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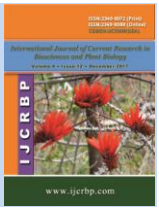

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Effect of Plant Growth Regulators on Indirect Organogenesis of *Trichosanthes cucumerina* L.

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Abstract

The snake gourd (*Trichosanthes cucumerina* L.) is an important cucurbit reported for its medicinal value, therapeutic potential, and as a popular delicacy (especially in Indian cuisine). Being nutritive and desirous, it has potential to feed the nations and addresses their nutritional security and economic prosperity. The plant is usually vegetatively propagated and cultivated for fruits during summer and rainy seasons. The limited supply of planting material, limits cultivation and production. The present study was in anticipation for callus induction and indirect organogenesis from leaf and internode explants of *T. cucumerina*. In this study, the MS medium supplemented with 2.5 mg/L BA and 2.5 mg/L 2iP was found to be most efficient for callus induction, followed by 2.0 mg/L 2,4-D and 1.5 mg/L Kin. The embryogenic callus was developed by sub-culturing of leaf and inter node callus in MS medium supplemented with 2iP and Kin in combination with BAP ranging from 1.0 – 2.0 mg/L. Through indirect organogenesis, the leaf and inter node explants have responded to produce true-to-type plants for propagation. It was observed that MS supplemented with 1.5 mg/L 2iP was efficient for shoot proliferation, and 1.5 mg/L NAA was found more efficient for root development. The rooted plantlets were successfully acclimatized in sand, soil and vermiculite and subsequently established in the field with 85% survival.

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Introduction

Plant regeneration through tissue culture can be accomplished by enhanced axillary branching from shoots or lateral buds. Organogenesis is the process of shoot meristem organization and development, eventually leading to shoot elongation. Plant regeneration of cucumber via organogenesis in cotyledon explants (Gambley and Dodd, 1990; Ali et al., 1991; Colijn-Hooymans et al., 1994; Burza and Malepszy, 1995; Kim et al., 2000), leave (Malepszy, 1983) and hypocotyl (Trulson and Shanin, 1986).

In organogenesis of Cucurbitaceae crops, shoots grow out from a source tissue explant and are excised and rooted to obtain an intact plant. The specific route of regeneration is primarily dependent on the explant source and media used for tissue culture manipulations. Kim et al. (2000) reported that shoot induction rate in cucumber was higher in explant of 3rd-5th day than in 7th-9th day seedling. Also, In more developed cotyledon, the regeneration capacity was drastically reduced (Colijn-Hooymans et al., 1994). Organogenesis is influenced by the nature and developmental stage of

the explants (Chraibi et al., 1992), *in vitro* medium components such as growth regulators (Pugliesi et al., 1993) and gelling agents (Babbar and Jain, 1998). Cotyledon have long been a favored explant for shoot regeneration on culture media rich in cytokinin (Mante et al., 1989; Sharma et al., 1990; Knittle et al., 1991), and excised cotyledon of Cucurbitaceae species often have been a source for high efficiency shoot regeneration. Organogenesis of plants has been reported from cotyledon of cucumber (Trulson and Shahin, 1986; Cade et al., 1987; Punja et al., 1990; Saurabh et al., 2017; Rajender et al., 2017), watermelon (Blackmon and Reynolds, 1991; Compton et al., 1996; Jaworski and Compton, 1997; Compton, 2000; Chaturvedi and Bhatnagar, 2001), and melon (Orts et al., 1987; Chee, 1991; Gaba et al., 1994; Leshem et al., 1994; Molina and Nuez, 1995). But reports of organogenesis from cotyledon explant on Cucurbitaceae showed differences in genotypes, optimum growth regulator concentrations and combinations, and age of the explants. Also, the choice of gelling agent is also important for plant in organogenesis (Singhas, 1984; Pochet et al., 1991).

Cultivation of the plant has started in a limited way, but the non-availability of quality planting material and inconsistency in seed germination are major constraints in extending the cultivation of this species. Vegetative propagation has not yet been successful. Micropropagation appears as the most suitable alternative for large-scale clonal propagation of this species and several other species of *Trichosanthes* have been micropropagated successfully (Hoque et al., 1998; Mythili and Thomas 1999; Zhang et al., 2000). The aim of this study was to develop a viable multiplication technology through indirect organogenesis of *T. cucumerina* for large scale production of quality planting material, and to test field performance of micropropagated plants for feasibility of cultivation.

Materials and methods

Plant material and explant sources

The mature seeds of *T. cucumerina* collected from Agriculture University, Coimbatore District, Tamil Nadu, India. Plantlet obtained from *in vitro* germinated seeds, leaves and inter nodes were served as explants sources. All the explants collected from 15-20 days old plantlets. Seeds were decocted, washed with 5% (v/v) detergent solution (Teepol Qualigen, India) for 5 min and 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

Induction of organogenic callus and proliferation

Leaf and inter node explants were used as explants source for callus induction. Callus induction in snake gourd was also age and developmental stage dependent. Both the explants were placed in the callus induction medium which possessed MS medium salts and varying concentrations of BAP (0.5-2.5 mg/L), KIN (0.5-2.5 mg/L), 2,4-D (0.5-2.5 mg/L) and 2iP (0.5-2.5 mg/L) for organogenic callus induction. After the optimization of suitable concentrations of hormones for callus induction, combinations of different concentrations of NAA (0.5-2.0 mg/L) were tested for enhanced callus formation. After 6 weeks of culture, callus formation was observed from the cut end of explants. From the obtained mass, the organogenic nature of the callus was identified by the presence of green colour with compact nodular texture (CN). The organogenic portions were isolated and sub cultured in the same medium. Greenish friable (GF), brown compact (BC), brown friable (BF), and yellow green friable (YGF) colored non-organogenic callus were also observed and they were not selected and discarded for further studies due to nil response. The selected organogenic callus was weekly sub cultured for another two weeks for the induction, maximum of 50 explants were tested and these experiments were repeated for three with five replicates.

Adventitious shoot proliferation

The organogenic callus obtained from two different explants, were transferred to shoot bud induction medium. Normally shoot induction medium cytokinins we used BAP and 2iP, though we have described in the previous chapter. The organogenic calli were sub cultured on shoot induction medium, where the medium supplemented with 2iP and BAP. After 1-2 weeks the shoot buds induced and shoots are developed. The developed shoots are excised from the culture tubes and transferred to fresh medium containing the same combination of hormones and concentration for further

development. These shoots are further transferred to shoot elongation medium containing GA3 (0.5 to 2.5 mg/L).

Root induction and hardening

Root induction from the elongated shoots, were obtained from the MS medium fortified with NAA, IAA and IBA (1.0-2.0 mg/L), sucrose 15 g/L and agar (0.8 %). After the complete regeneration of roots with tertiary roots (30-40 days after root induction) the regenerated plants were transferred to plastic pots containing sand, soil and vermiculate in 2:1:1 ratio for hardening. The hardened plants were maintained in Plant Environmental Growth Chamber (Sanyo, Japan) for proper acclimatization. After two weeks in Environmental Growth Chamber, the plantlets transferred to green house condition for 15 days and then successfully transferred to field condition. The survival percentage of all the hardened plants was recorded regularly. For root induction studies, 50 elongated shoots were tested for each concentration. Experiments were repeated for three times with five replicates.

Statistical analysis

Mean and standard error were used throughout the study and the values were assessed by using parametric modes median test (Snedecor and Cochren, 1989). The data were analyzed for variance by Duncan's Multiple Range Test (DMRT) using SAS Programme (SAS Institute, Cary, N.C). For multiple shoot induction from the leaf and inter node explants, 50 explants tested with 5 replicates and each experiment was repeated three times. During root induction 30 elongated shoots were tested for each experiment was repeated 3 times with 5 replicates.

Results and discussion

Callus induction

The leaf and inter node explants were obtained from the germinating seedling (7 pieces) and were placed on MS basal medium supplemented with hormones like BAP, 2iP, 2,4-D and KIN concentration ranging from 0.5 to 2.5 mg/L (Table 1).

Table 1. Effect of cytokinins and auxins on organogenic callus induction from leaf and inter node explants on mMS medium supplemented with B5 vitamins.

Hormone concentration (mg/L)	Leaf explants		Cotyledonary inter node explants	
	Percentage of response	Callus morphology	Percentage of response	Callus morphology
BAP				
0.5	65.50 ± 0.5^{f}	GC	64.00 ± 0.5^{f}	GC
1.0	66.66 ± 0.0^{de}	GC	65.00 ± 0.0^{de}	GC
1.5	67.25 ± 0.5^{bc}	GC	65.25 ± 0.5^{cd}	GC
2.0	69.50 ± 0.4^{a}	GC	66.66 ± 0.2^{a}	GC
2.5	68.00 ± 0.2^{b}	GC	65.00 ± 0.0^{de}	GC
2iP				
0.5	63.25 ± 0.3^{lm}	GW	62.00 ± 0.2^{j}	GW
1.0	64.50 ± 0.5^{ij}	GW	63.50 ± 0.5^{gh}	GW
1.5	65.00 ± 0.2^{gh}	GW	64.00 ± 0.5^{f}	GW
2.0	67.00 ± 0.0^{d}	GW	66.00 ± 0.2^{b}	GW
2.5	66.50 ± 0.4^{e}	GW	65.50 ± 0.5^{c}	GW
2,4-D				
0.5	63.50 ± 0.5^{l}	GY	62.50 ± 0.2^{i}	GC
1.0	64.75 ± 0.2^{hi}	GY	64.00 ± 0.0^{f}	GC
1.5	65.25 ± 0.5^{fg}	GY	64.00 ± 0.0^{f}	GC
2.0	66.66 ± 0.2^{de}	GY	65.50 ± 0.2^{c}	GC
2.5	64.00 ± 0.5^{k}	GY	62.00 ± 0.4^{j}	GC
KIN				
0.5	62.00 ± 0.4^{m}	GF	61.50 ± 0.5^{k}	GF
1.0	63.50 ± 0.5^{l}	GF	62.50 ± 0.5^{i}	GF
1.5	64.50 ± 0.5^{ij}	GF	63.75 ± 0.0^{fg}	GF
2.0	65.00 ± 0.5^{gh}	GF	62.00 ± 0.2^{j}	GF
2.5	63.00 ± 0.3^{lm}	GF	59.50 ± 0.5^{l}	GF

GC-Green Compact, GF-Green Friable, GW-Green White; Values are means ± SE of three repeated experiments. Each treatment consisted of four replicates. Means within a column followed by the same letters are not significant at *P*=0.05 according to DMRT.

Table 2. Combined effect of some cytokinins on organogenic callus induction from leaf and inter node explants on mMS medium supplemented with B_5 vitamins.

Hormone concentration (mg/L)	Leaf explants		Cotyledonary inter node explants	
	Percentage of response	Callus morphology	Percentage of response	Callus morphology
BAP+2iP				
1.0+1.0	75.00 ± 0.5^{c}	GC	74.00 ± 0.5^{c}	GC
2.0+1.0	79.00 ± 0.0^{a}	GC	78.00 ± 0.5^{a}	GC
1.0+2.0	78.00 ± 0.5^{ab}	GC	77.00 ± 0.5^{ab}	GC
BAP+KIN				
1.0+1.0	71.00 ± 0.4^{de}	GC	70.00 ± 0.4^{de}	YF
2.0+1.0	72.00 ± 0.5^{d}	GC	71.00 ± 0.2^{d}	YF
1.0+2.0	69.50 ± 0.0^{ef}	GC	67.50 ± 0.5^{f}	YF

GC-Green Compact, YF-Yellow Friable; Values are means ± SE of three repeated experiments. Each treatment consisted of four replicates. Means within a column followed by the same letters are not significant at P=0.05 according to DMRT.

For leaf explants, from the different concentrations of BAP and Kin, combined effect of BAP and KIN tested, combination of BAP (2.0 mg/L) and 2iP (1.0 mg/L) showed maximum response of 79 % (Table 2). In the case of BAP with KIN the combined effect gave 72% response. The combined effect of BAP and 2iP a green compact callus produced at the concentration of 2.0+1.0 mg/L. But in the case of inter node explants BAP alone produces the organogenic callus at 2.0 mg/L concentration with maximum of 70% of response. The photograph of our results showed the response on callus induction by the hormones BAP and 2iP (Fig. 1 c; Fig. 2 b, c). 2,4-D, NAA, 2,4,5-T, IBA and IAA (0.5 or in combination with BAP and Kn (0.5 optimum phytohormone concen callus induction. Calli were maintained on MS medium supplemented with same concentrations of hormone. 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; Zakaria et al., 2012) 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.The first report on callus induction in wild *T. cucumerina* made by Fassuliotis (1975) and then achieved in cultivated varieties. They used 2iP with IAA for callus induction and organogenesis. The type and concentration of a given growth regulator in association to specific genotypes can cause significant differences in the morphogenetic responses of *T. cucumerina* (Kamat and Rao, 1978). The use of younger tissues significantly increases the frequency of callus induction thus indicating that the process is age dependent. Cotyledons from 30 days old seedlings are ideal time and source to give satisfactory results in taking the initial segments for callus induction. The differences of callus induction from both types of explants were significant thus indicating that the genotype does not influence the process. The morphogenetic response of both explants on different plant growth regulators. The increase of hormone concentration will leads to over growth of callus formation and multiplication, but the callus will become unable to produce multiple shoots. But the shoot production frequency will be reduced (Moreira – Dias et al., 2000).

We used BAP, 2iP, KIN and 2,4-D for callus induction, among these, the combination of 2iP and BAP produces the maximum response nearly about 78%. Most of the reports on *Solanum melongena* state that 2iP as the most successful hormone for callus induction and organogenesis. The combination of BAP and NAA has been a successful for callus induction. Molina (2004) reported earlier on *Salvia canariensis*, BAP with NAA produced high frequency of callus induction. But in the case of BAP, the individual treatment not produced appreciable results. Callus was more frequently produced from the petiole segments not in the stem segments. The colour and the texture of the callus are important to further proceed. Green and compact texture of the callus should be ideal for organogenesis or shoot induction. We have tried 2,4-D alone, and combination with other hormones for callus induction. In the case of *Tridax procumbens* the combination of 2,4-D with BAP produced organogenic callus and multiple shoot (Minal et al., 2010). Sreedhar et al. (2008) has reported that the combination of 2,4-D and BAP was a successful for high frequency callus induction in *Solanum nigrum.* As the concentration of 2,4-D increases the colour of the callus darkend (Brown) and the texture of the callus also changes as a fragile. Response in callus induction is highly genotype dependent.

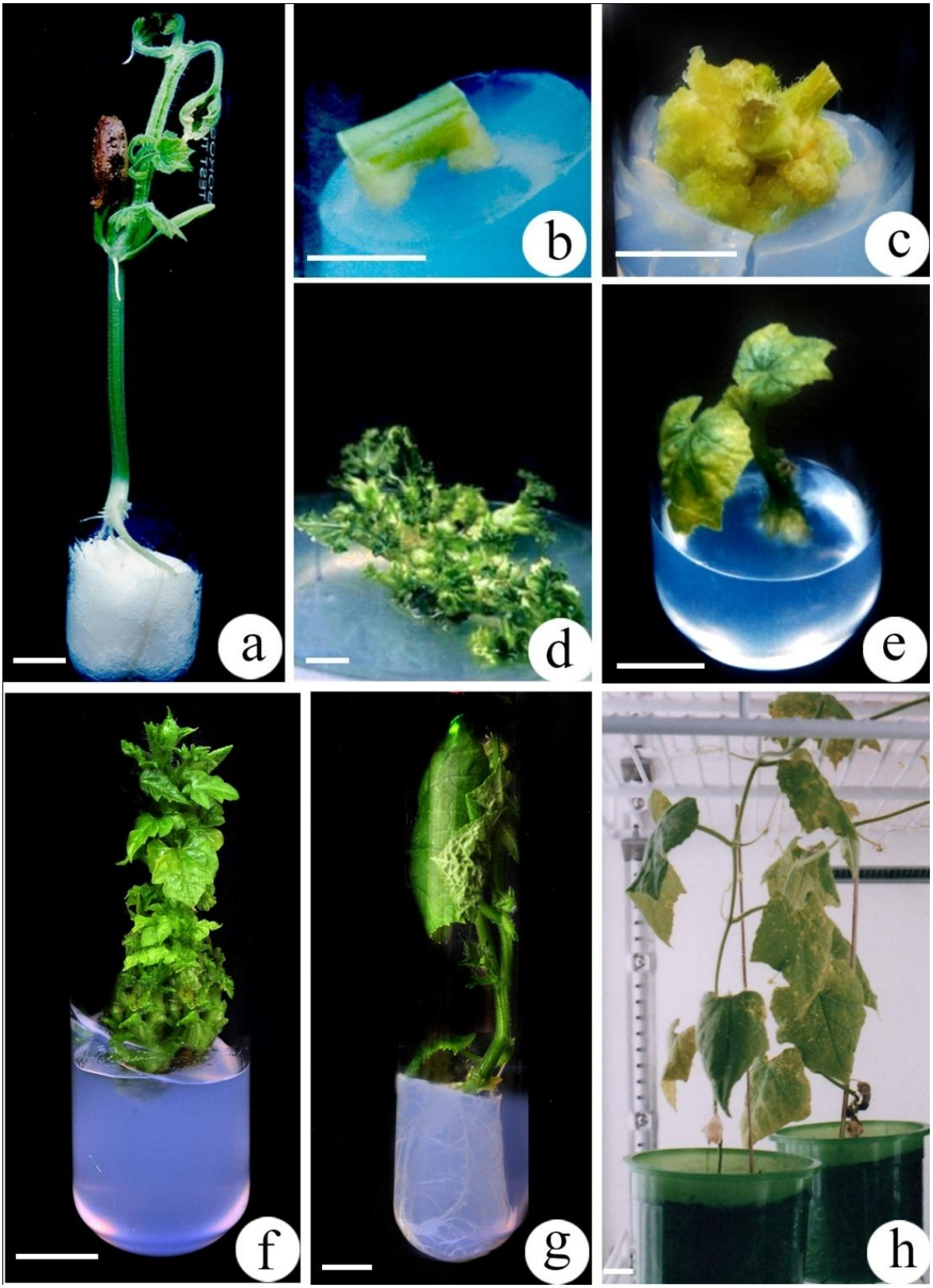

Fig. 1: Indirect organogenesis from leaf explant of *Trichosanthes cucumerina* L. a. Aspetic plant, b. Callus initiation from inter nodal explant, c. Callus proliferation d. Shoot bud initiation and proliferation, e and f. Shoot elongation, g. Root initiation, h. Hardened plants.

We spotted that the selected ranges of phytohormones had significant effect on callus induction observed. In chick pea, callus induction achieved by using hypocotyls explants on MS medium with 2,4-D and NAA with compact texture for about 68% of response. But, in combination of BAP with NAA also produced the more or less similar response of 66%.

In *Withania somnifera* both plant growth regulators (BAP and 2iP) have been tried for callus induction nearly 54% of response has documented (Gita rani et al., 2003). The callus induction initiated by 2,4-D alone and with NAA in many cases, for example, in *Glycine max* produced 100% frequency of callus induction (Joyner et al., 2010). Earlier, in tobacco the MS medium supplemented BAP and 2iP individually tested by Constantin et al. (1977). From their report we came to know that the induction of callus may increase as the concentration of 2iP increases, and in the case of BAP decreases the induction of callus as the concentration of hormone increases. Experiments in *Allium*, most of the species responded well in the supplementation of 2,4-D and BAP for callus induction, morphology of the callus (texture and color) suitable for organogenesis and shoot induction (Das et al., 2010). White or pale green, while somewhere brown color callus will be ideal for organogenesis. The browning of the callus is generally observed in the media containing 2,4-D or NAA in conjunction with any of the cytokinins.

The beneficial effect of the presence of a auxin and a cytokinin in the culture medium for stimulating the callus induction from leaf explants of various plants have been reported by various authors (Rey et al., 2000) have reported. The marphogenetic responses were greatly affected by medium employed for callus induction from the leaf explants in *Arachis pintoi* (Rey et al., 2000). In the case of cotyledonary explants Agrawal and Sardar (2006) reported that BAP and 2,4-D are the ideal combination for the callus induction for multiple shoot production in *Cassia angustifolia*.

Shoot induction from callus

At the time shoot proliferation, all the plant growth regulators like BAP, 2,4-D, 2iP (0.5 to 2.0 mg/L) with NAA (1.0 mg/L) showed shoot proliferation from obtained leaf and cotyledonary inter node explant derived callus cultures. Auxin particularly, NAA tested with combination of cytokinins for shoot induction and multiplication. After 30 days, callus from cotyledonary inter node and leaf explants has been successfully regenerated and produced maximum of 8-10 and 7-9 average shoots per callus culture of inter node and leaf explants respectively (Table 3). In the combination of 2iP and NAA produced nearly 9 average numbers of shoots per callus culture of leaf and in inter node explants 7 average number of shoots has been produced. Meanwhile BAP-NAA combination showed a moderate response for multiple shoot induction. Between 6-7 shoots has been produced on MS medium supplemented with KIN and NAA for both the explants derived callus.

The effect of some cytokinins for shoot induction and multiplication from organogenic callus was observed. Shoot induction and multiplication was achieved by incorporating 2iP, BAP and KIN individually with NAA, among these hormones 2iP was the most successful hormone with maximum of 79% of response has been achieved and 9.1 mean numbers of shoots has been produced. The other two hormones (BAP and KIN) did not given that much of appreciable results for leaf explant. For cotyledonary inter node explants KIN with NAA reached 69% of response and 6.6 mean numbers of shoots has been produced (Table 3). Other then these hormones 2iP has been tried with some amino acids for shoot multiplication. Serine with 2iP at 3.0 mg/L concentration produces 65% of response with 6.1 mean numbers of shoots produced and Glutamine at 3.0 mg/L concentration produced 63% of response and 5.9 mean numbers of shoots in leaf explants derived callus and in catyledonary inter node derived callus, serine produces 64% of response and 5.7 mean number of shoots per experimental unit. Glutamine has not produced any appreciable results for shoot induction and multiplication (Table 3).

Most of the researchers worked on *T cucumerina* mainly used BAP, 2,4-D for callus induction and BAP, 2iP for shoot induction and multiplication (Kashyap et al., 2002). In earlier days Organogenesis has been successfully achieved in cultivated and wild varieties as well as their hybrids (Fassuliotis, 1975). Fassuliotis was first to report regeneration in *S. sisymbriifolium* Lam., a wild species of *T. cucumerina*. He used a combination of two hormones like 2iP and IAA for shoot induction and multiplication. Other wild species in which regeneration studies have been carried out include *S. aviculare*, *S. gilo* (Kashyap et al., 1999), *S. khasianum* (Kowalozyk et al., 1983), *S. indicum* and *S. torvum* (Kashyap et al., 1999). Besides *T. cucumerina*, other crops like *Triticum aestivum* has produced positive results on MS medium

supplemented with 2iP (Rashid et al., 2009) and then replaced with its cheaper synthetic analogue BAP (Varshney et al., 1997). For shoot induction from callus has been achieved by supplemented with 2iP in MS medium in *Solanum melongena* and *Solanum torvum* (Kashyap et al., 2002).

Shoot elongation by GA3

For shoot elongation we have checked GA3 from 0.5 - 2.5 mg/L concentration, among these concentration 2.0 mg/L produced 76 % of response with 4.8 mean number of shoot length (Table 4).

Table 3. Effect of different cytokinins on multiple shoot induction after callus formation through leaf and inter node explants.

Hormone concentration (mg/L)	Leaf explants			Cotyledonary inter node explants		
	Percentage of response	Mean number of shoots	Average length of shoots in cm	Percentage of response	Mean number of shoots	Average length of shoots
BAP						
0.5	66.66 ± 0.0^{fg}	7.0 ± 0.5^{ef}	3.2 ± 0.5^{f}	62.00 ± 0.5^{g}	6.5 ± 0.2^{cd}	2.8 ± 0.3^{g}
1.0	67.00 ± 0.5^{ef}	7.1 ± 0.6^{de}	3.5 ± 0.5^{d}	63.25 ± 0.2^{ef}	6.9 ± 0.3^{a}	3.5 ± 0.2^{a}
1.5	69.00 ± 0.5^{c}	7.3 ± 0.2^{c}	3.7 ± 0.3^{b}	65.00 ± 0.5^{c}	6.7 ± 0.3^{b}	3.4 ± 0.3^{b}
2.0	67.50 ± 0.2^{de}	6.9 ± 0.5^{fg}	3.3 ± 0.2^{e}	62.00 ± 0.2^{g}	6.5 ± 0.5^{cd}	3.2 ± 0.5^{d}
2iP						
0.5	63.50 ± 0.5^{ij}	6.1 ±0.5^{h}	2.8 ± 0.2^{h}	59.00 ± 0.5^{ig}	5.5 ± 0.5^{h}	2.8 ± 0.5^{g}
1.0	64.50 ± 0.3^{hi}	7.2 ± 0.3^{cd}	3.1 ± 0.3^{g}	61.50 ± 0.2^{gh}	6.0±0.5^{f}	3.1 ± 0.2^{e}
1.5	79.00 ± 0.2^{a}	9.1 ± 0.5^{a}	3.8 ± 0.5^{a}	65.00 ± 0.5^{c}	6.3±0.3^{e}	3.3 ± 0.3^{c}
2.0	75.00 ± 0.2^{b}	8.5 ± 0.6^{b}	3.5 ± 0.5^{d}	64.00 ± 0.3^{de}	6.5±0.2^{cd}	3.1 ± 0.6^{e}
KIN						
0.5	62.00±0.5^{k}	6.9 ± 0.5^{fg}	3.1 ± 0.5^{g}	62.00 ± 0.2^{g}	5.9 ± 0.3^{fg}	2.9 ± 0.3^{f}
1.0	63.50±0.5^{ij}	7.0 ± 0.3^{ef}	3.5 ± 0.5^{d}	67.00 ± 0.5^{b}	6.3 ± 0.2^{e}	3.1 ± 0.6^{e}
1.5	68.00±0.4^{cd}	7.3 ± 0.2^{c}	3.6 ± 0.3^{c}	69.00 ± 0.2^{a}	6.6 ± 0.5^{bc}	3.5 ± 0.3^{a}
2.0	65.00±0.0^{h}	7.1 ± 0.5^{de}	3.2 ± 0.2^{f}	64.50 ± 0.5^{cd}	6.5 ± 0.6^{cd}	3.4 ± 0.2^{b}

Values are means ± SE of three repeated experiments. Each treatment consisted of four replicates. Means within a column followed by the same letters are not significant at *P*=0.05 according to DMRT.

Table 4. Effect of GA3 for Shoot elongation by different concentrations.

Hormone concentration (mg/L)	Percentage of response	Average length of elongation
GA3		
0.5	70.00 ± 0.5^{e}	3.5 ± 0.5^{e}
1.0	73.00 ± 0.2^{cd}	4.1 ± 0.6^{cd}
1.5	75.00 ± 0.5^{ab}	4.4 ± 0.3^{c}
2.0	76.00 ± 0.2^{a}	4.8 ± 0.2^{ab}
2.5	74.00 ± 0.5^{bc}	5.1 ± 0.2^{a}

Values are means ± SE of three repeated experiments. Each treatment consisted of four replicates. Means within a column followed by the same letters are not significant at *P*=0.05 according to DMRT.

Root induction and hardening

In *in vitro* plant propagation, induction of root is important step and essential too. The traditional root induction method uses a shock of high auxin concentration. The elongated shoots are transferred rooting medium supplemented with auxins for shoot induction. We have tried three auxins for root induction and elongation namely NAA, IAA and IBA. All the three auxins at a series of concentration from 1.0 mg/L to 2.0 mg/L have been experimented (Table 5). Among these three auxins, NAA was the successful auxin for root induction and elongation at concentration of 1.0 mg/L. Like our experiment, Agrawal and Sardar (2007) experimented with these three auxins for root induction and elongation in *Cassia angustifolia*. But in their case, IBA was the most successful hormone for root induction and elongation. In *Phaseolus angularis* different concentrations of IBA has been used for root induction and elongation, IBA produced nearly 74% of rooting response with 4.5µM concentration supplemented with MS medium (Varisai et al., 2006).

Normally, root induction and elongation in *T. cucumerina* achieved by incorporating NAA in MS medium with sucrose and agar. Many reports suggested that NAA is the ideal hormone for root induction and

elongation in *T. cucumerina* (Franklin and Lakshmi Sita, 2004). In contrasting to our results, Rahman et al. (2006) reported that instead of NAA, replaced with IBA in MS medium and produced nearly 15 roots per shoot. Our study provides one of the ideal protocols for indirect organogenesis without any disturbances like basal callus formation and recallusing from cotyledonary inter node and leaf explants. Root induction was highly increased when the medium supplemented with NAA as rooting hormone. The increased root length leads to increase in the survival percentage. For hardening process sand, soil and vermiculite were used in 2:1:1 ratio (Fig. 1 h, and Fig. 2 h and i). The hardened plants were kept in controlled environmental growth chamber for 2-4 days for acclimatization. After four days the plantlets transferred to pot and shifted to greenhouse and then transferred to field condition in green house.

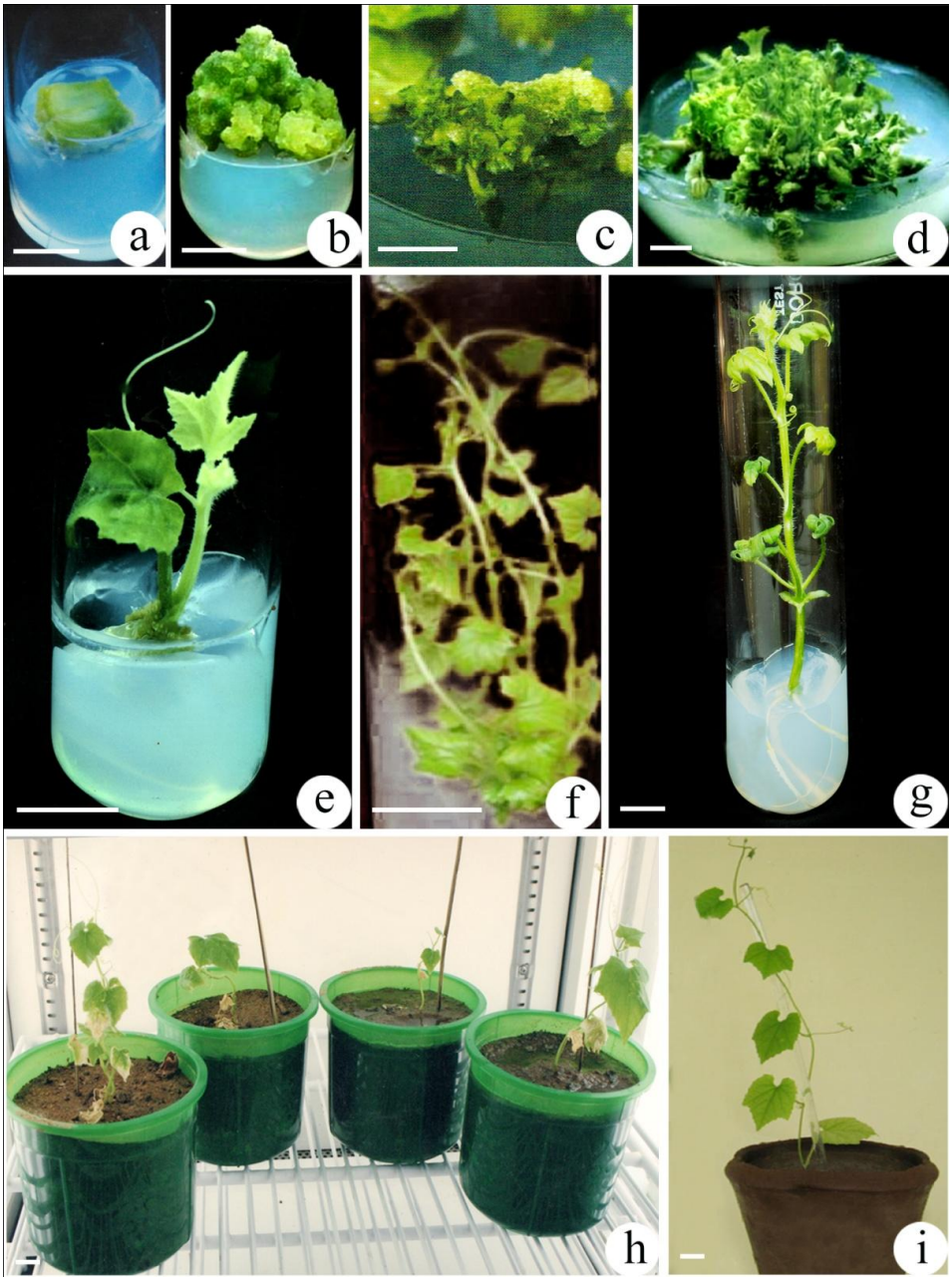

Fig. 2: indirect organogenesis from leaf explants of *Trichosanthes cucumerina* L. a. Callus initiation from leaf explant, b. Callus proliferation, c. Shoot bud initiation, d. Multiple shoot initiation, e and f. Root initiation (0.5 x), g. Hardening of *in vitro* derived plants (0.1 x and 0.2 x), h. Hardening plant.

Table 5. Influence of auxins in the induction of root with various concentrations supplemented on half strength MS medium containing B5 vitamins.

Hormone concentration(mg/L)	Percentage of response	Average number of roots	Average root length	Basal callus
NAA				
1.0	70 ± 0.0[a]	4.5 ± 0.5[a]	4.9 ± 0.3[a]	-
1.5	68 ± 0.5[b]	4.1 ± 0.6[b]	4.1 ± 0.5[b]	-
2.0	65 ± 0.0[c]	3.9 ± 0.2[bc]	3.5 ± 0.5[g]	-
IAA				
1.0	55 ± 0.5[d]	2.5 ± 0.2[d]	4.1 ± 0.5[b]	-
1.5	54 ± 0.4[ef]	2.3 ± 0.3[de]	3.8 ± 0.6[d]	-
2.0	50 ± 0.2[g]	2.0 ± 0.3[g]	3.7 ± 0.5[e]	-
IBA				
1.0	50 ± 0.5[g]	2.1 ± 0.2[fg]	3.9 ± 0.3[c]	-
1.5	54 ± 0.2[ef]	2.2 ± 0.6[ef]	3.6 ± 0.2[f]	-
2.0	55 ± 0.4[d]	2.2 ± 0.5[ef]	3.4 ± 0.5[h]	-

Values are means ±SE of three repeated experiments. Each treatment consisted of four replicates. Means within a column followed by the same letters are not significant at *P*=0.05 according to DMRT.

Conclusion

From this study we can conclude that simple and efficient protocol for *in vitro* adventitious shoot multiplication from callus cultures, and whole plant regeneration has been described. This protocol for organogenesis optimized by validating different concentrations of plant growth regulators for enhanced shoot multiplication. To conclude, this protocol explained in this paper provides a rapid regeneration system from callus for snake guard and the well developed plantlets were successfully transferred to the natural condition.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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