



IN VITRO CLONAL PROPAGATION OF VULNERABLE ETHNOMEDICINAL CUCURBIT, RED BALL SNAKE GOURD (*TRICHOSANTHES TRICUSPIDATA* LOUR.)**RAJENDER. G, D. BHEEMANNA, P. SREENU, B. PRASAD^A,
K. RAJENDER AND T. CHRISTOPHER REUBEN***

Department of Botany, Kakatiya University, Warangal-506 009 Telangana State
^ADepartment of Biotechnology, Kakatiya University, Warangal-506 009 Telangana State

ABSTRACT

Protocol for *in vitro* clonal propagation through shoot proliferation of nodal explants of a vulnerable ethnomedicinal Cucurbit, *Trichosanthes tricuspidata* Lour., was developed. Maximum number of shoots (20.16 ± 0.79) and shoot length (4.16 ± 0.79) was obtained on MS medium supplemented with BAP (4.0 mg/L) + TDZ (0.5 mg/L), after four weeks of culture. Microshoots cultured on MS medium supplemented with IBA (1.5 mg/L) produced adventitious roots. Cytological examination of root tips of plantlets (R_1) obtained in this study did not reveal mitotic anomalies and the R_1 plantlets were morphologically similar to mother plant. About 70% of R_1 plantlets transferred to field condition survived and the vine grew well. These results demonstrated that *in vitro* clonal propagation of *T. tricuspidata* is technically viable.

KEYWORDS: *Trichosanthes tricuspidata* Lour., Clonal Propagation, TDZ, Aceto-Orcein.

**T. CHRISTOPHER REUBEN***

Department of Botany, Kakatiya University, Warangal-506 009 Telangana State

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INTRODUCTION

Trichosanthes tricuspidata Lour., is an important ethnomedicinal plant belonging to Cucurbitaceae family¹. It is known by various vernacular names as Red ball snake gourd in English, Lal Indrayan in Hindi, Avuduta in Telugu, Kaundal in Marathi, Khe-Ka- Daeng in Thai and Indreni in Nepal, which shows its medicinal usage by indigenous communities. The plant distribution ranges from eastern Himalayas in India, Southern China through southern Japan and Malaysia to tropical Australia². In Ayurveda, the fruits are used for the treatment of Asthma, Ear ache and Ozoena (inter-nasal crusting, atrophy & fetid odor)² and in Unani system of medicine the fruits are used as carminative, purgative, abortifacient to lessen inflammation³, in treatment of stomatitis, cold and influenza to cure migraines and reduce heat of the brain, in treatment of ophthalmia, leprosy, epilepsy and rheumatism and as well as other uses². In Bastar district, Chhattisgarh State, India, the plant is used for curing snake bite and the juice of the plant is applied externally for skin eruptions². The seeds are put in wine which is taken to treat stomach ache⁵ and also used as purgative⁴. In Thai traditional system of medicine, the plant is used as anti-fever remedy, as a laxative, as an antihelminthic and for treatment of migraine⁴. In Nepal, roots are used for to cure bleeding in chicken². The seed paste is used for hoof & mouth disease in cattle⁶. Methanolic leaf extract of *T. tricuspidata* was examined for anti-convalescent activity in pilocarpine induced oxidative stress in mice hippocampus⁷. The fruit extract of *T. tricuspidata* showed cytotoxic effect against KB cell line⁸, anti-inflammatory, analgesic activity and anti bacterial activity⁸. The roots of *T. bracteata* have exhibited anti-oxidative effect on sildenafil induced migraine in albino mice⁹, anti pyretic effect on albino rats was investigated^{10, 11, 16}. Ethanol based root extract of *T. tricuspidata* was reported to possess anti- diabetic effect¹², anti- oxidant, anti-bacterial and anti-fungal¹³. The fruit extract of *T. tricuspidata* showed moderate larvicidal effect on mosquito *Culex quinquefasciatus* Say¹⁵. The cytotoxic effect of cucurbitacin from pericarps of *T. tricuspidata* fruits was investigated¹⁷. Trichosanthin isolated from *T. tricuspidata* induced apoptosis of Leukemia K56 cells⁹, anti-pyretic effect on albino rats was investigated^{18, 10, 11}. Trichosanthin is undergoing trials as a possible remedy for AIDS¹⁴. *T. tricuspidata* is little experimented plant with immune's medicinal potential¹⁹. Indiscriminate collection owing to its immense medicinal value by pharmaceutical companies and herbal medical practitioners is leading to make it as exhaustion of the plant material. Adding to the distress, low percentage of seed germination (> 10%) and relatively very small output of propagules using conventional methods of propagation has resulted in restricted distribution and low population density of this vulnerable plant²⁰. Tissue culture technique is a viable alternate propagation of this invaluable medicinal plant and musters the conservation goal. To the best of our knowledge, this is the first report on *in vitro* clonal propagation of vulnerable medicinal cucurbit, *T. tricuspidata* Lour.

MATERIAL & METHODS

Plants (vines) of *Trichosanthes tricuspidata* Lour., were collected from Laknepally (Village), Narsampet (Mandal), Warangal (District), (District geographical location- 17.9689° N & 79.5941°E), Telangana (State), India during 2015 and planted in medicinal garden, Department of Botany, Kakatiya University, Warangal (District.), Telangana (State), India. The plant material was authenticated as *Trichosanthes tricuspidata* Lour., by Dr. Md. Mustafa, Department of Botany, Kakatiya University, Warangal. The plant material is preserved as herbarium specimen in Department of Botany, Kakatiya University, Warangal, Telangana State, India. Nodal explants admeasuring 2.0 cm in length were collected from three months-old *T. tricuspidata* Lour., and were washed under running tap water for 15 minutes, followed by several changes of sterile distilled water and then surface sterilized with 0.1 % (w/v) mercuric chloride (HgCl₂) for 3 minutes and finally rinsed in several changes of sterile distilled water. (MS)²¹ medium fortified with 3 % sucrose (w/v) was adjusted to pH 5.8 with 1 N NaOH, solidified with 0.8 % agar-agar (w/v) and was autoclaved for 15 lbs for 20 minutes. Nodal explants were cultured on MS medium supplemented with 1.0-5.0 mg/L Benzylaminopurine (BAP) and 0.1-1.0 mg/L Thidiazuron (TDZ) individually and in combination for shoot proliferation. Single shoot (microshoot) was cultured on MS medium supplemented with 1.0-5.0 mg/L Indole-3-Butyric Acid (IBA) for adventitious root induction. All cultures were maintained under white fluorescent light (80μ EM²S⁻¹) at 25 ± 2°C under 16 hour's photoperiod. Complete plantlets were transferred to pots containing sterile soil and compost (1:1) and grown in green house for four weeks and then transferred to research field.

Cytological procedure

Roots of randomly selected 12 plantlets (R₁) were fixed in ethanol and acetic acid (3:1) for 24 hours and then preserved in 70% ethanol until further use. The translucent root tips were transferred to watch glass containing 1.0 N HCl and 2 % Aceto-Orcein (9:1) and heated until effervescence. The root tips were placed on a clean glass slide and squashed with a drop of 45 % acetic acid and covered with a cover slip. The mitotic stages were observed under a Magnus MLX compound microscope at 60 X magnification²².

STATISTICAL ANALYSIS

Data on shoots (number and length in cm) was scored after 4 weeks of culture of 12 replicates and was subjected to statistical analysis including mean (\bar{x}) and standard deviation (SD).

RESULTS AND DISCUSSION

The quiescent axillary buds on nodal explants sprouted and proliferated into actively growing shoots when cultured on MS medium supplemented with BAP or TDZ either alone or in combination, after 4 weeks of culture (Fig. 1. A, B, C). Among BAP & TDZ tested, TDZ at 0.5 mg/L induced more shoots (10.0 ± 0.66) than BAP (5.0 ± 0.66), however the shoots were stunted on TDZ and elongated on BAP. Maximum number of shoots (20.16 ±

0.79) with maximum shoot length (4.16 ± 0.79) was observed on combination treatment of MS + BAP (4.0 mg/L) + TDZ (0.5 mg/L) (Fig. 2). TDZ is popular in inducing more number of shoots in contrast to BAP as in other Cucurbits like *Cucurbita pepo*²³, *Melothria maderaspatana*²⁴; *Citrullus colosynthis*²⁵ and *Luffa cylindrica*²⁶. TDZ mode of action maybe to check the action of cytokinin oxidase, which in turn modulates the level of endogenous cytokinins²⁷ or varied translocation rates to the meristematic region and metabolic processes, in which cytokinin maybe degraded or get conjugated with sugars or amino acids to form biologically inert compounds²⁸. TDZ induced more number of shoots (20.16 ± 0.79) compared to BAP (5.0 ± 0.66), nevertheless, TDZ induced shoots are small in size and stunted in growth as opposed to BAP induced shoots which are large in size and robust in growth. Similar results were reported in *Zingiber montanum*²⁹; *Aframomum corrorima*³⁰. Microshoots

obtained from combination treatment of MS + BAP 4.0 mg/L + TDZ 0.5 mg/L developed adventitious roots on MS+IBA (1.0-5.0 mg/L), after 4 weeks of culture (Fig. 1 D, E). Best rooting response (90%) was observed in 1.5 mg/L IBA (Fig. 3). R_1 plantlets were found to be diploid ($2n=22$) and devoid of any chromosomal anomalies. Similar results were obtained in *Luffa cylindrica*²⁶, *Momordica dioica*³¹ and *M.charantia*³² and complete plantlets (R_1) were transferred to pots containing sterile soil and compost (1:1) and placed in green house (Fig. 1 F). Percentage of plantlets (R_1) that survived in field conditions was approximately 70%. The importance of this study is the development of a protocol for rapid *in vitro* clonal propagation which can be safely used for genetic transformation studies for enhancing Trichosanthin, a pharmaceutically important bioactive compound and this method is advantageous over seed propagation because it maintains genetic fidelity.

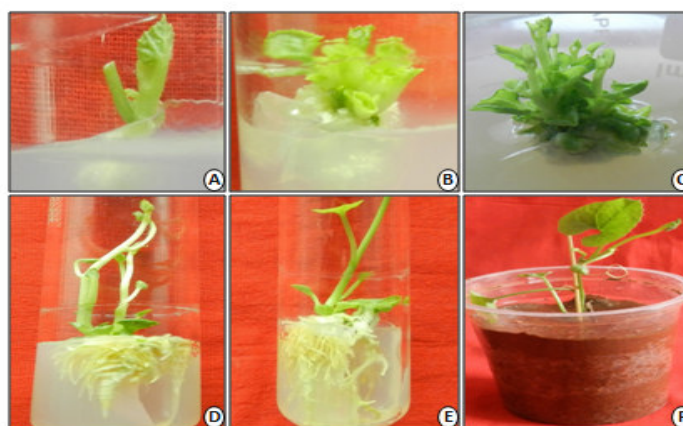


Figure 1

- A Fresh nodal explant cultured on MS+ BAP (4.0 mg/L) + TDZ (0.5 mg/L).
 B Miniature shoots emergence on MS+ BAP (4.0 mg/L) + TDZ (0.5 mg/L) after two weeks of culture.
 C Elongated microshoot proliferation on MS+ BAP (4.0 mg/L) + TDZ (0.5 mg/L) after four weeks of culture.
 D & E. *In vitro* root induction in microshoot on MS+ IBA (1.0 mg/L) after four weeks of culture.
 F Complete plantlets growing in pots containing sterile soil and compost (1:1).

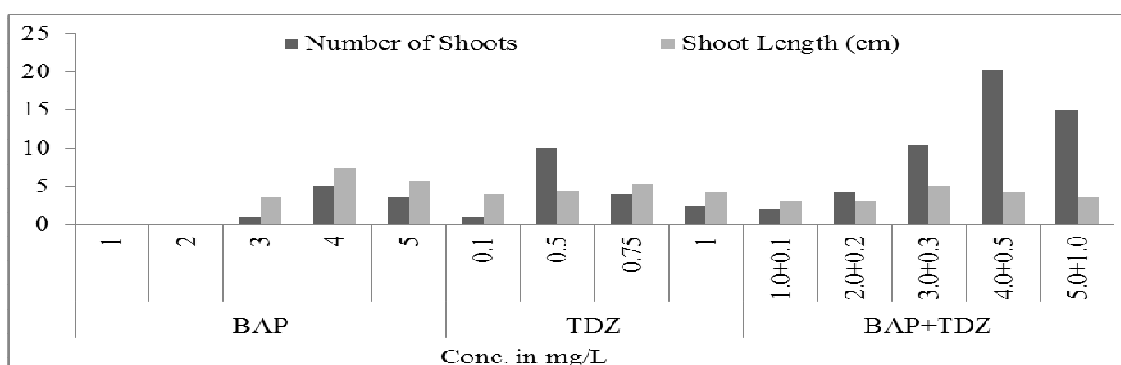


Figure 2

In vitro shoot proliferation in nodal explants of *T. tricuspidata* cultured on MS + BAP (1.0 -5.0 mg/L); TDZ (0.1 to 1.0 mg/L) and their combination

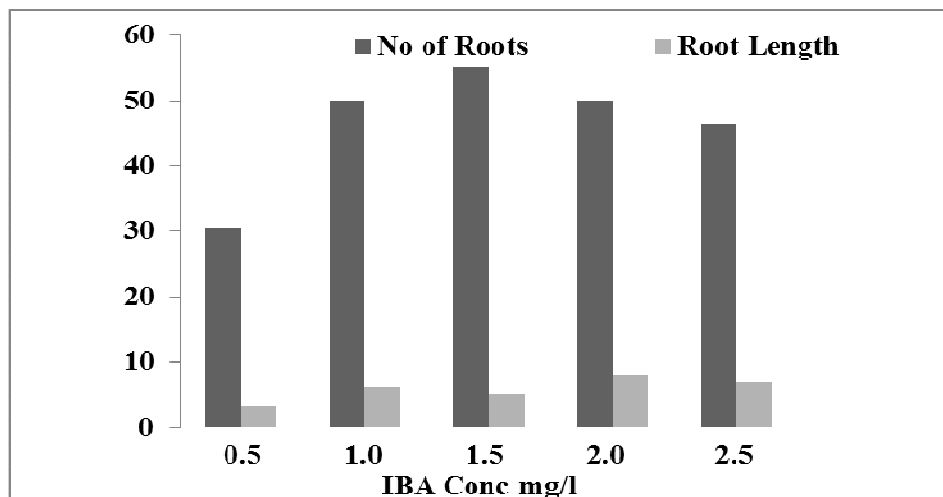


Figure 3

In vitro root induction in microshoots of *T. tricuspidata* on MS+ IBA (0.5 to 2.5 mg/L)

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CONFLICT OF INTEREST

Conflict of interest declared none.

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Reviewers of this article

Dr. MD. Mustafa M.Sc. Ph.D

Assistant Professor, Department of Botany,
Kakatiya University, NH-563,
Vidyaranyaपुरi, Hanamkonda, Warangal,
Telangana 506009



Prof. Dr. K. Suri Prabha

Asst. Editor, International Journal
of Pharma and Bio sciences.



**Mr. Anubrata Paul M.Sc. Biotech
(Research)**

Department of Biotechnology, Natural
Products Research Laboratory, Centre for
Drug Design Discovery & Development (C-
4D), SRM University,
Delhi-NCR, Sonapat, India



Prof. P. Muthu Prasanna

Managing Editor, International
Journal of Pharma and Bio sciences.

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