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IN VITRO PROPAGATION IN *CLINACANTHUS SIAMENSIS* BREMEK- A MEDICINAL TAXON OF WESTERN GHATS, INDIA

BEENA PRABHA AND N. SAVITHRAMMA

Dept. of Botany, S.V.University, Tirupati- 517 502, Andhra Pradesh, India.

ABSTRACT

In vitro propagation through nodal explants by indirect organogenesis of *Clinacanthus siamensis* Bremek. Nodal segments were surface sterilised in NaOCl for 5 min. This is followed by treating it with 0.1% $Hgcl_2$ for 8 min. Different concentrations of BAP and 2-P were added to full strength MS medium for culture initiation. Multiple shoots were formed in MS medium augmented with various concentrations of BAP and a maximum number of 5.8 ± 0.37 after 30 days with a mean length of 7.38 ± 0.17 cm. *In vitro* derived shoot segments approximately of 1.0 cm length were dissected and cultured in full strength MS medium augmented with various concentrations of 2,4- D and 2,4,5- T. Observations were made after 4 weeks of culture and found that three types of calli were formed. Further, shoot regeneration is induced from 500 mg fresh weight of friable callus in MS medium with different concentrations and combinations of 2,4-D and BAP, which induced maximum shoot multiplication followed by elongation after 4weeks of culture. Rooting was maximum with combinations of 2,4-D and IBA and IBA alone. These *in vitro* regenerated plants were successfully acclimatized and transferred to green house. Hardened plants were transplanted into sand and soil (1:1).

KEYWORDS: ***Clinacanthus siamensis* Bremek, nodal explants, BAP, 2iP, 2,4-D, 2,4,5- T, IBA, regeneration.**

*Corresponding author

BEENA PRABHA
Dept. of Botany, S.V.University, Tirupati- 517 502, Andhra Pradesh, India.

INTRODUCTION

Medicinal plants are extensively used in the tribal, ayurvedic and unani medicines. Increasing utility of medicinal plants for its extracts and research purposes has endangered the perpetuation of native species. Because of this utilization and also depletion of habitat, medicinal plants are on the verge of extinction. Hence, the conservation of these valuable genotypes is imperative. Plant tissue culture technology holds great promise for micropropagation, conservation, and enhancement of the natural levels of valuable secondary metabolites and to meet pharmaceutical demands and reduce the *in situ* harvesting of natural forest resources[1].*Clinacanthus siamensis* Bremek belonging to the family Acanthaceae grows as a perennial under shrub in Western Ghats [2] . It grows up to 2 meters in height. Leaves simple, opposite, acute or acuminate and slightly serrated, crowded at the end. Stem round, green colored, and turns grey when old. Plant pacifies vitiated kapha, pitta, poison bites, inflammation, traumatic oedema and swelling due to poison stings [3]. Used as anti snake venom, to treat diabetes, herpes infection, effective against influenza, gives immune response [4,5]. It also has anti-inflammatory and antiarthritic properties [6] Useful part : Leaves, Root and Stem. The purpose of the study was to develop a reliable and effective protocol for *in vitro* propagation of this important species.

MATERIALS AND METHODS

The plants were collected from the natural habitats of Western Ghats and compared with the species of Botanical Garden of Panchakarma Ayurvedic Research Centre, Thiruvananthapuram. The plant was planted in S.V.U. Botanical Garden. Further, Taxonomic identification of the plant was studied with the help of Gamble [7].Nodal explants of 1 cm length were cut and washed under running tap water and with teepol solution (6% v/v) for 15 minutes followed by 4 - 5 washing with distilled water. For surface sterilization, the explants were treated with 5% NaOCl and 0.1% $HgCl_2$ for time period ranging from 0-8 minutes. Infection free explants were more when they were treated with NaOCl for 4 minutes and 0.1% $HgCl_2$ for 8 minutes. The mortality rate was zero at this time (Table 1).

Table-1
Data on surface sterilisation of field grown mature nodal segment explants of Clinac**anthus siamensis.* **Each treatment consists of 12 explants. Observations were made after 15 days of culture.

Targeted plant	Sterilant/detergent used-Treatment duration (min)		Number of explants (%)		Number of explants responded (%)	Mortality (%)
	0.1% $HgCl_2$	5% NaOCl	Infection free	With Infection		
Clinacanthus siamensis	4	0	03(25.00)	9(75.00)	3(25.00)	0(0.00)
	8	0	07(58.33)	5(41.67)	6(50.00)	1(8.33)
	4	2	08(66.67)	4(33.33)	6(50.00)	2(16.67)
	8	4	12(100.0)	0(00.00)	12(100.0)	0(0.00)
	8	6	12(100.0)	0(00.00)	6(50.00)	6(50.00)

The surface sterilised explants were implanted in the MS medium [8] augmented with different concentrations of BAP and 2iP. The shoot segments so formed are selected for callus initiation in full strength MS medium supplemented with different concentrations of 2,4-D and 2,4,5-T. The friable callus formed was used for shoot regeneration and multiplication in full strength MS medium supplemented with different concentrations and combinations of 2, 4- D and BAP. Rooting was done in half strength MS medium with 2,4- D and IBA.

RESULTS AND DISCUSSION

Initiation

MS medium containing major and minor salts, vitamins, FeEDTA and inositol (100mgl^{-1}) was prepared and P^{H} adjusted. The medium was augmented with different concentrations of BAP and 2iP separately. The surface sterilised explants were implanted in the medium and incubated in a culture room at 25 ± 2^{o} C with a relative humidity of 50% to 60% and 16 h photoperiod at a photon flux density of 15-20µ Em^{2} /s^{-1}. For each treatment 12 explants were used and the experiment was repeated thrice. The cultures were examined periodically.MS medium augmented with different concentrations of BAP showed a maximum response when 0.5mgl^{-1} of BAP was used ie: 5.8± 0.37 shoot buds induced after 30 days (Figure 1,2). Elongated shoots of maximum length 7.38 ± 0.17 cm were harvested after 60 days. The number of shoots formed were less when MS medium augmented with 2iP was used (Table 2). No shoot induction took place when nodal explants were cultured in basal medium without any PGR supplement. Rapid shoot regeneration has been achieved with a wide range of species with initial explants being taken from normal aerial shoots of field grown herbaceous medicinal plant species [9,10,11,12].

Table-2

Data on culture initiation of Clinacanthus siamensis in full strength MS medium supplemented with different concentrations of PGRs. Each treatment consists of 12 explants and the experiments repeated thrice. ± indicates Standard Error.

PGRs (mgl^{-1})		Percentage Response (%)	Mean number of multiple shoots (After 30 days)	Mean length of multiple Shoots in cm (After 60 days)
BAP	2-iP			
0.0		6(50.00)	0.6 ± 0.24	7.64 ± 0.27
0.2		8(66.67)	2.8 ± 0.37	7.26 ± 0.37
0.5		12(100.0)	5.8 ± 0.37	7.38 ± 0.17
1.0		11(91.67)	4.4 ± 0.51	6.78 ± 0.24
1.5		10(83.33)	3.4 ± 0.51	6.04 ± 0.16
2.0		8(66.67)	2.8 ± 0.37	5.66 ± 0.19
	0.2	9(75.00)	1.8 ± 0.37	7.96 ± 0.19
	0.5	7(58.33)	3.4 ± 0.40	7.62 ± 0.22
	1.0	11(91.67)	2.4 ± 0.40	6.90 ± 0.33
	1.5	12(100.0)	2.0 ± 0.32	6.50 ± 0.32
	2.0	10(83.33)	1.8 ± 0.37	6.34 ± 0.24

Callus Initiation

Shoot segments of approximate length of 1.0 cm were cut from this in vitro derived shoots and cultured in full strength MS medium supplemented with different concentrations of 2,4 D and 2,4,5 T. Observations were made on 4 weeks after the culture. There was formation of compact, friable and semi friable callus (Table 3). The three types of calli were formed together. 33.75 ± 0.75 percentage of friable callus formation was observed when MS medium was supplemented with 1 mg l^{-} of 2,4- D. The friable callus so formed was then used for shoot regeneration and multiplication (Figure 3).

Table 3

Callus induction from in vitro derived shoot segments cultured on full strength MS medium supplemented with different concentrations of 2,4-D and 2,4,5-T. Observations were made 4 weeks after culture. Values are ± SE of the mean value.

PGR' s (mgl^{-1})		Percentage Callusing		
2, 4- D	2, 4, 5- T	Compact	Friable	Semi Friable
0.2		12.5 ± 1.19	10.25 ± 0.85	24.0 ± 0.82
0.5		10.25 ± 0.86	18.75 ± 1.49	29.0 ± 1.36
1		53.75 ± 1.14	33.75 ± 0.75	12.25 ± 1.55
1.5		67.25 ± 1.25	21.75 ± 0.86	11.0 ± 1.46
2		64.5 ± 1.26	24.5 ± 0.5	9.75 ± 1.55
	0.2	41.25 ± 0.75	23.5 ± 0.65	7.5 ± 1.45
	0.5	61.5 ± 0.65	23.75 ± 0.75	14.75 ± 0.86
	1	80.75 ± 0.48	13.75 ± 0.48	5.5 ± 0.65
	1.5	72.5 ± 1.19	14.5 ± 1.04	12.75 ± 1.25
	2	72.0 ± 0.71	23.75 ± 0.48	4.25 ± 0.86

Shoot multiplication

500 mg fresh weight of friable callus were then cultured in full strength MS medium supplemented with different concentrations and combinations of 2,4-D and BAP . Observations were made after 4 weeks of culture. 0.05 mg l^{-1} BAP showed the optimum concentration to develop maximum number of shoots (14.2 ± 0.86). Also 2,4- D and BAP (0.2 mg l^{-1} and 0.5 mg l^{-1}) proved to be the optimum concentration when combined (11.8 ± 0.58) (Table 4) (Figures 4,5). The combination of BA and NAA proved optimum concentration for shoot regeneration in *Dysopylla myosuroides* [13]. BAP is considered to be one of the effective cytokinins for induction of organogenesis and thereby effective micropropagation of plants [14] and a combination of BAP and NAA is most often used for shoot organogenesis [15]. High frequency shoot regeneration was reported in *Talinum cuneifolium* (vahl.) Willd when BA alone and BA in combination with Kn was used [16]. A combination of BAP, 2iP and IAA was successfully used for shoot regeneration in *Adathoda beddomei* [17].

Table 4

Shoot regeneration from 500mg fresh weight of friable callus cultured on MS medium supplemented with different concentrations and combinations of PGR' S. Observations were made after 4 weeks of culture. Values are ± SE of the mean value.

PGRs (mgl^{-1})		Percentage Response (%)	Mean No. of shoots	Mean Length of Shoot (cm)
2,4-D	BAP			
	0.2	50.00	4.8 ± 0.66	8.8 ± 0.17
	0.5	100.00	14.2 ± 0.86	8.0 ± 0.35
	1	83.33	10.6 ± 1.02	6.9 ± 0.48
	2	66.67	6.4 ± 0.75	6.3 ± 0.39
0.2	0.2	58.33	5.2 ± 0.66	6.2 ± 0.44
0.2	0.5	91.66	11.8 ± 0.58	6.3 ± 0.14
0.2	1	66.67	8.0 ± 0.71	5.3 ± 0.19
0.5	0.2	41.66	4.8 ± 0.38	5.4 ± 0.13
0.5	0.5	33.33	3.8 ± 0.58	5.3 ± 0.15
0.5	1	33.33	3.4 ± 0.39	5.1 ± 0.13
1	0.2	25.00	3.0 ± 0.45	4.9 ± 0.21
1	0.5	25.00	3.0 ± 0.32	4.5 ± 0.21
1	1	25.00	3.0 ± 0.32	2.7 ± 0.61

Rooting Half strength MS medium with various concentrations and combinations of 2,4- D and IBA showed effective rooting of the multiple shoots. 9.8 ± 0.73 number of roots responded with 1.0 mgl^{-1} IBA and 10 ± 0.71 number of roots responded with a combination of 1.0 mgl^{-1} IBA and 0.2 mgl^{-1} 2,4-D (Table 5) (Figure 6). In the micropropagation of *Talinum cuneifolium* IBA was found to be more effective in comparison with IAA and NAA [18]. Effectiveness of IBA in rooting has been reported in medicinal plants viz. *Gymnema sylvestre* [19] and *Hemidesmus indicus* [20]. The effectiveness of NAA in rooting has been reported for a few plant species such as *Decalepis hamiltonii* [21]. NAA or IBA alone induced rooting in *Cassia alata* and *Albizzia amara* [22,23]. Formation of a maximum of 25 roots were observed when IAA and BAP was combined in the case of rooting in *Cochlospermum religiosum* [24]. MS medium supplemented with (2.0mg/L) IBA was used to induce roots in *Vigna mungo* L [25].

Table 5

Root regeneration from the multiple shoots cultured on Half Strength MS medium supplemented with different concentrations and combinations of PGRs. Observations were made after 4 weeks of culture. Values are ± SE of the mean value.

PGRs (mgl^{-1})		Percentage Response (%)	Mean No. of roots	Mean Length of root (cm)
2,4-D	IBA			
	0.2	75.00	5.0 ± 0.45	3.02 ± 0.17
	0.5	83.33	6.2 ± 0.73	3.44 ± 0.35
	1	100	9.8 ± 0.73	2.66 ± 0.48
	1.5	91.67	6.8 ± 0.58	2.44 ± 0.24
0.2	0.2	58.33	3.6 ± 0.51	3.2 ± 0.22
0.2	0.5	91.67	7.4 ± 0.51	3.32 ± 0.25
0.2	1	100	10.0± 0.71	3.54 ± 0.33
0.5	0.2	58.33	4.0 ± 0.71	2.64 ± 0.25
0.5	0.5	91.67	7.2 ± 0.58	3.56 ± 0.24
0.5	1	66.7	5.4 ± 0.51	2.74 ± 0.28
1	0.2	33.33	3.4 ± 0.39	3.42 ± 0.43
1	0.5	25.00	3.0 ± 0.32	3.08 ± 0.35
1	1	58.33	3.8 ± 0.37	2.3 ± 0.28

Hardening and Acclimatization

Plantlets with 5 to 10 fully expanded leaves and well developed roots were successfully acclimatized and eventually established in the soil. The *ex vitro* survival rate of the plants after transfer to fine garden soil: sand (1:1) was 80%. The regenerated plants did not show detectable variation in morphology and growth when compared with that of donor plant (Figure 7,8).

Figure 1, 2
Stages of shoot initiation in MS medium

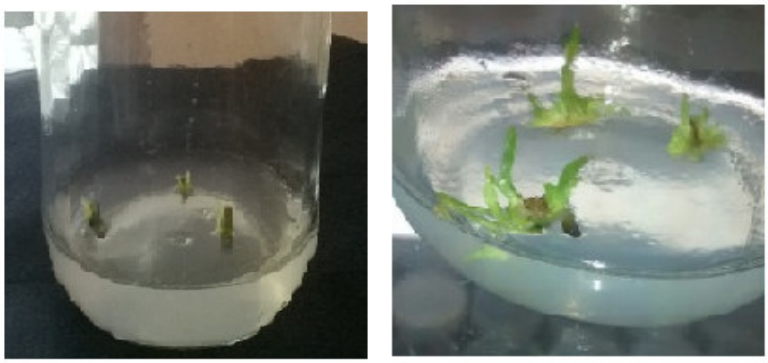

Figure 3
Callus formation

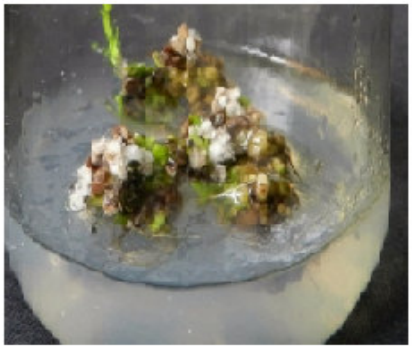

Figure 4, 5
Shoot multiplication stages

Figure 6
Root regeneration

Figure 7
Plantlets

Figure 8
Acclimatization

CONCLUSION

The research and results presented here show that the nodal explants of *Clinacanthus siamensis* has high potential of shoot regeneration and a large number of platelets can be formed from callus within a short period of time when cultured in appropriate growth regulators.

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