– **Short communication**

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Effect of Different Plant Growth Regulators on Direct Regeneration of Watermelon (*Citrulus lanatus* Thumb.)

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Watermelon (*Citrulus lanatus* Thumb.) is an economically important crop and a valuable alternative source of water in desert areas. 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. The soluble fiber in watermelon may help to reduce cholesterol and risk of heart diseases. It is a good source of fiber, which is important for keeping digestive tract operating properly by preventing constipation, hemorrhoids and diverticular disease. It is an excellent source of important minerals. It is also rich in vitamin C and potassium. In propagation the introduction of new characters into watermelon by means of genetic manipulation is of great potential value, specially of the traits that would confer resistance to diseases and pests. Less attention has been given to tissue culture of watermelon than its closely related taxa, such as cucumber and melon (Dong and Jia 1991). Ahad et al. (1994) reported that establishment of an efficient protocol for plant regeneration from its immature and mature embryo axis explants of watermelon. The present investigation was conducted to establish a suitable regeneration protocol for *Citrulus lanatus* growing in Bangladesh.

Shoot tips were collected from the field grown five-day-old plants of *Citrulus lanatus* and washed repeatedly with distilled water and finally treated with $HgCl_2$ (0.1%) for 4 min in a laminar flow cabinet and washed three times with autoclaved distilled water to remove any trace of $HgCl_2$.

After surface sterilization, shoot tips were excised at the base and divided into pieces as explants of size 25 - 30 mm. The basal medium used for all the experiments was MS mineral formulation containing standard salts and vitamins, 30 mg/l sucrose and 7 g/l agar. Media were variously supplemented with BA either individually or in different combinations with auxin, NAA.

The pH was adjusted to 5.7 ± 0.1 before adding agar and the media were autoclaved for 30 min at 121_C under 1.1 kg/cm^2 pressure. Cultures were incubated at 25 ± 1_C with a photoperiod of 16 h at 2000 - 3000 lux cool white fluorescent light.

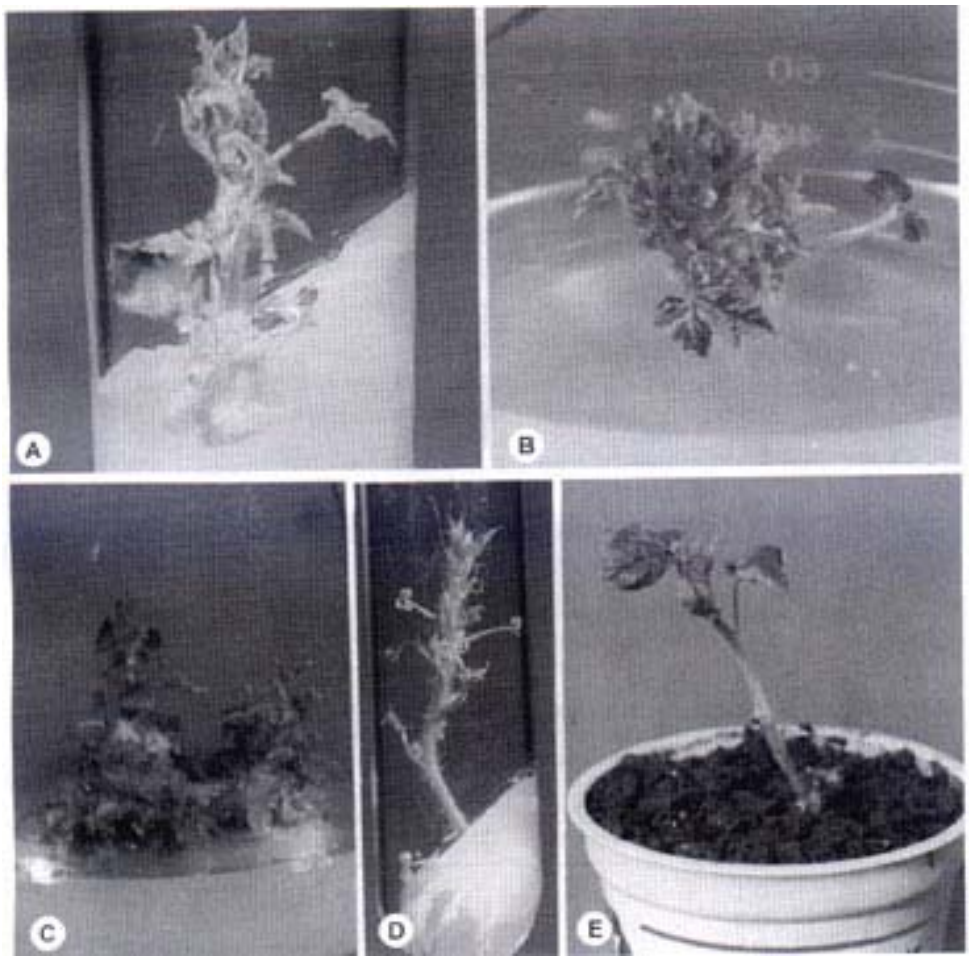
The primary shoots regenerated from explants, after one week of culture on MS supplemented with 1.0 mg/l BA and 0.2 mg/l NAA. They were isolated and subcultured on fresh medium of the same constituents for two weeks and thereafter the regenerated micro-shoots were excised several times and cultured on the same medium.

In rooting experiments 2 - 3 cm long shoots were excised from multiplication cultures and implanted to the rooting medium consisting of half strength MS micro- and macronutrients in glass tubes. The media were variously supplemented with NAA, IBA and IAA (0.5 - 1.0 mg/l). Rooted shoots from one-month-old cultures on half strength MS + 0.1 mg/l NAA were transferred to pots after *in vitro* hardening.

About 90% contamination free cultures were obtained when the shoot tip explants of five-day-old plants were treated for 4 min with 0.1% $HgCl_2$. These explants remained green and showed healthy growth and proliferation of axillary shoots. For the primary establishment of *in vitro* culture surface sterilization of the explants from the *ex vitro* grown plants was essential.

As surface of young shoots of *Citrulus lanatus* is hairy and waxy use of a sterilant like $HgCl_2$ was essential.

Different experiments were conducted with a view to finding out optimum culture condition for shoot regeneration from cultured explants. Multiple shoots were found to develop from shoot tip explants of five-day-old plants. Initiation of multiple shoots in most of treatments was observed within three weeks of culture (Fig. A). In shoot tip explatns the best shoot induction was observed in MS + 1.0 mg/l BA + 0.2 mg/l NAA. In the case of shoot tips culture, 100% of the explants developed shoot, number of shoots per culture was 6.10 ± 0.15 and average length of shoots per culture was 4.50 ± 0.17 on the above medium (Fig. B). The combinations of BA with NAA were found superior to BA only and the combination of 1.0 mg/l BA + 0.2 mg/l NAA was superior to all other combinations of BA with NAA (Table 1). Lauzer and Vieth (1990) reported that shoot tip explants of *Cyndara scolymus* (Green Globe) seedlings showed the best shoot regeneration and multiplication on MS supplemented with BA and NAA. Effectiveness of BA + NAA for *in vitro* shoot regeneration and multiplication from shoot tip cultures was reported in several other plants (Conver and Lits 1987, Tokuhara and Mii 1993).



Figs. **A - E** : Regeneration of plantlets *in vitro* from shoot tip explants obtained from field grown watermelon seedlings. **A**. Development of axillary shoots from shoot tip explants after three weeks of culture. **B**. Development and multiplication of the axillary shoots on MS containing 1.0 mg/l BA + 0.2 mg/l NAA after five weeks of culture. **C.** Adventitious root formation on regenerated shoots. **D**. Establishment of *in vitro* grown watermelon plantlets in outside pot.

Root formation was induced in the *in vitro* regenerated shoots by culturing them on half strength of MS medium with 0.1 - 1.0 mg/l either of NAA, IBA and IAA. Among the three types of auxin, NAA was found to be most effective at different concentrations tested for producing roots on the cut margin of the shoot and 0.1 mg/l NAA found to be the best concentration of auxin for proper rooting in which 100% shoots rooted within six weeks of culture (Table 2).

Table 1. Effect of different concentrations of BA and in combinations with NAA in MS on shoot proliferation from field grown seedling (shoot tip) of watermelon.

Growth regularors (mg/l)	% of shoot formation	No. of total shoots/culture	Average length of shoots/culture (cm)
0.5	60	2.60 ± 0.26	2.10 ± 0.21
1.0	70	3.00 ± 0.18	2.20 ± 0.23
1.5	90	4.40 ± 0.19	3.00 ± 0.26
2.0	100	5.20 ± 0.23	3.50 ± 0.14
2.5	75	4.00 ± 0.17	2.50 ± 0.21
BA + NAA			
0.5 + 0.1	50	2.55 ± 0.17	3.45 ± 0.16
0.5 + 0.2	55	2.65 ± 0.15	3.50 ± 0.29
0.5 + 0.5	60	3.10 ± 0.15	3.80 ± 0.23
1.0 + 0.1	75	3.05 ± 0.26	4.00 ± 0.13
1.0 + 0.2	100	6.10 ± 0.15	4.50 ± 0.17
1.0 + 0.5	90	4.00 ± 0.25	4.50 ± 0.18
1.5 + 0.1	70	2.95 ± 0.13	3.90 ± 0.18
1.5 ± 0.2	80	3.20 ± 0.15	4.20 ± 0.29
1.5 + 0.5	65	2.86 ± 0.23	3.75 ± 0.28
2.0 ± 0.1	60	2.90 ± 0.13	3.80 ± 0.16
2.0 ± 0.2	65	3.00 ± 0.14	3.85 ± 0.16
2.0 + 0.5	70	3.10 ± 0.24	3.80 ± 0.23

Table 2. Effect of different concentrations of auxins on adventitious root formation from the *in vitro* grown shoot cultured on half strength of MS. There were 15 - 20 microcuttings in each treatment.

Types of auxin	Different concentrations	% of shoots rooted	Number of roots/shoot	Average length of the root (cm)
NAA	0.1	100	4.50 ± 0.30	2.85 ± 0.20
	0.2	80	2.30 ± 0.21	2.50 ± 0.23
	0.5	55	1.65 ± 0.12	2.45 ± 0.21
	1.0	-	-	-
IBA	0.1	85	2.50 ± 0.32	2.10 ± 0.25
	0.2	70	1.95 ± 0.25	1.85 ± 0.45
	0.5	45	1.53 ± 0.15	1.80 ± 0.13
	1.0	-	-	-
IAA	0.1	65	2.12 ± 0.25	2.00 ± 0.20
	0.2	40	1.55 ± 0.25	1.85 ± 0.15
	0.5	-	-	-
	1.0	-	-	-

Among the three types of auxin used, NAA was found to be the best for root induction (Fig. C). The findings are in agreement with those observed in other plant species such as *Caphaelis ipecacuanha* (Jha and Jha 1989), *Plantago ovata* (Wakhlu and Barna 1989).

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