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In vitro propagation of *Citrullus lanatus* Thumb. from nodal explants culture

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Abstract

A standard protocol was established for rapid *in vitro* propagation of watermelon (*Citrullus lanatus* Thumb.) from nodal explants of field grown plant. Multiple shoot proliferation was achieved from nodal explants on MS medium supplemented with 1.0 mg/l BAP + 0.2 mg/l NAA within 30 days of inoculation. The elongation of shoots was obtained on the same medium. Highest percentage of root induction was achieved on MS medium supplement with 1.0 mg/l IBA within 25 days of culture. Well rooted plantlets were transferred to small pots and after proper acclimatization the plantlets were transplanted in the field condition, where 80% plantlets were survived and grew successfully.

Keywords: *In vitro* regeneration, Nodal explant, *Citrullus lanatus*

Introduction

Watermelon (*Citrullus lanatus* Thumb.) is one of the most common and popular summer fruit crops with major economic importance in Bangladesh including other tropical and sub- tropical countries. The crop is widely grown in the tropics and subtropics especially in most part of Southeast Asia, Africa, the Caribbean and southern part of the United States of America. It is one of the members of Cucurbitaceae family. Although, it's fruit is usually eaten fresh but it is also eaten as cooked vegetable in Africa (Wehner, 2005). It is used as thirst quenching super food, found to function as natural viagra and is also used for preventing cancer, stroke, and heart disease. It is beneficial for lycopene and rich in nutrition. Seeds are usually propagating material of the crop. Because of limited supply of seeds, the farmers generally produce seeds from the crops of previous years which results in decrease in size, weight, food values of fruits of the crop (Ahad *et al*. 1994). Micropropagation is an alternative way to have quality seedling in the crop. Therefore, the present research work was undertaken with a view to establish a protocol for large scale *in vitro* multiplication of plantlets from nodal explants that shows the highest number of shoots and maximum root elongation in the culture than that of shoot tip explants used in the study.

Materials and Methods

The nodal explants of *Citrullus lanatus* were collected from field grown plants from the field of University of Rajshahi, Rajshahi-6205, Bangladesh. The explants were surface sterilized. The nodal segments were rinsed in 90% ethyl alcohol for one minute and washed with sterile distilled water for seven times to remove traces of alcohol. This was followed by treating the materials with 0.1% (W/V) $HgCl_2$ for three minutes. Finally, the explants were washed thoroughly using autoclaved distilled water for several times to remove the traces of $HgCl_2$. The nodal segments were cut into 1-1.5cm and transferred into culture vessels containing full strength MS (Murashige and Skoog, 1962) medium with 3% (W/V) sucrose which was solidified with 0.7% (W/V) agar. All these operations were done under a Laminar Airflow Hood. The pH of the medium was adjusted to 5.7 and then autoclaved at 121^0C for 20 minutes. MS medium was also supplemented with different concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l), KIN (0.2, 0.5, 1.0, 2.0, 3.0, 4.0 mg/l) , BAP+NAA (0.5+0.2, 0.5+0.5, 1.0+0.2, 1.0+0.5, 2.0+0.5, 3.0+0.5 mg/l) , KIN+IBA (0.5+0.5, 1.0+1.0, 1.0+2.0, 2.0+0.5, 2.0+1.0, 2.0+2.0 mg/l) in combinations used for shoot proliferation. *In vitro* regenerated shoots were cultured on MS medium supplemented with different concentrations of IBA (0.1, 0.5, 1.0, 1.5, 2.0 mg/l) for rooting. The cultures were incubated in a culture room at 25 ± 2^0C with a photoperiod of 16 hour at 3000 lux light intensity provided by cool white fluorescent tubes. Ten explants were used in each treatment and all experiments were repeated thrice. Visual observation of culture was made in every week. Data on shoot and root induction were recorded after 25 and 30 days of inoculations respectively and was used for statistical analysis. Well developed plantlets were removed carefully from

the culture vessels, washed gently under running tap water and planted in plastic pots containing a potting mixture of sand, soil and farmyard manure in the ratio of 1:1:1. The potted plantlets were covered by polythene sheet to maintain suitable humidity. After sufficient acclimatization, the plantlets were transplanted in the field in the pots.

Results and Discussion

Nodal segments of *C. lanatus* were cultured on MS media supplemented with various concentrations of BAP, KIN, BAP with NAA and KIN with IBA for shoot induction. It revealed that proliferation of multiple shoots was observed with high frequency from the explants. The highest percentage of multiple shoot induction was 90% on MS medium supplemented with 1.0 mg/l BAP + 0.2 mg/l NAA (Fig. A; Table 1). Maximum mean number of shoots per explants (5.0) ware recorded from MS medium having 1.0 mg/l BAP + 0.2 mg/l NAA (Fig. B; Table 1) followed by the treatment consisting of 2.0 mg/l KIN + 0.5 mg/l IBA. The tallest shoots were also found in the same medium. MS medium supplemented with 1.0 mg/l BAP + 0.2 mg/l NAA found to be the best for maximum multiple shooting. Similar findings were reported by many workers in various plants viz. Lee and Thomas (1985) in *Cucurbita foctidissima* , Moreno *et al*. (1985) in *Cucumis melo* and Brandt and Hess (1994) in Chickpea .Well developed *in vitro* regenerated shoots were isolated and cultured on MS medium having different concentrations of IBA for root induction which showed profound response of rooting in *Citrullus lanatus* . The highest percentage (100) of root induction was recorded from MS medium having 1.0 mg/l IBA (Fig. C and D; Table 2) and the mean number of roots per shoot (12) and the longest roots (7.0 cm) were also observed in the same medium which was followed by MS medium with 1.5 mg/l IBA. From the investigation, it was observed that 1.0 mg/l IBA was an ideal treatment for root induction as well as elongation. These results are in agreement with many scientists, such as, Lin *et al*. (2003) in *Polygonum multiflorum*, Rani *et al*. (2006) in *Coleus blumei*, Kaliamoorthy *et al*. (2008) in *Harpagophytum procumbens*, Wakhlu and Sharma (1998) in *Heracleum candicans* and Hasan *et al*. (2008) in *Cassia alata* Thiruvengadam and Jayabalan (2000) in *Vitex negundo*, Sikdar *et al*. (2003) in *Momordica charantia* , Karuppusamy *et al*. (2006) in *Vanasushava pedata* , Binita *et al*. (2006) in *Plumbaga zelanica* and Biswas *et al*. (2007) in *Abrus precatorius* obtained the best results in the same hormonal treatments. When the plantlets were 8-10 cm long and had developed a good root system, they were ready for hardening and transplantation into pots. The cups of the culture vessels containing the plantlets were removed and the plantlets were kept in growth room for two days. Then the cultures were transferred gradually from growth room to open room and kept there for four days. Then the rooted plantlets were regularly sprayed with water using a hand sprayer and were covered with polythene bags to maintain high humidity around juvenile plants (Fig. E). Plantlets were subsequently transferred to larger pots and gradually acclimatized to outdoor condition. The ultimate survival rate of the transferred plantlets to soil was 80% and their growth in natural condition was satisfactory (Fig. F).

Table 1. Effect of different concentrations and combinations of phytohormones on multiple shoot from nodal segment explants of *Citrullus, lanatus* (Data collected after 30 days of culture)

Hormonal supplements (mg/l)	Percentage of culture response (M ±SE)	Mean no. of shoots per explants (M ±SE)	Mean shoot length (cm) (M ±SE)
BAP			
0.5	40 ±1.7320	2.0±0.5773	2.1±0.1154
1.0	50 ±2.3094	2.1±0.6082	2.0±0.5773
1.5	60 ±2.3094	3.0±0.5773	2.5±0.1443
2.0	70±1.1547	4.0±1.1547	2.5±0.1443
2.5	60±2.8867	3.0±0.5773	2.0±0.5773
3.0	50±1.7320	2.0±0.5773	1.8±0.0577
KIN			
0.2	30±1.7320	1.0±0.2886	2.0±0.5773
0.5	50±1.1547	2.0±0.5773	2.5±0.1443
1.0	60±2.3094	3.0±0.5773	2.9±0.1154
2.0	70±1.1547	4.0±0.5773	3.7±0.1732
3.0	50±2.3094	2.0±0.5773	2.6±0.1732
4.0	40±2.3094	2.0±0.5773	2.1±0.1154
BAP+NAA			
0.5+0.2	40±2.3094	3.0±0.5773	4.0±0.5773
0.5+0.5	50±2.3094	4.0±0.5773	4.0±0.5773
1.0+0.2	90±1.1547	5.0±0.5773	5.0±0.5773
1.0+0.5	80±2.3094	4.0±0.5773	4.0±0.5773
2.0+0.5	70±1.1547	3.0±0.5773	4.1±0.1154
3.0+0.5	60±1.1547	2.0±0.5773	3.0±0.5773
KIN+IBA			
0.5+0.5	30±1.1547	2.0±0.5773	2.5±0.1443
1.0+1.0	40±1.1547	3.0±0.5773	3.5±0.1443
1.0+2.0	50±2.8867	4.0±0.5773	3.9±0.0577
2.0+0.5	80±1.1547	4.8±0.9237	4.2±0.1154
2.0+1.0	70±1.1547	3.0±0.5773	3.2±0.2309
2.0+2.0	50±2.3094	1.0±0.2886	2.0±0.5773

Here: M = Mean and SE = Standard error

Table 2. Effect of IBA on root induction in regenerated shoots (Data collected after 25 days of culture)

IBA mg/l	No. of explants inoculation	Days of root initiation	Percentage root induction (M ± SE)	Mean no. of root per explants (M ±SE)	Mean length of longest root (cm) (M ±SE)
IBA					
0.1	20	20-22	50 ± 2.8867	4.5±0.1443	4.5±0.1443
0.5	20	10-11	57 ± 1.7320	7.0±0.5773	4.9±0.1154
1.0	20	7-8	100 ± 00	12.0±0.5773	7.0±0.5773
1.5	20	9-10	80 ± 1.1547	11.0±0.5773	6.0±0.5773
2.0	20	15-20	70 ± 1.1547	6.5±0.1443	3.7±0.1154

Here: M = Mean and SE = Standard error

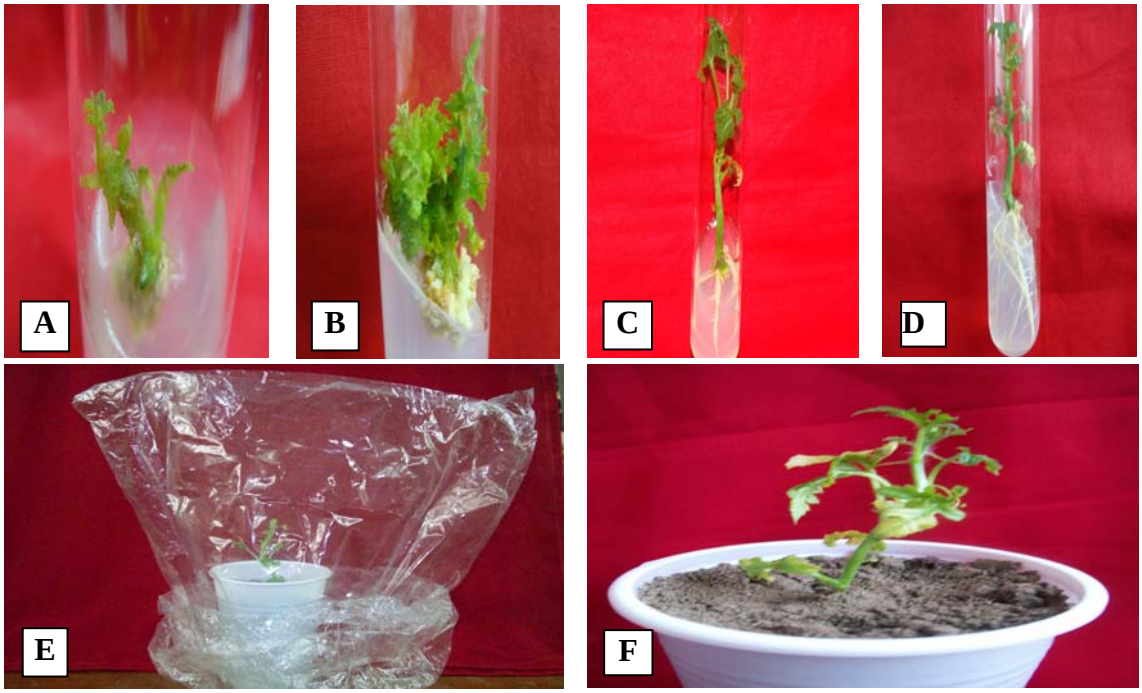

Fig. A. Induction of multiple shoot from nodal explants in MS medium with 1.0 mg/l BAP+0.2 mg/l NAA
Fig. B. Elongation of multiple shoot in the same medium
Fig. C. Induction of roots in medium having 1.0 mg/l IBA
Fig. D. Elongation of roots in the same medium
Fig. E. Acclimatization of plantlet covered by polythene bag
Fig. F. Plantlet regenerated under natural condition transferred to pot

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