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RESEARCH ARTICLE

CALLUS INDUCTION FROM LEAF AND SHOOT PROLIFERATION FROM NODAL EXPLANTS OF CLERODENDRUM SERRETUM L, A THRETENED MEDICINAL PLANT

T.N. Swamy[1], Bylla Prasad[2], M.O.Ghani[1],V. Suresh[1] and Md.Mustafa[1*]

[1]Department of Botany Plant Tissue culture and Molecular Taxonomy laboratory Kakatiya University Warangal, Telangana State, India

[2]Department of Biotechnology, Plant Tissue culture and Molecular Taxonomy laboratory Kakatiya University Warangal, Telangana State, India

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ABSTRACT

Protocol has been developed for the induction of callus and shoot proliferation from leaf and nodal explants of *Clerodendrumm serretum* L. These explants were inoculated on MS medium fortified with different combinations of 2,4-D and BAP for callus induction and BAP and L-Gluatamic acid for the shoot proliferation. After one month period leaf and nodal explants were proliferated into Green compact callus on MS medium supplemented with 2.0mg/lBAP +0.5mg/l2,4-D.Where as an optimum shoot proliferation was recorded from nodal explants on MS medium supplemented with 3.0mg/lBAP+1.0mg/L-Glutamic acid.Callus has been used for various biochemical studies and shoots were rooted on ½ strength MS medium with 3.0mg/lIBA.

INTRODUCTION

Clerodendrum.serretum L, is an important medicinal plant belongs to the family verbenaceae. The plant is distributed in WesternGhats of India (Manjuntha *et al.,* 2004).The genus belongs to tropical and warm temperate regions of the world(Mebberley *et al* ., 2008).

As per the traditional assert, roots were potential source of drugs and they were used in curing diseases like syphilis,cancer, snakebites, inflammation, epilepsy, malaria,ulcer, wound,hypertenion (Mukesh *et al.,*2012) asthama and rheumatism (Shah 2003; Krishna *et al.,*2007).Roots are also significantly used against Alzheimer's disease in mice(Babenko *et al.,* 2008,Fuchs *et al.,* 1993). Root bark contains mainly sapogenins (Rangaswari and Sarangam, 1969).It is one of the ingredients of the ayurvedic drug 'Kasa damana'an effective expetorant and antitussive remedy (CSIR,2001).Reports on *in vitro* propagation procedures with in the genus *Clerodendrum* are scanty only one *in vitro* propagation method of *C.colebrakianum* has been reported. *In view* of its medicinal importance and lack of tissue culture studies. There is an urgent need to dvelop a protocol for the micropropagation of this thretened mecdicinal plant for future use for that we have attemped to develop a protocol for the induction of callus and shoot proliferation from different explants.

MATERIAL AND METHODS

Clreodendrum serretum plants were collected from Cherla and Venkatapur mandals of Khammam District, Telangana State. This species was identified with the help of "Flora of presidency of Madras" (Gamble1935).These plants were grown in our research field, department of Botany and voucher specimens were deposited in the departmental herbarium.

Leaf and nodal explants were thoroughly washed under running tap water for 10 minutes and surface sterilized with 0.1% $Hgcl_2$ for 8-10 minutes, rinsed 3-4 times with sterilized distilled water. The sterilized leaf and nodes were cut into small pieces and inoculated on MS medium supplemented with various concentration of BAP + 2, 4-D for callus induction, BAP + L-Glutamic acid for regeneration and IBA for rooting with 30% sucrose and 0.9% agar-agar, pH was adjusted to 5.8 and autoclaved at 121^0c.The cultures were incubated in chambers where 16 hrs photoperiod of light and 25 ± 2^otemperature have been maintained. The cultures were responded after 40 days of culture and results were recorded with different interval of time.

Corresponding author:* **T.N. Swamy
Department of Botany Plant Tissue culture and Molecular Taxonomy laboratory Kakatiya University Warangal, Telangana State, India

RESULTS AND DISCUSSION

The leaf explants (0.5cm) were inoculated on MS medium fortified with various concentration and combinations of BAP+2,4-D.Green compact callus formation was obsereved on MS medium supplemented with 2.0mg/lBAP+0.5mg/l2,4-D after four weeks of culture (Table-1,fig-a).The callus growth was enhanced after first passage of 24 days. Callus turned to black when the concentration of BAP was increased (Table-1, fig-b).Green friable callus was reported by reducing the concentration of BAP from 2.0 mg/l to 1.0mg/l.The higher concentration of 2,4-D suppressed the growth of the callus and lead to scenscence.

When the nodal explants were inoculated on MS medium supplemented with various concentration and combination of BAP +L-Glutamic acid, little amount of callus with few shoots proliferation was observed on MS medium supplemented with 0.5mg/lBAP+1.5mg/l L-Gluatmic acid(fig-c).When the concentration of L-Glutamic acid was increased to 2.5mg/l the mean number of shoots were increased along with percent response of cultures(Table-2).Shoot proliferation and elongation was observed on MS medium supplemented with 1.0mg/l BAP+0.5mg/l L-Glutamic acid (Table-2,fig-d).The shoots were subcultured on the same medium significantaly increased the number of auxillary bus.The further increase concentration of amino acid and BAP did not favour the positive results.Shoot proliferation from nodal cultures was increased by increasing the cocentration of BAP (2.0mg/l) and L-Glutamic acid (2.0mg/l) (Table-2).This combination has been proved as better for more number of shoot proliferation after 28 days of culture.Nodal explants responded positively with highest number of shoots proliferation on MS medium supplemented with 3.0mg/l BAP and 2.0mg/l L-Glutamic acid. (Table-2,Fig-e). Only the callus was induced from the leaf explant MS meddium with BAP and 2,4-D.Callusing of *Clerodendrum serretum* was optimized at the concentration of 2.0mg/l BAP +0.5mg/l2,4-D but failed to produce shoots.A similar type of response was observed in the spceies of *Discorea zingiberensis*(Shu *et al.*,2005 and vidya *et al.*, 2005).It is well documented that globular calli with nodular structures are the best material to regenerate plantlets (Leupin *et al.*, 2000).Further observstion showed that compact callus resemble embryogenic tissue in *Vetiveria* and was proved as highly regenerate.Similar results were also reported by (Vengadesan *et al.*, 2000). Our results strongly supporting that BAP in combination with 2,4-D promoted the formation of Green compact callus as in *Acacia sinuata*(Vengadesan *et al.*, 2000 and *Cucumis* Tawfik and Naga.,2000). Nodal explants cultured on MS medium supplenmented with varius combinations of BAP and L-Glutamic acid.It was observed that higher and highest number of shoots were proliferated on MS medium fortified with 2.0mg/lBAP +2.0mg/l L-Glutamic acid and 3.0mg/lBAP +2.0mg/l L-Glutamic acid respectively.It was observed that 2-3 shoots were proliferatied during first culture,where as number of shoots were increased after Ist passage on the same medium.There are similar results have been reported in *Clerodendrum colebrookiamum* by Mao *et al* .,1995. Number of shoots proliferatied from nodal explants was very low in *Clerodendrum indicum*(Mukhery *et al.*, 2005).Where as we could achive the highest number of shoots from the nodal explants after one passage by the addition of L-Glutamic acid in combination with BAP.Similarly shoot length was also incresed in this PGR combination. Addition of L-Glutamic acid in the adventitious shoot regeneration medium has greatly enhanced the production of shoots from callus. This is an agreement with the finding of (Selvaraj *et al.*, 2002).Adding nontoxic glutamine to the medium maintain a high growth rate of cells for a longer period. Few amino acids have proved to be most effective for the growth of excised embryos (Matsubara,1964: Monnier,1978).Sander and Burkhoder (1948) tested a mixture of 20 amino acids in the propagation in which they occur in casein Hydrolysate. In addition to ten of these amino acid, which had been earlier found to be beneficial for plant growth were also listed individually and collectively. Glutamic acid is one the tested amino acid.

Table1 Induction of callus from leaf explants of C serretum on MS medium supplemented with different concentrations of 1BAP+ 2.4-D.

BAP+2.4-D(mg/l)	% of response	Morphogenetic response
0.5+0.5	8	White friable
1.0+0.5	10	Green friable
1.5+0.5	20	Green callus
2.0+0.5	50	Green compact callus
2.5+0.5	30	Black friable callus
3.0+0.5	20	Black friable callus
0.5+1.0	10	White callus
1.0+1.0	30	Brown callus
1.5+1.0	20	Brown friable
2.0+1.0	40	Light green with brownpatchs
2.5+1.0	50	White friable
3.0+1.0	60	Black friable

*Data was collected after 4 weeks of cultures.

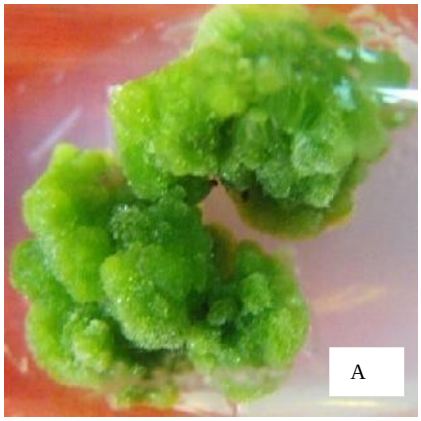
Table2 Shoot proliferation of *C serretum* on MS medium supplemented with different concentrations of BAP+L-Glutamic acid.

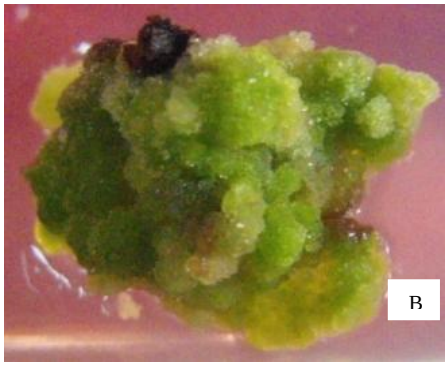
S.No	BAP+L-Glutamic acid(mg/l)	% of Response	Mean. No of shoots	Mean. No of shoot length
1	0.5+0.5	8	1.8±0.11	2.2±0.06
2	0.5+1.0	10	2.1±0.14	4.1±0.05
3	0.5+1.5	20	3.9±0.12	2.8±0.05
4	0.5+2.0	40	4.4±0.16	1.5±0.06
5	0.5+2.5	50	5.5±0.23	3.2±0.06
6	0.5+3.0	40	4.3±0.32	2.4±0.08
7	1.0+0.5	30	2.3±0.17	2.6±0.04
8	1.0+1.5	30	2.3±0.17	3.6±0.04
9	1.0+2.0	50	4.6±0.22	3.7±0.06
10	1.0+2.5	40	5.1±0.17	2.2±0.08
11	1.0+3.0	60	6.1±0.14	2.8±0.08
12	2.0+0.5	30	7.8±0.17	3.0±0.07
13	2.0+1.5	60	8.4±0.21	2.4±0.06
14	2.0+2.0	50	9.0±0.25	1.8±0.04
15	2.0+2.5	40	8.6±0.24	2.2±0.06
16	2.0+3.0	50	6.7±0.25	2.6±0.07
17	3.0+0.5	50	6.0±0.35	2.0±0.08
18	3.0+1.5	70	9.0±0.36	2.0±0.08
19	3.0+2.0	80	13.1±0.35	3.2±0.06
20	3.0+2.5	40	7.0±0.35	3.0±0.05
21	3.0+3.0	40	9.0±0.40	2.5±0.04

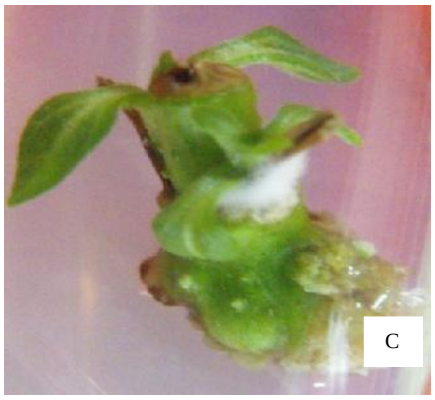
*Data was collected after Ist passage of nodal explants after 24 days.

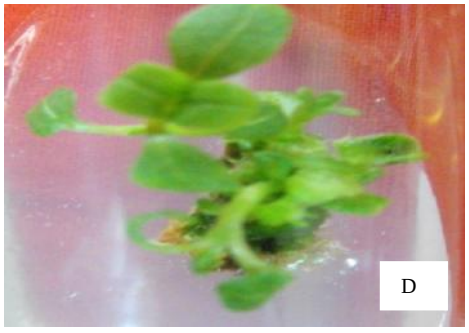
Instances, in literature are available where supplementing the media with mixture of amino acid enhance the growth and differentiation of tissue of *Pteris* (Krishnasagar and Mehtha,1978)and *Torenia*(Taminoto and Harada,1982). The

proliferated shoots plantlets were separated and inoculated on MS halfstrength medium supplemented with 3.0mg/l IBA.
Each shoot produced 4 -7 roots after 20 days of culture.Superior effect of IBA has been suggested by Bansal and Bansal(1995),Ancheral *et al*,.(1981),Rathor *et al*,. (2010).The combination of IBAand way of its application also has a significant effect on the root induction (Vander krieken *et al*., 1993).









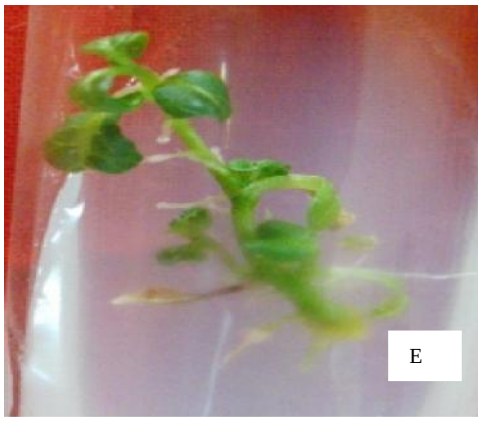


Fig: a: Green compact callus MS medium supplemented with 2.0 mg/l BAP+0.5mg/l 2,4-D from leaf explants. Fig: b: Callus turning to black on MS medium supplemented with 3.0 mg/l BAP+0.5mg/l 2,4-D from leaf callus .Fig: c: Nodal explants producing little amount of callus few shoots on MS medium supplemented with 0.5mg/l BAP +1.5 L-Glutamic acid. Fig: d: Highest number of shots proliferation on MS medium supplemented with 3.0mg/l BAP+2.0mg/l L-Glutamic acid.
Fig: e: Rooting of plantlets on half strength MS medium with 3.0mg/l IAA.

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