CHARACTERISATION OF LONG PEPPER (*Piper longum* L.) GENOTYPES USING MORPHOLOGICAL, ANATOMICAL AND MOLECULAR MARKERS

By

JITHA JALEEL

THESIS

submitted in partial fulfillment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

Department of Plant Breeding and Genetics

COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

2006

DECLARATION

I hereby declare that the thesis entitled **"Characterisation of long pepper (*Piper longum* L.) genotypes using morphological, anatomical and molecular markers"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellanikkara
29-12-05

Jitha Jaleel

CERTIFICATE

Certified that this thesis entitled **"Characterisation of long pepper (*Piper longum* L.) genotypes using morphological, anatomical and molecular markers"** is a record of research work done independently by **Mrs. Jitha Jaleel** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, associateship or fellowship to her.

Vellanikkara
29.12.06

Dr. K. Nandini
(Chairperson, Advisory Committee)
Associate Professor,
Dept. of Plant Breeding & Genetics,
College of Horticulture,
Vellanikkara.

CERTIFICATE

We, the undersigned members of the advisory committee of **Mrs. Jitha Jaleel,** a candidate for the degree of **Master of Science in Agriculture,** with major field in **Plant Breeding and Genetics,** agree that the thesis entitled **"Characterisation of long pepper (*Piper longum* L.) genotypes using morphological, anatomical and molecular markers"** may be submitted by **Mrs. Jitha Jaleel,** in partial fulfilment of the requirement for the degree.

29/12/06

Dr. K. Nandini
(Chairperson, Advisory Committee)
Associate Professor (Pl. Physiology),
Dept. of Plant Breeding & Genetics,
College of Horticulture,
Vellanikkara.

29/12

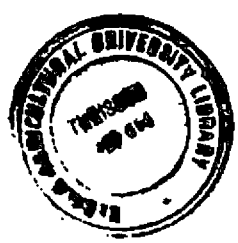
Dr. V. V. Radhakrishnan
(Member, Advisory Committee)
Associate Professor & Head
Dept. of Plant Breeding & Genetics
College of Horticulture,
Vellanikkara.

29/12/06

Dr. P.A. Nazeem,
(Member, Advisory Committee)
Associate Professor & Head,
Centre for Plant Biotechnology and
Molecular Biology,
College of Horticulture,
Vellanikkara.

29/12/06

Dr. R. Sujatha,
(Member, Advisory Committee)
Assistant Professor
Dept. of Plant Breeding & Genetics
College of Horticulture,
Vellanikkara.



29/12/06

External Examiner

ACKNOWLEDGEMENT

I would like to record a word of gratitude to all those helping hands and well wishers who have helped me to successfully complete this endeavour.

I humbly bow my head before the ALMIGHTY, for the blessings that helped me to complete this endeavour successfully amidst the difficulties.

I record my deep sense of gratitude and indebtness to my chairperson, Dr. K. Nandini who was in all means my leading light with her valuable advice, everwilling help, abiding patience, motherly affection and unstinting support rendered at all stages of work contributed most to the completion of the study. It is her empathetic approach, good will and lively inspiration that gave me confidence at every phase of this research. It is my privilege to work under her.

Dr. V.V.Radhakrishnan, Head Of Dept, Plant Breeding And Genetics, and member of advisory committee was there at every phase of this work with his valuable guidance and constructive criticisms in preparing the manuscript. Without his support the work at the medicinal garden would not have finished successfully. I express my unreserved gratefulness to him.

I place a deep sense of gratitude to Dr. P.A.Nazeem, Associate Professor & Head, CPBMB and member of my Advisory Committee for the help, and constructive suggestions received from her in spite of her busy schedule. I thank her for giving me an opportunity to work at the CPBMB lab.

I express my sincere thanks to Dr. R. Sujatha, Assistant Professor, Dept Of Plant Breeding And Genetics and member of my advisory committee for her kind concern and valuable suggestion for the betterment of the manuscript.

I sincerely thank Dr. Prasannakumary.K.T., Dr. Dijee Bastian, Dr. C . R. Elsy, Dr. Arya, K. from Dept Of Plant Breeding And Genetics who had helped me in several ways for the completion of this venture.

I place on record my profound sense of gratitude to Dr. E.V. Anoop Assistant Professor, College Of Forestry who had given me the opportunity to use the facilities at the anatomy lab and for his valuable suggestions.

May I also take this opportunity to thank Dr. T. Girija , Dr. Mini and Dr. Laly John who showed ardent interest and gave encouragemet at different phases of the study.

I wish to acknowledge my sincere thanks to Sunandachechi, Sathichechi, Vargheesechettan, Vidhuchechi, Sanalchettan, and Research Assistants in the Dept. of Plant Breeding & Genetics for all the help rendered.

I am extremely thankful to Blessy Paul who helped and gave necessary suggestions for the work at the CPBMB lab. My profound thanks to all the research assistants and my friends, Smini, Smita Nair, Kukku, Resmi and Mable for the help and concern.

I wholeheartedly thank my friends Gayathri , Ambika , Smitha, Lekha, Femina, Mohan, Simi, Malini to name a few; seniors Divya, Anisha, Chandrasekhar, and juniors Ashwathy, Sani, Vishnu , Marimuthu, Sameera, Likhitha, and Nidhi for making this tenure a memorable one. Words cant express my thanks to Ampily for all her help and affection.

I am also grateful to Karuppaiyan Sir and Joshi, who helped me in the statistical works.

My wholehearted thanks to Manila, Valsala chechi and all the labourers of medicinal garden of KAU for the help rendered..

The award of KAU Junior Research Fellowship is duly acknowledged

I also thank Santhoshchettan of Students Computer Center, College of Horticulture for the valuable help rendered in the typesetting of the thesis.

I am in dearth of words to express my indebtness to my parents, in laws, brothers and sisters for their boundless affection, care, encouragement, support and prayers. It was my husband and little daughter who faced my negligence most. My work would not have been fruitful without his support, co-operation, inspiration and love. His help too is acknowledged with love.

Jitha Jaleel

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Introduction

1. INTRODUCTION

Piper longum L. (long pepper) is an important medicinal plant belonging to the family Piperaceae. The genus Piper consists of around 100 species including important cash crops like black pepper *(P. nigrum)* and betel vine *(P. betle)*. Apart from *Piper longum*, *P. chaba, P. peepuloides, P. mullesuae and P. hapnium* are the other wild species of this genus, which have medicinal values and sold as long pepper.

Long pepper of commerce is the dried mature spike of female plants. The dried roots and lower stems of male plants constitute Piplamool which also has medicinal properties. On distillation, long pepper yield essential oil and alkaloids piperine, piplartine, piperidine, piperlonguimine *etc.* which imparts its medicinal property. The medicinal uses of *Piper longum* in Ayurvedic, Unani and Sidha preparations had been described by several workers (Kirtikar and Basu, 1935; Suseelappan, 1991; Joseph and Skaria, 2001; Sasikumar, 2004). It forms an important component in Ayurveda preparation such as Trikadu (dry ginger –black pepper- long pepper) and Panchakolam. It is known as a rejuvenating and revitalizing drug in Ayurveda and is mainly used for the treatment of bronchial asthma, dyspepsia, amoebiasis, as memory enhancer and aphrodisiac.

Long pepper is known as *thippali* or Pippali in Sanskrit. *Piper longum* is a native of Indo-Malaya origin and is found growing wild in the tropical rain forests of India, Nepal, Malaysia, Sri Lanka, Rio, Timor, and the Philippines. In India it grows wild in the Western Ghats, North East and Himalayan regions.

The plant is a slender perennial creeping undershrub of dioecious nature with stout, cylindrical and thickened nodes. The stem exhibits dimorphic branching. On the vegetative branch leaves are cordate and petiolate whereas on fruiting branches leaves are mostly sessile and lanceolate.

Piper longum is propagated mainly by cuttings, requires optimum shade and heavy organic manuring for proper growth and productivity. It starts yielding from first year during which the dry spike yield is 400 kg per hectare. The yield is maximum in the second year (1000 kg) and then it declines. The average yield of

dry roots is 500 kg per hectar. ...eculiar climatic requirement it is not recommended as a sole crop in ... intercrop in irrigated coconut plantations. (Viswanathan, 1995).

The crop fetches a price of Rs. 115-... kg and at least 176 tonnes of thippali is used in North Kerala annually by Ayurvedic pharmaceutical companies (Sasidharan and Muraleedharan, 2000). A survey conducted by the State Government in 1987 revealed that the annual requirement pf *thippali* in Kerala is 313 tonnes and the demand for this crop is increasing day by day. The demand for ayurvedic medicine in the state is growing at a rate of 10-12 percent per annum .A similar trend is expected globally also. However there is no published estimate for area under cultivation of medicinal plants. The area under cultivation is very limited and the productivity of cultivated crop is very low. So only 10% of our requirement is met domestically and the rest is imported from countries like Srilanka, Malaysia and Indonesia.

However Kerala is blessed with wide variability in *thippali* and more than 67 accessions have been identified based on different morphological characters at All India Network Programme on Medicinal & Aromatic Plants at College of Horticulture, Vellanikkara. These identified accessions have been grouped into three categories based on morphological similarity (Biennial Report, 2000). In order to have a comprehensive study on the performance of these three groups, eight accessions were selected based on morphological characters and detailed investigations were carried out to achieve the following objectives,

- To characterise the morpho-physiological attributes favouring productivity
- To understand the variation in anatomical characters of stem, root, leaf and spike in male and female accessions
- To detect genetic polymorphism between selected accessions using molecular markers
- To identify the physiological maturity of spike in relation to quality

2. REVIEW OF LITERATURE

This chapter reviews the relevant literature available in India and abroad in various aspects related to the present study under the following heads.

2.1 Taxonomy and Distribution

2.2 Morphological characters

2.3 Anatomical characters

2.4 Molecular markers

2.5 Chemical composition

2.1 Taxonomy and Distribution

The earliest record of the description of Piper of Indian subcontinent was by Rheede (1678) in his *Hortus Indicus Malabaricus*, wherein he described five types of wild pepper including black pepper and long pepper. Hooker (1886) divided the order Piperaceae into two tribes namely Saurureae and Piperae. Piperaceae is further divided into genus Piper and Peperomia. The genus Piper is further divided into six sections namely Muldera, Cubeba, Chavica, Pseudochavica, Eupiper and Heckeria. *P. longum* comes under the section chavica. This section includes fifteen species other than *P. longum*. Ravindran (1990) had classified *Piper* based on erect or pendent spike as Pippali and Maricha.

Indian long pepper is mostly derived from wild plants, the main source of supply being Assam, West Bengal, Nepal and Uttar Pradesh. Small quantities are also available from evergreen forests of Kerala, and certain parts of Andhra Pradesh (Grieve, 1977). A survey conducted by Rahiman *et al.* (1979) revealed the presence of *P. longum* in the forests of Karnataka. Manilal and Sivarajan (1982) reported the distribution of *P. longum* in Calicut. Sivarajan and Indira (1995) reported Western Ghats as the natural habitat of *P. longum*. According to Ravindran (2000) it is common in low land forests.

2.2 Morphological characters

2.2.1. Vegetative characters

2.2.1.1 Stem characters

Piper longum

Hooker (1886) described *P. longum* as creeping with jointed stems thickened at nodes. *P. longum* is a creeper whose runners struck roots and branches at every node, the branches were erect and scandent, the stems smooth, green and comparatively very thin. (Rahiman *et al.*, 1979). Ravindran (2000) described *P. longum* as a slender, perennial creeping undershrub, which is dioecious. Vegetative branches creep and spread on the ground, fruiting branches erect. Young branches are puberulous, hairs minute, multicellular and deciduous. Older branches are totally glabrous.

In the biennial report of All India Co-ordinated Research Project on Medicinal & Aromatic Plants (2000) it was reported that 67 accessions of *P. longum* were characterized based on morphological characters.

Genotypic and morphogenetic differences among three female varieties of *P. longum* were studied. Morphogenetic potential of node, internode and leaf explants from all three varieties were compared by Shaji *et al.* (2000) and developed an efficient protocol for plant regeneration from leaf calli.

Piper nigrum

Kanakmony *et al.* (1985) proposed a key for the identification of different cultivars of black pepper. Forty five *P. nigrum* cultivars were grouped according to a key based on the nine vegetative characters. The characters studied included internodal length in orthotrope and plageotrope, thickness at node and internode, petiole length, leaf area, leaf shape and leaf tip.

Stem characteristics like internodal length, branching nature, direction of growth of branches etc were reported to vary with cultivars. (Ibrahim *et al.*, 1986, Sujatha and Namboodiri, 1995).

Two interspecific hybrids, *P. nigrum* × *P. attennuatum* and *P. nigrum* × *P. barberi* were characterized using morphology, anatomy, isozymes, cytology, and function and the hybrids were reported to exhibit distinct morphological and anatomical features. (Sasikumar *et al.*, 1999).

Piper betle

In *Piper betle* 16 cultivars were characterised using morphological characters like plant height, branch and node numbers, internodal length and petiole length as well as biometrical traits by John (1996).

Genetic divergence in a set of 16 genotypes of betel vine was measured using Mahalanobis D^2 technique by Das *et al.* (2000). Wide range of variation was observed in the cluster mean values in respect of number of leaves per vine, leaf area, petiole length, inter nodal length, leaf length, leaf breadth, number of laterals per vine, vine length and diameter of inter node.

Mithila *et al.* (2000) studied the growth and morphology of vegetative and reproductive branches in betel vine. Data indicated that growth rates in terms of branches produced were higher in vegetative branches compared with reproductive branches. Vegetative branches showed higher petiole and internodal lengths. Leaves produced by reproductive branches were higher than vegetative branches.

Other crops

Sharma *et al.* (1997) classified 36 genotypes belonging to three series of the genus *Cymbopogon* using D^2 analysis based on the characters like plant height, number of tillers per plant, leaf area, leaf length, width, chlorophyll a and b content, herb yield, and oil content. The morphometric classification was compared with taxonomic classification and found that it does not seem to question the taxonomic boundaries.

2.2.1.2 Leaf characters

Piper longum

To compare the leaf area, a constant for the genus *Piper* was derived as 0.70. (Rao *et al.*, 1994).

The leaves of *Piper longum* are petiolated, cordate, glabrous, alternate, acute, entire and smooth. (Hooker, 1886). According to Rahiman *et al.* (1979) the leaves are membranous ranging in size upto $13\times6.5cm^2$, ovate or ovate oblong, young leaves asymmetrically cordate, anterior most pair of veins deviates form the leaf base. One of the two lobes of leaf base is large over the petiole; leaves on creeping stems are cordate with equal lobes. He gave an account of morphology of nine species of family *Piperaceae* including *Piper longum* based on the following vegetative characters *viz* habit, size of leaf, shape of leaf, leaf base and anterior most part of veins.

Manuel (1994) evaluated five accessions of *thippali* using morphological and biochemical characters to study the effect of growth as intercrop in coconut gardens.

Ravindran (2000) reported that leaves of *Piper longum* were distinctly dimorphic in whole plant; those on creeping shoots cordate, glabrous, petioles very long, grooved and approximately $7\times5cm^2$ area. Leaves on the fruiting branches were oblong lanceolate, base unequally cordate with pronounced auricle,

tip acuminate, 3-4 pairs of lateral ribs arise right from the base, lower side puberulous or downy when young, petioles very short or even absent.

Piper nigrum

Studies on shape of leaf, leaf area, petiole characters, texture and colour of leaf were reported useful in varietal identification of varieties in pepper (Purseglove, 1969; George and Mercy, 1978; Nambiar *et al.*, 1978).

With respect to leaf shape, *P. nigrum* cultivars were grouped into three classes namely elliptic, ovate and cordate. (Kanakamony *et al.*, 1985)

Variability in open pollinated seedlings of black pepper was studied by Ravindran and Sasikumar (1993) using morphological variation in height, leaf number, leaf width, leaf length, internodal length and stem girth.

Forty four cultivars and seven wild black pepper accessions were subjected to single linkage and centroid linkage cluster analysis using twenty-two morphological characters. Out of the 11 groups identified, a single group consisted of 28 cultivars which showed that majority of common cultivars resemble each other closely and probably have a common origin (Ravindran *et al.*, 1997).

Piper betle

Thirteen cultivars of betelvine were grouped into four based on the morphological and physiological characters. (Devi *et al.*, 1992)

Reddy (1996) examined fourteen betelvine genotypes for variation in 10 morphological characters. Varietal differences for number of leaves, 100 leaf weight, leaf colour, leaf shapes, and pungency aided in classification.

Studies on genetic variability, coefficient of variation, genetic advance and character association for 12 characters in 16 genotypes of *P. betle* showed that the number of leaves per vine had positive correlation with leaf length, leaf breadth, leaf area, number of laterals per vine, vine length, diameter of internode, chlorophyll a and b content and 100 leaf weight. (Das *et al.*, 1999).

2.2.2 Productive characters

Piper longum

The inflorescence of *P. longum* is erect and cylindrical as observed by Rahiman *et al.* (1979). He also described the spike characters of *P. longum* which included the average spike length, spike shape, spike surface, bract and number of stamens.

Ridley (1983) observed spike characters and reported that the male spikes were slender and about 1 to 3 inches long.

Comparative evaluation studies of four selected geographical races of *P. longum viz* Cheematippali, Panniyur, Pattambi and Kanjur based on green spike yield and dry spike yield revealed that Cheematippali recorded the maximum green spike yield (624.92 kg per ha) followed by Pattambi (407.82 kg per ha), Kanjur (401.34 kg per ha) and Panniyur (28.7 kg per ha). (Anon, Progress Report, AICRP on M & AP, 1993).

The female spikes were short, sessile, opposite to the leaf, cylindrical with a rounded base and blunt tip. The flowers were numerous but the bracts were orbicular and fruits were minute, drupe embedded in the fleshy spike. The whole spike of fruit forms a cylindrical mass, broadest at the base and when dry was of grey colour and very pungent. Spikes cylindrical, erect about 2 to 4 cm long, creamy white to yellowish white when young with peduncle about 1 to 2 cm long. Male spikes much longer (6-10 cm), yellow on maturity. Bracts peltate, orbicular,

glabrous, pedicellate, flowers laterally fused. Stamens 3 to 4, carpel single, ovary obovate, style absent, stigma 3 to 4 lobed, papillate. Fruits very small fused laterally, spicy and pungent. Seeds very small. Spikes on ripening turns from green to black. (Ravindran, 2000)

Piper nigrum

Nambiar *et al.* (1978) compared six Malabar and twelve Travancore cultivars of black pepper on the basis of spike characters *viz,* mean spike length, mean number of developed berries on the spike, mean weight of 100 green berries, mean volume of 100 green berries, percentage of dry to green weight of pepper and mean number of days taken for emergence of spike.

The productive characters of some of the popular cultivars of black pepper were described by Ravindran and Nair (1984) which included range of spike length, mean length of spikes, size of berries, colour of berries, driage percentage and yield.

Kanakamony *et al.* (1985) while preparing a key for the identification of forty five cultivars of black pepper studied the following spike characters *viz* spike length, number of well developed berries, number of underdeveloped berries, 1000 berry weight in the fresh condition and dry condition.

Mathai (1986) conducted growth and yield analysis in black pepper varieties under different light conditions. It was found that Panniyur-1 bore more laterals, spikes and berries, a higher mean berry weight and higher rate of photosynthesis than other varieties.

Mathew *et al.* (2001) reported graph clustering of 57 cultivars *P nigrum* using Graph theory model based on 27 morphological characters including both qualitative and quantitative traits.

Piper betle

Shivasankara *et al.* (2000) studied the effect of different light intensities on growth and yield of betelvine. It was found that optimum light intensity for maximum betelvine growth and yield was 36 percent.

Other crops

Ram *et al.* (1996) classified the cultivars of menthol based on growth parameters of suckers like length, girth number of nodes, weight per sucker and yield of suckers.

Joy *et al.* (1998) evaluated 34 accessions of *Cinnamomum verum* for growth, yield and quality parameters. The yield of eugenol and leaf oil were primarily associated with leaf yield and positively correlated with canopy spread and plant height.

Two diploid and tetraploid cultivars of *Chamomile* were compared to determine the variation in morphology, yield and essential oil component at two harvest times. An analysis of variance was performed and means were compared using Duncan's test. Andrea (2002) reported there was no statistical difference on oil percentage among the four cultivars and between harvest times.

Asensio *et al.* (2002) characterized nine white grape vine cultivars by morphological and amino acid analysis. The morphology of various plant organs was described at several phenological stages. The results showed that the two methods were complementary and could be used to differentiate varieties.

Labro *et al.* (2004) compared the *Ocimum* cultivars based on the morphological, essential oil and molecular characters. They found that a combined analysis of these represent optimal approach to verify taxonomy and correlate it with agronomic traits.

2.3 Physiological parameters

Metabolite partitioning studies in five each of high and low yielding black pepper accessions revealed that during juvenile stage, stem had higher reducing sugars and lower total carbohydrates as compared to leaves. High yielding accessions had higher starch levels compared to low yielder. In general, high yielder had higher photosynthetic rate and higher transpiration compared to low yielder. (Research Highlights, IISR 2004-2005).

The study on oilpalm germplasm for abiotic stress revealed that higher photosynthetic rates were recorded in Guinea Bissau germplasm, which coincided with their high stomatal conductance to diffusion of carbondioxide. The leaves of Guinea Bissau duras maintained less temperature, indicating their better tolerance to water stress (ICAR, Annual Report 2004-2005).

Patel *et al.* (2005) found that weed species had higher rate of photosynthesis, transpiration rate, stomatal conductance and water use efficiency than crops and were more efficient. Several workers also have reported significant variation in the rate of photosynthesis amongst the different crops. (Basuchoudhari and Dasgupta, 1987; Kumar *et al.*, 1998; Timmannavar and Patil, 2000).

2.4. Anatomy

2.4.1 Stem

The anatomy of most of the *Piper* species is similar with minor variations. The anatomy of stem of *P. nigrum* was described by Metcalfe and Chalke (1950) as dicot stem with anomalous secondary growth. Murthy (1959) noticed the absence of mucilage canal in *P. longum* whereas Pal (1981) reported presence of it. According to Dutta (1970) *Piper* stem exhibited anomalous secondary growth due to the presence of distinct medullary and cortical bundles. Cortical bundles of

different sizes occur in large numbers towards the periphery in an irregular ring. The cortical bundles were bounded internally by a broad wavy band of thick walled and lignified cells called the conjunctive tissue.

Shylaja and Manilal (1992) studied the bark anatomy of four species of *Cinnamomum* and found that they differ in many characters such as nature of sclerenchymatous groups in pericyclic region, nature of phloem rays, distribution of phloem fibres and presence of crystalline inclusions. The differences in the bark structure were reported to be useful in distinguishing the genuiness of true *Cinnamomum* bark and also in the taxonomy of genus.

Ravindran (2000) reported that the T.S. of the orthotropic stem of *P. nigrum* revealed an epidermis made of rectangular cells over which there was a corrugated layer of cuticle. Below the epidermis there were 2-3 layers of collenchymatous hypodermis with many sclerieds distributed in between the cells. A discontinuous band of sclerenchyma was seen inner to collenchymatous hypodermis. Just below the sclerenchymatous band 2-3 layers of chlorenchymatous cells and 4-6 parenchymatous layers were present. The most distinguishing feature of stem anatomy was the distribution of vascular bundles. There was an outer ring of vascular bundles (cortical or peripheral) and an inner ring of medullary bundles. The outer ring consisted of small and large bundles arranged alternatively which were collateral and open. They had a sclerenchymatous cap at the phloem end, below which lie phloem, cambium and xylem. The medullary bundles were larger than cortical bundles. In *P. longum* the epidermis had minute hairs, which were unicellular or multicellular, uniseriate. Below the epidermis, there were patches of sclerenchyma forming a broken ring. There were 18 to 20 peripheral and six medullary bundles. In the middle there was a prominent mucilage canal.

Remashree *et al.* (2005) used anatomical markers to identify the genuine raw drug from *Asoka* and its adulterant *Polyalthia longifolia*. According

to them this method can be used for the floor level checking of raw drugs used in Ayurveda, which will in turn lead to the standardization and quality control.

2.4.2 Root

Aiyer and Kolammal (1966) described the root anatomy of *P. longum* with cork as the outermost tissue, cortex having stone cells and secretory cells, endodermis consisting of rectangular cells, pith in the centre, surrounded by wedge shaped vascular tissue. Medullary ray cells were loaded with starch grains; some have calcium oxalate and oil globules. According to them presence of large quantity of starch grains was peculiar to *P. longum.*

The anatomy of rhizomes of four economically important species of *Curcuma viz. C. longa, C. aromatica, C. amada* and *C. zedonia* showed that all had similar anatomical characters. Variations were noticed in the number and arrangement of primary and secondary vascular bundles, orientation of endodermoid layer, number and shape of starch grains and curcumin containing cells. The number of companion cells was directly proportional to metabolic translocation and deposition of reserve substances, which can be considered as identifying character among the species (Sherlija *et al.*, 1998)

According to Ravindran (2000) the underground root of *P. longum* had a typical dicot structure. The aerial root differs from underground in having more number of xylem and phloem groups and the cortex was made of irregularly shaped closely packed cells.

2.4.3 Leaf

Basically all the plants of the family *Piperaceae* share the same anatomical features. The differences noticed are only minor ones. According to Dutta (1970) *P. longum* had a single layered epidermis which were elliptical and papillose on the abaxial surface of major veins. Hypodermis and palisade were

also unilayered. Spongy tissue was compact with small intercellular spaces. There was a multilayered chlorenchyma and abaxial sclernchyma cap was present. Vascular bundles were open and collateral. Cambial zone was 3-4 layered. Phloem and xylem members were equal in proportion and central mucilage canal was absent. Leaf epidermal studies by Samuel *et al.* (1984) showed that epidermal cells of *P. longum* were larger than *P. nigrum.*

Dagade (2004) reported the anatomical basis of resistance to foot rot disease by *Phytophthora* in *P. nigrum* cultivars *viz* Panniyur – 1 which was susceptible, Kalluvally that was tolerant and *P. colubrinum*, which was immune.

2.4.4 Spike

Farooqi (2000) had reported that the spike of *P. longum* was circular in outline with tangentially elongated parenchymatous tissue filled with mostly starch grains. Vascular strands were occassionly found in this tissue.

2.5 Molecular Markers in Diversity Analysis

Biodiversity of plant species existing in the Indian subcontinent not only have to be build up and maintained but also need an in depth characterization from conventional taxonomic to cellular and molecular levels. The introduction of molecular markers, which include biochemical constituents (e.g. secondary metabolites) and macromolecules, *viz,* proteins and DNA have revolutionized the entire scenario of biological sciences. The discovery of PCR brought about a new class of DNA profiling markers which facilitated the development of marker based tags, variability studies, phylogenetic analysis, marker assisted selection etc.

2.5.1 RAPD Markers

Randomly Amplified Polymorphic DNA (RAPD) is an arbitrary sequence marker developed by Welsh and Mc Clelland in 1991. The application of RAPD and their related modified markers in variability analysis and individual specific genotyping has been largely carried out. RAPD technique has been used for the analysis of diversity and in longer term, the establishment of core collections and identification of duplicates within germplasm collection (Virk *et al.*, 1993).

Piper longum

Parani *et al.* (1997) reported the application of RAPD fingerprinting in selection of micro propagated plants of *P. longum* for conservation. RAPD analysis of twenty micro propagated plants and the mother plant was done using ten random decamer primers. It was reported that 18 micro propagated plants formed a major cluster with the mother plant and others were molecular off types (somaclonal variants).

The molecular basis of genotypic differentiation between male and female plants were studied using RAPD technique in *P. longum* by Banerjee *et al.* (1999). Twenty five females and six males were analysed using 40 decamer primers. It was reported that two RAPD bands consistently appeared only in the plants showing male genotype suggesting the male associated nature of the DNA markers.

The genotypic and morphogenetic differences among three female varieties of *P. longum*- Assam, Calicut and Viswam were reported by Philip *et al.* (2000). RAPD analysis revealed that these varieties were genetically different. Nine decamer RAPD primers were used for the study, and revealed 95% similarity between Viswam and Calicut.

RAPD profiles of thirteen *Piper* species using 20 random primers were used to form genetic similarity indices. The dendrgogram had four clusters; *P. nigrum* and *P. longum* formed two separate clusters. *P. colubrinum* and *P. attenuatum* in third cluster and the fourth cluster had *P. chaba, P. betle, P. arboreum* in it (Murugan, 2002).

The molecular basis of genotypic differentiation between male and female *P. longum* plants using RAPD technique and the development of sex associated markers were reported by Manoj *et al.* (2004).

Kesavachandran *et al.* (2005) conducted genetic fingerprinting of *P. nigrum* and *P. longum* cultivars using RAPD markers. Fourteen land races and three advanced cultivars of *P. nigrum* and eleven land races and one advanced cultivar of *P. longum* were used. Ten primers were used for the study. Cultivar specific single bands were obtained for a few landraces and accessions of both *Piper* sp. RAPD analysis indicated that accession analysed could be differential based on their RAPD profile.

Piper nigrum

Babu (2000) conducted a study to assess the genetic stability in tissue culture derived black pepper plants using RAPD analysis with three selected primers. He observed monomorphic banding pattern for the tissue culture regenerants with their respective source plants.

Pradeepkumar *et al.* (2001) characterized 24 black pepper accessions including 13 landraces and nine advanced cultivars using RAPD markers. Out of 34 primers screened 24 were selected. Cultivar specific bands were obtained for all cultivars except for Panniyur 1, 2 and 3.

RAPD analysis was conducted in 22 cultivars of *P. nigrum* from S. India and one accession each of *P. longum* and *P. colubrinum*. *P. colubrinum* is the

most distinct of the three species. Genetic proximity was found among *P. nigrum* cultivars. Greater divergence was observed among landraces than among advanced cultivars. (Pradeep *et al.*, 2003).

Genetic variability of 49 varieties of black pepper was studied using RAPD and AFLP analysis by Nazeem *et al.* (2005). The dendrogram revealed an average similarity of 63 percent among accessions. Five distinct clusters were observed with 34 varieties, other varieties stood separately which indicated a close genetic relationship among the varieties studied. Among 100 primers screened, 20 were selected.

Piper betle

RAPD analysis using ten primers conducted in selected cultivars of betel vine Kapoori and Bangla were more heterogenous. Bangla cultivars were mostly similar to each other. Only six bands out of total 60 bands were found to be common to cultivars of both types (Ranade *et al.*, 2002).

Verma *et al.* (2004) reported genetic variations within and among the four groups of betelvine- Kapoori, Bangla, Sanchi and others based on RAPD analysis. Kapoori group is the most diverse. Eleven RAPD primes were used for the study.

Other crops

Four elite genotypes of *Vetiveria zizanoides* were analysed by RAPD profiling to develop unique pattern of these genotypes and to assess the genetic diversity. Twelve decamer primers were screened and nine were selected. A divergence of 29-35 percent was estimated to exist in the form of DNA polymorphism among accessions (Shasany *et al.*, 1998)

Eighteen commercial mango cultivars grown in India were assessed for genetic relatedness by Ravishankar *et al.* (2000) through RAPD analysis using 30 primers. Twenty seven primers were found to amplify the DNA.

Sharma *et al.* (2000) used RAPD, protein and morphological markers for the variability analysis in *Podophyllum hexandrum* Royle, an endangered medicinal herb of North West Himalaya. Morphologically the selected 30 accessions were divided into four variant groups. SDS page of root protein regrouped the accessions into two groups with subgroups. RAPD analysis revealed high inter and intra population genetic diversity. The study revealed the inability of protein markers to delineate the accessions into region specific groups and suggested that RAPD was more suitable for the elucidation of genetic diversity.

Ushavani (2003) used morphological, biochemical and molecular markers to fingerprint selected cashew genotypes. RAPD analysis using four primers was done and two primers could distinguish two varieties.

Darokar *et al.* (2003) studied the germplasm diversity in *Aloe* species using RAPD and AFLP analysis. Twenty one accessions of four species were used for the study and RAPD analysis revealed comparable inter and intra specific variations with AFLP results.

Twenty one accessions of *Vetiver* sterile, oil type and non oil type were analysed using RAPD. Nineteen of the accessions clustered around the cultigen, sunshine. (Adams *et al.*, 2003)

Kumar *et al.* (2003) reported a comprehensive account of genetic relationships obtained in the genotype of *Terminalia arjuna* by RAPD analysis. Eighty primers were screened and 65 primers were selected.

Gunter (2003) reported the use of RAPD markers in assessing the genetic similarity among *Alamo* switch grass seed lots from alternate propagation sources. About more than 95% similarity was obtained among all the sources.

RAPD analysis of 10 accessions of *Centella asiatica* collected from different geographical locations of Kerala, grouped them into five clusters which had two groups of three accessions and rest forming each individual clusters. Forty decamer primers were screened and four primers were selected (Krishnan, 2005).

Nair (2005) conducted variability studies in the medicinal plant chakkarakkolli (*Gymnema sylvestre* R.BR.) using morphological, biochemical and molecular markers. Out of the 93 accessions studied for morphological and biochemical characters, eighteen accessions showing high variability were subjected to RAPD analysis.

2.6 Chemical composition

The fruits of *P. longum* contain alkaloids piperine and piplartine (Atal *et al.*, 1975; Narasimham, 2003; Pradeep, 2003)

The essential oil of *P. longum* from berries was found to be a greenish yellow liquid with a yield of 0.6 percent. Caryophyllum had been found to be the major constituent of the oil on chemical analysis. A sesquiterpene hydrocarbon has been isolated and the presence of a compound has been established (Nigam *et al.*, 1968)

Five types of *P. longum* namely Cheematippali, Panniyur, Mala, Pattambi and Kanjur were evaluated for total alkaloid content in the dried spikes of each of the five types. The study revealed that Panniyur possessed the maximum alkaloid content (3%) followed by Pattambi and Kanjur each of which recorded 2.9 percent, Cheematippali (2.83%) and Mala 2.8 percent (Annual report, AICRP on M& AP, 1993)

Shankaracharya *et al.* (1997) reported that *P. longum* contains 1 percent volatile oil, 1.25 percent of piperine and 40 percent starch. The oil was dextrorotatory. The three major compounds of the oil were β-caryophyllene (17%), pentadecane (17.8%) and β-bisabolene (11.16%).

P. longum contain an alkaloid pipernonaline which has mosquito larvicidal activity (Lee, 2000; Yang, 2002)

Madhusudhanan and Vandhana (2001) isolated tetrahydropiperine by column chromatography from the methanol extract of *P. longum* dried fruits. It is the first report of an aryl pentanamide from a natural source.

Natural habitat analyses as well as domesticated trials carried out on the medicinal properties of *P. longum* revealed that wild sample yielded a higher percentage of crude extract than domesticated ones. The content of soluble sugars and starch was more in domesticated samples (Raj *et al.*, 2001).

Anuradha *et al.* (2004) reported that the hexane extract of dried fruits of *P. longum* on fractionation yielded a new alkaloid, isohydro piper longumininine and 2 phenyl propanoic acid derivatives. These alkamides possess insecticidal and anti asthmatic activity.

Long pepper on distillation gave about 0.7 to 0.8 percent essential oil. The oil consists of n-hexadecane, n-heptadecane, n-octadecane, n-nonadecane, ciocosane, n-hencosane, n-thujene, terpenole, zingiberine, p-cymene, p-methoxy acetophenone, dihydrocarveol, phenyl ethyl alcohol etc. Petroleum ether extraction gave sylvatin, sesomin and dieudesmin (Sasikumar, 2004).

Singh and Panda (2005) reported that long pepper contains resin, volatile oil, starch, gum, fatty oil, inorganic matter and alkaloids piperine (1-2%), piperlongumine, piperlonguminine in roots and stem, pipataline, peepuloidin, pipemonaline, feruperine, dihydroferuperine in fruits hydrocinnamic acid and beta sitosterol in leaves.

3. Materials and Methods

The experiment was conducted in the Department of Plant Breeding and Genetics, College of Horticulture during 2003 to 2005. Seven genotypes of *Piper longum* selected from the germplasm maintained at All India Network Programme on Medicinal and Aromatic Plants (AINP on M and AP) and one from Aromatic and Medicinal Plants Research Station (AMPRS), Odakkali were used for the study.

3.1 Experimental Material

In an earlier study, long pepper germplasm maintained at the AINP on Medicinal and Aromatic Plants were grouped into three clusters based on D^2 analysis (Biennial Report, 2000). From these clusters, seven genotypes were selected. They were as follows.

Accession	Male/Female
Assam	Female
Pattambi	Female
Kanjur	Female
Maharashtra	Female
Viswam	Female
NL-84-68	Female
Nilambur	Male
Odakkali	Male

Odakkali, a male genotype was collected from AMPRS, Odakkali. (Plates 1, 2 & 3)

Plate 1: View of the experimental plot

Assam

Kanjur

Maharashtra

NL - 84 - 68

Viswam

Pattambi

Plate 2: The six female accessions used for the study

Nilambur

Odakkali

Plate 3: The two male accessions used for the study

3.2 Methods

Three noded cuttings of the selected genotypes were kept for rooting in polybags during March 2004 in polyhouses. During June 2004 the rooted cuttings were transferred to main field (2 cents). Eight ridges were taken and in each ridge one accession was planted at the rate of three rooted cuttings per pit. About 15 to 20 cuttings were planted in a ridge. Recommended package of practices were followed to establish good crop stand.

3.2.1 Morphological Characters

3.2.1.1 Vegetative Characters

3.2.1.1.1 Stem characters

a. Internodal length

The length in centimeter of two internodes of the main stem selected at random from 10 plants in each accession was measured and mean arrived at.

b. Angle of insertion of reproductive branch

The angle subtended by the spike bearing branch with the main stem was measured in degrees with the help of protractors. Observations were recorded from ten spike bearing branches per accession.

c. Length of reproductive branch

The length of reproductive branch was measured from the base to the tip from ten random plants of each accession and mean arrived at.

3.2.1.1.2 Leaf Characters

Leaf characters like length, width, area, petiole length, colour, texture and shape of leaves of both vegetative and reproductive branches were taken. Ten leaves were chosen at random from all accessions as per the descriptor for medicinal and aromatic plants (Singh *et al.*, 2004)

a. Lamina length

Measured from the base of the midrib to the tip of well developed basal leaves at initial flowering stage and the mean of the leaves arrived at and expressed in centimeter.

b. Lamina width

The maximum width of lamina was measured in centimeter and the mean arrived at.

c. Length to breadth ratio

The length to breadth ratio of the leaves of both vegetative and reproductive branch was calculated from the length and width of the lamina and mean arrived at.

d. Area of leaf lamina

Determined through graph paper method. The leaf boundaries were drawn on the graph paper and area determined by counting the number of squares and expressed in square centimeter.

e. Petiole length

Petiole length was measured in centimeter and mean arrived at.

f. Petiole circumference

Petiole circumference was measured in centimeter and mean arrived at.

g. Shape of lamina, lamina base, lamina margin

Lamina shape was recorded by visual observation as ovate, oblanceolate, obcordate, cordate or other shapes.

Lamina base shape was recorded as truncate, round, cordate or others by visual observation.

The margin was recorded by visual observation as even or wavy.

h. Lamina pubescence

Presence or absence of hairs was scored as 0 for absence and 1 for presence.

i. Lamina texture

Texture was recorded by visual observation as membranous, coriaceous or others.

j. Lamina colour

The colour of lamina was recorded by visual observation.

3.2.1.2 Reproductive Characters

3.2.1.2.1 Spike characters

Spike length, diameter, spike orientation, spike shape, spike colour, fresh weight and dry weight, were recorded for 10 spikes selected at random.

a. Spike length

Spike length was measured in centimeter and mean arrived at.

b. Spike diameter

Spike diameter was measured at the basal region of fully developed spike with the help of strip of paper calibrated in millimetres and centimeters and expressed.

c. Number of spikes on reproductive branch

The number of spikes on reproductive branch was counted and the average was worked out.

d. Days to spike initiation

Number of days taken from planting to spike initiation was recorded.

e. Days to maturity of spike

Ten randomly selected spikes after emergence were tagged, indicating date of emergence on the label. When the spikes attained maturity, the date was noted. From that number of days required for the maturity of spike arrived at.

f. Fresh weight of spike

The weight of spikes immediately after harvesting is recorded.

g. Dry weight of spike

The harvested spikes were dried in partial shade for a period of four to five days and their weight was recorded.

h. Percentage loss in weight after drying

Percentage loss in weight after drying was calculated as loss in weight after drying on fresh weight basis.

i. Spike shape, orientation, and colour

Spike shape was recorded as oblong, cylindrical or others by visual observation.

Spike orientation was recorded by visual observation as erect or suberect.

Spike colour was recorded as reddish brown, grayish brown or greenish black after one year of transplanting when spikes are fully developed.

3.2.2 Statistical analysis

The details of the statistical methods used are given below.

3.2.2.1 Analysis of variance

The data collected were subjected to analysis of variance as given by Panse and Sukhatme (1974).

3.2.3. Physiological parameters

The physiological parameters like rate of photosynthesis, transpiration rate and stomatal conductance were recorded using LICOR-6400 portable photosynthetic system from randomly selected plants of each accession and mean arrived at.

3.2.4. Anatomical Characters

Thin hand section of uniformly aged stem, root, leaves and spikes of eight accessions were taken and stained with saffranine. The sections were mounted in a microscopic slide and covered with cover glass. Then it was viewed to study the anatomical difference under compound microscope equipped with image analyzing software Digi prov. 2.0.

3.2.5 Molecular Characterization

The molecular characterization was done at the Molecular Biology lab of Centre for Plant Biotechnology and Molecular Biology, College of Horticulture. The laboratory equipments used for the study are given as Appendix I.

3.2.5.1 DNA extraction

The DNA was extracted using the Doyle and Doyle (1987) method.

A. Reagents

1. Extraction Buffer (4x)
2. Lysis Buffer
3. TE Buffer
4. Iso-propanol
5. Chloroform: Isoamyl alcohol mixture (24:1, v/v)
6. 5% Sarcosin
7. Ethanol 100% and 70%

Composition of reagents are given in Appendix II.

B. Procedure

Leaf sample (0.5g) was ground in pre-chilled mortar after freezing with liquid nitrogen using a pestle and 3ml of 1X extraction buffer was added. The homogenate was then poured into a 50ml centrifuge tube containing 7.5ml lysis buffer and 1.25ml sarcosin. Then it was incubated at 65°C for 15-20 minutes. The content was mixed well by inversion. Equal volume of chloroform: isoamyl alcohol (24:1) mixture was added to the tube, mixed gently by inversion and centrifuged at 10,000 rpm for 10 minutes at 4°C. The upper aqueous phase was pippetted out and transferred to a 50ml centrifuge tube. 2/3 volume of chilled isopropanol was added into the tube containing the aqueous phase. The contents were mixed gently by inverting and kept at –20°C for half an hour to precipitate DNA. The DNA was pelleted by centrifuging at 10,000 rpm for 5 minutes at 4°C. DNA was washed with 70% and absolute alcohol by centrifuging at 5000 rpm for

2 minutes. The pellet was then allowed to air dry and resuspended in 250µl of TE buffer or sterile milli Q water.

3.2.5.2 Estimation /Quantification of DNA

The quality and quantity of DNA was estimated by electrophoresis and by spectrometry.

3.2.5.2.1 Agarose gel Electrophoresis

A. Reagents

1. Agarose
2. 50X TAE buffer
3. Tracking dye (6X)
4. Ethidium bromide

Composition of reagents is given in Appendix III.

B. Procedure

For preparing 1X TAE buffer 1 ml was taken from the stock solution (50X) and volume was made upto 50ml with sterile water. 30ml TAE buffer was taken in a beaker; 300 mg agarose was added to make 1% gel and boiled in microwave oven for one minute. After cooling 2µl of ethidium bromide was added to this and mixed well. The open end of the gel casting tray was sealed with cellotape and the tray was placed on a horizontal surface. The comb was placed properly and the dissolved agarose was poured into the tray and kept for half an hour at room temperature for solidification. The comb was removed after half an hour. The gel was kept in the electrophoresis unit and the tank was filled with 1X TAE buffer. The well side was directed towards of the cathode. 5µl of DNA sample was pipetted out onto parafilm and mixed well with 4µl of tracking dye. The samples were then loaded carefully into the well using micropipette. The λDNA/*Eco*RI/*Hind*III Double Digest (Bangalore Genei) was used as the molecular weight marker. The cathode and the anode of the electrophoresis unit

were connected to the power pack (Hoefer, USA) Electrophoresis was carried out at a constant voltage of 70V, till the loading dye had covered two third length of gel.

3.2.5.2.2 Gel Documentation

The gel was taken from the electrophoresis unit and viewed under UV light in UV transilluminator. The DNA fluoresces under UV light on account of intercalating ethidium bromide dye. The image was documented and stored using the 'Quantity One' software of the gel documentation system (Biorad).

3.2.5.2.3 Spectrophotometer determination

The DNA samples were diluted 10 times using sterile water and the absorbance read at two specific wavelengths *viz*; 260nm and 280nm. The 260/280 ratio was calculated to check the purity. Pure DNA gives a ratio of 1.8. The DNA in the sample was quantified as per the equation OD of one at 260nm = 50µg/ml DNA.

3.2.5.3 RAPD Analysis

Single decamer primers were used to amplify random sequences in total genome DNA. PCR amplification process involves repeated thermal cycles. The programme standardization involves

a) Denaturation at 94°C for 1 minute

b) Primer annealing at 37.5°C for 1 minute

c) Primer extension at 72°C catalysed by Taq DNA ploymerase enzyme for 2 minutes

d) Cycle repeated 40 times

The resulted products were resolved with electrophoresis technique and visualized by ultraviolet illumination of ethidium bromide stained gels.

The reaction mixture consisted of the following

i) Template DNA – 25ng

ii) dNTP's – 1.5µl/tube

iii) Primer – 1.5µl/tube

iv) 10X assay buffer – 2.5µl/tube

v) $MgCl_2$ – 1 µl /tube

vi) Taq DNA polymersae – 1µl/tube

vii) Sterile milli Q water – to make the volume to 25µl

The reaction mixture was prepared as a master mix for the required number of reactions. The aliquot of the mastermix (dNTPs 1.5µl/tube, primer 1.5µl/tube, 10× assay buffer 2.5µl/tube, $MgCl_2$ 1µl/tube, Taq DNA polymersae – 1µl/tube) was dispensed to 0.5ml PCR tubes into which template DNA 25ng and sterile water were added. The control samples were run without template DNA. The reaction mixture was centrifuged in a micro centrifuge for mixing the components. PCR tubes were loaded in a thermal cycle (PTC-200 MJ Research, USA), which used a preheating lid.

3.2.5.4 Primer screening

Twenty decamer primers obtained from Operon Technologies USA were screened using genomic DNA from Assam. Those primers which gave maximum number of reproducible bands were selected and further used.

3.2.5.5 Statistical methods

The data obtained were analysed using the following methods

i) Similarity coefficient

Pair wise similarity between genotypes (primer wise and pooled) were calculated using Jaccard's similarity coefficient. (SIMQUAL Programme)

$G\ S_{ij} = a/a+b+c$

$G S_{ij}$ – Measure of genetic similarity between individuals i and j.

a – number of polymorphic bands shared by i and j

b – number of bands present in i and absent in j

b – number of bands present in j and absent in i

ii) Construction of Dendrogram

The DNA finger print data were used to construct dendrogram through Unweighted Pair Group Method of Arithmetic Average cluster method of computer software The Numerical Taxonomy System of Multivariate Statistical Programme (NTSYS) pc 2.0 programme (Rohlf, 1998).

3.2.6 Quality components

3.2.6.1 Estimation of essential oil

The essential oil was estimated using Clevenger apparatus Five grams of powdered dry spikes were mixed with 250ml water and kept for distillation for three hours. The amount of oil collected was read from the calibrated tube and expressed as percentage.

3.2.6.2. Estimation of piperine

100 mg of long pepper powder was mixed with100 ml acetone and kept in dark for 2 hours. Aliquote (0.1ml) was taken and diluted to 5 ml with acetone. Absorbance was noted at 337nm in a UV spectrophotometer. Standard curve was prepared with piperine and the content of piperine in the sample was calculated and expressed as percentage.

4. RESULTS

Results of observations on the morphological, anatomical, molecular markers and quality components of the eight accessions *viz.*, Assam, Nilambur, Kanjur, Maharashtra, NL-84-68, Viswam, Odakkali and Pattambi are presented in this chapter.

4.1 Morphological characters

The results of the analysis of variance of the morphological characters are given on Appendix 4.

4.1.1 Vegetative characters

4.1.1.1 Stem characters

a) Length of reproductive branch

Analysis of variance showed that the vine length of the spike bearing branch of the eight accessions differ significantly with respect to each other as given in Table 1. Maharashtra recorded the maximum vine length of 26.60 cm and Viswam recorded the minimum vine length of 18.10 cm. The male accession Nilambur which had a length of 23.95 cm was on par statistically with Kanjur (25.9 cm). The other accessions Pattambi (21.5 cm), Assam (21.2 cm) and Odakkali (21.2 cm) were on par statistically. The mean vine length for female accessions was 22.35cm and for male accession it was 22.58 cm.

b) Internodal length

The mean data on internodal length are given in Table 1. Analysis of variance showed that the internodal length of the eight accessions differ significantly. NL-84-68 had the maximum internodal length of 10.08 cm and Assam recorded the minimum internodal length of 6.82 cm. The internodal length of Viswam (7.23 cm) and male accession Odakkali (6.93 cm) were on par statistically with Assam. The other accessions Pattambi, Nilambur, Kanjur, and

Table 1. Mean of different accessions for various quantitative traits of stem

Accession	Vine length(cm)	Angle of insertion of reproductive branch(degrees)	Internodal length(cm)
Assam	21.20^{bc}	73.20^{a}	6.82^{c}
Kanjur	25.90^{ab}	61.00^{a}	8.14^{b}
Maharashtra	26.60^{a}	56.50^{a}	8.10^{b}
NL 84-68	20.80^{bc}	59.50^{a}	10.03^{a}
Pattambi	21.50^{bc}	62.00^{a}	8.48^{c}
Viswam	18.10^{c}	69.50^{a}	7.23^{c}
Odakkali	21.20^{bc}	58.40^{a}	6.93^{c}
Nilambur	23.95^{ab}	57.00^{a}	8.21^{b}
Mean for female accessions	22.35	63.62	8.13
Mean for male accessions	22.58	57.70	7.57
Grand mean	22.41	70.89	7.99
Min	18.10	56.50	6.82
Max	26.60	73.20	10.03
CD 5%	4.649	68.38	0.7924

Treatments with the same alphabet do not differ significantly

Maharashtra recorded internodal length of 8.48 cm, 8.21 cm, 8.14 cm and 8.1 cm respectively and they did not differ significantly. The female accessions recorded a mean value of 8.13 cm and male accessions recorded a mean value of 7.57 cm.

c) Angle of insertion of reproductive branch

Angle of insertion of reproductive branch did not reveal any significant difference among eight long pepper accessions (Table 1). The mean values ranged from 56.5° (Maharashtra) to 73.20° (Assam). In general the male accessions Nilambur and Odakkali recorded lower values (57° and 58.4° respectively) for this character when compared to female accessions.

4.1.1.2 Leaf characters

The observations for various leaf characters on the vegetative and reproductive branch are given in Table 2. Plates 4 and 5 show the leaves on vegetative and reproductive branch of the different accessions.

a) Lamina length

The length of leaf on vegetative branch differ significantly among eight accessions. The mean length of lamina was maximum for NL-84-68 (11.25 cm) and minimum for Viswam (6.37 cm). The male accession Odakkali had a mean of 10.16 cm and Nilambur recorded 9.11 cm lamina length. Kanjur which recorded a lamina length of 9.98 cm was on par with these male accessions. The other female accessions Pattambi, Maharashtra and Assam recorded lamina length of 8.81 cm, 8.8 cm and 8.51 cm respectively.

Analysis of variance showed that the eight accessions differ significantly for lamina length of leaves produced by reproductive branch. The longest leaves were observed for the male accession, Nilambur (8.97cm) and Pattambi recorded the shortest lamina length (4.49cm) followed by Viswam (5.14cm). The other male accession Odakkali exhibited a mean length of 6.78 cm,

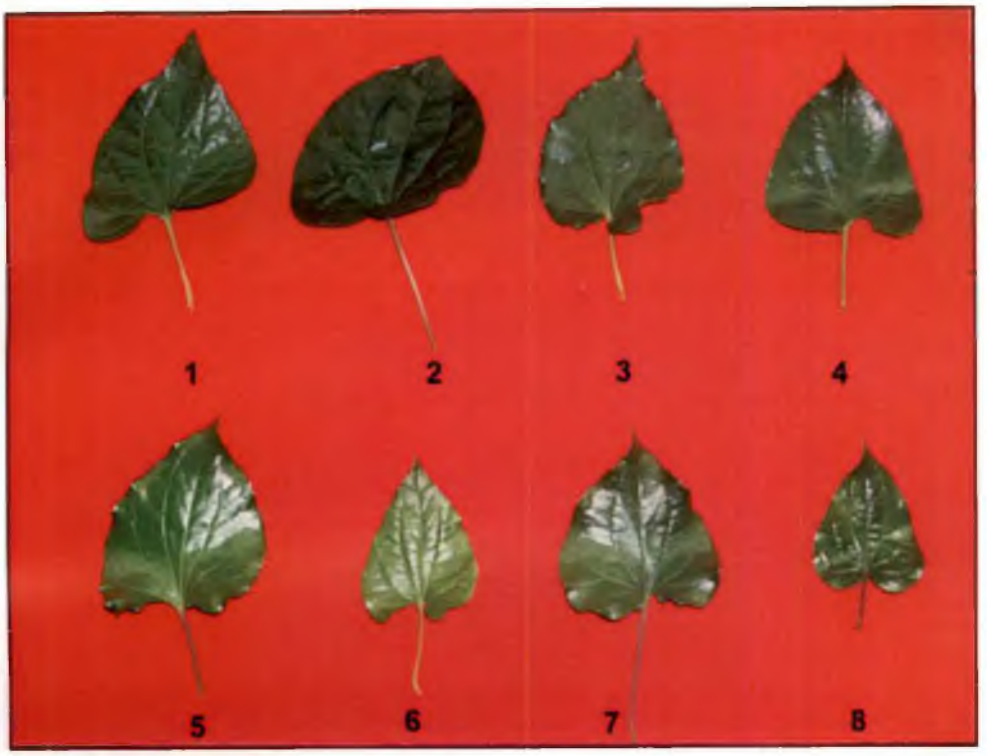


Plate 4: Morphological variations for the leaves of vegetative branch of the eight accessions

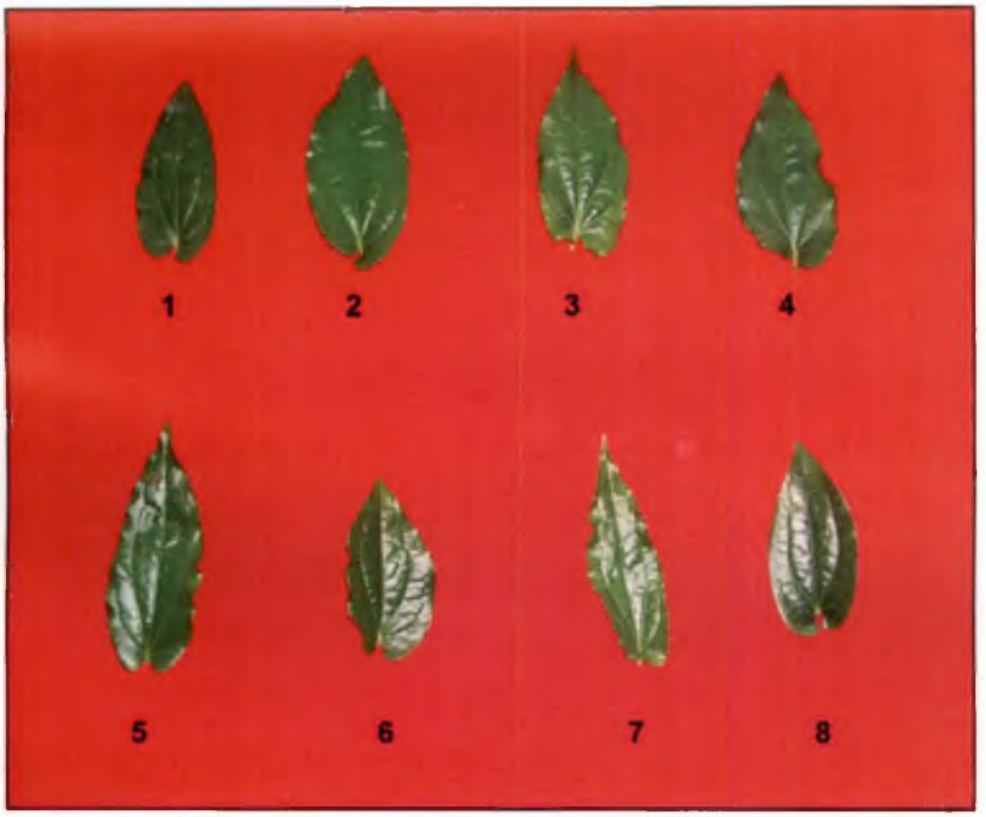


Plate 5 : Morphological variations for the leaves of reproductive branch of the eight accessions

1. Assam	2. Nilambur	3. Kanjur	4. Maharashtra
5. NL - 84 - 68	6. Viswam	7. Odakkali	8. Pattambi

which was on par with Assam (6.61 cm). The mean lamina length of Maharashtra was 6.13 cm.

b) Lamina width

Data on lamina width of leaf of vegetative branch indicated statistically significant difference between the eight accessions as given in Table 2. The maximum width was recorded by NL-84-68 (10.15 cm) and lowest width was recorded by Viswam (6.41 cm). Kanjur with a mean lamina width of 9.39 cm was on par statistically with NL-84-68 and male accession Nilambur (9.25 cm). Assam recorded 8.7 cm mean lamina width, which was on par statistically with male accessions Nilambur (9.27cm), Odakkali (9.03 cm) and the female accessions Pattambi (8.2 cm) and Maharashtra (8.05 cm).

The lamina width of leaf of the reproductive branch also differs significantly for the eight accessions (Table 2). The highest mean width of 4.06 cm was recorded by NL-84-68 and lowest by male accession Odakkali and female accession Kanjur (2.5 cm). The female accession Pattambi and Maharashtra recorded a mean width of 2.64 cm and 2.61 cm respectively and were on par with Odakkali and Kanjur for this character. Nilambur had a mean of 3.79 cm whereas Assam and Viswam recorded a lamina width of 3.39 cm and 3.14 cm respectively.

The overall results on leaf lamina width indicated that the leaves on reproductive branch recorded lower values when compared to leaves on vegetative branch.

c) Length to breadth ratio

The length to breadth ratio of leaves of vegetative and reproductive branch is given in Table 2. The results indicate that leaves of reproductive branch had higher length to breadth ratio than the leaves of vegetative branch. The length to breadth ratio was highest for the male accession, Odakkali for both the leaves of vegetative branch and reproductive branch. Analysis of variance showed that the accessions differ significantly at 5 percent level for the l/b ratio of leaves of vegetative branch. For the leaves of reproductive branch it was significant at both

1 and 5 per cent levels. The lowest ratio for leaves of vegetative branch was recorded by Assam (0.979) and for the leaves of reproductive branch the lowest ratio was recorded by Viswam (1.667). NL –84-68, though ranked second in l/b ratio (1.111) for the leaves of vegetative branch was statistically on par with the other accessions Maharashtra (1.095), Pattambi (1.079), Kanjur (1.069), Viswam (1.001) and Nilambur (0.998). The female accession Kanjur and the male accession Nilambur recorded a ratio of 2.521 and 2.442 respectively for the leaves of reproductive branch. These two accessions were on par statistically with Odakkali and Maharashtra (2.386). Assam and Pattambi recorded a ratio of 1.972 and 1.782 for the same character and were on par statistically with NL-84-68 (2.056) and Viswam.

d) Area of leaf lamina

Analysis of variance showed that the eight accessions differed significantly for the area of lamina of leaf on vegetative branch as given in Table 2. The highest mean value for this character was observed in NL-84-68 (96.25 cm^2) where as the lowest value was recorded by Viswam (40.43 cm^2). The male accession Nilambur recorded an area of 87.80 cm^2, which was on par with NL-84-68 and Kanjur (83.07 cm^2). Maharashtra recorded a mean leaf area of 67.69 cm^2, was on par statistically with Assam, which had a leaf area of 64.79 cm^2. Kanjur and Maharashtra was on par statistically with the male accession Odakkali (75.34 cm^2) for this character.

The leaves on the reproductive branch showed significant difference for leaf area for all the eight accessions as given in Table 2. The area was maximum for the male accession Nilambur (28.0 cm^2) and the lowest area was recorded by Pattambi (10.53 cm^2). NL-84-68 recorded an area of 25.42 cm^2 which was on par with Nilambur. Assam recorded a mean area of 19.44 cm^2 followed by the female accessions, Maharashtra and Kanjur with a mean area of 14.52 cm^2 and 13.74 cm^2 respectively. The reproductive leaf area for Odakkali was 13.28 cm^2 and it was on par with Viswam (13.04 cm^2).

Table 2. Mean of different accessions for various quantitative traits of leaf

Accession	Petiole circumference(cm)	Petiole Length(cm)	Vegetative leaf				Reproductive leaf			
			Length (cm)	Width (cm)	Area (cm^2)	Length/ Breadth	Length (cm)	Width (cm)	Area (cm^2)	Length/ breadth
Assam	0.86^{bc}	5.14^{de}	8.51^{d}	8.70^{bcd}	64.79^{cd}	0.979^{c}	6.61^{cd}	$3.39b^{c}$	19.44^{b}	1.972^{cd}
Kanjur	1.03^{a}	5.51^{cd}	9.98^{bc}	9.39^{ab}	83.08^{b}	1.064^{abc}	6.2^{d}	2.50^{d}	13.74^{c}	2.521^{ab}
Maharashtra	0.82^{c}	6.57^{bc}	8.80^{d}	8.05^{d}	67.69^{cd}	1.095^{ab}	6.13^{d}	2.61^{d}	14.52^{c}	2.386^{b}
NL 84-68	1.01^{a}	7.58^{ab}	11.25^{a}	10.15^{a}	96.26^{a}	1.111^{ab}	8.31^{b}	4.06^{a}	25.42^{a}	2.056^{c}
Pattambi	0.94^{ab}	4.29^{e}	8.81^{d}	8.20^{cd}	59.81^{d}	1.079^{abc}	4.49^{f}	2.64^{d}	10.53^{d}	1.782^{cd}
Viswam	0.80^{c}	4.67^{de}	6.37^{e}	6.41^{e}	40.43^{e}	1.001^{bc}	5.14^{e}	3.14^{c}	13.04^{cd}	1.667^{d}
Odakkali	0.96^{a}	5.54^{cd}	10.16^{b}	9.03^{bc}	75.34^{bc}	1.127^{a}	6.78^{c}	2.50^{d}	13.28^{cd}	2.722^{a}
Nilambur	1.00^{a}	7.81^{a}	9.11^{cd}	9.27^{b}	87.80^{ab}	0.998^{bc}	8.97^{a}	3.79^{ab}	28.00^{a}	2.442^{ab}
Mean for female accessions	0.91	5.63	8.95	8.48	68.68	1.05	6.15	3.06	16.12	2.06
Mean for male accessions	0.98	6.68	9.64	9.15	81.57	1.06	7.88	3.15	20.64	2.58
Grand mean	0.93	5.89	9.12	8.65	71.90	1.057	6.61	3.08	17.25	2.193
Min	0.80	4.29	6.37	6.41	40.43	0.979	4.49	2.50	10.53	1.667
Max	1.03	7.81	11.25	10.15	96.26	1.111	8.97	4.06	28.00	2.722
CD 5%	0.08915	1.051	0.9393	0.6171	12.44	0.1016	0.5488	0.4367	2.749	0.2957

Treatments with the same alphabet do not differ significantly

e) Petiole length

Analysis of variance showed that the petiole length of the eight accessions showed significance with respect to each other (Table 2). Nilambur recorded the maximum petiole length of 7.81 cm and the minimum petiole length was recorded by the female accession, Pattambi (4.29 cm). NL-84-68 recorded a mean value of 7.58 cm, which was on par statistically with Nilambur and Maharashtra, (6.57 cm). Odakkali and Kanjur recorded mean petiole length of 5.54 cm and 5.14 cm respectively and were on par statistically. Assam recorded a mean petiole length of 5.14 cm and Viswam had a mean of 4.67 cm, which were on par with Pattambi (4.29 cm), Kanjur and Odakkali.

The petioles on the leaves of reproductive branch were either too short or absent.

f) Petiole circumference

The mean values for the petiole circumference of the eight accessions are given in Table 2. The analysis of variance showed that they did not differ significantly. Kanjur recorded the maximum petiole circumference (1.03cm) followed by NL-84-68 (1.01 cm). The male accessions Nilambur and Odakkali recorded a mean of 1 cm and 0.96 cm respectively. Maharashtra recorded a mean of 0.82 cm followed by Viswam, which had the lowest mean petiole circumference of 0.80 cm .The female accession Assam (0.86cm) was on par with Pattambi, Maharashtra and Viswam for this character.

g) Shape of lamina, lamina base, and margin

The qualitative characters of the leaf like lamina shape, lamina base shape, margin, lamina pubescence, lamina texture and lamina colour are given in Table 3 and 4.

The shape of the lamina for all the eight accessions for the leaves of the vegetative branch was cordate, whereas it was lanceolate for the leaves of the reproductive branch.

Table 3. Qualitative characters of leaves of vegetative branch

Accession	Shape of		Margin	Pubescence	Texture	Colour
	Lamina	Lamina base				
Assam	Cordate	Cordate	Even	Glabrous	Membranous	Dark green
Kanjur	Cordate	Cordate	Wavy	Glabrous	Membranous	Dark green
Maharashtra	Cordate	Cordate	Even	Glabrous	Membranous	Dark green
NL 84-68	Cordate	Cordate	Wavy	Glabrous	Membranous	Dark green
Pattambi	Cordate	Cordate	Even	Glabrous	Membranous	Dark green
Viswam	Cordate	Cordate	Slightly wavy	Glabrous	Membranous	Medium green
Odakkali	Cordate	Cordate	Wavy	Glabrous	Membranous	Dark green
Nilambur	Cordate	Cordate	Even	Glabrous	Membranous	Dark green

Table 4. Qualitative characters of leaves of reproductive branch

Accession	Shape of		Margin	Pubescence	Texture	Colour
	Lamina	Lamina base				
Assam	Lanceolate	Unequally cordate	Slightly wavy	Glabrous	Membranous	Dark green
Kanjur	Lanceolate	Unequally cordate	Wavy	Glabrous	Membranous	Dark green
Maharashtra	Lanceolate	Unequally cordate	Wavy	Glabrous	Membranous	Dark green
NL 84-68	Lanceolate	Unequally cordate	Wavy	Glabrous	Membranous	Dark green
Pattambi	Lanceolate	Unequally cordate	Even	Glabrous	Membranous	Dark green
Viswam	Lanceolate	Unequally cordate	Wavy	Glabrous	Membranous	Dark green
Odakkali	Lanceolate	Unequally cordate	Wavy	Glabrous	Membranous	Medium green
Nilambur	Lanceolate	Unequally cordate	Even	Glabrous	Membranous	Dark green

Cordate shaped lamina base was observed for male and female accessions for the leaves of vegetative branch. It was unequally cordate in the case of leaves of the reproductive branch for all the accessions.

Assam, Nilambur, Maharashtra, Pattambi had even margin for the leaves of the vegetative branch. The margin was wavy for Kanjur, NL-84-68 and Odakkali, where as Viswam exhbited slightly wavy margins.

Only Pattambi had even margin for the leaves of reproductive branch. Kanjur, Maharashtra, NL-84-68, Viswam, and Odakkali had wavy margin. The margin was slightly wavy for Assam and the male accession Nilambur.

h) Lamina pubescence

Glabrous leaves were produced by both vegetative and reproductive branch of all accessions.

i) Lamina texture

The leaves of the vegetative and reproductive branches had membranous texture for all the accessions studied.

j) Lamina colour

All the accessions had dark green colour for the leaves of the vegetative branch except Viswam, which had medium green colour. In the case of leaves of reproductive branch all accessions except Odakkali (medium green) recorded dark green colour.

4.1.2 Reproductive characters

4.1.2.1 Spike characters

The spikes of the different accessions are given in Plate 6.

a) Spike length

Analysis of variance showed that there was significant difference for spike length among the accessions as given in Table 5. The male accessions

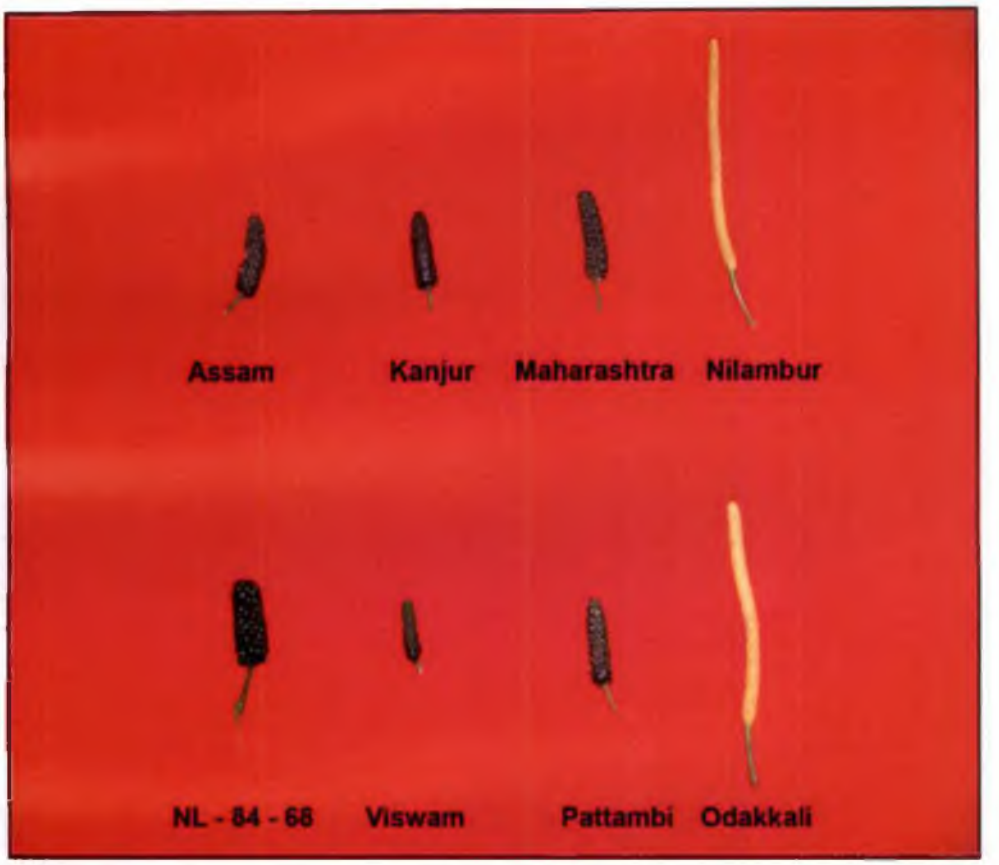


Plate 6 : Comparitive study of spikes of the eight accessions

Nilambur and Odakkali produced longest spike with a mean length of 7.55 cm and 7.31 cm respectively. Among the female accessions, NL-84-68 recorded the longest spike length (4.23cm), followed by Maharashtra (3.77cm), Assam (3.68 cm), Pattambi (3.68cm), Kanjur (3.5cm) and they were on par statistically. Viswam recorded the shortest spike length (2.4cm).

b) Spike diameter

The mean data on spike diameter is given in Table 5. The analysis of variance showed that the accessions differ significantly for the spike diameter. The female accession NL-84-68 recorded the maximum diameter of 3.59cm. The male accessions Nilambur and Odakkali recorded minimum spike diameter of 1.31 cm and 1.4 cm respectively. The female accession Viswam that recorded a spike diameter of 1.53 cm was on par with the male accessions. The other female accessions Kanjur, Maharashtra, Pattambi and Assam recorded spike diameter of 2.41cm, 2.32 cm and 2.23 cm respectively.

NL-84-68 has the boldest spike in terms of spike length and spike diameter. The male spikes though longest had low mean values for spike diameter (1.38cm) compared to the female accessions (2.39 cm).

c) Number of spikes per spike bearing branch

Analysis of variance showed that there was no significant difference among the accessions for the number of spikes per spike bearing branch. A maximum of four spikes per branch was recorded for Assam followed by Viswam and male accession Odakkali (3.6 spikes). Pattambi recorded a mean of 3.1 spikes, which was on par statistically with the other accessions Kanjur and Nilambur, both recorded 3 spikes per spike bearing branch. Maharashtra recorded 2.9 spikes and NL-84-68 recorded the minimum number of spikes per spike bearing branch (2.6).

d) Days to spike initiation

The mean data for the number of days taken from planting to spike initiation was given in Table 5. There was significant difference among the

accessions for the character. NL-84-68 took the maximum number of days (178) for spike initiation. The male accessions took the minimum days for spike initiation. Nilambur took 132 days whereas Odakkali took 135 days and they were on par statistically. Assam was on par statistically with NL-84-68 and it took 175 days for spike initiation. The female accession Pattambi recorded 162 days for spike initiation. Kanjur which took 157 days was on par statistically with Pattambi, Viswam (156 days) and Maharashtra (152 days) for spike initiation. The female accessions took more days (163.7) compared to male accessions (133.2) for spike initiation.

e) Days to maturity of spike

Analysis of variance showed that there was significant difference among the accessions for days to maturity of spike as given in Table 5. NL-84-68 recorded the maximum number of days for maturity of spike (69.6). The male accessions Odakkali and Nilambur which took 56 days for maturity recorded the minimum days. Pattambi and Viswam recorded 65 days for maturity of spike followed by Maharashtra (64 days), Assam (63 days) and Kanjur (62 days). In this case also female accessions required more days for maturation (64.87) compared to male accessions (56.3).

f) Fresh weight of spike

The mean weight of the spikes immediately after harvest was recorded and is given in Table 5. The analysis of variance showed that the eight accessions differed significantly for the character. NL-84-68 with 1.639g fresh weight recorded the maximum mean spike weight. Maharashtra recorded 1.58g and the male accession Nilambur had a mean fresh weight of 0.963g. The other accessions Pattambi, Odakkali, Assam recorded a mean of 0.7384g, 0.6775g, 0.59g respectively, were on par statistically. Viswam recorded the lowest fresh weight of 0.36g per spike.

Table 5. Mean of different accessions for various quantitative traits of spike

Accession	Number of days for		Spike number	Spike length (cm)	Spike diameter (cm)	Fresh weight (g)	Dry weight (g)	Loss in weight after drying (%)
	Spike initiation	Spike maturation						
Assam	175.3[a]	63.3[bc]	4.00[a]	3.68[b]	2.23[b]	0.590[d]	0.083[d]	85.11[a]
Kanjur	157.4[bc]	61.9[c]	3.00[bc]	3.5[b]	2.41[b]	0.761[d]	0.194[b]	74.91[b]
Maharashtra	152.4[c]	64.1[bc]	2.90[bc]	3.77[b]	2.32[b]	1.158[b]	0.204[b]	82.82[a]
NL 84-68	178.3[a]	69.6[a]	2.60[c]	4.23[b]	3.59[a]	1.639[a]	0.271[a]	83.34[a]
Pattambi	162.4[b]	65.3[b]	3.10[bc]	3.68[b]	2.23[b]	0.738[d]	0.098[cd]	85.56[a]
Viswam	156.4[c]	65.0[b]	3.60[ab]	2.4[c]	1.53[c]	0.362[e]	0.107[cd]	69.93[c]
Odakkali	134.8[d]	56.3[d]	3.60[ab]	7.31[a]	1.45[c]	0.677[d]	0.107[cd]	84.22[a]
Nilambur	131.6[d]	56.3[d]	3.00[bc]	7.55[a]	1.31[c]	0.963[c]	0.132[c]	86.28[a]
Mean for female accessions	163.70	64.87	3.20	3.54	2.39	0.87	0.16	80.28
Mean for male accessions	133.20	56.30	3.30	7.43	1.38	0.82	0.12	85.25
Grand mean	156.08	62.73	3.23	4.515	2.13	0.861	0.149	81.52
Min	131.6	56.3	2.60	2.4	1.31	0.362	0.083	69.93
Max	178.3	69.6	4.00	7.55	3.59	1.639	0.271	86.28
CD 5%	5.09	2.77	0.7648	0.7721	0.5832	0.1738	0.03987	3.461

Treatments with the same alphabet do not differ significantly

g) Dry weight of spike

The eight accessions differed significantly for the dry weight of spike as shown by analysis of variance. The mean data is given in Table 5. The maximum dry weight was recorded by NL-84-68 (0.271g) followed by Maharashtra (0.204 g) and Kanjur (0.194 g). Nilambur recorded a dry weight of 0.132g. The other male accession Odakkali, and the female accession Viswam recorded 0.107g of dry weight each. Pattambi (0.098g) was on par statistically with Odakkali and Viswam.

h) Percentage loss in weight after drying

Analysis of variance showed that the eight accessions differ significantly for the percentage loss in dry weight. The maximum percentage loss in weight was recorded by the male accession Nilambur (86.28%) and the minimum by the female accession, Viswam (69.93%). Nilambur was followed by Pattambi (85.55%), Assam (85.11%), Odakkali (84.22%), NL-84-68 (83.34%), and Maharashtra (82.81%) and were on par statistically. Kanjur recorded 74.91 percent loss in weight after drying.

i) Spike shape, orientation, and colour

The qualitative characters of the spike like spike shape, orientation and colour are given in Table 6.

All the male and female accessions had cylindrical spikes.

Erect spikes were observed in male accessions whereas the female accessions had suberect spikes. (Plate 7)

At early stages of spike growth, creamy yellow colour is observed for female accessions whereas for male accessions the colour is green. Later, on maturity the spikes were greenish black for all the female accessions and yellow in colour for the male accessions.

Table 6. Qualitative characters of spike

Accession	Sex	Shape	Orientation	Colour at initiation	Colour at maturity
Assam	Female	Cylindrical	Sub erect	Yellow	Greenish black
Kanjur	Female	Cylindrical	Sub erect	Yellow	Greenish black
Maharashtra	Female	Cylindrical	Sub erect	Yellow	Greenish black
NL 84-68	Female	Cylindrical	Sub erect	Yellow	Greenish black
Pattambi	Female	Cylindrical	Sub erect	Yellow	Greenish black
Viswam	Female	Cylindrical	Sub erect	Yellow	Greenish black
Odakkali	Male	Cylindrical	Erect	Green	Yellow
Nilambur	Male	Cylindrical	Erect	Green	Yellow

Sub erect spike of the female accession Assam

Erect spike of the male accession Odakkali

Plate 7: Comparison of the spike orientation of male amd female accessions

4.2. Physiological parameters

a. Rate of photosynthesis

Data on the mean rate of photosynthesis for different accessions are given in Table 8. The analysis of variance showed that the eight accessions differ significantly for the character. The highest photosynthetic rate was observed in the female accession, Viswam (12.15 μmol m^{-2} s^{-1}) followed by Assam (11.75 μmolm^{-2} s^{-1}), Kanjur (11.55 μmol m^{-2} s^{-1}) and NL-84-68 (11.3 μmol m^{-2} s^{-1}). Among the female accessions, Pattambi recorded the minimum photosynthetic rate (8.15 μmol m^{-2} s^{-1}). The lowest rate was recorded by the male accession, Nilambur (7.5 μmol m^{-2} s^{-1}) whereas Odakkali recorded a photosynthetic rate of 10.48-μmol m^{-2} s^{-1}. The mean photosynthetic rate for the male accessions (9.03 μmol m^{-2} s^{-1}) was lower to female accessions (10.82 μmol m^{-2} s^{-1}).

b. Transpiration rate

Data on the mean value for transpiration rate for the eight accessions are given in Table 8. Analysis of variance showed significant difference among the accessions for transpiration rate. The mean transpiration rate for the female accessions was recorded as 3.66 millimol m^{-2} s^{-1} and ranged between 2.52 milli mol m^{-2} s^{-1} (Viswam) to 5.28 millimol m^{-2} s^{-1} (Kanjur). Odakkali and NL-84-68 recorded a transpiration rate of 4.12 and 3.87 millimol m^{-2} s^{-1} respectively. The female accession Maharashtra and the male accession Nilambur, both recorded transpiration rate of 3.69 millimol m^{-2} s^{-1} followed by Assam (3.65millimol $m^{-2}s^{-1}$) and were on par statistically with NL-84-68 and Pattambi (2.95millimol m^{-2} s^{-1}).

c. Stomatal conductance

Analysis of variance showed that the eight accessions differ significantly for this character (Table 7). The maximum conductance was recorded by Kanjur (0.27 mol m^{-2} s^{-1}), NL-84-68 and Maharashtra recorded 0.18 mol m^{-2} s^{-1} and they were on par statistically with the female accession, Viswam (0.13 mol m^{-2} s^{-1}). Assam, Pattambi and the male accessions Nilambur, Odakkali

Table 7. Mean of different accessions for various physiological parameters

Accession	Rate of Photosynthesis $\mu molm^{-2}s^{-1}$	Stomatal Conductance $molm^{-2}s^{-1}$	Transpiration rate $millimolm^{-2}s^{-1}$
Assam	11.75^{a}	0.17^{b}	3.65^{bc}
Kanjur	11.55^{a}	0.27^{a}	5.28^{a}
Maharashtra	10.04^{b}	0.18^{b}	3.69^{bc}
Nl-8468	11.30^{a}	0.18^{b}	3.87^{b}
Pattambi	8.15^{c}	0.17^{b}	2.95^{cd}
Viswam	12.15^{a}	0.13^{b}	2.52^{d}
Odakkali	10.48^{b}	0.22^{a}	4.12^{b}
Nilambur	7.57^{d}	0.17^{b}	3.69^{bc}
Mean for female accessions	10.82	0.18	3.66
Mean for male accessions	9.03	0.20	3.91
Grand mean	10.37	0.19	3.72
Min	7.57	0.13	2.52
Max	12.15	0.27	5.28
CD 5%	0.8872	0.05242	1.101

Treatments with the same alphabet do not differ significantly

had a conductance of 0.17 mol m^{-2} s^{-1}. The mean stomatal conductance for male accessions was 0.20 mol m^{-2} s^{-1} and for female accessions, it was 0.18 mol m^{-2} s^{-1}.

4.3 Anatomy of *Piper longum*

The stem of *P. longum* exhibit anomalous secondary growth. It has medullary bundles in the centre which are large and cortical bundles which are small towards the periphery. Cortical bundles though small are numerous in number. The medullary and cortical bundles are separated by a layer of sclerenchymatous tissue, which forms a wavy ring. The root of *P. longum* has a dicot root structure. The upper leaf epidermis consists of an outer layer of cuticle and epidermal cells with trichomes. The mesophyll region consists of round to ovoid mesophylls, spongy parenchyma and chlorenchymatous palisade tissue. Open, collateral vascular bundles with xylem phloem and cambium are observed in the midrib region. Stomata were seen in the lower epidermis.

4.3.1. Comparative evaluation for anatomical variability

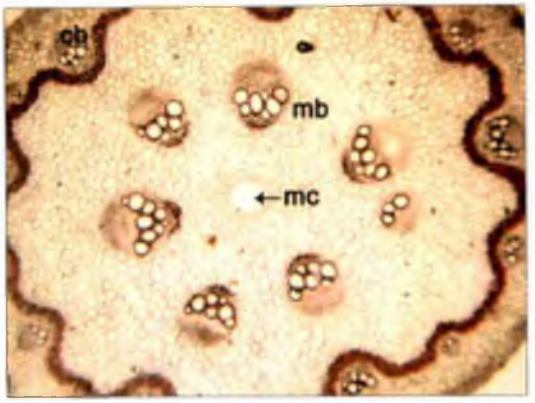
All the accessions have the same anatomical features for stem, root, and leaf (Plate 8, 9 and 10) except for the number of cortical and medullary bundles in the stem and root as well as the presence of mucilage canal in stem (Table 8). However the male and female spikes showed different anatomical features (Plate 11 and 12)

4.3.1.1. Stem

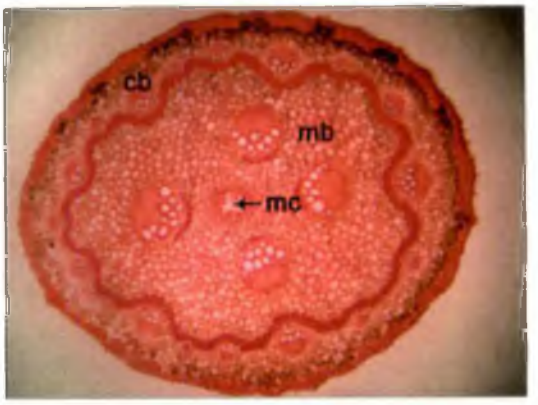
Viswam had the maximum number of medullary (7) and cortical bundles (20) for the vegetative branch. A minimum of four medullary bundles was observed for the female accessions Assam, Pattambi, NL-84-68 and the male accessions Nilambur and Odakkali. Maharashtra and Kanjur had six medullary bundles each. Maharashtra had the minimum number of cortical bundles (15) for the vegetative branch. There were seventeen cortical bundles for the female

Table 8. Observations on the anatomy of stem, root, leaf

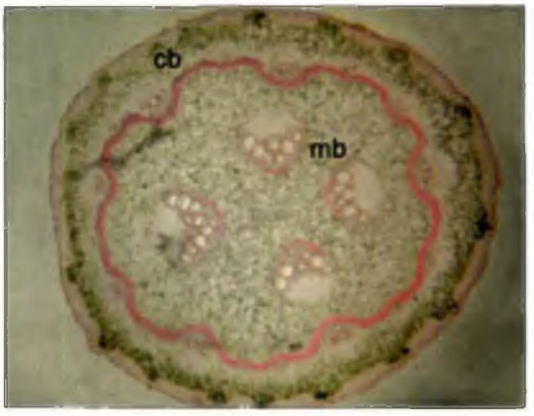
Accession	Vegetative stem		Reproductive stem		Root	Mucilage canal	
	Medullary bundles	Cortical bundles	Medullary bundles	Cortical bundles	Vascular bundles	Stem	Root
Assam	4	17	5	15	6	Present	Absent
Kanjur	6	19	5	15	9	Absent	Absent
Maharashtra	6	15	6	16	10	Absent	Absent
NL 84-68	4	19	5	15	7	Present	Absent
Pattambi	4	17	4	16	5	Absent	Absent
Viswam	7	20	6	15	7	Present	Absent
Odakkali	4	17	6	16	10	Absent	Absent
Nilambur	4	19	4	16	10	Absent	Absent



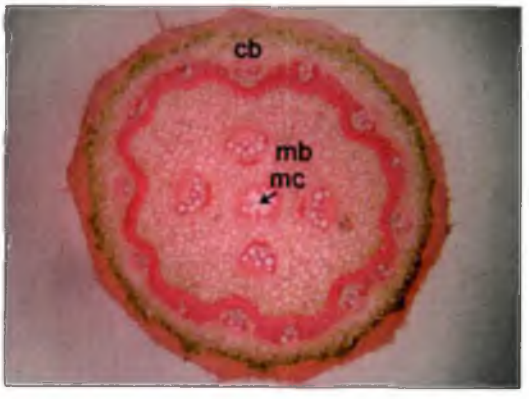
Viswam



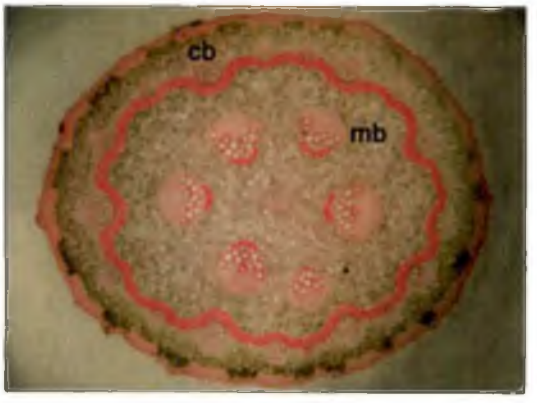
NL- 84-68



Nilambur (Male)



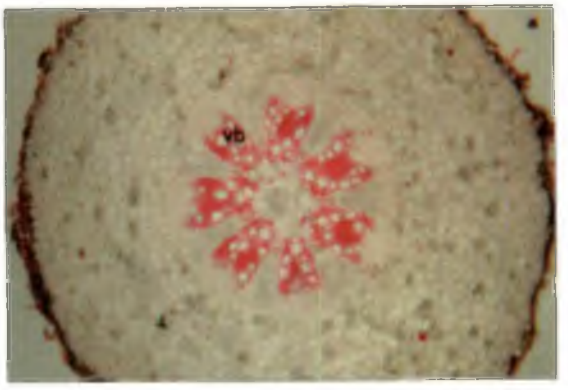
Assam



Maharashtra

Plate 8: T.S. of the Vegetative stem of different accessions

mc- mucilage canal mb- medullary bundle cb- cortical bundle



Viswam

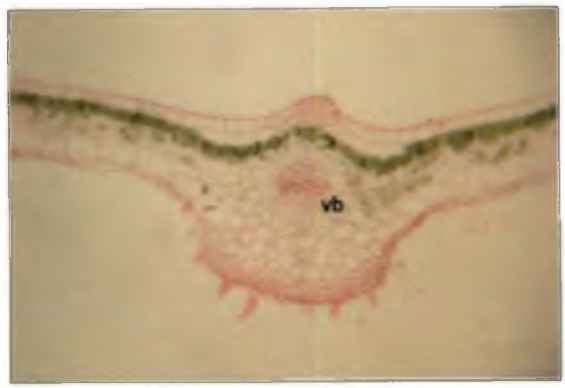
Maharashtra

Assam

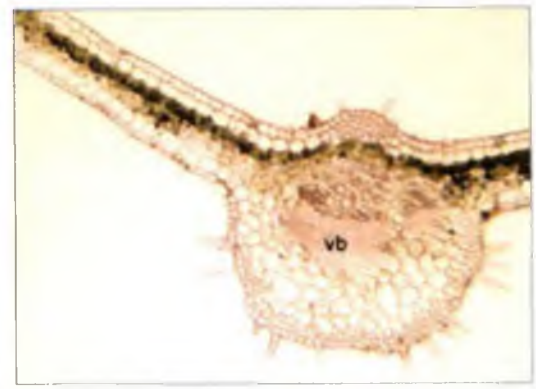
Nilambur (Male)

Plate 9: T.S. of the root of different accessions

vb- vascular bundles

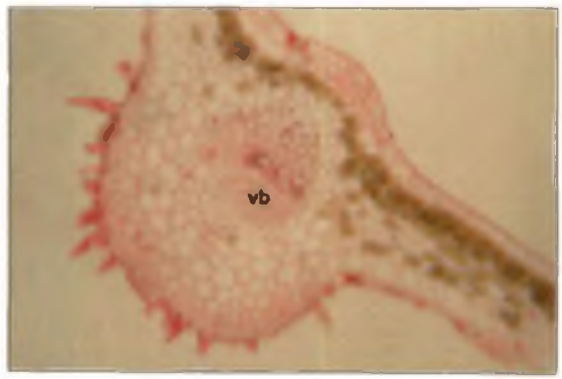


Viswam

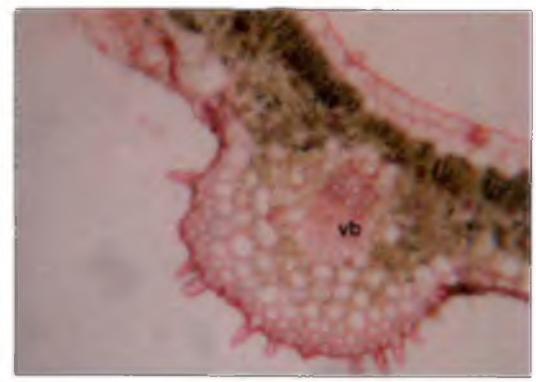


NL-84-68

Nilambur (Male)



Assam



Maharashtra

Plate 10: T.S. of the Vegetative leaf of different accessions

vb- vascular bundles

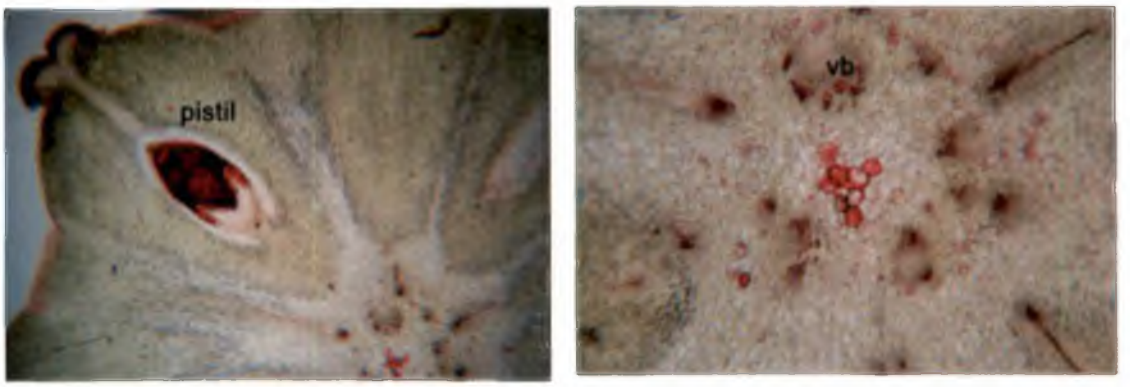


Plate 11: T.S. of the female spike

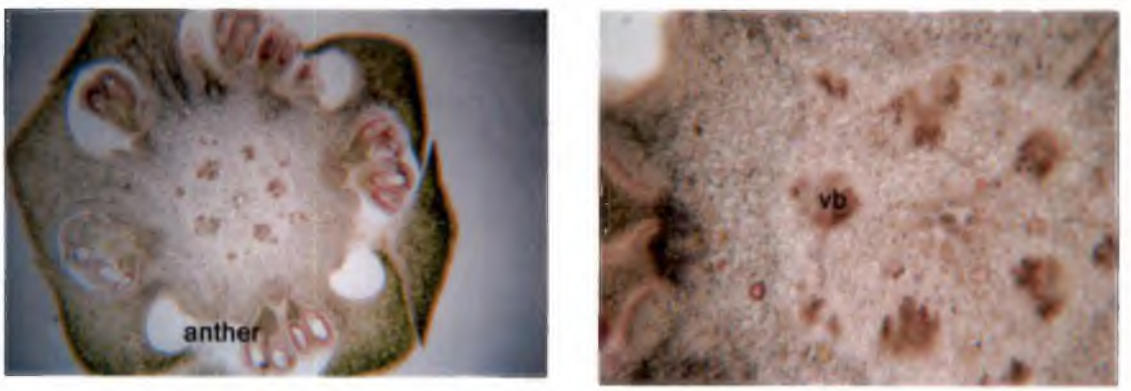


Plate 12: T.S. of the male spike

vb- vascular bundles

accessions, Assam, Pattambi, and the male accession Odakkali. Nilambur, NL-84-68 and Kanjur recorded nineteen cortical bundles each.

The T.S of the reproductive stem of the eight accessions showed that the number of medullary bundles ranged between four to six and for the cortical bundles, it was 15-16. Nilambur and Pattambi had four medullary bundles each whereas Assam, Kanjur and NL-84-68 recorded five medullary bundles. The maximum number of medullary bundles (6) was for Maharashtra, Odakkali and Viswam. Assam, Kanjur, NL-84-68 and Viswam had fifteen cortical bundles each whereas Maharashtra, Nilambur, Odakkali and Pattambi had sixteen cortical bundles each.

Mucilage canal was present in the centre for the female accessions NL-84-68, Assam and Viswam. Mucilage canal was not seen for the other accessions on the vegetative stem.

4.3.1.2 Root

The number of vascular bundles varied for the different accessions. A maximum of ten vascular bundles were recorded by the accession Maharashtra and the male accessions Nilambur and Odakkali. Pattambi recorded the minimum number of vascular bundles (5). Viswam and NL-84-68 had seven bundles each whereas Assam recorded six vascular bundles.

4.3.1.3. Leaf

The leaf epidermis of all the accessions was unilayerd. There was no difference among the leaves of male and female accessions.

4.3.1.4. Spike

Anatomical differences were noticed for male and female spikes. The T.S of the female spike clearly showed the pistil with the persistent stigma and vascular bundles in the middle. The anthers and vascular bundles were present in the case of the male spikes. Outermost layer is single layered epidermis and

parenchymatous cells are tangentially elongated. These cells are filled with fine starch grains.

4.4. Molecular characterization

4.4.1. Genomic DNA isolation

The protocol suggested by Doyle and Doyle (1987) was tried for the extraction of DNA. The quality of DNA isolated was assessed using agarose gel electrophoresis. Good quality genomic DNA was obtained which was evident by the distinct nature of band without any smears (Plate 13). The quantity of DNA in samples was analysed using spectrophotometry. The ratio of absorbance at 260 nm and 280 nm was around 1.80 indicating good quality of DNA for all the accessions.

4.4.2 RAPD assay

4.4.2.1 Random Primer Screening

Randomly selected primers of Operon Primer Kit (Operon Technologies, USA) were screened using genomic DNA isolated from Assam. Out of the twenty primers screened five were selected for further analysis based on maximum number of reproducible bands. The primers screened were OPF-3, OPF-5, OPF-6, OPAH-6, OPAH-13, OPP-4, OPP-5, OPP-6, OPAZ-2, OPAZ-3, OPAZ-4, OPAZ-5, OPAZ-6, OPAZ-7, OPAZ-8 and OPA 1 to OPA-5. The number of bands varied with each primer and they gave average, poor and good amplification. The amplification pattern obtained for the selected primers are given in Table 9.

4.4.2.2. Molecular characterization using selected primers

The RAPD profile obtained for the eight selected accessions using the five selected primers, OPAZ 4, OPAH 6,OPF 5,OPAZ 5 and OPF 3 are given in Plates 14,15,16&17.

Table 9. Number of amplification products and polymorphic bands produced by the five selected primers.

Sl. No.	Primer	Sequence	No. of Amplicons	No. of Polymorphic bands	Percentage Polymorphism
1.	OPAZ 4	CTCCCCAGAC	7	5	71
2.	OPAZ 5	TTGCAGGCAG	7	6	85.7
3.	OPAH 6	GTAAGCCCCT	6	4	66.7
4.	OPF 3	CCTGATCACC	9	7	77.7
5.	OPF 5	CCGAATTCCC	6	4	66.7

Plate 12: Genomic DNA of the eight accessions of *P. longum*

Plate 13: RAPD profile of the accessions using the primers OPAZ- 4 & OPAH - 6

M - Marker 1-Assam 2- Pattambi 3- Nilambur 4- Kanjur
5- Maharashtra 6- NL-84-68 7- Viswam 8- Odakkali C- Control

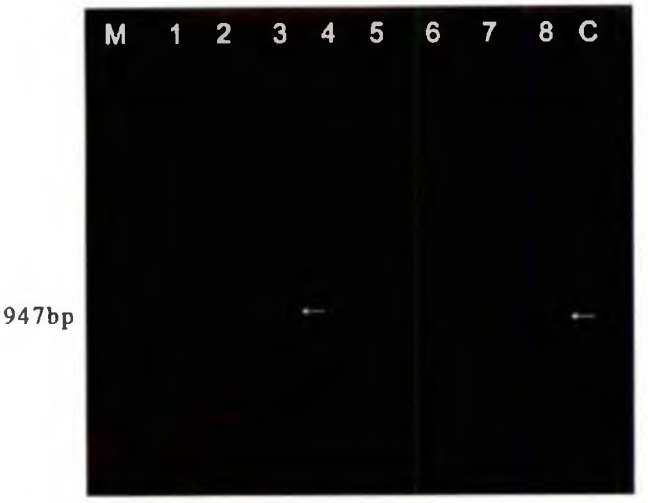


Plate 14: RAPD profile of the accessions using the primer OPF 5

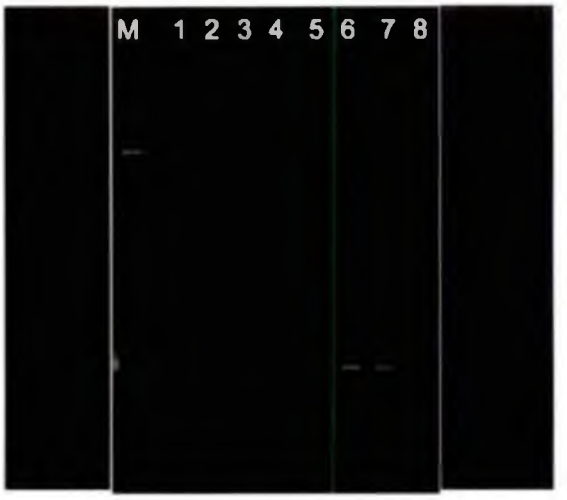


Plate 15: RAPD profile of the accessions using the primer OPAZ 5

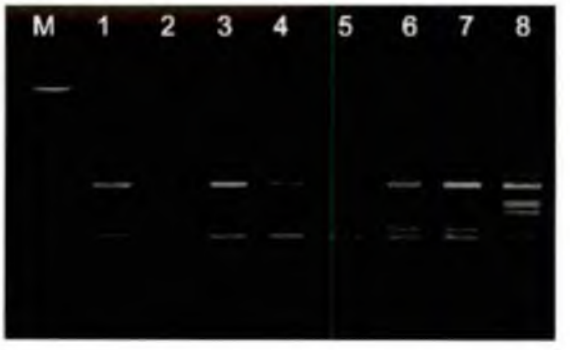


Plate 16: RAPD profile of the accessions using the primer OPF 3

M - Marker 1-Assam 2- Pattambi 3- Nilambur 4- Kanjur
5- Maharashrta 6- NL-84-68 7- Viswam 8- Odakkali C- Control

Pattambi, Kanjur, Maharashtra and Odakkali produced four bands for the primer OPAZ-4. Viswam had the maximum number of bands (7). Of the total seven bands five were polymorphic and two were monomorphic.

The primers OPF-5 produced six bands of which four were polymorphic bands. All the accessions shared four out of six bands for the primer OPF-5. Amplification with OPF-5 provided bands (947bp) specific for male accessions. Band one was present only for the accession, Maharashtra.

OPAH-6 also produced six bands and four of them were polymorphic. Viswam and Maharashtra had only two bands each for the primer OPAH-6. Fifth band was present only for the accessions NL-84-68 and Odakkali. Five bands were observed for the accessions Assam, Nilambur, Kanjur and Odakkali.

The primer OPAZ-5 produced a total of seven bands, of which one band was monomorphic. The number of bands ranged from two for Kanjur to five for NL-84-68. Fourth band was absent in only one accession, Kanjur whereas fifth band was present only for NL-84-68.

The primer OPF-3 generated two monomorphic and seven polymorphic bands. Third band was unique to the accession, Odakkali. Maharashtra and Pattambi had only two bands for this primer.

On the whole the five selected primers generated a total of twenty six polymorphic bands for the eight accessions, out of the total 35 bands.

4.4.2.3 Genetic Analysis

The RAPD data was used to generate a similarity matrix using the SIMQUAL programme. Based on estimated Genetic Similarity Matrix (Table 10) the maximum percentage of similarity was 85 percent between the male accessions Odakkali and Nilambur. The minimum percentage of similarity was 41 percent between Maharashtra, and the male accession Odakkali. NL-84-68 and Viswam had 81 percent similarity. Kanjur had 61 percent similarity with Viswam and 59 percent similarity with NL-84-68. Assam and Pattambi shared 67 percent

Table 10. Similarity values based on RAPD profiling of *P. longum* accessions

	Assam	Pattambi	Nilambur	Kanjur	Maharashtra	NL-84-68	Viswam	Odakkali
Assam	1.00000000000000							
Pattambi	.66666666666667	1.00000000000000						
Nilambur	.678571428571429	.640000000000000	1.00000000000000					
Kanjur	551724137931034	560000000000000	.642857142857143	1.00000000000000				
Maharashtra	.461538461538462	.600000000000000	.444444444444444	.541666666666667	1.00000000000000			
NL-84-68	.620689655172414	.518518518518518	.500000000000000	.586206896551724	.560000000000000	1.00000000000000		
Viswam	.586206896551724	.600000000000000	.566666666666667	.607142857142857	.583333333333333	.807692307692308	1.00000000000000	
Odakkali	.580645161290323	.535714285714286	**.851851851851852**	.548387096774194	**.413793103448276**	.515151515151515	.531250000000000	1.00000000000000

similarity. Maharashtra had 60 percent similarity with Pattambi and 58 percent similarity with Viswam.

4.4.2.4. Dendrogram

Genetic distances were used to construct the dendrogram (NTSYS software) as given on Fig 1. In the dendrogram the eight accessions were classified to two major clusters 1 and 2 at 51 percent similarity. Maharashtra formed a separate cluster and all the other seven accessions were grouped in the other major cluster. The other cluster had two subclusters A_1, A_2. A_1 cluster had four accessions in it; of which Assam and Pattambi formed one group and the male accessions Nilambur and Odakkali formed another group. The other subcluster had three accessions in it. NL-84-68 and Viswam were grouped together and the accession, Kanjur joined this cluster separately.

4.5 Quality components

4.5.1 Essential oil content

The female accessions Assam, Kanjur and Maharashtra and the male accessions, Nilambur and Odakkali yielded 0 percent oil on distillation of spikes with 25-30 days maturity (immature). Viswam recorded 0.26 percent, Pattambi 0.2 percent and NL-84-68 yielded 0.13 percent of oil at this stage.

The yield of oil was maximum when the spikes attained full maturity at 60-70 days (Table 11). The analysis of variance showed that there was significant difference among the cultivars for the yield of oil. NL-84-68 and Viswam yielded the maximum quantity of oil (1.87%). The second highest oil content of 1.47 percent was recorded by Pattambi followed by Assam (1.13%) and Kanjur (1%). The female accession Maharashtra yielded only 0.8 percent oil. The male accessions Nilambur and Odakkali recorded the minimum yield of 0.47 percent and 0.33 percent respectively.

There was a slight decline in the yield of oil when spikes were harvested after 75 days (over ripe). NL-84-68 (1.73%) and Viswam (1.67%)

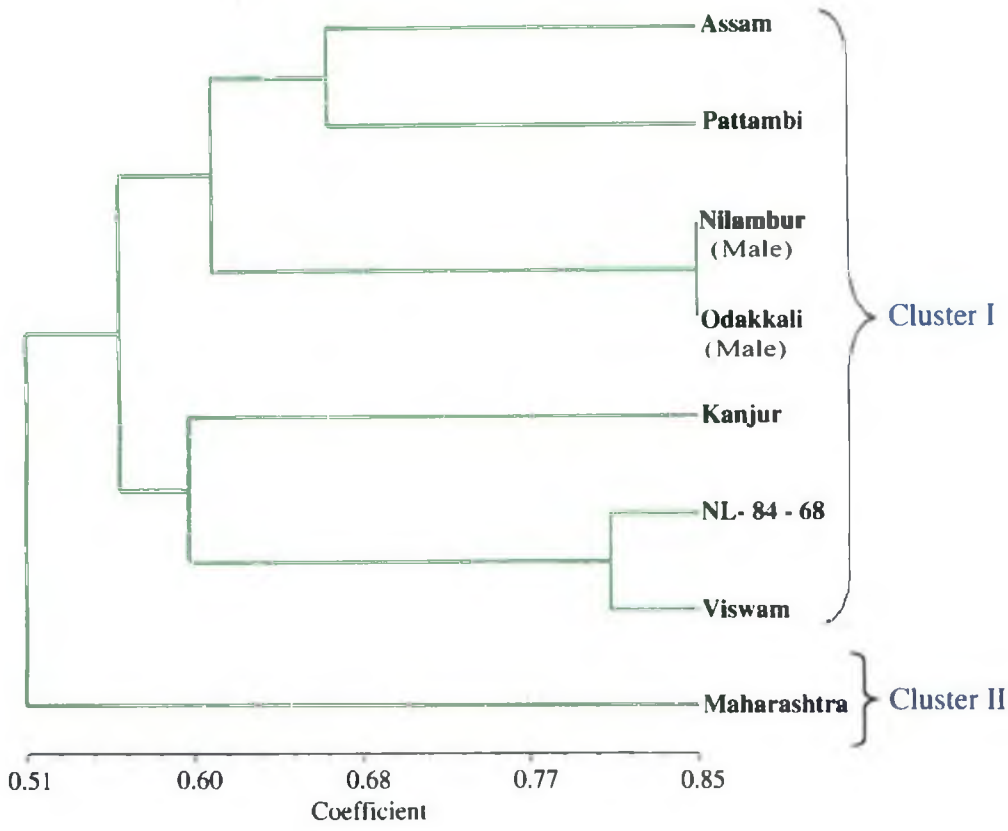


Fig 1. Dendrogram derived from the analysis of eight *P.longum* accessions using five random primers

recorded the maximum quantity here also. They were followed by Pattambi, Assam, Kanjur, and Maharashtra which yielded 1.2%, 1.07%, 0.93% and 0.53% of oil respectively. The male accessions Nilambur and Odakkali yielded only 0.2% of oil at this stage.

4.5.2 Piperine content

The piperine content of all the accessions was maximum when spikes were harvested at full maturity i.e. after 60-70 days of their formation. Assam recorded the maximum piperine content of 1.97 percent when it was harvested after 25-30 days followed by Viswam (1.09 %) piperine and NL-84-68 (0.78 %). The other female accessions Maharashtra, Pattambi and Kanjur recorded a yield of 0.57, 0.51 and 0.1 percent respectively. The male spikes Odakkali (0.08%) and Nilambur (0.07%) also yielded piperine.

Analysis of variance showed significant difference among the accessions for the piperine content when the spikes were harvested 60-70 days after their formation (Table 11). The maximum piperine content was for Assam (5.57%) and the minimum for the male accessions Nilambur (0.45%) and Odakkali (0.53%), which recorded 0.45 and percent respectively. Viswam recorded 3.7 percent piperine at full maturity. Maharashtra and NL-84-68 recorded 2.72 and 2.59 percent respectively and were on par statistically. Pattambi recorded 1.2 percent piperine at full maturity.

After 75 days, the piperine content of the spikes declined slightly, but the decline showed the same trend as that of spikes at full maturity. Here also Assam yielded the maximum of 3.9 percent followed by Viswam with 2.9 percent piperine. Maharashtra and NL-84-68 recorded 2 and 1.93 percent respectively. Pattambi recorded 0.35 percent piperine at this stage. The male accessions recorded the lowest piperine content of 0.39 (Odakkali) and 0.35 percent (Nilambur).

Table 11. Mean of different accessions for oil and piperine content

Accession	Piperine (%)			Oil (%)		
	25-30 days	60-70 days	After 75 days	25-30 days	60-70 days	After 75 days
Assam	1.97^{a}	5.57^{a}	3.90^{a}	0.00	1.13^{bc}	1.07^{b}
Kanjur	0.10^{e}	0.53^{e}	0.39^{e}	0.00	1.00^{c}	0.93^{b}
Maharashtra	0.57^{d}	2.72^{c}	2.00^{c}	0.00	0.80^{cd}	0.53^{c}
NL 84-68	0.78^{c}	2.59^{c}	1.93^{c}	0.13	1.87^{a}	1.73^{a}
Pattambi	0.51^{d}	1.20^{d}	0.35^{d}	0.20	1.4^{b}	1.20^{b}
Viswam	1.09^{b}	3.70^{b}	2.90^{b}	0.26	1.87^{a}	1.67^{a}
Odakkali	0.08^{e}	0.24^{e}	0.13^{e}	0.00	0.33^{e}	0.20^{d}
Nilambur	0.07^{e}	0.45^{e}	0.35^{e}	0.00	0.47^{de}	0.20^{d}
Mean for female accessions	0.84	2.72	1.91	0.10	1.35	1.19
Mean for male accessions	0.08	0.35	0.24	0.00	0.40	0.20
Grand mean	0.65	2.12	1.49	0.07	1.12	0.94
Min	0.07	0.24	0.13	0.00	0.33	0.20
Max	1.97	5.57	3.90	0.26	1.87	1.73
CD 5%	0.172	0.324	0.503		0.371	0.338

Treatments with the same alphabet do not differ significantly

5. DISCUSSION

An assessment of variability existing in a germplasm is the prime prerequisite of any breeding programme. Characterizations at morphological, anatomical, physiological and molecular level for both qualitative and quantitative characters are the various tools to understand the variability present in a germplasm. A total of 26 morphological characters (15 vegetative characters and 11 reproductive characters), three physiological characters, anatomy of stem, leaf, root and spike, molecular characteristaion and quality components of the six female and two male accessions were studied and results obtained are discussed under the following heads.

5.1 Morphological characters

Morphological architecture of eight accessions revealed a significant variation for different vegetative and reproductive characters.

5.1.1 Vegetative characters

There was no significant difference between male and female accessions for the various stem characters studied. The maximum vine length was recorded by Maharashtra with lowest angle of insertion of reproductive branch.

Among vegetative characters vine length, internodal length, leaf area of both vegetative and reproductive leaf and l/b ratio were found to be favouring productivity. This was evident from the female accession NL-84-68, Viswam, and Assam. NL-84-68 recorded the maximum internodal length, area for leaves of vegetative and reproductive branch, which was manifested in the spike size. On the other hand, Viswam recorded the minimum vine length, and area for leaves of vegetative and reproductive branch, l/b ratio for reproductive leaf and more number of spikes per spike bearing branch. The two accessions exhibited different morphological characters clearly indicating their separate entity. Hence these

morphological characters can be used to identify the varieties. Supporting evidences were reported by Ram *et al* (1996) and Reddy M.L.N (1996) in mint and betel vine. Assam expressed a medium character between the two accessions contributing better productivity. In general the higher leaf area is contributed through high length and breadth of leaf in all the accessions studied.

Significant difference exists for length, breadth and l/b ratio for leaves on vegetative and reproductive branches. In general, the vegetative branches had large leaves with petiole when compared to leaves of reproductive branches (Fig 2). Reproductive branches produced smaller leaves with lowest values for length, breadth and rudimentary petiole clearly indicating morphological variation between leaves on vegetative and reproductive branches. The higher petiole length in vegetative leaves provide better exposure of the leaf to sunlight and higher leaf area contribute more photosynthetic rate which is useful for production of more reproductive branches and other vegetative structures at early stages of growth favouring better yield. This was in agreement with the findings of Manuel (1994) and Viswanathan (1995) in *P. longum*. The male and female accessions did not differ significantly for the characters like leaf area, length, breadth, l/b ratio of leaves of vegetative and reproductive branches.

With respect to qualitative characters leaf lamina was cordate for leaves of vegetative branch, whereas it was lanceolate for leaves of reproductive branch. Lamina base was cordate for leaves of vegetative branch and unequally cordate for reproductive leaf, which can be used as a diagnostic tool for identification of vegetative and reproductive branches. All the accessions could be grouped into two as even and wavy based on the leaf margins. Leaf pubescence and texture remained the same for all accessions. Leaf colour was dark green for all accessions except Viswam, which had medium green leaf of vegetative branch. Since wide variation was not observed in different accessions for qualitative morphological characters like leaf pubescence, texture and colour these cannot be used as a diagnostic tool for proper identification of long pepper accessions. Earlier reports suggest that in pepper, betel vine, and curry leaf, characters like leaf shape, margin, base, colour, can be used for identification of different

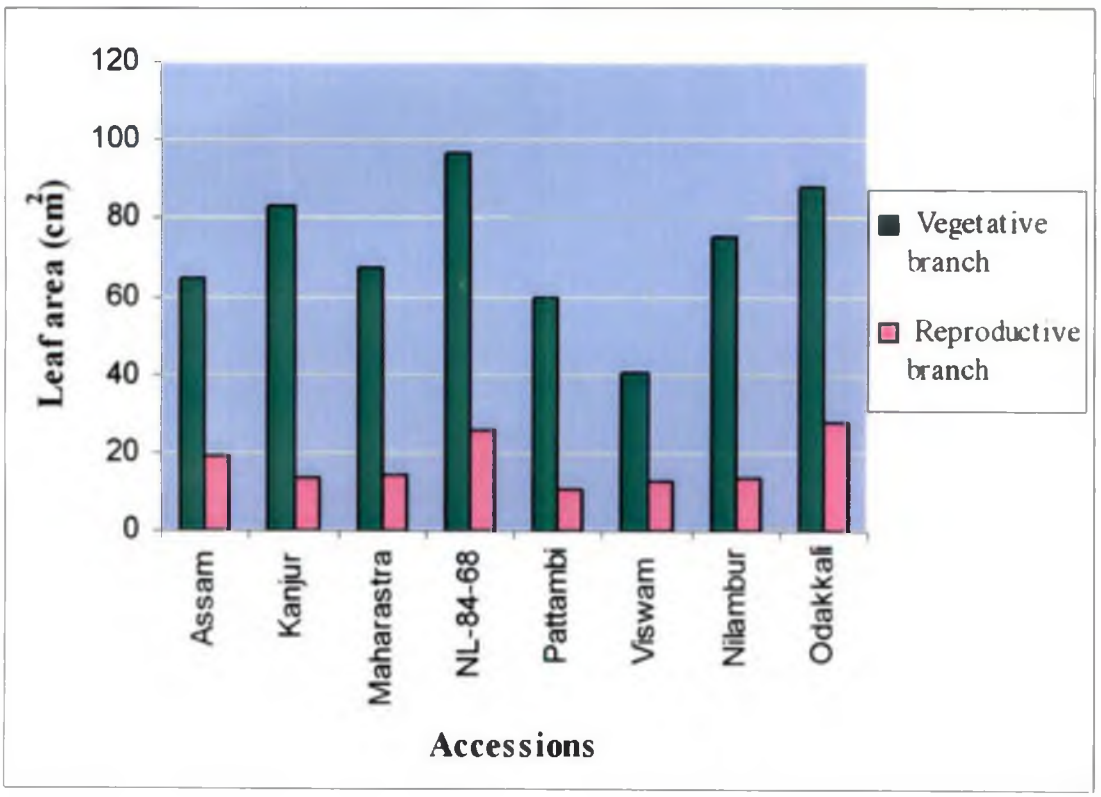


Fig. 2 Comparison of leaf area of vegetative and reproductive branch

accessions in these crops. (Kanakamony *et al*, 1985; Sujatha. 1991; Devi *et al.*, 1992; Lal. R. K, 2003)

5.1.2 Reproductive characters

All the accessions studied differ significantly for reproductive characters like spike length, spike diameter, days to spike initiation, days to maturity of spike, fresh weight of spike, dry weight of spike and percentage loss in weight after drying. Length and diameter of spike determines the size of the spike, which ultimately influences the yield. This is evident in the case of NL-84-68, which produced longest spike with maximum diameter among female accessions studied, and thereby maximum fresh weight of spike even though the spike number per spike bearing branch was not promising. This is not true in the case of male accessions though their spikes are much longer compared to the female accessions (Fig 3.)

Number of spikes per spike bearing branch is an important yield parameter in pepper as suggested by Ibrahim *et al.* (1985) and Sujatha and Namboodiri (1995). Observations revealed no significant difference between accessions for this character, though maximum number was recorded by Assam followed by Viswam.

Days to spike initiation is another important character, which expresses the earliness of the crop. There was significant difference for this character between male and female accessions. Male accessions took minimum days for spike initiation whereas female accession took maximum days for it. Among female accessions NL-84-68 recorded maximum days for spike initiation whereas Viswam and Maharashtra recorded minimum days.

With reference to days to maturity it ranged from 62-69.6 days in female accession, and 56 days in male accession. Generally male accessions exhibited short growth duration for spikes compared to female accessions. The late maturing female accessions were distinct in their growth habit also. Among female accessions, NL-84-68 recorded maximum days of maturity with minimum

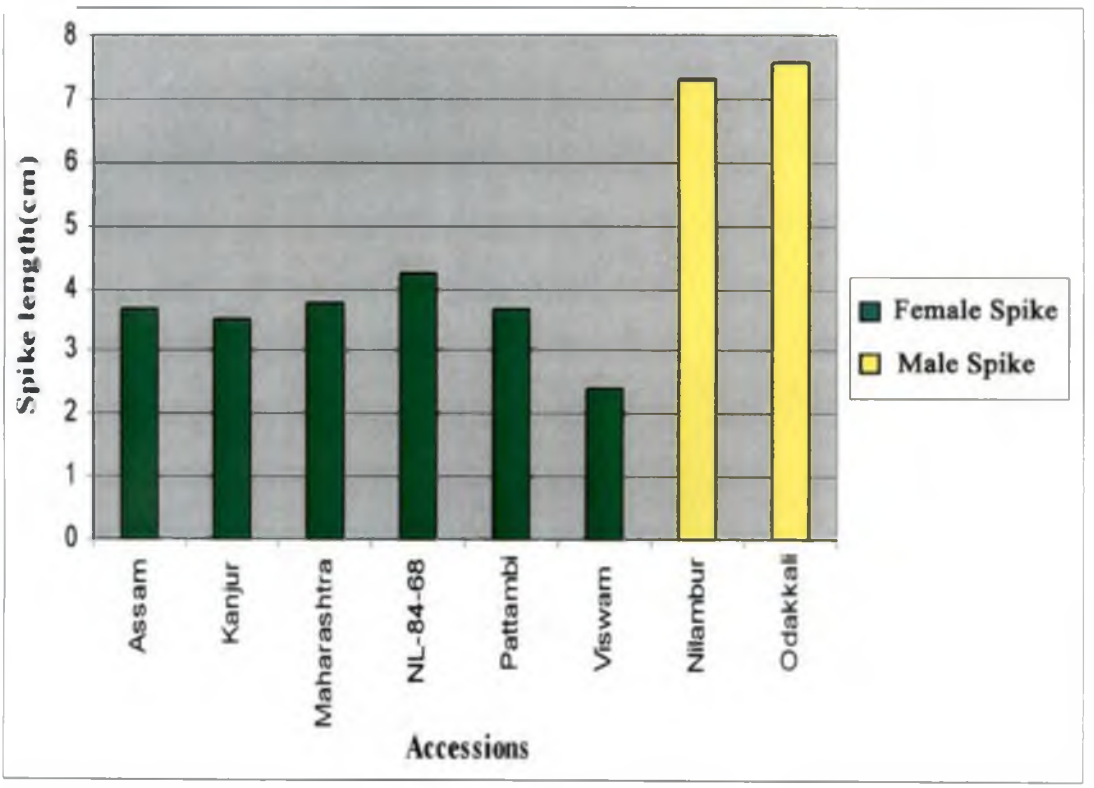


Fig. 3 Comparison of spike length of male and female accessions

spike number, whereas Kanjur, Assam and Viswam matured earlier than NL-84-68, which was expressed through more number of spikes per spike bearing branch. Earlier workers also reported the importance of early maturity in crop improvement programme because of their significance associated with yield (Singh *et al.*, 1996).

Percentage loss in weight after drying based on fresh and dry weight is also related to yield. In the present investigation minimum weight loss of 69.93 percent was observed in Viswam. Hence the lowest fresh weight recorded by this accession is compensated.

The qualitative character of spike was specific for different sexes with respect to orientation and colour. The orientation of spike was sub erect for females whereas erect for male and colour was greenish black for female and yellow for male accessions at maturity. At initial stages the colour of female spike is creamy yellow and that of male is dark green.

5.2 Physiological parameters

Photosynthetic rate per unit of leaf area varies significantly (Fig 4). Among female accession, Viswam recorded the highest photosynthetic rate with lowest stomatal conductance and transpiration rate. These characters exhibit a positive association with water use efficiency (Fig 5), which was calculated as described by Rosenberg and Kriiger (1993). The same trend was observed in female accessions, Assam and NL-84-68. Though the other female accession, Kanjur expressed high photosynthetic rate along with stomatal conductance and transpiration rate, it could not express promising yield characters. This may be due to more vegetative growth expressed by the accession and hence photo assimilates are used for vegetative growth rather than yield attributes. Yoshida (1972) had reported high rate of photosynthesis and its association with higher productivity. The three accessions Viswam, Assam and NL-84-68 were found promising with respect to photosynthesis and WUE. These two characters are important when long pepper is cultivated under irrigated conditions. The

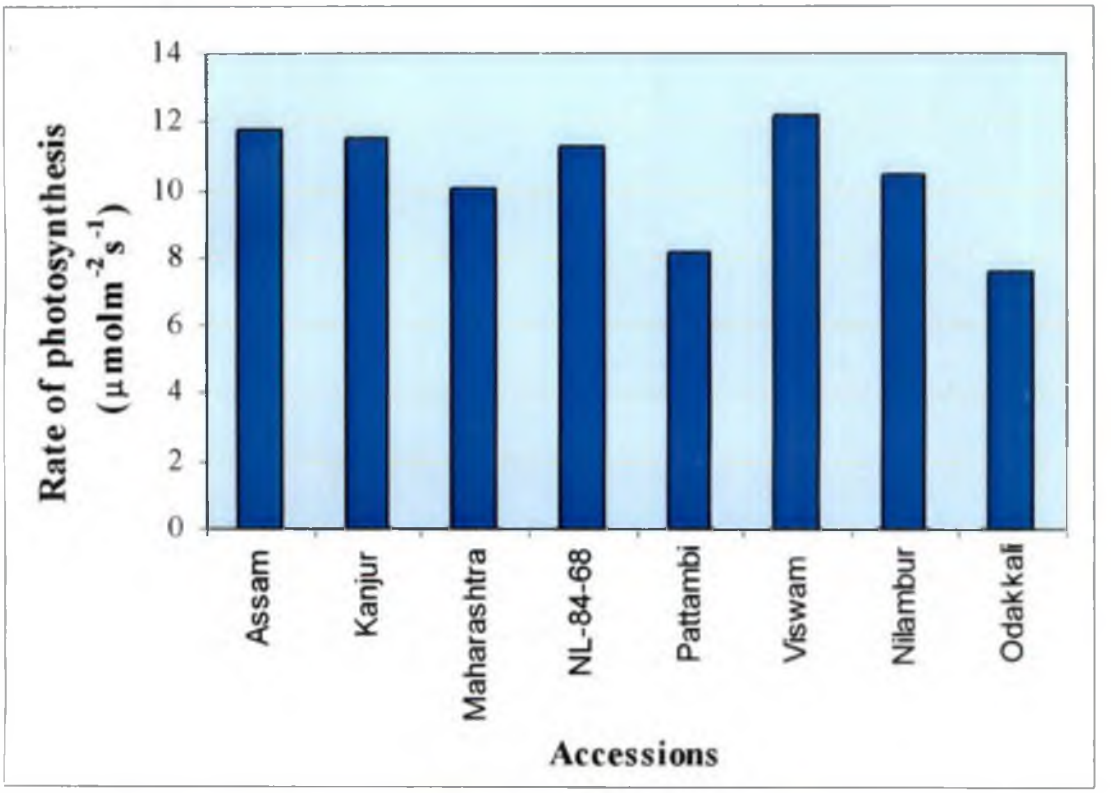


Fig. 4 Rate of photosynthesis of the different accessions

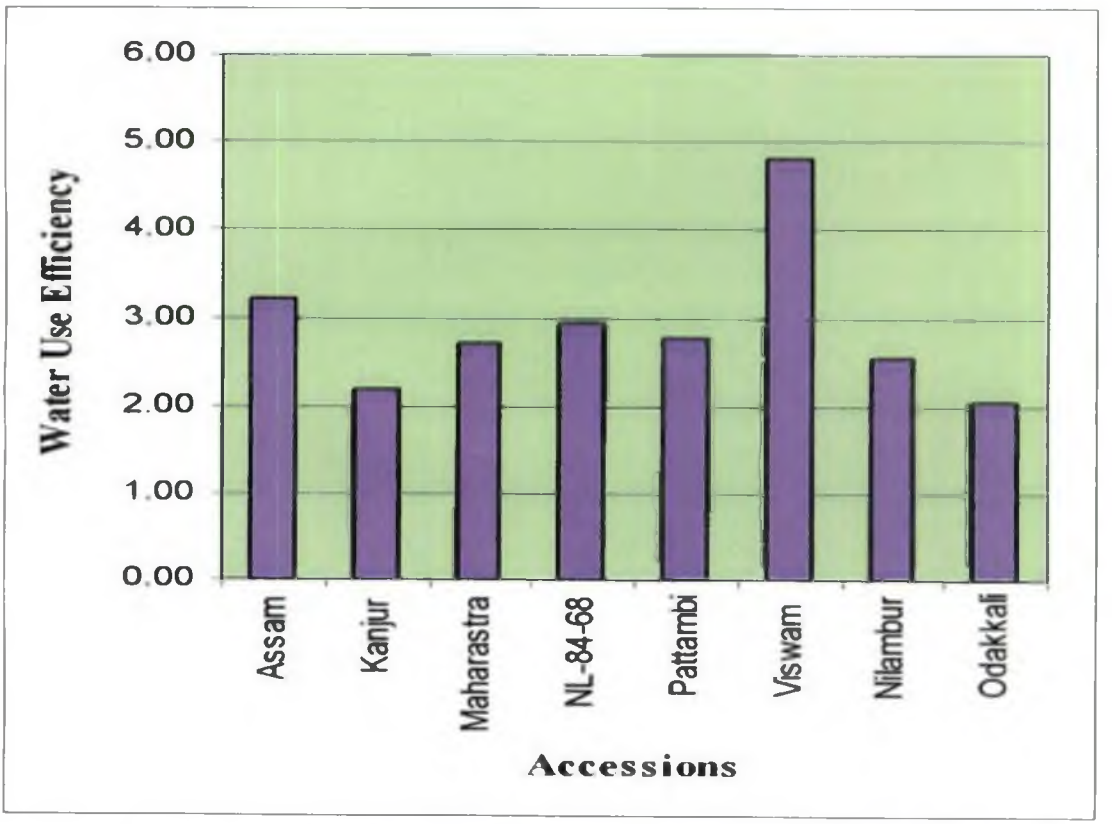


Fig. 5 Water Use Efficiency of the different accessions

photosynthetic rate and associated parameters were found to differ in crops (Patel *et al.*, 2005).

5.3 Anatomical characters

Anatomical characters are used as markers for identification of species (Menancherry, 1993; Sasikumar *et al*, 1999; Remashree *et al.*, 2005). The main anatomical difference could be observed only in the number of vascular bundles in vegetative and reproductive stem and root of different accessions. Mucilage canal was observed only in vegetative stem of female accessions *viz* Assam, NL-84-68 and Viswam. All other anatomical features of stem were same for male and female accessions. More number of vascular bundles and presence of mucilage canal is an indication of better translocation of metabolites which inturn enabled for better growth and development. These two anatomical characters are well expressed in the female accessions Assam, NL-84-68 and Viswam. Earlier work (Mithila *et al*, 2000) in betelvine supports this conclusion.

Root anatomy revealed variation in number of vascular bundles for female accession, Maharashtra and male accessions Odakkali and Nilambur. More number was observed in these accessions, which was reflected in their early spike initiation. All the above observations are supportive of Haberlandt (1884). Solereder (1908); Metcalfe and Chalk (1950); and Menacherry (1993) also reported variation in the number and arrangements of bundles and mucilage canal as distinguishing anatomical criteria for classifying *Piper* sp.

5.4 Molecular characterization

5.4.1 RAPD Analysis

Morphological and biochemical markers though widely used for characteristaion they are influenced by environmental factors. An efficient method

to fingerprint the genotypes without the effect of environment is the use of molecular markers.

Welsh and Mc Clelland in 1990 developed the RAPD technique which detects nucleotide sequence polymorphism in DNA by using a single primer of arbitrary nucleotide sequence. The RAPD technique is relatively simple and rapid approach. The use of RAPD for assessment of variability in different crops had been reported by several workers (Das *et al*, 2004; Darokar *et al*, 2003; Philip *et al*, 2002).

5.4.1 Primer Screening and RAPD profiling

Random decamer primers from Operon Technologies USA were used for the present study. Many workers have reported the use of Operon primers for their studies (Ravi *et al*, 2003; Harisankar *et al*, 2002; Uma *et al*, 2004). Twenty random primers from different series were used. Of these five primers that produced good amplification were selected for RAPD analysis. The presence of 16 unique RAPD markers and the absence of three unique markers obtained from 12 primers made it possible for Chen *et al* (2002) to discriminate 14 wild tea germplasm collections. Shasany (1998) used twelve primers to distinguish the vetiver genotypes.

Twenty six polymorphic bands were obtained from a total of 35 RAPD produced using five primers. The number of markers ranged from six to ten for the different primers. The primer OPF-5 had specific bands for the male accessions, Odakkali and Nilambur. Banerjee *et al* (1999) also had reported male sex associated RAPD markers for *P. longum*. Similar findings of sex linked RAPD marker in *Pistachio vera* was reported by Hormaza *et al* (1994).

5.4.2 Genetic analysis

The dendrogram constructed with the RAPD markers using the genetic similarity indices divided the eight accessions into two major clusters (Fig 1.).

The highest similarity of 85 percent was shared by the male accessions Odakkali and Nilambur, which was evident by their morphological characters also. Sanalkumar (2005) have reported the distinctness of Njavra rice genotypes from check varieties using the morphological and molecular markers. Viswam and NL-84-68, both shared similar values in their oil yield also were clustered together with a similarity index of 82% between them. Assam and Pattambi though had different morphological characters were grouped in one cluster, but has genetic relations with the male cluster Odakkali and Nilambur. This indicates that all these four accessions have a common ancestral origin. So also Maharashtra found a place very distinct from the other seven accessions. This may be due to its repeated cultivation in a different geographical area. A direct correlation of RAPD pattern to morphological characters could not be established in the present study. Royo and Itiosz (2004) observed in apple that the genetic relationships using RAPD and isozyme showed little congruence with the morphological relationship. A variability of 15-49% between the eight accessions was revealed by the RAPD analysis in *P. longum*. Since variability exists between the accessions the superior genotypes could be used for further breeding programmes.

5.5 Quality parameters

The quality of spike depends on piperine and essential oil content. Both these varied in different accession at different stages of spike maturity (Fig 6 & 7). However the maximum piperine and oil yield were obtained when the spikes were harvested at 60-70 days maturity and the yield declined thereafter. The minimum decline of 21 per cent piperine was obtained in Viswam and the maximum was observed in Assam (30%). Das, *et al* (2003) also obtained a similar result when oil and oleoresin yield of mature, ripe and overripe fruits of *P. chaba* were analysed. The female accessions Assam, NL-84-68, and Viswam recorded highest piperine content and oil content compared to other female accessions. These findings agree with the report of Viswanathan (1995). Radhakrishnan *et al* (2002) also have reported variation in piperine and oil content among the different

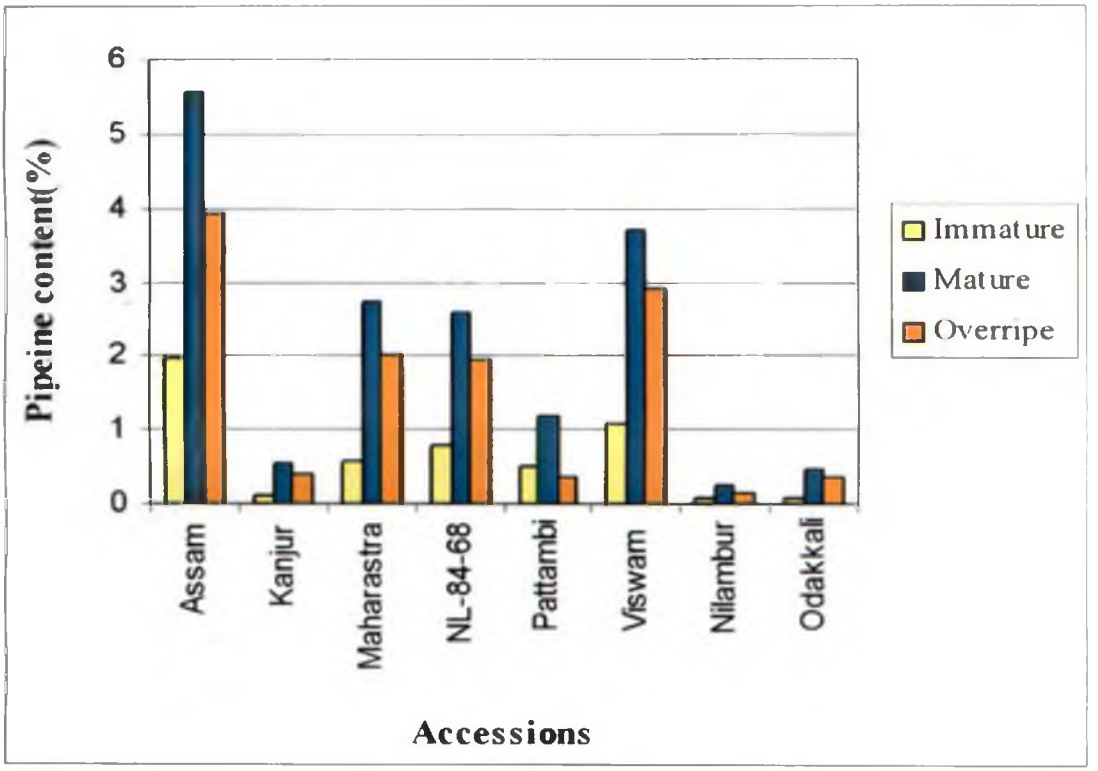


Fig. 6 Effect of maturity on piperine content of the different accessions

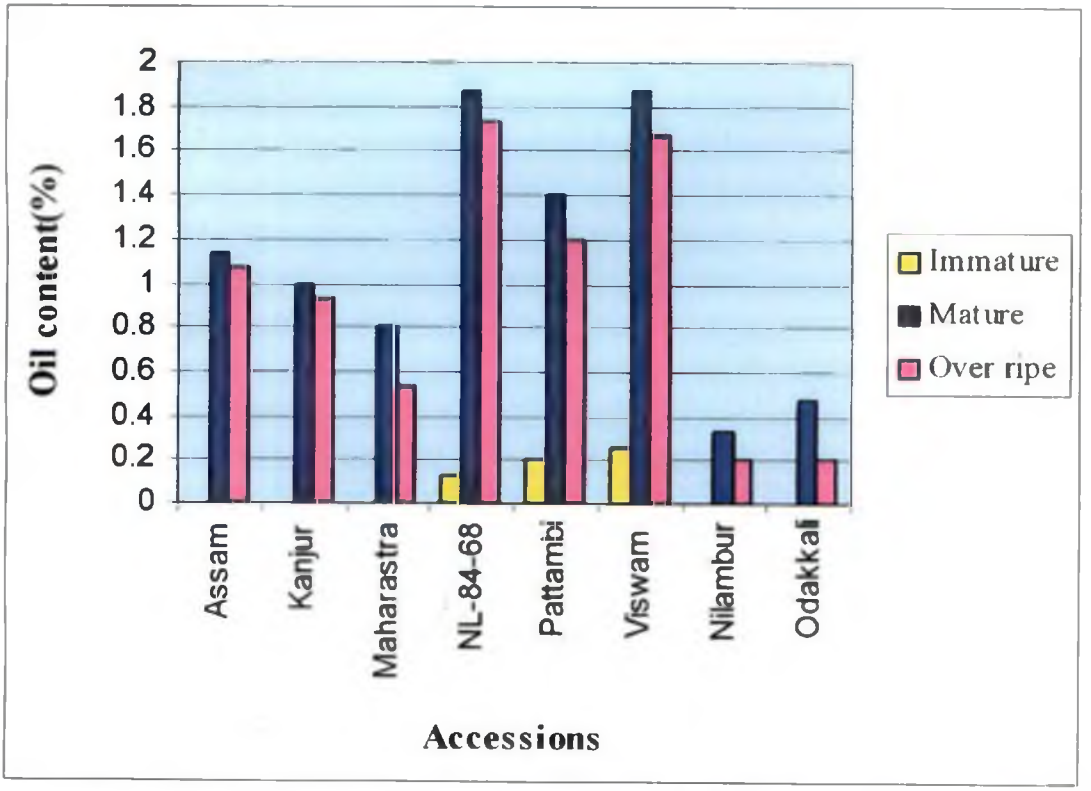


Fig. 7 Effect of maturity on oil content of the different accessions

varieties of pepper. The male spikes also yielded piperine and oil content though the quantity was less when compared to female accessions. *In P. longum* though the quality components of female spikes were well explained (Atal *et al.*, 1975; Narasimham, 2003) the quality aspects of male spikes are not yet reported. The quality components were maximum at 55-60 days in male accessions depending on their early maturity.

Based on different qualitative and visual characters of six female and two male accessions studied, the female accessions NL-84-68, Assam and Viswam were identified as accessions with desirable characters for crop improvement.

Summary

6. SUMMARY

The present investigation entitled "Characterisation of long pepper (*P. longum* L.) genotypes using morphological, anatomical and molecular markers" was undertaken at the Department of Plant Breeding and Genetics, College of Horticulture ,Vellanikkara during the period 2003-2006. Six female and two male accessions formed the material for the study and were subjected to morphological, anatomical, and molecular characterization. The salient features could be summarized as follows.

1. The vegetative and reproductive branches showed marked morphological difference in their leaf size, shape, and l/b ratio for male and female accessions.
2. Vegetative branches had large leaves with petiole whereas leaves of reproductive branches were small having rudimentary petiole.
3. The l/b ratio of leaves of reproductive branches were higher compared to leaves of vegetative branch.
4. The male and female accessions did not differ significantly for the characters like leaf area length, breadth and l/b ratio of leaves produced by vegetative and reproductive branch.
5. The spikes of male accessions were longer and slender, and turn yellow at maturity where as the female spikes were short, bold and greenish black on maturity. At initial stages of growth distinct colour difference exist between male (green) and female spike (yellow).
6. The anatomical features of stem, leaf, root and spike of the male and female accessions showed almost similar features. The difference was noticed in the number of medullary and cortical bundles and the presence and absence of mucilage canals.
7. The three female accessions, Assam, NL-84-68 and Viswam had mucilage canals whereas it was absent in all other accessions.
8. The male and female spikes showed variation in their anatomy.

9. The female accessions had higher photosynthetic rate compared to male accessions and Viswam had the highest photosynthetic rate.
10. Doyle and Doyle method was found suitable for extracting good quality of DNA.
11. Twenty primers were screened and five primers were selected for RAPD analysis.
12. Based on the similarity values obtained for the eight accessions from the RAPD profiles, they were grouped in to two major clusters.
13. The female accession, Maharashtra formed a separate cluster and all other male and female accessions were grouped in another cluster.
14. The male accessions Nilambur and Odakkali had the maximum (85 %) similarity.
15. Among the female accessions, Viswam and NL-84-68 had the maximum (82 %) similarity.
16. The eight accessions showed 15-49% variability between them at the molecular level.
17. The primer OPF 5 produced male specific bands.
18. The spikes were analysed for their quality on oil and piperine content to derive the physiological maturity.
19. It was found that all the accessions yielded maximum oil and piperine content at 60-70 days maturity when the spikes were greenish black and hard.
20. Among the female accessions, Viswam, NL-84-68 and Assam recorded highest piperine and oil content.
21. Low amounts of piperine and oil were found in male spikes also.
22. Based on the study three female accessions, Viswam, NL-84-68 and Assam were found to be promising types and further breeding programmes can be carried out in these accessions to improve their performance.

References

REFERENCES

Adams, R.P., Pandey, R.N., Dafforn, M.R. and James, S.A. 2003. Vetiver DNA-finger printed cultivars - Effects of environment on growth, oil yield and composition. *J. Essential Oil Res.* 15: 363-371

Aiyer, N. M. A and Kolammal, M.1966. *Pharmacognosy of Ayurvedic Drugs,* Dept. of Pharmacognosy, Kerala University,Thiruvananthapuram 1(9):52-57

Andrea, L.D. 2002. Variation of morphology yield and essential oil component in common chamomile (*Chamomilla reticulate* L.) cultivars grown in S. Italy. *J. Herbs Spices Medicinal Pl.* 94: 359-365

Anon. 1993. *Progress Report.* All India Co-ordinated Research Project on Medicinal and Aromatic Plants, College of Horticulture, Vellanikkara. 13p

Anuradha,V., Srinivas,P.V. and Rao, J.M. 2004. Isolation and synthesis of isodihydropiperlonguminine. *Natural Product Res.* 18(3): 247-251

Asensio, M.L., Valdes, E. and Cabello, F. 2002. Characterisation of some Spanish white grape vine cultivars by morphology and amino acid analysis. *Scientia Hort.* 93: 289-299

Atal, C.K., Dhar, K.L. and Singh, J. 1975. The chemistry of Indian *Piper* species. *Lloydia* 38(3): 256-264

Babu, T.P.H. 2000. RAPD analysis to assess the genetic stability in tissue culture derived black pepper. MSc. (Ag) thesis, Kerala Agricultural University, Thrissur, 86p.

Banerjee, N.S., Manoj, P. and Das, M.R. 1999. Male sex associated RAPD markers in *P. longum* L. *Curr. Sci.* 77(5): 693-695

Basuchoudhari and Dasgupta. 1987. Photosynthetic rate and yield relationship in some late duration high yielding rice. *Indian J. Plt. Physiol.* 30: 400-403

Chen, L. and Wang, P. S. 2002. Discrimination of wild tea germplasm resources using RAPD markers. *Agric. Sci. China.* 1(10): 1105-1110

Dagade, S.B. 2004. Anatomy of two *Piper* species with respect to Phytophthora foot rot disease. *J. Maharashtra agric. Univ.* 29(2): 178-182

Darokar, M.P., Rai, R., Gupta, A.K., Shasany, A.K., Rajkumar, S., Sundaresan, V. and Khanuja, S.P.S. 2003. Molecular assessment of germplasm diversity in Aloe species using RAPD and AFLP analysis. *J. Medicinal Aromatic Pl. Sci.* 25: 354-361

Das, A., Mondal, B., Sarkar, J. and Chaudhuri, S. 2004. RAPD profiling of some elite clones of mandarin orange (*Citrus reticulata* Blanco) in the North Eastern Himalayan Region of India. *J. hort. Sci. Biotech.* 79: 850-854

Das, A., Sarkar, J. and Mandal, B. 2004. Genetic diversity of citrus cultivars by RAPD markers. *Ind. J. Genet. Plt. Breeding.* 64 (4): 281-285

Das, P.H.A., Rajesh, P.N., Zachariah, T.J., Mathew, P.A. and Subramanian, S. 2003. Influence of harvesting stage and drying on quality of *Piper chaba* Hunter. *J. Plantn. Crops.* 31(1): 46-49

Das, R.C., Das, J.N. and Misra, P.K. 1999. Variation and character association of leaf yield and its component characters in betel vine (*Piper betle* L.). *Orissa J. Hort.* 27(2): 66-71

Das, R.C., Das, J.N. and Misra, P.K. 2000. Genetic divergence in betel vine (*Piper betle* L.). *Indian J. Hort.* 57(3): 259-263

Devi, D., Ravishankar, C. and Babu, M.K. 1992. Morphological and physiological characterization of betel vine cultivars. *S. Indian Hort.* 40(4): 213-217

Doyle, J.J. and Doyle, J.L. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissues. *Phytochem. Bull.* 19: 11-15

Dutta, A.C. 1970. *Botany for Degree Students*. Third edition. Bombay Oxford University Press. 324p.

Farooq, S. 2000. *555 Medicinal Plants; Field and Laboratory Manual (Identification with its Phytochemical and invitro studies data)*, International Book Distributors, Dehradun. 498p.

George, K.J., Ganga, G., Varma, R.S., Sasikumar, B. and Saji, K.V. 2005. Identification of hybrids in black pepper (*Piper nigrum* L.) using male parent specific RAPD markers. *Curr. Sci.* 88(2): 216-218

George, M. and Mercy, S.T. 1978. Origin and botany of pepper (*Piper nigrum* L.). Silver Jubilee Souvenir, Pepper Research Station, Panniyur. Pp.11-12

Grieve, M.1977. *A Modern Herbal.* Jonathan Cape Ltd., London, p.628

Gunter, L.E., Black, A.S., Ratnayeke, S., Tuskan, G.A. and Wullschieger, S.D. 2003. Assessment of genetic similarity among 'Alamo' switch grass seed lots using RAPD markers. *Seed Sci. Technol.* 31: 681-689

Haberlandt,G. 1884. *Physiological Plant Anatomy* (Drummond,M., trans.) Fourth edition. Indian Reprint 1965. Today and *Tomorrrows* Printers and Publishers, Delhi. 777p.(Translated from German).

Harisankar, P., Pillai, S.V., Sumarani, G.O. and Sundarsen, S. 2002. Isozyme and RAPD analysis of Cassava germplasm: Identification of duplicates in exotic collection. *Plant Cell Biotech. mol. Biol.* 3: 21-28

Hooker, J.D. 1886. *Flora of British India.* V.L. Reeve & Co. Ltd., London, p.83

Hormaza, J.L. Dollo, L. and Polito, V.S. 1994. Sex associated RAPD makers in *Pistachio vera. Theor. Appl. Genet.* 89:9-13

Ibrahim, K.K., Sukumara Pillai, V., Sasikumar, S. 1985. Genotypic and phenotypic correlation among yield and its components in black pepper (*Piper nigrum* L.) *Agric. Res. J. Kerala.*23 (2): 150-153

Ibrahim, K.K., Pillai, V.S. and Sasikumaran, S. 1986. Comparitive genetic variability within the open pollinated seedlings of certain varieties of black pepper (*P. nigrum* L.). *Agric. Res. J. Kerala.* 24(1): 74-76

ICAR 2005. Improvement and management of horticultural crops. *Annual Report 2004-2005.* Indian Council of Agricultural Research, New Delhi, 20p.

IISR 2006. *Research highlights 2005-2006.* Indian Institute of Spices Research, Calicut.14p.

John, S.A. 1996. Biometrical characterization of betel vine (*Piper betle* L.) collections. *J. Plantn. Crops.* 24(2): 115-118

Joseph and Skaria, 2001. *Piper* -a medicinal genus. *J. Spices Medicinal Aromatic Crops* 20: 12-14

Joy, P.P., Thomas, J., Mathew, S. and Ibrahim, K.K. 1998. Growth, leaf oil yield and quality investigation in cinnamon (*Cinnamomum verum*). *J. Medicinal Aromatic Pl. Sci.* 20: 441-450

Kanakamany, M.T., Namboodiri, K.M.N. and Babu, L.C. 1985. Key for identification of the different cultivars of pepper. *Indian Cocoa Arecanut Spices J.* 9(1): 6-11

Kesavachandran, R., Nazeem, P.A. and Karihaloo, J.K. 2005. Genetic finger printing of *P. nigrum* L. and *P. longum* cultivars using RAPD markers. National Symposium on Biotechnological Interventions for Improvement

of Horticultural Crops, 10-12 January 2005. Kerala Agricultural University, Thrissur. Abstract: 288

Kirtikar, K.R. and Basu, B.D. 1935. *Indian Medicinal Plants* (2nd ed.). Bishen Singh Mahendra Pal Singh, Dehradun, 2150p

Krishnan, G.R.P., Prasad, C.N.V., Rajmohan and Soni, K.B. 2005. RAPD analysis of *Centella asiatica* ecotypes. National Symposium on Biotechnological Interventions for Improvement of Horticultural Crops, 10-12 January 2005. Kerala Agricultural University, Thrissur. Abstract: 273

Kumar, A. and Puspangadan, P. 2003. Estimation of genetic relationship in *T. arjuna* Bedd. utilizing RAPD. 2nd World Congress in Biotechnological Developments of Herbal Medicine, NBRI, Lucknow, UP, 20-22 February 2003, Abst: 51

Kumar, P., Dube, S.D., Chauhan, V.S. 1998. Relationship among yield and some physiological traits in wheat. *Indian J. Plt. Physiol.*3: 229-230

Lal, R.K.2003. Diversity pattern in curry neem leaf (*Murraya koenigii*). *J.Medicinal Aromatic Crops*. 25(1): 13-28

Lee, S.E. 2000. Mosquito larvicidal activity of pipemonaline, a piperidine alkaloid derived from long pepper, *Piper longum. J. Am. Mosquito Control Ass.* 16(3): 245-247

Madhusudhan, P. and Vandana, K.L. 2001. Tetrahydropiperina, the first natural aryl pentanamide from *Piper longum. Biochemical Systematics Ecol.* 29(5): 537-538

Manilal, K.S. and Sivarajan, V.V. 1982. *Flora of Calicut.* Bishen Singh Mahendra Pal Singh, Dehradun, p.248

Murugan, C. 2002. Molecular characterization of *Piper* species using RAPD technique. M.Sc (Ag) thesis, Kerala Agricultural University, Thrissur, 80p.

Nair, S. 2005. Variability in chakkarakkolli (Gymnema sylvestre R.BR.) using morphological, biochemical and molecular markers. M.Sc. (Ag) thesis, Kerala Agricultural University,Thrissur, 75 p.

Nambiar, P.K.V., Pillai, V.S., Sasikumaran, S. and Chandy, K.C. 1978. Pepper Research at Panniyur - a resume. *J. Plantn. Crops* 6(1): 4-11

Narasimhan, S. and Mehrotra, S. 2003. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. *J. AOAC int.* 86(6): 1124-1127

Nazeem, P.A., Keshavachandran, R., Babu, T.D., Achuthan, C.R., Girija, D. and Peter, K.V. 2005. Assessment of genetic variability in balck pepper (*Piper nigrum*) varieties through RAPD and AFLP analysis. National Symposium on Biotechnological Interventions for Improvement of Horticultural Crops, 10-12 January 2005. Kerala Agricultural University, Thrissur. Abstract: 226

Nigam, S.S. and Radhakrishnan, C. 1968. Chemical examination of the essential oil derived from the berries of *P. longum* L. *Bull. Natn. Inst. Sci. India* 37: 189-196

NRCMAP (National Research Centre for Medicinal and Aromatic Plants) 2002. Collection and cataloguing of germplasm of long pepper. *Biennial Report 2000 - 2002.* National Research Centre for Medicinal and Aromatic Plants, Boriavi, Gujarat.332p.

Manoj, P., Soniya, E.V., Banerjee, N.S. and Ravindran, P. 2004. Recent studies on well-known spice, *Piper longum* L. *Natural Product Rodrance* 3: 222-227

Manuel, J. 1994. Comparative evaluation of selected types of *Piper longum* in coconut plantations. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 109 p.

Massino Labra, Mariangita, Fabrissio Grassi and Francesco Sula. 2004. Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. *Pl. Sci.* 167(4): 725-731

Mathai, C.K.1986. Growth and yield analysis in black pepper vaieties (*Piper nigrum*) under different light conditions. *Mysore, J. agric. Sci.* 41(5): 190-191

Mathew, P. J., Mathew, P.M., Kumar, V. 2001. Graph clustering of *Piper nigrum* L. *Euphytica* .118(3): 257-264

Menancherry, A.J.1993. Comparitive anatomical studies on five species of Piper (Piperaceae). Ph.D. thesis , Calicut university. 123p.

Metcalfe, C.R. and Chalk, L. 1983. *Anatomy of Dicotyledons*. Second edition. Clarendon Press, Oxford. 481p

Mithila, J., Shivasankara, K.S., Satyabrata and Maiti, S. 2000. Growth and Morphology of vegetative and reproductive branches in betel vine (*Piper betle* L.). *J. Plantn Crops* 28(1): 50-54

Murthy, K.S. 1959. Studies in order Piperales IV - A contribution to the study of vegetative anatomy of three species of piper. *Proceedings of National Institute of Scientific India*. 25: 31-38

Murugan, C. 2002. Molecular characterization of *Piper* species using RAPD technique. M.Sc (Ag) thesis, Kerala Agricultural University, Thrissur, 80p.

Nair, S. 2005. Variability in chakkarakkolli (Gymnema sylvestre R.BR.) using morphological, biochemical and molecular markers. M.Sc. (Ag) thesis, Kerala Agricultural University,Thrissur, 75 p.

Nambiar, P.K.V., Pillai, V.S., Sasikumaran, S. and Chandy, K.C. 1978. Pepper Research at Panniyur - a resume. *J. Plantn. Crops* 6(1): 4-11

Narasimhan, S. and Mehrotra, S. 2003. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. *J. AOAC int.* 86(6): 1124-1127

Nazeem, P.A., Keshavachandran, R., Babu, T.D., Achuthan, C.R., Girija, D. and Peter, K.V. 2005. Assessment of genetic variability in balck pepper (*Piper nigrum*) varieties through RAPD and AFLP analysis. National Symposium on Biotechnological Interventions for Improvement of Horticultural Crops, 10-12 January 2005. Kerala Agricultural University, Thrissur. Abstract: 226

Nigam, S.S. and Radhakrishnan, C. 1968. Chemical examination of the essential oil derived from the berries of *P. longum* L. *Bull. Natn. Inst. Sci. India* 37: 189-196

NRCMAP (National Research Centre for Medicinal and Aromatic Plants) 2002. Collection and cataloguing of germplasm of long pepper. *Biennial Report 2000 - 2002*. National Research Centre for Medicinal and Aromatic Plants, Boriavi, Gujarat.332p.

Pal, P.K. 1981. Developmental studies VI. The origin and courses of the vascular systems in the shoot apices of six species of the genus Piper (Piperaceae). *Bull. Bot. Soc.* (Bazil) 15: 17-29

Panse, V.G. and Sukhatme, P.V.1978. *Statistical Methods for Agricultural Workers.* ICAR, New Delhi,120p.

Parani, M., Anand, A. and Parida, A. 1997. Application of RAPD finger printing in selection of micropropagated plants of *Piper longum* for conservation. *Curr. Sci.* 73(1): 81-83.

Patel, D. P., Das, A., Kumar, R. and Munda, G. C. 2005. Comparitive study of photosynthesis and associated parameters in major crops and weed species in mid hills of Meghalaya. *Ann. Plt. Physiol.* 19(1):1-4

Philip, S., Banerjee, N.S. and Das, M.R. 2000. Genetic variation and micropropagation in three varieties of *Piper longum* L. *Curr. Sci.* 78(2): 169-174

Pradeepkumar, T., Karihaloo, J.K. and Archak, S. 2001. Molecular characterization of *Piper nigrum* L. cultivars using RAPD markers. *Curr. Sci.* 81(3): 246-248

Pradeepkumar, T., Karihaloo, J.K., Archak, S. and Baldev, A. 2003. Analysis of genetic diversity in *Piper nigrum* L. using RAPD markers. *Genet. Resour. Crop Evolution* 50(5): 469-475

Prasada Rao, G.S.L.H.V. and Sebastain, S. 1994. Estimation of leaf area in tree crops. *J. Plantn. Crops* 22(1): 44-46

Purseglove, J.W. 1969. *Tropical Crops Dicotyledons.* Longman, London. pp. 400-441

Radhakrishnan,V.V., Madhusoodanan,K.J., Priya,P. and Thomas,J. 2004. Performance evaluation of selected varieties of pepper in the high ranges of Kerala. *Indian J. Arecanut Spices Medicinal Pl.* 6(3): 87-88

Rahiman, B.A., Murthy, K.N., Nair, M.K. and Nayar, N.M. 1979. Distribution, morphology and ecology of *Piper* species in Karnataka. *Indian J. Plantn. Crops* 7(2): 93-100

Raj, N.M., Augustin, A. and Nybe, E.V. 2001. Biochemical characterization of medicinal plants in the wild and domestic environments. Proceedings of the National Seminar on the Frontiers of Research and Development in Medicinal Plants, 16-18 September 2000. CIMAP, Lucknow, India. 23-27

Ram, P., Kothari, S.K., Raja Ram and Kumar, V. 1996. Cultivar differences in the morphological characteristics of suckers and yield in the menthol mint *Mentha arvensis*. *J. Medicinal Aromatic. Pl. Sci.* 18: 15-16

Ranade, S.A., Verma, A., Gupta, M. and Kumar, N. 2002. RAPD profile analysis of betel vine cultivars. *Biologia Plantarum* 45(4): 523-527

Ravindran, P.N and Nair, M.K.1984. Pepper varieties. *Indian Cocoa Arecanut Spices J.* 7 (3): 67-69

Ravindran, P. N. 1990. studies in black pepper (piper nigrum L.) and some of its wild relatives. Ph.D. thesis, Calicut University, 336p.

Ravindran, P.N. and Sasikumar, B. 1993. Variability in open pollinated seedlings of black pepper. *J. Spices Aromatic Crops* 2: 60-65

Ravindran, P.N., Balakrishnan, R.O. and Babu, K.N. 1997. Morphometrical studies on black pepper (*Piper nigrum* L.) and cluster analysis of black pepper cultivars. *J. Spices Aromatic Crops* 6(1): 9-20

Ravindran, P. N. 2000. *Black Pepper (Piper nigrum* L.*)*. Harwood Academic Publishers, Amsterdam 553p.

Ravishankar, K.V., Dinesh, M.R. and Anand, L. 2000. Assessment of genetic relatedness among mango cultivars using RAPD markers. *J. hort. Sci. Biotech.* 75(2): 198-201

Reddy, M.L.N.1996. Morphological variation in betel vine (*Piper betle* L.) collections. *J. Plantn. Crops.* 24(2): 115-118

Remashree, A.B., Raja,S.S., Jayanthy,A., Unnikrishnan,K.P. and Balachandran,I. 2005.comparative anatomical and phytochemical markers to identify Asoka from its common adulterant. *Aryavaidyan* 19(1): 13-24

Rheede, H.V. 1678. *Hortus Indicus Malabaricus.* Vol. 7.Amstelodam, p.31

Ridley, H.N. 1983. *Spices*. Macmillan & Co. Ltd., London, 523p

Rohlf, F.J. 1998. NTSYS-PC Numerical taxonomy and multivariate analysis system 2.0. Exeter Publications, New York. *Euphytica* 68: 69-76

Rosenberg, L. V. and Kriiger, G .H. J. 1993. Comparitive analysis of differential drought stress induced suppression and recovery in CO_2 fixation, stomatal and non-stomatal limitation in *Nicotiana tabacum J.Plt.Physiol.* 142: 296-306

Royo, J.B. and Itoiz, R. 2004. Evaluation of discriminance capacity of RAPD, isoenzymes and morphologic markers in apple (*Malos domestica* Borkh) and the congruence among classifications. *Genet. Resour. Crop Evolution* 51: 153-160

Samuel, M.R.A., Balasubramanian, S. and Bavappa, K.V. 1984. Cytochemical and leaf epidermis studies in the genus *Piper. J. Plantn. Crops* 12: 56-63

Sanalkumar, P. 2005. Biochemical and molecular characterisation of *Njavara* genotypes of rice (*Oryza sativa* L.). Ph.D. thesis, Kerala Agricultural University, Thrissur, 214 p.

Sasidharan, K. and Muralidharan, P. K. 2000. KFRI Report No:193. Kerala Forest Resersch Institute,Peechi.

Sasikumar, B. 2004. Underutilized medicinal species. *Spice India* 17: 2-5

Sasikumar, B., Chempakam, B., George, J.K., Remashree, A.B., Devasahayam, S., Dhamayanthi, K.P.M., Ravindran, P.N. and Peter, K.V. 1999. Characterisation of two interspecific hybrids of piper. *J. hort. Sci. Biotech.* 74(1): 125-131

Shaji Philip, Banerjie, N.S. and Das, M.R. 2000. Genetic variation and micropropagation in three varieties of *Piper longum* L. *Curr. Sci.* 8(1): 169-173

Shankaracharya, N.B., Rao, L.J., Naik, J.P. and Nagalakshmi, S. 1997. Characterisation of chemical constitutents of Indian long pepper (*P. longum* L.). *J. Fd. Sci. Technol.*34 (1): 73-75

Sharma, J.R. and Ram, R.S. 1997. Morphometric divergence in *Cymbopogon* species. *J. Medicinal Aromatic Pl. Sci.* 19: 1009-1016

Sharma, K.D., Singh, B.M., Sharma, Y.R., Katoch, M. and Guleria, S. 2000. Molecular analysis of variability in *Podophyllum hexandrum* Royle. -an endangered medicinal herb of north west Himalayas. *Pl. Genet. Resour. Newsl.* 124: 57-61

Shasany, A.K., Lal, R.K., Khanuja, S.P.S., Darikar, M.P. and Kumar, S. 1998. Comparitive analysis of four elite genotypes of *Vetiveria zizanoides* through RAPD profiling. *J. Medicinal Aromatic Pl. Sci.* 20: 1022-1025

Sherlija, K.K., Remashree, A.B., Unnikrishnan, K. and Ravindran, P.N. 1998. Comparitive rhizome anatomy of four species of Curcuma. *J. Spices Aromatic. Crops* 7(2): 103-109

Shivasankara, K.S., Mithila, J. and Maiti, S. 2000. Effect of different light intensities on growth and yield of betel vine (*Piper betle* L.). *J. Plantn. Crops* 28(3): 196-200

Shylaja, M. and Manilal, K.S. 1992. Bark anatomy of four species of Cinnamon from Kerala. *J. Spices Aromatic Crops* 1(1): 84-87

Singh, A.K., Singh, J. and Sharma, S.1996. Plant character association and path analysis among yield and its component characters at different stages of growth in lavender,(*Lavandula officinalis*). *J. Medicinal Aromatic Plt.*18: 34-37

Singh,. B.M., Mahajan, R.K., Umesh, S. and Pareek, S.K. 2004. *Minimal Descriptors of Agri-Horticultural Crops. Part IV-Medicinal and Aromatic Plants*. National Bureau of Plant Genetic Resources, Pusa, NewDelhi. pp 295-300

Singh, J., Sinha, K., Mishra, N.P., Singh, S.C., Sharma, A. and Khanuja, S.P.S. 2004. Traditional uses of *Piper longum. J. Medicinal Aromatic Pl. Sci.* 26(1): 79-83

Singh, M.P. and Panda, H. 2005 *Medicinal Herbs with Their Formulations*. Daya Publishing House, New Delhi, 660p.

Sivarajan, V.S. and Indira, B. 1995. *Ayurvedic drugs and their plant sources.* Oxford & IBH 374-376

Solereder, H. 1908. *Systematic Anatomy of the Dicotyledons II.* Clarendon Press, Oxford, London. pp. 1108-1148

Sujatha, R. and Namboodiri, K.M.N. 1995. Influence of plant characters in yield in black pepper (*Piper nigrum* L.). *J. tropic. Agric.* 33: 11-15

Sujatha, R.1991. Variability in intervarietal F1 hybrids and open pollinated seed progenies of black pepper. M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 103 p.

Suseelappan, M.S. 1991. Medicinal uses of pepper in Ayurveda. *Indian Spices* 28(3): 25-26

Timmannavar, S.V. and Patil,B.C. 2000. Photosynthesis, light interception and seed cotton yield of different cotton species under rainfed condition. *Indian J. Plt. Physiol.*5: 236-249

Uma, S., Sudha, S., Saraswathi, M.S., Manickavasagam, M., Selvarajan, R., Durai, P., Sathiamoorthy, S. and Siva, S.A. 2004. Analysis of genetic diversity and phylogenetic relationships among indigenous and exotic Silk (AAB) group of bananas using RAPD markers. *J. hort. Sci. Biotech.* 79: 523-527

Ushavani, D. 2003. Morphological, Biochemical and molecular markers for the genetic analysis of cashew (*Anacardium occidentale* L.). M.Sc. (Ag) thesis, Kerala Agricultural University, Thrissur, 74 p.

Verma, A., Kumar, N. and Ranade, S.A. 2004. Genetic diversity among landraces of a dioecious vegetatively propagated plant, betel vine (*Piper betle* L.). *J. Biosciences* 29(3): 319-328

Virk, P.S., Lloyd, B.V., Jackson, M.T. and Newbury, J.J. 1993. Use of RAPD for the study of diversity within plant germplasm collections. *Heredity* 74: 170-179

Viswanathan, T.V. 1995. *Piper longum* (thippali) as intercrop in coconut plantations. *Spice India.* 8(6): 12-14

Welsh, J. and McClelland, M. 1990. Finger printing genomes using PCR with arbitrary primers. *Nucleic Acids Res.* 18: 7213-7218

Yang, Y.C., Lee, S.G. and Kim, M. 2002. A piperidine amide extracted from *P. longum* fruit shows activity against *Aedes aegypti* mosquito larvae. *J. agric. Fd. Chem.* 50(13): 3765-3767

Yoshida, S. 1972. Physiological aspects of grain yield. *Ann. Rev. Plt. Physiol.* 23: 437-464

Appendices

APPENDIX I

Laboratory equipments used for the study

Spectrophotometer	Spectronic Genesys – 5, Spectronic Instrument, USA
Refrigerated centrifuge	Kubota, Japan
Water purification system	Millipore, Germany
Thermal cycler	Peltier PTC 200, MJ Research, USA
Ice flaking machine	Ice matics
Gel documentation system	Biorad Alpha Imager

APPENDIX II

a) Reagents for DNA isolation as per Doyle and Doyle (1987)

I. Extraction Buffer (4x) - 1litre

Sorbitol – 25.6 gm

Tris – 48 gm

EDTA disodium salt – 7.4 gm

II. Lysis Buffer- 1litre

1 M Tris pH 8 – 200 ml

0.25 M EDTA – 200 ml

CTAB – 20 gm

III.TE Buffer

10 mM Tris (pH 8)

1 mM EDTA (pH 8)

IV. Iso-propanol

V. Chloroform: Isoamyl alcohol mixture (24:1, v/v)

VI. 5% Sarcosin

VII. Ethanol 100% and 70%

APPENDIX III

Reagents for agarose gel electrophoresis

I. Agarose

II. 50X TAE buffer

Tris Base - 242g

0.5M EDTA (pH 8) - 100ml

Glacial acetic acid - 57.1ml

III. Tracking dye (6X)

Bromophenol blue 0.25 %

Xylene cyanol FF 0.25%

Glycerol in water 30%

IV. Ethidium bromide

APPENDIX IV

Analysis of variance for various characters of the eight accessions

Character	Degrees of freedom	Mean sum of square	F value
Vine length	7	81.475	2.996**
Angle of insertion of Reproductive branch	7	5464.641	.929 NS
Internodal length	7	10.792	13.659**
Petiole circumference	7	0.08	8.274**
Petiole length	7	16.975	12.189**
Vegetative leaf length	7	20.702	18.605**
Vegetative leaf width	7	12.727	15.174**
Vegetative leaf area	7	3124.818	16.309**
Vegetative leaf l/b	7	0.032	2.443*
Reproductive leaf length	7	22.192	58.504**
Reproductive leaf width	7	3.788	15.747**
Reproductive leaf area	7	407.749	42.889**
Reproductive leaf l/b	7	1.428	12.961**
Number of days for Spike initiation	7	2815.107	86.11**
Number of days for Spike maturation	7	206.479	21.403***
Spike number	7	2.136	2.901**
Spike length	7	35.082	46.769**
Spike diameter	7	5.373	12.538**
Fresh weight	7	1.549	40.921**
Dry weight	7	0.044	22.012**
Loss in weight after drying	7	345.839	22.942**
Rate of Photosynthesis	7	1549.9	27.0680**
Stomatal conductance	7	0.048	20.2748**
Transpiration rate	7	20.242	22.9132**
Piperine (%) (25-30 days)	7	1.26	131.28**
Piperine (%) (60-70 days)	7	10.575	308.34**
Piperine (%) (after 75 days)	7	5.532	67.09**
Oil (%) (60-70 days)	7	1.028	22.96**
Oil (%) (after days)	7	1.072	28.87**

**Significant at 1% level

* Significant at 5% level

NS not significant

CHARACTERISATION OF LONG PEPPER (*Piper longum* L.) GENOTYPES USING MORPHOLOGICAL, ANATOMICAL AND MOLECULAR MARKERS

By

JITHA JALEEL

ABSTRACT OF THE THESIS

submitted in partial fulfillment of the requirement
for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University, Thrissur

Department of Plant Breeding and Genetics

COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680 656
KERALA, INDIA

2006

ABSTRACT

The present investigation entitled "Characterisation of long pepper (*P. longum* L.) genotypes using morphological, anatomical and molecular markers was undertaken at the Department of Plant Breeding and Genetics, College of Horticulture ,Vellanikkara during the period 2003-2006. Six female and two male accessions formed the material for the study and were subjected to morphological, anatomical, and molecular characterization.

The accessions showed variation in the morphology of vegetative and reproductive branches in leaf size, leaf shape and l/b ratio. Vegetative branches had large cordate leaves with petioles whereas reproductive branches had lanceolate leaves with rudimentary petiole. The l/b ratio of leaves of reproductive branches was higher compared to leaves of vegetative branch. The spikes of females were short, bold and greenish black on maturity whereas it was long, slender and yellow in the case of males. At early stages of spike growth, female spikes were creamy yellow and male green in colour.

The stem, leaf, and root anatomy of the male and female accession showed almost similar features. The difference was noticed in the number of medullary and cortical bundles and the presence or absence of mucilage canals. Mucilage canal was present only in the female accessions, Assam, NL-84-68 and Viswam. The male and female spikes differ in their anatomical features.

The physiological parameters revealed higher photosynthetic rate for the female accession, Viswam. The male accessions had lower photosynthetic rate compared to female accessions.

RAPD analysis done on the eight accessions using five selected primers revealed 15 to 49% variability among the accessions at the molecular level and the primer OPF 5 produced male specific bands.

The female accessions yielded maximum piperine and oil content at 60 -70 days maturity when they were greenish black and hard. Among the female accessions, Viswam, NL-84-68 and Assam recorded highest piperine and oil content. Low amounts of piperine and oil were found in male spikes also.

Based on the study three female accessions, Viswam, NL-84-68 and Assam were found to be promising types and further breeding programmes can be carried out in these accessions to improve their performance.