Callogenesis in *Tragia involucrata* L.-A potent tribal medicinal plant

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SUMMARY

Tragia involucrata L. is a well known medicinal plant used in tribal medicine of India .In this paper *in vitro* studies have been reported. Calli were initiated from leaf and stem segments on Murashige and Skoog's (MS) medium supplemented with 2, 4-D or Kinetin. Explant browning a major hurdle in the establishment of cultures was minimized by adding ascorbic acid (2mg/l) to the MS basal medium. The explants induce proliferated mass of callus on MS medium supplemented with 2, 4-D and Kn. Luxuriant mass of callus was achieved by sub culturing the calli on MS supplemented with BAP (3.0 and 5.0 mg/l) alone or in combinations with NAA (0.5,1.0 and 1.5 mg/l).By subculturng the callus on fresh medium at 15 days interval,browning of the callus was eliminated simultaneously the initiation of somatic embryos. But calli failed to induce somatic embryo genesis even addition of coconut water (10.20 and 25%) while sub culturing, it produce only whitish green, friable, soft callus. Rhizogenesis was achieved by subculturng calli on MS medium containing IAA (2.5 and 5.0 mg/l) and IBA (2.0 and 4.0mg/l) alone.

Key words : Leaf culture, Callus induction, Sub culture, Rhizogenesis

Tragia involucrata (Euphorbiaceae) is a perennial evergreen twiner with hispid, stinging bristles and widely used in traditional system of medicine for a variety of diseases. The roots have been reported to possess diaphoretic and alterative actions and the infusion is given when the extremities are cold during fever, skin infection and also for pains in the legs and arms. Root paste is applied for removal of guinea worms and it forms basis of an external application in leprosy. A decoction of the root (1 in 10) was found to be useful in relieving bronchitis and the attendant fever (Kirtikar and Basu, 1975; Chatterjii and Pakrashi, 1994 and Chopra *et al.*, 1956). The decoction of the leaves is being used by tribal people of western ghats of Tamilnadu (Kalrayan hills) for the treatment of skin infection, pain swelling, children scabies and eczema (Perumalsamy *et al.*, 2006c).

Callus formation is the fundamental stage for many tissue culture techniques such as organogenesis, somatic embryogenesis and protoplast culture. However callus formation can be especially difficult to attain in some species. We are not aware of any method that was available in the literature on the tissue culture studies in *T. involucrata*. Phytochemcally, the air-dried powder of alcoholic and ether fraction of root contains beta sitosterol and beta -sitosterol –beta- D -glycoside (Srinivasan, 1985). Leaf and root extracts show multiple pharmacological effects including antibacterial (Perumalsamy *et al.*, 2006b), wound healing (Perumalsamy *et al.*, 2006c), psychopharmacological (Dhara *et al.*, 2002) and a significant analgesic and anti inflammatory activities (Dhara *et al.*, 2000; Perumalsamy *et al.*, 2006a).

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MATERIALS AND METHODS

Leaf and stem parts of the plant were used as explants source. The explants were collected from in and around university campus and the explants were excised into1.0-1.5cm length and washed thoroughly under running tap water for 30 min to remove the adhered surface particle. Then, treated with a liquid detergent laboline (5% v/v) for 30 min followed washing under running tap water. The explants were surface sterilized with 0.1%(v/v)aqueous mercuric chloride for 10 min and finally rinsed with sterilized ,cooled distilled water aseptically and were carefully inoculated onto the Murashige and Skoog's(1962) basal media supplemented with 3% sucrose and solidified with bacteriological grade agar (0.8%) and various concentrations of growth hormones. The media pH was adjusted to 5.8 with 1N NaoH or 1N Hcl before autoclaving at 121ºC for 20 min at 15 lbs pressure. The cultures were incubated and maintained at 25±2ºC under 16/8 hr photoperiod of 2000lux light intensity provided by white, fluorescent tubes with 60-80% relative humidity.

Callus induction:

MS medium containing 2, 4-D (1.0, 2.0 and 5.0mgl) and kinetin (1.0, 2.0 and 5.0mg/l) were tested for callus induction from the explants.MS lacking growth

regulators served as control. The explants were inoculated on the slanted media.12 replicates were used per treatment.

RESULTS AND DISCUSSION

Both the explants such as leaf and stem segments respond to callogenesis after 15days of incubation. Browning of explants were major hurdle in establishment callus cultures here, to minimize explants browning ascorbic acid (2mg/l) was induced to the basal medium.

Leaf and stem explants induce callus after 15-20 days of incubation on MS medium supplemented with 2, 4-D (1.0, 2.0 and 5.0 mg/l) or kinetin (1.0, 2.0 and 5.0 mg/l). Callus was proliferated from the cut edge and margin of the leaves whereas in stem the explants bulging towards the cut ends and initiate callus development all along the explants. Growth and morphology of callus varies on different concentrations of growth regulators as shown in Table 1 and 2.

Table 1 : Effect of auxin on callus induction on leaf and stem explants

Auxin	Conc (mg/l)	% of Response		Nature of callus
		Leaf	Stem	
2,4-D	1.0	75	66.6	White greenish or
	2.0	83.3	91.6	light yellow,
	5.0	41.6	58.3	Granular or
				friable, transulent

Table 2 : Effect of cytokinin on callus induction from stem explants

Cytokinin	Conc (mg/l)	% of Response	Nature of callus
Kn	1.0	91.6	Greenish, nodular,
	2.0	91.6	compact
	5.0	83.3	
	10.0	58.3	

Subculture:

Initially the explants produce white greenish or light yellow, translucent, granular or friable soft callus. But, after 30 days of culture callus turns brown, this may be due to synthesis of phenolics and transfer of this callus to fresh medium reduced browning (Ghosh and Bannerjee, 2003) the same type approach was used in many species like *Euphorbia lathyris* (Ripley and Prece, 1986), *Pisonia alba* (Jagadishchandra *et al.*, 1999) and in *Sida rhombifolia* (Guha *et al.*, 2006). By this method, here also the problem was overcome and produced luxuriant proliferated callus through sub culturing of callus on MS medium supplemented with different concentrations and combinations of growth regulators such as BAP, NAA, CW, IAA and IBA at 15-20 days interval for callus proliferation and also for induce somatic embryogenesis. Different morphological characters were recorded as shown in Table 3.

In the majority of plant species the synthetic hormone 2, 4-D interacted with endogenous hormones of the explants and stimulated the cells to proliferate into callus mass (Narayanaswamy, 1994). As observed in the culture of *Tylophora indica* (Faisal and Anis, 2003), *Guizotia abyssinica* (Kumar *et al.*, 2000) and *Embelia ribes* (Shankaramurthy *et al.*, 2004) in *Tragia involucrata* proliferation of callus on leaf and stem explants observed at the range of 1.0, 2.0 and 5.0mg/l of 2,4-D. Highly proliferating white greenish or light yellow, translucent, granular or friable callus was observed in the medium containing lower concentrations of 2,4-D. At higher concentrations also proliferated callus turned brown and friable then growth of callus declined may be due to browning. The stem explants cultured on MS basal medium supplemented with kinetin (1.0, 2.0 and 5.0mg/l) produced greenish, nodular compact callus on lower concentrations, but at higher concentrations as usual like 2, 4-D.

The calli obtained on induction medium were sub cultured on MS medium supplemented with BAP, NAA, CW, IAA and IBA, showed high frequency of callus multiplication and luxuriant proliferation as shown in Table\ 3 and it failed to induce somatic embryos as reported in *Sida rhombifolia*, achieved different morphological characters of the callus by serial sub culturing on fresh medium but it fails to induce somatic embryos (Guha *et al.*, 2006).

Rhizogenesis was achieved by sub culturing callus on medium containing IAA or IBA.IBA (2.0 and 5.0mg/l) induced a mat of white roots. On IAA (2.0 and 4.0mg/l) supplemented medium a few roots were induced.IBA induced roots efficiently in case of *Swaisonia formosa* (Jusaitis, 1997) and *Cunila galoides* (Fracro and Echiverrigaray, 2001).

To conclude, we have initiated the *in vitro* studies of *T.involucrata* and further work is in progress for design a protocol for micropropagation of the plant.

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Table 3 : Luxuriant proliferation of callus on different plant growth regulators on subculture

Plant growth regulator	Conc (mg/l)	Callusing	Rhizogenesis	Nature of callus
BAP	1.0	++	-	Yellow, nodular, compact and
	2.0	++	-	soft
	3.0	+++	-	
BAP	5.0	+++	-	Brown, friable and soft
	10.0	++	-	
BAP+NAA	3.0+0.5	+++	-	Light yellow friable nodular
	3.0+1.0	+++	-	and soft
	3.0+1.5	++++	-	
Coconut water	5%	+++	-	White, transulecent friable and
	10%	++++	-	compact
	15%	++ +	-	
IAA	2.5	+++	++	Yellow, nodular and friable,
	5.0	+++	++	compact hard callus
IBA	2.5	+++	+++	
	5.0	+++	+++	

+ =slow growth, ++ =moderate, +++ =profuse mass, ++++ =luxuriant proliferation

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