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Indirect Propagation of *Solanum trilobatum* L using Leaf Explants

Kamalanathan Desingu and Natarajan Devarajan*

Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem, Tamil Nadu, India

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ABSTRACT

The present investigation was aimed to develop an efficient protocol for indirect organogenesis of Solanum trilobatum L. using leaf explants. Development of organs from callus and multiple shoot induction was achieved in the modified Murashige and Skoog (MS) medium enriched with NAA, 2, 4 – D alone and in combinations with various concentrations (0.5, 0.3, 1.0 + 1.0 mg L^{-1}). Morphogenic pale yellow green and greenish brown callus was obtained after 4 weeks of incubation. Multiple shoots were formed from the callus in the MS medium containing BAP combination with KIN at the concentration (1.0 + 0.3mg L^{-1}) after appropriate incubation period. The regenerated shoots were transferred to rooting medium (containing NAA + IBA with concentrations of 0.5 mg L^{-1} + 0.4 mg L^{-1}) exhibited better roots formation. The complete plantlets were transferred to the poly cups filled with sterile sand and red soil in the ratio of 1:1 and successfully acclimatized in the field.

Key words: Indirect organogenesis, MS medium, Plant Growth Regulators.

INTRODUCTION

Solanum trilobatum (Solanaceae) is an erect branching herb widely distributed throughout India and has long been used in traditional system of medicines to treat various diseases [1]. Calcium, iron, phosphorus, carbohydrates, protein, fat, crude fibre and minerals are present in leaves [2]. It is used as medicine for diabetes, asthma, vomiting with blood, bilious matter phlegmatic rheumatism and leprosy [3]. The biological properties of *S. trilobatum* showed potent antibacterial, antifungal, antimitotic, antioxidant and antitumouours activities [4, 5, 6, 7]. The major phytochemcials like Sobatum, β - solamarine, solaine, solasodine, glycoalkaloid and diosogenin and tomatidine were isolated [8, 9]. Several biochemical constituents are reported from *Solanum* species, which includes alkaloids, phenolics, flavonoides, steroidal saponins and their glycosides [10]. Two bioactive compounds i.e Soladunalinidine and tomatidine (alkaloids) were isolated from the leaf and stem of *Solanum* species [9].

Many researchers focussed on this genus *Solanum* and documented with the direct and indirect micropropagation protocols for their medicinal properties and considerable beneficial phytoconsitituents. Callus organogenesis in *Solanum tuberosum* [11], shoot tip and nodal culture of the same species *Solanum trilobatum*, [2, 12] in modified LS medium was reported. And this study was aimed to develop a rapid micropropagation protocol for the indirect organogenesis and multiplication of *S. trilobatum* using leaf explants.

MATERIALS AND METHODS

Plant materials

The fresh and healthy aerial parts of *S. trilobatum* were collected from the local garden and nomenclature was identified by Dr. D. Natarajan, Assistant Professor, Natural Drug Research Laboratory, Department of Biotechnology, Periyar University, Salem, Tamilnadu.

Induction of callus

In vitro grown leaf explants (two weeks matured) were cultured for callus induction in MS [13] media supplemented with 2% sucrose as carbon source and 0.7% agar (Hi Media) along with various concentrations of auxins (2, 4 – D (1.0 – 5.0 mg/L), NAA (1.0 – 5.0 mg/L) and combination of 2, 4 – D + NAA (0.5 + 0.5, 1.0 + 0.5, 1.0 + 1.0). The pH of the media was adjusted to 5.8 prior to autoclaving for 15 min at 121^{0}C. The cultures were maintained under cool-white fluorescent light at 24 ± 2°C with 16 h photoperiod. The callus cultures were maintained for about 4 – 7 weeks and repeatedly subcultured in the same hormonal medium.

Organogenesis

The callus cultures were subcultured for the embryogenic development of shoot induction in the MS medium supplemented with various concentration of cytokinins and auxins such as BA alone (0.4 – 2.5 mg/L), BA + KIN (1.0 + 0.1 – 0.5 mg/L) and BA + NAA (1.0 + 0.1 – 0.5 mg/L) and incubated. The developed auxiliary buds were isolated and repeatedly subcultured on same medium for the formation of multiple shoots. For root formation, well developed shoots (3 cm) were transferred to MS media supplemented with auxins IBA alone (0.4 – 2.0 mg/L) and combination of IBA + NAA (0.5 + 0.1 – 0.5 mg/L) and incubated. Each treatment consists of 3 replications and each replication, 14 explants were used. The data was recorded after 5 weeks of culture. The well developed plantlets were transferred to polycups containing sterile sand and red soil (1:1 ratio) and watered often and transferred to green house.

RESULTS

Growth of callus cultures

The explants of *S. trilobatum* were inoculated in MS medium supplemented with different concentrations of plant growth regulators such as NAA, 2, 4-D alone and its combination for callus regeneration. Formation of the morphogenetic varied characteristic callus cultures (like greenish brown, pale yellow green callus) was obtained within two weeks of inoculation in the medium containing hormones at the concentration of 3.0 mg L^{-1} 2, 4 - D and 5.0 mg L^{-1} NAA and combination of 2, 4 - D + NAA (1.0 + 1.0 mg L^{-1}). Whereas other combinations of 2, 4 - D and NAA yielded slightly friable and brownish callus. The results were observed for 4 – 8 weeks of time period without altering the culture conditions under light. The hormone 2, 4 - D + NAA combination developed better callus mass then 2, 4 – D and NAA alone at lower concentrations. The rate of growth of callus increased from the second week of culture initiation until the eighth week after the rate of callusing declined. Pale yellow green callus developed from the medium was repeatedly subcultured after every 4 weeks interval for shoot initiation (table 1; figures 1 a, b).

Table 1: Effects of plant growth regulators (NAA and 2, 4 – D) on callus formation from *in vitro* leaf explants of *S. trilobatum*.

Hormones	Concentration (mg/L)	Response of callus mass	Callus appearance
NAA	1.0	-	No callus mass
	2.0	++	Yellow white, smooth
	3.0	++	Yellow white, hard
	5.0	+++	Yellow, green hard
2, 4 – D	1.0	+	Pale brown hard
	2.0	+	Brown hard
	3.0	+++	Greenish Brown hard
	5.0	++	Brown hard
2, 4 – D + NAA	0.5 + 0.5	++	Pale Brown hard
	1.0 + 0.5	+++	Brown hard
	1.0 + 1.0	+++	Pale yellow green hard

+, ++, +++ indicates slight, moderate and considerable callusing, - indicates no response

Shoot multiplication

In vitro developed callus (4 – 7 weeks) were inoculated on MS medium fortified with different concentrations of BA (0.4, 0.8, 1.0, 1.5, 2.0 & 2.5 mg L^{-1}) alone and combination of BAP with KIN (1.0 + 0.1 – 0.5mg L^{-1}); with NAA (0.1 – 0.5 mg L^{-1}) for the development of shoots. The initiation of shoots from the callus cultures were observed after 3 – 4 weeks of incubation in the MS medium containing BA + Kin (1.0 + 0.3 mg/l). The regenerative response was noticed after 15 days of incubation. It was recorded that the growth of shoot length upto 5 - 6 cm long within 6 weeks period. The MS medium supplemented with BA (1.0 mg L^{-1}) alone and BA + NAA (1.0 + 0.5 mg L^{-1}) produced several number of multiple shoots within 3 weeks periods at the average of 85% each (Table 2) and about 80% of the shoots were continued to elongation about 3.43±0.3cm in the medium containing BA + Kin (1.0 + 0.3 mg/l). Few multiple shoots were developed at 1.0 mg L^{-1} BA + 0.5 mgL^{-1} KIN (80%). The average number of multiple shoots developed was higher in the concentration of 1.0 mg L^{-1} BA + 0.5 mg L^{-1} NAA (85%) with shoot length of about 3.02±0.2 cm. The overall response was moderate and lack of multiple shoot formation in other concentrations of medium. There was no morphological variation in the plantlets was observed after complete maturation (Figures 1 c, d).

Table 2: Effects of different concentrations of cytokinin and auxin in modified MS medium on *in vitro* shoot proliferation from embryogenic calli cultures of *S. trilobatum*.

Hormone	Concentration of hormones (mg/L)	Duration of Regenerative response (Days)	Average number of multiple shoot (%)	Shoot length (cm)
BA	0.4	20	65	2.64±0.3
	0.8	20	70	2.69±0.3
	1.0	15	85	3.01±0.8
	1.5	15	72	3.26±0.8
	2.0	20	65	2.91±0.1
	2.5	20	65	2.83±0.1
BA + KIN	1.0 + 0.1	20	65	2.78±0.3
	1.0 + 0.2	18	65	2.93±0.3
	1.0 + 0.3	15	75	3.43±0.3
	1.0 + 0.4	15	75	3.0±0.2
	1.0 + 0.5	15	80	3.06±0.3
BA + NAA	1.0 + 0.1	30	-	-
	1.0 + 0.2	30	-	-
	1.0 + 0.3	30	-	-
	1.0 + 0.4	23	75	2.89±0.3
	1.0 + 0.5	20	85	3.02±0.2

- *No response. The values are the mean ± standard deviation. Data represents the average of triplicates, each replicates consists of 14 cultures.*

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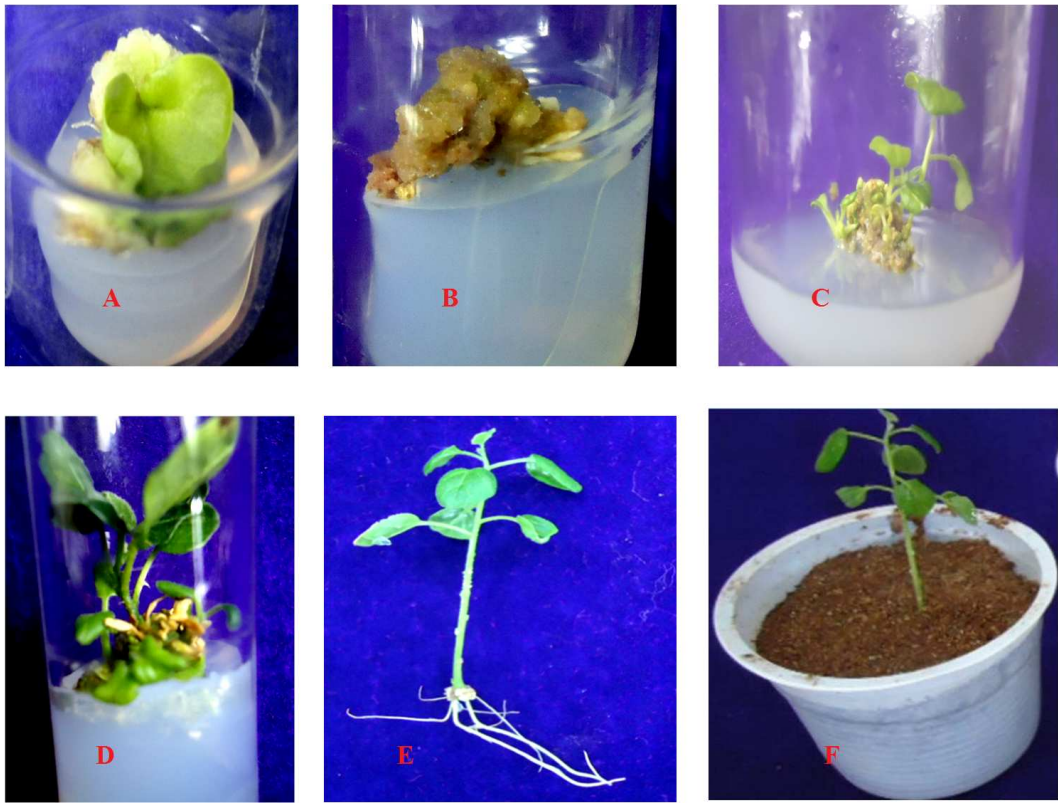

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Fig. 1: Indirect organogenesis of leaf explants of *Solanum trilobatum*. A. Callus initiation, B. embryogenic callus, C. organogenesis (shoot proliferation), D. multiple shoots, E. *invitro* developed roots, F. acclimatized plant.

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Rooting and Acclimatization

The shoots excised from the cultures (3cm length) were transferred to MS medium containing different concentrations of IBA (0.4 – 2.0 mg L^{-1}) and combinations with NAA + IBA (0.5 + 0.1 – 0.5 mg L^{-1}) for root induction. Maximum number of roots was formed in full strength MS medium at 0.5 + 0.4 mg L^{-1} IBA + NAA the average length of the developed roots was about 4.30±0.2 cm (Table 3). IBA + NAA showed better rooting formation than the IBA alone and more than 80% of multiple roots was observed (after 4 weeks) in higher concentration of IBA and combination IBA + NAA under controlled conditions (Figure 1e). The rooted plants were then maintained for maturation of the roots and it was further removed from the culture medium and transferred to the polycups containing sterile sand and red soil mixed in the ratio of 1:1 and watered frequently with half strength MS medium at the temperature of 24±0.2º C and gradually increase the humidity to strengthen the shoots in acclimatization. The rate of successful acclimatization was achieved as 78%.

Table 3. Effect of different concentrations of individual and combinations of IBA and NAA on root formation of *S. trilobatum*.

Hormones	Concentration of hormones (mg/L)	Duration of root formation (Days)	Formation of multiple roots (%)	Root length (cm)
IBA	0.4	30	-	-
	1.0	25	-	-
	1.5	15	80	3.06 ± 0.07
	2.0	15	82	3.05 ± 0.1
IBA + NAA	0.5 +0.1	30	45	2.86 ± 0.2
	0.5+0.2	20	70	2.90 ± 0.1
	0.5+0.3	20	76	3.18 ± 0.5
	0.5+0.4	15	85	4.30 ± 0.2
	0.5+0.5	15	80	3.55 ± 0.1

No response. The values are the mean ± standard deviation. Data represents the average of triplicates, each replicates consists of 14 cultures.

DISCUSSION

Reliable callus induction and regeneration of viable plants considered as a limiting steps to the successful use of modern techniques in genetic improvement of the major crop [14]. Many researchers observed 2, 4 – D is the best hormone for callus induction in monocot and dicot plants [15]. The results of present study showed 2, 4 – D and combination of 2, 4 – D + NAA yielded high degree of callus mass.

The auxin 2, 4-D, by itself or in combination with cytokinins, has been widely used to enhance callus induction and maintenance [16]. Moreover, depending on 2, 4-D concentration there was a range of variations in callus initiation, percentage of explants developed from callus, callus texture, callus colour and degree of callus formation was noticed. Callus initiation on cut ends of *in vitro* cultured explants could be observed in all 2, 4-D and 2, 4 – D + NAA levels after 7- 17 days. Similar findings were reported by Yasmin *et al.,* (2003) [17].

The callus mass was subcultured in MS medium containing cytokinins for shoot induction. The BAP + KIN (0.5+ 1 mg/l) produced more number of multiple shoots (3.0±0.6 and shoot length of about 3.5 ± 0.2) and other concentration of cytokinins like BAP, BAP + KIN produced considerable amount of shoot formation. Similar observations about the role of cytokinins in induction of shoots from callus cultures were recorded in *Solanum* species [18, 19]. The rooting response from the shoots cultured in MS medium supplemented with auxins IBA + NAA (at the concentration of about 0.5 + 0.4 mg/l) produced maximum length of roots (4.3±0.2 cm) and 85% of multiple roots. Similarly [11, 20, 21] were also noticed similar results in developing callogenesis and somatic embryogenesis of *Solanum tuberosum.* Kumari *et al,* (2008) [22] reported similar kind of organogenic green callus obtained from *Ricinus communis* oil seed plant, *Eclipta alba* [23]. Similarly direct regeneration of the same species of plant was reported by Jawahar et al. (2004) [2] using different concentration of 6 – BA (8.88 µM/l) and KIN (9.28 µM/l) for shoot regeneration and root formation (at 9.48 µM/l IBA).

The present work concluded the indirect organogenesis protocol for the rapid *in vitro* proliferation of *Solanum trilobatum* L. an important medicinal plant of India using leaf explants. The induction of callogenesis was strongly dependent on the auxin and cytokinin concentrations used during the subcultures. This study will support improvement of the genetic characteristics of the species and further continuation for large and commercial scale production of the plant and this study helps to avoid further loss of species from natural environment.

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