Teasle gourd, In vitro, Regeneration, Callus, Micropropagation.

I. INTRODUCTION

Teasle gourd (*Momordica dioica* Rox.) locally called kakrol belongs to the family Cucurbitaceae. Among the summer vegetables it has high food value. It is rich in protein, carotene, carbohydrate [13], vitamins (vit C) and minerals [3], [5]. Only 1.6 ton Kakrol is produced $acre^{-1}$ in^{-1} Bangladesh [4] whereas 10-12 tons of this vegetable may be produced if proper methodology of cultivation is evolved [15]. Kakrol is an important summer vegetable in Bangladesh. It is widely grown in Comilla, Brahmanbaria, Rangpur, Bogra, Norshingdi and Sylhet districts of Bangladesh and has high economic value with export potential. Its cultivation is gaining popularity for its high economic return as compared with that of cereals [6], [2]. It has better self life and being exported to Middle East, UK and other countries thereby earning foreign currencies. However, as dioecious nature of the plant, the propagation entirely depends on underground tuberous roots. Fruit quality of kakrol deteriorates due to presence of large number of seeds. Seedless or less seeded teasle gourd may be obtained by applying mutagens during the study. Maintenance of quality of tuberous roots in the field as well as in storage condition is difficult. Micropropagaiton and use of mutagens may help to overcome these problems to a great extent by producing new variants. It has good nutritional value as well as having 33 mg Ca, 42 mg P, 4.6 mg Fe and 1620 µg carotene 100^{-1} g of edible portion and a high amount of vitamin C [5]. Germination of seeds is very difficult or impossible because of the hard seed coat [14]. Induction of somaclonal variation through callus culture may provide some seedless plants. Use of mutagens through biotechnoligcal techniques may create heterogenecity, from which seedless teasle gourd may be achieved.

However there have not been many studies on micropropagation of teasle gourd in Bangladesh or in neighboring countries. Till now, there is no adequate information on tissue culture on teasle gourd as it is considered to be a minor vegetable and as such it receives no research priority. To day, it is needed to findout the improve production technology for this crop. 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 vitro* propagation.

II. MATERIALS AND METHODS

Internodes, nodes, leaves, shoot tips and cotyledones of teasle gourd were used as experimental materials. The true seeds and other plant parts were taken from the field growing plants as well as mature plants from the experimental field of Crop Botany Department, Bangladesh Agricultural University, Mymensingh. Experimental materials were throughly washed in running tap water for 10-15 minutes. The explants were treated with 70% alcohol for 30 second and 0.1% mercuric chloride ($HgCl_2$) for 5-7 minutes along with 1-2 drops of tween-20 for surface sterilization. The sterilized explants were then rinsed 4-5 times with sterile distilled water to remove all traces of $HgCl_2$ under laminar airflow cabinet. The sterilized explants were cut into small pieces (2-3 mm) aseptically using fine sterile forceps and scalpel. The explants were then inoculated/cultured on MS [11] medium with different concentation and combination of BA and NAA and with different concentration of BA singly for callus initiation. In case of seeds, they were decoated, washed thoroughly and then cotyledon placed on MS medium following the above procedure. After induction of callus they were subcultured in shooting medium with

different concentration and combinations of BA, NAA and with different concentration of BA. Each adventitious shoot was cut from the basal end and subcultured again for further multiple shoot induction. Subcuture were maintained at an interval of 30 days. Regenerated multiple shoots were cut and individual shoots were placed in half MS media with different concentration and combinations of IBA and IAA for root induction. The pH of the medium was adjusted to 5.8±0.1 using 0.1N NaOH or 0.1N HCl. In order to solidify the media, laboratory grade agar was added to the solution @ 8 gL^{-1}. The culture media were sterilized at 1.06 kg^{-1} cm^2 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 explants were placed on the solidified medium carefully. The cultures were incubated in the growth room at a temperature of 25±1°C with a light intensity of 2000-3000 lux provided by florescent tuber light under a photoperiod of 16 hours light and 8 hours dark with a relative humidity of 60-70%. The experiment was conducted under controlled conditions following complete randomized design (CRD). All the experiment were repeated three times with 10 replicates per treatments. Duncun's Multiple Range Test (DMRT) was used with the help of MSTAT software to test the variability among the treatments.

III. RESULTS AND DISCUSSION

Callus induction

Cotyledons showed high performance in callus induction than internode, node, leaf and shoot tip (Table 1-3 and Fig. 1). Callus initiation was induced by 1.0 mg L^{-1} BA + 0.5 mg L^{-1} NAA in internode explant in shortest period (14.21 days) but cotyledon showed higher performance of callus induction (98.33%) by 1.0 mg L^{-1} BA at 15.33 days (Table 3). [12] had more or less similar observation in kakrol. They found that the combination of 1.0 mg L^{-1} BA + 0.1 mg L^{-1} NAA in MS media was the most suitable for callus induction from cotyledons.

TABLE I. EFFECT OF EXPLANT ON CALLUS INDUCTION

Explants	Days to callus initiation	Days to callus induction	Percentage of (%) callus induction	Weight of callus (g) 45 DAI
Internode	14.21 e	28.31 e	45.60 b	0.1436 b
Node	18.95 b	35.44 d	30.84 c	0.1364 b
Leaf	18.88 b	40.32 c	28.76 d	0.1367 b
Shoot tip	22.63 a	42.76 d	18.25 f	0.1336 b
Cotyledon	17.56 c	50.23 a	50.25 a	0.2596 a
CV%	4.89	5.59	6.98	10.23
$LSD_{(0.05)}$	0.3669	0.8814	0.9617	0.01327

TABLE II. EFFECT OF GROWTH REGULATORS ON CALLUS INDUCTION IN DIFFERENT EXPLANTS

Concentation of growth regulators (mg L^{-1})		Explant	Days to callus initiation	Days to callus induction	Percentage of callus induction	Weight of callus (g) 45 DAI
BA	NAA					
0	0	Internode	16.33 v-z	28.67z	21.33yz	0.09s-v
		Node	14.33 z	44.67f-h	25.00w-z	0.20e-i
		Leaf	14.67 z	30.33r-u	38.00n-s	0.12p-v
		Shoot tip	18.00 r-v	22.00x-z	37.67o-t	0.13j-v
		Cotyledon	23.67f-i	69.33c	35.33u-x	0.43b
1	0.5	Internode	9.67b	23.33e	52.33a	0.44b
		Node	17.33t-x	34.33w-y	51.00gh	0.08uv
		Leaf	15.67x-z	22.33x-z	40.67j-l	0.20e-j
		Shoot tip	19.67z	23.67f-i	39.33l-p	0.13l-v
		Cotyledon	22.00i-m	80.67a	82.67a	0.54a
	1.0	Internode	13.00z	29.67z	67.33b	0.19f-o
		Node	18.33r-u	36.67r-v	45.67i	0.15h-t
		Leaf	19.67n-r	50.33h	43.00hi	0.08uv
		Shoot tip	17.67z	21.00k-o	36.00s-w	0.13j-v
		Cotyledon	21.00k-o	65.00d	34.00w-y	0.23d-f
	1.5	Internode	11.33z	30.33z	80.67a	0.18f-q
		Node	19.00p-t	34.33w-y	40.33jk	0.13l-v
		Leaf	19.33o-s	45.67i	48.67bc	0.15h-t
		Shoot tip	15.67a-e	22.67h-k	37.33p-u	0.14i-v
		Cotyledon	23.00f-i	64.00de	30.33z	0.32c
	2.0	Internode	16.67u-y	32.33yz	70.33c	0.11r-v
		Node	21.33k-n	49.67ab	20.67z	0.14i-v
		Leaf	22.33i-l	40.00j-l	51.33a	0.14i-v
		Shoot tip	14.33de	19.67n-r	60.67j-l	0.15h-t
		Cotyledon	21.00k-o	60.00e	35.67t-w	0.13m-v
CV%			4.82	6.84	2.80	9.73
$LSD_{(0.05)}$			1.53	3.88	1.84	0.05

TABLE III. EFFECT OF DIFFERENT CONCENTRATION OF BA ON COTYLEDON

Concentration of BA (mg L^{-1})	Days to callus initiation	Days to callus induction	Percentage (%) of callus induciton	Weight of callus (g) 45 DAI
0.0	0.00d	0.00d	0.00d	0.00e
0.5	17.33b	57.00b	95.00b	0.23c
1.0	15.33c	51.67c	98.33a	0.43a
1.5	15.67bc	55.33b	97.00ab	0.15d
2.0	23.00a	60.00a	93.33c	0.32b
CV (%)	5.79	2.31	1.93	0.92
$LST_{(0.05)}$	1.87	2.34	0.95	0.07

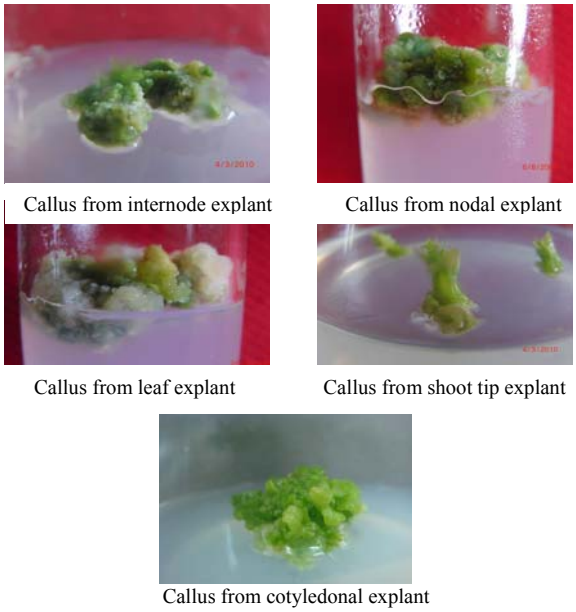


Figure 1. Callus induction from internode, node, leaf, shoot tip explants cultured on MS medium supplemented with 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} NAA and cotyledon explant cultured on MS medium supplemented with 1.0 mg L^{-1} BA at 45 days

Shoot induction

The different concentrations and combinations of BA & NAA had significant influence on shoot regeneration from internode, node, leaf, shoot tip and cotyledons (Table 4-6 and Fig. 2) and with different concentrations of BA only on cotyledon had significance influence of producing multiple shoots. The results indicated that (Table 4) the cotyledon explants were more capable of producing multiple shoots compared to other explants. Among the various concentrations of (BA 0.5, 1.0, 1.5, 2.0 & NAA 0.5, 1.0, 1.5, 2.0) and (BA 0.5, 1.0, 1.5, 2.0); BA alone showed highest frequency of shoot formation (89.67%) and maximum number of shoots explant^{-1} (5.33) and longest shoots (0.9 cm) from cotyledonal explants at 1 mg L^{-1} (Table 6).

TABLE IV. EFFECT OF EXPLANTS ON NUMBER OF SHOOT INDUCTION

Explants	Days to shoot induction	Number of shoots/callus	Shoot length (cm) after 30 DAI
Internode	17.00e	1.33d	0.35c
Node	36.00b	2.67ab	0.64b
Leaf	27.33d	1.67cd	0.43d
Shoot tip	31.67c	2.33bc	0.53c
Cotyledon	51.00a	3.33a	0.83a
CV%	2.69	27.90	1.86
LSD $_{(0.05)}$	1.54	0.92	0.05

TABLE V. EFFECT OF DIFFERENT CONCENTRATIONS AND COMBINATIONS OF BA AND NAA ON SHOOT REGENERATION FROM NODAL CALLUS

Concentration of hormone (mg L^{-1})		Days to shoot induciton	Percent shoot induction	Shoot length at 30 DAI (cm)
BA	NAA			
1.0	0.0	17.00g	26.00i	0.10i
	0.5	32.33e	54.67a	0.48c
	1.0	34.33bcd	39.67b	0.14h
	1.5	35.33bc	34.67cd	0.37f
	2.0	38.00a	31.00ef	0.42d
CV (%)		3.92	8.55	12.87
LST $_{(0.05)}$		1.30	2.27	0.05

TABLE VI. EFFECT OF DIFFERENT CONCENTRAITONS OF BA ON SHOOT REGENERATION FROM COTYLEDONAL CALLUS

Different concentration of BA (mg L^{-1})	Days to shoot initiation	Number of shoot callus^{-1}	Percent shoot induction	Shoot length at 30 DAI (cm)
0	0.00d	0.00	0.00b	0.00c
0.5	58.67a	2.33cd	74.67a	0.50b
1.0	51.67c	5.33a	89.67a	0.90a
1.5	55.33b	3.67ab	84.67a	0.60b
2.0	57.00ab	3.33bc	80.67a	0.40b
CV (%)	2.54		6.52	3.12
LST $_{(0.05)}$	2.57	0.93	0.74	0.23

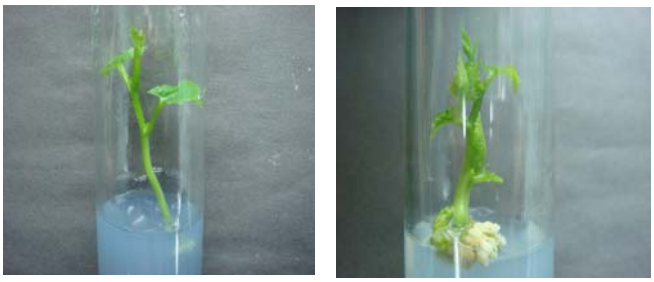


Figure 2. Shoot induction from nodal explant cultured on MS medium supplemented with 1.0 mg L^{-1} BA + 0.5 mg L^{-1} NAA at 30 days and shoot induction from cotyledon explant cultured on MS medium supplemented with 1.0 mg L^{-1} BA at 30 days.

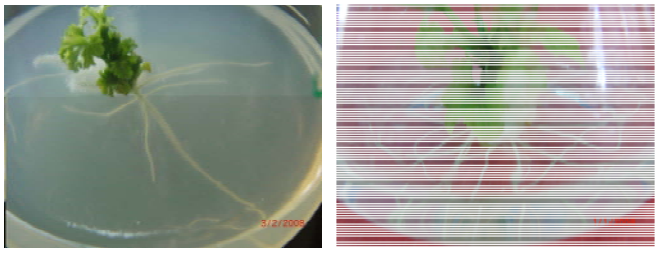
Root induction

Successful micropropagation depends on healthy and strong root system. In vitro multiple shoots were excised and cultured on half strength of MS supplemented with IBA and IAA for rooting. Response of different concentrations of IAA (0.1, 0.2, 0.3 mg L^{-1}) and IBA (0.1, 0.2, 0.3 mg L^{-1}) on in vitro adventitious root formations were observed (Fig. 3.). Rooting was found only with IBA at 0.3 mg L^{-1}. The maximum number of roots (13) and maximum length (8.1

cm) after 30 days were observed at cotyledonous shoots (Table 7-8). The present investigation revealed that auxin; IBA had better effect on root induction. Agarwal (2004) obtained similar results in *M. charantia* and [7] in *M. dioica.*

Acclimatization

For the establishment of regenerated healthy rooted plantlets were placed at room temperature for one week. Then the plantlets were removed from the culture bottle and carefully removed adhering agar from the plantlets. Plantlets were planted on sterilized soil to observe the accomplishment of the plant in earthen pot (Fig.4).



Rooting from nodal shoot Rooting from cotyledonal shoot

Figure 3. Root induction from nodal and cotyledonal shoots cultured on half MS medium supplemented with 0.3 mg L^{-1} IBA at 30 days after inoculation

Figure 4. Hardening of plantlets

TABLE VII. EFFECT OF DIFFERENT CONCENTRATIONS OF IAA AND IBA ON HALF STRENGTH MS FOR NUMBER OF ROOTS PLANT^{-1}

Explants	Concentration of growth regulators (mg L^{-1})					
	IBA			IAA		
	0.1	0.2	0.3	0.1	0.2	0.3
Cotyledon	0	0	13.00 a	-	-	-
Node	0	0	6.67 b	-	-	-
CV%			23.49			
LSD $_{(0.05)}$			0.5562			

TABLE VIII. EFFECT OF DIFFERENT CONCENTRATIONS OF IAA AND IBA ON HALF STRENGTH MS FOR LENGTH OF ROOTS (CM)

Explants	Concentration of growth regulators (mg L^{-1})					
	IBA			IAA		
	0.1	0.2	0.3	0.1	0.2	0.3
Cotyledon	0	0	8.10 a	-	-	-
Node	0	0	6.2 b	-	-	-
CV%			3.07			
LSD $_{(0.05)}$			0.06105			

Cotyledon showed higher percentage of callus induction (98.33%) by 1.0 mg L^{-1} BA at 15.33 days (Table 3). Proliferated calli were cultured on MS medium for shoot initiaiton. [10] obtained the highest frequency of shoot formation (78%) with 7.9 shoots per explants in MS supplemented with 2.0 mg L^{-1} BA. This inference was nearer to the findings of the present investigation. [8] also obtained the highest shoot regeneration rate (88%) of teasle gourd with 8.8 shoots explant^{-1} in MS supplemented with 1.5 mg L^{-1} BA and 0.1 mg L^{-1} NAA. The best shoot elongation was obtained by [9] on pointed gourd (*Trichosanthus dioica* Roxb.) in MS supplemented with 1.0 mg L^{-1} BA, 0.1 mg L^{-1} NAA and 1.0 mg L^{-1} adenine sulphate. The cotyledon explants had produced higher frequency of shoot formation (89.67%) with maximum number of shoots explant^{-1} (5.33) and longest shoots was 0.9 cm on MS medium containing 1.0 mg L^{-1} BA (Table 6). The shoots were subcultured on MS medium supplemented with IBA and IAA for rooting. Sufficient roots were induced on half MS medium containing only 0.3 mg L^{-1} IBA.

IV. CONCLUSION

In the present findings; the performance of cotyledon explants resulted best for callus, shoot and root induction of teasle gourd over the other plant parts. Thus, a protocol of regeneration of teasle gourd has been developed via callus formation using cotyledon explants.

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