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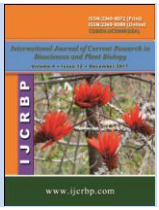

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In Vitro Plant Regeneration through Different Explant of *Trichosanthes cucumerina* L.

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Abstract

An efficient *in vitro* plant regeneration protocol was developed for *Trichosanthes cucumerina* L. from leaf and shoot tip explant. Explants were cultured on MS medium supplemented with 6-benzyl amino-purine (BAP) thidiazuron (TDZ) and kinetin (Kin) ranging from 0.5-3.0 mg/L alone and in combination with gibberellic acid (GA_3) ranging from 0.2-1.0 mg/L and produced multiple shoots. The highest percentage of response 96.3 percentage of response in leaf and 39.33 percentage of response in shoot tip explant were noticed on 0.6 mg/L GA_3 in combination with 2.5 mg/L BA. The 84.38 and 24.96 percentage of response were noticed in leaf and shoot tip explants respectively in 2.5 mg/L. The regenerated shoots were then transferred to MS medium fortified with different concentrations of indole acetic acid (IAA), 1-naphthalene acetic acid (NAA) and indole butyric acid (IBA) (0.5-3.0mg/L) alone for root induction. The maximum percentage of response 96.63 with 9.66 cm root length per shoot was obtained from 1.5 mg/L NAA. The rooted plantlets were successfully acclimatized in red soil, farmyard manure and sand mixture and subsequently established in the field with 90% survival.

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Keywords

Growth hormones
Leaf explant
Multiple shoot
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Introduction

A complete understanding of medicinal plants involves a number of factors like botany, chemistry, genetics, quality control and pharmacology. In addition there is a large wealth of knowledge in the medicinal and other properties of plants from generation to generation by the tribal societies. *Trichosanthes cucumerina* L. var. *cucumerina* belongs to Cucurbitaceae and is distributed in throughout India, Bangladesh, Sri Lanka, Burma, Malaysia, and Australia. It is a well known plant, the fruit of which is mainly consumed as a vegetable. It is a perennial climber with an attractive white flower. It is highly bitter in taste which may be supposed to contain medicinal properties (Pillai et al., 2008).

The seed posses anthelmintic and antifibrile properties. The seeds are haemoagglutinating (Chakravarty, 2010) Trichosanthin is an antiviral protein purified from the root of *T. kirilowii* Maxim. It is an active component of Chinese medicine and is still being used in midterm abortion and to treat carcinoma. Karasurin is another new abortificient protein isolated from root of *T. kirilowii* (Toyokawa et al., 1991) and it is appetizer, laxative, aphrodisiac, blood purifier (Shivarajan and Indira, 1994) and anti-inflammatory (Kolte et al., 1997). Trichosanthin shows inhibition of human immuno

deficiency virus (HIV) because of its ribosome inactivating activity (Jian-Hua Wang et al., 2003). Seed is a good source of nutrients (Adebooye, 2008). Root is used to cure bronchitis, headache and boils. Leaves, for biliousness, emetic, externally applied over bald patches of alopecia to reduce congestion on congestive cardiac failure (Pullaih, 2006). Isolation and characterization of galactose specific lactin from the seed. It is used as one of the important ingredients in 16 commercially available herbal products in India.

Biotechnology has opened up new vistas for crop improvement. Biotechnological tools like *in vitro* propagation, genetic engineering and molecular biology has helped over coming constraints of conventional breeding, and identification and introduction of useful genes that confer resistance to pests and diseases, and tolerance to abiotic stresses in snake gourd. Although some developments in the field of biotechnological applications have taken place, the full potential is yet to be exploited for improvement of snake gourd.

Plant regeneration studies have contributed much to the crop, as there are extensive studies on regeneration *via* organogenesis and somatic embryogenesis. The understanding of specific metabolic pathways directly or indirectly involved in plant morphogenesis has helped in understanding and improving the regeneration potential of snake gourd genotypes. Improvement of organogenesis and somatic embryogenesis by manipulating the polyamines metabolism has opened new vistas not only in snake gourd tissue culture but also tissue culture of other crops.

Materials and methods

Collection of seeds

The snake gourd seeds (Co-1) were procured from Tamil Nadu Agriculture University, Coimbatore District, Tamil Nadu, India. The seeds were grown on the field, in the Department of Plant Science, Bharathidasan University for the collection of explants.

In vitro seed germination

The seeds were washed in running tap water to remove dust particles and the chemical reagents used for storage of seeds, rinsed with four drops of commercial soap solution (Teepol) for 5 mins and then kept under running tap water for one hour. The seeds were 0.1 % Mercuric chloride ($HgCl_2$) for 3 mins aseptically to remove the microbes adhered on the surface of the seeds followed by five times rinse with sterile distilled water to remove the sterilant and used for inoculation. The sterilized seeds were kept on moistened cotton and half strength MS medium containing 0.8 g/L Agar as gelling agent and 15 g/L Sucrose as carbon source in MS medium without hormones for germination. This setup kept to 16 h L/D photoperiod with low light intensity (1,000 Lux). Seeds germinated after two weeks, and the germinated seedlings produced leaves and shoot tips after three weeks which served as explant source. The main reason for opting *In vitro* seed germination is to avoid the contamination.

Culture media and conditions

Explants were cultured in Borosil tubes (130 mm height and 6.5 mm) containing MS medium (Murashige and Skoog, 1962) fortified with 3% sucrose, 0.8 % (w/v) agar and different concentrations of cytokinins and auxins for shoot induction and multiplication. The pH of the media were adjusted to pH 5.8 before and autoclaved at 121°C and 125 kPa for 15 min. All the cultures were maintained at 25±2°C under 16h of light at an intensity of 2,000-3,000 lux provided by cool white fluorescent tubes (Philips, India).

Shoot induction and multiplication

Sterilized leaf and shoot tip segments were cultured on MS medium supplemented with various concentration of (BA) 6-benzyladenine, Thidiazuron (TDZ) and Kinetin (Kin) alone at 0.5 – 3.0 mg/L for shoot induction. The shoot buds were further sub-cultured on the same medium for shoot multiplication.

Shoot elongation

The developed shoots were excised from the explant and immediately shifted to elongation medium for shoot elongation, and elongation medium were supplemented with Gibberellic acid (GA_3) (0.2 - 1.0 mg/L) and cytokinins like BA, TDZ and kin (2.5) on MS medium with agar and sucrose.

Rooting

In vitro raised shoots were excised from shoot cluster and transferred to half-strength MS medium supplemented with indole-3- acetic acid (IAA) and

indole butyric acid (IBA) 1-naphthalene acetic acid (NAA) at 0.5-3.0 mg/L either alone or in combination for root induction.

Hardening and acclimatization

The rooted plantlets were carefully taken out from the cultures tube and washed thoroughly with distilled water to remove the culture medium. Subsequently, they were transferred to paper cups containing sterile red soil, farmyard manure and sand in the ratio of 2:1:1. Initially the plantlets were maintained at 25±2°C with 16h of light at an intensity of 2,000-3,000 lux provided by cool white fluorescent tubes in an artificial plant growth chamber (Sanyo, Japan). After about one month, the hardened plants were transferred to polybags containing sand, soil and vermiculite (2:1:1). Then it was transferred in to green house finally to experiment field.

Statistical analysis

The analysis of variance (ANOVA) appropriate for the design was carried out to detect the significance of differences among the treatment means were compared using Duncan's Multiple Range Test (DMRT) at 5% level of significance (Gomez and Gomez, 1976). For multiple shoot induction from the shoot tip and shoot tip 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

In vitro seedlings

The primary establishment of *in vitro* cultures from field grown plants is difficult because of profuse surface contamination attached to explants. To overcome this problem, surface sterilized seeds were sown in test tube on moistened cotton and this germinated seeds were used as explant sources for further study. Surface sterilization procedure was very effective with 97% culture proved clean and only 3% of the total initiated plants were contaminated by fungus. The 90% seeds germination was noted after 7 days on sterile moistened cotton. A high percentage of seed germination (90%) has been reported on sterile moistened filter paper or cotton in Petri dishes or test tubes in various crop species, including snake gourd (Pillai et al., 2008; Devendra et al., 2008 and 2015); *Brassica oleracea* (Finch-Savage et al., 2006) and *Triticum aestivum* (Lehner et al., 2006) *Ophiorrhiza mungos* (Namdeo et al., 2012), *Ipomoea sepiaria* (Cheruvathur et al., 2015). In the present study, the higher regeneration efficiency was observed in leaf explants excised from 7-10 day-old seedlings (Gogoi et al., 2017), whereas in earlier reports cotyledon explants used were older than 3-4 days (Prem Kumar et al., 2015). Therefore, seeds were germinated on sterile moistened cotton and used for all the experiments in the present study.

Shoot multiplication from leaf and shoot tip explants

The shoot tip explants were collected from 7-10 day old *in vitro* grown seedlings. All healthy explants were cultured on MS medium supplemented with BA (2.5 mg/L) for effective shoot induction. Medium without plant growth regulators was used as the control. The leaf and shoot tip explants were cultured on MS medium supplemented with different concentration of (0.5-3.0 mg/L) BA, TDZ and Kin in alone. The maximum numbers of shoots were obtained in MS medium supplemented with BA (2.5 mg/L), with a mean of 84.38 shoots per leaf explant and 24.96 shoots from shoot tip explant (Table 1; Figs. 1 and 2).

The highest number of shoots were obtained in Ms medium supplemented with GA_3 (0.6 mg/L) in combination with BA (2.5mg/L) with mean percentage of 96.3 shoots per leaf explant and 39.33 shoots were obtained from shoot tip explant (Table 2). TDZ and Kin treatments showed moderate response and they produced lower number of shoots when compared to BA (Table 2). Higher concentrations of BA were noticed to inhibit the formation of shoots and the shoots so formed were short and thick. Similar findings have also been reported in *Amomum subulatum* (Purohit et al., 2017).

Other workers have also reported similar thick rosette types of shoots formation in higher concentrations of BA in case of *Gossypium hirsutum* (Prem Kumar et al., 2015; *Commiphora wightii* (Mohan et al., 2017). The shoot multiplication significantly varied according to the explant type. A similar phenomenon was also reported in *Gymnema sylvestre* (Komalavalli and Rao, 2000), *Fagopyrum esculentum* (Lee et al., 2003) *Swertia lawii* (Kshirsagar et al., 2015), *Amomum subulatum* (Purohit et al., 2017), *Strobilanthes tonkinensis* (Srikun, 2017).

Table 1. Effect of different concentration of cytokinin on multiple shoot induction *Trichosanthes cucumerina* cultured on MS medium.

Concentrations of PGR (mg/L)	Percentage of response (Mean ± SE) leaf	Percentage of response (Mean ± SE) shoot tip
Control*	34.06 ± 0.93^{l}	7.55 ± 0.41^{o}
BA		
0.5	75.64 ± 0.54^{e}	16.35 ± 0.29^{h}
1.0	76.95 ± 0.19^{de}	17.67 ± 0.48^{g}
1.5	79.05 ± 0.32^{cd}	20.90 ± 0.23^{d}
2.0	81.37 ± 0.28^{bc}	22.33 ± 0.38^{bc}
2.5	84.38 ± 0.67^{a}	24.96 ± 0.33^{a}
3.0	82.63 ± 0.65^{b}	23.02 ± 0.57^{b}
TDZ		
0.5	69.65 ± 0.33^{h}	12.36 ± 0.41^{kl}
1.0	72.32 ± 0.28^{g}	14.69 ± 0.29^{ij}
1.5	78.62 ± 0.19^{d}	18.09 ± 0.37^{fg}
2.0	80.98 ± 0.47^{c}	20.34 ± 0.31^{de}
2.5	82.69 ± 0.62^{ab}	22.04 ± 0.51^{c}
3.0	72.61 ± 0.54^{f}	19.39 ± 0.54^{e}
Kin		
0.5	65.34 ± 0.46^{j}	10.30 ± 0.22^{m}
1.0	68.68 ± 0.52^{hi}	12.69 ± 0.38^{k}
1.5	70.64 ± 0.56^{gh}	15.39 ± 0.41^{i}
2.0	72.34 ± 0.61^{fg}	17.30 ± 0.64^{gh}
2.5	75.02 ± 0.60^{ef}	18.39 ± 0.66^{f}
3.0	62.68 ± 0.46^{k}	10.03 ± 0.45^{mn}

***Control** - Treatment without PGR's; Data recorded after 4 weeks of culture; Values are mean 30 explants per treatment and repeated three times.
Mean values within a column followed by different letters are significantly different from each other at 5% level comparison by Duncan's Multiple Range Test (DMRT).

Table 2. Effect of GA_3 in combination with BA, Kin and TDZ on elongation of shoots in *Trichosanthes cucumerina* L. cultured on MS medium.

Concentrations of plant growth regulators (mg/L)	Percentage of response (Mean ± SE) leaf	Percentage of response (Mean ± SE) shoot tip
Control*	35.69 ± 0.55^{k}	14.09 ± 0.22^{k}
GA_3 + BA		
0.2 ± 2.5	90.93 ± 0.66^{b}	17.08 ± 0.47^{c}
0.4 ± 2.5	94.66 ± 0.39^{ab}	28.34 ± 0.55^{b}
0.6 ± 2.5	96.30 ± 0.23^{a}	39.33 ± 0.32^{a}
0.8 ± 2.5	88.60 ± 0.20^{bc}	26.92 ± 0.19^{d}
1.0 ± 2.5	87.04 ± 0.55^{c}	16.68 ± 0.60^{de}
GA_3 ± Kin		
0.2 ± 2.5	63.33 ± 0.45^{i}	15.96 ± 0.33^{fg}
0.4 ± 2.5	67.60 ± 0.17^{gh}	25.69 ± 0.67^{g}
0.6 ± 2.5	68.93 ± 0.30^{fg}	36.03 ± 0.43^{f}
0.8 ± 2.5	61.30 ± 0.51^{ij}	25.39 ± 0.70^{gh}
1.0 ± 2.5	59.09 ± 0.69^{j}	14.63 ± 0.54^{j}

Concentrations of plant growth regulators (mg/L)	Percentage of response (Mean ± SE) leaf	Percentage of response (Mean ± SE) shoot tip
GA_3 ± TDZ		
0.2 ± 2.5	75.62 ± 0.44^{e}	15.03 ± 0.24^{i}
0.4 ± 2.5	83.36 ± 0.29^{d}	26.60 ± 0.35^{e}
0.6 ± 2.5	86.09 ± 0.25^{cd}	36.93 ± 0.69^{cd}
0.8 ± 2.5	70.93 ± 0.31^{f}	25.30 ± 0.33^{h}
1.0 ± 2.5	68.33 ± 0.54^{g}	15.09 ± 0.51^{hi}

*__Control -__ Treatment without PGR's; Data recorded after 2 weeks of culture;
Values are mean 30 explants per treatment and repeated three times. Mean values within a column followed by different letters are significantly different from each other at 5 % level comparison by Duncan's Multiple Range Test (DMRT).

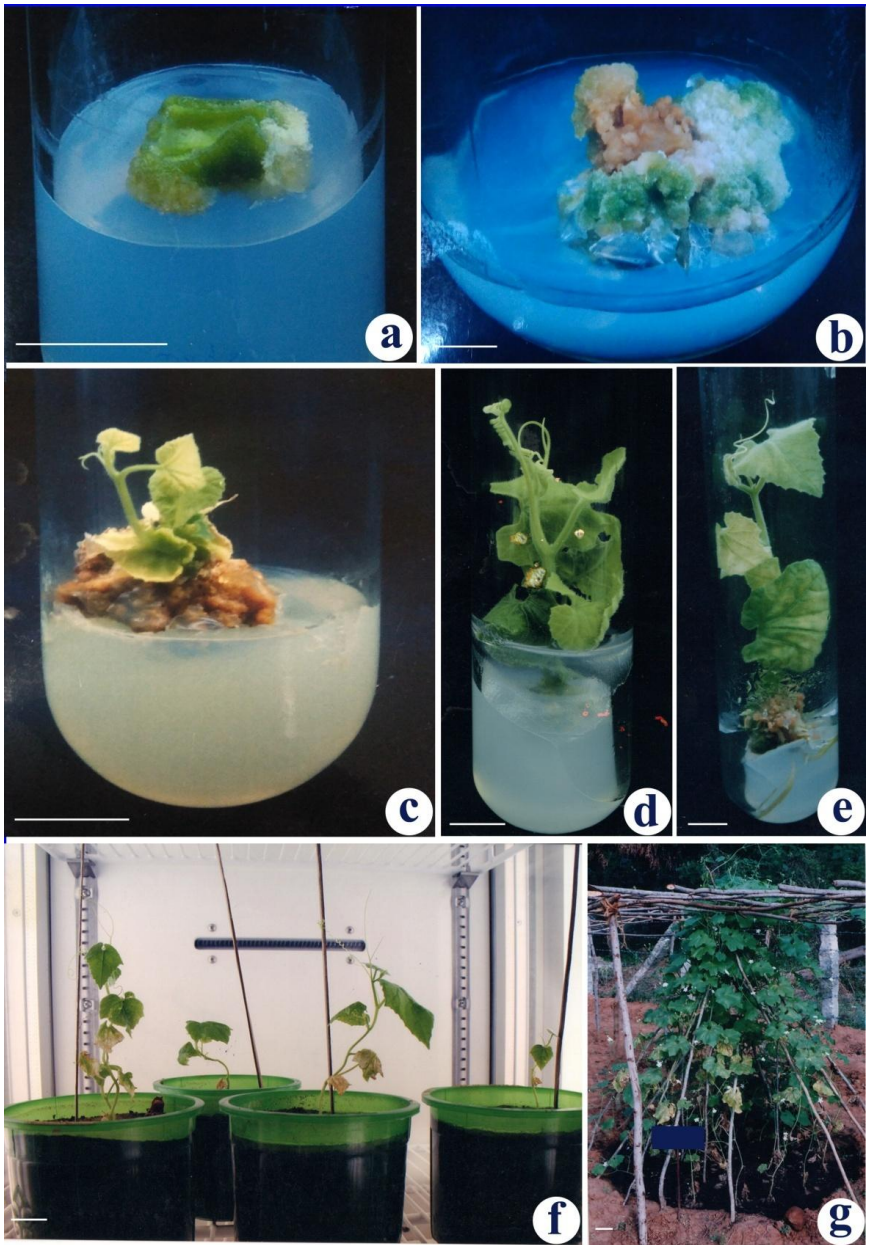

Fig. 1: Micropropagation of *Trichosanthes cucumerina* L. using leaf explant. a. Leaf explant, b. shoot bud proliferation, c. multiple shoot initiation, d. shoot elongation, e. plantlet on rooting medium, f. hardened plant under plant growth chamber, g. potted plant under natural conditions. Bar = 10 mm.

Table 3. Effect of different concentrations of auxins on root induction from elongated shoots obtained from leaf explants cultured on the MS medium.

Concentrations of Auxins (mg/L)	Percentage of Response (Mean ± SE)	Number of roots per plantlet (Means ± SE)
Control*	27.33 ± 0.60^{n}	3.61 ± 0.54^{p}
IBA		
0.5	65.07 ± 0.30^{jk}	4.65 ± 0.44^{m}
1.0	66.94 ± 0.69^{ij}	5.30 ± 0.58^{jk}
1.5	69.06 ± 0.50^{h}	4.96 ± 0.34^{kl}
2.0	67.33 ± 0.45^{i}	3.60 ± 0.36^{pq}
2.5	66.68 ± 0.22^{j}	5.33 ± 0.60^{j}
3.0	67.94 ± 0.19^{hi}	5.03 ± 0.39^{k}
NAA		
0.5	85.05 ± 0.66^{d}	8.30 ± 0.55^{c}
1.0	92.65 ± 0.70^{b}	8.66 ± 0.21^{b}
1.5	96.63 ± 0.38^{a}	9.66 ± 0.48^{a}
2.0	92.36 ± 0.43^{bc}	8.09 ± 0.17^{cd}
2.5	84.98 ± 0.20^{de}	7.93 ± 0.59^{d}
3.0	82.38 ± 0.52^{e}	7.03 ± 0.67^{e}
IAA		
0.5	54.91 ± 0.49^{m}	4.30 ± 0.55^{o}
1.0	73.02 ± 0.28^{g}	5.96 ± 0.69^{hi}
1.5	79.30 ± 0.30^{ef}	6.64 ± 0.29^{f}
2.0	69.63 ± 0.19^{gh}	6.39 ± 0.70^{fg}
2.5	60.97 ± 0.60^{l}	6.09 ± 0.19^{h}
3.0	58.04 ± 0.47^{lm}	4.61 ± 0.38^{mn} `

***Control -** Treatment without PGR's; Data recorded after 2 weeks of culture.
Values are mean 30 explants per treatment and repeated three times. Mean values within a column followed by different letters are significantly different from each other at 5 % level comparison by Duncan's Multiple Range Test (DMRT).

In vitro rooting of shoots

Well-developed shoots were transferred to MS medium with different concentration of NAA, IBA and IAA in the range of 0.5-3.0 mg/L individually for root induction. Auxins (IAA, IBA and NAA) supplemented media significantly increased the rooting frequency when compared to the auxin free MS medium (Table 3). First single root emerged after a week in the presence of NAA that continued its linear growth with further production of lateral roots. Of the three auxins tested, NAA was found to be the most effective than the IAA and IBA supplemented medium (Table 3). Similarly, Purohit et al. (2017) reported that NAA was effective for root induction in *Amomum subulatum*. In contrast, IBA was more effective than IAA and NAA for root induction in other plant species, *Swertia lawii*, *Strobilanthes tonkinensis* (Parthraj et al., 2015; Srikun, 2017). Among the auxins, NAA at 1.5 mg/L showed maximum response (96.63%) with 9.66 roots per shoot and attained 5.93 cm root length. However, IBA (1.5 mg/L) showed the lowest response (69.06%) and 4.96 roots per shoot. The frequency and the number of roots varied depending upon the concentration and type of auxins (Table 3). High concentration (> 2.5 mg/L) of NAA, IAA and IBA produced simultaneous rooting and callusing at the thick end root base. Similar observation has been reported in *Ophiorrhiza mungos* (Namdeo et al., 2012). The callus was highly friable, it could be easily removed from shoots before transferring to field. Rooting was declined vigorously when increasing (> 2.0 mg/L) concentrations of NAA. Similar results were also noticed in *Sesamum indicum* (Raja and Jayabalan, 2011), *Solanum surattense* (Rahman et al., 2011) and *Gossypium hirsutum* (Prem Kumar et al., 2015).

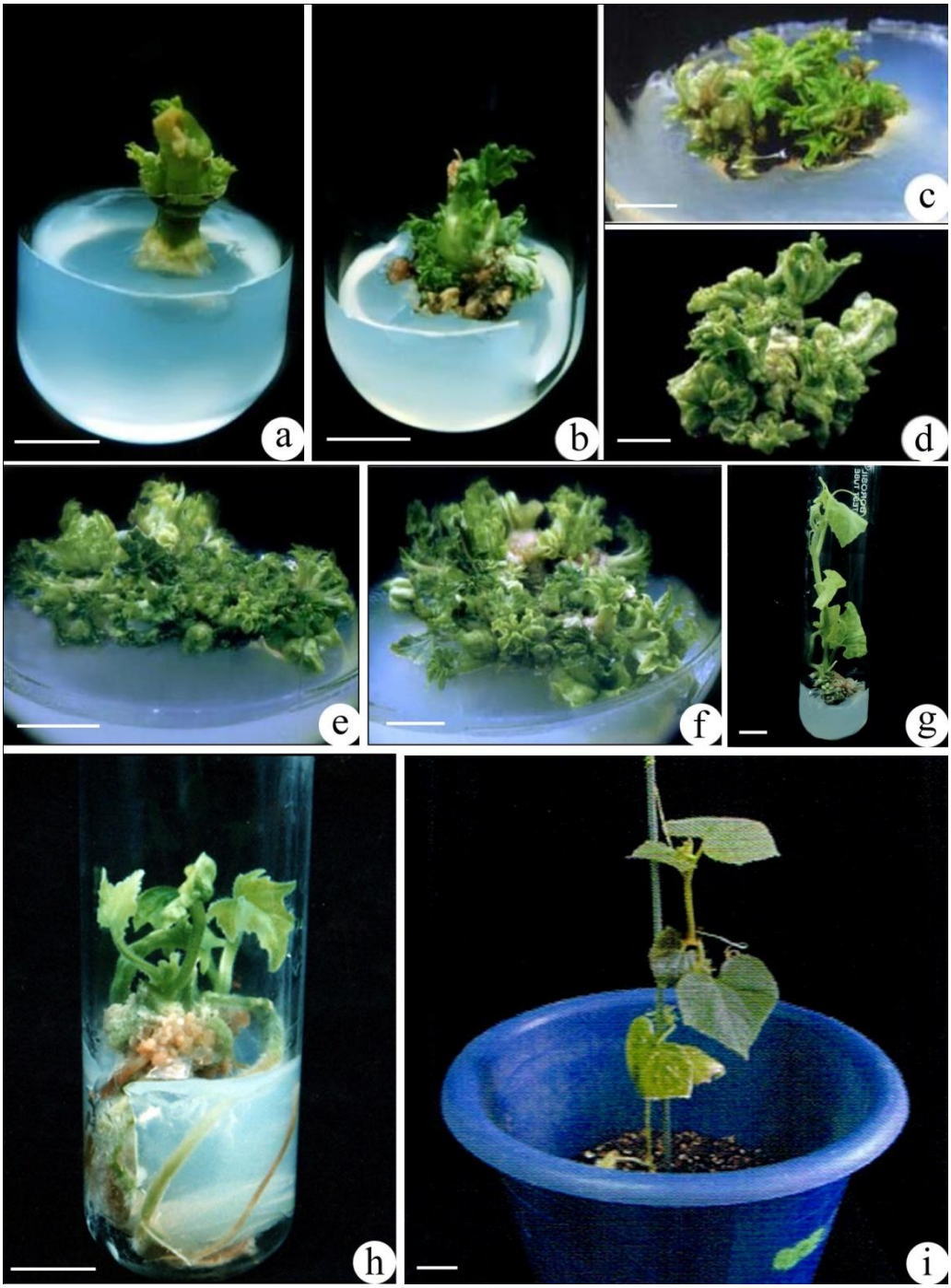

Fig. 2: Micropropagation of *Trichosanthes cucumerina* L. using shoot tip explant. a and b. Shoot explant; c and d. shoot bud proliferation; e and f multiple shoot initiation; g. shoot elongation; h. plantlet on rooting medium; i. Potted plant under natural conditions. Bar = 10 mm.

Hardening and acclimatization

The plantlets were successfully acclimatised inside the versatile plant growth chamber (Sanyo, Japan) than, the hardened plants were transferred to polybags containing sand, soil and vermiculite (2:1:1). Then it was transferred in to green house finally to experiment field with 90% survival.

Conclusion

The present study provides an efficient protocol for

mass propagation of *T. cucumerina* direct organogenesis through leaf and shoot tip explant. The highest number of shoot bud induction was obtained from the leaf explants. BA in combination with GA_3 responded maximum shoot induction and multiplication, and the shoots were effectively rooted on NAA in alone. Well-developed plantlets were successfully transferred to the field condition with maximum survival.

Conflict of interest statement

Authors declare that they have no conflict of interest.

References

Adebooye, O.C., 2008. Phytoconstituents and anti-oxidant activity of the pulp of snake tomato (*Tricosanthes cucumerina*). Afr. J. Trad. Compl. Alt. Med. 5(2), 173-179.

Chakravarty, B., Pruski, G.W., 2010. Rapid regeneration of stable transformants in cultures of potato by improving factors influencing Agrobacterium-mediated transformation. Adv. Biosci. Biotechnol. 1, 409-416.

Cheruvathur, M.K., Abraham, J., Thomas, T.D., 2015. *In vitro* micropropagation and flowering in *Ipomoea sepiaria* Roxb. An important ethanomedicinal plant. Asian Pac. J. Rep. 4(1), 49-53.

Devendra, N. K., Vijaykumar B. Malashetty., Seetharam, Y. N., 2015. Indirect organogenesis in wild snake gourd (*Trichosanthes cucumerina* L. var. *cucumerina*). Int. J. Curr. Res. 7(2), 12685-12690.

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

Finch-Savage, W.E., Leubner-Metzger, G., 2006. Seed dormancy and the control of germination. New Phytol. 171(3), 501-23.

Gogoi, G., Borua, P.K. and Al-Khayri, J.M., 2017. Improved micropropagation and *In vitro* fruiting of *Morus indica* L.(K-2 cultivar). J. Gen. Eng. Biotechnol. 15, 249-256.

Jian-Hua Wang., Hui-Ling Nie., Hai Huang., Siu-Cheung Tam., Yong-Tang Zheng., 2003. Independency of Anti–HIV-1 activity from ribosome–inactivating activity of Trichosanthin. Biochem. Biophys. Res. Commun. 302, 89-94.

Kolte, R.M., Bisan,V.V., Jangde, C.R., Bhalerao, A.A., 1997. Anti-inflammatory activity of root tubers of *Trichosanthes cucumerina* (LINN) in mouse's hind paw oedema induced by carrageenin. Ind. J. Indigen. Med. 18(2), 117-121.

Komalavalli, N., Rao, M.V., 2000. *In vitro* micropropagation of *Gymnema sylvestre*–A multipurpose medicinal plant. Plant Cell Tiss. Organ Cult. 61(2), 97-105.

Kshirsagar, P.R., Chavan, J.J., Umdale, S.D., Nimbalkar, M.S., Dixit, G.B. and Gaikwad, N.B., 2015. Highly efficient *In vitro* regeneration, establishment of callus and cell suspension cultures and RAPD analysis of regenerants of *Swertia lawii* Burkill. Biotechnol. Rep. 6, 79-84.

Lee, Y.K., Chung, W.I., Ezura, H., 2003. Efficient plant regeneration via organogenesis in winter squash. Plant Sci. 164, 413-418.

Lehner, B., Doll, P., Alcamo, J., Henrichs, T., Kaspar, F., 2006. Estimating the impact of global change on flood and drought risks in europe: a continental. Integ. Anal. Clim. Change. 75, 273-299.

Mohan, C., Naresh, B., Kumar, B.K., Reddy, V., Manjula, P., Keerthi, B., Sreekanth, D., Manzelat, S.F. Cherku, P.D., 2017. Micropropagation studies and phytochemical analysis of the endangered tree *Commiphora wightii*. J. Appl. Res. Med. Arom. Plants. 6, 70-79.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.

Namdeo, A.G., Priya, T., Bhosale, B.B., 2012. Micropropagation and production of camptothecin form *in vitro* plants of *Ophiorrhiza mungos*. Asian Pac. J. Trop. Biomed. 2(2), S662-S666.

Parthraj, R., Kshirsagar, P.R., Chavan, J.J., Umdale, S.D., Nimbalkar, M.S., Dixit, G.B., Gaikwad, N.B., 2015. Highly efficient *in vitro* regeneration, establishment of callus and cell suspension cultures and RAPD analysis of regenerants of *Swertia lawii* Burkill. Biotechnol. Rep. 6, 79-84.

Pillai, G.S., Martin, G., Raghu, A.V., Lyric, P.S., Balachandran, I., Ravindran, P.N., 2008. Optimizing culture conditions for in vitro propagation of *Trichosanthes cucumerina* L.: An important medicinal plant. J. Herbs Spices Med. Plants. 14(1-2), 13-28.

Prem Kumar, G.P., Sivakumar, S., Siva, G., Vinoth, S., Vigneswaran, M., Kanakachari, M., Senthil Kumar, T., Jayabalan, N., 2015. Evaluation of different carbon sources for high frequency callus culture with reduced phenolic secretion in cotton (*Gossypium hirsutum* L.) cv. SVPR-2. Biotechnol. Rep. 7, 72-80.

Pullaih, T., 2006. Encyclopaedia of World Medicinal Plants. Vol-IV, Regency Publication New Delhi.

Purohit, S., Nandi, S.K., Paul, S., Tariq, M. and Palni, L.M.S., 2017. Micropropagation and genetic fidelity analysis in *Amomum subulatum* Roxb.: A commercially important Himalayan plant. J. Appl. Res. Med. Arom. Plants. 4, 21-26.

Rahman, M., Amin, M., Islam, M., Sultana, R., 2011. Mass propagation of *Solanum surattense* Bum. using direct adventitious shoot organogenesis from intershoot tip. Acta Agric. Slov. 97(1), 11-17.

Raja, A., Jayabalan, N., 2011. *In vitro* shoot regeneration and flowering of Sesame (*Sesamum indicum* L.) cv. SVPR - 1. J. Agric. Technol. 7(4), 1089-1096.

Shivarajan, V.V., Indira, B., 1994. Ayurvedic drugs and their plant sources. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi.

Srikun, N., 2017. *In vitro* propagation of the aromatic herb *Strobilanthes tonkinensis* Lindau. Agric. Natural Resour. 51(1), 15-19.

Toyokawa, S., Takeda, T., Ohigara, Tukio., 1991. Isolation and characterization of a new abortificientprotein, Karasurin, from root tubers of *Trichosanthes kirilowii* Max. ver. *japonicum* Kitam. Chem. Pharm. Boll. 39 (3), 716-719.

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