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IN VITRO REGENERATION OF MULTIPLE SHOOTS FROM THE NODAL EXPLANTS OF *DRYMARIA CORDATA* (L.) WILLD. EX. ROEM. AND SCHULT

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ABSTRACT
Drymaria cordata commonly known as West Indian chick weed is widely exploited for its medicinal properties. An efficient protocol for *in vitro* multiplication of *Drymaria cordata* using nodal explants is developed. Nodal cultures were raised on Murashige and Skoog's medium (MS) supplemented with auxins and cytokinins either alone or in combination with various auxins. Maximum number of shoots (19.51 ± 0.18) with high frequency of shooting response (76.6%) was obtained in nodal cultures on MS medium fortified with BAP (4.44μM) alone. The *in vitro* regenerated shoots were rooted in MS + IBA (0.49 μM). The plantlets obtained were successfully hardened and established in soil. Nearly 90% of survival was recorded.

INTRODUCTION

Many valuable medicinal plants are under the verge of extinction because of various reasons including deforestation, urbanization and unscientific collection. Demand for the herbal medicines is steadily increasing in both developed and developing countries. It has been estimated that in developed countries, plant drugs constitute as much as 25% of the total drugs, while in fast developing countries, the contribution is as much as 80% (Joy *et al.,* 1998). With an ever increasing global inclination towards herbal medicines, the overexploitation of medicinal plants will be continued. *In vitro* micropropagation is now established as an alternate strategy for the mass multiplication of economically important plants (Murashige, 1974).

Drymaria cordata (L.) Willd. ex. Roem. and Schult., a member of the family Caryophyllaceae is an annual herbaceous plant which is found distributed widely in the Tropical regions of Asia, Africa, Central and South America (Dequan and Gilbert, 2001) and in Tropical and Sub Tropical India extending into the Himalayas up to a elevation of 2100 metres. General survey and ethnobotanical studies have shown that the plant is utilized by the tribal people as it has a number of medicinal properties and is used by the local folk to cure a number of ailments. It is used to treat snake bites, and is applied topically for burns and skin diseases (Rao, 1981). Studies on antitussive activity (Mukherjee *et al*., 1997a), antibacterial efficacy (Mukherjee *et al*., 1997b) and anti-inflammatory effects (Mukherjee *et al*., 1998) of *Drymaria cordata* has been investigated. Due to its medicinal properties, a number of biologically active compounds have been isolated from the leaves. Cyclopeptides (Ding *et al*., 2000), Flavonoid glucosides (Ding *et al*., 1999), norditerpenes and norditerpene glycosides (Vargas *et al*., 1988) have been isolated from the leaves. A new alkaloid, drymaritin exhibiting anti HIV properties in H9 lymphocytes has been isolated from *Drymaria diandra* (Hsieh *et al*., 2004).

Low germination rate and poor seed viability (Ghimire *et al*., 2010) are the main reasons for its large scale propagation and conservation. Hence, an alternative propagation method has become a necessity. Large scale rapid production of clonal plants through *in vitro* culture of single node stem segments and shoot to shoot proliferation was achieved in several medicinal plants (Chaturvedi *et al.,* 2007). About 2,56,6000 *in vitro* plants were obtained from the initial culture of single node in *Dioscorea floribunda* (Chaturvedi, 1975; Chaturvedi and Sinha, 1979). Thus the present investigation elucidates an *in vitro* multiple shoot regeneration through nodal segments of *Drymaria cordata* for mass propagation and also preservation of this valuable germplasm through direct organogenesis from nodal explants.

MATERIALS AND METHODS

Plant material and explant source

The plant was collected from Kodagu District, Karnataka State, India. The identification of the taxon was authenticated by BSI, Coimbatore, South India. They were maintained in the

green house, Department of Botany, Bangalore University. Nodes of about 1 to 1.5 cm were excised from healthy plants and used as explants.

Surface sterilization

Nodal explants were washed thoroughly in running tap water for 10 min, then in 1% Tween 20 solution for 5 min and washed repeatedly with sterile distilled water. For surface sterilization, explants were treated with 70% alcohol for 10 sec and washed with sterile distilled water for 3-4 times after which they were treated with 0.1% $HgCl_2$ for 4 ½ min. The explants were thoroughly washed with sterile double distilled water to remove traces of $HgCl_2$.

Culture medium

The nodal explants were trimmed and then inoculated on Murashige and Skoog's medium (1962) fortified with various cytokinins (BAP and KN) individually. Sucrose (3%) and bacteriological grade agar agar (0.8%) were used as carbon source and gelling agent respectively. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.8 using 0.1N NaOH or 0.1N HCl before gelling with agar. About 20ml of the medium was dispensed in each culture bottle and autoclaved at 121ºC for 15 min. The cultures were incubated at 25 ± 2ºC with 16h photoperiod. The cultures were illuminated by white fluorescent tubes at light intensity of 25μmolm^{-2}s^{-1}.

Multiple shoot induction and multiplication

For multiple shoot induction, the young nodal explants were cultured on MS medium fortified with different concentrations of BAP (2.22 - 17.76μM) and Kn (2.32 - 18.59μM) individually. After four weeks of incubation, well developed multiple shoots were subcultured on fresh MS medium consisting of optimal concentration of cytokinin and auxin for further shoot elongation and multiplication. Experiments were repeated thrice and results observed at regular intervals and tabulated.

Rooting and plant regeneration

Following multiple shoot elongation, the healthy shoots (3-4cm) were transferred onto MS medium supplemented with various concentration of IBA individually. Observations were taken at the end of the 4th week.

Well rooted plantlets were washed in sterile water and transferred to plastic cups containing sterilized mixture of cocopeat: soilrite and perlite (1:1:1). Plantlets were nourished with ½ strength MS liquid medium. The cups were covered with plastic cover and kept in the growth room maintained at 25±2ºC under cool fluorescent light (25μmolm^{-2} s^{-1}) with 16 h photoperiod. After three weeks, the hardened plants were transferred to pots containing garden soil and maintained in the green house for acclimatization.

Data analysis

Data obtained was subjected to one way analysis of variance (ANOVA) and means were compared using student t test at 0.05% probability level.

RESULTS AND DISCUSSION

Nodal explants when cultured on MS basal medium supplemented with different plant growth hormones, more than two shoots were obtained. This indicates the necessity for supplementation of plant growth regulators externally for induction of multiple shoots (Sasikumar *et al*., 2009).

All concentrations of BAP and Kn facilitated shoot bud differentiation. Within four weeks, multiple shoot bud development occurred directly from the explants. The response was good at 4.44μM BAP where 19.51 ± 0.18 shoots developed, while there was a decrease in the number of shoots as the concentration increased (Table 1, Fig. 1). Jeyakumar and Jayabalan (2002) observed the multiplication of shoots on BAP (2.22μM) in plant *Psoralea corylifolia*. Similar effects of BAP were observed in *Dianthus caryophyllus* (Ali *et al.,* 2008) and *Spilanthus acmella* (Haw and Keng, 2003). The shoots showed stunted and bushy growth.

There was a reduction in number of shoots on Kn supplemented medium, but showed long internodes with effective rooting. Similar effect of cytokinin is reported by Beena

Table 1: Effect of BAP and Kn on multiple shoot regeneration. Data (Mean ± SE) recorded after 4 weeks

Growth regulators (μM)	% of Shooting response	Mean number of shoots produced /nodal bud explants (Mean ± SE)
BAP		
2.22	66.66	13.77 ± 0.28^{d}
4.44	76.66	19.51 ± 0.18^{a}
6.66	66.66	14.93 ± 0.40^{b}
8.88	53.33	14.36 ± 0.13^{c}
11.10	63.33	8.04 ± 0.20^{e}
13.32	60.00	4.58 ± 0.21^{g}
15.54	56.66	4.00 ± 0.10^{h}
17.76	56.66	3.44 ± 0.28^{i}
Kn		
2.32	70.00	2.05 ± 0.23^{k}
4.65	70.00	2.56 ± 0.14^{j}
6.97	70.00	2.95 ± 0.34^{i}
9.29	73.33	3.08 ± 0.19^{i}
11.62	80.00	3.37 ± 0.12^{i}
13.94	83.33	5.57 ± 0.07^{f}
16.26	70.00	3.79 ± 0.21^{h}
18.59	63.33	2.91 ± 0.18^{j}

CD = 0.5 Means followed by the same letter does not differ significantly by student t test at 0.05% probability level.

Table 2: Rooting Response. Data recorded after 4 weeks

Combination	Average Root length (cm)	% ofRooting	Slight Callus	Remarks
½ MS	4.53 ± 0.06^{c}	100%	-	Less roots developed and slow growth
½ MS+0.49 μM IBA	4.67±0.17^{c}	100%	-	Thin fragile roots
½ MS+2.46 μM IBA	4.32±0.13^{d}	100%	+	Thin Fragile roots
Full strength MS	5.16 ± 0.17^{b}	100%	-	Healthy roots
MS+0.49μM IBA	6.93 ± 0.04^{a}	100%	-	Profuse and healthy rooting
MS+ 2.46μM IBA	6.79 ± 0.06^{a}	100%	+	Profuse and healthy rooting

CD = 0.29 Means followed by the same letter does not differ significantly by student t test at 0.05% probability level

Figure 1: Multiple shoots from nodal explants on MS + BAP (4.44μM)

et al., (2003) in *Ceropegia candelabrum*. BAP appeared to be more effective hormone than Kn at inducing multiple shoots as was reported in several other medicinal plants (Karthikeyan *et al*., 2009; Sasikumar *et al*., 2009; Sharma *et al*., 2010).

Further shoot multiplication (30-40 shoots) and elongation were observed when the developed shoots from BAP (4.44μM) were transferred onto fresh medium containing BAP (4.44μM) in combination with IBA (0.49μM) (Fig. 2). While BAP (4.44 μM) in combination with NAA (0.54μM) and IAA (0.57μM) resulted in slow growth. However, Ghimire *et al*. (2010) have obtained highest number of shoots on MS supplemented with BAP and NAA from leaf cultures. A low auxin concentration in combination with a high concentration of cytokinin is the most suitable combination for the proliferation of shoots (Tejavathi and Gayathramma, 2005).

The healthy shoots (3-4cm long) when cultured on MS supplemented with IBA (0.49μM) individually proved to be efficient in producing elongated roots without the formation of basal callus. In *Drymaria cordata*, rooting was observed in the absence of auxins also, but the best rooting was observed on full strength MS medium with IBA (0.49μM) with a maximum root length of 6.93 $\pm$ 0.04 cm (Table 2, Fig. 3). Similar effects of IBA were also observed in *Psoralea corylifolia* (Jeyakumar and Jayabalan, 2002), *Solanum trilobatum* (Arockiasamy *et al*., 2002), *Bacopa monnieri* (Sharma *et al*., 2010), *Morus indica* var. Mysore local (Tejavathi *et al*., 2009)

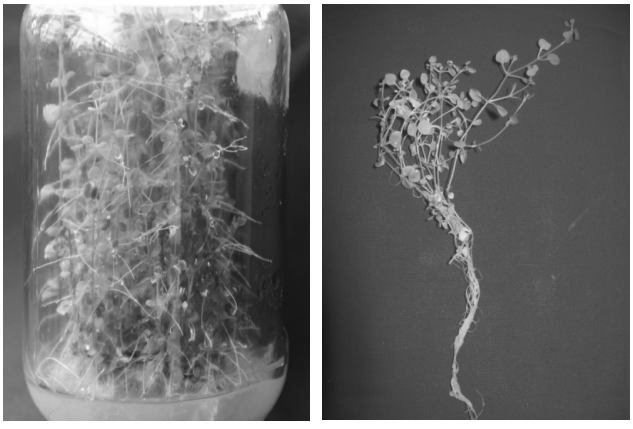

Figure 2 and 3: (2)Multiple shoot formation and elongation on MS + BAP (4.44μM) + IBA (0.49μM) (3) Plant with well developed Roots

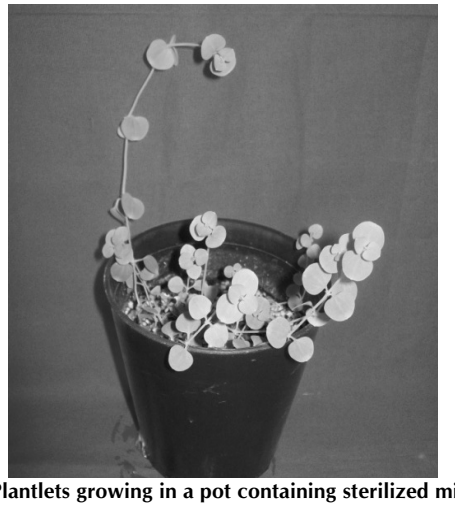

Figure 4: Plantlets growing in a pot containing sterilized mixture of cocopeat: soilrite and perlite (1:1:1).

and *Pluchea lanceolata* (Arya *et al*., 2008). Increase in the auxin concentration induced callusing at the base. The selection of auxin at a particular concentration that can promote only the growth of shoots without callus induction is found to be a significant aspect to get clones through micropropagation.

The regenerated plants were washed in sterile water and transferred to plastic cups containing sterilized mixture of cocopeat: soilrite and perlite (1:1:1). Plantlets were nourished with ½ strength MS liquid medium. The cups were covered with plastic cover and kept in the growth room maintained at 25 ± 2ºC under cool fluorescent light ($25\mu molm^{-2}s^{-1}$) with 16h photoperiod (Fig. 4). After three weeks, the hardened plants were established in garden soil and maintained in the green house for acclimatization. The plants showed 90% survival rate (Fig. 5).

It is necessary to develop effective protocols for mass propagation to conserve the germplasm to make the availability of plants throughout the year to get resistant varieties, to get uniform plants of selected genotype and to obtain plants with changed genotypes (Bajaj *et al*., 1988). The *in vitro* production of plants through nodal culture is a most reliable method to get uniform plants of selected genotype (George and

Figure 5: Regenerated plant in pot containing garden soil

Sherrington, 1984). In conclusion, the single nodal culture method here offers an effective method of propagation and multiplication of *Drymaria cordata*.

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