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IN VITRO PROPAGATION OF FEW *PIMPINELLA SPECIES*: A REVIEW

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ABSTRACT: The genus *Pimpinella L.* is one of the largest genera in Apiaceae subfamily Apioideae, with approximately 150 species distributed in Asia, Europe, and Africa. A few species can also be found in South America and one can be found in the western part of North America. The present study includes four species of *Pimpinella* (*Pimpinella anisum, P. tirupatiensis, P. candolleana and P. brachycarpa*). Maximum of *Pimpinella* species can be annual, biennial or perennial. They are generally characterized by the presence of fibrous collars on the top of the rootstock and compound umbels. These plants usually grow on dry rocky places, rocky crevices, fields, meadows, mountain pastures, and grasslands. Majority species have more medicinal and Pharmacological properties, generally propagated through seeds. The seed germination in nature is very poor. So, it is difficult for propagating them rapidly to meet the market demand. Hence they are endangered and slowly becoming extinct. Therefore, plant tissue culture techniques may facilitate their propagation over a shorter period of time than conventional techniques. This may also be of value to the preservation and conservation of the genetic resources of this important family.

KEYWORDS: Apiaceae**,** Conservation, Micropropogation, *Pimpinella* species.

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1.INTRODUCTION

The genus *Pimpinella L.,* consists of 150 species, distributed throughout the world [1] is one of the largest genera of the family Apiaceae. About two dozen *Pimpinella* species occur in India. Out of these, eight species are largely confined to peninsular India, eleven occur in the Eastern Himalaya

and two are endemic to the western Himalaya [2]. These are annual, biannual, and perennial species usually growing on dry rocky crevices, fields, meadows, mountain pastures and grasslands[3]. *Pimpinella* belongs to the family Apiaceae and represents many important medicinally valued plants. These are used for pharmaceutical, cosmetics, perfumery and food industry. Some of the species of *Pimpinella* contains essential oil used as antispasmodic, antioxidant, antimicrobial, insecticidal and antifungal effects[4] .

Classification of *Pimpinella L.*

Kingdom : Plantae
Subkingdom : Tracheobionta
Super division : Spermatophyta
Division : Magnoliophyta
Class : Magnoliopsida
Sub class : Rosidae
Order : Apiales
Family : Apiaceae
Genus : *Pimpinella*

We checked scientific studies in various electronic databases (Medline, Pubmed, Science Direct, Scopus, Google Scholar websites, Books and Various Thesis) from 1993 to 2017. After a comprehensive search on the micropropagation aspects of Apiaceae family in India, we reviewed available publications that provided information about different applications of these plant species in human and livestock. In this article, scientific and author names of plant species were confirmed for latest changes according to "The plant list" (http://www.theplantlist.org)[25].

Morphology, Distribution and Micropropagation studies of *Pimpinella* species

***Pimpinella anisum* L.**

Pimpinella anisum L is an aromatic, annual grassy herb with 30-50 cm height, white flowers and small green to yellow seeds with secondary feather like leaflets of bright green, twice pinnate[5],[6].

Distribution

P. anisum present in Eastern Mediterranean region, West Asia, the middle East, Mexico, Egypt and Spain.

Micropropagation

"Somatic embryogenesis" from callus cultures has been reported [7]. A detailed description of *P. anisum* embryogenesis through seed culture has been reported [8]. The cell culture of *P.anise* was grown in the presence or absence of (2,4-D) [9]. Application of isopentenyladenine or isopentenyladenosine (4.10^{-8} to 4.10^{-7} M) to the proembryonic culture yielded an increase of the cell density, in contrast to a proembryonic culture grown without exogenous application of cytokinins. Embryogenesis was induced by transferring the cells to a hormone-free medium. Embryo

development was promoted by isopentenyladenine and isopentenyladenosine (5.10^{-8} to 5.10^{-7} M), higher concentrations (5.10^{-6} M) inhibited embryogenesis. The effect of cytokinins on embryogenesis was only promotive until the third day of culture, i.e. coincident with cell growth rather than differentiation. *In vitro* development of *P.anise* clonal lines, root explants cultured on Murashige and Skoog's media supplemented with 4 different growth regulators was reported [10]. The synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) induced somatic embryos from callus. Napthalenacetic acid (NAA), also a synthetic auxin, induced prolific root formation. The synthetic cytokinins 6-Benzylaminopurine (BAP) and Thidiazuron (TDZ) induced adventitious shoot formation. The 2,4-D induced somatic, embryogenesis system followed by plant regeneration was the best system for the establishment of clonal lines of *P.anise*. Simple *in vitro* protocol for large scale multiplication of plants from various explants of *P. anisum* has been developed [11]. High frequency of multiple shoots formation was achieved from callus cultures derived from shoot apices, root, and stem explants, and also from seed derived calli. Somatic embryogenesis was observed in callus cultures derived from seed and shoot apices. Complete plants developed from these embryoids. Direct regeneration of plantlets from shoot apices was also observed. Root formation occurred in all the cultures. The requirement for exogenous auxins and cytokinin for differentiation was found to be varying in different tissues. It was observed that hairy root cultures in *P.anisum* L. using cultures were grown in four different media, both under darkness and under photo period conditions of 16h light [12]. A high frequency organogenesis in anise (*P.anisum* L.) using hypocotyl explants from *in vitro* germinated seedlings was reported [13]. Both cytokinins, benzylaminopurine and kinetin induced shoot regeneration but the effect of Benzylaminopurine (BAP) was more pronounced. High frequency shoot regeneration (45 shoots explant-1) was obtained on BAP 1 mg l^1. Both cytokinins were also tested in combination with auxins (Naphthalene acetic acid /Indole acetic acid). Interaction of benzyl amino purine /kinetin with naphthalene acetic acid/ indoleacetic acid increased the length of regenerated shoots. Different kinds of callus morphology were observed but it had no relationship with regeneration potential. The regenerated plants were normal and healthy. *In vitro* micropropagtion of *P.anisum* L. by using various explants shoot tip, node, internode and seeds was done [14]. It was reported that mature seeds of *P.anisum* cultured on Murashige and Skoog's Basal medium (MSBM) fortified with GA3 (2.89 µM/L) was suitable for seed germination (77.3±1.0%). In shoot tip culture, shoot tip measuring 1-2 cm was excised from 20 days *in vitro* axenic plants of *P.anisum* and observed that cultured on MSBM with BAP (13.3µM) and NAA (5.37 µM) was the best medium for shoot tip initiation and formation of multiple shoots, MSBM with BAP (13.3µM) and NAA (5.37 µM) was the best medium for initiation and formation of multiple shoots from nodal explants, MSBM with 2,4,-D (2.26 µM) was the best medium for induction and growth of whitish green callus from the internodes as explants of *P.anisum*, when compared to the callus derived from MSBM with IAA and MSBM with NAA in different

concentrations. (Table: 1)

***Pimpinella tirupatiensi* Bal. & Sub.**

Pimpinella tirupatiensis is locally known as 'adavikothimeera' (Forest Coriander). It is a narrow endemic species of seasonal occurrence with underground tuberous root system [15]. It is an erect herb with perennial tuberous root stock, stem is simple, branched, terete, striate; branches alternate, bifurcate; branch lets glabrous, veins prominent, margins cartilaginously crenate - serrate. Basal leaves are simple, ovate obtuse or acute, deeply cordate, 1.7-3.8 x 1.3-3.8 cm, petiole 2.5-7.8 cm long, sheathing at base, cauline leaves palmately 3-partite . Flowers are white, 5-16, in compound umbles bracteoles 1-2, very small, linear. Petals are 1 mm long, glabrous, obovate, sub-orbicular, apex inflexed; styles small, slender, 1mm long; stylopod conical, yellowish brown, persistent. Fruits are 1.5 mm long, ovoid, Papillos - scrabrous [16]. The tuber consists of a tap root growing inside the soil up to about 50 cm. The tubers occur in carrot like pieces of varying length, measured to 30 cm long and 8 cm diameter.

Distribution

Pimpinella tirupatiensis is distributed on Tirumala hills and plants were confined to the Eastern Ghats of peninsular India.

Micropropagtion studies

"Somatic embryogenesis" has been reported in *P. tirupatiensis* [17]. Hypocotyl segments were excised from 4-week-old aseptic seedlings of *Pimpinella tirupatiensis*, a medicinal plant and were cultured on MS medium with TDZ (1 mg/l) and NAA (0.5 mg/l), which gave rise to friable, pink callus after 4 weeks of culture. Embryogenic callus on transfer to MS medium containing TDZ (1 mg/l) produced somatic embryos after 8 weeks having dark green shoots and white hairy roots. On MS medium with TDZ (1 mg/l) + BA (1 mg/l), somatic embryo formation was enhanced. Embryos isolated and germinated in the presence of MS medium with TDZ (1.0 mg/l) and GA3 (1.0 mg/l) showed normal flowering without any morphological variation on transplantation to soil. I*n vitro* micro propagation of *P. tirupatiensis* by using various explants shoot tip, axillary bud and seeds was reported [18]. It was reported that MS medium containing GA3 (1.0mg/l) has shown highest percent of seed germination (67.0%).When MS medium was supplemented with BAP alone (1.0mg/l) a maximum of 18.04 ± 3.24 shoot were obtained from axillary bud explants. A frequency of 48% and a mean shoot length of 4.12 cm were recorded .25.3 shoot were obtained in presence of (1.0mg/l) BA and (0.1mg/l) NAA with in a period of 6 weeks of culture. A frequency of 92% and a mean shoot length of 5.2 cm were recorded. "*In vitro flowering protocol* in *P. tirupatiensis"*, using multiple shoots was obtained from *in vitro* plants through direct regeneration from shoot tip explants[19]. Murashige and Skoog's medium supplemented with 6-benzyl amino purine (BAP) 2.22 and 4.44 µM, α-napthalene acetic acid (NAA) 0.54 µM and gibberellin (GA3) 1.44 and 2.89 µM was the best combination for initiation of *in vitro* flowering. As many as 6 umbels per explant

were noticed on MSBM supplemented with BAP (4.44 µM), NAA (0.54 µM) and GA3 (2.89 µM) and the least number of (3) umbels were observed on MSBM with BAP (2.22 µM), NAA (0.54 µM) and GA3 (2.89 µM). Flower maturation (35 days) and formation of the seeds (66 days) were observed early with BAP (4.44 µM), NAA (0.54 µM) and GA3 (2.89 µM).

***Pimpinella candolleana* Wight & Arn.**

Pimpinella candolleana (Umbelliferae) is one of the important medicinal plant, endemic to Western Ghats in India [2]. It is a slender, erect, branched and sparsely leafy above, densely shortly pubescent, usually 30-60 cm tall, perennial from a fusiform tap root. Basal leaves subrisulate, ovate – cordate, 2-6 cm long, simple, somewhat acute to obtuse at apex, deeply cordate, palmately 5-9 veined, pubescent on both surface but especially beneath; petiole slender, up to 10cm long, pubescent sheating at base; cauline leaves reduced upward. Peduncle few terminal or lateral, elongated. Involucres of 3-8 linear, pubescent bract up to 10 mm long. Long umbellets 10-15 flowered, the central often sterile, the mature pedicles pubescent. Flowers white; petals obovate, hirsute dorsally; calyx obsolete; stylopodium conical, the styles slender, reflexed. Fruit ovid, 2.5 mm long, a little narrow at apex, compressed laterally.

Distribution

P. candolleana is distributed in Western Ghats & Eastern Ghats, Moist Deciduous to Evergreen Forests. Endemic to Western Ghats.

Micropropagtion studies

A workup on the micropropagation protocol of *Pimpinella candolleana* was undertaken [20]. It was reported that high frequency plantlet regeneration was achieved from shoot tip explants of *P. candolleana*. Further studies conducted on effect of growth regulators in different concentrations and combination of BAP, KN, NAA, IAA and IBA on *in vitro* morphogenesis, observed that the highest regeneration response in Murashige and Skoog's Basal Medium (MSBM) fortified with BAP (13.31µM) and NAA (2.69 µM) where 94 % of the cultures responded with an average set number of 7.20 ±. 0.81 per explant in six weeks time. The best elongation response was noticed on MSBM supplemented with BAP 13.31µM + GA3 1.44 µM where 93% of the shoots attained the average height of 6.80 ± 0.50. Rooting was achieved on half strength MSBM with 9.8 µM IBA. The plantlets with well developed shoot and root system were hardened and acclimatized to the natural habitat with 90% survival frequency. In *vitro flowering* in *P. candolleana* , using multiple shoots obtained from *in vitro* plants through direct regeneration from shoot tip explants was reported[21]. Murashige and Skoog's medium supplemented with 6-benzyl amino purine (BAP) in different concentrations ranging from 2.22 µM, 4.44 µM,6.67 µM and 8.87 µM and Kn in different concentrations ranging from 2.32 µM,4.6 µM,6.97 µM and 9.2 µM separately to induce *in vitro* flowering. After 35 days of culture, multiple shoots obtained from shoot tip culture have shown flower bud initiation on all concentrations of cytokinins studies, which showed 2-8 umbels per

explant. More number of umbels (8) per explant were noticed on MSBM supplemented with BAP (2.22 µM) and the less number of umbels (2) were observed on MSBM fortified with Kn (9.29 µM). Flower maturation (20 days) and formation of the seed (45 days) were early with BAP (2.22 µM).The number of days for initiation, opening of flowers and seeds formation were increased when the concentration of BAP was increased (8.87 µM).

***Pimpinella brachycarpa* (Kom.) Nakai**

Pimpinella brachycarpa is commonly known as chamnamul and short-fruit *Pimpinella*, It is a scented plant with saw-toothed, oval leaves, which bears white flowers between June and August, and edible green leaves[22].The leafy vegetable frequently consumed in Korea. The plant species are commonly used to prepare Namul, a seasoned raw vegetable dish.

Distribution

Pimpinella brachycarpa is a widely distributed as vegetable plant in Europe, Africa, and Asia.

Micropropagtion studies of *Pimpinella brachycarpa*

A rapid protocol was establish for micropropagtion of *Pimpinella brachycarpa* via repetitive effective induction of "somatic embryogenesis" achieved on both MS and modified B5 media containing BAP+ 2,4-D (or) BAP+ 2,4-D + NAA under light condition [23]. Plantlets could be regenerated on MB5 basal medium containing 0.1mg/L NAA. Regenerated plantlets were maintained on MB5 (or) MS basal media for 4 to 6 more weeks and transferred to soil of an artificial mixture for acclimatization. Most plantlets survived (more than 97%) and grew without any deformity. It was stated that "somatic embryos" of *P. brachycarpa* cultured photoautotrophically at different CO concentrations, and conversion of the somatic embryos was compared to those cultured photomixotrophically [24]. The germination rate of somatic embryos in cotyledonary torpedo stage increased as CO concentration rate increased, showing that high concentration of CO increases the photoautotrophic ability. When transplanting plants germinated at photomixotrophic system to *ex vitro*, the survival rate at three days after *ex vitro* was 0%. All explants withered in 24 hours. However, the survival rate of plants germinated at photoautotrophic system after transplanting to *ex vitro* was 100%, and it was possible to transplant without acclimatization process. Photoautotrophic system was applicable to *P. brachycarpa* for development of micropropagation system using somatic embryos being provided with the CO concentration properly.

Table 1: List of *Pimpinella* species with source of explants

***Pimpinella* species**	**Source of Explant**	**References**
Pimpinella anisum L	Somatic embryogenesis from callus cultures.	[7]
Pimpinella anisum L	Somatic embryogenesis through seed culture.	[8]
Pimpinella anisum L	Cell culture	[9]
Pimpinella anisum L	Root explants	[10]
Pimpinella anisum L	Callus cultures derived from shoot apices, root, and stem explants, and also from seed derived calli.	[11]
Pimpinella anisum L	Hairy root	[12]
Pimpinella anisum L	Hypocotyl explants from in vitro germinated seedlings	[13]
Pimpinella anisum L.	Shoot tip, Node, Internode and seeds.	[14]
Pimpinella tirupatiensis Bal and Subr.	Somatic embryogenesis through hypocotyl segments.	[17]
Pimpinella tirupatiensis Bal and Subr.	Axillary bud explants	[18]
Pimpinella tirupatiensis Bal and Subr.	Shoot tip explants	[19]
Pimpinella brachycarpa Wight & Arn	Somatic embryogenesis	[23]
Pimpinella brachycarpa(Kom.) Nakai	Somatic embryos	[24]
Pimpinella candolleana Wight & Arn	Shoot tip explants	[21]

2. CONCLUSION

Due to scarce availably of plant material and difficulty in propagation through conventional propagation methods of these species viz., *Pimpinella anisum, P.tirupatiensis, P. candolleana and P. Brachycarpa,* micropropagation through somatic embryogenesis, shoot tip, and hairy root cultures is recommended, since these plants have great medicinal and pharmacological properties used all over the world. Propagation of these medicinally and aromatic important species through various tissue culture techniques would be highly relevant for the large scale multiplication for further commercial exploitation.

CONFLICT OF INTEREST

Authors don't have any conflict of interest.

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