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An efficient in vitro plant regeneration of *Dipteracanthus prostratus* (Poir.) Nees.- a medicinal herb

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ABSTRACT

Objective: This is the first attempt for an efficient plant regeneration protocol through in vitro direct organogenesis for a valuable medicinal plant, Dipteracanthus prostratus using nodal segment. **Methods:** Multiple shoots were induced from nodal explants cultured on Murashige and Skoog medium supplemented with kinetin (KIN), 6-benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA). **Results:** Maximum shoot responses (80%) were obtained with kinetin at 1.0 mg^{-1}. The rate of shoot multiplication was maintained in subsequent subculture on similar fresh culture medium. The highest shoot length (3.96cm) was obtained with seventy three percentages of shoots at 0.2 mg^{-1} NAA along with 1.0 mg^{-1} kinetin. Maximum length of root (3.63cm) was formed at 0.5 mg^{-1} IBA with significant responses (80%). Rooted plantlets were then transferred to perforated plastic cups and grown in the green house at 80% survival rate. **Conclusions:** The highest survival rate was noticed and this plant developmental protocol could be used for large- scale regeneration of *D. prostratus*.

1. Introduction

In general, medicinal plants are disappearing from natural habitats by rapid agricultural development, urbanization, indiscriminate deforestation and uncontrolled collection of medicinal plants. Industrialization coupled with urbanization is constantly putting pressure on natural and ecological resources. Due to over depletion and ruthless collection of medicinal plants are on the verge of extinction. Plant tissue culture technology holds great promise for micropropagation, conservation, and enhancement of the natural levels of valuable secondary plant products and to meet pharmaceutical demands and reduce the *in situ* harvesting of natural forest resources. For mass propagation of medicinal plant species in which conventional methods possess limitations, in vitro multiplication provides the way out. There are sufficient reports available about protocols on in vitro micropropagation of many threatened medicinal species [1, 2]. *In vitro* plant regeneration is a complex phenomenon involving different biochemical mechanisms for its progression[3]. *In vitro* propagation refers to true-to-type propagation of selected genotypes using in-vitro studies. Different explants such as single cells, protoplasts, pieces of leaves or roots can be used to generate a new plant on culture media with required nutrients [4].

Dipteracanthus prostratus (Poir.) Nees belongs to Acanthaceae family and very important indigenous medicinal plant, which present in moist shady places throughout India. In India, it is used as a remedy for ear diseases and believed to be anticancer against the epidermis of the nasopharynx region, slightly hypoglycemic, anti-inflammatory and antimicrobial. And also the leaves are eaten as vegetable [5, 6]. Therefore, the present study was conducted to develop an efficient protocol for direct organogenesis and establishment of complete plant from nodal segments of *D. prostratus*.

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2. Materials and methods

2.1. Chemicals

The hormones and chemicals were purchased from Hi media laboratories Pvt. limited, Mumbai, India.

2.2. Explants collection and inoculation

D. prostratus was collected from the campus of St. Joseph's College. The plant was identified by Dr.S.Soosai Raj, Department of Botany, St. Joseph's College, Tiruchirappalli. Nodal segments were excised and washed thoroughly in running tap water for ten minutes, then in 1% Teepol solution for five minutes and washed five times with sterile distilled water under aseptic condition. The explants were rinsed by 0.1% aqueous $HgCl_2$ solution for three minutes and rinsed with sterile distilled water five times to remove traces of $HgCl_2$ for surface sterilization. The explants were cultured on MS medium [7] fortified with various concentrations of KIN, BAP and NAA along with 3% sucrose (w/v) and 0.8% (w/v) of agar. The pH of the medium was adjusted to 5.7 using 0.1N NaOH or 0.1N HCl. Each culture tubes were sealed with non-absorbent cotton plugs prior to autoclaving at 121℃ for 15 min. All cultures were maintained at 16 h photoperiod with 3000 lux light intensity at 25±2℃.

2.3. Statistical analysis

All the results are expressed as mean±SE. The statistical analyses were performed by the Student's t-test with SPSS 11.0J (SPSS Japan Inc., Hiroo, Tokyo, Japan) and relationships were considered to be statistically significant when $P<0.05$.

3. Results

3.1. Shoot proliferation

In our present investigation, a successful regeneration of *D. prostratus* was accomplished through in vitro micropropagation techniques. The nodal part of *D. prostratus* was used as explants, which aseptically placed on MS medium with different concentrations of KIN, NAA and BAP. Of the three hormones, kinetin (1 mg^{-L}) was more effective for shoot proliferation than BAP and NAA (Table 1). Their morphological characteristics such as, percentage of shoot respond, number of shoots and their lengths were recorded. Various concentrations of kinetin exhibited significant influence on shoot number and best shoot length. In 1 mg^{-L} of KIN, the shoots (80%) formed along with 46.66% of root from the nodal explants (Table 1). And also, the highest mean shoot length (2.63±0.08 cm) was obtained (Fig.1d). At 1.5 mg^{-L} of KIN, the mean of 3.33 shoots/nodal explants was noticed (Fig.1e). The callus was formed at the proximal end of the regenerated stem which developed at 0.5 mg^{-L} of KIN (Fig.1c). There was no significant effect on multiple shoot regeneration in both 2 and 2.5 mg-L of kinetin.

The mean shoot responses (73.33%) with 2.13 cm height observed in 0.2 mg^{-L} of NAA, in which callus and root (33.33%) were formed at the proximal end of the stem and also 63.33% of shoot responded in 0.5 mg^{-L} of NAA (Fig.1a), respectively. When the concentration of NAA increased the percentage of shoot respond was decreased slightly. The mean of highest multiple shoots number (9.66) was proliferated in 2.5 mg-L of BAP (Fig.1f) with 53.33% shoots response. However, the highest length of shoot (2.56 and 2.46cm) obtained in both 0.5 and 1.0 mg^{-L} of BAP with 56.66% and 53.33% of shoots, respectively. Table 2 reveals that combination of 0.2 mg^{-L} of NAA and 1.0 mg^{-L} of KIN (Fig.1g) showed the mean of shoot responses (73.33%) with mean of significant number of shoots per explants (2.66) along with shoot length (3.96cm).

Table 1
Effects of phytohormones with MS medium on shoot proliferation of *D. prostratus* after 24 days

Plant Growth Regulators(mg^{-L})	Percentage of shoots responded	Number of shoots/ explant	Shoot length (cm)	Percentage of shoots rooted
Kinetin				
0.2	42.00±1.15	1.33±0.33	0.90±0.05	13.33±3.33
0.5	43.33±3.33	2.66±0.33	1.33±0.08	16.66±3.33
1.0	80.00±5.77	2.66±0.66	2.63±0.08	46.66±3.33
1.5	46.66±3.33	3.33±0.88	1.43±0.03	30.00±5.77
2.0	26.66±3.33	1.33±0.33	1.30±0.05	13.33±3.33
2.5	26.66±6.66	1.33±0.33	0.90±0.11	–
BAP				
0.2	26.66±6.66	2.33±0.33	1.23±0.08	–
0.5	56.66±3.33	1.66±0.33	2.56±0.14	–
1.0	53.33±8.81	1.00±0.00	2.46±0.08	–
1.5	33.33±8.81	1.33±0.33	1.26±0.14	13.33±3.33
2.0	26.66±3.33	1.66±0.33	0.96±0.17	16.66±6.66
2.5	53.33±8.81	9.66±1.20	2.06±0.06	26.66±3.33
NAA				
0.2	73.33±3.33	1.33±0.33	2.13±0.08	33.33±3.33
0.5	63.33±3.33	1.33±0.33	1.83±0.06	26.66±3.33
1.0	33.33±6.66	1.66±0.66	0.93±0.13	16.66±6.66
1.5	40.00±5.77	1.66±0.33	1.03±0.08	23.33±3.33
2.0	33.33±5.77	2.33±0.33	1.86±0.08	23.33±3.33
2.5	33.33±3.33	1.66±0.33	1.43±0.12	–

All values are expressed as mean±SE of ten replicates

Table 2
Effects of phytohormones combinations on shoot regeneration of *D. prostratus* using MS medium

Plant Growth Regulators (mg^{-L})	Percentage of shoots responded	Number of shoots/ explant	Shoot length (cm)	Percentage of Shoots rooted
NAA + KIN				
0.2 + 0.5	36.66±8.81	1.33±0.33	2.43±008	16.66±6.66
0.2 + 1.0	73.33±3.33	2.66±0.66	3.96±012	56.66±6.66
0.2 + 2.0	60.00±5.77	1.66±0.33	2.13±014	26.66±3.33
0.5 + 0.5	46.66±6.66	2.33±0.33	1.13±014	20.00±10.00
0.5 + 1.0	56.66±3.33	1.66±0.66	1.76±008	–
0.5 + 2.0	26.66±8.81	2.66±0.33	1.23±008	–

All values are expressed as mean±SE of ten replicates

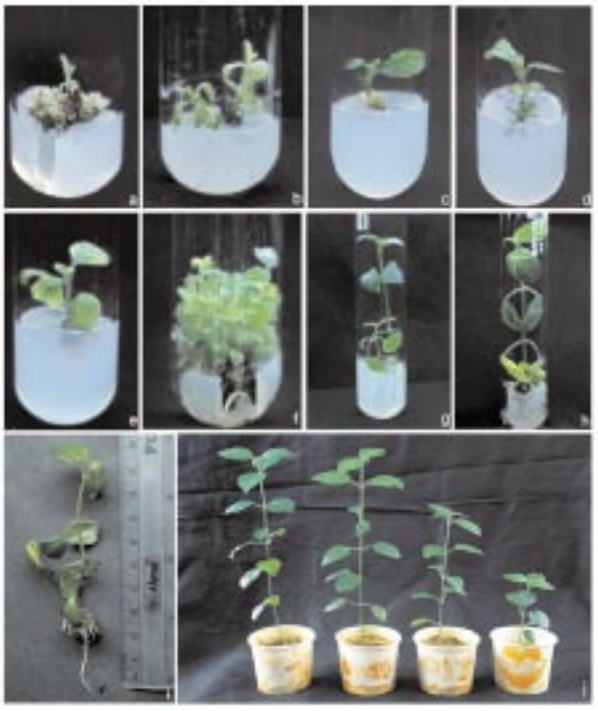

Fig 1. *In vitro* regeneration of Dipteracanthus prostratus Nees from nodal explant
a) Callus development and shoot proliferation on MS+0.5 mg-L NAA
b)Induction of shoot buds on MS+ 2.0 mg-L NAA
c)Callus formation at the proximal end of shoot on MS+ 0.5 mg-L KIN
d)Shoot formation on MS+1.0 mg-L KIN
e)Proliferation of shoot with in vitro root on MS+ 1.5 mg-L KIN
f)*In vitro* shool multiplication with root on MS+ 2.5 mg-L BAP
g)Shoot elongation with in vitro root on MS+ 0.2 mg-L NAA+1.0 mg-L KIN
h)*In vitro* roots on MS+0.5 mg-L IBA
i)Well developed roots on MS+0.5mg-L IBA
j)Hardened of in vitro raised plant

3.2. Root induction

For root induction, in vitro developed shoots were transferred to MS medium supplemented with different concentrations of IBA and NAA (0.2- 1.0 mg^{-L}). One excised shoot was placed in a tube (25×150 mm) containing 10 ml of the culture medium with root induction hormone. The root lengths were varied in all MS basal strength with IBA and NAA concentrations (Table 3). However, maximum percentage of rooting (80%) of the excised shoot along with 3.63cm of root length was achieved within twenty four days in MS medium fortified with 0.5 mg^{-L} IBA (Fig. 1h, i).

3.3. Hardening

Complete plantlets were taken out from the test tubes and rinsed with double distilled water to remove the medium. Finally, in vitro raised plantlets were transplanted to pot containing sterile mixture of sand, soil and cow dung manure in the ratio of 1:1:1 (v/v) and kept in a polythene house maintained in 80% relative humidity for acclimatization. The water was poured in planted pots whenever required. The rooted plantlets were transferred to greenhouse conditions where they showed about 80% survivals (Fig. 1j). The in vitro grown plants did not show any detectable variations on their morphological characteristics when compared with the parent plant.

Table 3
Effects of NAA and IBA in MS medium on root induction of *D. prostratus* after 24 days

Plant Growth Regulators (mg^{-L})	Root responded (%)	Root length/Explants (cm)
NAA		
0.2	43.33±3.33	1.66±0.08
0.5	63.33±8.81	2.26±0.14
1.0	53.33±3.33	2.63±0.06
IBA		
0.2	36.66±6.66	1.06±0.08
0.5	80.00±0.00	3.63±0.08
1.0	33.33±8.81	1.33±0.08

All values are expressed as mean±SE of ten replicates

4. Discussion

In general point of view, the nodal segment has been reported earlier as a significant source of in vitro shoot proliferation in Andrographis neesiana [8], *Centella asiatica* (9) and *Aegle marmelos* [10]. Our present study proved previous reports that kinetin which increases the number of shoots and its length [10, 11, 12]. As reported by Jeyachandran *et al.* [13], callus forms frequently at the basal cut end of nodal explants on cytokinin. The significant stimulation of shoot lets formation observed in MS medium fortified with various concentrations of both BAP and KIN. At the higher concentration of KIN, percentage of shoot responses, number of shoot per explants and its length were decreased with further increase in the concentration of KIN. Our findings are directly connected with earlier reports [14]. The present investigation was directly coinciding with previous work that shoots induction was found to be greater in BAP on *Centella asiatica* L. (9), Tuberaria major (15), Apple (16), *Maesa* (17), *Passiflora* (18), and *Metabriggsia* (19) *Gynura procumbens* (20), *Vitex negundo* (21). Earlier report explores that significant shoot formation and length can be achieved in the combination of NAA and KIN (22). Our investigations also proved the previous results [23, 24, 25, 26, 27, 28] that best rooting hormone is IBA than other auxins because of its stability against catabolism and inactivation by conjugation.

In conclusion, an efficient protocol was developed for the regeneration of *D. prostratus* using nodal explants. The results showed that node is suitable and reproducible capability explants for the in vitro propagation of *D. prostratus*. And also, the present efficient in vitro regeneration protocol could be used for large scale commercial production and also for development of elite genotypes for further pharmacological evaluations.

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Conflict of interest statement

We declare that we have no conflict of interest.

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