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In vitro Rapid Regeneration of Plantlets from Leaf Explant of Watermelon (*Citrullus lanatus* Thumb.)

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Abstract: Plantlets regeneration were achieved in callus culture of leaf segments from five days old field grown seedlings of *Citrulus lanatus* Thumb. Callus induction and plant regeneration at various frequencies were observed using different concentrations and combinations of growth regulators. The highest percentage of callus induction was observed in MS medium supplemented with 2.5 mg L^{-1} 2, 4-D. After transfer of this callus on MS medium supplemented with 1.0 mg L^{-1} BA+0.2 mg L^{-1} NAA induce adventitious shoots and developed into plant by further subculture in the same medium. NAA (0.1 mg L^{-1}) was found effective in the production of root. Plantlets were acclimatized and subsequently transferred to the field. Survival of the plantlets under *ex vitro* condition was 80%.

Key words: Regeneration, *in vitro*, leaf explant, *Citrullus lanatus*

INTRODUCTION

Watermelon (*Citrulus lanatus* Thumb.) belonging to the family cucurbitaceae, is a popular fruit which native to Central Africa, was first grown by ancient Egyptians and is believed to also have been cultured in Asia minor, Russia and the near and Middle East thousands of years ago. It is widely grown in the tropics and subtropics, most part of South East Asia, Africa, the Caribbean and the southern part of United States. Watermelon is an economically important crop and a valuable alternative source of water in desert areas. It is a good source of fiber, which help to reduce cholesterol, important for keeping our digestive tract operating properly, by preventing constipation, hemorrhoids and diverticular disease. It is a good source of vitamin C and important mineral. The nutritive value of seeds is due to their high oil and protein content.

Regeneration of plants from adventitious shoots is necessary for application of gene transfer technology and for screening plants for somaclonal variation. The latter purpose would be particularly interesting in situations in which conventional breeding programmes have had relatively little impact upon commercial production. In conventional method, watermelon is propagated by seed. But in this plant, there are some problems in their propagation. Low productivity, disease susceptibility and higher cost of production are major constraints faced by watermelon. In the recent years micropropagation techniques are being profitably used to overcome such constraints in various crops as well as ornamental and horticultural plants. Due to present demand (economic) and recover the propagation problem, it is necessary to develop a suitable protocol for mass clonal propagation of watermelon. The process of organogenesis via callus is a suitable method of micropropagation and has the protocol of mass propagation commercially at low cost per unit. There are some early reports on *in vitro* culture and its closely related taxa, like *Cucurbita pepo*[1], cucumber[2,3], squash[4,5], teasle gourd[6]. *Momordica charaantia*[7]. The potential use of tissue culture in watermelon was demonstrated by Dong and Jia[8] who reported complete plantlet regeneration from cotyledon explant via callus. Ahad *et al.*[9] reported the establishment of an efficient protocol for plant regeneration from its immature and mature embryo axis explants of watermelon. So far there is no report on plant regeneration via callus in leaf explant. Therefore, the present investigation was undertaken to establish protocols for regenerating large number of plantlets *in vitro* from the leaf-derived callus cultures. This is perhaps the first report on *in vitro* plant regeneration from leaf explant of watermelon in Bangladesh.

MATERIALS AND METHODS

Leaves were collected from five days old field grown plants and washed thoroughly under running tap water, then treated with 1% Savlon (v/v) for about 10 min. This is followed by successive three washings with distilled

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water to make the material free from Savlon. Surface sterilization was carried out with 0.1% $HgCl_2$ (w/v) for 4 min. followed by gently shaking. The material was then washed with autoclaved distilled water for five min giving four-five changes. The leaves were then sliced into about 5×3 mm long pieces and cultured on MS medium[10] supplemented with various concentrations of 2, 4-D alone or in combination with BA or KIN for callus induction. Seven weeks old callus were subcultured on MS medium containing different combinations of BA or KIN with NAA or IBA for organogenesis. The organogenic calli were subcultured at monthly intervals on same medium for development and elongation of complete plantlets.

In rooting experiments, 3-4 cm long shoots were excised from multiplication cultures and implanted on the rooting medium consisting of half strength MS medium contained in glass tubes. The medium was variously supplemented with NAA, IBA and IAA (0.05-1.0 mg L^{-1}). Rooted shoots from one month old cultures on half strength MS+0.1 mg L^{-1} NAA were transferred to pots after *in vitro* hardening.

The pH of all media was adjusted to 5.7±0.1 before addition of agar and sterilized by autoclaving for 20 min. at 1.1 Kg cm^{-2} pressure at 121°C. For solidifying the medium, 7-8 g L^{-1} agar. The tubes or flasks containing explants were incubated on culture racks in the growth chamber. The cultures were maintained at 25±2°C under the cool white fluorescent lights for 16 h photoperiod with a photon flux density of 2000-3000 lux.

RESULTS AND DISCUSSION

Induction of callus: Leaf segments were cultured on MS medium with various levels of 2, 4-D alone or in combination with BA or KIN for callus induction. After 4 weeks of culture incubation callus mass further increased (Fig. 1A). Morphogenic potentialities of the explant was found to differ depending up to growth regulator supplements are shown in Table 1.

Among the different concentrations of auxins tried, 2, 4-D was found highly effective for induction of callus. It was mostly soft, faster growing, light creamy-white and friable. The highest frequency of callus induction (100%) was recorded at the end of 7 weeks on medium containing 2.5 mg L^{-1} 2, 4-D. Similar observation were made earlier Bhattacharya and Dasgupta[11] on *Bauhinia variegata,* by Begum *et al.*[12] and Karim *et al.*[13] on sugarcane. The combination of 2.5 mg L^{-1} 2, 4-D+0.5 mg L^{-1} BA produced small amount of callus. Vincent *et al.*[14], Gliozeris *et al.*[15] and Mathukumar *et al.*[16] reported the positive effects of 2, 4-D+BA combination on callus induction in *Kaempferia galanga*, *Caladium hortulanum* and *Datura metel*, respectively.

Table 1: Effect of different concentration of 2, 4-D alone or in combination with BA or KIN in MS basal medium for callus induction field-grown seedling (leaf segments) of watermelon. Data were recorded 7 weeks after culture and each treatment consisted of 15-20 test tube

Growth regulators (mg L^{-1})	% of explants induced callus	Callus colour	Degree of callus formation
2, 4-D			
1.0	50	W	*
2.0	75	Cr	**
2.5	100	Crw	***
3.0	90	Cr	**
3.5	65	W	**
4.0	40	W	*
2, 4-D+BA			
2.5+0.2	70	Cr	**
2.5+0.5	85	Crw	***
2.5+1.0	60	W	*
3.0+0.2	55	W	*
3.0+0.5	75	Cr	**
3.0+1.0	50	W	*
2, 4-D+KIN			
2.5+0.2	50	Cr	**
2.5+0.5	60	Crw	***
2.5+1.0	45	W	*
3.0+0.2	40	W	*
3.0+0.5	50	Cr	**
3.0+1.0	35	W	*

(*) slight callusing; (**) considerable callusing and (***) profuse callusing Cr = Creamy; Crw = Creamy-white; W = White

Table 2: Effect of cytokinin (BA, KIN) with auxin (NAA, IBA) at different concentrations and combinations in MS medium on shoot regeneration from the callus tissue of watermelon. Data were taken after 8 weeks of culture

Growth regulators (mg L^{-1})	% of shoot formation	No. of total shoots per culture	Average length of shoots per culture
BA+NAA			
0.5+0.1	55	4.05±0.24	3.00±0.18
0.5+0.2	60	4.20±0.16	3.15±0.20
0.5+0.5	50	4.10±0.12	2.45±0.26
1.0+0.1	65	4.55±0.32	3.21±0.26
1.0+0.2	70	5.85±0.26	3.54±0.24
1.0+0.5	50	4.05±0.18	2.65±0.18
BA+IBA			
0.5+0.1	40	3.90±0.31	2.45±0.19
0.5+0.2	50	4.10±0.25	3.00±0.12
0.5+0.5	35	3.75±0.10	2.42±0.16
1.0+0.1	50	4.00±0.21	2.65±0.23
1.0+0.2	60	4.68±0.23	3.10±0.15
1.0+0.5	45	3.28±0.24	2.40±0.25
KIN+NAA			
0.5+0.1	40	3.45±0.21	2.95±0.13
0.5+0.2	55	3.68±0.22	3.00±0.22
0.5+0.5	40	3.30±0.10	2.40±0.20
1.0+0.1	50	4.15±0.24	3.11±0.12
1.0+0.2	60	4.52±0.25	3.35±0.21
1.0+0.5	45	3.85±0.12	2.55±0.15
KIN+IBA			
0.5+0.1	35	3.75±0.23	2.40±0.17
0.5+0.2	40	4.00±0.20	2.85±0.18
0.5+0.5	30	3.45±0.18	2.24±0.12
1.0+0.1	45	3.85±0.24	2.55±0.29
1.0+0.2	50	4.20±0.22	3.05±0.18
1.0+0.5	40	3.20±0.26	2.22±0.20

Organogenesis of the shoots from callus tissue: All these studies indicated that watermelon explant requires

Table 3: Effect of different concentrations of auxins on adventitious root formation from the *in vitro* grown micro-cutting cultured on ½ MS medium. There were 15-20 micro-cuttings in each treatment. Data were recorded after 4-6 weeks of culture

Types of auxin	Different concentration of auxin (mg L^{-1})	% of micro-cutting rooted	Number of root per micro-cutting	Average length of the root (cm)
NAA	0.05	75	3.45±0.27	2.55±0.25
	0.10	95	4.65±0.35	2.60±0.22
	0.20	80	2.85±0.25	2.40±0.16
	0.50	60	2.40±0.18	1.85±0.24
	1.00	-	-	-
IBA	0.05	60	3.00±0.27	2.40±0.25
	0.10	80	4.10±0.35	2.50±0.22
	0.20	55	2.55±0.25	2.25±0.16
	0.50	45	2.25±0.18	1.55±0.24
	1.00	-	-	-
IAA	0.05	-	-	-
	0.10	45	2.00±0.24	2.10±0.21
	0.20	65	2.20±0.20	2.30±0.10
	0.50	40	1.85±0.15	1.45±0.22
	1.00	-	-	-

higher contractions of 2, 4-D for callus induction. Various concentrations of cytokinins (BA, KIN) and auxins (NAA, IBA) were used in different combinations for shoot regeneration. During this investigation shoot formation was highly influenced by concentrations and type of the growth regulators used in the experiment (Fig. 1B). Among the different concentrations and combinations of cytokinins and auxins for shoot multiplication, best performance was showed in MS medium supplemented 1.0 mg L^{-1} BA+0.2 mg L^{-1} NAA (Table 2). On this combination the percentage of explant produced shoots was 70. The number of shoots and average length shoots per culture was 5.85±0.26 and 3.54±0.24, respectively (Fig. 1C and 1D).

The results of the present investigation agree with the findings of Hoque *et al.*[17]. They found that a combination of 1.5 mg L^{-1} BA and 0.1 mg L^{-1} NAA was more suitable for adventitious multiple shoot formation in teastle grourd whereas in this experiment 1.0 mg L^{-1} BA+0.2 mg L^{-1} NAA was observed to be best for the production of multiple shoots in watermelon. There are some reports on callus culture and organogenesis of plant of several related species. Halder and Gadgril[18] obtained callus and shoot but development when cotyledons were cultured in MS medium supplemented with 0.1-1.0 mg L^{-1} NAA and 3.8 mg L^{-1} adenine. Wehner and Lockly[19] achieved adventitious shoot formation from the callus of cotyledon culture of *Cucumis sativas*.

Rooting of micro-shoots: Different types of auxins were used at different concentrations to regenerate adventitious root. Among different concentration of auxins, NAA was found to be comparatively better response than IBA and IAA for producing roots. Best rooting was observed with half strength of MS medium supplemented with 0.1 mg L^{-1} NAA (Table 3) and highest number roots per microcuttings were 4.65±0.35 (Fig. 1F). The findings are in agreement with those observed in other plant species such as *Caphaelis ipecacuanha*[20], *Plantago ovata*[21]. The plantlets with well developed roots were successfully transplanted in soil and the percentage of survivability was 70 (Fig. 1F).

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