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Somatic Embryogenesis and Micropropagation in Teasle Gourd

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Abstract.-In vitro **somatic embryogenesis and subsequent plant regeneration was achieved in callus cultures derived from internode, node, shoot tip, petiole and leaf explant of teasle gourd plant on semi-solid Murashige and Skoog (MS) basal salts and growth regulators supplemented with 1.0mgl^{-1} BAP, 0.1mgl^{-1} NAA and 30 g l^{-1} (w/v) sucrose. Somatic embryos proliferated rapidly by somatic embryogenesis after 4 weeks. The embyogenic callus germinated on MS salts and growth regulators supplemented with 1.0mgl^{-1} BAP and 0.1mgl^{-1} NAA. The embryo-derived plantlets were transfered on half MS media with 0.3 mgl^{-1} IBA and sufficient rooting was achieved. Plantlets were acclimatized in the controlled environment.**

Keywords-**Callus culture, Explant, Somatic embryogenesis & Teasle Gourd.**

I. INTRODUCTION

Teasle gourd *(Momordica dioica* Roxb.) locally known as kakrol is a popular summer vegetable in Bangladesh. Recently teasle gourd has become a major vegetable in Bangladesh because of its high export potential and demand in the internal market. It has good nutritional value as well as having 33 mg Ca, 42 mg P, 4.6 mg Fe and 1620 µg carotene per 100 g of edible portion and a high amount of vitamin C[2]. As a crop, kakrol has a number of problems, including poor natural pollination of female flowers and low yield. Fruits become inedible at maturity owing to the presence of large number of hard seeds. Production of tuberous roots plant^{-1} is low; 10-20 tuberous root pieces are produced plant year^{-1}. Germination of seeds is very difficult or impossible because of its hard seed coat[4]. Moreover, it is impossible to predict the sex of seed-produced plants before flowering. As dioecious nature of plant it requires artificial transfer of pollen from male to female flower. Bees, insect and wind being natural pollinators, hand pollination is necessary.

Fruit quality of kakrol deteriorates due to the presence of large number of seeds. Seedless or less seeded teasle gourd may be obtained by applying radiations or chemical mutagens during tissue culture. Induction of somaclonal variation through callus culture may lead to the production of plants giving seedless or less seeded fruit. Use of radiation through tissue culture technique may create heterogenecity, from which seedless teasle gourd may be achieved. Unfortunately there have been very few reports on tissue culture of kakrol in home and abroad. The present study was, therefore carried out to develop a protocol of *in vitro* regeneration that would be used for the improvement of this crop.

Somatic embryogenesis has a tremendous potential tool for large scale production of plant material[5] and is considered as an effective aid genetic transformation study. It represent an alternative for massive clonal propagation and also appears to be a potential solution to the problem of field propagation, especially in area with frequent diseases transmission and maintenance of cultivars that have been selected for their important genetic characteristics[1]. Unfortunately, there have been no reports of somatic embryogenesis in teasle gourd. Therefore, the objective of the study was to study the applicability of somatic embryogenesis as a tool of micropropagation.

II. MATERIALS AND METHODS

Different explants of teasle gourd like internode, node, petiole, shoot tip and leaf segments were collected from tender and actively growing vines available in the experimental field of Crop Botany Department, Bangladesh Agricultural University, Mymensingh, Bangladesh. Explants were thoroughly 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 $HgCl_2$ inside the clean bench.

The sterilized explants were cut in to small pieces (2-3mm) aseptically using fine sterile forceps and scalpel. The explants were then inoculated in MS[9] medium with different concentration and combination of BAP (0.0, 0.5, 1.0, 1.5 & 2 mgl^{-1}) and NAA (0.0, 0.1, 0.2 & 0.3 mgl^{-1}) for callus initiation. After induction of callus they were subcultured in shooting medium with different concentration and combination of BAP (0.0, 0.5, 1.0, 1.5 & 2 mgl^{-1}) and NAA (0.0, 0.1, 0.2 & 0.3 mgl^{-1}). Each adventitious shoot was cut from the basal end and subcultured again for further multiple shoot induction. Subcultures 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 combination of IBA and IAA. The pH of the medium was adjusted to 5.8 ± 0.1 using 0.1N NaOH or 0.1N HCI. In order to solidify the media, laboratory grade agar was added to the solution @ 7gl^{-1}.

The culture media were sterilized at 1.06 kg/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 fluorescent tube lights. A photoperiod of 16 hours light and 8 hours dark with a relative humidity of 60-70%. The experiment was conducted under controlled

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conditions following complete randomized design (CRD) with ten replications. Duncan's Multiple Range Test (DMRT) was used with the help of MSTAT software to test the variability among the treatments.

III. Results and Discussion

A. Callus Induction

The response of Internode, leaf, petiole, shoot tip and nodal segments of teasle gourd to different concentrations of BAP and NAA was observed (see Table I, II). Callus initiation was achieved in shortest time (12.95 days) on the medium containing 1.0 $mg1^{-1}$ BAP + 0.1 $mg1^{-1}$ NAA from internode explant and showed higher percentage of callus induction (75%) and the colour of calli was light green and the texture was friable (Fig. 5). Most suitable callus induction on MS medium from different explants of teasle gourd was achieved in combination of 1.0mgl^{-1} BAP and 0.1 mgl^{-1} NAA[8].

Table I: Effect of explants on callus induction

Explants	Days to callus initiation	Days to callus induction	% of callus induction	Colour of calli	Morphological features of calli
Inter node	12.95de	29.35d	75a	Light green	Friable
Node	14.27c	29.92cd	575b	green	Friable
Leaf	19.77a	31.92ab	25e	Light green	Friable
Petiole	16.4b	32.8a	325d	green	Compact
Shoot tip	13.18d	30.48c	50c	Light green	Friable
LSD	1.02	1.24	0.01		

B. Shoot Induction

The different concentrations and combinations of BAP and NAA had significant influence on shoot regeneration from shoot tips, internode, leaf and nodal segments (see Table III, IV). The results indicated that the nodal explants were more capable of producing multiple shoots compared to other explants. 1.0 $mg1^{-1}$ BAP + 0.1 $mg1^{-1}$ NAA produced shoots in shortest time (15 days). Among the various concentrations of NAA at (0.1, 0.2, 0.3) & BAP at (0.5, 1.0, 1.5, 2.0), 1.0 $mg1^{-1}$ BAP + 0.1 $mg1^{-1}$ NAA resulted maximum number of shoots $explants^{-1}$ (5.1) and length of longest shoots of explant (0.9 cm) after 30 days from nodal segments. Highest frequency of shoot formation (78%) from *M. charentia* with 7.9 $shoots\ explants^{-1}$ in MS medium supplemented with 2.0 mg/l BA[7]. Multiple shoot regeneration of *Cucumis melo* was achieved using shoot tips as explant on the MS medium supplemented with 2.5 mg/l NAA and 1.0 mg/l BAP was obtained[10].

Table II: Effect of growth regulators on callus induction in different explants

Concentration of growth regulator (mg/l)		Explant	Days to callus initiation	Days to callus induction	% of callus induction
NAA	BAP				
0.0, 0.1	0.0, 0.0	Internode	0.0	0.0	0
		Node	0.0	0.0	0
		Leaf	0.0	0.0	0
		Petiole	0.0	0.0	0
		Shoot tip	0.0	0.0	0
0.1	0.5	Internode	15.2g	28.8f	70b
		Node	14.0h	28.1fg	50d
		Leaf	18.0d	30.1de	20g
		Petiole	17.6de	30.8d	30f
		Shoot tip	14.9g	31.9cd	50d
0.1	1.0	Internode	10.2j	27.1g	80a
		Node	13.1i	27.9fg	60c
		Leaf	20.3b	29.4ef	20g
		Petiole	14.1h	29.8e	40e
		Shoot tip	10.2j	28.4f	60c
0.1	1.5	Internode	12.2i	30.1de	70b
		Node	14.9g	31.6d	60c
		Leaf	19.1c	33.1c	30f
		Petiole	16.0f	33.3c	30f
		Shoot tip	13.7h	29.5ef	50d
0.1	2.0	Internode	14.2h	31.4d	80a
		Node	15.1g	32.1c	60c
		Leaf	21.7a	35.1b	30f
		Petiole	17.9de	37.3a	30f
		Shoot tip	13.9h	32.1c	40e
		LSD	0.96	1.17	0.01

Table IIII: Effect of explants on shoot induction

Explants	Days to shoot induction	Number of shoots/ callus	Shoot length (cm) after 30 days
Internode	17.3c	1.3d	0.56c
Node	15d	5.1a	0.90a

Leaf	34b	1.59b	0.55d
Petiole	0.0e	0.0e	0.0e
Shoot tip	47a	1.7c	0.66b
LSD 5	1.91	0	0.11

Table IV: Effect of growth regulators on different concentrations and combinations for shoot induction

Concentraion of growth regulator growth regulator (mg/l)		Explant	Days to shoot induction	Percentage of shoot regeneration	Shoot length (cm) after 30 days
NAA	BAP				
0.0, 0.1, 0.1, 0.1	0.0, 0.0, 0.5, 1.5	Internode	0	0	0
		Node	0	0	0
		Leaf	0	0	0
		Petiole	0	0	0
		Shoot tip	0	0	0
0.1	1.0	Internode	17.3c	11c	0.56c
		Node	15.0d	51a	0.90a
		Leaf	34.0b	10c	0.55c
		Petiole	0.0e	00d	0.00d
		Shoot tip	47.0a	16b	0.66b
		LSD 5	0.53	1.9	0.04

C. Somatic embryogenesis

All factors (treatment and explant) had significantly influenced on effects on somatic embryo formation and maturation. In somatic embryogenesis experiment, some embryo like bodies were observed on the different medium supplemented with concentrations of growth regulators. Internode calli showed better performance in the formation of somatic embryo. It was evident that calli influenced on the formation of somatic embryo and organogenesis in plants. Somatic embryo formation was observed when calli were exposed to medium containing $1mgl^{-1}$ BAP & $0.1 mgl^{-1}$ NAA. *Brassica napus* showed the highest rate of mature embryo formation when it was exposed to higher concentration of ABA for 30 days[6]. Garlic root tips induced to regenerate shoots either directly to form plantlets and bulblets or indirectly through callus culture and somatic embryogenesis to obtain plantlets. Explants from plantlets taken 15 to 18 days after sprouting showed 95% shoot initiation when cultured on MS medium supplemented with $1mgl^{-1}$ NAA and $10mgl^{-1}$ BA[3]

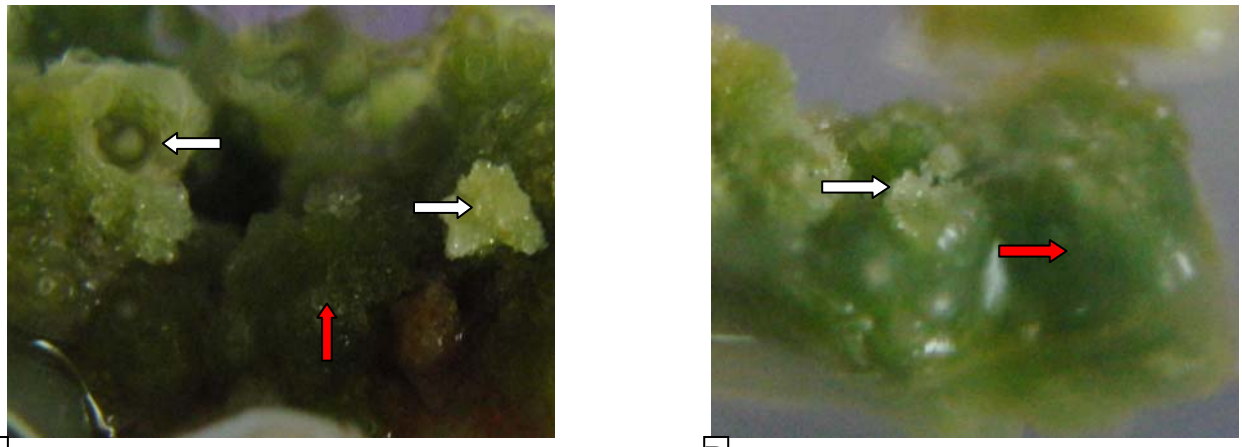

Fig 1: Somatic embryos induced from Nodal (A) & internodal (B) callus segment cultured on $1mgl^{-1}$ BAP & $0.1mgl^{-1}$ NAA after 30 days. Each (A & B) callus was composed of embryogenic (white arrow) & non-embryogenic (red arrow) (Arrow= 2mm).

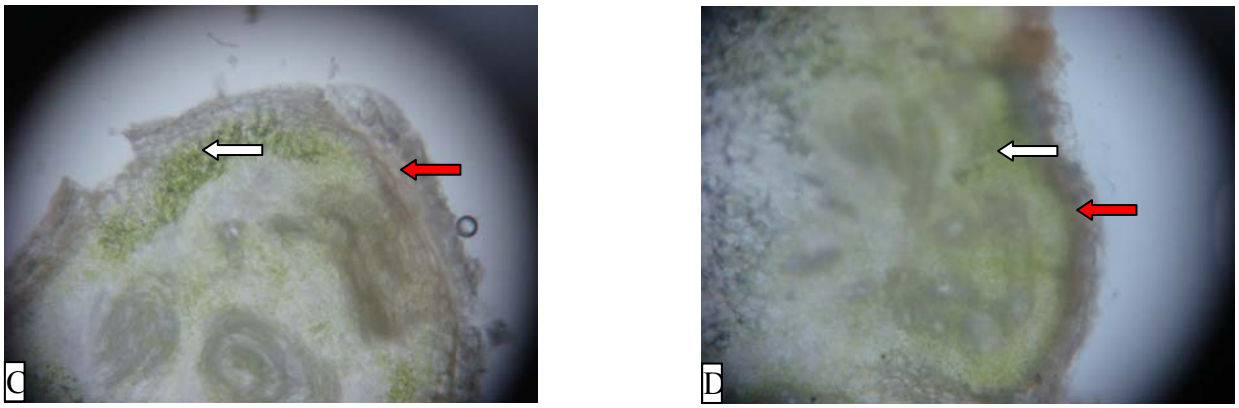

Fig 2: Somatic embryos of *momordica dioeca* Roxb from nodal (C) and internodal (D) callus shows shoot regeneration at 1mgl^{-1} BAP & 0.1 mgl^{-1} NAA on MS media. Green colour shows chlorophyll cell (white arrow) and parenchyma cell and many epidermal layers (red arrow) in stem tip. (400X magnifying)

D. Root Induction

In vitro rooting from shoot is an essential step in micropropagation. For successful micropropagation, healthy and strong root system is essential. Different concentrations of IAA (0.1, 0.2, 0.3 & 0.4 mg1^{-1}) and IBA (0.1, 0.2, 0.3 & 0.4 mg1^{-1}) was used for root formation (Fig-8). Rooting was found only with IBA at 0.3 mg1^{-1}. The number of root were 12.8 and length 8.1 cm after 30 days (seeTable-V).

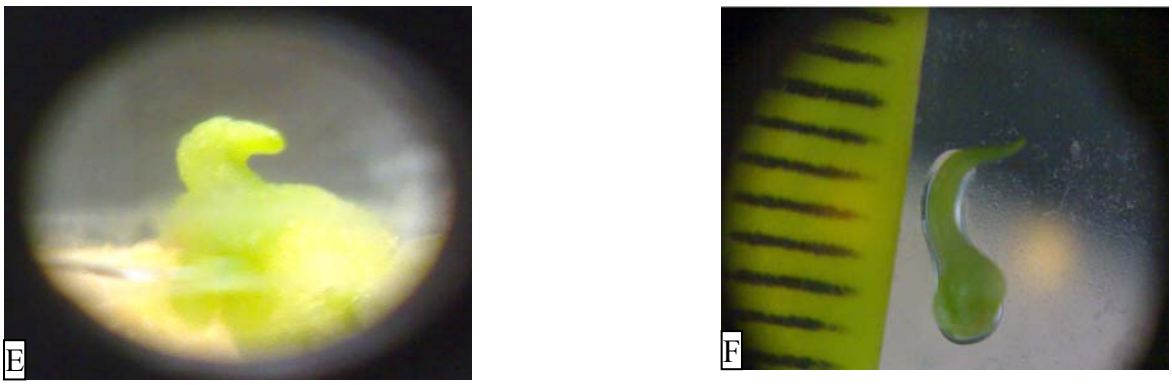

Fig 3: (E) Germination of somatic embryo on germination medium (medium contain 1mgl^{-1} BAP & 0.1 mgl^{-1} NAA). (F) An embryo isolated from germination medium.

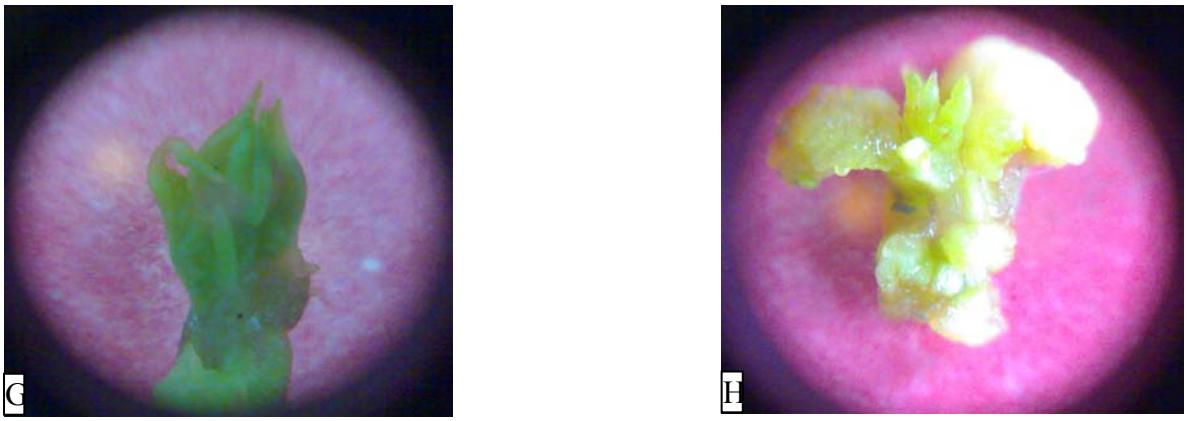

Fig 4: Germination of single somatic embryo G & H.

Fig5: Callus induction from **Internode** cultured on MS medium supplemented with 0.1mgl-1 NAA + 1 mgl-1 BAP at 30 days after inoculation.

Fig6: Shoot induction from **Node** segments cultured on MS medium suplimented with 0.1 mgl^{-1} NAA $^{+1}$.0 mgl-1 BAP at 40 days after inoculation.

Table V: Effect of different concentration of IAA & IBA on half strength MS for root induction

xplants	Concentrations of growth regulators (mg l^{-1})		Number of roots / plantlets	Length of roots (cm)
	IAA	IBA		
Node	0.1, 0.2, 0.3, 0, 0	0, 0, 0, 0.1, 0.2	-	-
	0	0.3	12.8	8.1
LSD			2.1	1.3

Fig7: Root induction from **node** segments cultured on half MS medium suplimented with 0.3 mgl^{-1} IBA at 30 days after inoculation.

Fig8: Hardening of plantlets

IV. Acclimatization

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

V. Conclusion

The present study was conducted to establish an effective protocol of plantlet regeneration through somatic embryogenesis in teasle gourd. The optimum concentration of BAP and NAA (1.0 & 0.1mgl^{-1}) had an effect on callus and embryo formation. This may help large scale propagation of teasle gourd through *in vitro* regeneration & mutagenesis and to obtain seedless or less seeded fruits of kakrol.

Reference

[1] E. De Garcia and Martinez S. Somatic embryogenesis in solanum tuberosum L. cv. Desiree from stem nodal sections. J. Plant Physiol. 1995, 145: 526-530.
[2] M.R.H. Bhuiya, A.K.M.A. Habib and M.M. Rashid. Content of loss of vitamin C in vegetables during storage and cooking. Bangladesh Hortic. 1977, 5: 1-16.
[3] M.S. Haque, Wada, T. and Hattori, K. Efficiant plant regeneration in garlic through somatic embryogenesis from root tip explants. Plant Prod. Sci. 1998, 1(3): 216-3-222.
[4] M.M. Rashid. Vegetable of Bangladesh. BARI, Joydebpur, Gazipur. 1976b, pp: 494.
[5] P.V. Amirato and Styer D.J. Stretegies for large scale manipulationof somatic embryos in suspension cultures. Biotechnology in plant Science. Academic Press, New York. 1985, 161-178.
[6] R. Angoshtry, T.R. Afshari, S. Kalantari and M. Omidi. Effect of Abscisic Acid on Somatic Embryogenesis and induction of Desiccation Tolerence in brassica napus. Asi. J. of Plant Sci. 2009, 8(4): 276-284.
[7] R. Islam Sarker, K.P. Naderuzzaman A.T.M. and O.I. Joarder. In vitro regeneration of plants from cotyledons of Momordica charantia L. Plant Tissue Cult. 1994, 4(2) : 105 - 109.
[8] S. A. Nabi., M. M. Rashid, M. Al-Amin and M. G. Rasul. Organogenesis in Teasle Gourd (Momordica dioica Roxb.). Plant Tissue Cult. 2002, 12(2) : 173-180.
[9] T. Murashige, and F. Skoog. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962, 15: 473-497.
[10] V. M. I. Moreno, B.Gareia-Sogo, Granell, Carcia-Sopgo and L. A. Roig. Plant regeneration from calli of melon (Cucumis melo L. cv. Amarillo Orl). Plant Cell Tiss. Organ Cult. 1985, 5 : 139 - 146.