ISSN-1991-3036 (Online) & ISSN-2075-1621 (Optical)
Int. J. Sustain. Crop Prod. 5(4):25-29(November 2010)

IN VITRO PLANT REGENERATION FROM COTYLEDON AND INTERNODES DERIVED CALLUS IN WATERMELON (*Citrulus lanatus* Thumb.)

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Accepted for publication on 15 October 2010

ABSTRACT

Khatun MM, Hossain MS, Khalekuzzaman M, Rownaq A, Rahman M (2010) *In vitro* plant regeneration from cotyledon and internodes derived callus in watermelon (*Citrulus lanatus* Thumb.). *Int. J. Sustain. Crop Prod.* 5(4), 25-29.

A study was carried out to observe the callus induction and subsequent regeneration potentiality of watermelon (*Citrullus lanatus* Thumb.) from cotyledon and internodes of this plant. Greenish compact callus was achieved from cotyledon on MS medium supplemented with 1 mg/l 2, 4-D within one week of inoculation. The callus produced large number of shoots when cultured on MS medium with1.0 mg/l BAP+0.2 mg/l NAA. Rooting rate was 100% when shoots from second subculture were cultured in medium with 1.0 mg/1 IBA and the shoots were more prone to rooting by increasing subcultures. *In vitro* grown plantlets with well root system were successfully established in natural condition through successive phases of acclimatization. The regenerated plantlets were healthy, uniform and identical to donor plants and survival percentage was 80%.

Key words: *callus induction, plant regeneration, cotyledon and internodes, Citrullus lanatus*

INTRODUCTION

Watermelon (*Citrullus lanatus*) is an economically important and most common fruit crops. It is used as edible seeds, a dessert food (edible flesh) and animal feed. Although, it is primarily eaten fresh, 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 of this crop enrich the sperm quality and leaf juice increases the blood quantity in human. Its total world production in metric ton is the third largest among the vegetable species. The total area of its cultivation is 4.5k hectare of land and second in ranking after tomato, which is grown on 4.8 k hectare in Bangladesh (Anonymous 1992). The crop is widely grown in the tropics and subtropics specially in most part of Southeast Asia, Africa, the Caribbean and Southern part of the United States of America. In Bangladesh, watermelon is propagated by hybrid seeds imported every year from abroad because of limited supply of hybrid seeds. The farmers generally grow seeds from the crops of previous years, which results in the decrease in size, weight and food value of fruits of this crop (Ahad *et al*. 1994). This investigation deals with the standardization of a technique for micropropagation through callus induction. The protocols provide rapid proliferation of shoots from cotyledon and internodes derived callus with comparatively a reduced requirement of plant growth regulators and successful acclimatization of plants in the soil.

MATERIALS AND METHODS

The present experiment was carried out at the Professor Ali. Md. Eunus Laboratory, Dept. of Genetic Engineering & Biotechnology, University of Rajshahi, Rajshahi-6205, Bangladesh during October to February. The seeds of the crop (*Citrullus lanatus* Thumb.) was collected from seed market, Siddique Bazar, Dhaka. These seeds were packed by TAKII and CO., LTD. Kyoto. Japan. The seeds were taken in a conical flask and thoroughly washed under running tap water for 30min. to reduce the level of surface microorganisms and to loose the seed coat. Then the seeds were taken in reagent bottle containing distilled water with few drops of tween-80 (wetting agent) and 2-3 drops of savlon for about 10-12 minutes rinsed with constant shaking. This was followed by a second washing with distilled water to remove all traces of above chemicals. Surface sterilization was carried out by 0.1% $HgCl_2$ with gentle shaking for solution for 5 minutes. Then the sterilized seeds were washed 5-7 times with sterile distilled water immediately to remove all traces of $HgCl_2$. This procedure was carried out in aseptic condition of laminar airflow cabinet. The sterilized seeds were then inoculated into MS medium (Murashige and Skoog, 1962). After one week, *in vitro* grown seedlings were the ready sources of different kinds of explants like, shoot tips, nodal segments cotyledons, and internodes which were free from contamination. In the present study, cotyledon and internodal explants were collected from aseptically germinated seedlings for callus induction and plant regeneration. The explants were cultured on MS medium with 3% (W/V) sucrose which was solidified with 0.7% (W/V) agar. The pH of the media was adjusted to 5.7 prior to autoclaving at 121^0C for 20 minutes. 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. For each treatment, 10 replications were used and all experiments were repeats thrice. Visual observation of culture was made in every week. Data on callus, shoot and root induction were recorded after 30, 35 and 25 days of inoculation respectively. The medium supplemented with different concentrations of NAA (Naphthalene acetic acid) and 2, 4-D (2,4-Dichlorophenoxy acetic acid), in order to obtain the most suitable culture media for induction of

high frequency of callus. Once the callus developed, they were further cultured for regeneration and elongation of shoots in the medium having different concentrations of auxins and cytokinins. Elongated shoots were rooted on MS medium supplemented with different concentrations of auxins (Naphthalene acetic acid and Indole-3 butyric acid) singly. After proper rooting, the plantlets were gradually exposed to normal conditions for acclimatization and transferred to natural condition where the plantlets were grown successfully.

RESULTS AND DISCUSSION

Callus induction

For callus induction, cotyledon and internodes of watermelon were cultured on MS medium supplemented with different concentrations and combinations of 2, 4-D and NAA and callus induction was observed within seven to 30 days of culture from the cut surface of the cotyledon and internodes (Table 1). The highest 90% callus formation from cotyledon was obtained in the MS medium in combination with 1.0 mg/l 2, 4-D. These calli were greenish in colour and compact (Fig.1.A). But in case of internodes, highest 80% creamy white callus formation was found in medium having 2.5 mg/l 2, 4-D and the second highest percentage (70) of callus formation was observed from both cotyledon and internodal explants with 2.0 mg/l 2, 4-D. The lowest 10% callus formation was obtained from cotyledon in MS medium with combination of 0.5 mg/l BAP + 0.1 mg/l 2, 4-D. Between the two explants, cotyledon derived explants was suitable for callus induction (Table 1). There are many reports on callus induction from cotyledon explants (Rao *et al*. 1982 in some tropical fruits) and from internodal explants (Moore 1986 in *Citrus* root stocks and Belaizi *et al*. 1991 in apple). Again, the table 1 showed that 2, 4-D was the best auxin for callusing. Similar results have been obtained by other workers in monocot and even in dicot (Nadel *et al*. 1989 and Chee 1990).

Table 1. Effect of different concentrations and combinations of phytohormones on callus induction from cotyledon and internode explants of *Citrullus lanatus* (Data collected after 30 days of culture)

Hormonal supplements (mg/l)	Sources of explants					
	Cotyledon			Internode		
	Days to callus initiation	Percentage of callus induction (M ±SE)	Callus colour	Days to callus initiation	Percentage of callus induction (M ±SE)	Callus colour
NAA						
0.2	10-12	60±2.3094	GC	24-25	20±1.1547	Cr
0.5	8-9	65±2.8867	GC	23-24	30±1.1547	Cr
1.0	10-12	60±2.3094	GC	22-23	40±0.5773	Cr
1.5	12-14	50±2.3094	Cr	18-20	50±2.3094	Cr
2.0	20-24	40±0.5773	Cr	16-18	60±2.3094	CrW
2, 4-D						
0.2	20-22	40±0.5773	Cr	22-23	30±1.1547	Cr
0.5	10-12	60±2.3094	Cr	18-20	40±0.5773	Cr
1.0	**7-8**	**90±1.1547**	**GC**	16-18	50±2.3094	Cr
1.5	9-10	80±1.1547	GC	14-15	60±2.3094	Cr
2.0	10-11	70±1.1547	GC	10-12	70±1.1547	CrW
2.5	15-16	50±2.3094	Cr	**8-10**	**80±1.1547**	**CrW**
BAP+2,4-D						
0.5+0.1	25-30	10±2.8867	Cr	20-22	40±0.5773	Cr
1.0+0.1	25-30	20±1.1547	Cr	16-17	50±2.3094	Cr
2.0+0.1	20-22	30±1.1547	Cr	9-10	65±2.8867	GrW
2.0+0.5	16-18	40±0.5773	Cr	12-13	60±2.3094	Cr
2.0+1.0	12-15	60±2.3094	Cr	15-17	60±2.3094	Cr

Note: Here, each value represents an average of 10 replicates and each experiment was replicated thrice following RCBD trial and Cr=Creamy, GC=Greenish Compact, Cr W=Creamy White and M = Mean and, SE = Standard Error

Shoot regeneration from induced callus

Cotyledon and internodes derived callus were cultured in BAP + NAA media composition and different concentrations (Table 2). The highest 80% calli induced from cotyledon produced shoots when the calli cultured in medium containing 1.0 mg/l BAP+0.2 mg/l NAA (Fig.1.B). Where as, only 20% calli produced shoots on MS medium supplemented with 2.0 mg/l BAP + 1.0 mg/l NAA. The highest mean number of shoots per culture was 5 recorded in medium containing 1.0 mg/l BAP + 0.2 mg/l NAA (Fig.1.C) and the highest mean length of longest shoot was 6 cm in the same medium. Again, 2.0 mg/ BAP + 1.0 mg/l NAA showed the lowest no. of shoots and lowest in shoot elongation both in cotyledon and internodal callus. On the contrary, the highest 70% calli produced shoots in the medium consisting of 1.0 mg/l BAP+0.1 mg/l NAA. Maximum number of shoots and elongation of shoots were also recorded in the same medium. Similar findings were also reported by Haque *et al.* (1995) in kakrol (*Momordica dioica* Roxb.) Thus, the cotyledon induced callus was better than that of internodal callus for shoot multiplication in the present study and it can also be concluded that MS medium supplemented with 1.0 mg/l BAP + 0.2 mg/l NAA was suitable for shooting from cotyledon induced callus.

Table 2. Effect of different concentrations of BAP+ NAA on shoot regeneration via callus derived from cotyledon and internode of *Citrullus lanatus.* (Data collected after 35 days of culture)

Hormonal supplements (mg/l)	Sources of explants					
	Cotyledon			Internode		
	Percentage of no. of calli regenerated shoot (M ±SE)	Mean number of shoot per callus (M ±SE)	Mean length shoot in cm (M ±SE)	No. of calli regenerated shoot (%) (M ±SE)	Mean number of shoot per callus (M ±SE)	Mean length shoot in cm (M ±SE)
BAP+NAA						
0.5+0.1	30±1.1547	3.0±0.5773	4.0±0.5773	40±1.7320	4.0±0.5773	3.0±0.5773
1.0+0.1	50±2.3094	4.0±0.5773	4.5±0.1443	**70±1.1547**	**5.0±0.5773**	**5.0±0.5773**
1.0+0.2	**80±1.1547**	**5.0±0.5773**	**6.0±0.5773**	50±2.3094	4.0±0.5773	4.0±0.5773
1.5+0.5	40±1.7320	4.0±0.5773	5.0±0.5773	40±1.7320	3.0±0.5773	3.0±0.5773
2.0+0.5	30±1.1547	3.0±0.5773	5.0±0.5773	30±1.1547	2.5±0.1443	2.5±0.1443
2.0+1.0	20±1.1547	2.0±0.5773	3.0±0.5773	20±1.1547	2.0±0.5773	2.0±0.5773

Note: Here, each value represents an average of 10 replicates and each experiment was replicated thrice following RCBD trial and M= Mean, SE = Standard error

Root induction and acclimatization

In vitro regenerated shoots collected from cotyledon induced callus was isolated and inoculated for root induction in MS medium supplemented with different concentrations of NAA and IBA singly (Table 3).

Table 3. Effect of different concentration of auxins on rooting from regenerated shoot from cotyledon derived callus. (Data collected after 25 days of culture)

Hormonal supplements mg/l	No. of explants inoculation	Days to root initiation	Percentage root induction (M ± SE)	Mean no. of root per explants (M ±SE)	Mean length of longest root (cm) (M ±SE)
NAA					
0.1	20	20-21	30 ±1.1547	3.0±0.5773	3.4 ±1.332
0.2	20	10-12	50 ±2.8867	6.5±0.1443	4.2 ±0.975
0.5	**20**	**8-9**	**80 ±1.1547**	**9.0 ±1.023**	**6.0±0.5773**
1.0	20	10-11	60 ±2.3094	6.5±0.1443	4.9±0.1154
1.5	20	15-20	50 ±1.1547	4.8 ±0.9237	3.5 ±1.1102
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

Note: Here, Each value represents an average of 10 replicates and each experiment was replicated thrice and M = Mean, and SE = Standard error

The highest number of roots per shoot was 100% observed in MS medium containing 1.0 mg/l IBA (Table 1, Fig.2.A) followed by 80% found in the media having 1.5 mg/l IBA, where as, highest 80% root induction was observed in the media having 0.5 mg/l NAA. On the other hand, only 30% rooting frequency was observed in media having 0.1 mg/l NAA. The highest number of roots (12) and elongation of roots were also observed in media containing 1.0 mg/l IBA. In the present study, IBA was found to be more effective than that of NAA between two auxins for root induction and growth of roots in *Citrullus lanatus.* These results are a good agreement with Ahad *et al.* (1994) in watermelon, Rani *et al*. (2006) in *Coleus blumei*, Kaliamoorthy *et al*. (2008) in *Harpagophytum procumbens*, and Hasan *et al*. (2008) in *Cassia alata.* When the plantlets were 8-10 cm long and had developed a good root system, they were gradually transferred from growth room to the small pots and kept there for seven days. Then the plantlets were regularly sprayed with water using a hand sprayer covered with polythene sheet to maintain high humidity around juvenile plants. Plantlets were subsequently transferred to larger pots (Fig.2.B) and gradually acclimatized to outdoor condition. The survival rate of the transferred plantlets to soil was 80% and their growth in natural environment was satisfactory.

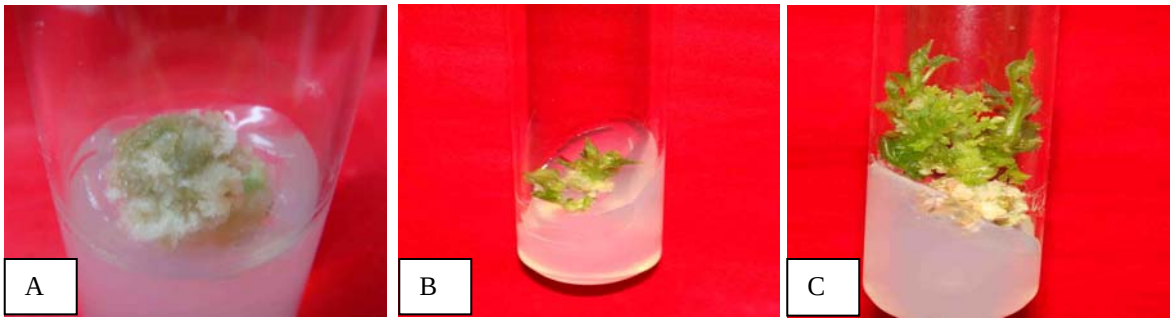

Fig. 1. *In vitro* callus induction and shoot proliferation from cotyledons derived callus
A. Mature callus, B. Shoot initiation, C. Multiple shoots

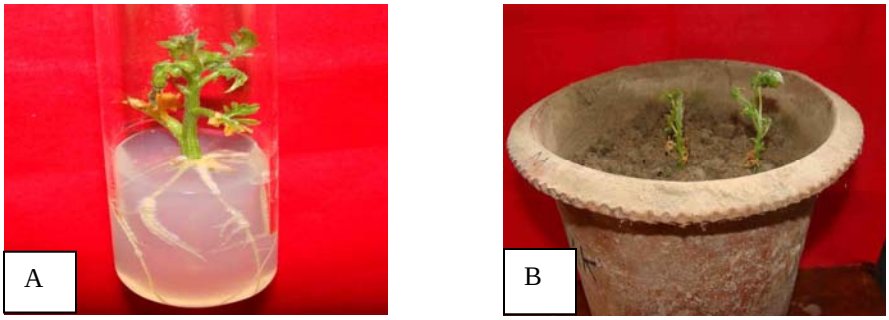

Fig. 2. Root induction and establishment of *in vitro* grown watermelon
A. Root induction, B. Regenerated plantlet under natural condition in the larger pot

CONCLUSION

In the present study, it was observed that 2, 4-D was found as the best auxin for callus induction in *Citrulus lanatus* Thumb. Again, the cotyledon induced callus performed better than the internodal callus for shoot multiplication which justified its further use in root induction. These results indicated that cotyledon might be used for suitable source of explants for the improvement of this crop through biotechnological approaches.

REFERENCES

Ahad A, Islam R, Hossain M, Khalekuzzaman M, Joarder OI (1994) Plant regeneration from immature and mature embryo axes of watermelon. Plant Tissue Cult. 4, 39-44.

Anonymous (1992) Yearbook of Agri. Stat. Bangladesh, Min. of Plann., Govt. of the Peoples Repub. Bangladesh, Dhaka.

Belaizi M, Paul H, Sangwan RS, Sangwan-Norrel BS (1991) Direct organgenesis from internodal segments of in vitro grown shoots of apple cv. Golden delicious. Plant Cell Rep. 9, 471-474.

Chee PP (1990) High frequency of somatic embryogenesisi and recovery of fertile cucumber plants. *Hort. Sci.* 25, 792-793.

Haque A, Islam R, Joarder OI (1995) *In vitro* plantlets differentiation in kakrol (*Momordica dioica* Roxb.). *Plant Tiss. Cult*. 5, 119-124.

Hasan MF, Das R, Rahman MS, Hossain MS, Rahman MR (2008) Micropropagation from shoot tips and nodal segments of *Cassia alata* L. *Intl. J. Bio Res.*, 4(4), 70-74.

Kaliamoorthy S, Naidoo G, Achar P (2008) Micropropagation of *Harpagophytum procumbens*. *Biol. Plantarum*. 52 (2), 191-194.

Moore GA **(**1986) *In vitro* propagation of *Citrus* rootstocks. Hort. Sci. 21, 300-301.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Plant Physiol*. 15, 473–497.

Nadel BL, Altman A, Ziv M (1989) Regulation of somatic embryogenesis in celery cell suspension. *Plant Cell Tiss. Org. Cult.* 18, 181-189.

Rani G, Talwar D, Nagpal A, Virk GS (2006) Micropropagation of *Coleus blumei* from nodal segments and shoot tips. *Biol.Plantarum*. 50(4), 496-500.

Rao AN, Yeon MS, Khothagoda N, Hutchinson JF (1982) Cotyledon tiss. Cult. of some tropical fruits. In: COSTEd Symp. on Tissue Culture of Economically Important Plants, AN Rao (Ed.), Singapore, pp.124-137.

Wehner TC (2005) Dept .of Hort. Sci., North Carolina State Univ., Releigh, NC: 27695-7609.