Article

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.

IPC code; Int. cl.[7] — A01H4/00, A23L1/22, A61K35/78

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* (Telegu), *Pippali*,

Fig. 1: Male plant

Magadhi, Kana, *Ushana* (Sanskrit) (Sumy *et al*, 2000). It is a product either of ***P. longum*** or ***P. peepuloides*** **Roxb.** while the Indonesian or Jawa Long Pepper is from ***P. retrofractum*** **Vahl,** which has rods a little bit smaller than ***P. longum***. In Western countries, mostly the latter is available. The species ***P. longum*** is of South Asian origin (Deccan peninsula), whereas ***P. retrofractum*** comes from South East Asia and is mostly cultivated in Indonesia and Thailand. Both species are often not clearly distinguished in the spice trade.

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, Saimhali* and *Gajapippali* (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. *Gajapippali* 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 *Gajapippali* (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 *Gajapippali* (Sivarajan & Balchandran, 1994).

Fig. 2: Female plant

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 *p*H 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 inter-crop 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 × 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 *p*H 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). 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 30μl 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 37°C for 45 sec and polymerization at 72°C for 90 sec followed by final extension at 72°C for 7 min. The resulted products are analysed after agarose gel electrophoresis. Considering the presence or absence of gel bands, genetic variation and DNA polymorphism within and between the plant accessions are studied.

For identifying sex related genetic difference, male and female plant genomic DNA were analysed individualy. The PCR program consisted of initial denaturation at 94°C, for 5 min, 40 cycles of denaturation at 94°C for 1 min, annealing at 40°C for 1 min and extension at 72°C for 2 min 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 piperlonguminine, 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, piperlongumine 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*, *Chyavanaprasam, 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 anti-amoebic 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-hydroxy-tryptamine 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). Dehydropipernonaline 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 defenitely help in understanding the genetic mechanism of sex determination in this dioecious plant.

References

1. Amit A, Saxena VS, Pratibha N, D'Souza P, Banchi M, Bagchi D and Stohs SJ, Mast cell stabilization, lipoxygenase inhibition, Lyaluronidase inhibition, anti-histaminic and anti-spasmodic activities of Aller-7, a novel botanical formulation for allergic rhinitis, *Drugs Exp Clin Res*, 2003, **29** (3), 107-115.
2. Anshuman PS, Sing KP and Aasra KG, Effect of Vardhaman pippali (*Piper longum*) on patients with respiratory disorders, *Sachitra Ayurv*, 1984, **37** (1), 47-49.
3. Bai YF and Xu H, Protective action of Piperine against experimental gastric ulcer, *Acta Pharmacol Sin*, 2000, **21**(4), 357-359.
4. Banerjee NS, Manoj P and Das MR, Male sex associated RAPD markers in *Piper longum* Linn., *Curr Sci*, 1999, **77**(5), 693-695.
5. Bhat SR, Chandel KPS and Malik SK, Plant regeneration from various explants of cultivated Piper species, *Plant Cell Rep,* 1995, **14**, 398-402.
6. Charaka Samhita: ed.GS Pandeyya, Chowkhambha Sanskrit Sansthan, Varanasi, 1983.
7. Chatterjee S, Bronchodilatory and anti-allergic effect of Pulmoflex- A proprietary herbal formulation, *Indian J Physiol Pharmacol*, 1999, **43**(3), 486-490.
8. Chopra RN, Nayar SL and Chopra IC, Glossary of Indian medicinal plants, CSIR, New Delhi, 1956, 224.
9. Chunekar KC, *Bhavaprakasanighantu* of Sri Bhavamisra Commentary, Varanasi (in Hindi), 1982, 21.
10. Dahanukar SA, Karandikar SM and Desai M, Efficacy of *Piper longum* in childhood asthma, *Indian Drugs*, 1984, **21**(9), 384-388.
11. Ghoshal S, Prasad BN and Lakshmi V, Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolytica* in *in vitro* and *in vivo*, *J Ethnopharmacol*, 1996, **50**(3), 167-170.
12. Kholkute SD, Kekare MB and Munshi SR, Antifertility effects of the fruits of *Piper longum* in female rats, *Indian J Exp Biol*, 1979, **17,** 3, 289-290.
13. Koul IB and Kapil A, Evaluation of the liver protective potential of Piperine, an active principle of black pepper

and long pepper, *Planta Med*, 1993, **59**(5), 413-417.

14. Kurup PNV, Ramdas VNK and Joshi P, Handbook of Medicinal Plants, New Delhi, 1979, 166.
15. Mooss NS, Ganas of vahata, Kottayam, 1980, 86.
16. Murashige T & Skoog F, A revised medium for rapid growth and bioassays with tobacco tissue culture, *Physiol Plant*, 1962, **15**, 473-497
17. Nadkarni AK, Indian Materia Medica, Bombay, 1954, 1117.
18. Nair KKN, Manual of Non-wood forest produce plants of Kerala, Kerala Forest Research Institute, Kerala, 2000, 268-270.
19. Neelam and Krishnaswamy Kamala, Nutritive value of Pepper, *In*: Pepper market review, National Institute of Nutrition, Hyderabad, May, 2001, 1-5.
20. Philip S, Banerjee NS and Das MR, Genetic variation and micropropagation in three varieties of *Piper longum* Linn., *Curr Sci*, 2000, **78**(2), 357-359.
21. Reddy Srinivasa P, Kaiser Jamil, Madhusudhan P, Anjali G and Das B, Antibacterial activity of isolates from *Piper longum* and *Taxus baccata*, *Pharm Biol,* 2001, **39**(3), 236-238.
22. Rogers SO and Bendich AJ, Extraction of DNA from plant tissues, *In*: Plant molecular biology manual, SB Gelvin and RA Shilperoot (eds), Kluwer Academic Publishers, Dordrecht, 1988, pp 1-10.
23. Sambrook J, Fritsch EF and Maniatis T, Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York, 1989.
24. Sarasan V and Nair GM, Tissue culture of medicinal plants: morphogenesis, direct regeneration and somatic embryogenesis, Horticulture — New Technologies and Applications, J Prakash and RLM Pierik (eds), Kluwer Academic Publishers, 1991, 237-240.
25. Sarasan V, Thomas E, Lawrence B and Nair GM, Plant regeneration in *Piper longum* Linn. (*Piperaceae*) through direct and indirect shoot development, *J Spices Arom Crops,* 1993, **2**, 34-40.
26. Sharma PV, *Dravyaguna vijnana*, Varanasi (in Hindi), 1983, 276.
27. Shoji N, Umeyama A, Saito N, Takemoto T and Kajiwar O, Dehydropipernonaline, an amide-possessing coronary vasodilating activity isolated from *Piper longum* Linn., *J Pharm Sci,* 1986, **75**(12), 1188-1189.
28. Sivarajan VV and Balachandran Indira, Ayurvedic drugs and their plant sources, Oxford and IBH Publishing Co.Pvt.Ltd, New Delhi, 1994, 374-376.
29. Soniya EV and Das MR, *In vitro* micropropagation of *Piper longum*, an important medicinal plant, *Plant Cell Tissue Organ Cult*, 2002, **70**, 325-327.
30. Sumy Oommen, Ved DK and Krishnan R, Tropical Indian Medicinal Plants, propagation methods, 2000, 268-269.
31. Sunila ES and Kuttan G, Immunomodulatory and anti-tumour activities of *Piper longum* Linn. and piperine, *J Ethnopharmcol*, 2004, **90**(2-3), 339-346.
32. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products — Raw Materials Series, Vol. VIII,96-99, 1969, Publications and Information Directorate, Council of Scientific & Industrial Research, New Delhi.
33. Tripathi DM, Gupta N, Lakshmi V, Saxena KC and Agrawal AK, Antigiardial and immunostimulatory effect of *Piper longum* on giardiasis due to *Giardia lamblia*, *Phytother Res*, 1999, **13**(7), 561-565.
34. Vaidya KM, The Ashtangahridayakosha with the Hridaya Prakasha commentary, Trichur (In Sanskrit), 1936, 181.
35. Viswanathan TV, Medicinal and Aromatic Plants, KL Chadha and R Gupta (eds), Malhotra Publishing House, New Delhi, 1995, 373-383.

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).