INTERNATIONAL JOURNAL OF SCIENCE AND NATURE

www.scienceandnature.org

MICROPROPAGATION AND POLLEN STUDIES OF *PEUCEDANUM DHANA* VAR *DALZELLII*, A THREATENED UMBELLIFER

Babu, T. P & Sreenath, K. P.
Department of Botany, Bangalore University, Bangalore, Karnataka 560 056, India

ABSTRACT
Peucedanum dhana, var *Dalzellii* (Apiaceae) is an endangered & endemic species of South India that has not received any scientific attention. The plant is spotted to inhabit in Karnataka University campus Dharwad. In the present studies, the endemic & endangered status of this rare plant is revealed; micropropagation is implemented along with pollen studies. Micro propagation is succeeded from nodal explants inoculated on MS media supplemented with 0.3 mg/l BAP + 0.1 mg/l IAA for multiple shoot initiation and root initiation with half strength MS media supplemented with 0.3 mg/l IBA. Hardening was succeeded & the propagated plant was reintroduced in its natural habitat. The pollen morphology was studied by light microscope. The result of the pollen samples confirms the perprolate, trizonocolporate, operculate, isopolar, bilaterally symmetrical and subrectangular.

KEYWORDS: *Peucedanum dhana*, Micropropagation, Pollen

INTRODUCTION: The family Apiaceae **(**Umbelliferae) is annual herbaceous plants usually aromatic. The members are maximum restricted to frigid zone to tropical, mainly north temperate. In India, most native species are distributed in high altitude regions. There are 68 genera and 240 species of which 8 genera and about 60 species are endemic (Mukherjee, 1993). Peucedanum dhana var. dalzellii C. B. Clarke is endemic to Central India, Konkan region, Belgaum and north Karnataka districts and few districts of Andhra Pradesh. The plant is a perennial glabrous weak herb with pale yellow flowers in perfect umbels. Traditionally the root of this plant is known for nutritional value. The occurrence of this plant is very rare in Karnataka and lacks any scientific information.

The plant is a slender, erect, few branched, glabrous, 15-60 cm tall, from a stout taproot. Leaves clustered at base, 3-parted to pinnate, Coriaceous, oblong to oval, pinnately or ternately parted to simply pinnate, the 3-5 leaflets are linearly oblong or lanceolate-elliptic to orbicular, acute at apex, cuneate at base, entire; petiole slender narrowly sheathing at base. The Umbellets 15-20-flowered, the pedicels are slender, unequal. The flowers are yellow, regular: petals ovate with a narrower inflexed apex: calyx teeth mostly obsolete; stylopodium low conical; ovaries glabrous. Fruit subquadrate to elliptic, often emarginate at both apex and base 7-8 mm long & 5 mm broad, glabrous. Vittae 2-6 and bipartite carpophore. The flowering and fruiting occurs Mar – May & July – Aug respectively. Representative specimens: Orissa: Delaisara, Mooney 3784 (DD); Jashpur, Mooney 1333 (DD). Andhra Pradesh: Araku Valley, Suneraj 21,325 (MH). Madhya Pradesh: Jubbalpur, Beddome (CAL). Maharashtra: Bombay, Dalzell (CAL, DD); Belgaum, Talbot 3811 (CAL).

Scarcity of this plant specimen in natural habit and its rare availability has promoted us to initiate invitro propagation. Previously Micropropagation is succeeded in certain species of endemic & endangered Apiaceae species. In *Vanasushava pedata, m*icropropagation is achieved on MS supplemented with synergistic combination of BA (5.0 mg/l), IAA (0.1 mg/l) and 3 % sucrose that promoted maximum number of shoots as well as enhanced shoot lengths. Rooting was highest (100%) on half strength MS containing IAA at 2.0 mg/l (S. Karuppusamy, 1997). Micro propagation in *Daucus carota* is succeeded from the nodal explants cultured in MS medium supplemented with 0.5 mg/l of 6-benzylaminopurine (BAP). The nodal explant also induced same number of multiple shoot in MS medium supplemented with 2 mg/l of BAP and 1 mg/l of α-nephthalene acetic acid (NAA). Root and leaf explants induced roots and callus when cultured on MS medium with NAA at the rate of 1 mg/l and 2 mg/l. Similarly, stem explants also induced roots and callus in the same concentration of hormones whereas few multiple shoot were induced when cultured on MS supplemented with 2 mg/l of BAP and 1 mg/l of NAA (Pant & Manohar, 2007). In vitro propagation in Hydrocotyl conferata is achieved through axillary bud multiplication is succeded on MS media supplemented with 6.66 µM benzyl adenine (BA) & 5.37 µM naphthalenacetic acid (NAA). The roots were best observed in half strength MS media supplemented with 0.54 µM NAA. (S. Karuppusamy, 2007).

Umbelliferae pollen are easily recognised. They are isopolar, radiosymmetrical, generally tri-colporate, sub- to per-prolate, and the tectum which is usually smooth overlies bacula which in most cases are simple (Erdtman, 1952; Cerceau, 1959, 1962). Despite such homogeneity the characters of the grains vary from genus to genus, and certain features, including the shape imparted by the endexine and the structure of the apertures, are diagnostic at the generic level. There are abundant palynology studies in the family Apiaceae (Erdtman, 1952; Cerceau-Larrival, 1962, 1963, 1965; Aytuğ *et al*., 1971; Cerceau-Larrival, 1971; Ferreira and Purper, 1972; Cerceau-Larrival and Roland Heydacker, 1976; Herrnstadt and Heyn, 1977; Moore and Webb, 1978; Punt, 1984).

MATERIALS & METHODS

The plant specimen was expelled from the soil along with rhizome (whole plant). Its morphological and floral characters were studied using several standard Flora. The specimens collected for planting purpose were transferred in polythene bags and brought as quickly as possible to the selected field and planted there. Few flowering specimens of the species were collected separately for the purpose of identification, specimens were processed to make herbarium sheets and housed in the department herbarium. The plant identification was done by reference to standard flora. The literature survey on this plant by reference of various journals was made, but very less scientific information is available.

Micro propagation

Young plants of about 4-5 weeks old were collected from the natural habitat of Dharwad University campus. The collected plants were washed thoroughly in running 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 5 - 7 min and finally rinsed thoroughly with sterile distilled water under Laminar airflow cabinet. 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 (supplemented with respective growth regulators) 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 about 30 ml of phytohormones enriched media. The mouth of culture bottles are 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 obtained for every four weeks intervals.

To obtain multiple shoots as well as roots, the nodal explants were cultured on solidified MS media supplemented with various combinations of phytohormones. The hormones such as Indole Acetic Acid (IAA), Indole Butyric Acid (IBA) & 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, subcultured for the purpose of 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 after washing the roots gently under running tap water, plantlets were transferred to plastic pots (10 cm diameter) containing autoclaved chopped peatmoss 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 morphological characteristics were examined.

Pollen studies

The flowers bearing mature pollens were taken from plant specimens in the field. The pollen grains taken from these flowers were prepared according to the Erdtman (1960) method and then left to dry in centrifuge tubes. Different pollen parts such as polar diameter (P), equatorial diameter (E), colpus length (Clg) or pore width and colpus width (Clt) were measured by means of light microscopy. About 10 pollens were used for obtaining their measurement values.

RESULTS AND DISCUSSION

Pollen studies: According to light microscope investigations, the pollen grains of the *Peucedanum dhana* are perprolate, trizonocolporate, operculate, isopolar and bilaterally symmetrical, subrectangular in equatorial view, triangular in polar view, with a rugulate-striate exine sculpture. There is a thickening around the aperture of exine (costae) with a decreasing diameter towards the poles. Apertures are on the same plane in the equatorial region.

Pollen grains of the Umbelliferae show uniformity to some extent. Detailed palynogical investigations have been carried out by Cerceau-Larrival (1962, 1963, 1965, 1971). As a result of these investigations, five basic types, namely subrhomboidal (Rh), subcircular (C), ovoid (O), subrectangular (Rg) and equatorially constricted (E), were defined according to inner outline of the endexine (nexine). On the basis of Cerceau-Larrival's classification, all studied pollen grains of the present study were consistent with subrectangular pollen type.

Polar diameter (P) µm	Equatorial diameter (E) µm	Ratio of polar axis & polar diameter (P/E)	Colpus length (Clg) µm	Colpus width (Clt) µm
41.6 ± 2.5	18 ± 1.2	(P/E)2.3	26.2 ± 2.3	(Clt)0.6 ± 2

Micro propagation

Nodal explants of Peucedanum dhana 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 that is depicted in table 1.

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)
Cytokinin (BAP) mg/l	**Auxin (IAA)** mg/l		
0.1	0	0	0
0.3	0	0	0
0.5	0	6 - 8	2.5 – 5.5
1.0	0	0	0
2.0	0	0	0
0.1	0.1	0	0
0.3	0.2	8 – 10	3.5 – 7.5
0.5	0.3	8 – 10	3.5 – 7.5
1.0	0.4	6 – 8	2.5 – 3.5
2.0	0.5	0	0
	Auxin (IBA)		
0.1	0.1	0	0
0.3	**0.2**	**12 – 16**	**6.5 – 11.5**
0.5	0.3	8 – 10	3.5 – 7.5
1.0	0.4	8 – 10	2.5 – 5.5
2.0	0.5	0	0

From the above observations, IBA was found to be more effective than IAA. The shoot length was highest at 11.5 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.

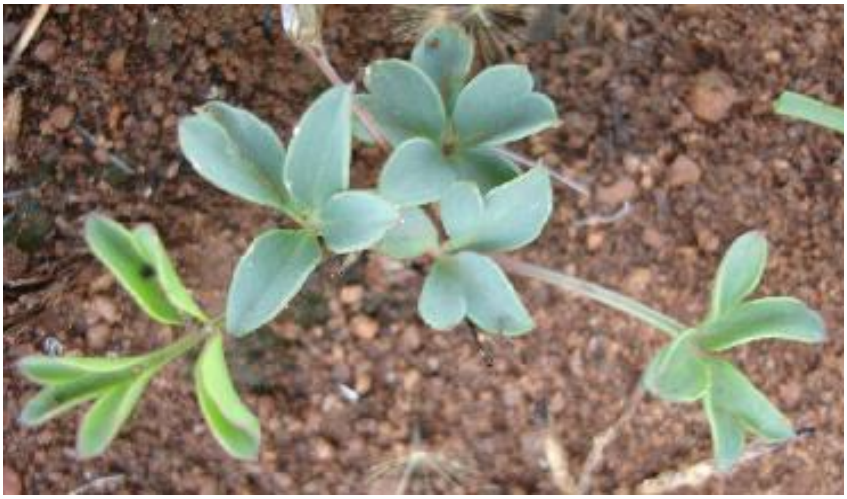
The explants inoculated on MS media 0.2 mg/l NAA + 0.1 mg/l Kinetin exhibits less shoot proliferation but produces callus at the basal region of explants. The proliferated shoots were excised from culture tubes and inoculated on half strength MS media supplemented with different concentrations and combinations IAA and IBA. The percent of root frequency, number of roots per shoots, the length of the roots were recorded after 4 – 5 weeks of culture maintenance. 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$\pm$ 2^0C for two weeks. Elongation of leaves was prominently observed. Such plantlets were transferred to polybag containing forest humus and garden soil in the ratio 1:1 and maintained for three weeks and finally transferred to natural habitat.

In vitro regenerated Peucedanum dhana plants grow normally without exhibiting any morphological variations from the plants grown in natural habitat. This protocol may remain useful for the conservation of other rare & threatened Umbellifer plants.

1

2

3



4

FIGURE-1, 2, 3 & 4 Pollen, callus, multiple shoots & Hardened plant

REFERENCES

Cooke, T. (1967) Flora of Presidency of Bombay. Botanical Survey of India. Calcutta. Second reprint edition.

Karuppusamy, S., C. Kiranmai, V. Aruna and T. Pullaiah. (2006) Micropropagation of *Vanasushava pedata* – An Endangered Medicinal Plant of South India. Plant Tissue Cult. & Biotech. 16(2): 85-94.

Karuppusamy, S., C. Kiranmai, V. Aruna and T. Pullaiah. (2007) In vitro propagation of an endemic Umbellifer, Hydrocotyle conferta. *Indian Journal of Biotechnology* Vol. 6: 541- 544.

Mukherjee, P. K. & Constance (1993) Umbelliferae (Apiaceae) of lndia, American Institute of Indian Studies & Oxford & IBH publishing Co. New Delhi.

Nath S and Buragohain A K (2003) *In vitro* method for propagation of *Centella asiatica* (L.) Urban by shoot tip culture. J. Plant Biochem & Biotech.12: 167-169.

Pant, B. and S. Manandhar. (2007) In vitro propagation of carrot (Daucus carota) L. Scientific World, Vol. 5 (5).

Pullaiah.T, (1997) Flora of Andhra Pradesh. Scientific Publishers. Jodhpur.

Sevil Pehlivan, Birol Başer and Evren Cabi (2009) Pollen morphology of 10 taxa belonging to *Prangos* lindl. and *ekimia* h. duman & M. F. Watson (Umbelliferae) from Turkey and its taxonomic significance. Bangladesh J. Plant Taxon. 16(2): 165- 174.

Yousefzadi, M.,Azizian, D., Sonboli, A. and Mehrabian, A. R. (2006) Palynological studies of the genus Tetrataenium (Apiaceae) from Iran. Iran. Journ. Bot. 12(1).