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EFFECT OF COLCHICINE APPLICATION ON *IN VITRO* REGENERATION OF TEASLE GOURD PLANTLETS

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ABSTRACT

Ahmed SSU, Karim MA, Haque MS, Ullah MA (2015) Effect of colchicine application on *in vitro* regeneration of teasle gourd plantlets. *Int. J. Sustain. Crop Prod.* 10(3), 45-50.

To develop *in vitro* regeneration protocol several explants (Node, internode, leaf, leaf petiole, shoot tip and cotyledon) and growth regulators (BA, NAA, IBA, IAA) were used for callus, shoot and root induction from teasle gourd (*Momordica dioeca*) plant. Cotyledon was found to be the best explants and BA at 1.0 mgL^{-1} was found to be the best for callus initiation and shoot regeneration. IBA at 0.3 mgL^{-1} was the best for root initiation in ½ MS medium with 3% sucrose. Mature calli were soaked in colchicine solution of 0.05% concentration for short duration (1, 2, 4 & 8 hours). Simultaneously, 4 different concentrations of colchicine (10, 20, 30 & 40 mgL^{-1}) at longer durations (3, 6 & 9 days) were used. Low concentration at short duration (10 mgL^{-1} for 3 days) had high percentage (90%) of survivability. The highest size (4.0 cm) and weight (5.4 g) of calli was found in 10 mgL^{-1} colchicine treatment for 3 days. Also highest shoot germination from callus (60%), maximum shoot per flask (32), shoot length (3.0 cm), maximum root per plant (24) and length of root (2.8 cm) was found in this treatment. Four hour treatment of colchicine at 0.05% concentration resulted in vigorous vegetative growth. Rooted plantlets of about 4-6 cm height (2-3 nodes) were subsequently transferred to different media for acclimatization. Plants grown in mixture of soil and cowdung at the proportion of 3:1 and showed 100% of survival rate during rainy season.

Key wards: *teasle gourd, cotyledon, callus, colchicine, explants, acclimatization*

INTRODUCTION

Teasle gourd (*Momordica dioica* Roxb.) belongs to the family Cucurbitaceae is one of the popular vegetables in Bangladesh. Green fruits of teasle gourd are used as vegetable. In winter, a good number of vegetables are available in Bangladesh but in summer it is scanty. Among the summer vegetables, teasle gourd is an important one, which has a high economic value with export potentials in the local market. The average yield of teasle gourd is only 1.66 tons per acre which is too low. Whereas 10-12 tons per acre of this vegetable may be produced if proper production technology is followed (Rahman *et al.* 2004). Lack of high yielding varieties is one of the factors for poor performances. Hence development of improved cultivars becomes the top priority to overcome the poor yield levels. Few land races are available for commercial cultivation in Bangladesh (Mian *et al.* 2001) but their yield is low. Farmers generally desire to get higher number of larger fruits per plant. Variety improvement in teasle gourd is handicapped by its dioecious nature. Vegetative propagation of teasle gourd has blocked its variety improvement in traditional breeding. It may be possible to improve teasle gourd by applying some mutagens during tissue culture technique. Induction of somaclonal variation through callus culture may also provide high yielding plant. Use of mutagens (colchicine) during tissue culture method may bring heterogenecity, which may be a mean of producing improved plant. Therefore the present study was undertaken to obtain the various regenerants from the colchicine treated calli.

MATERIALS AND METHODS

Twenty three teasle gourd (*Momordica dioica* Roxb.) accessions were collected from different agro-ecological zones of Bangladesh for this study. Field experiments were conducted at the field of Crop Botany Department, Bangladesh Agricultural University, Mymensingh during the period from 2008 to 2010 to study 33 morphological characters with the objective to find out the best accession. To develop *in vitro* regeneration protocol, internode, node, leaf, petiole, shoot tip, cotyledon, epicotyle, endosperm and anther explants were used from accession # TG0022. Fast growing callus (4 weeks older after explant culture) was obtained from cotyledon of immature fruit (18 days old fruits after anthesis) from the teasle gourd. Sterilization of colchicine was made by using filter (Pore size 0.22 µ mesh) and mix with sterile water. Soaking of calli in colchicine solution (0.05%) for 0, 1, 2, 4 and 8 hours. The flasks containing 20 calli pieces (around 5mm diameter) per flask soaked by the liquid colchicine medium. After treatment for a varying length of time, surviving calli were washed several times with sterilized distilled water and transferred to solid regeneration medium containing 1 mgL^{-1} BA. Another treatment was soaking of calli by colchicine at four concentrations (10, 20, 30 & 40 mgL^{-1}) for three different durations (3, 6 & 9 days). Filter sterilized colchicine was mixed with MS medium (after sterilization of medium when medium temperature is around 50°C) at the rate of 10, 20, 30 & 40 mgL^{-1}. After 3, 6 and 9 days the calli were subcultured in MS medium with 1 mgL^{-1} BA. MS medium were adjusted to p^H 5.8 prior to sterilization. Cultures were kept at 24 ± 2ºC.

RESULTS AND DISCUSSION

Effect of colchicine on calli at 1, 2, 4 & 8 hours

The different soaking duration in 0.05% aqueous solution of colchicine for 1, 2, 4 and 8 hours had significant influence on calli growth after 30 days of treatment (Table 1). The survivability of the control was noticed to be 95 percent. Lower duration (1h) and higher duration (8h) of colchicine showed 56% and 13% survivability, respectively (Fig. 1). Hamill *et al.* (1992) found that 90% survivability of shoot tip explants of banana at 0.05% aqueous solution of colchicine for 4h.

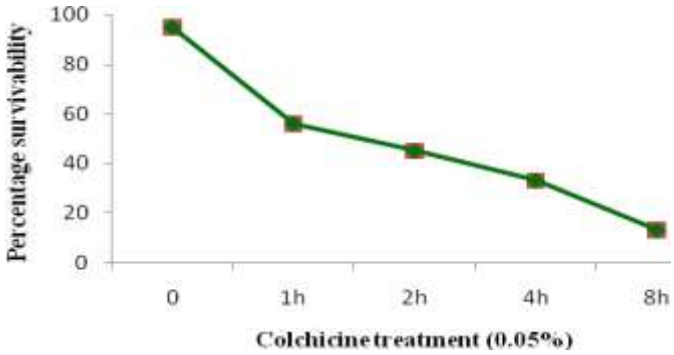

Fig. 1. Effect of colchicine over the periods on survivality of calli

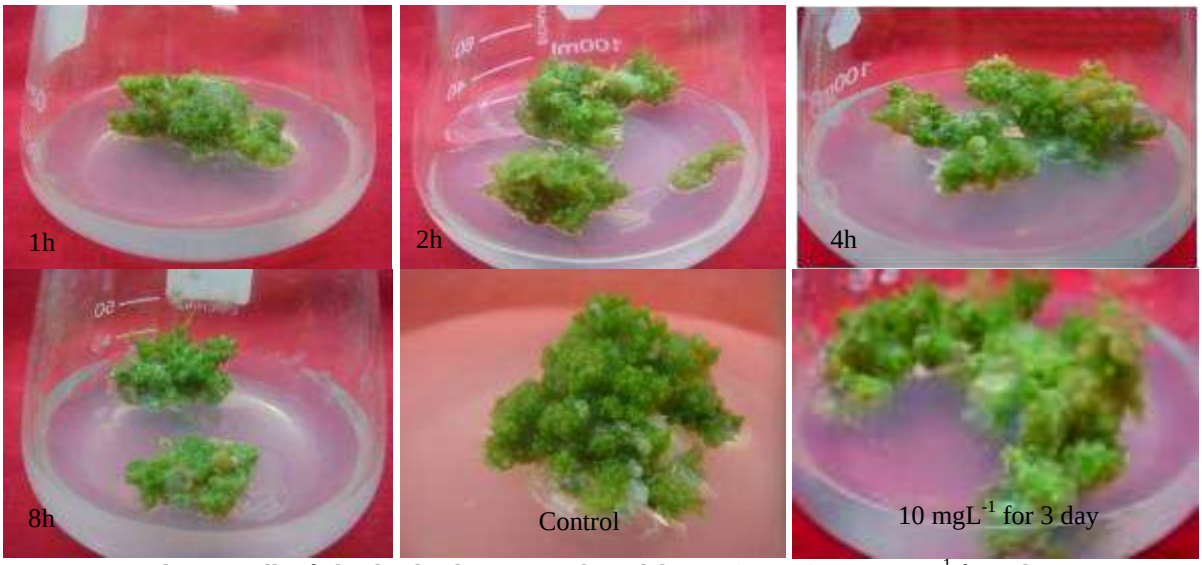

Plate 1. Calli of 1h, 2h, 4h, 8h treatment by colchicine (0.05%) & 10 mgL^{-1} for 3 day

Maximum size (3.0 cm) and weight (3.3 g) of calli were obtained in control. Minimum size (1.1 cm) and weight (1.4 g) of calli were found in 8h treated explants. The color of calli was deep green in control and 4h treatment (Plate 1). Saradhuldhat and Silayoi (2001) reported that the survivality of plantlets of banana was 61.11%, 50% and 39.89% with soaking duration 2.5, 5 and 7.5 hours by colchicine, respectively.

Table 1. Effect of colchicine on callus at different hours

Soaking duration in colchicine (0.05%)	Callus Treated	Callus survived	Size (cm)		Weight (g)		Color
			Initial size of explant	Size of callus after 30 days	Initial weight of callus	Weight of callus at 30 days	
Control	20	19.0a	0.5	3.0a	0.25	3.3a	Deep green
1 hour	20	11.2b	0.5	1.3b	0.25	2.0c	green
2 hour	20	9.0c	0.5	1.3b	0.25	2.4b	green
4 hour	20	6.6d	0.5	1.4b	0.25	2.6b	Deep green
8 hour	20	2.6e	0.5	1.1b	0.25	1.4d	green
Level of significance		***		***		***	

Mean values for 5 replicates of each type of hormone concentration.
Mean values followed by same letter(s) do not differ significantly. *** $P < 0.001$ (0.1%)

Effect of colchicine on calli at 3, 6 & 9 days duration

The effect of different concentrations and time had significant variation on percent survivability of calli. Colchicine treated calli resulted vigorous and enlarged growth. Low concentration at short duration had high percentage of survivability. Maximum survivalily (95%) of calli was observed in control treatment whereas minimum (12%) was observed in 40 mgL^{-1} for 6 days treatment (Fig. 2). Effect of colchicine (0, 10, 20, 30 & 40 mgL^{-1}) on calli regeneration of gerbera which declined with increasing concentration of colchicine and finally there was no germination in 40 mgL^{-1} at presoaked seeds (Altaf *et al.* 2009).

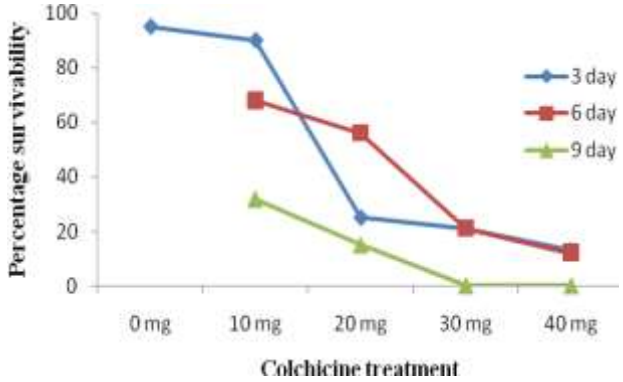

Fig. 2. Effect of colchicine over the periods on survivality of calli

The highest size (4.0 cm) and weight (5.4 g) of calli was found in 10 mgL^{-1} colchicine treatment for 3 days and this concentration of colchicine was better perform among the treatment (Table 2, Plate 1). Chane and Kallemeyn (1978) found that in daylily callus treatment at 10, 20, and 40 mgL^{-1} for 3 days resulted 50% of the plants initiated were completely tetraploid and 20 mgL^{-1} treatment appeared to be the most effective in production of tetraploid.

Table 2. Effect of colchicine on calli at different time and concentrations for the growth of calli

Colchicine treatment		Treated callus	Callus survived	Size (cm)		Weight (g)	
Duration (Day)	Conc. of colchicine (mgL^{-1})			Initial size of explant	Size of callus after 30 days	Initial weight of callus	Weight of callus after 30 days
Control	0	20	19.0a	0.5	3.0b	0.25	3.3b
3	10	20	18.0a	0.5	4.0a	0.25	5.4a
	20	20	5.0de	0.5	2.1cd	0.25	2.6c
	30	20	4.2def	0.5	1.7de	0.25	2.2c
	40	20	2.6f	0.5	1.3f	0.25	1.2d
6	10	20	13.6b	0.5	1.3ef	0.25	1.5d
	20	20	11.2c	0.5	2.0cd	0.25	2.4c
	30	20	4.2def	0.5	1.5ef	0.25	1.6d
	40	20	2.4f	0.5	1.2f	0.25	1.3d
9	10	20	6.4d	0.5	2.2c	0.25	2.2c
	20	20	3.0ef	0.5	2.2c	0.25	2.3c
	30	20	-	0.5	-	0.25	-
	40	20	-	0.5	-	0.25	-
Level of significance			***		***		***

Mean values for 5 replicates of each type of hormone concentrations.
Mean values followed by same letter(s) do not differ significantly. *** $P < 0.001$ (0.1%)

Effect of colchicine on shoot induction at 1, 2, 4 & 8 hours treatment

Different soaking time duration in 0.05% aqua's solution of colchicine for 1, 2, 4 and 8 hours showed significant differences on percent shoot regeneration, days required for shoot initiation, number of shoot $flask^{-1}$ and shoot length at 60 days after treatment. The highest shoot regeneration frequency (94%) was found in control which differed significantly from all other treatments. The lowest percentage of shoot regeneration (20%) was found at 1 h treatment which was significantly different from all treatments (Table 3). Minimum days (21.6) required for shoot initiation was found in 4h treatment. Whereas, maximum days (49.0) required for shoot initiation was observed in control.

Plate 2. Effect of colchicine on shoot growth
(A) Shooting from 4 h treatment & (B) Shooting from 10 mgL^{-1} for 3day treatment

Significantly highest number of shoots $flask^{-1}$ (19.1) and shoot length (2.0 cm) was found in control treatment. The maximum number of shoots $flask^{-1}$ (9.6) and shoot regeneration (73%) was observed at 4h treatment which was better then others treatment. Maine and Simpson (1999) found that four different times (1, 5, 10, 24 h) for three colchicine concentration (0.01, 0.05 & 0.07%) on potato callus resulted reduced shoot growth by increasing time of treatment.

Table 3. Effect of colchicine on shoot growth at different time duration

Soaking duration (0.05%)	Callus for shooting	Callus generated shoots	% shoot regeneration after 60 days	Days to shoot initiation after treatment	Shoot $flask^{-1}$ after 60 days	Shoot length at 30 days (cm)
Control	20	18.8a	94a	49.0c	19.1a	2.0a
1h	20	4.0d	20d	48.8c	1.8d	0.4c
2h	20	9.0c	45c	42.2b	5.0c	0.5bc
4h	20	14.6b	73b	21.6a	9.6b	0.8b
8h	20	-	-	-	-	-
Level of significance		***	***	***	***	***

Mean values for 5 replicates of each type of hormone concentration.
Mean values followed by same letter(s) do not differ significantly. *** $P < 0.001$ (0.1%)

Effect of colchicine on shoot growth at different days (3, 6 and 9 days)

Different colchicine treatments (10, 20, 30 & 40 mgL^{-1}) for different number of days (3, 6 and 9 days) showed significant differences on percent shoot regeneration, days required for shoot initiation, number of shoot $flask^{-1}$ and shoot length at 90 days after treatment. The highest shoot regeneration frequency 62%, lowest days required (48.8 days) for shoot initiation and highest shoot per flask (32) was found in 10 mgL^{-1} for 3 days treatment than other colchicine treatment (Table 4). Whereas the lowest frequency (12%) was recorded from 20 mgL^{-1} for 9 days treatment. Mujib (2005) conducted an experiment on pineapple calli and treated by colchicine 100 mgL^{-1} for 7, 8 and 10 days in liquid medium on a rotary shaker (120 rpm). A few calli showed early death and found 60.1% shoot regeneration. Raza *et al.* (2003) found the effect of different concentrations of colchicine (0.01, 0.05 & 0.1%) on watermelon seeds and found that 17.6, 10.2 & 7.6% shoot induction for 4 days treatment and 10.2, 5.0 & 3.7% shoot induction for 7 days treatment, respectively.

Table 4. Effect of colchicine on shoot growth at different days

Colchicine treatment		Callus for shooting	Callus generated Shoot	% Shoot regeneration	Days to shoot initiation	Shoot $flask^{-1}$ after 90 days	Shoot length (cm) after 90 days
Duration (Days)	Conc. (mgL^{-1})						
3	Control	20	18.8a	94a	49.0d	23.1b	3.0b
	10	20	12.4b	62b	48.8d	32.0a	3.0b
	20	20	8.6c	43c	63.2c	15.8c	0.2d
	30	20	2.8de	14de	82.0a	1.6e	2.6bc
	40	20	-	-	-	-	
6	10	20	3.2de	16de	78.0ab	7.2d	2.6bc
	20	20	7.8c	39c	72.4b	21.2b	1.7c
	30	20	-	-	-	-	
	40	20	-	-	-	-	
9	10	20	4.8d	24d	51.6d	8.8d	4.8a
	20	20	2.4e	12e	58.0c	6.8d	3.4b
	30	20	-	-	-	-	-
	40	20	-	-	-	-	-
Level of significance			***	***	***	***	***

Mean values for 5 replicates of each type of hormone concentration.
Mean values followed by same letter(s) do not differ significantly. *** $P < 0.001$ (0.1%)

Effect of colchicine on root induction at 1 hr, 2 hrs, 4 hrs treatment

Shoots were cultured on half strength MS medium supplemented with 0.3mgL^{-1} IBA (Table 5). The control required lowest number of days (17.2) for root induction which was similar to 2h and 4h treatments, respectively. The maximum days (29.2) were required for root induction was in 1h treatment. The highest number of roots (22.0) was observed in 4h treatment (Plate 3) which was similar to the control. Whereas, the minimum roots (2.2) was observed in 1h treatment. The highest length of roots (5.6 cm) was observed in control and the minimum length of root (0.9 cm) was observed in 1h treatment.

Table 5. Effect of colchicine on root induction at different time duration

Soaking duration (0.05%)	Days to root induction	Number of roots Plantlets^{-1} after 15 days	Length of roots after 15 days (cm)
Control	17.2b	21.4a	5.6a
1h	29.2a	2.2c	0.9d
2h	20.4b	15.8b	2.3c
4h	17.6b	22.0a	3.6b
8h	-	-	-
Level of significant	***	***	***

Mean values for 5 replicates of each type of hormone concentration.
Mean values followed by same letter(s) do not differ significantly. *** $P < 0.001$ (0.1%)

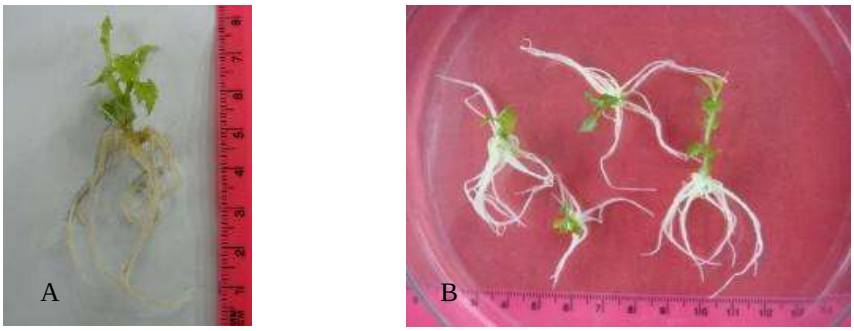

Plate 3. Effect of colchicin on root growth
(A) Plantlet of 4h treatment & (B) Plantlet of 10 mgL^{-1} for 3 day treatment

Effect of colchicine on root growth at 3, 6 & 9 days treatment

The highest number of roots (24.6) was observed in treatment of 10 mgL^{-1} for 3 days which did not differ significantly with the control whereas the lowest number of roots (10.2) was found in10 mgL^{-1} for 6 days treatment (Table 6, Plate 3). The maximum length of roots (7.4 cm) was observed in treatment of 10 mgL^{-1} for 9 days. Raza *et al.* (2003) observed the effect of different concentrations of colchicine (0.01, 0.05 & 0.1%) on watermelon seeds and found 2.18, 2.08 and 2.13 number of roots at 4 days and 1.08, 1.0 and 0.98 roots at 7 days, respectively.

Table 6. Effect of colchicine on root induction at different da

Colchicine treatment		No. of roots plantlet^{-1} after 15 days	Length of roots (cm) after 15 days
Duration (Day)	Conc. of colchicine (mgL^{-1})		
	Control	21.4ab	5.6b
3	10	24.6a	2.8c
	20	19.4b	2.4c
6	10	10.2d	0.9d
	20	20.0b	4.8b
9	10	15.0c	7.4a
	20	10.6d	2.4c
Level of significant		***	***

Mean values for 5 replicates of each type of hormone concentration.
Mean values followed by same letter(s) do not differ significantly. *** $P < 0.001$ (0.1%)

CONCLUSION

It was concluded that 10 mgL^{-1} for 3 days colchicine treatment showed the best performance among the treatments. The highest survival percentage, highest callus size and weight, shoot regeneration, number of shoot per callus, shoot length also quick shoot formation and highest root formation was found in this treatment compeer with other treatments.

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