Establishment Of An Efficient In Vitro Regeneration System Of Ridge Gourd (*Luffa Acutangula* L. Roxb) From Immature Embryo And Cotyledon Explants

F.T. Zohura, M.E. Haque, M.A. Islam, M. Khalekuzzaman, B. Sikdar

Abstract: Ridge gourd (*Luffa acutangula* L. Roxb) is a popular and nutritious vegetable in Bangladesh. The present investigation was undertaken to establish an efficient in vitro regeneration system of ridge gourd from immature embryo and cotyledon explants. Immature embryos and cotyledons were collected from immature seeds and culture on MS medium supplemented with various concentration and combination of growth regulators for primary development and multiple shoot formation. The highest result of direct shoot multiplication was observed from immature embryo and cotyledon on MS with 1.0 mg/l BAP + 0.2 mg/l GA3 and 2.0 mg/l Kin + 0.3 mg/l GA3 respectively. The highly regenerative callus with light green compact structure was obtained from cotyledon explants using 1.5 mg/l BAP + 1.0 mg/l NAA and effective regeneration from callus was found on MS medium supplemented 3.5 mg/l BAP + 0.2 mg/l GA3. In vitro regenerated shoots were subcultured on ½ strength MS medium containing different concentrations of IBA and NAA for successful root induction and the efficient result was found using 0.5 mg/l IBA.

Key words: *In vitro*, Regeneration, Ridge gourd, *Luffa acutangula* L., Immature embryo, Cotyledon

1 INTRODUCTION

Ridge gourd (*Luffa acutangula* L. Roxb), popularly known as Kalitori also called angled gourd, angled loofah, belongs to genus Luffa of Cucurbitaceae family and has chromosome number 2n = 26. Ridge gourd is a monoecious viny vegetable. It contains a gelatinous compound called luffein and has medicinal importance. It is a nutrition powerhouse, an ideal weight loss food and strengthens immune system. The juice prepared from ridge gourd is a natural remedy for jaundice. It is a natural detoxifier and thus helps in purifying blood, contains insulin-like peptides, alkaloids and charantin, which help to lower blood and urine sugar levels without altering blood insulin levels. The fruit contains protein (0.5%), carbohydrate (3%), carotene (37 mg) and vitamin C (18 mg) per 100 g of edible portion. Many works have been reported on in vitro regeneration of different cucurbitaceous species in Bangladesh, such as in vitro regeneration of pumpkin [1], cucumber [2], ridge gourd [3] and snake gourd [4] however, there are no available reports on immature embryo and cotyledon culture of ridge gourd till now. Male sterility is a common problem for plant species in hybridization [5] although it has an important role in keeping newly-introduced plant species from becoming invasive [6]. Maintenance of male sterile line is a major challenge. Micropropagation is the only viable approach for maintaining this unique source as the genotype can be fixed without any genetic change [7]. So, new source of male sterility in ridge gourd can be maintained through in vitro culture. Therefore, the present investigation was undertaken to establish an efficient regeneration system in ridge gourd from immature embryo and cotyledonary node.

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2 MATERIALS AND METHODS

Immature seeds were collected from immature fruits of ridge gourd. Seeds were taken in a conical flask and thoroughly washed under running tap water for 20-30 minutes to reduce the level of surface microorganisms. Then they were taken in autoclaved conical flask containing distilled water with few drops of tween-80 and 2-3 drops of savlon with constant shaking and kept for about 10-15 minutes following this seeds were washed thoroughly by sterile distilled water. Finally surface sterilization was carried out by dipping 0.5% $HgCl_2$ solution with gentle shaking for 2-2.3 minutes at laminar air flow cabinet. Then the sterilized materials were washed 4-5 times with autoclaved distilled water immediately to remove all traces of $HgCl_2$. Seeds were de-coated and the immature embryo axes and cotyledons were excised from immature seed of ridge gourd using sharp scalpel. Then the excised embryos and cotyledons were inoculated in semisolid MS medium [8] supplemented with various concentrations and combinations of growth regulators such as: 6-benzylaminopurine (BAP), Kinetin (Kin), Gibberelic acid-3 (GA_3), Indole-3-buteric acid (IBA), ∝-Naphthalene acetic acid (NAA), and 2, 4-D for embryo development and multiple shoot formation, callus induction and shoot initiation from cotyledons and root induction. The pH of the medium was adjusted 7.0 and 10 ml of medium was taken in each test tube which was covered by nonabsorbent cotton plug. After preparation of the medium, it was autoclaved for 21 min at the pressure of 1.5 kg/cm2 at 121ºC. The inoculated cultures were kept at dark condition for a couple days prior to take in the growth chamber. The condition of the growth chamber was light 2500-3000 lux

and temperature 22±2ºC. Every subculture was carried out after 21 days of previous culture with recording data and each of the experiment was repeated two times. After complete rooting, the micro plantlets were acclimatized in sandy soil with carefully maintenance.

3 RESULTS AND DISCUSSION

3.1 Direct Regeneration from Immature Embryo and Cotyledon:

Immature embryo and cotyledon of *Luffa acutangula* were cultured on MS medium containing different concentrations and combinations of BAP, Kin, GA3, and NAA for multiple shoot formation. Different concentrations of BAP (0.50 mg/l – 3.0 mg/l) in combination with Kin (0.20 mg/l – 2.0 mg/l), NAA (0.20 mg/l-0.50 mg/l) and GA3 (0.1 mg/l-0.3 mg/l) were used for multiple shoot formation (table 1). The highest mean number of shoots (5.70±0.69) and the mean length of shoots (3.8±0.39) were recorded from cotyledon explants on MS + 2.0 mg/l Kin + 0.30 mg/l GA3 (fig-E, F, G & H). In case of immature embryo the best combination for embryo development, primary root and shoot formation and shoot multiplication were MS + 0.5 mg/l BAP + 0.1 mg/l GA3 and 1.0 mg/l BAP + 0.20 mg/l GA3 respectively, where the mean number of shoots per culture was 5.20±0.94 (Fig- A, B, C & D). MS with only BAP observed the highest frequency (93%) of multiple shoot regeneration from cotyledon explants of Garden baslam [9]. However in our investigation, we have found a little response of multiple shoot regeneration on MS with BAP singly. Cytokinin at lower concentration has been reported for multiple shoot induction in different plant species [10] and [11]. In another investigation, the highest multiple shoot formation from the cotyledons was reported in Citrullus lanatus (Thunb.) in the hormonal combination of BA with 2- ip [12].

3.2 Indirect Regeneration from Cotyledon:

Indirect regeneration of ridge gourd was obtained via callus induction in various concentrations and combinations of BAP (0.5- 2.0 mg/l), 2, 4-D (0.1-0.5 mg/l) and NAA (0.2-1.5 mg/l) (table 2). Among different combinations, we found better callus induction on MS with 1.5 mg/l BAP + 1.0 mg/l NAA (fig. I) rather than BAP singly or the combination of BAP with 2, 4-D. In this study, maximum percentage of callus formation was obtained after 2-3 weeks of inoculation and the morphology of callus was different in different nutrient media. The light green compact type callus was found in better regeneration on MS with 3.5 mg/l BAP + 0.2 mg/l GA3 where the average number of shoots per culture was 4.10±0.21 (table 3) (fig. J). Auxin has been reported to induce callus formation in tissue culture technique on plant [13] in which NAA and IAA promoted excessive callus formation in watermelon [14]. MS with NAA singly was reported better for callus induction from cotyledon and embryo axis in squash (C. melo) [15], from cotyledon and hypocotyl explants in *Cucumis metuliferus* [16]. According to [17] the cotyledon showed the best performance in callus induction of teasle gourd.

3.3 *In Vitro* Root Formation and Establishment of Microplants in Soil:

Root induction was accomplished from the in vitro developed shoots on ½ strength MS supplemented with different concentrations of IBA (0.1-1.0 mg/l) and NAA (0.1-1.0 mg/l). Among different concentration of IBA and NAA, efficient rooting was achieved on ½ strength MS medium supplemented with 0.5 mg/l IBA and 80% micro shoots produced root (table 4), (fig. K). In case of NAA the highest percentage (70%) of root induction was observed on ½ strength MS with the same concentration of IBA. Several reports have been published on root induction of many cucurbitaceous plant species using IBA [18], [19], [20]. After sufficient root formation, the plantlets were removed from agar medium and washed carefully with distilled water. Plantlets were acclimatized in sandy soil containing humus and the pots were covered by polythene and kept growth chamber. After 7 days, acclimatized plantlets were transferred in natural environment for establishment where 70 plantlets were found successfully survived (fig. L).

TABLE 1

EFFECTS OF DIFFERENT CONCENTRATIONS AND COMBINATIONS OF GROWTH REGULATORS ON DIRECT MULTIPLE SHOOTS FORMATION FROM IMMATURE EMBRYO AND COTYLEDON OF *LUFFA ACUTANGULA*

Used Growth regulators (mg/l)	% of explants responded		Mean no. of Shoots/ culture (M ± SE)		Mean length of shoots/culture (cm) (M ± SE)	
	Immature embryo	Cotyledon	Immature embryo	Cotyledon	Immature embryo	Cotyledon
BAP						
0.50	40	60	2.60±1.03	3.60±1.00	1.5±0.59	3.6±0.95
1.00	20	30	1.90±0.90	2.20±1.07	1.4±0.88	1.6±0.01
2.00	40	50	2.80± 0.20	3.40±0.82	1.8± 0.90	2.7±0.70
BAP + GA3						
0.50 + 0.10	50	60	2.90±0.03	3.70±0.55	2.8±0.65	3.4±0.72
1.00+ 0.20	80	70	5.20±0.94	4.80±0.73	3.9±0.18	3.5±0.22
1.00+ 0.30	-	-	-	-	-	-
BAP + NAA						
1.00 + 0.20	20	35	1.80±0.32	2.30±1.40	1.7±1.00	2.4±0.69
2.00 + 0.20	35	30	3.20±0.55	2.20±0.40	2.6±0.85	2.8±0.80
3.00 + 0.50	30	40	2.30±0.48	2.40±0.55	1.4±0.92	1.8±0.86
BAP + Kin						
0.50 + 0.20	50	60	2.40±0.89	3.70±0.60	2.6±0.77	2.8±1.00
0.50 + 0.50	40	55	2.30±0.49	3.40±0.73	1.5±0.58	1.7±0.69
1.00 + 1.00	25	40	2.20±0.03	2.50±1.0	1.3±0.55	1.8±0.78
Kin + GA3						
0.50 + 0.20	50	75	2.90±0.12	3.50±.36	2.41±.043	3.1±0.15
2.00 + 0.30	70	95*	3.90±0.50	5.70±0.69	3.6±0.58	3.8±0.39
2.00 + 0.30	50	60	3.30±0.12	3.50±1.32	1.4±0.59	1.7±0.90

TABLE 2

EFFECTS OF DIFFERENT CONCENTRATIONS AND COMBINATIONS OF GROWTH REGULATORS ON CALLUS INDUCTION FROM COTYLEDON OF LUFFA ACUTANGULA

Concentrations (mg/l)	% of Callus induction	colour of Calli	Morphological features of calli
BAP			
0.50	0	-	-
1.00	30	Light Green	compact
1.50	60	Light Green	compact
2.00	40	Light Green	compact
BAP + 2,4-D			
1.50 + 0.10	40	brownish	Friable
2.00 + 0.10	60	brownish	Friable
2.00 + 0.20	70	Brown	Friable
2.00 + 0.50	70	brown	Friable
BAP+NAA			
1.00 + 0.20	40	Green	Compact
1.50 + 0.50	70	Green	Compact
1.50 + 1.00	85*	Light Green	Compact
2.00 + 1.50	60	Green	Compact

TABLE 3

EFFECTS OF DIFFERENT CONCENTRATIONS AND COMBINATIONS OF GROWTH REGULATORS ON SHOOT BUD FORMATION AND DEVELOPMENT OF SHOOTS FROM CALLUS OF *LUFFA ACUTANGULA*

Growth regulators (mg/l)	% of Callus induced shoot	Mean number of shoots/culture
BAP		
0.50	0	-
1.00	0	-
1.50	10	1.10±0.02
2.00	15	1.10±0.12
BAP + GA3		
1.00 + 0.10	-	-
2.00 + 0.10	20	2.00±0.27
2.50 + 0.20	35	2.10±0.07
3.00 + 0.20	40	3.75±0.14
3.50 + 0.20	55	4.10±0.21
4.00 + 0.20	50	2.50±0.19
BAP+NAA		
1.00 + 0.20	-	-
1.50 + 0.20	15	1.20±0.14
1.50 + 0.50	30	2.10±0.02
2.00 + 0.50	25	1.70±0.24

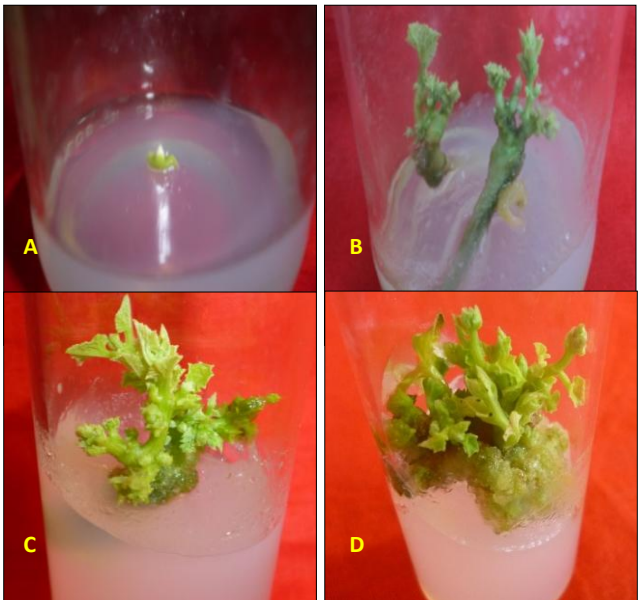

Fig. A & B. Development of immature embryo, primary root and shoot formation on MS + 0.5 mg/l BAP + 0.1 mg/l GA_3; **C & D.** Shoot multiplication and elongation on MS with 1.0 mg/l BAP + 0.2 mg/l. GA_3 from immature embryo of ridge gourd

TABLE 4

EFFECTS OF AUXIN IN ½ STRENGTH OF MS MEDIA ON ROOT INDUCTION IN IN VITRO ESTABLISHMENT OF IMMATURE EMBRYO AND COTYLEDON OF *LUFFA ACUTANGULA*

Growth regulators (mg/l)	Number of explants cultures	% of shoot produced root	Mean number of roots/culture
IBA			
0.1	10	20	2.25±0.12
0.3	10	30	4.20±0.25
0.5	10	80	7.07±0.14
1.0	10	60	5.99±0.21
NAA			
0.1	10	-	-
0.3	10	30	4.10±0.23
0.5	10	70	4.22±0.21
1.0	10	50	3.51±0.24

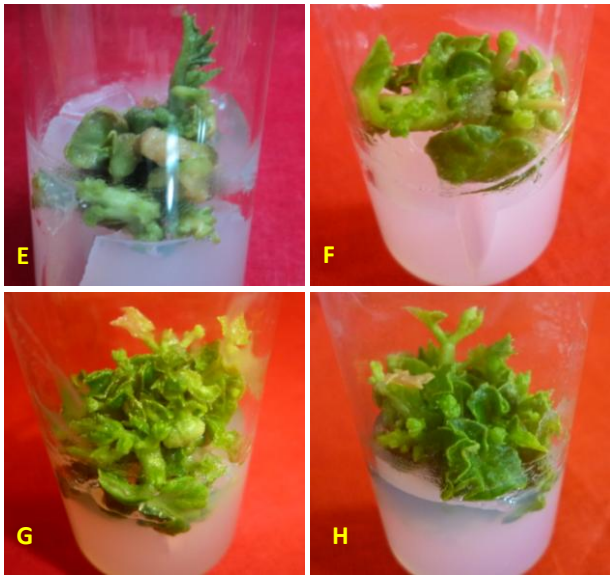

Fig. E & F. Shoot bud formation; **G & H.** Multiple shoot formation and development of shoots directly from cotyledon explants on MS with 2.0 mg/l Kin + 0.3 mg/l GA_3

4 CONCLUTION

Effective regeneration system in ridge gourd (Luffa acutangula L. Roxb) has been established through tissue culture technique. This in vitro regeneration system will be helpful for better improvement of ridge gourd.

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Fig. I. Callus induction from cotyledon on MS with 1.50 mg/l BAP + 1.00 mg/l NAA; **J.** Shoot formation from callus on MS with 3.50 mg/l BAP + 0.20 mg/l GA_3 ; **K.** root induction in In vitro developed shoot on MS containing 0.5 mg/l IBA and **L.** establishment of plantlet on soil after 25 days of transplantation

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