Recent studies on well-known spice, *Piper longum* Linn.

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**Abstract**

The fruits of *Piper longum* Linn. are very well-known medicine for diseases of the respiratory tract, viz. cough, bronchitis, asthma, etc.; as counter-irritant, analgesic when applied locally for muscular pains and inflammation and as general tonic and hematinic. They are carminative and known to enhance the bioavailability of food and drugs. In this paper recently developed micropropagation method by tissue culture and molecular basis of genotypic differentiation between the male and female plants, using Randomly Amplified Polymorphic DNA (RAPD) technique and development of sex associated DNA markers have been discussed along with some medicinal and pharmacological properties of the spice.

**Keywords:** *Piper longum*, Long pepper, *Pippali*, Tissue culture, RAPD, DNA markers, Medicinal uses.

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**Introduction**

*Piper longum* Linn. (*Piperaceae*) is the accepted source of the drugs *Pippali* and *Pippalimulam* throughout the country. *Pippali* is the dried ripe fruits; *Pippalimulam* is the roots of this plant (Sivarajan & Balachandran, 1994).

The plant is a dioecious slender aromatic climber with perennial woody roots, or a perennial creeping under shrub. Branchlets erect, glabrous with swollen nodes; roots clasping at nodes, which help to get attached to the host trees; leaves alternate, ovate, cordate; apex acute to acuminate, margin entire, glabrous. The male and female plants are morphologically very similar till the formation of spikes. Male spikes greenish-yellow, fleshy, cylindrical, with minute flowers, female spikes erect, yellow. Mature female spikes, known as long pepper are shorter and thicker than the male spikes (Figures 1 & 2). Fruit spikes cylindrical, oblong, berries red or black when ripe, globose with aromatic odour and pungent taste (Sumy *et al*, 2000; Banerjee *et al*, 1999; Viswanathan, 1995).

*P. longum* is a native of North East India. It occurs in the hotter parts of India, from Central Himalayas to Assam, Khasi and the Mikir hills, the lower hills of West Bengal, and the evergreen forests of the Western Ghats from Konkan to Travancore. It has also been recorded in the Car Nicobar Islands and is also cultivated. Globally the species is distributed in the Indo-Malesian region and Sri Lanka (Sumy *et al*, 2000; Sivarajan & Balachandran, 1994).

Indian Long Pepper is also known as *Pipli*, *Pipar*, *Pipal* (Hindi), *Hippali*, *Thippali balli* (Kannada), *Tippali*, *Pippali* (Malayalam), *Pimpli* (Marathi), *Pippli*, *Tippili* (Tamil), *Pippallu*, *Pippali* (Telugu), *Pippali*, *Pipali* (Telegu), *Pippali*.
Long pepper probably came to Europe before the now dominant black pepper. It was highly priced during the Roman Empire i.e. about three times the price of Black pepper. With its taste pungent and sweet at the same time, it was preferred for Roman cookery. Long pepper is also known and popular in parts of Africa, mainly in the Islamic regions of North and East Africa.

In Ayurvedic literature, Rajanighantu there is a mention of four types of Pippali namely Pippali, Vanapippali, Sainmhal and Gajapipali (Sivarajan & Balchandran, 1994). Sharma (1983) has equated the former three with *P. longum*, *P. sylvaticum* Roxb. and *P. retrofractum* Vahl, respectively. Of these, *P. sylvaticum* is a Himalayan species. However, Kerala physicians do not make a distinction between the three and *P. longum* is accepted for all. Gajapipali is considered as a different drug but its identity is highly controversial. According to some it is the fruits of *P. chaba* Hunter, a species known under cultivation in India and Malaysia (Chunekar, 1982; Sharma, 1983). But, Kerala physicians equate this species with a different drug, Chavya. Yet, others have accepted the spikes of *Scindapsis officinalis* Schott of Araceae as Gajapipali (Vaidya, 1936; Nadkarni, 1954; Chopra et al, 1956; Mooss, 1980).

However, market survey has revealed that a root-parasite, *Balanophora fungosa J.R. & G. Forst.* (Balanophoraceae) which superficially resembles the inflorescence of *Scindapsis officinalis* and chopped stem of *Raphidophora pertusa* Schott (Araceae) are also used as Gajapipali (Sivarajan & Balchandran, 1994).

### Cultivation

Indian Long pepper is mostly derived from the wild plants, the main sources of supply being Assam, West Bengal and Uttar Pradesh. Small quantities are also collected from evergreen forests of Kerala, West Bengal and certain parts of Andhra Pradesh. It is reported to be cultivated at low elevations in Annamalai hills and parts of Assam particularly in the Cherrapunji area (Wealth of India, 1969). As the plants are excessively extracted from its natural source, the species has now become very rare in the forests of Kerala (Nair, 2000).

Long pepper is typically found in tropical humid climate and prefers shady moist conditions, well-drained sandy soil of pH range 5.5 to 8.5 with rich humus. Laterite soils with organic matter content and good water holding capacity are suitable. Areas with good rainfall and high relative humidity are suitable for its successful growth. It is a shade loving plant but, for better fruiting, 50 per cent shade is best (Sumy et al, 2000). It grows well in the shade of trees in areas of abundant rainfall and can also be grown as an intercrop in coconut plantations in the plains, though altitude of 900-1500 m above sea level is recommended.

### Vegetative propagation

It grows as a bushy runner and can be propagated using vegetative means like mature branches or by suckers planted at the beginning of the rainy season.

1. **Through cuttings** — Semi-hard stem cuttings, 10 to 12 cm long with at least 3 nodes are planted in shaded nursery beds with the upper most bud exposed. A spacing of 12 to 15 cm should be provided between each pair of cuttings. The cuttings root in 10 to 15 days and success is 60 to 70 per cent.

2. **Through tillers** — The tillers arising from the base of a mature plant can be separated and planted individually.

   The sprouted cuttings can be planted in the field at a spacing of $2 \times 2m$ in 20 cm$^3$ pits. These plants are prone to many diseases and care should be taken to prevent their occurrence (Sumy et al, 2000).
**Tissue culture**

For developing rooted plantlets by tissue culture technique, excised shoot tips, leaves and stem pieces are washed first under running tap water for an hour, then immersed in 70% alcohol for 2 min before surface sterilization with 0.1% aqueous mercuric chloride for 3-4 min. After washing several times with sterile distilled water, shoot tips, leaf discs and stem segments are excised and implanted to basal medium consisting of MS (Murashige & Skoog, 1962) salts and vitamins with 3% sucrose and 0.8% agar. Basal medium is supplemented with various concentrations of growth hormones, Kinetin (K) and 6-Benzyladenine (BA) for axillary shoot multiplication and Indole-3-acetic acid (IAA), Naphthalene acetic acid (NAA), and Picloram for direct regeneration. The pH of the medium is adjusted to 5.72 and the medium is dispensed in culture tubes before autoclaving at 121°C for 15 min. All cultures are maintained in 16 hr day length at 26°C in a culture room. Shoot tips cultured on MS medium supplemented with BA and K develop multiple shoots after 20 days (Soniya & Das, 2002). Multiple shoots directly from leaf explants using combinations of BA, K and coconut water in MS medium have also been developed (Sarasan & Nair, 1991; Sarasan et al, 1993). Multiple shoots directly from leaf explants using combinations of BA, K and coconut water in MS medium have also been developed (Sarasan & Nair, 1991; Sarasan et al, 1993). Stem pieces are difficult to initiate shoots. When the regenerated shoots are 2-3 cm long, they are separated and transferred to MS medium containing Indole-3-butyric acid (IBA) for rooting. The rooted plantlets are transferred to greenhouse conditions where about 90% seedlings survive.

**Molecular characterization of genotypes**

**Randomly Amplified Polymorphic DNA (RAPD) Technique**

Very little is known regarding the genetic identity of different cultivars of *P. longum* at the molecular level. In this decade, RAPD has emerged as a very convenient tool for genotyping closely related accessions, particularly plant species. Such an effort might prove useful for the molecular characterization of *P. longum* genotypes.

Total genomic DNA was isolated from young healthy leaves by standard protocol (Rogers & Bendich, 1994). The purified genomic DNA was subjected to polymerase chain reaction (PCR) for RAPD analysis using random decamer oligonucleotide primers from M/s Operon Inc. (USA). The PCR reaction consisted of 20 ng genomic DNA, 200µM each of dNTPs (dATP, dGTP, dCTP and dTTP), 15 picomole primer, 1X Taq DNA polymerase buffer and 0.5 unit of Taq DNA polymerase (M/s Bangalore Genei, India) in a final volume of 20 µl in sterile ultrapure water overlayed with 30 µl mineral oil. The PCR was performed in Perkin-Elmer PE480 thermal cycler.

For the detection of genetic difference among the individual plants the following program is used: initial denaturation at 94°C for 4 min, 40 cycles of denaturation at 94°C for 45 sec, annealing at 40°C for 1 min and extension at 72°C for 90 sec followed by final extension at 72°C for 7 min. The PCR products were visualized with 1.2% agarose gel electrophoresis in 1X Tris-borate–EDTA buffer (Sambrook et al, 1989), ethidium bromide staining and documented under ultraviolet light. RAPD profile of the male plants generated by certain random primers is distinctly different from the females by the presence of prominent male plant associated bands (Banerjee et al, 1999).

**Phytochemistry**

The fruits contain 1% volatile oil, resin, alkaloids, piperine and pipelongumine, a waxy alkaloid N-isobutyldeca-trans-2-trans-4-dienamide and a terpenoid substance. The pungency of the fruits is mainly due to the piperidine alkaloid piperine. The fruits also contain calcium, 1230; phosphorous, 190; and iron, 62.1mg/100g.

Roots contain piperine, pipelongumine or piplartine and dihydrostigmasterol (Neelam & Krishnaswamy, 2000).

**Medicinal uses**

The fruits are used as spice and also in pickles. They have a pungent taste and cause salivation and numbness of the
mouth (Neelam & Krishnaswamy, 2000). Since Long pepper is more pungent than Black pepper, it must be used with care, unless fiery food is demanded. Since terpene components are missing in its aroma, Long pepper cannot be substituted by Black pepper. Its hot and sweet taste goes well with spicy cheese specialities or wine sauces.

In traditional medicines, mature spikes of female plants, thick stems, roots and leaves are extensively used in the treatment of bronchial diseases, dyspepsia, worms, amoebiasis and aphrodisiac agent. Pippali is an important drug capable of improving intellect and memory power and also to regain health by dispelling diseases. It is reportedly acrid, hot, light, digestive, appetizer and tonic.

It cures cough, dyspnoea, ascites, leprosy, diabetes, piles, colic indigestion, anaemia, thirst and dispels cardiac and spleen disorders, chronic fever and loss of appetite. It rehabilitates vitiated vata and kapha. Dried ripe fruits and roots are the officinal parts (Viswanathan, 1995). It is also used as an antidote to snake-bite and scorpion-sting (Sumy et al, 2000). It is carminative, sedative, emollient, demulcent, general tonic and hemantinic. It enhances thermogenic response or release of metabolic heat energy.

Long pepper causes high pitta but no information about the safety of this herb is available. However, it is not advisable to take P. longum, except under the supervision of a qualified professional.

The fruits and roots of Long pepper are used as snuff in coma and drowsiness, as sedative in insomnia and epilepsy, as cholagogue in obstruction of bile duct and gall-bladder, as emmenagogue, abortifacient and as anthelmintic (Neelam & Krishnaswamy, 2000). Clinical studies have revealed that Pippali is very effective in the treatment of bronchial asthma in children (Dahanukar et al, 1984; Anshuman et al, 1984). The important formulations using the drug are: Abhayaristam, Draksaristam, Chyavanaprasham, Pippalyasavam, etc. (Sivarajan & Balachandran, 1994).

The drug is used in Ayurvedic treatment for abdominal tumours and distention, to improve the digestive fire, kapha disorders, flatulence, gout, laryngitis, paralysis, rheumatic pain, sciatica, worms, and for the immune system. It is used in manufacturing cold relief balm, pain balm, joint care balm and in heart and geri/stress care and cough syrups.

Pharmacological activities

In view of the commercial, economic and medical importance of Piper longum, several workers have investigated the species pharmacognostically, chemically and also pharmacologically (Neelam & Krishnaswamy, 2000).

Antibacterial activity

Long pepper exhibits antibacterial activity; its isolates are active against Gram positive bacteria and moderately active against Gram negative bacteria. Each isolate is highly active against at least one particular species of bacteria: piperlonguminine against Bacillus subtilis and piperine against Staphylococcus aureus (Reddy et al, 2001).

Antiallergic activity

The fruit effectively reduce passive cutaneous anaphylaxis in rats and protect guinea pigs against antigen-induced bronchospasm; a 30% protection of mast cells was observed in an in vitro study (Chatterjee, 1999; Dahanukar et al, 1984). Aller-7, a combination from seven medicinal plants including Long pepper is used for allergic rhinitis, as antihistaminic and as antispasmodic (Amit et al, 2003).

Antitumour activity

Protective action of piperine against gastric ulcer was observed (Bai & Xu, 2000). Immunomodulatory and anti-tumour activities of Piper longum fruits and piperine are reported in mice (Sunila & Kuttan, 2004).

Intestinal disorders

The fruit extract exhibits antimicrobial activity against Entamoeba histolytica in rats. The ethanolic extract and piperine, a pure compound, from this plant material cured 90% and 40% of rats with caecal amoebiasis, respectively (Ghoshal et al, 1996). The drug is also reported to show anti-giardial and immuno-stimulatory activity in mice infected with Giardia lamblia trophozoites (Tripathi et al, 1999).

Hepatitis

The hepatoprotective effect of fruits has been reported in carbon tetrachloride induced liver damage in rats. Along with P. nigrum it has been useful in viral hepatitis (Koul & Kapil, 1993).
Respiratory disorders

Decoction of immature fruits and roots is used in chronic bronchitis, cough and cold (Sumy et al., 2000). Clinical studies have revealed that Pippali is very effective in the treatment of bronchial asthma in children (Dahanukar et al., 1984; Anshuman et al., 1984).

Studies conducted on children revealed that long-term use of fruits decreased (58.3%) severity of bronchial asthma attacks. Piperine decreased the rate and amplitude of respiration and showed nonspecific blockade of acetylcholine, histamine 5-hydroxytryptamine induced spasm on isolated guinea pig and rabbit intestine.

Other activities

Some compounds isolated from fruits were found to possess antitubercular activity (Kurup et al., 1979). Dehydropiperonaline obtained from the dried fruits displayed coronary vasodilating activity (Shoji et al., 1986). Antifertility effects of the fruits in female rats were also reported (Kholkute et al., 1979).

Conclusion

Red list status of Piper longum has been assessed as endangered for Tamil Nadu and lower risk for Kerala. The species is not evaluated for Karnataka as its wild presence is not recorded in this state. Conventional propagation is beset with problems of poor seed viability, low percentage of germination and scanty, delayed rooting of vegetative cuttings. Therefore, there is a need for alternative propagation methods. Tissue culture technique might be applied to generate large number of clonal propagules. Authors could establish protocols for in vitro propagation of P. longum through shoot multiplication and direct regeneration, which offers a potential system for improvement, conserving and mass propagation of this important medicinal plant.

A molecular marker system to identify different varieties of P. longum and also to identify plants producing male/female spikes has also been developed. Screening of more markers associated with sex in P. longum plants, identifying the genes involved and full length sequencing of the genome will definitely help in understanding the genetic mechanism of sex determination in this dioecious plant.

References

In a single-dose human study, piperine, a chemical found in black pepper and long pepper (Piper nigrum, Piper longum), was reported to increase blood levels of propranolol, which could increase the activity and risk of side effects of the drug (http://www.netrition.com/cgi/healthnotes_display_for_print.cgi?content_id=1472000).