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IN VITRO CLONAL PROPAGATION OF VULNERABLE ETHNOMEDICINAL CUCURBIT, RED BALL SNAKE GOURD (*TRICHOSANTHES TRICUSPIDATA* LOUR.)

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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***.

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INTRODUCTION

Trichosanthes tricuspidata Lour., is an important ethnomedicinal plant belonging to Cucurbitaceae family[1]. 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 Australia[2]. 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 inflammation[3], 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 uses[2]. 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 eruptions[2]. The seeds are put in wine which is taken to treat stomach ache [5] and also used as purgative [4]. In Thai traditional system of medicine, the plant is used as anti-fever remedy, as a laxative, as an antihelmenthic and for treatment of migraine[4]. In Nepal, roots are used for to cure bleeding in chicken [2]. The seed paste is used for hoof & mouth disease in cattle [6].Methanolic leaf extract of *T. tricuspidata* was examined for anti-convalescent activity in pilocarpine induced oxidative stress in mice hippocampus [7]. The fruit extract of *T tricuspidata* showed cytotoxic effect against KB cell line[8], anti-inflammatory, analgesic activity and anti bacterial activity [8].The roots of *T. bracteata* have exhibited anti-oxidative effect on sildenafil induced migraine in albino mice[9], anti pyretic effect on albino rats was investigated [10, 11, 16]. Ethanol based root extract of *T. tricuspidata* was reported to possess anti- diabetic effect [12], anti- oxidant, anti-bacterial and anti-fungal[13].The fruit extract of *T. tricuspidata* showed moderate larvicidal effect on mosquito *Culex quinquefasciatus* Say [15]. The cytotoxic effect of cucurbitacin from pericarps of *T. tricuspidata* fruits was investigated [17]. Trichosanthin isolated from *T. tricuspidata* induced apoptosis of Leukemia K56 cells [9], anti-pyretic effect on albino rats was investigated [18, 10, 11]. Trichosanthin is undergoing trials as a possible remedy for AIDS [14]. *T. tricuspidata* is little experimented plant with immune's medicinal potential [19]. 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 plant [20].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 cuccurbit, *T.tricuspidata* Lour.

MATERIAL & METHODS

Plants (vines) of *Trichosanthes tricuspidata* Lour., were collected from Laknepally (Village),Narsampet (Mandal), Warangal (District), (District geographical location- 17.9689^0 N & 79.5941^0E), 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 ($HgCl_2$) 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 EM^2S^{-1}) at 25 ± 2^0C 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 (R_1) 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 magnification[22].

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 cytokinins [27] 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 compounds [28]. 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.

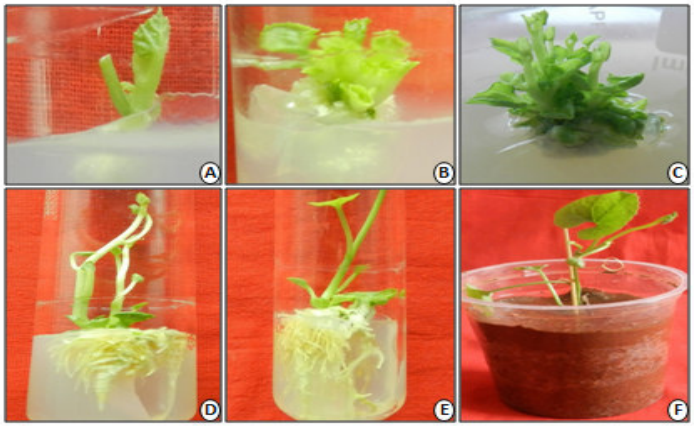

Figure 1

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).

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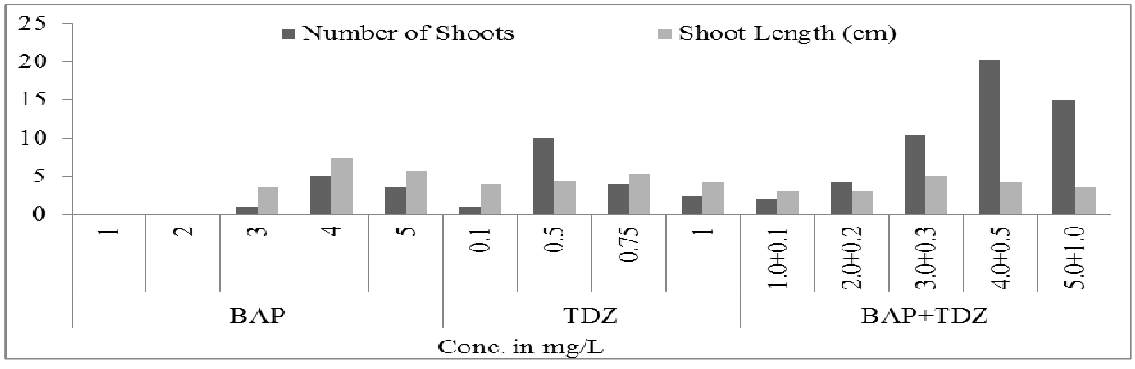

Figure 2
***In vitro* shoot proliferation in nodal explants of *T. tricuspidata* cultured on MS + BAP (1.0 -5 .0 mg/L; TDZ (0.1 to 1.0 mg/L) and their combination**

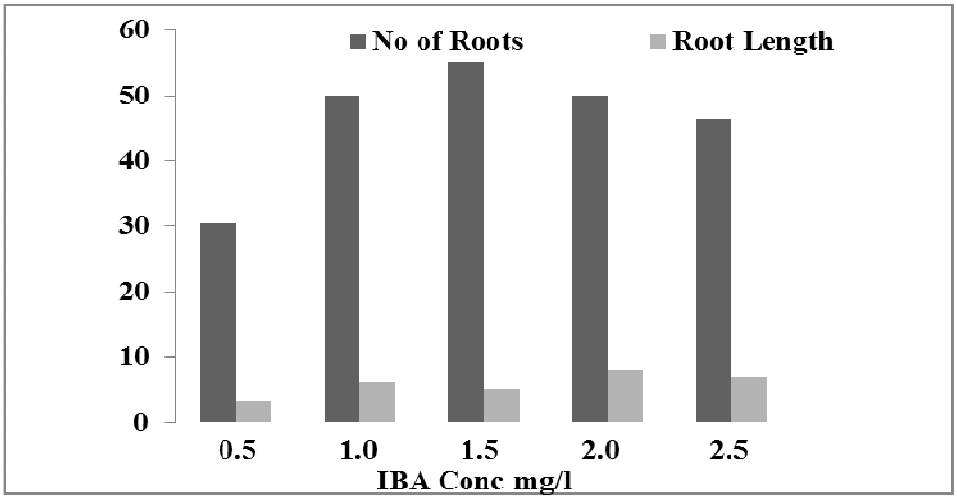

Figure 3
***In vitro* root induction in microshoots of *T. tricuspidata* on MS+ IBA (0.5 to 2.5 mg/L)**

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CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Vartak VD, Gadgil Madhav. Studies on sacred groves along the western ghats from Maharashtra and Goa. Role of beliefs and folklore. In. SK Jain (editor) Glimpses of Indian Ethno botany. New Delhi: Oxford and IBH Publishing Co; 1981, p.272-278.
2. Bhandari S, Upama D, Mamta S, Bisht NS. *Trichosanthes tricuspidata*: A Medicinally Important Plant. Trees for Life Journal. 2008 Nov 10; 3(5):1-6.
3. Chopra RN, Nayar SN, Chopra TC. Glossary of Indian medicinal plants, New Delhi: Council of Scientific and Industrial Research; 1956, p. xx-329.
4. Kanchanapoom T, Ryoji K, Yamasaki K. Cucurbitane, hexanorcucurbitane and octanorcucurbitane glycosides from fruits of *Trichosanthes tricuspidata*. Phytochemistry. 2001 Oct 7; 59: 215-228.
5. Perry LM. Medicinal plants of East and South East Asia: Attributed properties and uses. London: The MIT Press Cambridge; 1980 Feb 28, p.119.
6. Gaur RD. Flora of the District Garhwal North West Himalaya, Srinagar, Uttarakhand (India), Transmedia Publishers; 1999, p.811.
7. Smilin Bell Aseervatham G, Thilagar Sivasudha, Mohan Suganya, Angappan Ramesh Kumar, Ramachandran Jeyudevi. *Trichosanthes tricuspidata* Modulative Oxidative Toxicity on Brain Hippocampus against Pilocarpine Induced Status Epileptics in Mice. Neurochem Res.2013 May18; 10(1):1064-1075.
8. Mai, le Phuong, Guenard, Daniel, Franck, Marc, Van Tri, Mai, Christiane, Servant Thierry. New cytotoxic cucurbitacins from the *Trichosanthes tricuspidata* Fruit; Natural Product Research. 2010 Oct 27; 16 (1):15-19.
9. Nithiya P, Mohan K. Antioxidative effect of *Trichosanthes tricuspidata* root extract on sildenafil induced migraine in albino mice. Pharma. Res. 2010 Jan 2; 1:402-405.
10. Verma P, Kamboj V, Ranjan S, Mahesh K. Anti-Inflammatory and analgesic activity of aqueous extract of *Trichosanthes bracteata* fruit in animal model. Pharmacology online. 2010; 1: 8-16.
11. Manivel K, Rajangam P, Muthusamy M, Rajashekar S. Evaluation of anti-pyretic effect of *Trichosanthes tricuspidata* Linn on albino rats. Int. J. Res. Pharma Biomed Sci. 2011 Dec; 2(4): 25-32.
12. Srinivasan Kulandaivel, Pawan Bajpai, Thangavel Sivakumar. Anti Hyperglycemic activity of *Trichosanthes tricuspidata* root extract. Bangladesh. J. Pharmacol; 2013 July 8;8:305-310.
13. Shweta S. Saboo, Priyanka K. Thorat, Ganesh G, Tapadiya Khadabadi SS. *In vitro* Anti- Oxidant and Antifungal activity of chloroform extract of *Trichosanthes tricuspidata* Lour roots. Int. J. Pharm. Sci. Rev. Res. 2013 Sep 30; 22(2): 264-268.

14. Duvey BK, Goyel R, Parashar B, Verma D, Hitesh D, Sharma D. *Trichosanthes tricuspidata*: exploration of its medicinal value. Asian J. Pharm. Tech. 2012 March 12; 2 (1): 26-28.
15. Sonwalkar RP, Kadam PS. *Trichosanthes tricuspidata* methanol and petroleum ether extract effect on culex. Weekly science. 2013 Aug 29; 1 (6): 1-3.
16. Mohamed PA. Isolation of Trichotetrol-A new tetrahydroxy pentacyclic triterpene from *Trichosanthes bracteata* (Cucurbitaceae), Linn; Voight, Syn. *T. palmate* (Roxb.) Current Science 1999; 43: 116.
17. Mai IeP, Guenard D, Frank M, Van TM, Gaspard C, Sevenet T. New cytotoxic cucurbitacins from the pericarps of *Trichosanthes tricuspidata* fruits. Natural Product Letters. 1994; 16(1):15-19.
18. Kasai R, Sasaki A, Hashimoto T, Ohtani K and Yamasaki K. Cycloartane glycosides from *Trichosanthes tricuspidata*. Phytochemistry. 1999 July; 51: 803- 808.
19. Sadangi N, Padhy RN, Sahu RK. A Contribution to medico-ethno botany of kalahandi district, orissa on ear and mouth disease. *Anci. Sci. Life* 2005 Jan; 24(3): 160-163.
20. Ananthi P, Kala A, Soosairaj S. Factors influencing seed dormancy and germination of *Trichosanthes tricuspidata*- A vulnerable plant. Int. J. Inte. Sci. Inno. Technol. Sec. 2012 Aug; B 1(3):19-23.
21. Murashige T, Skoog T. A revised medium for rapid growth and bioassay with tobacco plant. Plant Physiol. 1962; 15:475-479.
22. Christopher T, Rajam MV Effect of genotype, explant and medium on *in vitro* regeneration of red pepper. Plant Cell Tiss Organ Cult. 1996 Sep; 46:245-250.
23. Pal SP, Alam I, Anisuzzaman M, Saraker KK, Sharmin SA, Alam MF. Indirect organogenesis in summer squash (*Cucurbita pepo* L). Turk. J. Agric. For. 2007; 31:63-70.
24. Baskaran P, Velayutham P, Jayabalan N. *In Vitro* regeneration of *Melothria maderaspatana* via indirect organogenesis. *In Vitro* cell. Dev. Biol. Plant. 2009 Aug 1; 45: 407-413.
25. Savitha R, Shasthree T, Sudhakar J, Mallaiah B. High frequency of plantlet regeneration and multiple shoot induction from leaf and stem explant of *Citrullus colosynthis* (L) schrad, an endangered medicinal cucurbit. Int. J. Pharma. Biol. Sci. 2010; 6(2):1-8.
26. Sujatha D, Ravi CH, Raghuvardhan L, Prasad B, Gulab Khan R, Sadanandam A, Christopher T. *In vitro* plantlet regeneration and genetic transformation of sponge gourd (*Luffa cylindrical* L). Afr. J. Plant Sci. 2013 June; 7 (6):244-252.
27. Hare PD, Van Staden J. Cytokinin oxidase: Biochemical features and physiological significance. Physiol. Plant. 1994 May; 91:128-136.
28. Kaminek M. Progress in cytokinin research. Trends Biotechnol. 2003 July 31; 10:159-162.
29. Hamirah MN, Sani HB, Boyce PC, Sim SL. Micropropagation of red ginger (*Zingiber montanum Koenig*), a medicinal plant. Afr J. Biotechnol. 2010; 18(1):127-130.
30. Tefera W, Wannakrairoj S. Synergistic effects of some plant growth regulators on *in vitro* shoot proliferation of Korarima (*Aframomum corrorima* (Braun) Jansen). J. Biotechnol. 2006 Oct 16; 5(10):1894-190.
31. Mustafa Md., Swamy TN, Raju S, Peer Mohammad SK, Suresh V. Regeneration of plantlets from nodal cultures of *Momordica dioica* Roxb. Int J Pharm Bio Sci. 2012 Oct; 3(4): (B) 92 - 96.
32. Agarwal M, Kamal R. *In vitro* clonal propagation of *M. charantia*. Ind. J. Biotechnol. 2004 July; 3: 426-430.

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