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Clonal propagation of an endangered Umbellifer, *Polyzygus tuberosus* from South India

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Abstract

Polyzygus tuberosus Dalzell ex Walp. is a critically endangered and endemic species of South India that has received very little attention scientifically. The plant inhabits in a small patch of natural habitat in the field of Bangalore University, Jnanabharathi campus. In the present studies, the endemic and threatened status of this rare plant is revealed. Clonal propagation was succeeded from nodal explants inoculated on MS media supplemented with 0.3 mg/l BAP + 0.2 mg/l IBA for multiple shoot initiation. The nodal explants also induced multiple shoots on MS media supplemented with 0.5 mg/l BAP. The root and nodal explants induced callus on MS media supplemented with 2 mg/l NAA. Roots were initiated in half strength MS media supplemented with 0.3 mg/l IAA + 0.5 mg/l IBA. The hardened plants were reintroduced in its natural habitat and observed normal growth characters without any morphological changes

Keywords: Clonal propagation, *Polyzygus tuberosus*, Apiaceae

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Introduction

The family Apiaceae comprises annual herbaceous plants usually aromatic. The members are restricted to both tropical and temperate regions. There are 68 genera and 240 species of which 8 genera and about 60 species are endemic to India. *Polyzygus tuberosus* Dalzell ex Walp. is monotypic and endemic to South India (Heywood, 1971). The plant occurrence is reported in Flora of Bangalore, Andhra Pradesh, Bombay presidency, Madras presidency, Flora of British India and Umbelliferae of India (Mukherjee, 1993) Most Apiaceae species are distributed in high altitude regions with very few exceptions such as this species. Scientific data on this rare species is very meager.

The literature survey reveals *Polyzygus tuberosus* enlisted as threatened plant of India in Red data book of Indian plants (Nayar and Sastry, 1990).

In the natural field of Bangalore University, Jnanabharathi campus, this species inhabits in a very small population amidst the grassy slopes and gravellery soil. The plant is a slender, weak stemmed, erect, 10-30 cm tall. It bears very small tuberous root. The leaves are clustered at base, 3-parted to pinnate, oblong and entire. Petiole slender with narrowly sheathing at base. The Umbellets 15-20 flowered. The flowers are whitish yellow & regular. The flowering and fruiting occurs during February – March. The observed unique features of this specimen are growing

in dry vegetation, bearing thick leathery non-aromatic leaves and flowering during the onset of summer. These features reflect the drought and stress tolerance in this species, which deviates from most native species of the family Apiaceae.

Since 2008, the plant occurrence is carefully observed and noticed that there is a drastic decline in the population and in its natural habitat. Hence, our main objective was to initiate a conservation protocol through Clonal propagation.

The literature survey of this species provides very meager information on Cytology (Basappa, 1991), essential oil (Janardhan, 2001) and Karyomorphological studies (Janardhan, 2003).

The literature survey reveals the clonal propagation have been succeeded in few closely related species such as *Heracleum candicans* (Wakhlu *et. al.*, 1998) in *Thapsia garganica* (Makunga *et. al.*, 2004); in *Vanasushava pedata* (Karuppusamy *et. al.*, 2006); in *Hydrocotyle conferta* (Karuppusamy *et. al.*, 2007) and in *Daucus carota* (Pant *et. al.*, 2007).

Materials and Methods

The plant specimens were collected from the natural habitat of Bangalore University, Jnanabharathi campus. Its morphological and floral characters were studied using several Flora of South India. The specimens collected for planting purpose were transferred in polythene bags and planted in selected field. Few flowering specimens of the species were collected separately for the purpose of identification. Selected specimens were processed to make herbarium sheets and housed in the department herbarium. The specimen was also confirmed by matching with the voucher specimens deposited in Madras Herbarium, Botanical Survey of India, Coimbatore, Tamil Nadu.

For clonal propagation, young plants of about 2 months old were selected, washed thoroughly in flowing tap water for 30 min followed by treatment with a solution of 3 % Tween 20 (v/v) for 15 min and thereafter washed three to five times with sterile distilled water. The explants were then treated with 0.1 % (w/v) aqueous mercuric chloride solution for 3 - 5 min and finally rinsed thoroughly with sterile distilled water under laminar airflow. The nodal segments were trimmed at both ends prior to inoculation on the culture media.

Single nodal segments were cultured on MS basal medium supplemented with 3 % (w/v) sucrose for culture initiation which also served as explant sources for subsequent experiments. The pH of the medium was adjusted to 5.7 with 1N NaOH or 1N HCl before gelling with 0.8 % (w/v) agar. The medium was dispensed into culture vessels and autoclaved at 15 Pounds pressure and 121^{O}C for 15 min. The surface sterilized explants were implanted vertically on the culture bottle containing 30ml medium and its mouth tightly covered with airtight lid. All the cultures were incubated at 25 ± 2 ^{O}C under 16 h photoperiod irradiance provided by cool white fluorescent tubes. The relative humidity of 60 - 65 % was maintained. All subsequent subcultures were maintained for every four weeks of interval.

To obtain multiple shoots as well as roots, the nodal explants were cultured on solidified MS media supplemented with various combinations of phyto-hormones. The hormones such as Indole Acetic Acid (IAA), Indole Butyric Acid (IBA) and Benzyl Amino Purine (BAP) was used in full or half strength MS media containing 3 % (w/v) sucrose and 0.8 % (w/v) agar. Elongated multiple shoots were excised from each culture, sub-cultured for the purpose of propagation. Few

propagation. Few explants were transferred to culture bottles with rooting media. The culture bottles were maintained in culture room for about three to four weeks.

Plantlets with well-developed roots were removed from the culture medium and gently washed under flowing tap water. These plantlets were transferred to plastic pots (10 cm diameter) containing autoclaved chopped peat moss and farmyard manure (1:1). Each was irrigated with half-strength MS basal salt solution devoid of sucrose and inositol every fourth day for two weeks. The potted plants were covered with porous polythene sheets for maintaining high humidity and were maintained inside the culture room conditions. The relative humidity was reduced gradually, and after 30 days the plantlets were transplanted to pots (25 cm diameter) containing forest humus and garden soil (1:1). The pots were transferred to greenhouse for further growth and development. Well acclimatized *in vitro* raised plants were transferred finally to its original habitat. The growth and morphological characteristics was examined.

Result and Discussion

Reporting the existence of *Polyzygus tuberosus*, a rare, endemic, threatened and red list plant in Bangalore University, Jnanabharathi campus. Botanical Survey of India has enlisted this species in threatened plant list of Karnataka & Maharashtra along with Sahyadri database maintained by Indian Institute of Science.

The plant is found in countable number of around 40 – 50 plants distributed fragmentally around 10 meters in radius. The location of the plant remains highly vulnerable. The plants grow in dry region amidst gravellry soil & grasses. The vegetative growth resumes from November - December, It starts flowering from mid February onwards and fruiting occurs from end of February to end of March. It was also noticed that by end of February, these plants are exposed to burning of dry mulch layer and in short time the plant resumes its vegetative growth followed by immediate flowering and fruiting. This reveals the resistance ability of the underground tuber towards fire; while the burned seeds are unable to germinate perhaps this remain as one of the reason why the species is unable to propagate naturally. Nodal explants of *Polyzygus tuberosus* were cultured on MS media supplemented with various concentrations of BAP, IAA & IBA individually for shoot regeneration. Among the different concentrations of phytohormones used, MS media supplemented with 0.3 mg/l BAP + 0.2 mg/l IBA exhibits maximum percent of multiple shoots (Fig - 1) followed by even in 0.5 mg/l BAP also (Fig - 2). The growth observations on various hormone concentration enriched media are shown in the Table - 1.

From the above observations, IBA was found to be more effective than IAA. The shoot length was highest at 10 cms in 0.3 mg/l BAP + 0.2 mg/l IBA. About 80% of nodal explants produced shoots after 40 days from the day of inoculation with an average of 12 – 16 shoots per explants. The explants maintained on MS media supplemented with only IAA and IBA did not provide shoot regeneration. Among the combination of two phytohormones, increased in the concentration of auxins affected the shoot proliferation significantly.

The explants inoculated on MS media supplemented with 0.2 mg/l NAA produces callus at the basal region of explants (Fig 3). The proliferated shoots were excised from culture tubes and inoculated on half strength MS media supplemented with different concentrations and combinations IAA and IBA. The percent of root frequency, number of roots per shoots

Table 1: Effect of different growth regulators on axillary shoot proliferation in Polyzygus tuberosus

Growth regulator			Number of shoot per explants	Length of shoot (cm^3)
Cytokinin (BAP) mg/l	Auxin (IAA) mg/l	Auxin (IBA) mg/l		
0.1	0	0.1	0	0
0.3	0	0.2	12 - 16	6.5 – 10.0
0.5	0	0.3	4-5	0.5 – 1.0
1.0	0	0.4	0	0
2.0	0	0.5	0	0
0.1	0.1	0	0	0
0.3	0.2	0	4 – 8	1.5 – 2.5
0.5	0.3	0	0	0
1.0	0.4	0	0	0
2.0	0.5	0	0	0
0.1	0	0	0	0
0.3	0	0	0	0
0.5	0	0	6 - 8	4.5 – 6
1.0	0	0	0	0
2.0	0	0	0	0

Table 2: Effect of growth regulators on root formation in *Polyzygus tuberosus* cultured upto 5 weeks

Growth regulator		Rooting Frequency %	Root length (cm)	Days to Root formation
Auxin (IAA) mg/l	Auxin (IBA) mg/l			
0.1	0.3	0	0	0
0.3	0.5	66.66	0.8 ± 0.1	20 – 25
0.5	1	40	0.6 ± 0.2	25 – 20
0.1	1	0	0	0
0.3	**0.5**	82.28	1.2 ± 0.2	20 – 25
0.5	0.3	30.33	0.6 ± 0.2	25 – 30

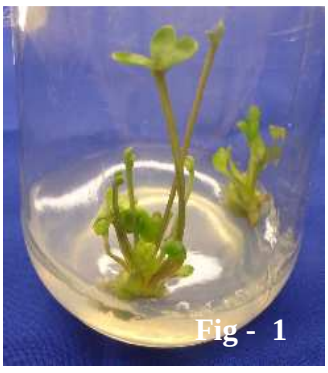
Fig - 1

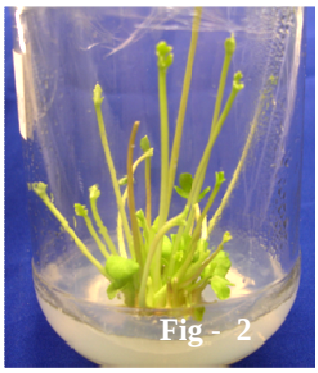
Fig - 2


Fig - 3


Fig - 4

Fig 1 & Fig 2 – Multiple shoots of Polyzygus tuberosus from nodal explants on MS media with 0.3 mg/l BAP + 0.2 mg/l IBA and 0.5 mg/l BAP. Fig - 3 – Callus; Fig - 4 – Hardened in vitro raised plant.

and the length of the roots were recorded after 4 – 5 weeks of culture maintenance and the results are as shown in table 2. The rooted plants were transferred to small polythene cups containing autoclaved peat moss and farmyard manure in the ratio 1:1. Acclimatization was allowed at 25± 2^0C for two weeks. Elongation of leaves was prominently observed. Such plantlets were transferred to poly bag containing forest humus and garden soil in the ratio 1:1 and maintained for three weeks and finally transferred to natural habitat. Clonal propagated plants grow normally without exhibiting any morphological variations from the plants grown in natural habitat.

Conclusion

Reporting the existence of an Apiaceae species *Polyzygus tuberosus* Dalzell ex Walp from Bangalore University, Jnanabarathi campus. The specimen is found growing amidst the dry gravellary soil and monitoring their existence reveals their gradual decline in the population. The unique feature of this plant is growing in dry habitat, bearing thick leathery non-aromatic leaves and flowers during the onset of summer. These features reflect the drought and stress tolerance of this species that deviates from most native species of family Apiaceae. Our observations with respect to the available literature, It is in agreement with the observations made by Botanical Survey of India.

Clonal propagation of this species is achieved by culturing nodal explants on MS media supplemented with 0.3 mg/l BAP + 0.2 mg/l IBA. This concentration initiates multiple shoots followed by even in 0.5 mg/l BAP. The explants inoculated on MS media supplemented with 0.2 mg/l NAA produces callus. Maximum percent of rooting was observed in half strength MS media supplemented with 0.3 mg/l IAA + 0.5 mg/l IBA. Clonal propagated plants grow normally without exhibiting any morphological variations from the plants grown in natural habitat.

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