

***In Vitro* Multiplication from Stem node Explants of *Trichosanthes anguina* L. A Medicinal important Plant.**

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ABSTRACT

Multiplication of genetically identical copies of a cultivar by asexual reproduction is called clonal propagation and a plant population derived from a single individual by asexual reproduction constitutes a clone. In nature, clonal propagation occurs by apomixis (seed development without meiosis and fertilization) and/or vegetative reproduction (regeneration of new plants from vegetative parts). MS basal medium supplemented with various Auxons/Cytokinins BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots. Addition of BAP at 3.0 mg/l and NAA at 2.0 mg/l to the MS basal medium, induced regeneration from the Stem node segments. With an increase in the level of BAP 2.0 - 3.0 mg/l the percentage of explants producing shoots also increased. The number of shoots developed on the stem node segments ranged from 1-4 to 2-3 by the addition of BAP at concentration of 2.0 mg/l or NAA at 2.0 mg/l. Among the three concentrations of coconut milk used i.e, 6, 10 and 15% of coconut milk along with 0.5 mg/l BAP proved to be ideal for multiple shoot induction. MS medium fortified with 2.0 mg/l BAP 1.0 mg/l L-3.0mg/l Kn or 3.0 mg/l L-Glutamic acid also induced shoot buds on Stem node segments. Regeneration shoots from Stem node explants of callus and *In Vitro* multiple shoots were obtained on MS Medium within BAP, NAA, L-Glutamic acid and Kinetin, Coconut milk (CM). High frequency plant regeneration from leaf explants of *S. Nigrum* by Ugander *et al* (2010) Callus induction and base explants of *Aloe vera* R. Prasad, Venkateshwarlu M *et al* (2018).

Keywords : Stem node Explants, L-Glutamic acid, BAP, *Trichosanthes anguina* L, NAA

INTRODUCTION

Since most fruit trees and ornamental plants are highly heterozygous their seed progeny is not true to type. Asexual reproduction, on the other hand, gives rise to plants which are genetically identical to the parent plant and, thus, permits the perpetuation of the unique characters of the cultivars. In the present paper, a simple and reproducible procedure was devised to obtain multiple shoots from Stem node segments of *Trichosanthes anguina* L on MS medium fortified with plant growth regulators along with coconut milk and amino acids. The main objective of clonal propagation is to establish plants that are uniform and predictable for selected qualities. Growth or *in vitro* propagated plants is often stronger than in those cloned *in vitro* phyto chemical analysis and biological activities in *Mimosa pudica* Venkateshwarlu *et al* (2011). The plants of Cucurbitaceae suffer from several diseases including the water melon mosaic virus Wayne *et al* (2011) Cucumber green mottle mosaic virus Wayne *et al* (2011) and *Solanum nigrum* also suffers from downy and powdery mildews which seriously limits the crop production. Axillary buds from pump-kin were reported by Ugander *et al* (2019) & Rathore (2010).

MATERIALS AND METHODS

The explant material is then surface sterilized, usually in multiple courses of bleach and alcohol washes and finally rinsed in sterilized water. This small portion of plant tissue, sometimes only a single cell, is placed on a growth medium, typically containing sucrose as an energy source and one or more plant growth regulators (plant hormones). They were cultured on MS medium containing 2.5% sucrose and 0.8% Agar- Agar and different concentrations of BAP, NAA and L-Glutamic acid. Stem node segments of *Trichosanthes* were cultured and surface sterilized with 0.1% HgCl₂ for 5-6 minutes and rinsed with sterile distilled water. Cultures were incubated under 16 hrs illumination (250 lux) at 25± 2°C temperature. Each treatment consisted of 10-15 replicates. The data was recorded at the end of eighth week in vitro propagation of *Zyglis Sudhershana* et al (2000) cloning protocol Campstrini (2006). The pH of the medium was adjusted to 5.8 and later was autoclaved at 120 °C for 17 minutes. Rajendraprasad, Venkateshwarlu M (2018) experimental mutagenesis on cicer tissue culture studies stem node explants, multiple shoots in *cucumis* Venkateshwarlu M (2019) and (2020).

RESULTS AND DISCUSSION

The medium is sterilized during preparation to prevent fungal and bacterial contamination, which can outgrow and smother the growing explant. Autoclaves and filter sterilization are used to remove potential contaminants; under smaller scales of production a pressure cooker is often used. The plant tissue grows and differentiates into new tissues depending on the medium. For example, media containing cytokinins are used to create branched shoots from plant buds. The results of the study have shown the initiation of shoot buds and formation of multiple shoots from stem node segments. Addition of NAA failed to produce many shoots, but enlarged the leaf segments. Lower levels of coconut milk (4 & 8%) induced callus formation. Leaf explants were inoculated on MS basal medium fortified with various Auxins cytokinins i.e., BAP and NAA. Coconut water also had a role in triggering the formation of multiple shoots Kanna *et al* (2005) *In Vitro* micropropagation *Solanum nigrum* Ram *et al* (2002). The mean number of shoots developed on the stem node segments ranged from 1- 4 to 2 - 3 by the addition of different concentrations of BAP and NAA the level of SAP (3.0 mg/l to 4.0 mg/l) resulted in an increase in the percentage of shoots developed with 10, 15, 20% of coconut milk also triggered the induction of multiple shoots (Plate I). Low concentration of L- Glutamic acid (0.5 - 3.0 mg/l, along with SAP (1.0 mg/l, produced significant mean number of multiple shoots that ranged from 2-3 to 5- in the stem node segments. Shoot multiplication was obtained from stem node explants cultured on MS Medium supplemented with 1.0 to 3.0 mg/l BAP. Raising the level of BAP (0.5 to 2.0 mg/l) resulted in an increase in the number of shoots from stem node segments of *Trichosanthes anguina* L suggested that the formation of multiple shoots at the stem node region of the stem node of soybean indicated the existence of totipotency in this region which can be activated with the addition of BAP. (Table-1)

(Plate-I) In the final stage of plant micropropagation, the plantlets are removed from the plant media and transferred to soil or (more commonly) potting compost for continued growth by conventional methods. This stage is often combined with the "pretransplant" stage. Tissue culture has become a popular method for vegetative propagation of plants. The most significant advantage offered by this aspectic method of clonal propogation, popularly called ‘micropropagation’, over the conventional methods is that in a relatively short time and space a large number of plants can be produced starting from a single individual. For orchids, micro propogation is the only commercially viable method of clonal propagation

Table-I Multiple shoots from Stem node explants of *Trichosanthes anguina* L

Growth Regulators	Stem node Explants	
	% Frequency of Shoots	Mean no. of Shoots
MS + 0.5 mg/l BAP + Kn	45	Callus
MS + 1.0 mg/l BAP + Kn	40	Callus
MS + 2.0 mg/l BAP + NAA L-Glutamic acid	35	Shoots (2-4)
MS + 3.0 mg/l BAP + NAA L-Glutamic acid	30	Shoots (2-3)
MS + 0.5 mg/l NAA + Kn+CM	22	Callus+ Small buds
MS + 1.0 mg/l NAA + Kn+CM+L-Glutamic acid	20	Callus + Small buds
MS + 2.0 mg/l NAA + Kn+CM+L-Glutamic acid	25	Shoots (2-4)
MS + 3.0 mg/l NAA + L-Glutamic acid	20	Shoots (4-6)
MS + 4.0 mg/l NAA + L-Glutamic acid	20	Shoots (2-4)

Plate-I **Muliple shoots from stem node explants of *Trichosanthes anguina* L.**



Conclusion:

The technique developed by Morel was rapidly adopted by orchid growers because of its tremendous practical applications. Today, tissue culture is the most popular method for clonal propagation of a Medicinal important Plants.

REFERENCES:

1. Kanna T.M.S., SM Nagarajan and S. Kulothungan (2005). Micropropagation of *Solanum nigrum* L a medicinal herb plant Archies: 609-305.

2. Rathore, MS and Shekawat N.S. (2010). *Ex Vivo* implication of Phytohormonous on various *in vitro* responses in *Leptadenia reticulata* (Retz) night Am-An endangered plant Envi. Exp. Bilogy 49: 215-220.
3. Sudarshan L, Aboel MN and hussain J (2000). In Vitro propagation of *Ziziphus maritiana* cuctivar umrdu by shoots tip and nodal multiplication. Curr. Sci. 80(2) 290-292.
4. Waynem, Watt JM and MG. Breyer-Brandwijk (2011) *Solanum nigrum* L in the medicinal and poisonous plants of Southern and Eastern Africa. PP-996-1000.
5. Venkateshwarlu M, N Raju Odelu G, Srilatha T, Ugender T (2017). Studies on phytochemical analysis and biological activities in *Mamordica dioica* Roxb through Fruit. The pharma Innovations and Journal 6(12) 437-440.
6. Ugender T Venkateshwarlu M Anitha Devi U Srilatha T and Prameela K (2019). In Vitro plantlet regeneration from Cotyledonary explants of *Solanum torvum* (Swartz) a medicinal important plant. International multi disciplinary E – Research Journal PP – 99-106.
7. Rajendra Prasad, Venkateshwarlu m, Rajesham and N Raju (2018). High frequency callus induction from shoot base explants of *Aloe Vera* (L) Burm F An Important plant, medicinal plant European Journal of Bio-Medical & Pharmaceutical Sci. Vol. 5 Issue-01. PP. 363-373.
8. Ugender T Shekar GPV and Manjula P (2010). High frequency plant regeneration from leaf explants of *Solanum nigrum* advances in plant Sci. 23(1) 15-17.
9. Rao C.S. Eganathan P Anand A, BalaKrishna P and Reddy TP (1998) protocol for in vitro propagation of *Excocaria agallocha* a medicinal important plant Mangrove Sps. Plant Cell Rep. 17: 861-865.
10. Ram D, Kalloo G Banerjee M.K. (2002). Popularing Kakrol and Kartolo. The indiegenous nutrition S vegetables Indian Hort. Vol. 9: 6-9.
11. Rajashekar S, Sivaghanam K and Subramanian S (2006). Modulatory effect of *Aloe vera* leaf get extraction oxidative stress in rats treated with sterptosotocin J Pharma and Phamacol 52(2) 241-246.
12. Campestrini Ach, Kuhnen S Lemos M (2006). Cloving protocol of *Aloe vera* as study case for the Tailormade biotechnology to small farmers J. F. Tech Management and Innovation 1(5) 76-79.
13. Mandalaju Venkateshwarlu (2019). Tissue culture studies callus treatment on stem node explants of *Citrullus vulgaris* L perpex Indian Journal Research Vol. (8) ISS-12 No 2280-1991.
14. Rajendra Prasad Venkateshwarlu M Odelu G B Madan Mohan and Babu Rao M (2018). Studies on experimental Mutagenesis on chick pea (*Cicer aritinum* L Induced by UV rays and EMS Ejbps Vol.5 ISS-08 pp 506-511
15. Mandalaju Venkateshwarlu (2020). Hormonal Differentiation and plantlet regeneration from stem node explanats of *Cucurbita maxima* (L) - Avegetable crop plant. International Journal of Innovative Science, engineering & Technology. Vol. 07, Issue-98, August 2020. PP-187-190.