

In Vitro Micropropagation Studies from Stem Node Explants of Clematis Gouriana (Roxb Ex DC)

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Abstract: Multiple shoot proliferation from stem/shoot tip explants Venkateshwarlu M (2012). MS media with different combinations and concentrations of hormones supplemented with BAP, Kn, NAA and 2,4-D. Regeneration callus, from stem node explants of *Clematis gouriana*. Economically important fruity crop plant and its fruits is the commercial part, harvested for extracting the vegetable and its use are hypnotic, sedative, and specific in insanity, reduce blood pressure, digestions and uterine contractions. *In vitro* organogenesis and embryogenesis on the other hand must undergo developmental changes which usually involve the formation of Callus with shoots, subsequent recognition into plantlets. The demonstration to the orchids, tuber crop plants, a novel method of clonal propagation was revealed by Moral (1965) method that quickly become standard among the tissue culturists. Supplement of cytokinin was reported in the medicinal species *curcuma*. Sahoo and Chand (1998). It has become an endangered species due to its over exploitation and it is generally propagated by seeds, but propagation by seeds is not satisfactory owing to highly variable germination rates and rate of reproduction of these plants are poor. Therefore, there is an urgent need to develop tissue culture and micro propagation methods for the mass propagation and conservation of this threatened species. Hassanenin et al (2000) plant tissue & protoplast cultures. The night shade family has plants with many different habits. Taxonomus C section Edmonds (1977), Plant regeneration from leaf explants Patak (2014) Medicianl Meena et al (2010) and Harmonal differentiation *In vitro* culture regeneration Venkateshwarlu (2020), Venkateshwarlu et al (2018) Plant regeneration Shahzad et al (1999).

Keywords: In Vitro, Regeneration, Stem Node, BAP, NAA, IAA, Kn

Introduction

The production of plants from stem node buds or shoots has proved to be most generally applicable and reliable method of true to type *In vitro* propagation. *Clematis gouriana* Roxb ex DC Ranunculaceae is a large vine capable of climbing tall trees. Recently shoot meristem tips have also been used direct delivery of desired genes in soybean. Cotton and Sorghum Bhaskaran et al (1992), Balachandran S M (1990), Geetha S et al (2000). In view of the importance of mericlone technology in phyto pathology and genetic engineering experiment were conducted to achieve direct multiple shoots regeneration from *Clematis gouriana*. (Sweet potato) al (1995), Bais et al (2000) The biotechnological approaches for improvement will have to be *in vitro* selection techniques which have been successfully attempted in *Clematis gouriana* for recovery of anthracnose resistant somatic embryos after dual culture of embryogenic suspensions with culture filtrates from infected leaves and fruits. Considerable progress has been made in the propagation of this plant through *in vitro* cultures of *Clematis gouriana* made a successful induction of callus from shoot tip explants Somatic embryogenesis Pathak (2010). The improvement of *Clematis gouriana* through transformation with the help of selectable marker genes will depend upon advances in research on cloned genes having horticultural importance. Production of homozygous breeding lines the potential of haploid regeneration for other cultures or from irradiated ovules should be explored. Plant regeneration from shoot tip explants of soybean (T.U. & Venkateshwarlu M (2011). Diverse biological properties of *Clematis* spices are due to many secondary metabolites found in different parts of plants. In the Indian system of medicine Ayurveda the Ascarbic acid (97.28%) plant is used to eliminate malarial fever and headaches. Culture of shoot meristem, especially through enhanced braiding permits rapid clonal propagation land a high degree of genetic uniformity of progeny. Handique & Sunitha (2000). T Ugendar & Venkateshwarlu M (2012). Phyto chemical screening solaum sps.T.ugender and M.Venkateshwarlu(2019).

Material and Methods

The stem node explants were collected from Healthy plants washed in a 0.1% Mercuric Chloride (HgCl₂) solution for 3-4 minutes were washed thoroughly with sterile distilled water before the inoculation. Young

stem node were excised from the mature plants of *Clematis gouriana* and washed thoroughly in Tween 20 followed by rinsing in running water for 10 min. Biological activities of leaves of *Clematis gouriana* Venkateshwarlu *et al* (2018). It was followed by a dip in a 0.1% (w/v) mercuric Chloride (HgCl₂) Solution for 1-2 minutes. Finally the stem node explants were washed thoroughly with sterile water before the inoculation on the sterilized nutrient MS Medium culture tubes. All the above operations were performed under aseptic condition in a laminar air flow cabinet. The stem node explants were surface sterilized with HgCl₂ (0.1%) for 5 min, rinsed 3-4 times with sterile double distilled water. Stem node of 5 mm diameter were cultured with their surface on modified Murashige and Skoog's basal medium (MS) (Murashige, T. and Skoog, F. 1962) supplemented with 3.0 mg/l sucrose and 0.5-3.0 mg/l Benzyladenine (BAP and Kinetin (Kn). Multiplications of shoots were tested in the same media or by adding a-naphthalene acetic acid (NAA), IAA 1.0-3.0 mg/l and roots are obtained from half strength MS medium supplemented with 0.5-1.0 mg/l, MS medium with 0.5 mg l⁻¹ IBA and half strength MS basal and liquid medium. All the culture tubes were incubated under 16/8h light/dark photoperiod at 25 ± 2°C a light intensity of 40h Mol.M-2 was provided by cool-white florescent light. MS medium supplemented with combination 1.0 mg/l – 5.0 mg/l BAP, NAA, Kn, IAA and incubated under the same culture conditions. All cultures were maintained at 25 ± 2°C with 70 ± 5% Relative humidity on a 16-hour photoperiod under cool white fluorescent light of about 3000-lux for 16 h per day. Treatments were replicated three times and each replicates contained 20 cultures. MS medium with 0.5 mg l⁻¹ of NAA, IAA and BAP were the most effective giving high shoot regeneration frequencies associated with high number of shoots per stem node explants Venkateshwarlu M (2017). Young plants of *Clematis gouriana* collected from outside grown under partly shade conditions in the experimental garden of the Aromatic and medicinal plants.

Results and Discussions

The Result of present investigation show that the stem node explants from mature plants of *Clematis gouriana* could be induced to produce multiple shoots *in vitro* maximum number of shoots were (2-4 to 4-6) induced on MS medium fortified with various concentrations of BAP, NAA and Kn. The present study demonstrates the successful stem node regeneration from the *in vitro* cultured shoot tip explants of *Clematis gouriana* and the efficacy of the plant growth regulators was assessed by counting the number of shoots per stem node callus as well showed that 3.0 mg/l NAA and 2.0 mg/l BAP was found best for callus induction and growth. But in the present experiment, a higher level of NAA (3.0 mg/l) and BAP (3.0 mg/l) was found best for callus induction. *In vitro* regeneration trails followed by *in vivo* plant shoot tips acclimatization. The results showed a variable shoot forming capacity depending on the combination of growth regulators used in the culture medium. The number of shoots produced increased with the concentration of BAP and Kn until 1.5 mg/l or 0.5 mg/l of the cytokinin and showed high frequency of explants exhibiting compact green callus with shoots (4-6), growth and also for shoot induction. MS medium supplemented with 2.0 or 1.0 mg l⁻¹ of BAP and Kn shoot regeneration was obtained within 15-20 days and proliferation was also observed in the same concentration of medium Upadhyay *et al* has also showed that 1.0 mg/l of cytokinin (BAP and Kn) was found best for stem node regeneration and shoot proliferation. MS medium supplemented with different concentrations of BAP, NAA, IAA and Kn is presented. The medium was dispensed into culture tubes each containing 15ml of the culture medium capable with non-absorbent cotton and auto cloved at 121°C for 15minutes. Multiple shoot initiation from stem node explants was observed within 15-20 days after inoculation. (Plate-1, Table-1. fig -1 explant, fig -2 callus, fig -3 plantlet, fig -4 filed plant).

Table 1: In vitro Micro propagation studies from stem node explants of *Clematis gouriana* (Roxb ex DC)

Growth regulators (mg/ l)	Stem node explants showing callus response	No. of nodal explants
NAA (1.0) + BAP (1.0)	35	Callus
NAA (2.0) + BAP (1.5)	30	Callus
NAA (1.5) + BAP (2.0)	25	Shoots(2-4)
NAA (2.0) + BAP (3.0)	20	Shoots(4-6)
IAA (2.0) + BAP (2.0)	15	Shoots+Callus
2,4,D (1.0) + BAP (2.0)	10	Shoots (1-2)
Kn (1.0) + BAP (1.0)+IAA	30	Green Callus
Kn (2.0) + BAP (2.0)+IAA	25	Callus with shoot
Kn (3.5) + BAP (3.0)+IAA	20	Shoots (2-4)+roots
Kn (4.0) + BAP (4.0)	15	Shoots (2-3)+roots

Pate- 1. *In vitro* Micro propagation studies from stem node explants of *Clematis gouriana* (Roxb ex DC)



Conclusion

Multiple shoot production was observed from stem node explants of *Clematis gouriana*. This type of clonal propagation has advantage by producing *In vitro* culture shows the considerable importance of large scale propagation. The plantlets were transferred to poly pots containing pre-soaked vermiculite and maintained inside a growth chamber set at 28°C and 70-80% relative humidity. After two weeks they were transplanted to poly bags containing mixture of 1:1:1 ration of soil+sand+manure and kept under shade house for a period three weeks. Rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar. The shoot then hardened and later transferred to soil under greenhouse condition. Regenerated plants were transferred to pots from poly cups with 60-70% survival along with seed raised controls. Stem node explants *Clematis gouriana* cultured on various concentrations of hormones differentiated developed green callus *Clematis gouriana* with shoots culture on the MS media.

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