Micropropagation and Conservation of an Endemic Medicinal Plant *Tylophora subramanii* A. N. Henry

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Abstract

A standard protocol has been established for the *in vitro* propagation of an endemic medicinal plant *Tylophora subramanii* A. N. Henry, using nodal and shoot tip explants. The surface sterilized explants were inoculated on Murashige and Skoog (MS) medium containing various concentrations and combinations of BAP, KIN 0.5-3.5 mg/l and NAA, 2,4-D 0.2-1.2 mg/l respectively. The cultures inoculated on MS medium with BAP 1.5 mg/l and NAA 0.4 mg/l showed maximum number (4.0±0.8) of shoot induction. The shootlets thus derived were inoculated on ½ MS medium provided with NAA and IBA 0.2-1.2 mg/l respectively and the maximum number (3.8±1.8) of roots were induced from the shootlets with IBA 0.6 mg/l.

Keywords:

Tylophora subramanii, Medicinal plant, Nodal explants, Micropropagation

Introduction

Plants are the basic source of knowledge of modern medicine that have been utilized for basic preventive and curative healthcare. All over the world, various medicinal plants have been used for years to treat various diseases (Fransworth and Soejarto, 1991). In the Indian context, it is estimated that about 43% of the total flowering plants are reported to be of medicinal importance (Pushpangadan, 1995). Conservation of these medicinal plants is therefore important to ensure sustainable human development.

Mass propagation of plant species through *in vitro* culture is one of the best and most successful ways of commercial application of plant tissue culture technology. In a special way nodal and shoot culture, which is often utilized to maintain clonal fidelity would be of special advantage. With this background, the present study was undertaken aiming at multiplying the ornamental, medicinal as well as indigenous endemic plant *Tylophora subramanii* A. N. Henry belonging to Asclepidaceae.

Materials and Methods

The endemic medicinal plant *Tylophora subramanii* was collected from the field area in Gangaikondan and subjected to *in vitro* propagation for the purpose of *ex situ* conservation (Plate-1). It is an endemic climbing herb belonging to the family Asclepiadaceae found growing along the Southern Western Ghats. The major alkaloid tylophorine has been reported to have immune suppressive, anti-inflammatory and antitumor properties. The powdered leaves, stems and roots also contain other minor alkaloids, including cryptopleurine, antofine and ficuseptine C which are pharmacologically active.

The explants like node, shoot tip and leaves were collected from the disease-free mother plants and excised into desirable sizes. Then they were washed well in running tap water for 15 minutes to remove the soil or sand particles and also to reduce the microbial load on the surface of the explants. After which they were treated with Tween -20 and rinsed in running tap water for 15 minutes. Once again, the explants were treated with fungicide and rinsed in distilled water for 15 minutes. The rest of the procedure was done in inside the laminar airflow hood. In the next step, explants were treated with 70% ethanol for 20 seconds and washed with sterile distilled water for 4 times. Thereafter, the explants were treated with 0.1% (w/v) mercuric chloride for 2-3 minutes. Finally, they were thoroughly washed in distilled water for four times before inoculating on the MS culture medium with 3% sucrose, 0.6% (w/v) agar containing various concentrations and combinations of BAP, KIN 0.5-3.5 mg/l and NAA, 2,4-D 0.2-1.2 mg/l respectively for multiple shoot induction.

Regenerated multiple shoots were transferred to ½ MS media supplemented with various concentrations and combinations of NAA and IBA 0.2-1.2 mg/l so as to induce roots. The cultures were then incubated in the culture room at 25±2 °C for 12 hours photoperiod with 3,000 lux light intensity. The plantlets with well-developed roots were taken from culture tubes and agar was removed carefully by washing the roots under the running tap water. Then, they were planted separately in poly cups filled with sterilized soil and maintained in the culture room for 3-4 weeks. While hardening, the plants were covered with perforated polythene bags to keep the moisture. Then the plants were transferred to 1.5 cm dia pots with potting mixture of sterilized soil, sand, farmyard manure (1:1:1) and maintained in the green house with a relative humidity of 80-85%.

Results and Discussion

The Nodal explants inoculated on MS media supplemented with different concentrations and combinations of cytokinin and auxin for the purpose of shootlet initiation yielded desired results. Of the various combinations of plant growth regulators tested, 90% of the explants developed maximum number (4.0±0.8) of shootlets in the hormone combination of BAP 1.5 mg/l and NAA 0.4 mg/l (Table-1 & Plate-2). Higher concentrations of hormones paved way for the development of calli rather than shootlets. The present study indicates the need for cytokinin along with auxin for the adventitious shoot induction. Likewise, the results obtained by Daniela *et al.* (2009) emphasize the need for the combination of BAP and NAA that yielded maximum number of shootlets from the nodal explants of *Neoglaziovia variegate* on MS media fortified with NAA 0.5 mg/l and BAP 4.4 mg/l. Also, Sazdur *et al*. (2013) were able to obtain maximum shoot length of 4.9 cm when the explants were inoculated on BAP 3.0 mg/l and NAA 1.0 mg/l. Also, the induction of callus and subsequent differentiation and organogenesis was accomplished by the differential application of growth regulators such as BAP, Kin and NAA in the culture medium. Among the growth regulators tested BAP 2.0 mg/l and NAA 0.5 mg/l induced maximum frequency of shoot regeneration in *Achyranthes aspera* (Kumer Sen *et al*., 2014).

Similar to the present investigation, Shinso Yokota *et al.* (2007) was able to successfully micropropagate *Aralia elata* and *Phellodendron amurense* from nodal segments, on MS medium supplemented with BAP 2.0 μM and NAA 0.5 μM respectively. However, there is a report from Purohit and Kukda (2004) who were able to induce multiple shoots from nodal explants of a 30 year-old tree of *Wrightia tinctoria* on MS medium supplemented with BAP 2.0 mg/l which affirms that BAP alone may induce multiple shoots. But then the shoot tip culture yielded maximum percentage (80%) of multiple shoot induction on MS media augmented with BAP 1.2 mg/l and NAA 0.5 mg/l and the maximum number of (3.0±0.4) shoots. Das *et al*. (2008) could also successfully induce multiple shoots from cotyledons and shoot tips of wood apple (*Aegle marmelos*) on MS medium augmented with BAP 2.0 mg/l and NAA 0.2 mg/l within fourteen days of inoculation. This report confirms the similar combination of plant growth regulators used for the present study.

Initiation of roots at the base of *in vitro* grown shootlets is essential and indispensable step to establish a tissue culture derived plantlets to the soil. The most effective auxin for rooting is IBA (Uddin *et al.*, 2005). The multiple shootlets derived from the cultures were separated individually and subjected to rooting on ½ MS medium provided with NAA and IBA 0.2-1.2 mg/l. Root induction could be seen within 15 days and after that prolific roots were initiated within a period of two weeks. About 80% of rooting was observed on MS media fortified with IBA 0.6 mg/l only (Table 2).

Almost similar results were observed by Animesh *et al.* (2007) who developed suitable protocol for the *in vitro* propagation of *Abrus precatorius* and the microshoots were rooted in MS containing IBA1.0 mg/l and Pandey *et al*., (2013) reported that 95% shoots were able to produce roots with an average number of 6.8 roots per shoot on MS medium supplemented with IBA 2.5 mg/l.

On the contrary, there are several reports in which the *in vitro* raised shootlets when transferred to MS media augmented with different concentrations and combinations of IBA with NAA gave good results. Mehbooba *et al.,* (2011) was able to induce multiple roots on MS medium supplemented with IAA 1.0 mg/l and IBA 0.8 mg/l in *Morus nigra* and Shinso *et al.,* (2007) were able to induce rooting on the *in vitro* raised shootlets of *Aralia elata* and *Phellodendron amurense* when they were inoculated on MS medium augmented with NAA 2.0 mg/l for *A. elata* and MS medium with IBA 2.0 mg/l that produced maximum percentage and number of rooting. Also, Jadimath *et al.* (1998) could induce rooting on the microshoots of *Guizotia scabra* and *G. abyssinca* on MS medium supplemented with 0.2 mg/l NAA alone. Ogunsola and Ilor (2008) investigated the *in vitro* propagation of miracle berry through embryo and nodal explants using different combinations of auxins and cytokinins in MS medium. They were able to generate rooting of the embryo regenerated plantlets with IBA 1.0 - 2.0 mg/l + BAP 0.1 mg/l. It is interesting to note that BAP alone could induce rooting as reported by Senthilkumar (2007) who observed root initiation and the higher root growth in the basal medium containing BAP at 3.0 and 2.5 mg/l in the shootlets of *Acmella calva*.

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Table-1: Effect of PGRs on shoot multiplication from the nodal cultures of *Tylophora subramanii*

Plant Growth Regulators (mg/l)				Number of explants studied	Shooting response %	Average Number of shoots per explant
BAP	NAA	2,4-D	KIN			
0.5	0.0			10	20	1.5± 0.4
1.0	0.2			10	60	2.8± 1.2
1.5	0.4			10	90	4.0± 0.8
2.0	0.6			10	80	3.0± 1.1
2.5	0.8			10	70	3.3± 1.4
3.0	1.0			10	-	+++
3.5	1.2			10	-	+++
		0.0	0.5	10	20	1.5± 0.4
		0.2	1.0	10	30	2.8± 1.2
		0.4	1.5	10	40	3.0± 1.1
		0.6	2.0	10	60	3.4± 0.8
		0.8	2.5	10	-	+++
		1.0	3.0	10	-	+++
		1.2	3.5	10	-	+++

+++: High frequency of callus.

Table-2: Effect of PGRs on roots induction from *in vitro* derived shoots of *Tylophora subramanii* (½ MS medium)

NAA	IBA	Rooting response %	Average Number of roots induction per shoots	Average roots length (cm)
0.2		-	-	-
0.4		-	-	-
0.6		30	2.3±1.6	2.0±0.2
0.8		50	2.3±1.4	2.4±1.6
1.0		60	1.8±0.1	1.1±1.0
1.2		50	2.3±1.4	2.7±1.0
	0.2	40	1.8±0.1	3.0±0.2
	0.4	60	2.3±1.4	2.7±1.0
	0.6	80	3.8±1.8	3.5±0.2
	0.8	40	1.8±0.1	3.5±0.2
	1.0	20	2.3±1.4	2.4±1.6
	1.2	20	1.4± 0.2	1.0±1.6

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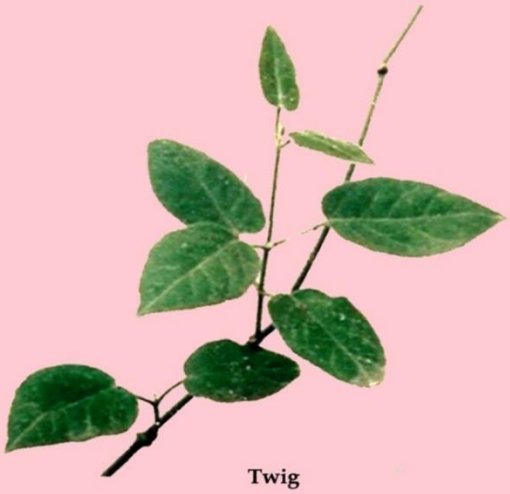
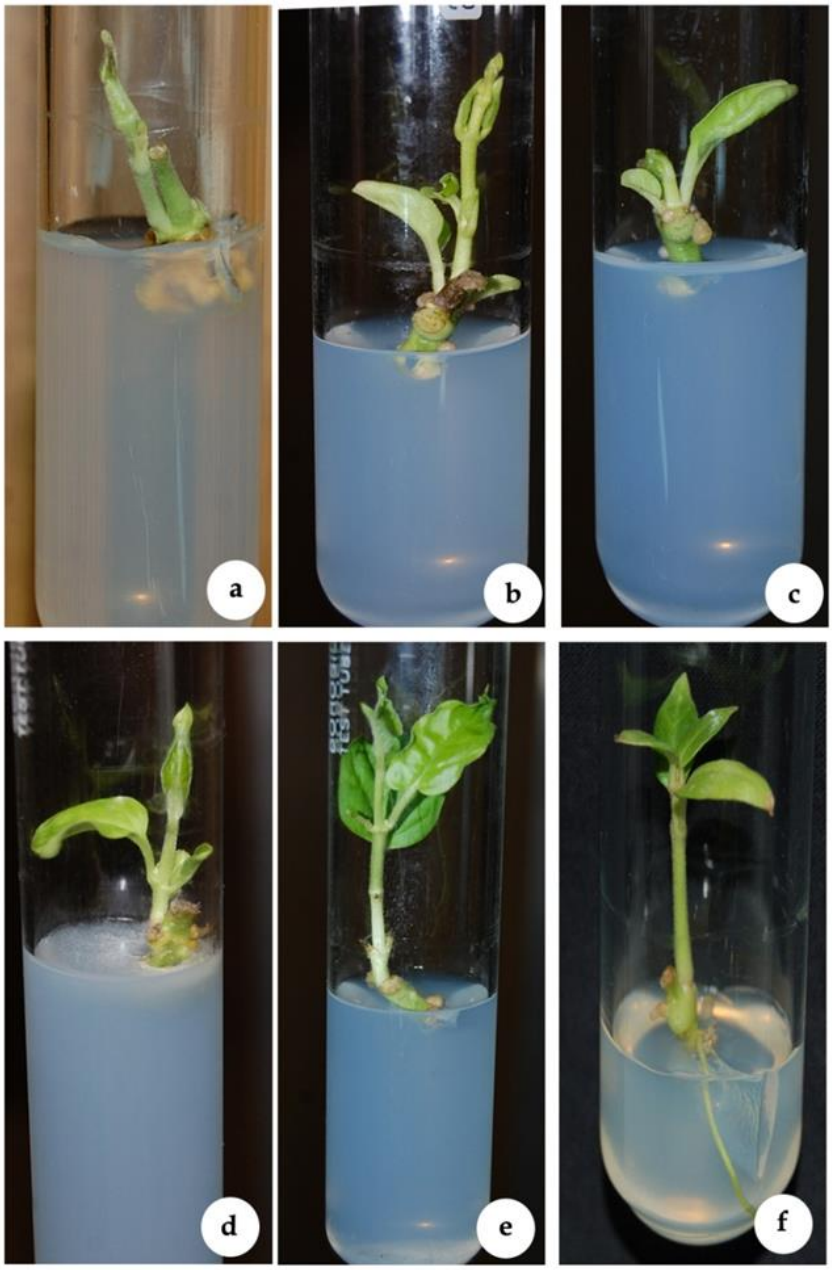
Plate 1 : ***Tylophora subramanii*** Henry.



Twig

Habit of *Tylophora subramanii*

Plate 2 : *In vitro* propagation of *Tylophora subramanii* Henry.



a) Shoot initiation from nodal explant ; b - e) shoot elongation ; f) Root initiation and elongation.