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RESEARCH ARTICLE

INDIRECT ORGANOGENESIS IN WILD SNAKE GOURD (*TRICHOSANTHES CUCUMERINA* L. VAR. *CUCUMERINA*)

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ABSTRACT

Efficient plant regeneration via organogenesis was established for medicinally important *Trichosanthes cucumerina* L. var. *cucumerina* using hypocotyl and leaf derived calli. Seeds were surface sterilized in 0.1% $HgCl_2$ for two minutes and germinated *in vitro* in MS media without plant growth regulators. The maximum morphogenic callus induction rate (86%) was observed from a hypocotyl explant by culturing in MS medium supplemented with 0.5 mg^{-1} 2, 4-D + 1.0 mg^{-1} BAP. Calli size and fresh weight increased substantially through sub culturing. The highest percentage of regeneration (85%) and highest mean number of shoots 15.2 per culture were obtained with 1.5 mg^{-1} BAP + 0.5 mg^{-1} GA_3. Hypocotyl explants were more responsive than leaf explants in terms of callus induction and subsequent plant regeneration. Regenerated shoots were rooted in MS ½ strength medium supplemented with 1.0 mg^{-1} IBA. About 80% of regenerated plantlets were survived and showed new branch development under *ex vitro* condition.

INTRODUCTION

Trichosanthes cucumerina L. var. *cucumerina* Linn. is medicinally significant plant belongs to the family Cucurbitaceae and is distributed in throughout India, Bangladesh, Sri Lanka, Burma, Malaysia, Australia (**Chakravarty 1982**). It is perennial climber with an attractive white flower. It is highly bitter in taste the bitter taste may suppose to contain medicinal properties (**Choudhary 1967**), as it has proved as an anti-diabetic (**Kiran and Srinivasan 2008**); an antimicrobial agent (**Devendra *et al.*, 2009**); traditional system of medicines (**Devendra *et al.*, 2008**) and other significant Ayurvedic preparations of *T. cucumerina* L. var. *cucumerina* are Patoladi-kvatha, Patoladi gana kvatha, Kalingadi kvatha, Patolakaturohinnyadi kashayam, Mahatiktaka ghratam, Vajrakam kasayam, Mahatiktaka kasayam, more than 16 commercially important drugs are available in the market in which *T. cucumerina* L. var. *cucumerina* is one of the important ingredient. Because of its wide usage and unscientific method of harvestment, plant population is declining drastically and included in the threatened list (**Parvathi menon, 2003**).

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MATERIALS AND METHODS

Plant material and explant sources

The mature seeds of *T. cucumerina* L. var. *cucumerina* were collected from Khanapur forest of Bidar District. Karnataka, India. Seeds were decoated, washed with 5% (v/v) detergent solution (Teepol Qualigen, India) for 5 min. and then in running tap water for 15 min, surface sterilization was done with freshly prepared 0.1% (w/v) aqueous mercuric chloride solution for 3 min. followed by repeated washing with sterile distilled water, these sterilized seeds were placed on a germination medium containing MS basal salts, vitamins and 3% (w/v) sucrose. The pH of the medium was adjusted to 5.7 before the addition of 0.8% agar.

Callus induction and proliferation

The leaf, cotyledon, epicotyl and hypocotyl explants were obtained from the germinating seedling (7-9 days old) cut in to pieces and were placed on MS basal medium supplemented with 2,4-D, NAA, 2,4,5-T, IBA and IAA (0.5-2.5 mg^{-1}) alone or in combination with BAP and Kn (0.5-2.5 mg^{-1}). Once the optimum phytohormone concentration had been established for callus induction. Calli were maintained on MS medium supplemented with same concentrations of hormone.

For each subculture ~250 mg of fresh callus was transferred. Media were adjusted to pH 5.6 - 5.8 and autoclaved at 15 psi pressure and 121^{0}C for 20 min.

Organogenesis

Fragments of callus varying 200 to 250 mg derived from leaf and hypocotyl explants was transferred on to MS basal medium supplemented with BAP (0.5-2.5 mg^{-l}) and Kn (0.5-2.5 mg^{-l}) individually and kept under culture room with light of 2000 lux with 85% relative humidity and 25±2^{0}C for plant regeneration. The average number of shoots and shoot length was recorded after 30 days of incubation. The developed shoots were further cultured for *in vitro* rooting to develop a complete plantlet.
Induction of rooting and acclimatization

The elongated shoots (4-5 cm) were excised from callus and than transferred to half strength MS basal semisolid medium supplemented with different concentrations of IAA (0.5, 1.0 and 1.5 mg^{-l}), IBA (0.5, 1.0 and 1.5 mg^{-l}) and NAA (0.5, 1.0 and 1.5 mg^{-l}) with 3% (w/v) sucrose tested individually for root initiation. One excised shoot was cultured in each tube (25x150 mm) containing 15 ml of culture medium. Temperature and photoperiod were same as for shoot multiplication. Rooted micro shoots were thoroughly washed to remove the adhering gel and planted in earthen pots containing a mixture of soil, sand and farmyard mixture in the ratio of 1:1:1 and grown in the green house for acclimatization. Watering was made at 2-day intervals. Percentage of survival was recorded 1 month after transfer.

Data analysis

All the experiments were repeated three times with 10 replicates per treatments, Data on bud proliferation state, percentage of regeneration, number of shoots per explants and shoot length were statistically analyzed using the procedure of SPSS package version X. Shoot regeneration efficiency was calculated as the percentage of the number of explants that regenerated shoots out of the total number of explants cultured in each treatment. Shoot number per explant was calculated as the number of regenerated shoots from each explant with bud in each treatment.

RESULTS AND DISCUSSION

Callus was induced on medium containing 2,4-D and IBA from both leaf and hypocotyl explants. The highest frequency of callus induction was observed in MS medium containing 0.5 mg^{-l} 2,4-D and 1.0 mg^{-l} IBA. Among both the explants hypocotyl was more responsive than leaf. Levels of 2,4-D and IBA above or below this gradually decreased the frequency of callus induction (Table 1).

The callus produced was compact, friable, and whitish to yellowish mass developed from leaf and hypocotyl explants. Callus initiated from 3rd day in leaf explants and from 5th day in hypocotyl on MS medium supplemented with lone concentration of IBA (1.0mg^{-l}) and 2,4-D (0.5mg^{-l}). From the literature it is evident that 2,4-D is the most widely used auxins for *in vitro* callus induction in a wide range of plant species. In our study, successful induction of potentially organogenic callus from leaf and hypocotyl was achieved using 2,4-D. similar results were reported previously (**Leljak-Levenic *et al*., 2004**) in *C. pepo* callus morphology was also in agreement with **Thomas and Sreejesh (2004)** as in *B. hispida* and with **Brachard and Chateau (1988)** in melon calli, as we obtained. Beyond 3.0mg^{-l} the percent frequency of callus induction was very poor and non-significant.

Table 1. Effect of various concentrations of auxins alone for callus induction from different explants of *T. cucumerina* L. var. *cucumerina*

S.No.	Hormone (mgl^{-l})	% explants producing callus			
		Cotyledon	Leaf	Hypocotyl	Epicotyl
1.	2,4-D (0.5)	60	90	100	20
2.	NAA (0.5)	40	60	50	40
3.	2,4,5-T (2.0)	40	70	40	20
4.	IAA (2.5)	20	40	20	10
5.	IBA (1.0)	60	100	100	50

Effect of IBA with BAP/Kn on growth of callus

The callus obtained from leaf and hypocotyl explants (about 250mg) was inoculated on MS medium supplemented with IBA and different concentrations of BAP for studying the effect of these hormones on further growth of callus by keeping the IBA concentration constant at 1.0mg^{-l}. Higher concentrations of IBA did not support the callus growth. From the data it is clear that 1.0mg^{-l} IBA + 0.5mg^{-l} BAP promoted the maximum proliferation and growth of callus with fresh weight 4822 ± 46.07 and dry weight of 350 ± 21.21, Where as IBA 1.0mg^{-l} + BAP 1.0mg^{-l} produced moderate amount of callus followed by IBA 1.0mg^{-l} + BAP 1.5mg^{-l}. As the concentration of BAP was increased from 0.5 to 2.5mg^{-l} there was a sudden decrease in the callus growth and produced minimum amount of callus (Table 2). As the growth period increased the amount of callus also increased at 30 days the amount of callus was maximum in all the concentrations tried and the callus produced was yellowish and friable in nature.

Whereas 1.0mg^{-l} IBA + 1.5mg^{-l} Kn also promotes good amount callus followed by 1.0mg^{-l} IBA + 1.0mg^{-l} Kn but it was less porliferative and compact and at 1.0mg^{-l} IBA + 0.5mg^{-l} Kn the amount of callus was very poor (Table 2).

Effect of 2,4-D with BAP/Kn on growth of callus

The callus initiated from hypocotyl explant (about 200 mg) was inoculated on MS basal medium supplemented with different concentrations of 2,4-D 0.5 mg^{-l} and BAP 0.5-2.5 mg^{-l} for studying the effect of these hormones on further growth of callus (Table 3). Watery spongy, very compact brown dead portions of calli were discarded during every sub culture. 2,4-D 0.5mg^{-l} and BAP 1.0mg^{-l} favored the maximum growth of callus and was found to be best in terms of obtaining proliferative and maximum amount of callus with fresh weight 4810±103.92 and dry weight of 344±9.27 (Fig. 1, a). Where as 0.5 mg^{-l} 2,4-D + 1.5 mg^{-l} Kn also promotes good amount callus followed by 0.5 mg^{-l} 2,4-D + 1.0 mg^{-l} Kn but it was less porliferative and compact and at 0.5mg^{-l} 2,4-D + 2.5mg^{-l} Kn the amount of callus was less (Table 3).

Table 2. Effect of IBA and BAP/ Kn on growth of callus derived from leaf and hypocotyls explants of *T. cucumerina* L. var. *cucumerina*

S.No.	Hormone (mgl^{-1})	Leaf		Hypocotyl	
		Fresh weight mg/culture	Dry weight mg/culture	Fresh weight mg/culture	Dry weight mg/culture
1.	IBA (1.0) + BAP (0.5)	4822±46.07	350 ±21.21	2446±30.43	190±3.16
2.	IBA (1.0) + BAP (1.0)	3108±12.4	258±5.9	2966±17.20	226±5.08
3.	IBA (1.0) + BAP (1.5)	2950±5.77	230±5.77	1912±53.41	168±8.60
4.	IBA (1.0) + BAP (2.0)	2163±8.81	190±5.77	1764±6.78	134±5.09
5.	IBA (1.0) + BAP (2.5)	1876±13.33	166±6.66	1506±14.00	116±5.09
6	IBA (1.0) + Kn (0.5)	2846±12.01	246±8.8	1874±9.27	144±5.09
7	IBA (1.0) + Kn (1.0)	3576±8.81	270±5.77	2196±53.81	202±8.60
8	IBA (1.0) + Kn (1.5)	4043±23.33	323±8.81	2558±15.62	226±5.06
9	IBA (1.0) + Kn (2.0)	3250±23.09	233±12.01	1972±3.74	174±5.09
10	IBA (1.0) + Kn (2.5)	2980±5.77	230±5.77	1538±6.63	130±3.1

Date represents average of three replicates; each replicate consists of 10 cultures.
Mean ± Standard error.

Figure 1. *In vitro* shoot regeneration from leaf callus culture of *Trichosanthes cucumerina* L var. *cucumerina* on MS medium

a. Callus obtained from hypocotyl after 30 days
b. Organogenic calli showing green point on MS medium containing BAP 1.5 mg^{-1}
c. Development of plantlet from callus on MS medium containing BAP 1.5 mg^{-1} after 30 days
d. Elongation of shoot on MS medium containing BAP 1.5 mg^{-1} + GA_3 1.0 mg^{-1}.
e. Rooting from micro shoot on MS medium containing IBA 1.0 mg^{-1}.
f. Completely hardened plantlet in the earthen pot

Table 3. Effect of 2,4-D and BAP/ Kn on growth of callus derived from leaf and hypocotyl explants of *T. cucumerina* L. var. *cucumerina*

S.No.	Hormone (mgl^{-1})	Leaf		Hypocotyl	
		Fresh weight mg/culture	Dry weight mg/culture	Fresh weight mg/culture	Dry weight mg/culture
1.	2,4-D (0.5) + BAP (0.5)	3874±6.78	288±3.74	4650±14.91	314±7.48
2.	2,4-D (0.5) + BAP (1.0)	3480±7.07	266±8.71	4810±103.92	344±9.27
3.	2,4-D (0.5) + BAP (1.5)	2962±9.48	214±5.09	4364±14.35	278±5.83
4.	2,4-D (0.5) + BAP (2.0)	2668±8.0	184±5.08	3838±14.69	260±7.07
5.	2,4-D (0.5) + BAP (2.5)	2618±9.1	154±7.48	3810±15.93	244±5.09
6	2,4-D (0.5) + Kn (0.5)	2636±12.08	258±5.8	3044±9.27	214±5.07
7	2,4-D (0.5) + Kn (1.0)	2930±27.0	176±8.7	3264±16.00	218±3.74
8	2,4-D (0.5) + Kn (1.5)	3052±13.92	224±6.0	3446±13.63	234±8.12
9	2,4-D (0.5) + Kn (2.0)	1762±17.1	124±6.7	3262±8.60	212±3.74
10	2,4-D (0.5) + Kn (2.5)	1574±10.77	112±5.83	3170±7.07	186±5.09

Date represents average of three replicates; each replicate consists of 10 cultures.
Mean ± Standard error.

Table 4. *In vitro* shoot regeneration from callus cultures of *T. cucumerina* L. var. *cucumerina*

S.No.	Hormone (mgl^{-1})	Leaf		hypocotyl	
		Average number of shoots	Average shoot length (cm)	Average of shoots	Average shoot length (cm)
1.	BAP (0.5)	3.6 ± 0.42	2.0 ± 0.47	4.3 ± 0.27	1.6 ± 0.08
2.	BAP (1.0)	5.6 ± 0.33	2.2 ± 0.34	7.5 ± 0.86	2.7 ± 0.86
3.	BAP (1.5)	6.25± 0.41	3.1 ± 0.32	10.6 ± 0.27	2.8 ± 0.16
4.	BAP (2.0)	4.9 ± 0.16	2.6 ± 0.14	6.6 ± 0.14	2.3 ± 0.16
5.	BAP (2.5)	4.3 ± 0.39	3.2 ± 0.49	5.8 ± 0.41	1.7 ± 0.39

Date represents average of three replicates; each replicate consists of 10 cultures. Mean ± Standard error.

Table 5. Effect of GA_3 on shoot elongation of multiple shoots derived from callus cultures of *T. cucumerina* L. var. *cucumerina*

S.No.	Hormone (mgl^{-1})	Leaf		hypocotyl	
		Average shoot length (cm)	Average shoot length (cm)	Average shoot length (cm)	Average shoot length (cm)
1.	BAP(1.5) + GA_3(0.5)	8.4 ± 0.16	10.3 ± 0.36	15.2 ± 0.66	10.4 ± 0.65
2.	BAP (1.5) + GA_3(1.0)	7.8 ± 0.25	11.2 ± 0.68	13.3 ± 0.36	10.6 ± 0.27
3.	BAP(1.5) + GA_3(1.5)	7.5 ± 0.41	11.5 ± 0.14	12.3 ± 0.30	13.9 ± 0.24

Date represents average of three replicates; each replicate consists of 10 cultures. Mean ± Standard error.

Table 6. Effect of culture media (half strength MS + different concentrations of auxins + 3% (w/v) sucrose) on rooting response of *T. cucumerina* L. var. *cucumerina*, after 4 week of culture

Type of auxins	Concentrations of auxins (mg^{-l})	Percentage of shoots rooted	Days to rooting	No. of roots per shoot	Average length of root in (cm)
IAA	0.5	43.3	13	4.00 ±1.04	3.14 ± 0.88
	1.0	66.6	9-10	13.71 ± 0.83	7.42 ± 0.57
	1.5	56.6	12-13	3.57 ± 0.71	3.28 ± 0.60
IBA	0.5	46.6	10	4.87 ± 1.45	3.12 ± 0.95
	1.0	73.3	7-8	20.87 ± 0.97	8.75 ± 0.45
	1.5	63.3	9-10	7.50 ± 1.65	5.75 ± 1.29
NAA	0.5	63.3	16-17	3.00 ± 0.75	2.25 ± 0.53
	1.0	70.0	12-13	7.62 ± 0.46	4.25 ± 0.53
	1.5	66.6	11-12	4.37 ± 0.80	2.75 ± 0.42

Among the cultures derived from leaf and hypocotyls explants, leaf derived callus was more proliferate yellowish and friable than that of hypocotyls derived callus which is whitish and moderately friable.

In vitro shoot regeneration from calluses

The friable, nodular calli were assumed potentially organogenic were obtained from $1.0mg^{-l}$ IBA + $0.5mg^{-l}$ BAP (leaf) and from 2,4-D $0.5mg^{-l}$ and BAP $1.0mg^{-l}$ (hypocotyls) were selected for maintenance and regeneration. For adventitious shoot induction, calli were cultured in MS medium supplemented with varying concentrations of BAP was studied. After 2-3 weeks of culture on regeneration medium organogenesis was observed on calli induced from hypocotyls first. In this case, green spots and development of several spots were observed gradually from a single callus mass.

The shoot like structures could be distinguished by the presence of green, opaque and compact nodules. Distinct shoot tip like structures were observed as shown in Fig-1,b indicating a monopolar structure characteristic of shoots. Such structures have been described for organogenesis in water melon (**Yalcin-Mendi *et al*., 2003; Krug *et al*., 2005**). Table 4, represents data obtained on average number of shoots and shoots length from leaf and hypocotyls derived callus after 30 days of incubation.

The pale yellow callus was sub cultured on cytokinin alone. It proliferated rapidly and became organogenic within 3 weeks. Among the cytokinins used BAP responded positively to induce organogenic callus through shoot regeneration, but Kn did not shown any organogenic response (in both leaf and hypocotyls derived callus) in any of the concentration tested for three successive subcultures. Hence, it is a poor hormone for organogenesis.

BAP at 0.5mg^{-1} failed to induce potent organogenic callus, when the concentration of BAP was increased to 1.5mg^{-1} callus turned to greenish yellow with occurrence of green organogenic spots as meristematic center and can be predicted its capacity to produce shoots (**Nabors *et al*., 1982; Ishii 1982**). Similar observations were reported on cotyledon explant of *Momordica dioica* (**Hoque *et al*., 2000; Devendra *et al*., 2009**) and hypocotyl in *Cucumis melo* cv. pusa madhuras by **Singh *et al*. (1990)**. Among the various concentrations of hormone treated BAP (0.5-2.5mg^{-1}), 1.5mg^{-1} was successful in initiating maximum number of shoots 6.25 ± 0.41 with an average shoot length of 3.1 ± 0.32 cm from leaf derived callus, where as an average of 10.6 ± 0.27 shoots with average shoot length of 2.8 ± 0.16 cm produced from hypocotyls derived callus respectively (Fig.1, c). The potential of medium with only cytokinin for the induction of shoots from callus has reported in *Cucumis melo* cv. pusa madhuras (Singh *et al*., 1990). BAP is generally regarded as most effective cytokinin for shoot differentiation. Table 5, shows that, Addition of 0.5mg^{-1} GA_3 favors not only for internodal elongation but also increases the number of shoot production 8.4 ± 0.16 with an average length of 10.3 ± 0.36cm in leaf derived callus and 15.2 ± 0.66 shoots were produced with an average shoot length 10.4 ± 0.65 in hypocotyl derived callus (Fig.1, d). Increase in the concentration of GA_3 1.0 mg^{-1} fails to increase in the shoot production but produces elongated shoots.

In vitro induction of organogenesis depends on the endogenous concentration of plant growth regulators, their distribution in the cultured tissue and interaction with exogenously supplied growth regulator. Good shoot regeneration was observed using BAP in combination with GA_3 from hypocotyl derived calli. A similar effect of this combination was noted previously by Ananthakrishnan *et al*. (2003) in *C. pepo*. It is suggested that GA_3 along with cytokinin stimulates the development of shoots (Molvig and Rose 1994). GA_3 promotes seed germination in *T. cucumerina* L. var. *cucumerina* (Devendra *et al*., 2008), stimulates the production of numerous enzymes, notably α-amylase, in germinating cereal grains, otherwise requires cold stratification or light for inducing seed germination (Davies 1995). Experimental result suggests that the gibberlin responsive gene (*HvGR*) (located to pockets of sub epidermal cells from where shoot primordial originate) is responsible for GA_3 induced shoot induction. *HvGR* showed the highest level of expression in the regeneration stage of the initiated shoot where the signal was localized primarily in the developing shoot primordia (**Seong *et al*., 2004**).

Rooting of micro shoots

The previous report (**Devendra *et al*., 2008**) conveys that 1.0 mg^{-1} IBA with 3% sucrose is suitable for rooting of micro shoots (Table 6). The rooted plantlets (8-10cm) height was transferred to soil, sand and farmyard mixture. (1:1:1) about 80% have survived for 1 month after transfer. The acclimatized plant exhibited normal growth (Fig.1, e).

Conflict of interests

The authors report no conflicts of interest. The authors are merely responsible for the content and writing of the paper.

REFERENCES

Ananthakrishnan, G., Xia, X., Elman, C., Singer, S., Paris, H. S., Gal, O. A. and Gaba, V. 2003. Shoot production in squash (*Cucurbita pepo*) by *in vitro* organogenesis. *Plant Cell Rep* 21:739-746.

Brachard, M. and Chateau, M. 1988. Obtention de plantes de melon (*Cucumis melo*) par embryogenese somatique. C. R. *Acad. Sci. Paris* t.307 series III: 777-780.

Chakravarthy, H. L. 1982. Fascicles of flora of India. (Fascicle 11) Botanical survey of India, Howra

Choudhury, B. 1967. Vegetables. National Book Trust,New Delhi India, pp: 152-154.

Davies, P. J. 1995. The plant hormones: their nature, occurance, and functions. In: plant hormones : physiology, biochemistry and molecular biology (Ed. P. J. Davies). Kluwer Academic Publishers, Boston, pp. 13-38.

Devendra, N., Kage, Y. N., Seetharam, and Vijaykumar, B. M. 2009. *In vitro* Antimicrobial activity and Phytochemical Analysis of *Trichosanthes cucumerina* L. var. *cucumerina* An important medicinal plant. *Adv. Nat. Appl. Sci*., 3(3); 438-441.

Devendra, N. K., Rajanna, L., Seetharam, Y. N. 2008. Seed Dormancy Studies in *Trichosanthes cucumerina* L. var. *cucumerina*. *My Forest,* 44(3) 199-203.

Devendra, N. K., Rajanna, L., Sheetal, C. and Seetharam, Y. N. 2008. *In vitro* Clonal Propagtion of *Trichosanthes cucumerina* L. var. *cucumerina* Plant Tissue Cult and Biotech 18(2): 103-111.

Devendra, N. K., Subhash, B. and Seetharam, Y. N. 2009. Callus growth and plant regeneration in *Momordica dioica* (Roxb.) Willd. Cucurbitaceae, *Am. Eurasian J. Sustain Agric.,* 3(4): 743-748.

Hoque, A., Islam, R. and Joardar, O. I. 2000. *In vitro* plantlets differentiation in Kakrol (*Momordica dioica* Roxb.) *Plant Tissue Cult* 12: 173-180.

Ishii, C. 1982. Callus induction and shoot differentiation of wheat oat and barley. In fujiwara (edn.). Proceeding of the 5th International Congress of Plant Tissue Culture and Cell Culture Japan. 185-186.

Kiran, H. and Srinivasan, B. P. 2008. *Trichosanthes cucumerina* Linn. improves glucose tolerance and tissue glycogen in non-insulin dependent diabetes mellitus induced rat. *Indian J. Pharmacol.,* 40(3):103-108.

Krug, M. G. Z., Stipp, L. C. L., Rodriguez, A. P. M. and Mendes, B. M. J. 2005. *In vitro* organogenesis in water melon cotyledons. *Pesq Agropec Bras, Brasilia*, 40:861-865.

Leljak-Levenic, D., Baur, N., Mihaljevic, S. and Jalaska, S. 2004. Somatic embryogenesis in pumpkin (*Cucurbita pepo* L.); control of somatic embryo development by nitrogen compounds. *J. Plant. Physiol.,* 161:229-236.

Molvig, L. and Rose, R. J. 1994. A regeneration protocol for *Spinacia oleracea* using gibberlic acid. *Aust. J. Bot.,* 42:763-769.

Nabors, M. W., Kroskey, C. S., Mc Hugh, D. M. 1982. Green spots are predictors of high callus growth rates and shoot formation in normal and in salt stressed tissue culture of oat (*Avena sativa* L.). *Z Pltanzen Physiol.,* 105:341-349.

Seong, R. C., Kim, J, Y,, Haam, J, W, and Seo, Y. W. 2004. Isolation and characterization of a gibberllin responsive gene (*HvGR*) from initiated shoots from calli derieved from mature embryo of barley. In Procedings of the 4th international Crop Science Congress, Brisbane, Australia. Published on website. URL; www.cropscience.org. au/icsc2004.

Singh, M. N., Rekha, K. and Bhatnagar, S.P. 1990. Regeneration of plants from hypocotyl and cotyledon cultures of *Cucumis melo* cv. *Pusa Maduras.* Phytomorphology 40: 401-405.

Thomas, T. D. and Sreejesh, K. R. 2004. Callus induction and plant regeneration from cotyledonary explants of ash goard (*Benincasa hispida* L.) Sci. Hort., 100: 359-367.

Yalcin-Mendi, N. Y., Ipek, M., Kacan, H., Curuk, S., Sari, N., Cetines, S. and Gaba, V.A. 2003. A histological analysis of regeneration in water melon. *J. Plant Biochem. Biotech.,* 12: 147-150.

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