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RESEARCH ARTICLE

Micro propagation and optimization of protocol for medicinal important plant (*Clerodendrum viscosum)*

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SUMMARY

Plants of *Clerodendrum viscosum* Vent. (Verbanaceae) were regenerated by taking nodal segments containing apical and axillary buds as explants and isolated from field-grown mature plants. The apical and axillary buds were inoculated on MS media with 11 different concentration of BAP and adenine sulphate either alone BAP or combination of BAP and adenine sulphate. Best establishment was found in MS media within 5 – 7 days of inoculation. On subculturing well established explants on the same respective media treatments multiple shoot induction was highest in MS media supplemented with BAP (4mg/l). Five treatments of MS media and varying concentration of 2, 4-D for callus induction were taken. Callus induction was observed best in MS media supplemented with 2, 4-D (4mg/l) in 23-25 days. This protocol can be used to generate cost-effective protocol for large-scale *in vitro* multiplication of *Clerodendrum viscosum.*

Key Words : *Clerodendrum viscosum,* Medicinal plant, Micro propagation, Tissue culture

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Clerodendrum viscosum Vent. (Verbanaceae) are gregarious tawny-villous shrub found throughout India. The alcoholic extracts of the roots yields sterol glycosides which is mixture of β cytosterol and D^5-25- stigmastadien 3-β-ol. The aerial parts yield several steroids, *viz.*, clerosterol and its 22 E- dehydro derivative, 24 β-ethylcholesta-5, 22E, 25- trien-3 β-ol as major sterols in addition to campesterol, cholesterol, cytosterol, 24-α-stigmasterol and 24 β-stigmasterol. The leaves are bitter, acride, thermogenic, laxative, cholagogue, antiseptic, demulcent, anti-inflammatory, depurative, vermifuge, expectorant, antipyretic and are useful in vitiated condition in kapha, helminthiasis, ascarides, abscesses, tumors, leprosy, skin diseases, indolent ulcers, cough, bronchitis, inflammations, intermittent fevers, malaria fever, general debility and proctoptosis.

Ethno-medical importance of various species of *Clerodendrum* genus has been reported in various indigenous systems of medicines like Ayurveda, Siddha and Unani for treatments of various life threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension (Mukesh *et al*., 1969). The genus is being used as medicines specifically in Indian, Chinese, Thai, Korean, Japanese systems of medicine for the treatment of various life-threatening. Few species of the genus like *Clerodendrum inerme*, *C. thomosonae*, *C. indicum* and *C. speciosum* are ornamental and being cultivated for aesthetic purposes. The powder/paste form and the various extracts of root, stem and leaves are reported to be used as medicine for the treatment of asthma, pyreticosis, cataract, malaria and diseases of blood, skin and lung.

Many medicinal plant species are disappearing at an alarming rate due to rapid agricultural and urban development, uncontrolled deforestation, lack of conservation and

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indiscriminate collection. Destruction of natural habitat and indiscriminate collection of plants has endangered its survival. Therefore, it is necessary to develop and standardized the protocol for its large scale multiplication through micropropogation. The technique of plant tissue culture is also unique biotechnological tool for the study of wide ranging problems in the physiology and biochemistry of higher plants. It also offers an avenue to further evaluate and exploit the metabolic potentialities of higher plants for the bio-production of useful plant metabolites, particularly in plants of pharmaceutical significance, an interesting alternative for controlled production of plant constituents (Bhakuni and Jain, 1995).

MATERIAL AND METHODS

The explants like stems, leaves and shoot apices were taken from the plants growing under the *in vivo* conditions. The stem pieces, leaves, shoot apices were placed in different bottles and covered with net and washed for 30 minutes under running tap water to remove all the adhering dust particles and microbes from the surface. Explants were brought in laboratory and cut to 4-5 cm length and washed with mild detergent (tween 20) for another 15 minutes and then washed properly to remove the detergent. Under laminar flow hood, explants were treated with 1 per cent mercuric chloride solution for 5 minutes. The explants were then thoroughly washed (4–5 washings) with sterilized distilled water to remove the traces of $HgCl_2$. For establishment of culture, fresh cuts were given to the stem explants after sterilization up to the inoculation size *i.e.* 1-2 cm length with 1-2 leaf primordia. Each explant was inoculated in an erect position in the test tube. The different combinations of media used were MS_0 (MS basal as control), MS_1 (MS_0+15 mg/I adenine sulphate), MS_2 (MS_0+ 1 mg/l BAP), MS_3 (MS_0 + 2 mg/l BAP), MS_4 (MS_0+3 mg/l BAP), MS_5 (MS_0+4 mg/l BAP), MS_6 (MS_0+5 mg/l BAP), MS_7 (MS_0+2 mg/l BAP+15 mg/l adenine sulphate), MS_8(MS_0+3 mg/l BAP+15 mg/l adenine sulphate), MS_9 (MS_0+4 mg/l BAP + 15 mg/l adenine sulphate) and MS_{10} (MS_0 + 5 mg/l BAP+15 mg/l adenine sulphate). The medium was supplemented with 30 g/l sucrose and was solidified with 8 g/l agar. The pH of the medium was adjusted to 5.8 before autoclaving. The inoculated tubes were kept in an air conditioned culture room at a temperature of 25 ± 4^{0}C. The source of illumination consisted of 2.5 feet wide fluorescent tubes (40 watt) and incandescent bulb (25 watt). The intensity of illumination was 3500 lux at the level of cultures and a 12 hour light regime was followed by 12 hour darkness. For multiplication of shoots the established explants were sub cultured after 10 to 15 days for multiplication on the same media.

For callus culture, leaf internode and leaf disc of very small size were inoculated in variously augmented MS media. The medium employed was MS basal with different concentrations of 2, 4-D. The treatments comprised of M_0 (MS as basal as control), M_1 (MS_0 + 1 mg/1 2, 4-D), M_2 (MS_0 + 2 mg/l 2,4-D), M_3 (MS_0 + 3 mg/1 2,4-D) and M_4 (MS_0 + 4 mg/1 2,4-D). After inoculation the culture tubes were transferred to the incubation room and were incubated at 25± 2^{0}C in dark room.

RESULTS AND DISCUSSION

The apical and auxiliary bud explants were inoculated on MS media with 11 different concentration of BAP and adenine sulphate either alone BAP or combination of BAP and adenine sulphate. Among the various treatments the effective results were obtained from the combination given in Table 1 and bud initiation is shown in Fig. 1. From Table 1, it is observed that, the nodal explant showed well establishment recorded about 95 per cent on media M_0 containing only MS media within 5-7 days. MS media supplemented with BAP (4mg/l) showed late establishment of about 80 per cent within 10-12 days and further decrease

Table 1: Effect of different concentrations of plant growth regulators on *in vitro* shoot establishment in *Clerodendrum viscosum*

Sr. No.	Media treatments	MS media	BAP (mg/l)	Adenine sulphate (mg/l)	No of explants inoculated	Percentage of explants establishing
1.	M_0	MS	-	-	20	95%
2.	M_1	MS	-	15	20	40%
3.	M_2	MS	1	-	20	00%
4.	M_3	MS	2	-	20	00%
5.	M_4	MS	3	-	20	30%
6.	M_5	MS	4	-	20	80%
7.	M_6	MS	5	-	20	50%
8.	M_7	MS	2	15	20	30%
9.	M_8	MS	3	15	20	00%
10.	M_9	MS	4	15	20	35%
11.	M_{10}	MS	5	15	20	00%

in establishment was observed in M_6 containing MS media with BAP (5mg/l). Media M_4, M_7 and M_9 containing MS media with BAP (3mg/l), MS media with BAP (2mg/l) + adenine sulphate (15mg/l) and MS media with BAP (4mg/l) + adenine sulphate (15mg/l), respectively showed poor establishment. But, Sharma *et al*. (2009) reported bud-break within 15 days of culture when the nodal stem segments having axillary bud of *Clerodendrum serratum* (Linn.) inoculated on modified MS medium supplemented with 0.25 mg/l each of 6-benzylaminopurine and indole-3-acetic acid along with 15 mg/l adenine sulphate.

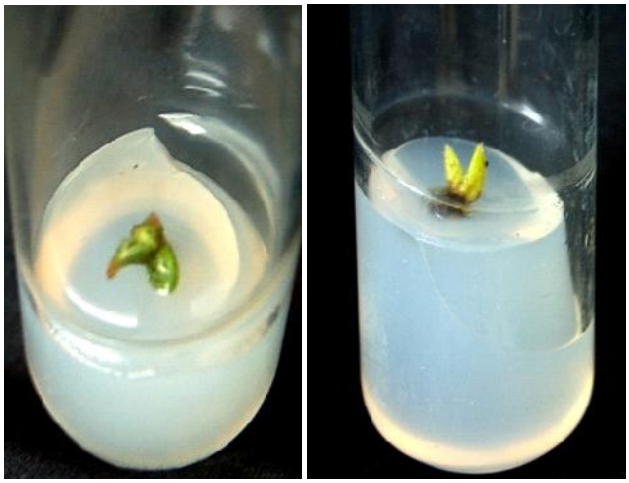

Fig. 1 : Well established explants on media M_0 as per treatment table 1within 5-7 days

After 10 to15 days, the established cultures were transferred to multiplication media having the same media components. Multiplication of shoot cultures was carried out by culturing the initiated shoots. The effect of different concentrations of plant growth regulators on *in vitro* shoot proliferation in *Clerodendrum viscosum* is given in Table 2 and the multiplication of shoot cultures is shown in Fig. 2. It was observed that sub culturing of established explants for multiplication on the same medium induced multiple shoots in 7 to 12 days. Up to 1 to 8 multiple shoots were obtained from single inoculated explants. The proliferating buds were well defined pale green to greenish and 0.5 – 1 cm long with bulbous base and pointed tips. The cluster formation initiated from the basal node; progressively increased in size from each subculture and the number of shoots initiated from the nodal portion varied from medium to medium. It was observed that during each passage, the number of leaves/

Fig. 2 : Media treatment M_5 showingh well proliferation and multiple shoot induction about 7-8 mutiple shoots were found in one week

Table 2: Effect of different concentrations of plant growth regulators on *in vitro* shoot proliferation from shoot tip explants of *Clerodendrum viscosum*

Sr. No.	Media treatments	MS media	BAP (mg/l)	Adenine sulphate (mg/l)	No of explants inoculated	No of shoots per explants
1.	M_0	MS	-	-	20	-
2.	M_1	MS	-	15	20	-
3.	M_2	MS	1	-	20	-
4.	M_3	MS	2	-	20	-
5.	M_4	MS	3	-	20	-
6.	M_5	MS	4	-	20	7-8
7.	M_6	MS	5	-	20	3-4
8.	M_7	MS	2	15	20	3-4
9.	M_8	MS	3	15	20	-
10.	M_9	MS	4	15	20	-
11.	M_{10}	MS	5	15	20	-

shoots had increased substantially along with the height of the shoots. From Table 2, it was observed that highest shoot multiplication was induced in media M_5 containing MS media supplemented with BAP (4mg/l) showing 7-8 multiple per cultured explant. Low multiplication rate was observed in media M_6 and M_7 with MS media supplemented with BAP (5mg/l) and MS media supplemented with BAP (2mg/l) and adenine sulphate (15 mg/l), respectively showing 3-4 multiples per inoculated explants. Other treatments did not show multiplication. However, Raaman *et al*. (2011) observed that the combined effect of BAP, KIN and NAA 2.5, 1.5 and 0.1 mg/L, respectively in the MS medium produced more multiple shoots (87%) in organogenic callus of *C. phlomidis*. Similar observation was reported by Sharma *et al*. (2009) while culturing the regenerated shoots of *Clerodendrum serratum* (L.) on modified MS medium supplemented with 0.25mg/l each of BAP and IAA along with 30mg/l adenine sulphate in presence of 0.5mg/l 2-chloroethyltrimethyl ammonium chloride and produced an average 4.98 new shoots per original shoot after 4 weeks of subculture. Similar results were also reported by Vidya *et al.* (2012) when stem derived callus was cultured on LM media fortified with 1.5 mg /l BAP and 0.3 mg /l and obtained the maximum numbers of shoots with maximum length in *Clerodendrum serratum* (L.).

Leaf disc and leaf internodal disc were inoculated for callus induction on MS media supplemented with different concentration of 2, 4-D. The explants enlarged within 12 to 14 days of inoculation; however, the callus formation started after 23 to 24 days at the tips of the explant (Table 3). The influence of different concentrations of plant growth regulators used for callus induction in *Clerodendrum viscosum* is given in Table 3 and callus initiation is shown in Fig. 3. From Table 3, it is observed that, the treatment M_4 (MS media supplemented with 2, 4-D (4mg/l)) exhibited callus induction whereas, other treatments did not callus induction. However, Raaman *et al*. (2011) have reported callus formation in *Clerodendron phlomidis* cultured on MS medium containing NAA at 3 mg/l showed effective callus production (97%) of leaf explants whereas MS medium containing combination of IAA, IBA and NAA at the level of 0.5, 0.1 and 0.1 mg/l, respectively, resulted in a significant callus production (51%) from the nodal and internodal explants. Whereas, Vidya *et al.* (2012) observed combined

Fig. 3 : Callus induction media M_4 showing brownish coloured callus from leaf disc explant within 23-25 days

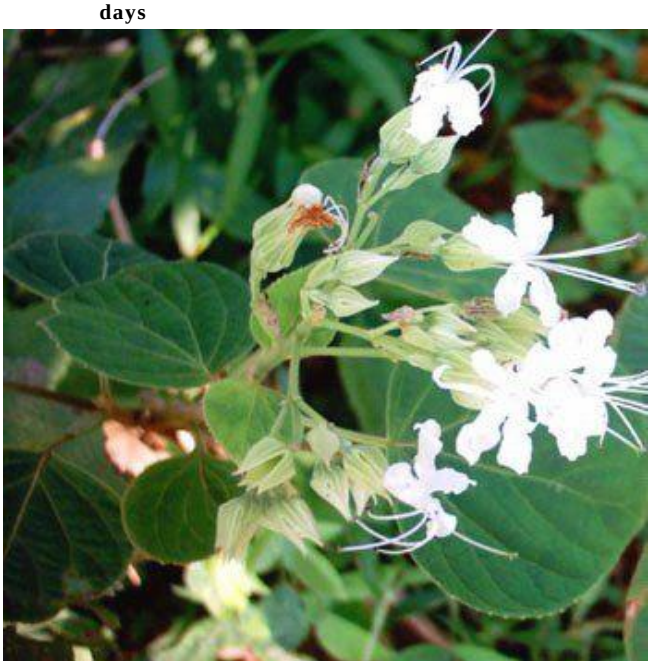

Fig. 4 : *Clerodendrum viscosum*

Table 3: Effect of different concentrations of plant growth regulators for callus induction in- *Clerodendrum viscosum*

Sr. No.	Media treatments	MS	2,4-D (mg/l)	Callus growth
1.	M_0	MS	-	-
2.	M_1	MS	1	-
3.	M_2	MS	2	-
4.	M_3	MS	3	-
5.	M_4	MS	4	++

effect of auxin and cytokinin for highest efficiency of callus initiation in *Clerodendrum serratum* (Linn.) when the explants was cultured on MS media containing 1 mg/l BAP and 0.1 mg/l NAA.

The present investigation was undertaken on an important, rare and endangered medicinal plant, *Clerodendrum viscosum* (Fig. 4) with a view to develop a reliable protocol for its clonal propagation under *in vitro* conditions. Hence, the outlined procedure can be used to generate cost-effective protocol for large scale *in vitro* production.

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