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Original Article

In vitro Multiplication and field Establishment of *Indoneesiella ecohides* L. - An Important Medicinal Plant

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Abstract

The present investigation was aimed at to develop a new micropropagation system for *Indoneesiella echohides*, using different explants. About 90% of the seeds were germinated on MS basal media followed by 80% on ½ MS media supplemented with BAP or Kinetin. The leaf explants were inoculated on MS media fortified with different concentrations of 2,4-D alone or in combinations with Kinetin. Green nodular callus was observed in media supplemented with 2.4-D in combination with Kinetin. Nodal and shoot tip explants were inoculated on to media supplemented with different concentration and combination of cytokinins. The number of multiple shoots developed per explant was registered as 6.0 ± 1.1 cm on medium supplemented with 8.88 µM l^{-1} BAP in combination with 0.53µM l^{-1} of NAA. Maximum number of roots was observed as 5.8 ± 1.1 in IBA 4.92 µM l^{-1} and root length recorded as 6.6 ± 1.1 cm on the same media. The in vitro derived plantlets were successfully transferred to soil after hardening, with a high rate of survival. The plants were comparable to natural population in growth and vigour.

***Key words*:** *In vitro*, Micropropagation, *Indoneesiella echohides*, Explants, Callus
Abbreviations: 2,4-D- 2,4-Dichlorophenoxy acetic acid, MS- Murashige & Skoog, NAA- Napthalic Acetic Acid, BAP – Benzyl Amino Purine, IBA – Indole Acetic Acid.

Introduction

An attempt has been made to micropropagate the medicinal herb *Indoneesiella echohides* L. It is an important medicinal plant belonging to the family Acanthaceae. It is rare in distribution. The whole plant parts are used to cure several diseases because of the presence of Echioidinin and Glucosidechidin. The herb is the well-known drug kalmegh (or) 'green Chirettia' and forms the principal ingredient of a reputed household medicine used as a bitter tonic and febrifuge. The high therapeutic value of kalmegh is due to its mechanism of the action, which is perhaps by enzyme induction. The herb is reported to possess astringent, anodyne, tonic and alexipharmic properties and helpful in dysentery, cholera, diabetes, consumption, influenza, bronchitis, swellings and itches, piles gonorrhea.

A decoction of the plant is a blood - purifier. It is used to cure torpid liver and jaundice. It forms the major constituent of the Ayurvedic drug SG -1 switradilepa that is effective in treating Vittiligo, a dermatological disease. The macerated leaves and juice together with certain species, such as cardamom, clove and cinnamom are made into pills and prescribed for relief from gripe and other stomach ailments in infants. A decoction of infusion of the leaves is useful in general debility and dyspepsia. The leaves and roots are also used as febrifuge, tonic stomachic.

Materials and Methods

Young healthy shoots of *Indoneesiella echoides* were collected during the month between June-July from 3 year old plant growing at the green house maintained by Sathyabama University, Chennai, Tamil Nadu, India. Fresh leaves were collected and washed in running tap water to remove debris and soil from their surface followed by soaked in 5 % (v/v) liquid detergent (Tween 20, Himedia, India) for 10 minutes, then washed under running tap water. Then the leaves were surface sterilized with 0.05% (w/v) $HgCl_2$ for 12-15 minutes and thoroughly rinsed with sterile distilled water for 4 or 5 times. The

explants (10 mm) were excised and placed on solid medium. The medium used in this experiment was MS (Murashige & Skoog, 1962) medium with 3% (w/v) sucrose and 0.6 % (w/v) agar (Himedia, India). Depending on the experiment, the basal medium was supplemented with various plant growth regulators as required. The pH of the media was adjusted to 5.8 ± 0.02 and dispensed into culture tubes (15 x 2.5 cm, Borosil, Inida) and plugged with non-adsorbent cotton prior to autoclaving at 121ºC for 15 minutes. Cultures in all experiments were incubated in the culture room maintained under 16/8 hrs (light/dark) photo period at 25 ± 1ºC under cool white fluorescent tubes (Philips, India) at an intensity of 50 µ mol m^{-2} s^{-1} and 85-90 % relative humidity. Each experiment was repeated thrice with 10 replicates per treatment. Various combinations of plant growth regulators were tried for shoot differentiation and rooting of the plant regenerated. The rooted plants were washed and shifted to green house condition in small poly cups (covered with a perforated plastic bag) containing soil and sand mixture (1:1). Subsequently they were transferred to the garden and after one month they were planted in the field.

Standard error is given to indicate the variation among the means of three experiments based on 10 replicates for each treatment. The data regarding shoots and roots were collected after 35 days and 25 days respectively, after inoculation and were analyzed by ANOVA with a confidence limit of 0.05.

Results

The seeds were inoculated on full and ½ MS media either in the presence or absence of plant growth regulators. About 90% of the seeds were germinated on MS basal media followed by 80% on ½ MS media supplemented with BAP or Kinetin. The results were represented in the table-1. The seeds cultured on the basal media were germinated and produced single shoot and root. Maximum shoot length was recorded as 6.7 ± 1.0 on ½ MS media supplemented with Kinetin 4.64 µM l^{-1}. The root length was measured as 5.0 ± 2.2 on MS basal media (Plate). The seeds inoculated on media fortified with cytokinins were produced only single shoot, it results in the poor development of root system. The shoots were excised and subjected for *in vitro* rooting. The *in vitro* seedlings emerged on basal media were subjected for hardening.

The leaf explants were inoculated on MS media fortified with different concentrations of 2,4-D alone or in combinations with Kinetin (Table-2). The callus pronunciation was observed on margin of the explants after 10 days. About 90% of the callusing was observed in almost all the media combination studied. Green nodular callus was observed in media supplemented with 2.4-D in combination with Kinetin. After 30 days, the regeneration of multiple shoots was observed from the nodular callus. Maximum of 5.8 ± 0.7 shoots were recorded from the callus culture after 45 days on media fortified with 4.82 µM l^{-1} (Plate-1).

Nodal and shoot tip explants were inoculated on to media supplemented with different concentration and combination of cytokinins. Out of the media combination tested, 90% of the explants were found to develop multiple shoots (Table-3). The number of multiple shoots developed per explant was registered as 6.0 ± 1.1 cm on medium supplemented with 8.88 µM l^{-1} BAP in combination with 0.53µM l^{-1} of NAA (Fig.1).

In vitro shoots measuring 3-4 cm were subjected to half strength MS medium with singular or combination of auxins. The initiation of roots was observed after 15-20 days. The number of length of the roots were recorded and represented in the table-4. Maximum percentage (90%) of rooting was observed on media supplemented with IAA 5.7 µM l^{-1}. Maximum number of roots was observed as 5.8 ± 1.1 in IBA 4.92 µM l^{-1} and root length recorded as 6.6 ± 1.1 cm on the same media. The *in vitro* seedlings as well as plantlets derived from the callus, node and shoot tips cultures were subjected for hardening in poly cups containing sterile soil. The plantlets were maintained in the culture room for 20 days at 25 ± 1ºC with 3000-lux photo intensity. Then, they were transferred to poly cups with garden soil and maintained in

the green house for 20-30 days. The percentage of survivability was registered as 90 % for the *in vitro* derived seedlings and 85% for the *in vitro* derived plantlets.

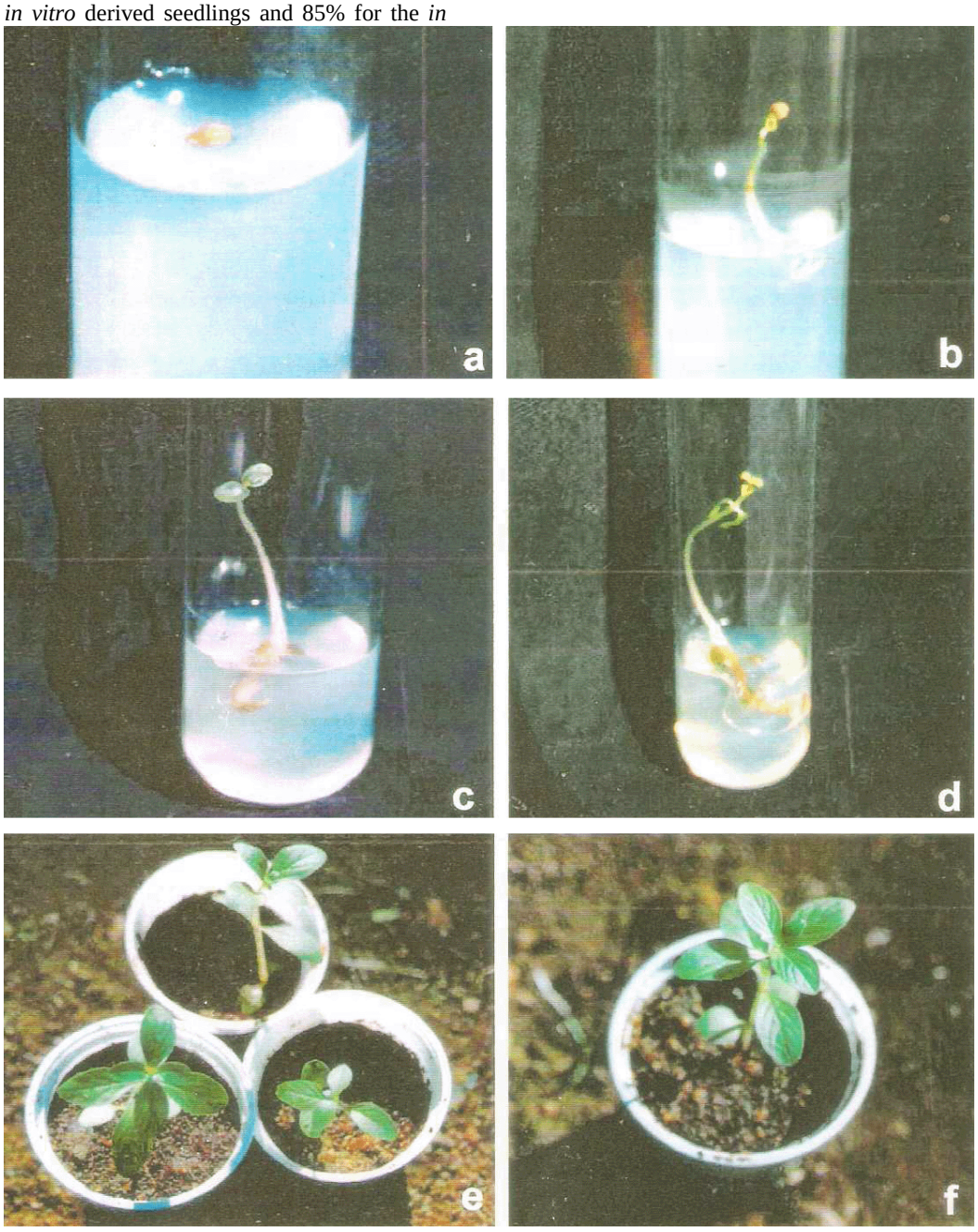

Fig. 1: Regeneration of *Indoneesiella ecohides* from nodal explants of *in vitro* raised seedling

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Table - 1: The effect of plant growth regulators on *in vitro* seedling of *Indoneesiella ecohides*

Sl. No	Media	Plant growth regulators (µM l^{-1})		Number of Seeds inoculated	% of response	Number of shoots proliferated / seed	Average Shoot length (cm)	Average root length (cm)
		BAP	KIN					
1	MS	-	-	20	90	1	3.8 ± 2.2	3.8 ± 2.2
2	½ MS	-	-	20	70	1	5.6 ± 1.5	5.0 ± 2.2
3	½ MS	4.44	-	20	80	1	6.1 ± 1.3	4.8 ± 1.1
4	½ MS	-	4.64	20	80	1	6.7 ± 1.0	4.5 ± 1.0

Table - 2: Effect of plant growth regulators on callus cultures and regeneration.

Sl. No	Plant growth regulators (µM l^{-1})		Number of explants inoculated	Percentage of callus observed	Type of callus
	KIN	2, 4-D			
1	-	4.52	20	80	Green colour
2	-	9.04	20	90	Green colour
3	2.32	4.52	20	80	Green nodular
4	4.64	2.68	20	80	Green nodular
5	4.64	9.04	20	80	Green nodular
6	9.28	4.82	20	80	Green nodular

Table - 3: Effect of plant growth regulators on shoot multiplication

Sl. No.	**Plant growth regulators (µM l^{-1})**			**Number of explants inoculated**	**% of response**	**Average number of shoot proliferation / explant**	**Average shoot length (cm)**
	BAP	**KIN**	**NAA**				
1	4.44	-	-	20	80	3.8 ± 1.2	4.1 ± 1.3
2	8.88	-	-	20	70	5.0 ± 1.1	5.8 ± 1.1
3	-	4.64	-	20	60	2.8 ± 0.7	4.8 ± 1.2
4	-	9.28	-	20	70	4.0 ± 0.8	4.5 ± 1.3
5	4.44	-	0.53	20	80	5.3 ± 1.4	4.4 ± 1.1
6	8.88	-	0.53	20	70	6.0 ± 1.0	5.5 ± 1.1
7	-	4.64	0.53	20	80	4.1 ± 0.9	5.1 ± 1.8
8	-	9.28	0.53	20	70	4.8 ± 1.1	4.7 ± 1.3

Table- 4: Effect of plant growth regulators on root formation

Sl. No.	**Plant growth regulators (µM l^{-1})**		**Number of explants inoculated**	**% of response**	**Average number of Root initiated/ explant**	**Average root length (cm)**
	IBA	IAA				
1	4.92	-	20	80	5.8 ± 1.1	6.6 ± 1.1
2	9.84	-	20	70	5.3 ± 0.8	6.2 ± 0.8
3	-	5.7	20	90	4.5 ± 1.3	5.1 ± 1.4
4	-	11.4	20	80	4.7 ± 0.9	6.3 ± 1.2
5	4.92	0.57	20	80	5.1 ± 1.4	5.6 ± 0.8
6	-	5.7	20	70	4.9 ± 1.1	5.8 ± 1.2

Discussion

In the present investigation, the most commonly used MS media was used to study the morphogenetic responses of this plant either in the presence or absence of plant growth regulators. The seeds cultured on hormone free medium produced short and pale green seedlings with well developed root system. The percentage of seed germination (90%) was observed and recorded. The same observation has been reported earlier in other plants including *Hemidesmus indicus* (Patnaik & Chand, 1996) and *Curculigo orchioides* (Raghu *et al.*, 2004). However, the seeds subjected to 2.22 µM l^{-1} BAP were produced only single shoot with poor root development.

Multiple shoots from the nodal and shoot tip cultures were recorded in almost all the media combinations studied. The results revealed that BAP alone in the medium is not sufficient to induce multiple shoot buds indicating the necessity of using combinations of auxins and cytokinins. The effect of NAA/IAA with BAP on adventitious shoot bud productions has been noticed in other species including *Rauwolfia* (Ghosh *et al*., 1998 and Mukhopadhay *et al*., 1991).

This present study also revealed that, the numbers of shoots were increased on media supplemented with BAP in combination with NAA. Maximum of 6.0 ± 1.0 shoots per explants was recorded on media fortified with BAP 8.88 µM l^{-1} and NAA 0.53 µM l^{-1}.

The establishment, growth and regeneration of plantlets from callus culture have been reported by several workers (Simmonds & Cummings, 1976 and Chen *et al*., 1988). In this present investigation also, the shoots were regenerated from the nodular callus derived from leaf explant on medium fortified with 2, 4-D in combination with Kinetin 9.28 µM l^{-1}.

The promotive effect of roots by IBA has been reported in many plants. In the present study, maximum number of roots was observed in IBA 4.92 µM l^{-1} and maximum root length also recorded on the same media. The rooted plantlets were transferred and established well in the green house. The *in vitro* grown plants exhibited high survival rate (75-80%) in the green house. It indicates that could be easily adopted for large-scale cultivation.

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