EVALUATION AND CHARACTERISATION OF PROMISING HYBRIDS OF LONG PEPPER (*Piper longum* L.)

By

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(2015-12-004)

THESIS

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COLLEGE OF HORTICULTURE

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DECLARATION

I, hereby declare that this thesis entitled **"Evaluation and characterisation of promising hybrids of long pepper (*Piper longum* L.)"** is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or society.

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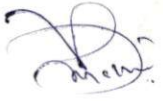
CERTIFICATE

Certified that this thesis, entitled **"Evaluation and characterisation of promising hybrids of long pepper (*Piper longum* L.)"** is a record of research work done independently by **Ms. Sruthy K.** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.



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We, the undersigned members of the advisory committee of **Ms. Sruthy K (2015-12-004),** a candidate for the degree of **Master of Science in Horticulture,** with major field in **Plantation Crops and Spices,** agree that the thesis entitled **"Evaluation and characterisation of promising hybrids of long pepper (*Piper longum* L.)"** may be submitted by **Ms. Sruthy K (2015-12-004),** in partial fulfillment of the requirement for the degree.

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Introduction

1. INTRODUCTION

Long pepper, *Piper longum* L., popularly known as thippali belongs to the family Piperaceae. Thippali or pippali is an important medicinal plant originated in South Asian countries like India, Srilanka, Nepal and Bhutan. Long pepper is used in traditional medicines in Asia and Pacific islands. It was used as a spice in ancient India. The family Piperaceae comprises 12 genera and about 1400 species, mainly found in tropical region (Barroso, 1978).

Long pepper has high potential as a medicinal plant and it is used in more than 320 classical medicinal formulations. Almost all parts of long pepper are medicinally important, especially in the treatment of respiratory tract disorders like bronchitis, asthma, cough etc. (Sivarajan and Balachandran, 1994). The principal pharmacological constituents are piperine and piplartine.

Dried spikes are the economic part which fetches good price in market as it is a main ingredient in many medicinal formulations especially in Ayurveda. Male and female plants are difficult to distinguish till they start flowering. Male spikes are long, slender and female spikes are short and fleshy. Female spikes are commercially exploited for medicinal purpose. However, staggered flowering makes harvesting difficult and labour intensive in long pepper.

Long pepper prefer humid tropical climate and it is suitable as an intercrop in coconut, arecanut and rubber plantations. *Piper longum* has very high demand in drug industry. Lack of high yielding varieties discourages farmers from its cultivation. Identification of high yielding varieties will attract many farmers to cultivation of long pepper so that they can earn more income by cultivating long pepper either as a monocrop or as an intercrop in coconut, arecanut and rubber plantations.

Kerala Agricultural University released a variety in long pepper, 'Viswam' in 1996. It is the only variety in long pepper released so far in *P. longum*. As a part of a KSCSTE funded project, hybridisation was carried out between female and bisexual types at College of Horticulture, Vellanikkara (Sujatha, 2009). Viable seeds and seedlings were produced in *Piper longum* for the first time. Out of the 794 seeds obtained 136 seeds germinated. Seedlings were raised in pots and evaluated for growth characters (Kanimozhi, 2010). High amount of variability was observed in the seedling population of thippali for number of main branches, length of the longest stem, length and width of leaves, length of petiole, number of spike bearing branches per stem, spike and pedicel growth, fresh weight, dry weight, yield and driage (Chandran, 2012). Sujatha (2016) evaluated ten selected accessions from the seedling population and found that four accessions were superior in yield.

The present study entitled "Evaluation and characterisation of promising hybrids of long pepper (*Piper longum* L.)" was taken up as a follow up of the earlier work with the objective of evaluating the four promising hybrids of long pepper, their performance at different shade levels for growth, yield and quality and to characterise the hybrids using molecular markers.

Review of literature

2. REVIEW OF LITERATURE

Piper longum L. is a popular medicinal plant used mainly for treating respiratory tract diseases like cough, bronchitis, asthma etc. It is also reported that it can be used as counter-irritant and analgesic when applied locally for muscular pains and inflammation. It can also be used as a general tonic and hematinic. Dried fruits of long pepper is pippali/thippali and the roots are pippalimulam. These Pippali and Pippalimulam are used in the drugs prepared from *Piper longum* (Sivarajan and Balachandran, 1994).

2.1 Origin and distribution

Piper longum is native to South Asian countries including India. In India, it is found from Central Himalayas to Assam, Khasi and Mikir hills and the lower hills of West Bengal. The species also occur in the evergreen forests of Western Ghats from Konkan to Travancore. It has also been recorded that Car Nicobar Islands is another region where *Piper longum* occur as well as cultivated. The species is distributed in the Indo-Malayan region and Sri Lanka (Sumy *et al.*, 2000; Sivarajan and Balachandran, 1994)

Piper longum has reached the other Asian and Mediterranean countries through "spice route" from India. It was used as a spice in all these regions (Ravindran and Balachandran, 2005). Long pepper probably came to Europe before black pepper and it was highly priced during the Roman Empire, almost three times more than black pepper. *Piper longum* was preferred for Roman cookery because of its pungency as well as sweetness. It was also popular in Africa, mainly in the Islamic regions of North and East Africa.

In the tribal belts of Andhra Pradesh, parts of Orissa, North West Bengal, Assam and in North Eastern states, long pepper is cultivated mainly for its fruits and

roots. It was used for flavouring various beverages and dishes in ancient India, as a source of pungency.

The Indian long pepper was said to be derived from two or three species. It is a product of either *Piper peepuloides* or *Piper longum*. The Java long pepper is from *Piper officinarum* (Khushbu *et al.*, 2011).

Indonesian or Java long pepper is from *Piper retrofractum*, synonym of *Piper officinarum*. In Western countries, mostly *Piper retrofractum* is available. This species comes from South East Asia and is mostly cultivated in Indonesia and Thailand. Both these species are often not distinguished clearly in the spice trade (Manoj *et al.*, 2004).

2.2 Cytology

Many researchers worked on cytological aspects of *Piper longum*. There was wide variation in the chromosome number reported. According to Tjio (1948) somatic chromosome number was 2n= 24 whereas Sampathkumar and Navaneethan (1981) reported that 2n=44. Other reports on somatic chromosome number of *Piper longum* includes 2n= 48 (Dasgupta and Datta, 1976); 2n= 52 (Mathew, 1958; Jose and Sharma, 1984); 2n= 53 (Samuel and Morawetz, 1989) and 2n= 96 (Sharma and Bhattacharya, 1959).

Anand (1997) studied Cyto- morphology in *Piper* species and standardised the procedure for mitotic studies. She has reported a new somatic chromosome number for *Piper longum*, i.e 2n= 32.

2.3 Molecular characterization

Application of RAPD fingerprinting of micro propagated plants of *Piper longum* was reported by Parani *et al.* (1997). Twenty micro propagated plants and the mother plant were subjected to RAPD analysis using ten random decamer

primers. They reported that 18 micro propagated plants along with the mother plant formed a major cluster and the others were molecular off types or somaclonal variants.

Banerjee *et al.* (1999) analysed polymorphism in the genomic DNA of 25 female and six male plants of *Piper longum* using RAPD. For that they used 40 decamer random oligonucleotide primers. Two RAPD bands consistently appeared only in the plants showing male genotype, suggesting male-associated nature of these DNA markers in dioecious *Piper longum*. This was the first report of molecular basis of dioecy in thippali.

High genetic variation among different species of *Piper* was found by Sen *et al.* (2010) in their study conducted using RAPD and this results helped in germplasm identification, management and conservation.

Genotypic and morpho-genetic differences among three varieties of long pepper, a variety from Assam, another one from Calicut and the released variety 'Viswam' were reported by Philip *et al.* (2000). A common and efficient method of plant regeneration was developed by them. RAPD analysis, using oligo nucleotide primers revealed that these three varieties were genetically different. Viswam and Calicut varieties were genetically more closer compared to Assam variety.

Murugan (2002) used RAPD profiles of thirteen *Piper* species using twenty random primers to form genetic similarity indices and based on this, four clusters were formed. Two separate clusters were formed by *P. nigrum* and *P. longum*, whille *P. colubrinum* and *P. attenuatum* formed third cluster. *P. chaba, P. betle* and *P. arboreum* were in the fourth cluster.

Two male associated RAPD markers were described by Manoj *et al.* (2005) in *Piper longum.* RAPD profiles of the male samples were easly distinguishable from those of females because of the presence of prominent male-associated bands

$OPA10_{827}$ and $OPA15_{744}$. Sequencing of male sex-associated RAPD bands for the development of male sex-associated SCAR markers was also done. They have converted RAPD marker $OPA10_{827}$ into a SCAR marker. In almost twenty percent of the female population of *Piper longum*, the SCAR developed based on the DNA sequence of the other RAPD marker was found. Based on this, and Southern hybridization experiments, it was clear that there was considerable homology between the chromosomes of male and female genotypes from which RAPD markers were derived. From the sequences, remarkable differences were obtained between the male and female plants of dioecious *Piper longum.*

Characterization of long pepper using morphological, anatomical and molecular markers in six female and two male accessions were done by Jaleel (2006). Variation in the morphology of vegetative and reproductive branches, in leaf size, leaf shape and l/b ratio were reported in the accessions studied. Vegetative branches possessed large cordate leaves. Leaves of reproductive branches had higher l/b ratio compared to that of vegetative branches. The spikes of male and female were morphologically distinct from each other. It was short, bold and greenish black at maturity for females whereas it was long, slender and yellow in case of males. Spikes of female were creamy yellow at early stages of growth whereas it was green for male. Differences in anatomy were found in case of the number of medullary and cortical bundles and the presence or absence of mucilage canals. Photosynthetic rate was higher for female accession, Viswam. RAPD analysis was done in eight accessions. Accessions showed 15-40 per cent variability at the molecular level. Male specific bands were produced by the primer OPF5.

Six *Piper* species were evaluated for their relatedness using RAPD. All the species were grouped into 3 clusters based on the number of bands. Maximum similarity was between *P. betel* and *P. longum* and also between *P. nigrum* and *P. mullesua* species, altogether forming one cluster in dendrogram (Chikkaswamy *et al.*, 2007).

Finger printing of *Piper longum* cultivars was reported by Keshavachandran *et al.* (2007). They used 11 land races and one advanced cultivar of long pepper for their study. Ten primers that yielded clear and dominant banding patterns were selected for the final analysis of 11 accessions. For a few land races and accessions, cultivar specific single bands were obtained.

Suma (2013) standardized the total genomic DNA isolation technique. Genetic diversity analysis using RAPD primers indicated that the accessions collected were genetically different and the four primers selected for the study were efficient in detecting the diversity in the group. Collections originating from various parts of the country did not form distinct groups and were interspersed with each other indicating no association between RAPD pattern and geographic origin of accessions.

Molecular characterisation of the four selected hybrids of *Piper longum* was made along with their parents and released variety 'Viswam' (Sujatha, 2016). Among forty three decamer primer screened, eleven primers showed difference between genotypes. Three primers *viz.*, OPM 20, OPB01 and OPAU03 showed polymorphism between the male and female types . Pl 140 was found to be most distinct from other hybrids. For the remaining hybrids, 2-3 primers showed polymorphism (Sujatha, 2016).

2.4 Isozyme analysis

Sebastian (1995) has done isozyme studies in *Piper* species which revealed that *Piper longum* and *Piper betle* are distinct from other *Piper* species and they stood individually having only low similarity index with others. Pooled analysis of similarity indices revealed that out of 11 species studied, nine could be grouped into three groups. First group consisted *Piper nigrum*, *P. pseudonigrum, P. bababudani* and *P. galeatum*. In second group, *P. argyrophyllum* and *P. attenuatum* are included. *P. chaba*, *P. hapnium* and *P. colubrinum* formed the third group.

2.5 Morphological characters

2.5.1 Stem characters

Stem of long pepper are numerous, ascending, cylindrical or globose. Swollen and irregularly knotty with each piece quarter inch long, irregularly thick, hard and brown coloured stems are found in long pepper. Branches are erect, prostrate or creeping, soft and grooved when dry. Also the whole stem was finely pubescent (Kumar, 1998).

Long pepper produces distinct dimorphic branches i.e., orthotropes and plagiotropes. Main branches which creep on the ground are orthotropes. Similarly, the axillary branches which grow erect are called plagiotropes. Orthotropes are vegetative and grow by the activity of the terminal bud while the short axillary branches are fruiting branches, which bear spikes on them and the growth is sympodial (Ravindran and Balachandran, 2005).

A comparative evaluation of selected types of *Piper longum* in coconut plantations was done by Manuel (1994). The study revealed that the five types of *Piper longum* differed for eleven vegetative characters namely length of the longest stem, number of vegetative branches per stem, length of leaf, width of leaf, length of petiole, spread of the plant, internodal length of main stem, number of spike bearing branches per stem and angle of insertion of spike bearing branch and for three productive characters namely number of spikes per spike bearing branch, yield of green spike and yield of dry spike at one or all of the stages for which observations were recorded. Of the above characters for which the five types differed significantly, eight characters showing high and significant correlation with yield were chosen for carrying out studies on intercorrelation among yield components and path analysis. Correlation studies and path analysis revealed that angle of insertion of spike bearing branch, number of stems per hill, number of spikes per spike bearing

branch and number of spike bearing branches per stem and yield green spike were the most important characters influencing dry spike yield.

Morphological, anatomical and molecular characterisation of eight accessions was done by Jaleel (2006). The study revealed that there was significant difference between these accessions for the length of spike bearing branch and internodal length. And there was no significant difference in angle of insertion of reproductive branches.

Sujatha and Nybe (2007) reported a bisexual variant in *Piper longum.* Joseph (2008) observed that number of vegetative branches per stem, number of spike bearing branches per stem, total number of leaves per hill, number of spikes per spike bearing branch, length of spike, girth of spike, fresh weight of spike and dry weight of spike had positive correlation with dry spike yield.

Chandran (2012) studied bisexual variants and evaluated superior types in thippali. The study revealed that the key morphological characters were same for all the accessions, which were typical for *Piper longum*. High amount of variability was observed for quantitative characters like number of main branches, length of longest stem and number of spike bearing branches per stem.

Nair (2015) studied 42 accessions of long pepper. Among the accessions studied, number of primary branches, spike bearing branches per primary branch and leaves per plant ranged from 1.00 to 8.00, 1.00 to 6.71 and 21.67 to 166.0, respectively. Plant height, petiole length, internodal length of spike bearing branches and leaf area ranged from 39.67 cm to 88.33cm, 1.11 cm to 7.56 cm, 1.86 cm to 7.38 cm and 25.98 cm^2 63.87 cm^2, respectively. Only 14 per cent similarity was found among accessions based on the quantitative data.

Sujatha (2016) evaluated promising hybrids under 25 per cent and 50 per cent shade levels and she found that at 25 per cent shade, Pl 141 possessed longest vine among all the hybrids studied. It was on par with female parent.

2.5.2 Leaf characters

Long pepper possess numerous leaves ranging in size from 6.0 - 9.0 cm, lower leaves are broadly ovate or cordate with broad rounded lobes at base (Kirthikar and Basu, 1935). Basal leaves are cordate whereas upper leaves are oblong-oval. All leaves are sub-acute, entire, glabrous, thin, bullate with reticulate venation, sunk above and raised below, dark green and shiny above, dull and pale beneath, petiole of lower leaves range in length from 5-7.5 cm and are stout. Petioles of upper leaves are very short or sometimes absent, stipules are membraneous, lanceolate, obtuse, soon falling, with a size of about 1.3 cm.

According to Chatterjee and Pakrashi (1997) leaves of *Piper longum* types were 5-9 cm long, 3-5 cm wide, subacute, entire, glabrous, cordate with broad rounded lobes at base.

Leaves in long pepper were numerous, simple, stipulate and petiolate or sessile according to their position on the plant (Kumar, 1998). We can see variation in shape of leaf in same plant. Leaves located in the upper region of plant are generally sessile, amplexicaul or stem creeping, ovate or ovate oblong, acute and most often unequally sided or unequally cordate at base. The study also shows that the leaves were 6.5-9 cm long and 3-5 cm wide. Lower leaves were broadly ovate, pale dull below; cordate at base. Length of petiole of lower leaves ranges from 0.5 to 7.5 cm and they were stout but the petioles of upper leaves were very short and sometimes absent. Stipules were membraneous, lanceolate, obtuse and they were about 1-3 cm and usually falling soon.

Nair (2015) reported that leaf area of the long pepper genotypes studied, was ranging from 25.98 cm^2 to 63.87 cm^2. She also reported that it took 77 to 146 days in female types for emerging spikes from planting and for emergence to maturity it was 60 to 80 days. Whereas it was 135 to 141 days and 61 to 64 days respectively for male types.

2.5.3 Inflorescence, flowers and spikes

Long pepper is usually dioecious, in which male and female flowers are produced in separate plants (Ravindran and Balachandran, 2005)

Piper longum produce spikate inflorescence, contain sessile flowers, usually unbranched, elongated, simple and intermediate (Kumar, 1998). Flowers are devoid of perianth and very densely packed in spikes. Kumar also reported that spikes were 5 cm long, cylindrical, solitary, pedunculate and upright. Flowers were unisexual, minute and sessile. Spikes were large, narrow and slender with narrow bracts in case of male. They were 1-3 inches long, peltate and bear two stamens. Female spikes were 1.3- 2.5 cm long and 4-5 mm in diameter, bracts circulate, flat and peltate, 3-4 stigma, very short and persisting. Season of flowering was July – August

Spikes of long pepper are cylindrical, oblong, berries red or black when ripe, globose with aromatic odour and pungent taste (Banerjee *et al.*, 1999; Viswanathan, 1995).

According to Kumar (1998) fruits in *Piper longum* were short, consists of multitude of minute buccate fruits closely arranged in a common axis, whole forming a spike, one and half inch length and quarter inch thickness, fruits were ovoid, crowned with stigma and arranged with small peltate bracts beneath each. Fruits were grayish green and nearly blackish and particularly sunk in fleshy axils at ripened stage. Season for fruiting was November to march. Seeds of long pepper were globose, testa thin with in the hardened periphery.

The male and female plants are morphologically very similar and difficult to distinguish till the formation of spikes (Manoj *et al.*, 2004). Very small spikes of size 2.0 - 3.0 cm was seen in female plants whereas male plant bears larger compared to female i.e, 6.0 - 7.5 cm long. Sujatha and Nybe (2007) identified a bisexual variant (Acc. P 25) with spikes as long as male spikes.

Flowers were arranged on a short cylindrical spike. Male spikes were much longer than the female spikes. Female spikes were stout and short in which flowers fused laterally. Female flowers possessed single ovary which arose from the axil of the bract (Ravindran and Balachandran, 2005). They also reported that fruits of long pepper were small and closely packed. Apomictic fruiting was observed in *Piper longum* and fruits were produced without pollination. Therefore male plants were not required for fruit production. Fruits of *Piper chaba* were larger, more conical and not cylindrical as in long pepper and become orange red on ripening. Spikes of *Piper chaba* were more pungent with less flavor. Spikes of *Piper peepuloides* were similar to *Piper longum*.

In all three sex forms, the spikes were cylindrical and erect. In female type, the spikes were creamy white until fruit set, later changed to green. In male and bisexual types, immature spikes were dark green, later turned to light yellow and further to dull yellow on maturity (Kanimozhi, 2010). It took 22 days for attaining full length of spike in female, 43 days in male and 46 days in bisexual types. Female flowers were represented by ovary and stigmatic lobe subtended by bract. Anthers covered by peltate bract represented the male flowers. Perianth was absent in male, female and bisexual types. In bisexual flowers, the stigmatic lobes varied from 2 to 6. Ninety per cent of the bisexual type possessed two stigmatic lobes as prominent. Four stigmatic lobes were common (74 per cent) followed by three lobes (20 per cent) in female type.

Kanimozhi and Sujatha (2016) reported that anthesis and anther dehiscence were between 7.30 am and 4.30 pm with a peak between 10.30 am to 12.30 pm. Maximum pollen fertility i.e., 42.54 per cent was reported at 9.30 am. It took seven days for complete opening of flowers within an inflorescence in male, female and bisexual types. In male and bisexual inflorescences, anther dehiscence completed in one week. Normally fruits didn't contain viable seeds. Viable seeds were produced on artificial pollination.

Nair (2015) reported that maximum inflorescence (more than 55 per cent) was produced during June, July and August. During December and January it was less than 5 per cent, i.e minimum. It was also reported that, flowering was extended during May to October in accessions like PL 42, PL 53 and PL 57. Coefficient of variation for year round flowering ranged from 7.34 to 46.32 per cent. Coefficient of variation for number of spikes per spike bearing branch ranged from 1.00 to 3.21. In female types spike length and girth varied from 0.90 cm to 3.10 cm and 3.75 mm to 8.86 mm respectively. Whereas it was 8.10 cm to 8.18 cm and 4 mm to 4.03 mm respectively in male accessions. For fresh and dry yield per plant, coefficient of variation was 122.45 per cent and 120.44 per cent respectively.

Jaleel (2006) reported that at 60 – 70 days maturity, when the spikes were greenish black and hard, yield of piperine and oil was maximum in female accessions.

2.6 Medicinal properties

The plant is said to be a good rejuvenator in Ayurvedic medicine. It helps in improving vitality and it can also used as a tonic to stimulate appetite. Thippali is known for its detoxifying activity of lungs. It helps in removing cold and congestion. Fruits and roots are the parts which possess medicinal property. It helps to expel out the mucus present in the respiratory tract. Long pepper is also being used against tumors, indigestion, epilepsy, gout, laryngitis, flatulence etc. It soothes and relieves

muscular pains and inflammation. Antibacterial and anthelminthic properties are identified for the oil extracted from *Piper longum*. Indian long pepper is a source of anti- HIV constituents (Hareesh *et al.*, 2006)

Roots and fruits of thippali are used as an antidote to snake bite and scorpion stings. It is also used to treat chronic bronchitis, cough and cold. Ripe fruits of thippali can be used as an alternative to tonic (Chahal *et al.*, 2011). Long pepper is an ingredient in health stimulants like 'Chawanaprash'.

Long pepper is one of the ingredient in Trikatu, a most popular formulation. It is used to cure the diseases due to kapha dosha and it also helps to digest amino acids. It increases the bioavailability of the drug, when it is used as a complementary medicine. Highest activity, almost equal to the standard ampicillin solution was reported for the extract of trikatu churna (Malvankar and Abhyankar, 2012).

On experimental level, Pippalimula or roots of thippali is reported having analgesic, antifungal, antimicrobial and anti-oxidant activities (Joshi *et al.*, 2013). Also, the fruit and root extract of long pepper along with ciprofloxacin showed good synergistic activity against MRSA. Roots of *P. longum* along with seeds of *E. ribes* showed antifertility activity in female albino rats.

2.6 Antimicrobial activity

Piper species like *P. cubeba, P. chaba* (syn. *P. retrofractum* Vahl), *P. longum* and *P. nigrum* were evaluated for their antimicrobial activity by Khan and Siddiqui (2007). Strong antibacterial and antifungal activity was reported for their crude extract. Significant antibacterial activity was reported for the fractions containing piperine, during the analysis of the bioactivity of fractions.

Pharmacognostic, phytochemical, physiochemical, chromatographic, and antimicrobial activities of *P. nigrum* and *P. longum* was studied by Trivedi *et al.*

(2011). They reported the antimicrobial activity against human pathogens for the aqueous and methanol crude extracts of *P. nigrum* and *P. longum.*

Ounchokdee *et al.* (2016) reported antifungal activity of *Piper longum* extracts. Extracts of dried fruits of thippali was taken as the plant material for the study. Potent antifungal activity against tested plant pathogens including *Colletotrichum capsici*, *C. gloeosporioides* and *Fusarium oxysporum* f.sp. *cubense* were reported by them.

2.7 Cultivation practices

2.7.1 Nursery management

Long pepper is usually propagated by stem cuttings of 3-4 nodes. Planting of cuttings in June showed approximately 70 per cent rooting. February planted cuttings exhibited very less or nearly zero per cent of rooting. Significantly higher percentage (78.33 per cent) of rooting was observed for cuttings planted along with leaves. Number of roots (11.70), root length (13.59 cm) and vine length (22.47 cm) was also higher for the cuttings planted with leaves than the cuttings without leaves (Bhuse *et al.*, 2002). Cuttings treated with 100 ppm IBA recorded highest rooting percentage (88.33 per cent), number of roots (13.60), root length (15.05 cm) and vine length (24.38 cm). Cuttings with leaves, treated with IBA at 100 ppm exhibited the highest rooting percentage of 96.66 per cent and number of roots (15.44) based on the treatment interactions.

Etampawala *et al.* (2002) studied performance of vegetative stem cuttings comprising the two uppermost nodes and cuttings obtained from vertically growing reproductive parts of long pepper, which exhibited the appropriate types for propagation. Performance of cutting was good under 50per cent shade compared to 25 and 75 per cent respectively. Sand, top soil and farm yard manure in the ratio of 1:1:1 was found best medium for the growth of long pepper. Fruit production was

found to be early in vertically grown reproductive branches compared to that of horizontally grown vegetative branches. They also reported that fifty per cent of the fruits were shed from the mother plant in about 22 days after their emergence.

A study conducted by Reghuvaran *et al.* (2010) on coir pith biodegradation with white rot fungus and nitrogen fixing bacteria for cultivation of medicinal plants including *Piper longum* showed that a proportion of 25 per cent garden soil and 75 per cent compost yielded an effective growth of all medicinal plants. They reported that composted coir pith with nitrogen fixing bacteria was an effective potting mixture for cultivation of medicinal plants.

Gogoi and Singh (2011) studied the effect of inoculation of different AM fungi on growth performance of long pepper plants. A positive effect on different growth parameters like root and shoot length, percent of root colonization, fresh and dry weight of root and shoot, total biomass and chlorophyll content were reported in long pepper plants inoculated with AM fungi. Significant increase in shoot length and biomass was reported in most of the cases, as a result of inoculation of *Glomus fasciculatum* followed by other five inoculants viz, *G. versiforme, Glomus sp., G. mosseae, G. geosporum and G. etunicatum,* respectively.

2.7.2 Planting in field

Piper longum is ideal for planting as an intercrop in coconut garden. It requires 25 to 50 per cent shade for growing. 60 × 60 cm spacing is ideal for planting in coconut gardens (Viswanathan, 1993). 60 × 30 cm or 30 × 30 cm can also be adopted to plant long pepper in pits on beds of 1m width and convenient length (KAU, 2016)

Performance of *Piper longum* under different shade levels was studied by Etampawala *et al.* (2002). Performance was good for the plants grown under fifty per cent shade (maximum instantaneous light intensity 850 µmol/m/s) compared to plants

grown under 25 and 75 per cent shade respectively. Sujatha (2016) studied performance of ten promising hybrids of long pepper for their growth and yield under 25 per cent and 50 per cent shade levels.

2.7.3 Training and pruning

Pathiratna *et al.* (2005) studied the effects of plant to pruning and training methods, shade and type of cutting on the production of reproductive branches and spikes. Three locally available selections like Selection 1, 2, and 3 were selected for the study to develop suitable cultural practices to get higher yields in long pepper. Increased production of reproductive branches and spikes were noticed in plants subjected to training as well as pruning of runners. More number of reproductive branches and spikes were obtained in Selection 1 after pruning of runners. In Selection 2, pruning of runner at a distance of 40 cm from the base of mother plant helped to develop more reproductive branches and spikes. In Selection 3, training of runners to erect supports was a successful method to encourage more number of reproductive branches. Fifty per cent shade under field condition was good for the growth and yield in all three selections. Cuttings collected from the reproductive branches of Selection 3 produced reproductive branches exclusively during a period of one year under observation.

2.7.4 Nutrient management

Growth and yield of thippali can be improved by proper nutrient management. Application of 2 kg FYM per pit is recommended by Kerala Agricultural University. Application of cow dung slurry once in two months followed by earthing up will enhance general growth and spike yield (KAU, 2016). Application of FYM @ 20 t ha^{-1} is recommended.

Application of organic manure will increase the water holding capacity of soil. Long pepper needs 15 to 25 tonnes of FYM for its growth (Viswanathan, 1995)

An optimum amount of FYM i.e, 20 t/ha was suggested by Sheela (1996) after analyzing growth and yield performance of *Piper longum* during initial 1 ½ years in coconut garden. Application of fertilizer @ 30:30:60 kg/ha NPK was also reported to have higher growth and yield during first year in long pepper grown as an intercrop in coconut garden.

Pande *et al.* (1995) reported increased yield in long pepper through urea application. Ayisha (1997) reported that the peak period of yield was 17 MAP whereas in dry months yield was poor. July-August and October-November were identified as two peak periods of bearing. Uptake of NPK was higher in plots supplemented with 20 t/ha organic manure and 30:30:60 kg/ha NPK, under 60 × 60 cm spacing. The economic analysis of long pepper cultivation revealed that it could be a profitable intercrop in coconut garden with the above recommendations.

2.7.5 Integrated nutrient management

The effect of inoculation of AM fungi on growth performance of *Piper longum* was evaluated by Seema and Garampalli, 2015 for their symbiotic response in greenhouse condition. Three AM fungi, *Glomus fasiculatum*, *Acaulospora fovata* and *Gigaspora margirata* selected from trap culture were used as inoculum. They reported an increase in number of leaves in plants inoculated with *Acaulospora fovata* compared to other inoculated plants and control. Compared to *G. fasiculatum*, *A. fovata* and *G. margirata* showed increased effect for dry weight of total biomass, mycorrhizal dependency and mycorrhizal inoculation.

Total fresh and dry spike yield as well as total alkaloid production were increased in long pepper grown in partial shade by following integrated nutrient management system involving application of vermi-compost @ 6.26 t/ha/yr and combined application of bio inoculants viz, *Azospirillum*, fluorescent pseudomonas and AMF (Krishnan, 2003).

Anilkumar *et al.* (2009) noticed enhancement of both total fresh and dry spike yield as well as total alkaloid production in long pepper grown as an intercrop in coconut garden as a result of following integrated nutrient management. It included incorporation of vermicompost @ 6.25 t/ha/yr, addition of NPK @ 30:30:60 kg/ha/yr and combined inoculation of *Azospirillum,* fluorescent pseudomonas and AMF.

Rao *et al.* (2010) studied integrated nutrient management in long pepper and they reported significant increase in dry spike yield due to integrated management of FYM and fertilizers. A significantly higher spike yield of 2412 kg/ha as well as increased piperine yield (32.3 kg/ha) were obtained by application of 40 t ha^{-1} FYM and 125:50:160 kg N, P_2O_5 and K_2O per hectare. Significantly higher growth, yield and quality were also reported with this combination.

2.7.8 Irrigation

Long pepper can be grown both as a sole crop and intercrop. If it is a sole crop, irrigation once in a week is sufficient. If it is grown as an intercrop irrigation is not necessary as it can meet its water requirement from the irrigation water supplied to the main crop. If water is not available to irrigate during summer season it is recommended to do mulching using dried leaves (KAU, 2016).

Roots of the *Piper longum* should be covered with straw during summer season so as to prevent them from damage due to hot sun. Viswanathan (1995) suggested that long pepper could be irrigated once or twice a week during hotter climate in Kerala, starting from January.

2.7.9 Plant protection

Leaf spot or leaf bilght caused by *Colletotrichum gloeosporoides* was reported in *Piper longum*. Spots are seen on leaf lamina. The infection is manifested by the presence of discolored areas at the tip or occasionally near the margin. Shriveling of

leaves followed by drying is the severity of the disease (Sathyarajan and Naseema, 1985)

Influence of relative humidity on fungal association and aflatoxin production in *Piper longum* fruits was reported by Chourasia and Roy (1989). They found that at 75- 76 per cent relative humidity the level of aflatoxin B1 production was higher. In stored samples, level of aflatoxin production was more when the incidence of fungus was higher.

Another experiment was conducted by Roy and Chourasia (1990) to study the effect of temperature on aflatoxin B1 production by *Aspergillus flavus* on *Piper longum* fruits. The highest level of Afl-B1 (1.25 µg/g) production was noticed at 30°C after three weeks of incubation. Range of aflatoxin level was 0.22 to 1.00 µg/g at 20, 25, 35 and 40°C. Whereas it was much lower (0.12 – 0.24 µg/g) at 15°C.

Abraharm (1991) reported occurrence of *Helopeltis theovora,* as a pest in *Piper longum*. He observed that damage could be reduced by 70 per cent using two per cent neem kernel suspension. A virus causing mosaic mottling in Indian long pepper, was reported by Bhat *et al.* in 2004. It was isolated and identified as an isolate of cucumber mosaic virus based on morphological, physio-chemical and serological properties. They also suggested that this virus is associated with Indian long pepper by studying the particle morphology, antigenic relationships and molecular weight of coat protein.

Incidence of *Meloidogyne arenaria* in thippali was first reported by Seena (2006) from Kerala. Application of bio agents like *Bacillus subtilis*, *Trichoderma viridae, Pseudomonas fluorescens* and AMF was found to be successful among various treatments studied for the management of root knot nematode. It improved the growth of long pepper with maximum vine length, number of leaves, number of branches, root length, root and shoot weight. Early spike formation and an increase in number of spike were observed in plants treated with bio agents, *Bacillus subtilis*

and *Pseudomona fluorescens* respectively. The study revealed that bio agents are effective in managing root knot nematode and it can be a better alternative to nematicides.

2.7.10 Harvesting and yield

Davies (1992) reported that vegetatively propagated crop of long pepper established well within six months. First harvesting could be done eight months after planting. Two harvest could be made during second year. A yield of 500 kg dried spikes per hectare during first year was reported from crop grown in irrigated coconut gardens. It was 750 kg during second year and 1000 kg during third year.

Viswanathan (1995) reported that long pepper will reach harvesting stage eight months after planting and 3-4 pickings could be made as and when the spike reaches maturity. The yield of spikes during first year was 400 kg/ha and it has increased to 1000 kg/ha in third year. After that the vines became less productive. Replanting was recommended after third year.

2.7.11 Drying and quality

Drying is one of the most critical and fundamental unit operation in the post harvest processing of medicinal plants. Dried spikes are the economic part of *Piper longum*. Muller and Heindl, 2006 reported that 50°C is the optimum temperature for drying since quality reduction due to discoloration occurs at higher temperature.

Abbasi *et al.* (2010) suggested that the essential oil and extracts derived from *P. chaba* might be a potential source of natural preservatives used in food industries. The first amide isolated from *Piper* species was piperine.

Piperine is a characterising compound present in fruits of black pepper and long pepper. It is used as a bioavailability enhancer. It has antioxidant and anti-inflammatory activity and could be extracted using soxhlet and supercritical fluid

extraction technique. Piperine is extracted by column chromatography. Characterisation was done using spectroscopic technique (Hamrapurkar *et al.*, 2011). Chandran (2012) reported that Acc. No. 9 contained more oleoresin than Viswam, released variety from Kerala Agricultural University, whereas oil and piperine content were lower.

Viswam, NL-84-68 and Assam recorded highest piperine and oil content (Jaleel, 2006). Eventhough the female spikes were rich in oil and piperine, male spikes were also a source of piperine and oil in low quantity.

Variability in oleoresin content and uniformity in essential oil content among hybrids of *Piper longum* was reported by Sujatha (2016). Essential oil content was 0.8 per cent in all the hybrids studied.

Materials and Methods

3. MATERIALS AND METHODS

The study entitled "Evaluation and characterisation of promising hybrids of long pepper *Piper longum* L." was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara and Department of Plant Biotechnology, College of Agriculture, Padannakad during the period 2015 to 2017.

3.1 Experimental materials

The experimental materials included four promising hybrids of long pepper selected from the hybrids developed by crossing female parent and bisexual types at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara as a part of KSCSTE funded project as well as the M.Sc. programme of Kanimozhi (2010). Female parent and released variety 'Viswam' were used as check. The details of experimental material are as follows;

1. Pl 9
2. Pl 63
3. Pl 140
4. Pl 141
5. Female parent
6. Viswam

3.2 Details of experiment

3.2.1 EXPERIMENT 1: EVALUATION OF PROMISING HYBRIDS IN POTS

This experiment was laid out in pots at Plantation and Spice farm, Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2015-2017. Four promising hybrids along with female parent and Viswam were evaluated for growth, yield and quality under three different shade levels such as zero per cent, twenty five per cent and fifty per cent.

Design : CRD

Treatments : 6

Replication : 4

Observations were made on the following parameters:

1. Plant height (cm)
2. Rate of growth of plant (%)
3. Number of primary branches per plant
4. Time taken for production of first lateral
5. Season of flowering and fruit set
6. Spike orientation
7. Spike shape
8. Immature spike color
9. Color change while fruit ripening
10. No.of spikes per lateral branch
11. Fresh spike yield per plant (g)
12. Dry spike yield per plant (g)
13. Quality parameters- essential oil (%), oleoresin (%) and piperine (%)

3.2.1.1 Time taken for production of first lateral

Number of days taken for production of first lateral was observed.

3.2.1.2 Plant height (cm)

Plant height/length was recorded at monthly intervals.

3.2.1.3 Rate of growth of plant (%)

Growth of plants based on the increment in vine length was recorded at monthly intervals.

3.2.1.4 Number of primary branches per plant

Number of primary branches were counted and recorded at monthly intervals.

3.2.1.5 Season of flowering and fruit set

Period of flowering and fruit set in different varieties under different shade levels were recorded.

3.2.1.6 Spike orientation

Orientation of spike of different accessions were recorded.

3.2.1.7 Spike shape

Shape of spike was recorded for selected hybrids, female and Viswam.

3.2.1.8 Immature spike color

Color of spike during initial stage i.e when immature, was observed for different varieties under study.

3.2.1.9 Color change while fruit ripening

Spike color during ripening was recorded by visual observation.

3.2.1.10 Number of spike per lateral branch

Spikes developed on laterals. It was recorded by counting number of spikes formed per lateral.

3.2.1.11 Fresh spike yield per plant (g)

Spikes were harvested from individual plants separately and fresh weight was recorded and expressed in gram.

3.2.1.12 Dry spike yield per plant (g)

Harvested spikes from individual plants were dried either by keeping under open sun or by artificial drying. Artificial drying was done by keeping spikes inside dryer at 50° C for three to four days until the dry weight become stable.

3.2.1.13 Quality parameters

3.2.1.13.1 Essential oil

Essential oil was extracted from long pepper. Four promising hybrids as well as female parent and Viswam were subjected to oil extraction. Two samples per treatment were analysed. It was done by using Clevenger apparatus [AOAC, 1980]. Dried samples of long pepper were powdered and 25 g of the powder was taken in a

round bottom flask and 300 ml of water was added into it. The apparatus was set up and started extraction at 100°C. Later the temperature was reduced to 70°C and the extraction was continued for two hours. Volume of oil extracted was noted down and per cent oil content calculated.

3.2.1.13.2 Oleoresin

Extraction of oleoresin was done using Soxhlet apparatus [AOAC, 1980]. Dried samples were powdered. Five grams of powdered sample was packed in a filter paper and tied and taken in Soxhlet apparatus. Added 150 ml acetone. Three to four siphonings were done. Extraction was continued for 3 to 4 hours till the solvent became colourless. Acetone was removed periodically and the final extract collected in volumetric flask was transferred to a preweighed beaker. It was kept overnight after covering the beaker with filter paper. Acetone was evaporated and oleoresin was collected in the beaker. Final weight of beaker was taken and the difference in initial and final weight of beaker gave the weight of oleoresin extracted. This was expressed in percentage.

3.2.13.3 Piperine

Piperine content was estimated by the method suggested by Soubhagya *et al.* (1990). Freshly powdered dried spikes were treated with 100 ml acetone in a volumetric flask and shaken for 2 hours at room temperature. 0.25 ml of clear solution was taken in a cuvette and made upto 5ml with 4.75 ml acetone. The solution was shaken well and absorbance was read at 337 nm in a UV spectrophotometer. Acetone was used as blank.

Preparation of the standard curve

Standard piperine solutions of different concentrations such as 0.4, 0.8, 1.2, 1.6 and 2 mg l^{-1} were prepared and their absorbance values at 337 nm were found out. The values were plotted on a graph. Concentration corresponding to the absorbance of the sample was observed and piperine content present in sample was worked out.

3.2.2 EXPERIMENT 2: EVALUATION OF PROMISING HYBRIDS IN FIELD

Four promising hybrids along with female parent and Viswam were evaluated in field for growth, yield and quality.

Design : RBD

Treatments : 6

Replication : 4

Observations were made on the following parameters:

1. Number of primary branches per plant
2. Number of spike bearing branches per plant
3. Internodal length of spike bearing branch and orthotropic branch (cm)
4. Leaf area (cm^2)
5. Length of petiole (cm)
6. Days from planting to emergence of spike
7. Number of spikes per plant
8. Fresh spike yield per plant (g)
9. Dry spike yield per plant (g)
10. Spike characters- Pedicel length (cm), spike length (cm), spike girth (cm)
11. Quality parameters- essential oil (%), oleoresin (%) and piperine (%)
12. Incidence of pest and diseases

3.2.2.1 Number of primary branches per plant

Number of primary branches per plant were counted at monthly intervals

3.2.2.2 Number of spike bearing branches per plant (laterals)

Number of spike bearing branches were counted at monthly intervals.

3.2.2.3 Internodal length of spike bearing branch and orthotropic branch (cm)

Observations on internodal length of spike bearing branch and orthotropic branch were recorded.

3.2.2.4 Leaf area (cm^2)

Leaf area was recorded using leaf area meter for leaves present on orthotropic branches and plagiotropic branches separately.

3.2.2.5 Petiole length (cm)

Length of petiole was measured for leaves of both orthotropic and plagiotropic branches.

3.2.2.6 Days from planting to emergence of spike

Number of days taken for spike emergence after planting was counted.

3.2.2.7 Number of spikes per plant

Number of spikes during each harvest was recorded and cumulative data for 11 months was taken for analysis.

3.2.2.8 Fresh weight of spikes per plant (g)

Fresh weight of spikes was taken from each plant separately during each harvest.

3.2.2.9 Dry weight of spikes per plant (g)

Dry yield of spikes per plant was recorded.

3.2.2.10 Spike characters

Spike characters like pedicel length (cm), spike length (cm) and spike girth (cm)

were measured for five spikes from each replication of all genotypes.

3.2.2.11 Quality parameters

Essential oil (%), oleoresin (%) and piperine (%) were estimated as per the procedure given in materials and methods of first experiment.

3.2.2.12 Incidence of pest and diseases

Occurrence of pest and diseases were observed and scored.

Both the experiments were conducted at the Plantation and Spice farm, Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara.

3.2.3 EXPERIMENT 3: MOLECULAR CHARACTERISATION OF PROMISING HYBRIDS AND PARENTS

The parents and selected hybrids along with the standard variety were characterised using the method of Random Amplified Polymorphic DNA. Genomic DNA was isolated from young leaf samples and subjected to amplification through polymerase chain reaction (PCR) using random decamer primers. Thirty primers were screened and ten best primers were selected for characterisation. The amplicons were separated on agarose gel and scored for uniqueness of hybrids. Characterisation of genotypes was done based on banding pattern.

The work was done at the Department of Plant Biotechnology, College of Agriculture, Padannakkad.

Observations were taken for the following parameters:

1. Quality and quantity of DNA isolated
2. No. of amplicons
3. No. of monomorphic and polymorphic bands

3.2.3.1 DNA extraction

The DNA was extracted using modified CTAB method (Doyle and Doyle, 1987).

A. Reagent

1. CTAB extraction buffer (100 mM Tris HCl - pH 8.0, 2 mM EDTA - pH 8.0, 1.4 M NaCl, 2% CTAB)
2. Chloroform: Isomyl alcohol mixture (24:1 v/v)

B. Procedure

Tender healthy leaves were collected and midribs were removed. One gram of leaves was taken and ground into a fine powder using liquid nitrogen in presence of a pinch of PVP or sodium metabisulphate. 10 ml CTAB buffer was added and transferred to oakridge tube. It was incubated by keeping in water bath at 65°C for 30 minutes. The content was gently mixed at 10 minutes interval. 4 ml chloroform: isomyl alcohol (24:1) was added into it. Then it was centrifuged at 12000 rpm for 15 minutes at 4°C. Supernatant was transferred to another oakridge tube. To precipitate the DNA, one and half times volume of chilled isopropanol and half volume of 5 M NaCl were added. It was again centrifuged at 10000 rpm at 4°C for 5 minutes. The supernatant was removed, DNA pellet was washed using 70 per cent ethanol, air dried, dissolved in 100 µL distilled water and stored at -20°C.

3.2.3.2 Quantification of DNA

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

3.2.3.2.1 Agarose gel electrophoresis

A. Reagents

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

B. Procedure

For preparing 1X TAE buffer, 2 ml stock solution (50X) was taken and volume made upto 100 ml with sterile water. 100ml TAE buffer was taken in a beaker, 0.8 g agarose was added to make 0.8 per cent gel and boiled in microwave oven for one minute. After cooling 6 µl of ethidium bromide was added to this and mixed well. The open end of the gel casting tray was sealed with cello tape and placed on a horizontal surface. Dissolved agarose was poured into the tray after placing the comb. It was kept at room temperature for half an hour for solidification. The comb was removed after solidification of the gel. The gel was placed in the electrophoresis unit containing 1X TAE buffer. The well side was directed towards the cathode. 5 µl of DNA sample was pippeted out onto Para film and mixed well with 1µl tracking dye. The samples were then loaded carefully into the well using micropipette. The cathode and the anode of the electrophoresis unit were connected to the power pack. Electrophoresis was carried out at constant voltage of 70 V, till the loading dye had covered two third length of the gel.

3.2.3.3 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).

3.2.3.4 Spectrophotometer determination

The DNA samples were diluted ten times using sterile water and the absorbance was 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 was quantified as per the equation OD of one at 260nm=50µg/ml DNA.

3.2.3.4 RAPD analysis

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

The steps involve,

a) Denaturation at 94°C for 1 minute
b) Primer annealing at 37.5°C for 1 minute
c) Primer extension at 72°C catalyzed by Taq polymerase enzyme for 2 minutes.
d) Cycle repeated 35 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:

The reaction mixture (25µL) was constituted with 20 ng DNA, 3U Taq DNA polymerase (0.3µL); 10X PCR buffer with $MgCl_2$ (2.5µL) and 100mM dNTPs (2µL), 10µM primers (2µL).

The dNTPs, buffers and taq polymerase were procured from Genei Biosciences, Bengaluru.

The reaction mixture was prepared as a master mix for the required number of reactions. The aliquot of the master mix was dispensed to 0.5 ml PCR tubes into which template DNA 20 ng 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 cycler.

3.2.3.5 Primer screening

Thirty decamer primers were screened using genomic DNA of female parent. Ten primers which gave maximum number of reproducible bands were selected and further used to characterise the four hybrids along with parents and 'Viswam'.

3.3 Statistical analysis of data

The data obtained were analysed using the OPSTAT and WASP 2.0 softwares for experiment 1 and 2 respectively. The DNA finger print data were used to construct dendrogram by employing Unweighed Pair Group Method of Arithmetic Average cluster method (UPGMA) using NTSYS programme (Rohlf, 2005) using Jaccard's similarity coefficient.

Results

4. RESULTS

The study entitled 'Evaluation and characterisation of promising hybrids of long pepper (*Piper longum* L.)' was conducted with the objective of evaluating the performance of four promising hybrids along with their female parent and released variety 'Viswam' at different shade levels for growth, yield and quality. Molecular characterisation of these hybrids and parents was also aimed at. The findings of the study are presented below.

4.1 EVALUATION OF PROMISING HYBRIDS IN POTS UNDER DIFFERENT SHADE LEVEL

To evaluate the performance of hybrids *viz,* Pl 9, Pl 63, Pl 140 and Pl 141 under different shade levels, an experiment was laid out in CRD with 6 genotypes (four hybrids, female parent and released variety Viswam) and four replications. Uniform sized cement pots were made ready by adding gravel at the bottom and mixture of soil, sand and FYM above. Planting was done in third week of June. The results of the study are explained here under.

4.1.1 Catalouging of *Piper longum* genotypes

Four promising hybrids along with the female parent and released variety Viswam were catalouged for vegetative and reproductive characters based on the descriptor developed for *Piper nigrum* by IPGRI (1995) with suitable modifications.

4.1.1.1 Leaf characters

Piper longum accessions used in the study showed variability in leaf characters. Leaf characters differed according to the orthotropic and plagiotropic shoots. As can be seen from the Table 1, the shape of leaf lamina varied from cordate to ovate-lanceolate or elliptic-lanceolate. Shape of leaf on plagiotropic shoots (laterals) were elliptic-lanceolate in all accessions. Except Pl 141, all the accessions

had cordate shaped leaves on their orthotropic shoots whereas it was ovate-lanceolate in Pl 141. In all genotypes studied, shape of leaf base was cordate and oblique in orthotropic and plagiotropic shoots respectively. Leaf colour was also varying from light green to dark green. The hybrids Pl 9, Pl 63 and Pl 141 had light green coloured leaves irrespective of the type of shoots. Similarly Pl 140 and Viswam possessed dark green coloured leaves on them. The leaf colour of female parent was green.

4.1.1.2 Spike characters

Piper longum accessions used in the study showed variability in spike characters also. Orientation of spikes was erect in all the accessions studied (table 1). Spike shape varied from cylindrical to conical in different accessions. Cylindrical spikes were seen in hybrids Pl 9, Pl 140, Pl 141 and Viswam. Cylindrical to conical spikes were observed in Pl 63 as well as in female parent.

Change in colour of spikes was observed during fruit ripening. As can be seen from table 1, Pl 141 and Viswam changed their spike colour from greenish yellow to dark green during ripening. Light yellow coloured spikes changed to dark green during ripening in all other accessions studied.

Table 1. Leaf and spike characters of *Piper longum* genotypes

Hybrid	Leaf shape		Leaf colour		Spike orientation	Spike shape	Immature spike colour	Colour change while fruit ripening
	Plagiotropic shoot	Orthotropic Shoot	Plagiotropic Shoot	Orthotropic Shoot				
Pl 9	Elliptic-lanceolate	Cordate	Light green	Light green	Erect	Cylindrical	Light yellow	Dark green
Pl 63	Elliptic-lanceolate	Cordate	Light green	Light green	Erect	Cylindrical-Conical	Light yellow	Dark green
Pl 140	Elliptic-lanceolate	Cordate	Dark green	Dark green	Erect	Cylindrical	Light yellow	Dark green
Pl 141	Elliptic-lanceolate	Ovate lanceolate	Light green	Light green	Erect	Cylindrical	Greenish yellow	Dark green
Female	Elliptic-lanceolate	Cordate	Green	Green	Erect	Cylindrical-Conical	Light yellow	Dark green
Viswam	Elliptic-lanceolate	Cordate	Dark green	Dark green	Erect	Cylindrical	Greenish yellow	Dark green

Plate 1. Leaf shape in *Piper longum*

Orthotropic shoot

Cordate

Ovate lanceolate

Plagiotropic shoot

Elliptic lanceolate

Plate 2. Shape of leaf base in *Piper longum*

Orthotropic shoot

Cordate

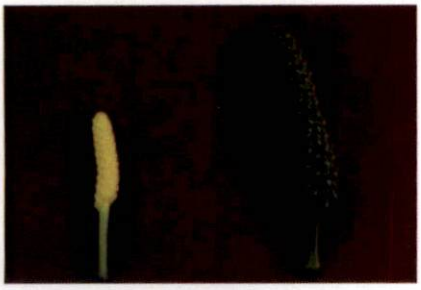
Plagiotropic shoot

Oblique

Plate 3. Color of immature and mature spikes in *Piper longum*

Pl 9

Pl 63



Pl 140

Pl 141

Female

Viswam

Plate 4. Orientation of spike in *Piper longum*

Erect

Plate 5. Shape of spikes in *Piper longum*

Cylindrical

Conical

4.1.2 Vegetative characters

Performance of hybrids for growth characters like plant height, rate of growth, time taken for production of lateral and number of primary branches per plant are discussed here under.

4.1.2.1 Plant height

Table 2 shows plant height at 11 months after planting. As can be seen from table, accessions differed in plant height at different shade levels. Female parent was significantly longer than all other genotypes at zero per cent and 25 per cent shade levels. Female was 460 cm long at 25 per cent shade and it was the maximum compared to other two shade levels.

Table 2. Plant height of *Piper longum* genotypes at various shade levels

Hybrids	Plant height/length (cm)			Mean
	0% shade	25% shade	50% shade	
Pl 9	94.75 (9.49)	178.75 (13.28)	138.00 (11.38)	137.17
Pl 63	153.50 (12.14)	175.50 (12.95)	112.00 (10.51)	147.00
Pl 140	128.50 (10.95)	273.50 (16.31)	88.50 (9.36)	163.50
Pl 141	238.00 (15.44)	198.50 (13.92)	83.00 (9.11)	173.17
Female	446.00 (20.21)	460.00 (21.38)	116.00 (10.78)	340.67
Viswam	97.50 (9.80)	70.25 (8.35)	122.50 (10.00)	96.75
Mean	193.04	226.08	110.00	
C.D (0.05)	5.65	3.98	NS	
C.D (0.05)				
Shade	59.37			
Hybrid	83.97			
Shade × Hybrid	145.43			

Note : * values in parenthesis are square root transformed

At zero per cent shade Pl 141 was 238 cm long. At the same time it had 198.50 cm and 83 cm long vines at 25 per cent and 50 per cent shade respectively. The promising hybrid Pl 9 was shorter (94.75cm) than all other genotypes at zero per cent shade level. The released variety Viswam was shorter than all other accessions at 25 per cent shade. At 50 per cent shade, Pl 9 was longer (138 cm) than all other genotypes studied.

4.1.2.2 Rate of growth of plant

The rate of growth of experimental plants was observed at monthly intervals for one year at three different shade levels viz, zero, 25 and 50 per cent. As can be seen from table 3, maximum growth rate was observed in Viswam at 3 MAP (69.2%). Except the hybrid Pl 63, all the genotypes showed higher growth rate during 3 MAP. But in Pl 63, higher growth rate was observed during 6 MAP (35.9%). At zero per cent shade Pl 140 had significantly higher growth rate (74.3%) at 3 MAP whereas it was for female (43.2%) at 6 MAP and Viswam (7.3%) at 11 MAP. At 25 per cent shade level Viswam, Pl 141 and Pl 9 had higher growth rate at 3 MAP, 6 MAP and 11 MAP respectively (table 3). Viswam had higher growth rate at 3 MAP (84.4%) and 11 MAP (13.5%) at 50 per cent shade level. At 6 MAP, Pl 63 (47.4%) recorded higher growth rate at 50 per cent shade level.

The details on monthly rate of growth of plant from August to May are presented in appendix I.

4.1.2.3 Time taken for production of first lateral

Number of days taken for production of first lateral varied from 44 (Pl 141) to 60.47 (Pl 9 and Female). It was also depended on the shade level in which the plants were grown. The performance hybrids at different shade levels were significantly different. Table 4 shows the time taken for production of first lateral

Plate 6. Performance of *Piper longum* accessions at different shade levels

Zero per cent shade

25 per cent shade

50 per cent shade

Table 3. Rate of growth of plant at different stages of plant growth at various shade levels

Hybrids	3 MAP				6 MAP				11 MAP			
	Rate of growth (%)			Mean	Rate of growth (%)			Mean	Rate of growth (%)			Mean
	0%	25%	50%		0%	25%	50%		0%	25%	50%	
Pl 9	31.90	25.30	25.30	27.50	13.80	27.20	38.60	26.50	4.30 (10.90)	31.00 (31.40)	3.00 (8.20)	16.80
Pl 63	55.00	18.30	10.60	28.00	6.80	53.50	47.40	35.90	2.30 (7.50)	2.50 (7.60)	2.90 (9.20)	8.10
Pl 140	74.30	23.00	4.40	33.90	20.80	38.80	11.00	23.50	2.70 (8.90)	8.00 (12.70)	5.10 (12.60)	11.40
Pl 141	55.60	35.10	8.70	33.10	22.80	58.30	14.40	31.80	6.40 (13.30)	8.30 (14.50)	11.20 (18.50)	15.40
Female	67.50	21.70	17.40	35.60	43.20	51.00	8.20	34.10	1.90 (7.30)	21.40 (25.20)	11.10 (17.60)	16.70
Viswam	33.50	89.70	84.40	69.20	31.20	7.70	5.60	14.80	7.30 (14.20)	3.40 (10.60)	13.50 (20.60)	15.10
Mean	53.00	35.50	25.10		23.10	39.40	20.90		10.40	17.00	14.40	
	C.D.				**C.D.**				**CD**			
Shade	NS				NS				NS			
Hybrid	NS				NS				NS			
Shade × Hybrid	NS				NS				NS			

Note : *Values in parenthesis are angular transformed

in different hybrids at different shade levels. As can be seen from table 4, Pl 140 took less number of days (44.80) to produce first laterals at zero per cent shade level. Pl 9, female and Viswam took 60 days to produce first lateral. At 25 and 50 per cent shade levels, Pl 141 took lesser number of days to produce first lateral.

Table 4. Time taken for production of first lateral at various shade levels

Hybrids	Number of days from planting to first lateral production			Mean
	0% shade	25% shade	50% shade	
Pl 9	60.00	60.00	61.40	60.47
Pl 63	55.00	54.40	55.00	54.80
Pl 140	44.80	44.60	45.00	44.80
Pl 141	46.80	44.00	41.20	44.00
Female	60.00	59.40	62.00	60.47
Viswam	60.00	57.80	60.80	59.53
Mean	54.43	53.37	54.23	
C.D (0.05)	1.60	1.32	1.44	
C.D (0.05)				
Shade	0.57			
Hybrid	0.81			
Shade × Hybrid	1.40			

4.1.5 Number of primary branches per plant

At zero per cent shade level maximum number of primary branches was produced in Pl 63 (33.75) followed by Pl 9 (24) (table 5). At 25 per cent shade number of primary branches varied from 5 (Pl 141) to 36 (Pl 63). At 50 per cent shade level, Viswam (9.25) produced significantly more number of primary branches compared to all other genotypes studied.

Table 5. Number of primary branches per plant

Hybrids	Number of primary branches per plant			Mean
	0% shade	25% shade	50% shade	
Pl 9	24.00 (4.80)	23.25 (4.90)	6.00 (2.60)	17.80 (4.10)
Pl 63	33.75 (5.90)	36.00 (6.10)	6.00 (2.60)	25.30 (4.80)
Pl 140	7.00 (2.80)	7.00 (2.80)	4.50 (2.30)	6.20 (2.60)
Pl 141	11.00 (3.40)	5.00 (2.40)	4.00 (2.20)	6.70 (2.70)
Female	12.25 (3.60)	8.50 (3.00)	4.50 (2.30)	8.40 (3.00)
Viswam	14.00 (3.80)	22.50 (4.80)	9.25 (3.10)	15.30 (3.90)
Mean	17.00 (4.10)	17.00 (4.00)	5.70 (2.50)	
C.D (0.05)	1.24	0.85	NS	
C.D (0.05)				
Shade	0.37			
Hybrid	0.52			
Shade × Hybrid	0.91			

Note : * values in parenthesis are sqaure root transformed

4.1.3 Reproductive characters

Reproductive characters like season of flowering and fruiting, number of spikes per lateral branch, number of spikes per plant, fresh weight of spikes per plant and dry weight of spikes per plant are discussed here under.

4.1.3.1 Flowering and fruiting season

Flowering was not synchronous in all the genotypes of *Piper longum* studied. Eventhough flowering was observed throughout the year, the peak season of flowering varied among genotypes. As can be seen from table 6, flowering in *Piper longum* accessions studied was from May to September. Peak flowering was during

June-July. In hybrid Pl 141 flowering was during August. In Viswam at zero per cent shade flowering was in September. Fruit set was late in Pl 141 compared to other accessions studied.

Table 6. Season of flowering and fruit set in experimental plants of *Piper longum*

Hybrid	Season of flowering			Season of fruit set		
	0% Shade	25% shade	50% shade	0% Shade	25% shade	50% Shade
Pl 9	May June-July	June-July	July	September-May	September-May	September-February
Pl 63	July	June-July	July	October-May	September-May	Sepember-March
Pl 140	June-July	July	August	September-May	October-May	September-May
Pl 141	August	August	August	December-April	November-May	February-May
Female	June-July	June-July	August	August-April	September-May	October-April
Viswam	September	June-July	July	December-May	October-May	October

4.1.3.2 Number of spikes per lateral branch

Spikes were produced on lateral branches. Accessions showed no significant difference for number of spikes per lateral at zero, 25 and 50 per cent shade level. However, Pl 9 produced maximum number of spikes per lateral compared to other accessions at zero and 25 per cent shade level. As can be seen from table 7, all accessions produced equal number of spikes per lateral at 50 per cent shade. Shade

level showed significant difference for number of spikes per lateral. Zero per cent and 25 per cent shade were on par with respect to number of spikes per lateral.

Table 7. Number of spikes per lateral at various shade level

Hybrids	No. of spikes per lateral			Mean
	0% shade	25% shade	50% shade	
Pl 9	1.75	1.25	1.00	1.33
Pl 63	1.50	1.00	1.00	1.17
Pl 140	1.50	1.50	1.00	1.33
Pl 141	1.00	1.25	1.00	1.08
Female	1.00	1.00	1.00	1.00
Viswam	1.25	1.25	1.00	1.17
Mean	1.33	1.21	1.00	
C.D (0.05)	NS	NS	NS	
C.D (0.05)				
Shade	0.21			
Hybrid	NS			
Shade × Hybrid	NS			

4.1.3.3 Number of spikes per plant

Fruits were harvested from September to April and number of spikes per individual plant was calculated at each harvest separately. A significant difference was observed in number of spikes per plant at different shades for different hybrids. As can be seen from table 8, at zero per cent shade level the highest number of spikes per plant was recorded in Pl 63 (47.5) followed by Pl 9 (44) and Pl 140 (40.5). At 25 per cent shade level Pl 140 (14.5) produced maximum number of spikes per plant

followed by Pl 63 (7.75), and Pl 9 (6). However, at 50 per cent shade, number of spikes per plant was maximum in Pl 9 (6) compared to other accessions studied. Performance of hybrids was better at zero per cent compared to 25 and 50 per cent shade.

Table 8. Number of spikes per plant at various shade levels

Hybrids	No. of spikes per plant			Mean
	0% shade	25% shade	50% shade	
Pl 9	44.00	6.00	6.00	18.67
Pl 63	47.50	7.75	1.50	18.92
Pl 140	40.50	14.50	2.00	19.00
Pl 141	1.00	1.00	0.75	0.92
Female	1.25	1.25	2.50	1.67
Viswam	1.25	1.75	0.50	1.17
Mean	22.58	5.38	2.21	
C.D (0.05)	20.38	7.33	3.31	
C.D (0.05)				
Shade	4.90			
Hybrid	6.93			
Shade × Hybrid	12.01			

4.1.3.4 Fresh weight of spike per plant in shade

Spikes were collected separately from each plant and fresh weight was taken. Total yield of fresh spikes per plant for one year was computed. Zero per cent shade was significantly superior over 25 and 50 per cent shade for fresh spike yield. As can be seen from table 9, maximum yield was observed in Pl 63 (44.23g) followed by Pl 9 (39.25g) at zero per cent shade. Hybrids Pl 9, Pl 63 and Pl 140 were on par with respect to fresh yield at zero per cent shade. At 25 per cent shade, Pl 9 and Pl 140 were on par with respect to fresh spike yield. At 50 per cent shade Pl 9 produced

maximum fresh spike yield (5.52g) compared to all other accessions studied. At zero and 25 per cent shade, fresh spike yield was equal in Pl 141 (0.85g). Yield was found to be increasing in female parent with increase in shade level. However, hybrids produced maximum yield at lower shade levels compared to the higher (50% shade).

Table 9. Fresh weight of spike per plant in shade

Hybrids	Fresh spike yield (g)			Mean
	0% shade	25% shade	50% shade	
Pl 9	39.25	5.79	5.52	16.85
Pl 63	44.23	4.88	1.28	16.79
Pl 140	25.48	12.06	1.48	13.00
Pl 141	0.85	0.85	0.56	0.75
Female	0.77	0.91	2.28	1.32
Viswam	0.83	0.90	0.40	0.71
Mean	18.57	4.23	1.92	
C.D (0.05)	19.56	7.01	2.98	
C.D (0.05)				
Shade	4.70			
Hybrid	6.65			
Shade × Hybrid	11.51			

4.1.3.5 Dry weight of spike per plant in shade

Spikes collected were dried and dry weight of spikes per plant was found out. Dry yield of spikes per plant was significantly higher at zero per cent shade compaed to 25 and 50 per cent shade level. As can be seen in table 10, Pl 63 had maximum dry weight (8.14) followed by Pl 9 (7.37g) and Pl 140 (4.45g) at zero per cent shade level. At 25 per cent shade level Pl 140 (2.01g) had maximum dry spike yield followed by Pl 63 (1.05g) and Pl 9 (0.95g). At zero per cent shade, Pl 9 and Pl 63 were on par. At 25 per cent shade, Pl 9, Pl 63 and Pl 140 were on par. At 50 per cent, Pl 9 produced maximum dry yield than all other accessions studied. However,

performance of hybrids in yield was significantly superior at zero percent shade compared to 25 and 50 per cent.

Table 10. Dry spike yield in *Piper longum* accessions at various shade levels

Hybrids	Dry spike yield (g)			Mean
	0% shade	25% shade	50% shade	
Pl 9	7.37	0.95	1.10	3.14
Pl 63	8.14	1.05	0.26	3.15
Pl 140	4.45	2.01	0.30	2.25
Pl 141	0.16	0.16	0.11	0.14
Female	0.14	0.16	0.46	0.25
Viswam	0.14	0.14	0.08	0.12
Mean	3.40	0.74	0.38	
C.D (0.05)	3.57	1.10	0.60	
C.D (0.05)				
Shade	0.85			
Hybrid	1.20			
Shade × Hybrid	2.08			

4.1.4 Quality parameters

Essential oil, oleoresin and piperine were estimated in the genotypes which gave analysable yield (all 6 genotypes at zero per cent shade level and Pl 9, Pl 63 and Pl 140 at 25 per cent shade level). As can be seen from table 11, at zero per cent shade, oil content was 0.8 per cent in all accessions except Pl 141, which had 0.83 per cent oil. At zero per cent shade Pl 9 (15.2 per cent) yielded more oleoresin followed by Pl 140 (12.37 per cent) and Viswam (12.24 per cent). In oleoresin content, Pl 9 was significantly superior over all other accessions studied. At zero per cent shade Pl 9 (3.47 per cent) showed more piperine content followed by Pl 141 (3.26) and Viswam (3.18 per cent). Pl 9 (3.47 per cent) and Pl 141 (3.26 per cent) had more piperine than Viswam (3.18 per cent). At 25 per cent shade, Pl 140 (0.86 per cent)

showed slightly higher essential oil content than Pl 9 (0.85 per cent). Oleoresin was also maximum in Pl 140 compared to Pl 9 and Pl 63 at 25 per cent shade (table 11).

Table 11. Quality parameters of *Piper longum* accessions in shade

Hybrid	Zero per cent shade			25 per cent shade	
	Oil (%)	Oleoresin (%)	Piperine (%)	Oil (%)	Oleoresin (%)
Pl 9	0.80	15.20	3.47	0.85	7.20
Pl 63	0.80	8.72	2.52	----	5.29
Pl 140	0.80	12.37	2.87	0.86	9.23
Pl 141	0.83	6.20	3.26	----	----
Female	0.80	5.00	3.11	----	----
Viswam	0.80	12.24	3.18	----	----
C.D (0.05)	NS	1.22	NS	NS	NS

4.2 EVALUATION OF PROMISING HYBRIDS UNDER FIELD CONDITION

To evaluate the performance of promising hybrids under field condition an experiment was laid out in RBD with six genotypes and four replications. Four promising hybrids (Pl 9, Pl 63, Pl 140 and Pl 141) were evaluated along with female parent and released variety Viswam as standard check. Each accession was planted in a bed of 3 m × 1 m and 15 cm height. The spacing given was 60 cm × 30 cm accommodating 6 plants per bed. Since weed growth was a big problem in thippali under open cultivation the beds were mulched with black bottom silver top mulching sheets. Field planting was done in the second week of June. Growth and yield of experimental plants were recorded at monthly intervals. The results of the study are presented here under.

4.2.1 Vegetative characters

4.2.1.1 Plant height/vine length (cm)

Significant difference was observed among accessions in vine length. As can be seen from table 12, female parent produced maximum vine length (221.75 cm) compared to all other accessions studied. Minimum vine length was observed in Pl 9 (80.75 cm).

4.2.1.2 Number of primary branches per plant

Accessions differed significantly with respect to number of primary branches per plant. As can be seen from table 12, number of primary branches was maximum in Viswam (31.67) compared to all other accessions studied. Viswam was found to be significantly superior to all other accessions in number of primary branches. Hybrids Pl 140 and Pl 141 produced lowest (6.50) number of primary branches.

Plate 7. Different stages of field experiment

Preparation of beds

Mulching

Early growth stage

Active growth stage

4.2.1.2 Number of spike bearing branches/laterals per plant

Accessions showed significant difference in number of laterals per plant. As can be seen from table 12, laterals per plant were maximum in Pl 9 and Pl 63. Hybrids Pl 9, Pl 63, Pl 140 and female parent were on par with respect to number of laterals per plant.

Details on monthly observations on plant height, number of primary branches per plant and number of laterals per plant are given in appendix II.

Table 12. Growth characters of *Piper longum* accessions in field

Hybrid	Plant height (cm)	Number of primary branches per plant	Number of laterals per plant
Pl 9	80.75 (8.90)	11.83 (3.31)	45.00 (6.29)
Pl 63	109.18 (10.40)	11.67 (3.42)	45.00 (6.52)
Pl 140	84.13 (9.06)	6.50 (2.52)	19.50 (4.32)
Pl 141	92.46 (9.39)	6.50 (2.52)	9.00 (2.93)
Female	221.75 (14.54)	13.54 (3.61)	24.00 (4.82)
Viswam	92.56 (9.62)	31.67 (5.61)	14.00 (3.49)
C.D (0.05)	3.07	0.95	2.41

Note : * values in parenthesis are square root transformed

4.2.1.3 Internodal length of orthotropic and plagiotropic branches (cm)

There was high variation in internodal length of orthotropic and plagiotropic shoots among the accessions studied (table 13). Among the genotypes Pl 141 had the shortest internode (4.83 cm) and the female had the longest internode (10.09 cm) in

orthotropic shoot. In the case of plagiotropic shoot also, shortest internodes were there for Pl 141 (2.68 cm) and the longest for female parent (5.92 cm).

4.2.1.4 Length of petiole (cm)

Leaves on plagiotropic shoots and orthotropic shoots showed slight variation in petiole length. However there was no significant difference for petiole length among the accessions studied. As can be seen from table 13, maximum length of petiole was recorded in female (2.13cm) and minimum in Viswam (1.19cm) in leaves of plagiotropic shoots. Petiole length was maximum in female parent (2.75cm) and minimum in Pl 63 (1.69cm) in orthotropic shoots.

4.2.1.5 Leaf area (cm^2)

Variation was observed in leaf area among the accessions in both orthotropic and plagiotropic shoots. As can be seen from table 13, maximum leaf area was recorded in Viswam (51.68 cm^2) in orthotrpic shoots and minimum in Pl 141(18.76 cm^2). It was maximum in female parent (39.04 cm^2) in plagiotropic shoots and minimum in Pl 141 (9.78cm^2).

4.2.2 Reproductive characters

4.2.2.1 Days from planting to emergence of spike

Flowering started in May–June and continued throughout the year. Numbers of days were counted from planting to emergence of spike. A significant difference was observed among genotypes for number of days to flowering. Hybrid Pl 140 was found to be earlier (50.21 days) in producing spikes (table 14). Significantly more number of days was taken by Viswam (66.38 days) to produce spikes.

Table 13. Stem and leaf characters of *Piper longum* accessions

Hybrid	Internodal length (cm)		Length of petiole (cm)		Leaf area ($cm^{2)}$	
	Orthotropic shoot	Plagiotropic shoot	Orthotropic shoot	Plagiotropic shoot	Orthotropic shoot	Plagiotropic shoot
Pl 9	6.31^{bc} (2.50)	3.92^{b} (1.98)	2.05	1.30	46.25^{a} (6.71)	27.00^{b} (5.18)
Pl 63	7.04^{b} (2.65)	3.58^{bc} (1.89)	1.69	2.00	39.68^{a} (6.27)	25.61^{b} (5.05)
Pl 140	5.52^{bc} (2.34)	3.17^{bcd} (1.77)	1.94	1.56	22.47^{b} (4.67)	16.01^{c} (3.99)
Pl 141	4.83^{c} (2.18)	2.68^{d} (1.63)	2.16	1.49	18.76^{b} (4.29)	9.78^{d} (3.12)
Female	10.09^{a} (3.16)	5.92^{a} (2.43)	2.75	2.13	50.86^{a} (7.06)	39.04^{a} (6.24)
Viswam	5.27^{bc} (2.29)	2.85^{cd} (1.68)	2.13	1.19	51.68^{a} (7.15)	32.56^{ab} (5.66)
C.D (0.05)	0.37	0.25	NS	NS	1.43	0.76

Note : * Values in parenthesis are square root transformed

4.2.2.2 Pedicel length

Significant difference was observed among genotypes for pedicel length. As can be seen from table 14, Pl 9 had longest pedicel (1.94cm) and Pl 141 had the shortest (0.87cm). Viswam was found on par with female, Pl 140 and Pl 141.

4.2.2.3 Spike length

As can be seen from table 14, maximum spike length was recorded in Pl 9 (4.60cm) and minimum in Viswam (3.22cm). Pl 9 and Pl 63 were found to be on par.

4.2.2.4 Spike girth

As can be seen from table 14, spike girth was found to be maximum in Female (2.91cm) and minimum in Pl 141 (2.11cm). Viswam was on par with female.

Table 14. Spike characters of experimental plants.

Hybrid	No.of days for emergence of spike (DAP)	Spike characters		
		Pedicel length (cm)	Spike length (cm)	Spike girth (cm)
Pl 9	55.17^{c}	1.94^{a}	4.60^{a}	2.54^{bc}
Pl 63	55.38^{c}	1.63^{b}	4.37^{ab}	2.42^{bc}
Pl 140	50.21^{d}	0.98^{d}	4.11^{abc}	2.29^{cd}
Pl 141	64.84^{b}	0.87^{d}	3.43^{cd}	2.11^{d}
Female	64.96^{b}	1.31^{c}	3.65^{bcd}	2.91^{a}
Viswam	66.38^{a}	1.07^{cd}	3.22^{d}	2.63^{ab}
C.D (0.05)	0.27	0.29	0.74	0.29

4.2.2.5 Yield per plant

As can be seen from table 15, number of spikes per plant ranged from 4.00 (Pl141) to 51.67 (Pl 9). Fresh yield of spikes ranged from 4g (Pl 141) to 44.87g (Pl 9). It was maximum in Pl 9 (44.87g) followed by Pl 63 (30.57g). Similarly dry yield of spikes per plant ranged from 0.82g (Pl 141) to 8.57g (Pl 9). Hybrids Pl 9 and Pl 63 were statistically on par with respect to number of spikes per plant and dry yield of spikes per plant (g).

Table 15. Yield per plant in field planted *Piper longum* accessions

Hybrid	Yield		
	Number of spikes per plant	Fresh weight of spikes per plant (g)	Dry yield of spikes per plant (g)
Pl 9	51.67^{a} (7.19)	44.87^{a} (6.70)	8.57^{a} (2.93)
Pl 63	50.00^{a} (6.86)	30.57^{b} (5.33)	6.18^{a} (2.38)
Pl 140	15.33^{b} (3.88)	7.52^{cd} (2.71)	1.39^{b} (1.16)
Pl 141	4.00^{c} (2.00)	4.00^{d} (2.00)	0.82^{b} (0.91)
Female	9.00^{bc} (3.00)	12.00^{c} (3.46)	2.37^{b} (1.54)
Viswam	9.33^{bc} (3.05)	5.82^{cd} (2.41)	0.94^{b} (0.97)
C.D (0.05)	1.59	1.36	0.68

Note : * values in parenthesis are sqaure root transformed

4.2.2.4 Influence of growth characters on yield

Correlation between growth characters and yield was studied. As can be seen from table 16, highly significant correlation was observed for characters like number of laterals and number of spikes per lateral on yield. Number of primary branches per plant and spike length also had significant correlation with yield.

4.2.3 Quality parameters

Essential oil, oleoresin and piperine were estimated in the accessions studied. As can be seen from table 17, there was not much variation in oil content among the accessions studied. All the accessions possessed 0.8 per cent oil except Pl 141, which had 0.83 per cent oil. However the oleoresin content of the genotypes were widely varying from 5 (Female) to 15.2 (Pl 9). Pl 9 was followed by Pl 140 (12.37) and Viswam (12.24) in oleoresin content. Piperine content was also maximum in Pl 9 (3.47 per cent) and was lowest in Pl 63 (2.52 per cent). Pl 141, female parent and Viswam also showed piperine content above three per cent.

Table 17. Quality parameters in experimental plants of *Piper longum*

Hybrid	Essential Oil (per cent)	Oleoresin (per cent)	Piperine (per cent)
Pl 9	0.80	15.20	3.47
Pl 63	0.80	8.72	2.52
Pl 140	0.80	12.37	2.87
Pl 141	0.83	6.20	3.26
Female	0.80	5.00	3.11
Viswam	0.80	12.24	3.18
C.D (0.05)	NS	1.22	NS

Table 16. Correlation between vegetative characters and yield

	No.of Primary branches per plant	No. of laterals	No. of spikes per lateral	Spike length	No. of spikes per plant	Fresh spike yield (g)	Dry spike yield (g)
No.of primary branches per plant	1						
No. of laterals	0.673**	1					
No. of spikes per lateral	0.178	0.544**	1				
Spike length	-0.178	0.335	0.450*	1			
No. of spikes per plant	0.426*	0.785**	0.638**	0.435*	1		
Fresh spike yield (g)	0.438*	0.834**	0.539**	0.446*	0.963**	1	
Dry spike yield (g)	0.422*	0.816**	0.540**	0.441*	0.968**	0.997**	1

Note : ** denotes correlation is significant at 0.01 level

* denotes correlation is significant at 0.05 level

4.2.4 Incidence of pest and diseases

Pest and disease incidence was recorded in *Piper longum* accessions planted in field. Attack of papaya mealy bug was observed during December- March. Pl 140 was the only accession affected by papaya mealy bug. In Pl 140, out the 24 plants only one plant was infested with papaya mealy bug. In the infested plant, nine spikes were infested by papaya mealy bug at varying levels (13.4 to 35 per cent). Thrips incidence was observed in female parent which resulted in premature drying and dropping of the spike. Incidence of fusarium wilt was observed during April-May. Per cent incidence ranged from zero in female to 25 per cent in Pl 141 and Pl 63 (table 18). Leaf spot caused by *Colletotrichum gloeosporoides* was also observed in the accessions. But it was not a serious problem.

Table 18. Incidence of fusarium wilt in field planted *Piper longum* hybrids

Hybrid	Percentage disease incidence (%)
Pl 9	16.60 (17.57)
Pl 63	25.00 (29.28)
Pl 140	8.33 (12.31)
Pl 141	25.00 (22.79)
Female	0 (0.59)
Viswam	8.33 (9.25)

Note : * Values in parenthesis are angular transformed

Plate 8. Incidence of pest and diseases in field

Fusarium wilt

(*Fusarium auxisporum*)

Leaf spot

(*Colletotrichum gloeosporoides*)

Papaya mealy bug

(*Paracoccus marginatus*)

4.2 MOLECULAR CHARACTERISATION OF PROMISING HYBRIDS AND PARENTS

4.2.1 DNA extraction

High molecular weight DNA was isolated from leaves of eight samples of *Piper longum* using CTAB method at the department of Plant Biotechnology, College of Agriculture, Padannakkad. The concentration and the quality of DNA were determined spectrophotometrically by measuring absorbance at 260 nm in bio photometer (Eppendorf). The quality and integrity of DNA was confirmed by means of agarose gel (0.8 per cent) electrophoresis and visualized using gel documentation system. Details of quantity of DNA isolated from eight samples of long pepper are given in table 19 below.

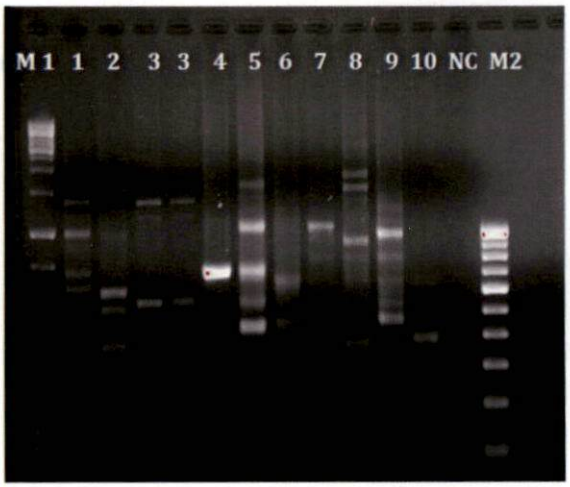
Table 19. DNA quantification results of eight long pepper samples

Samples	Optical density $A_{260/280}$	Optical density $A_{260/230}$	Quantity ng/µl
Bisexual type I	1.90	2.10	63.30
Bisexual type II	1.90	2.10	40.90
Female parent	1.90	1.90	26.10
Pl 9	1.85	2.07	36.40
Pl 63	1.85	1.94	28.40
Pl 140	1.90	2.20	43.50
Pl 141	1.65	1.80	66.60
Viswam	1.90	2.20	66.50

4.2.2 Developing RAPD profiles

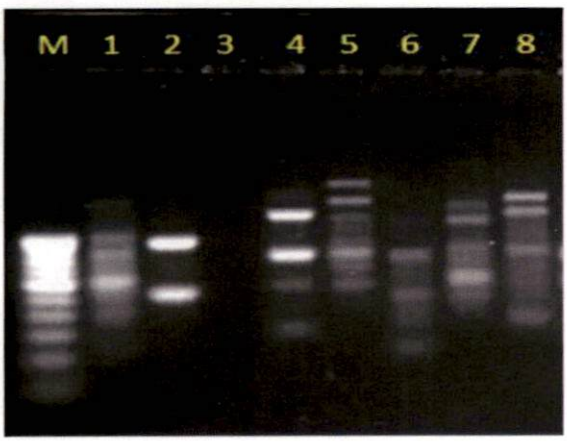
RAPD profiles were developed as per the standard protocol described by Williams *et al.* (1990). A total of 30 RAPD primers were used for the screening purpose (fig. 1 and 2). PCR amplicons were resolved by agarose gel (1.5 per cent) electrophoresis and then documented using Biorad gel documentation system. Thirty decamer primers generated 150 RAPD bands of different sizes.

Fig 1. Primer screening - RAPD profile of *Piper longum* using operon primers

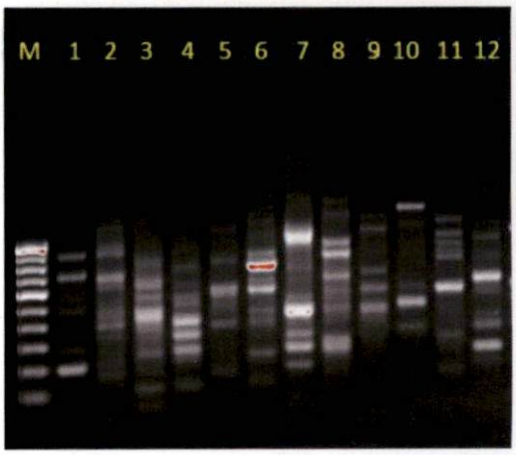


M1: 1kb ladder
M2: 100 bp ladder
NC: negative control (blank)

1. OPAU-04
2. OPAW-02
3.OPAW-16
4.OPAW-17
5. OPX-01
6. OPX-02
7.OPX -08
8. OPX-20
9.OPC-05
10.OPC-17



M: 100 bp ladder
1) OPM12
2) OPA20
3) OPA18
4) OPA17
5) OPA16
6) OPA11
7) OPA05
8) OPA08



M: 100 bp ladder
1) OPA12
2) OPAW09
3) OPAM1
4) OPM20
5) OPAU02
6) OPAW13
7) OPBA03
8) OPAW14
9) OPC10
10) OPA15
11) OPD7
12) OPM15

Robustness of amplification, clarity and scorability of banding patterns were the factors considered for selecting primers. Details of amplification pattern obtained with thirty RAPD primers are given in table 20.

Table 20. Details of amplification pattern obtained with 30 RAPD primers

SL. No	Primer	Amplification pattern			
		No. of Amplicons	Type of amplicons		Remarks
			Distinct	Faint	
1	OPAU-04	7	5	2	Selected
2	OPAW-02	6	4	2	Selected
3	OPAW-16	3	2	1	Rejected
4	OPAW-17	2	1	1	Rejected
5	OPX-01	6	5	1	Selected
6	OPX-02	4	2	2	Rejected
7	OPX-08	4	1	3	Rejected
8	OPX-20	7	4	3	Selected
9	OPC-05	9	5	4	Selected
10	OPC-17	2	1	1	Rejected
11	OPM-12	5	3	2	Rejected
12	OPA-20	2	2	0	Rejected
13	OPA-18	0	0	0	Rejected
14	OPA-17	5	2	3	Rejected
15	OPA-16	9	5	4	Selected
16	OPA-11	6	5	1	Selected
17	OPA-05	5	3	2	Rejected
18	OPA-08	4	3	1	Rejected
19	OPA-12	5	3	2	Rejected
20	OPAW-09	5	3	2	Rejected

21	OPAM-12	5	4	1	Rejected
22	OPM-20	5	3	2	Rejected
23	OPAU-02	7	5	2	Selected
24	OPAW-13	5	3	2	Rejected
25	OPBA-03	7	5	2	Selected
26	OPAW-14	5	4	1	Rejected
27	OPC-10	5	4	1	Rejected
28	OPA-15	5	2	3	Rejected
29	OPD-7	5	3	2	Rejected
30	OPM-15	5	2	3	Selected

Based on amplification, efficient primers giving six or more amplicons were selected (table 21) which were used to amplify the genomic DNA from parents, hybrids and Viswam.

Table 21. Primers selected for amplification and their sequence

SL. No	Primer	Sequence
1	OPAW-02	TCGCAGGTTC
2	OPAU-04	GGCTTCTGTC
3	OPX-01	CTGGGCACGA
4	OPX-20	CCCAGCTAGA
5	OPC-05	GATGACCGCC
6	OPA-16	AGCCAGCGAA
7	OPA-11	CAATCGCCGT
8	OPBA-3	GTGCGAGAAC
9	OPM-15	GACCTACCAC
10	OPAU-02	CCAACCCGCA

DNA isolated from *Piper longum* samples were subjected to PCR amplification. Among 1064 markers produced by ten primers 568 were polymorphic and 496 were monomorphic. Amplification products were separated by electrophoresis on 1.5 per cent agarose gels in 1X TAE buffer. Clear and well resolved amplicons were scored for presence (1) or absence (0). Same size bands across long pepper samples were treated as identical markers. The details of the bands or amplicons obtained in different primers are shown in table 22.

Table 22. Details of amplicons produced by selected primers from eight genotypes of *Piper longum*

Sl. No	RAPD Primer	Total No.of amplicons	No.of polymorphic amplicons	No.of monomorphic amplicons	PIC value (%)
1	OPAW-02	104	56	48	0.17
2	OPAU-04	104	48	56	0.16
3	OPX-01	128	88	40	0.29
4	OPX-20	144	112	32	0.30
5	OPC-05	168	120	48	0.28
6	OPA-16	96	48	48	0.15
7	OPA-11	64	56	8	0.24
8	OPBA-3	72	0	72	0.00
9	OPM-15	72	16	56	0.08
10	OPAU-02	112	24	88	0.10
Total		1064	568	496	

4.2.3 Polymorphic bands in *Piper longum* samples

4.2.3.1 Primer OPAW-02 (TCGCAGGTTC)

The primer gave good amplification in all the genotypes tested. In case of Viswam, a band was missing at 400 bp (indicated in blue arrow in figure 2) which was present in all the other genotypes. Though there was good amplification, most of the bands were monomorphic between male parents. Bands indicated by yellow

arrows in figure 2, showed polymorphism between the genotypes. Two bands were observed to be specific to Viswam at 300 bp and 2000 bp (indicated in red arrow). They were absent in all other genotypes.

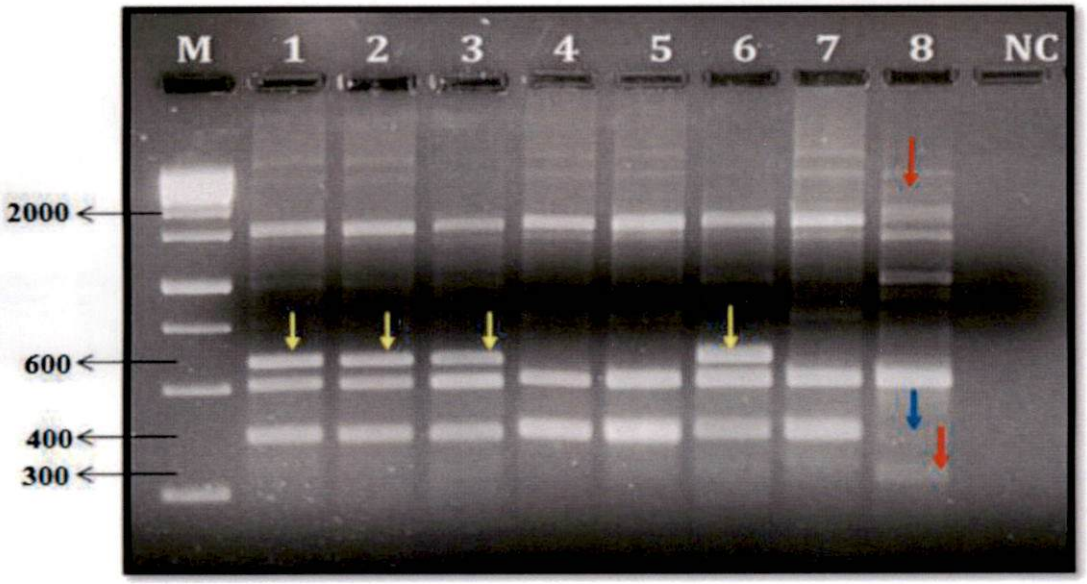
4.2.3.2 Primer OPAU-04 (GGCTTCTGTC)

Though the amplification was good in primer OPAU-04, most of the bands were monomorphic. In case of both male parents, Pl 140 and Pl 141 a common band was observed at 1500 bp (indicated in orange arrow in figure 3). Further in Pl 140, a band was seen at 650 bp which was also present in both male parents and female parent (indicated in yellow arrow in figure 3). A band was missing in Viswam at 1000 bp, which was present in all other genotypes (indicated in blue arrow in figure 3). In female parent, a specific band was observed at 250 bp and 450 bp which was absent in all other genotypes (indicated in red arrow in figure 3).

4.2.3.3 Primer OPX-01 (CTGGGCACGA)

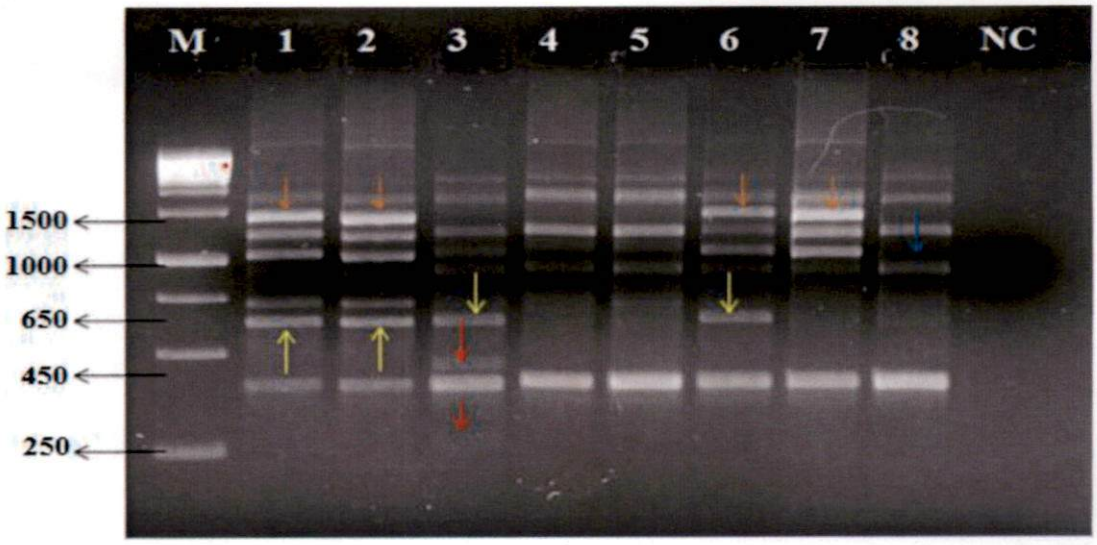
The primer gave good amplification in all the genotypes tested. There were more number of polymorphic bands compared to monomorphic bands. A common band was observed in both male parents, Pl 140 and Pl 141 at 2500 bp (indicated in yellow arrow in figure 4), which was absent in all other genotypes. Similarly another common band was present in Pl 140 and Pl 141 at 1250 bp (indicated in orange arrow in figure 4), which was not seen in other genotypes tested. Another band was found to be common in male parents, female parent and Pl 140 at 650 bp (indicated in red arrow in figure 4). A band was found to be missing in Viswam at 500 bp (indicated in blue arrow in figure 4) which was present in all other genotypes tested.

Fig 2. RAPD profile of *Piper longum* using the selected primer OPAW-02



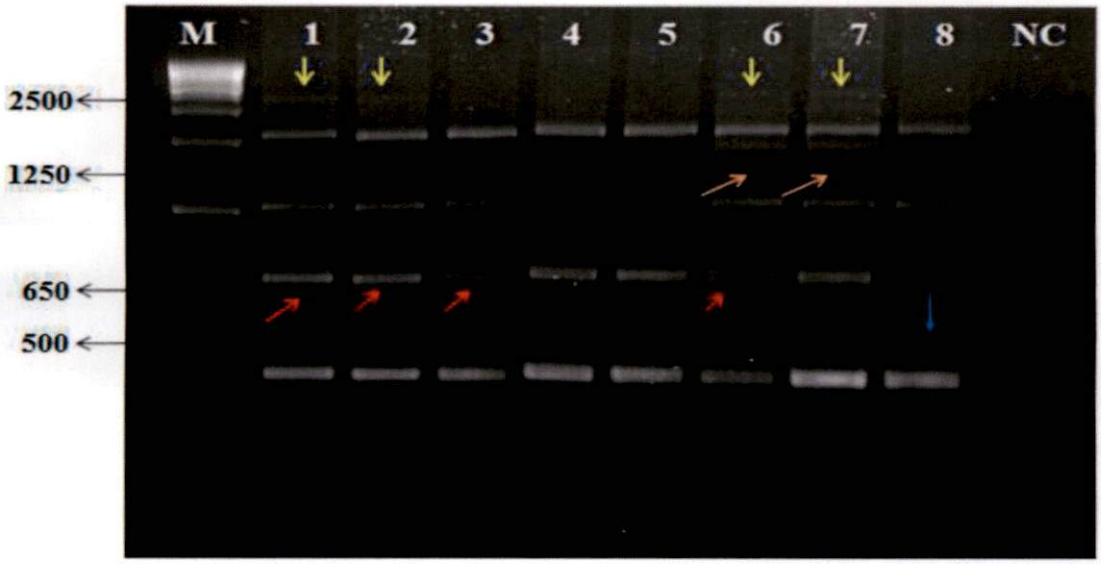
M: 1 kb ladder, 1: bisexual type I, 2: bisexual type II, 3: female parent, 4: Pl 9, 5: Pl 63, 6: Pl 140, 7: Pl 141, 8: Viswam, NC: negative control (blank)

Fig 3. RAPD profile of *Piper longum* using the selected primer OPAU-04



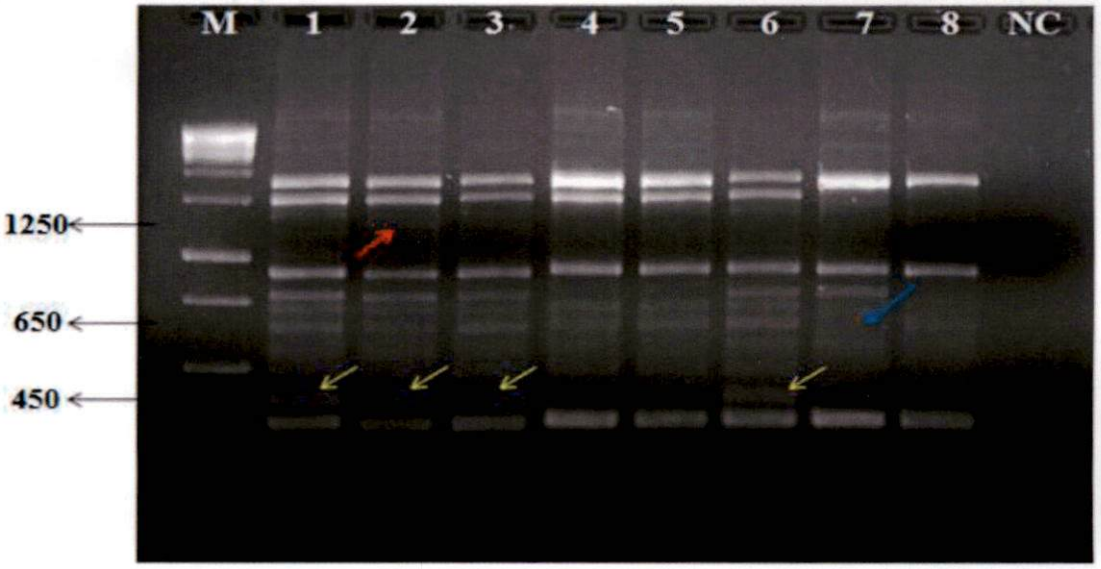
M: 1 kb ladder, 1: bisexual type I, 2: bisexual type II, 3: female parent, 4: Pl 9, 5: Pl 63, 6: Pl 140, 7: Pl 141, 8: Viswam, NC: Negative control (Blank)

Fig 4. RAPD profile of *Piper longum* using the selected primer OPX-01



M: 1 kb ladder, 1: bisexual type I, 2: bisexual type II, 3: female parent, 4: Pl 9, 5: Pl 63, 6: Pl 140, 7: Pl 141, 8: Viswam, NC: negative control (blank)

Fig 5. RAPD profile of *Piper longum* using the selected primer OPX-20



M: 1 kb ladder, 1: bisexual type I, 2: bisexual type II, 3: female parent, 4: Pl 9, 5: Pl 63, 6: Pl 140, 7: Pl 141, 8: Viswam, NC: negative control (blank)

4.2.3.4 Primer OPX-20 (CCCAGCTAGA)

The primer gave good amplification in all the genotypes tested. More number of polymorphic bands were produced in all the genotypes compared to monomorphic bands. At 1250 bp a specific band was found in male parent bisexual type II (indicated in red arrow in figure 5). At 650 bp a band was seen in all genotypes except Pl 141(indicated in blue arrow in figure 5). An equal sized band was found in parents as well as Pl 140 at 450 bp (indicated in yellow arrow in figure 5).

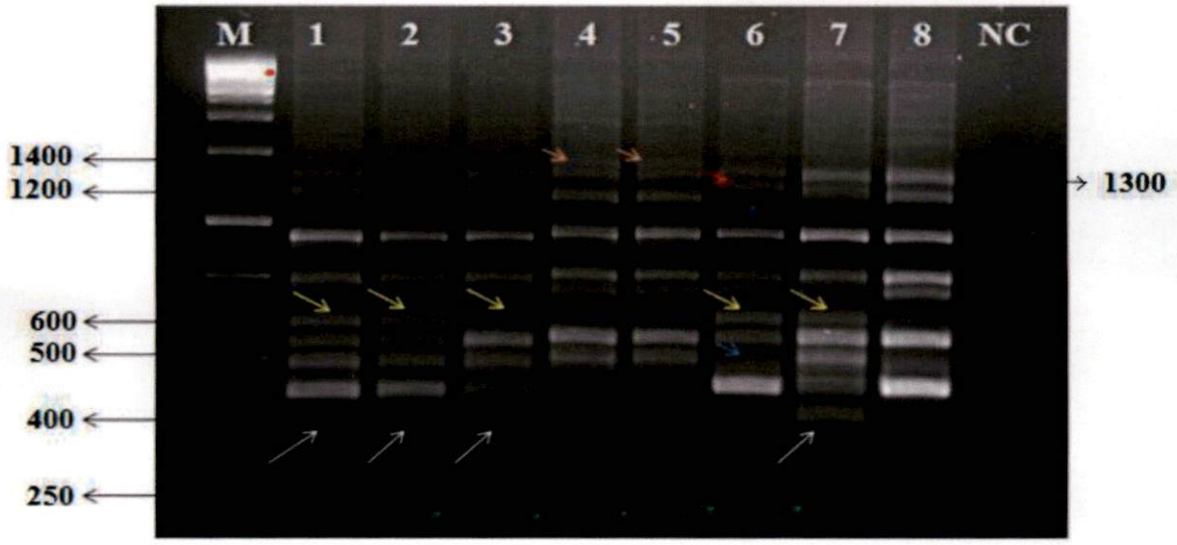
4.2.3.5 Primer OPC-05 (GATGACCGCC)

Primer gave good amplification in all the genotypes tested. Polymorphic bands were more in number compared to monomorphic bands in all genotypes tested. Pl 9 and Pl 63 shared a band of size 1400 bp, which was absent in all other genotypes tested. In Pl 140, a specific band was observed at 1300 bp (indicated in red arrow in figure 6). In all genotypes except Pl 140, a common band was observed at 1200 bp (indicated in blue arrow in figure 6). Further at 500 bp another band was missing in Pl 140 (indicated in blue arrow in figure 6). In all genotypes except Pl 9, Pl 63 and Viswam, a band was observed at 600 bp (indicated in yellow arrow in figure 6). At 400 bp a band was seen in parents as well as Pl 141 (indicated in white arrow in figure 6). In all promising hybrids and female parent a common band was observed at 250 bp (indicated in green arrow in figure 6).

4.2.3.6 Primer OPA16 (AGCCAGCGAA)

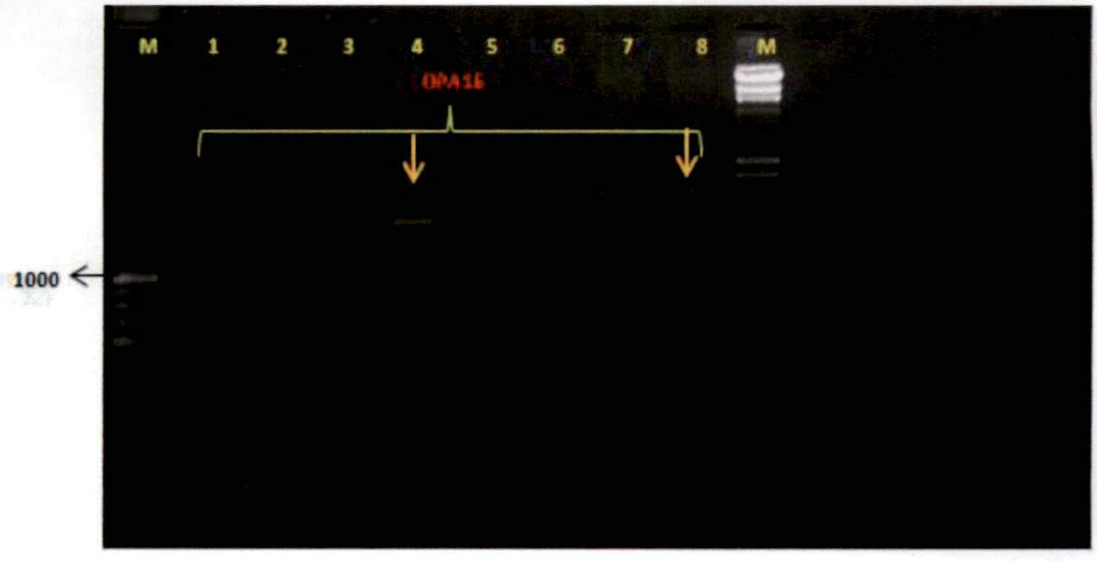
The primer gave good amplification in all the genotypes tested. The RAPD profile clearly shows the absence of a band above 1000 bp (indicated in yellow arrow in figure 7) in hybrid Pl 140 and Pl 141.

Fig 6. RAPD profile of *Piper longum* using the selected primer OPC-05



M: 1 kb ladder, 1: bisexual type I, 2: bisexual type II, 3: female parent, 4:Pl 9 5: Pl 63, 6: Pl 140, 7: Pl 141, 8: Viswam, NC: negative control (blank)

Fig 7. RAPD profile of *Piper longum* using the selected primer OPA-16



M: 100 bp ladder, Accessions: 1) bisexual type I, 2) female parent 3) bisexual type II 4) Pl 140, 5) Pl 9, 6) Viswam, 7) Pl 63 and 8) Pl 141

4.2.3.7 Primer OPA11 (CAATCGCCGT)

The primer gave good amplification in all the genotypes tested. The bands present at 900 bp and 1000 bp are present in all the genotypes except (indicated in orange arrow in figure 8) the hybrid Pl 9. Also a band at 750 bp was missing in hybrid Pl 140, but possessed a unique band at 800 bp (indicated in white arrow in figure 8).

4.2.3.8 Primer OPBA3 (GTGCGAGAAC)

The primer gave moderately good amplification in all genotypes tested. However no clear polymorphism was detected in the RAPD profile (figure 9).

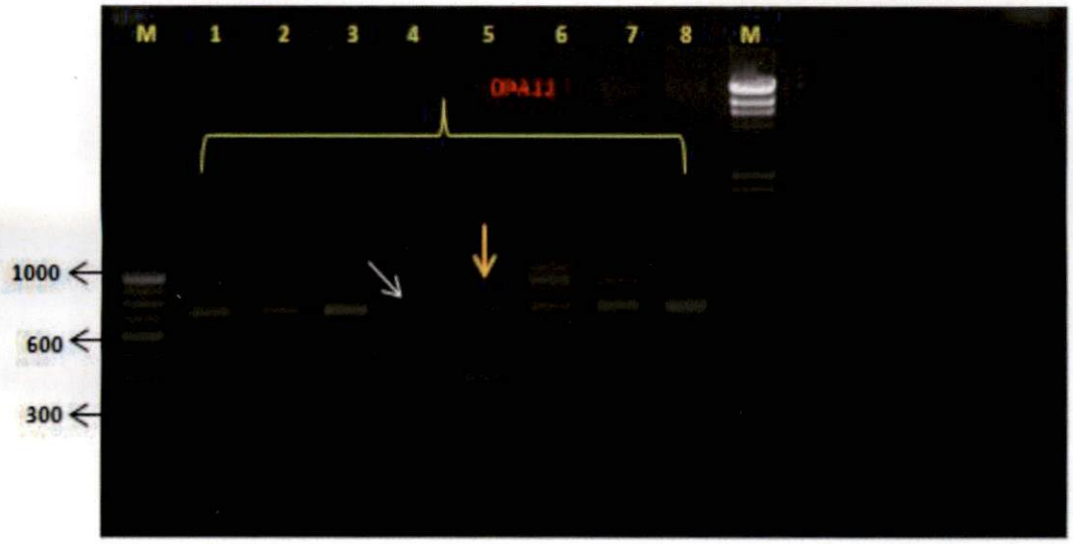
4.2.3.9 Primer OPM15 (GACCTACCAC)

The primer gave good amplification in all the genotypes tested. A band at 600 bp (indicated in red arrow in Figure 10) is missing in hybrids Pl 9, Pl 63 and Pl 141 respectively.

4.2.3.10 Primer OPAU02 (CCAACCCGCA)

The primer OPAU02 gave good amplification in all the accessions tested. A band was present (indicated in green arrows in figure 10) in parents (bisexual type I, II and female) at about 450 bp which was absent in all the hybrids.

Fig 8. RAPD profile of *Piper longum* using the selected primer OPA-11

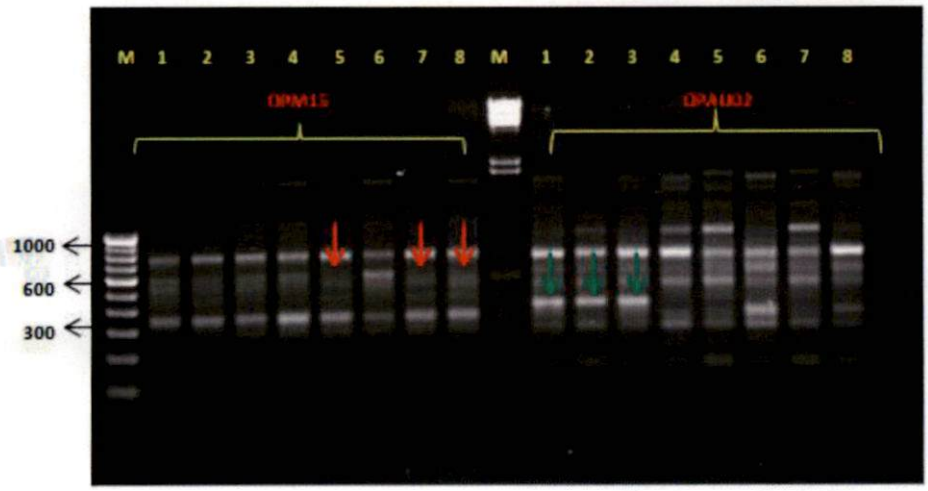


M: 100 bp ladder, Accessions: 1) bisexual type I, 2) female parent, 3) bisexual type II, 4) Pl 140, 5) Pl 9, 6) Viswam, 7) Pl 63 and 8) Pl 141

Fig 9. RAPD profile of *Piper longum* using the selected primer OPBA-3

M: 100 bp ladder, Accessions: 1) bisexual type I, 2) female parent 3) bisexual type II 4) Pl 140, 5) Pl 9, 6) Viswam, 7) Pl 63 and 8) Pl 141

Fig 10. RAPD profile of *Piper longum* using the selected primers OPM15 and OPAU-02

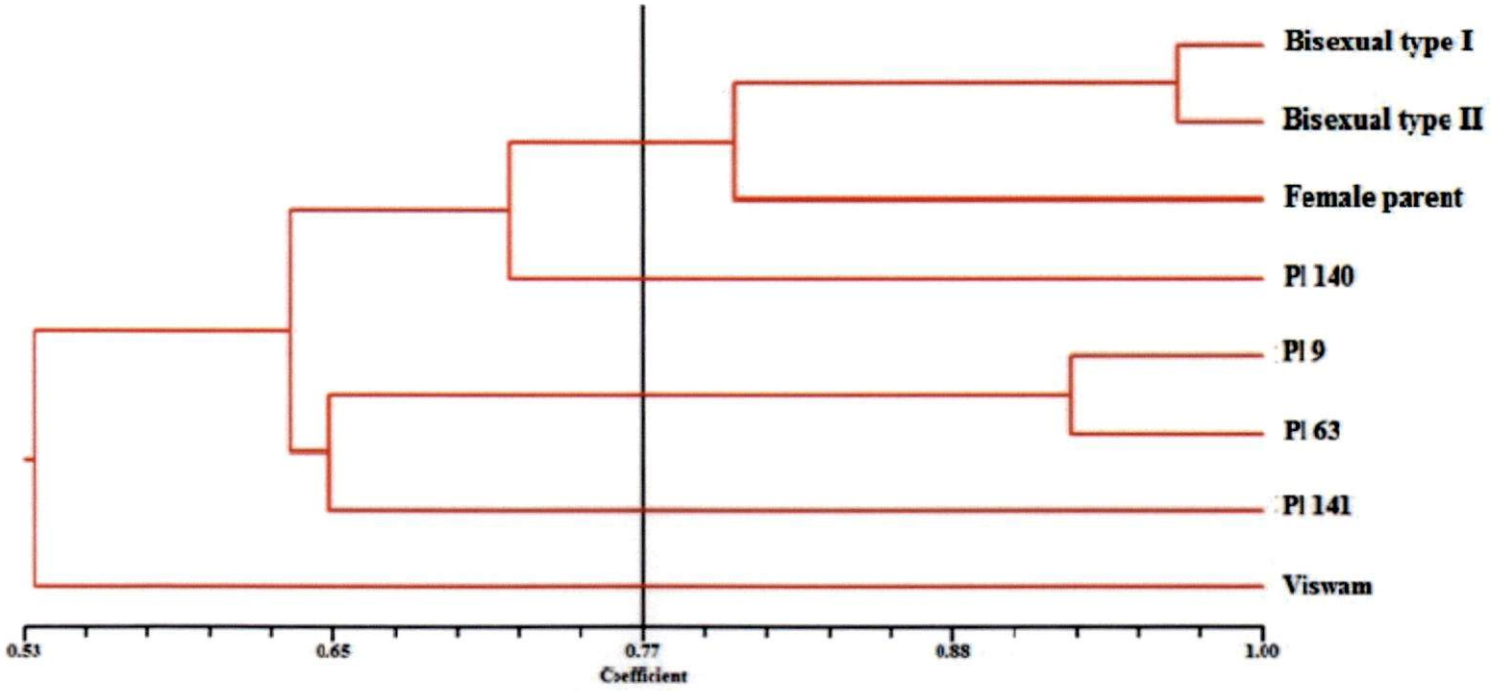


M: 100 bp ladder Accessions: 1) bisexual type I, 2) female parent, 3) bisexual type II 4) Pl 140, 5) Pl 9, 6) Viswam, 7) Pl 63 and 8) Pl 141

4.2.4 Relationship between parents and hybrids

Similarity and relationship between parents, hybrids as well as released variety 'Viswam' were studied by drawing UPGMA dendrogram using NTSYS. At Jaccard's similarity 0.77, eight genotypes of long pepper under study were grouped into five separate clusters. Both the male parents and the female parent formed a single cluster. Promising hybrids Pl 9 and Pl 63 were grouped in a separate cluster. The other two hybrids *viz.,* Pl 140, Pl 141 and Viswam had independent existence. Released variety Viswam was found to be most distant from all the promising hybrids and parents. Among the hybrids of *Piper longum* studied, Pl 140 was closer to parents compared to other three hybrids. Eighty per cent similarity was found between male and female parents. Almost 92 per cent similarity was observed between the two most promising hybrids *viz,* Pl 9 and Pl 63. UPGMA dendrogram showing the relationship between eight *Piper longum* genotypes taken for the study is depicted in figure 11.

Fig 11. UPGMA dendrogram showing relationship among eight *Piper longum* genotypes



Discussion

5. DISCUSSION

Piper longum is the genuine source of long pepper used extensively in many ayurvedic preparations. The species was reported to be dioecious. Female spikes are the commercial source of long pepper. Long pepper is well suited as an intercrop in coconut, arecanut and rubber plantations. However the farmers are not willing to cultivate thippali due to low return from the crop even if the market price is good. This is because of lack of high yielding varieties as well as short length of spikes in the ruling cultivars. To overcome this situation, high yielding varieties should be developed. An attempt was made to evolve superior types through hybridization and selection by utilizing the bisexual variant reported by Sujatha and Nybe (2007). Preliminary work on reproductive biology, standardization of pollination technique and development of hybrids were initiated by Kanimozhi (2010) and Sujatha (2009). Production of hybrids and back crosses were continued by Chandran (2012). Hybrids developed were evaluated in pots. Present study was taken up as a continuation of the earlier studies in *Piper longum* with the objective of evaluating four promising hybrids in pots under different shade levels as well as in field. Molecular characterisation of hybrids and parents were included in the present study. The results of the study are discussed in this chapter.

5.1 Evaluation of promising hybrids in pots under different shade levels

5.1.1 Cataloguing of *Piper longum* accessions grown in pots

Vegetative characters like leaf shape, leaf colour and reproductive characters like spike orientation, spike shape, immature spike colour, colour change while fruit ripening were analysed and catalogued based on the descriptor developed for *Piper nigrum* by IPGRI (1995) with necessary modifications.

Shape of leaf lamina was mostly cordate on the orthotropic shoots and only Pl 141 had ovate-lanceolate leaves. All the accessions studied had leaves with elliptic-

lanceolate shape on their plagiotropic shoots. Leaf color was varying from light green to dark green.

Spikes orientation was erect in all the genotypes of *Piper longum* studied. Spike shape varied from cylindrical to conical. All the genotypes except Pl 63 and female had developed cylindrical shaped spikes. Spikes were conical to cylindrical in Pl 63 and female. Variation was also seen in color of immature spikes among genotypes. It was varying from light yellow to greenish yellow. However, no variation was seen in spike color during ripening, it was blackish green in all the genotypes of *Piper longum* studied. Viswanathan, 1995 and Banerjee *et al.*, 1999 reported that spikes of long pepper were cylindrical, oblong, berries black when ripe, globose with aromatic odour and pungent taste. High variability in the seedling population of *Piper longum* L. was reported by Chandran (2012).

5.1.2 Vegetative characters

5.1.2.1 Plant height

Significant difference was observed in plant height among the hybrids at different shade levels. Among shade levels, zero and 25 per cent shade were more suitable for good vegetative growth. These were found to be significantly superior over 50 per cent shade. Among the genotypes, female parent produced maximum vine length. Results of the study indicated that maximum plant height was recorded in the female parent at 25 per cent shade level (fig. 12). Sujatha (2016) reported that at 25 per cent shade, Pl 141 was longest and it was on par with female parent.

5.1.2.2 Rate of growth of plant

The result showed that, rate of growth of plant was maximum at 25 per cent shade compared to zero per cent and 50 per cent shade (fig 13). Maximum growth rate was observed in Pl 141 and female at 25 per cent and zero per cent respectively. Rate of growth varied from 14.8 per cent to 35.9 per cent among the genotypes of

Fig 12. Plant height of *Piper longum* genotypes at various shade levels

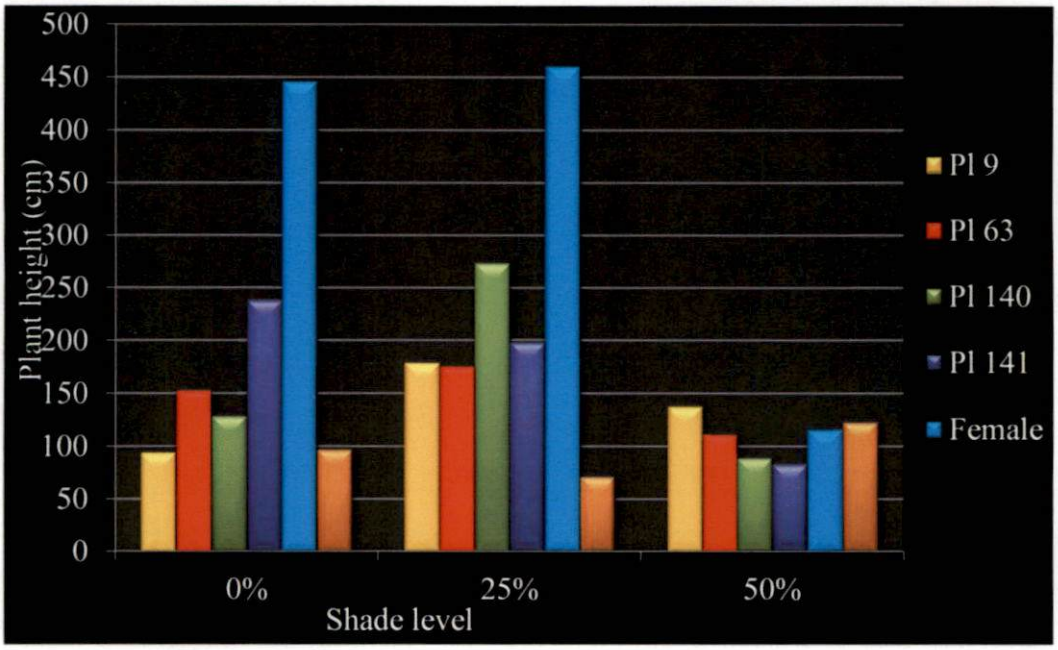
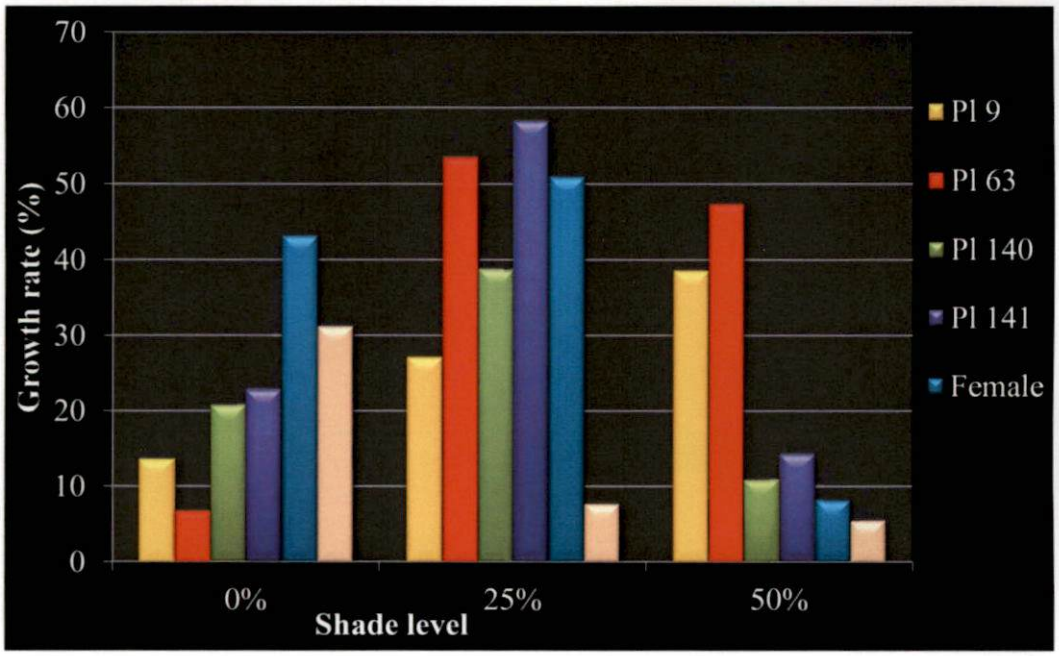


Fig 13. Rate of growth of plant at various shade levels



Piper longum. Mean monthly increment in height showed wide variation ranging from 0.56cm to 11.8cm in seedling progenies of *Piper longum* studied by Chandran (2012).

A slow growth pattern was observed at 50 per cent shade level. During initial stage of growth, the accessions grown at 50 per cent shade were affected with leaf rollers. Even though it was controlled by spraying dimethoate, it might have affected the vigour of plants.

5.1.2.3 Time taken for production of first lateral

Long pepper produced fruits on plagiotropic shoots or laterals. Therefore lateral production can influence yield in *Piper longum*. Time taken for production of first lateral was varying from one hybrid to another. It was also depended on the shade level at which the plants were grown. i.e. the performance of different hybrids at different shade levels were significantly different. As can be seen from table 4, Pl 140 took less number of days to produce first lateral at zero per cent shade. Pl 141 took lesser number of days to produce first lateral at 25 and 50 per cent shade levels. Time taken for production of first lateral varied from 41.2 (Pl 141) to 62 (female) days among the accessions studied. Chandran (2012) in initial evaluation of seedling progenies reported that time taken for lateral branch production varied from 109 to 260 days which is much higher than the present report. However, the accessions studied were different.

5.1.2.4 Number of primary branches per plant

Hybrids differed significantly in producing primary branches per plant. Number of primary branches went on increasing throughout the growing season. Number of primary branches showed variation among hybrids from 6.2 to 25.3 after one year of growth (table 5). Variability in this character was reported by Manuel (1994), Jaleel (2006), Joseph (2008) and Chandran (2012). At 6 months after planting, number of primary branches varied from 1.9 to 9. Nair (2015) studied 42 accessions of long pepper and reported that number of primary branches were ranging from 1.00 to 8.00 among accessions studied.

5.1.3 Reproductive characters

Flowering was observed throughout the year in *Piper longum* accessions studied. Hybrids Pl 9 and Pl 63 started flowering earlier (May-June) than other accessions. Viswam started flowering only during September at zero per cent shade. Maximum fruit set was during the month of November and February. Accessions grown at 50 per cent shade did not produce many flowers. Fruit set was also lesser at 50 per cent shade compared to lower shade levels. It may be because of the slow growth rate at 50 per cent shade. Nair (2015) reported that, maximum inflorescence was produced during June, July and August. Less than five per cent flowering was reported during December and January during her study.

Significant difference was observed among hybrids in number of spikes per plant and fresh and dry yield per plant. Pl 9, Pl 63 and Pl 140 were on par and significantly superior to all other accessions for yield. As can be seen from fig 14, at zero per cent shade, Pl 63 was found to be the most promising in yield attributes including number of spikes per plant, fresh weight (g) per plant and dry weight (g) per plant. Pl 63 was followed by Pl 9 at zero per cent shade for these characters. At 25 per cent shade Pl 140 yielded more than other genotypes studied (fig 15). At 50 per cent shade, Pl 9 produced maximum yield than other accessions studied (fig 16).

Fig 14. Yield characters of *Piper longum* accessions at zero per cent shade

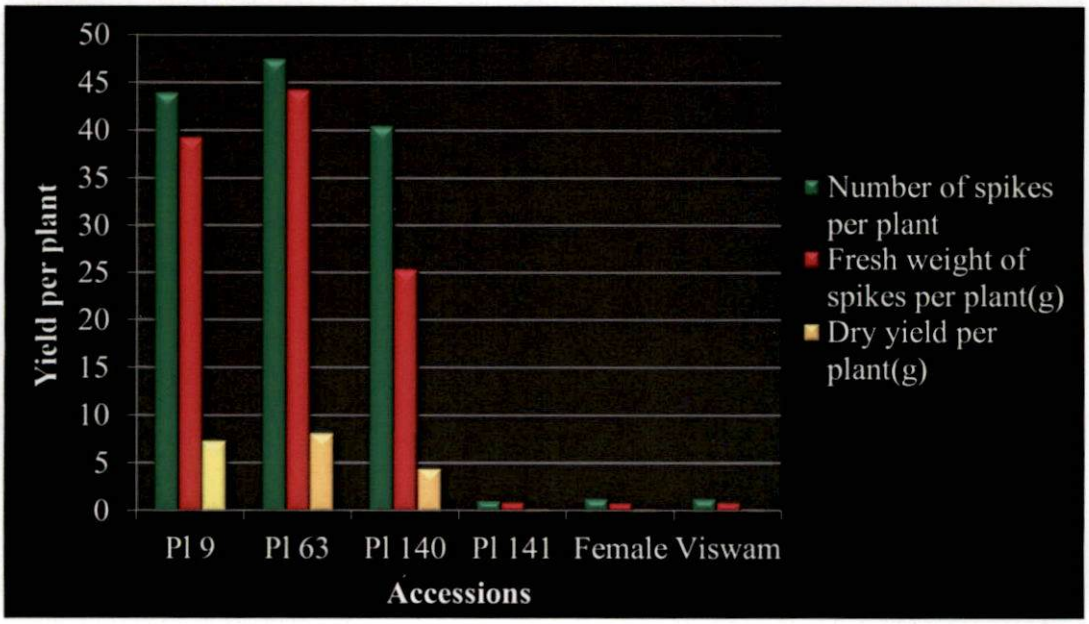
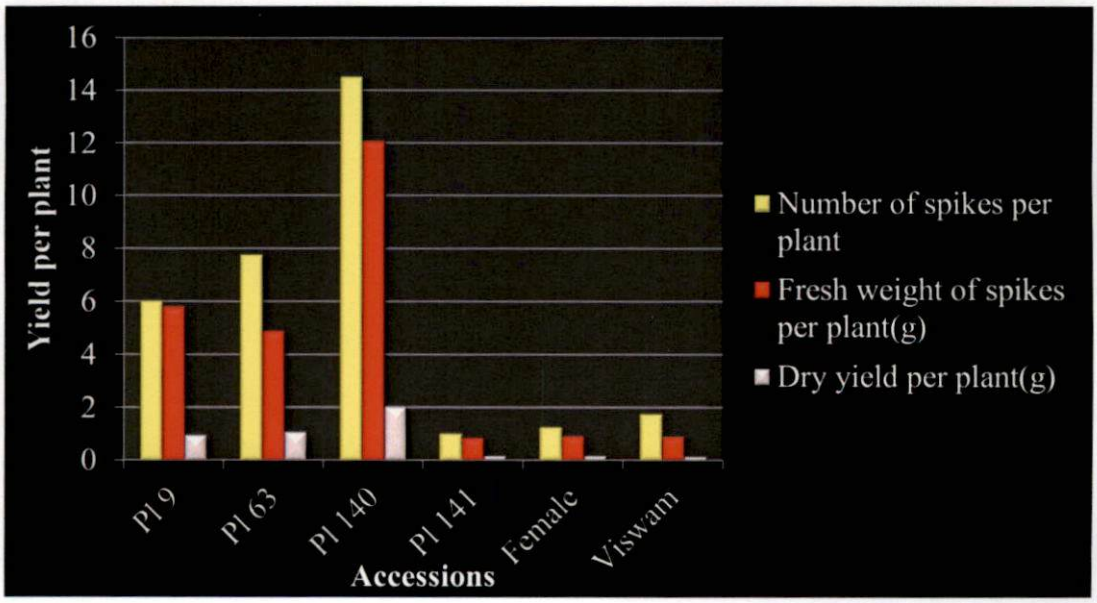


Fig 15. Yield characters of *Piper longum* accessions at 25 per cent shade



Mean value for yield was found to be decreasing with increasing shade levels from zero per cent to 50 per cent shade. Primary branches, laterals, number of spike bearing branches per plant and number of spikes per lateral branch significantly influenced yield. This was in agreement with the findings of Manuel (1994), Jaleel (2006) and Joseph (2008).

5.1.4 Quality parameters

Quality of spikes were analysed by estimating essential oil, oleoresin and piperine content in them. Essential oil, oleoresin and piperine were estimated in accessions studied. At zero per cent shade, in all genotypes, essential oil content was same (0.8 per cent) except Pl 141(0.83 per cent), in which a slightly higher percentage of oil was present. As can be seen from fig 17, Pl 9 contained more oleoresin than all other genotypes including Viswam. Chandran (2012) in an initial evaluation of *P. longum* seedlings reported that Pl 9 contained more oleoresin than released variety Viswam. Piperine was also found to be higher in Pl 9. At 25 per cent shade, Pl 140 (0.86 per cent) showed slightly higher essential oil content than Pl 9 (0.85 per cent). Oleoresin was also maximum in Pl 140 compared to Pl 9 and Pl 63.

High variability among accessions with respect to quality characters such as oleoresin and piperine content were observed during present study. This was in agreement with the earlier reports of Manuel (1994), Jaleel (2006), Joseph (2008), Chandran (2012) and Nair (2015).

Fig.16 Yield characters of *Piper longum* accessions at 50 per cent shade

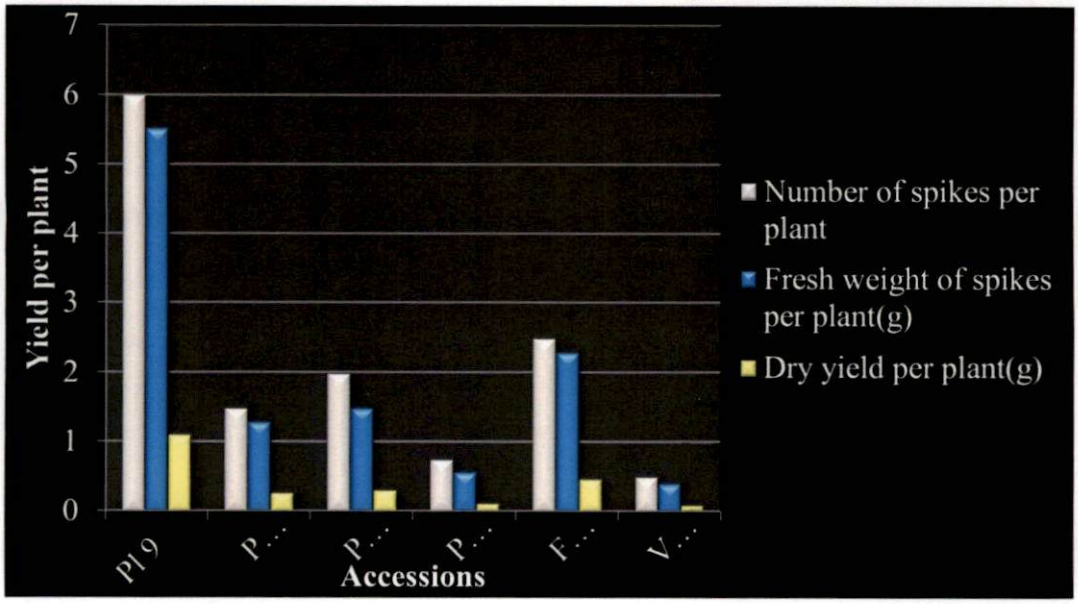
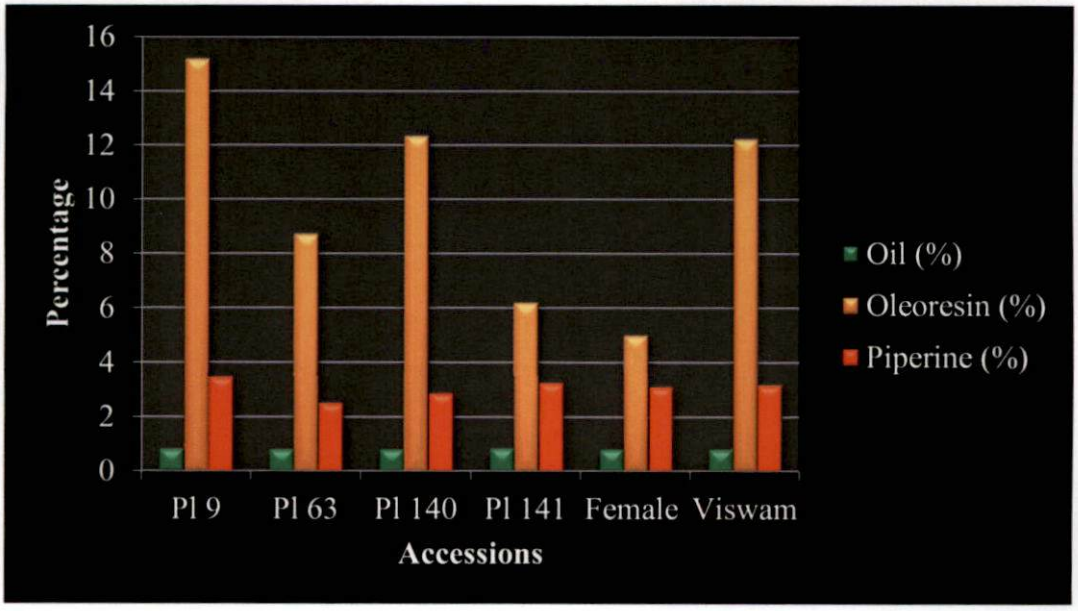


Fig 17. Quality parameters of *Piper longum* accessions at zero per cent shade



5.2 Evaluation of hybrids in field condition

In this experiment, four promising hybrids were laid out in randomized block design (RBD) in field with four replications. Female parent along with Viswam were used as check varieties.

All the accessions were evaluated for growth and yield characters. Significant differences were observed for the characters like number of primary branches per plant, internodal length of orthotropic as well as plagiotropic shoots, leaf area, number of spikes per plant, spike characters like pedicel length, spike length, spike girth, fresh weight of spikes per plant and dry spike yield per plant.

As can be seen from fig 18 and 19 internodal length and petiole length were more in orthotropic shoots compared to plagiotropic shoots in all the accessions studied. Among the accessions, female parent possessed maximum internodal length and petiole length in both orthotropic and plagiotropic shoots compared to other accessions. Accessions showed variation in internodal length and petiole length. Internodal length of spike bearing branches (Plagiotropic shoots) ranged from 2.68 cm to 5.92 cm. Nair (2015) reported that internodal length of spike bearing branches ranged from 1.86cm to 7.38cm.

Leaf area was recorded in all genotypes in present study. Significant variations were observed among accessions. Leaf area ranged from 18.76 cm^2 to 51.68 cm^2. Leaf area was reported to range from 25.98 cm^2 to 63.87 cm^2 among 42 accessions studied by Nair (2015).

As can be seen from fig 20, spike length was maximum in Pl 9 followed by Pl 63. Spike length ranged from 3.22 cm to 4.60 cm in present study. Spike length was reported to vary from 1.32 cm to 6.20 cm in a study conducted by Chandran (2012) to evaluate hybrids and back cross progenies of *Piper longum.*

Fig 18. Internodal length of orthotropic and plagiotropic shoots in *Piper longum* genotypes

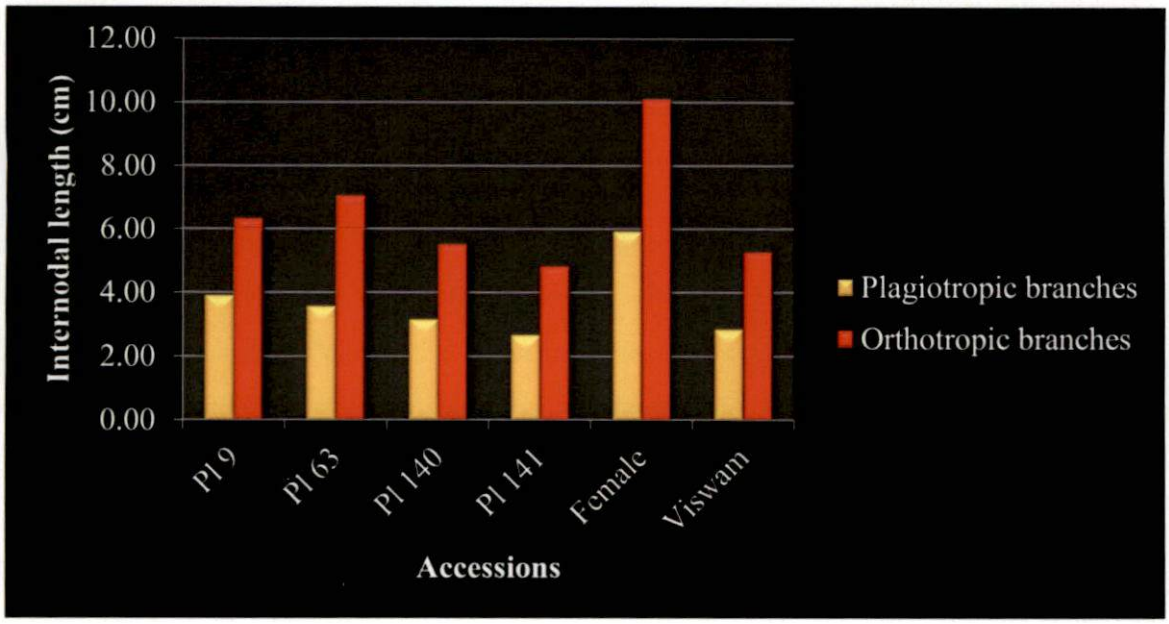
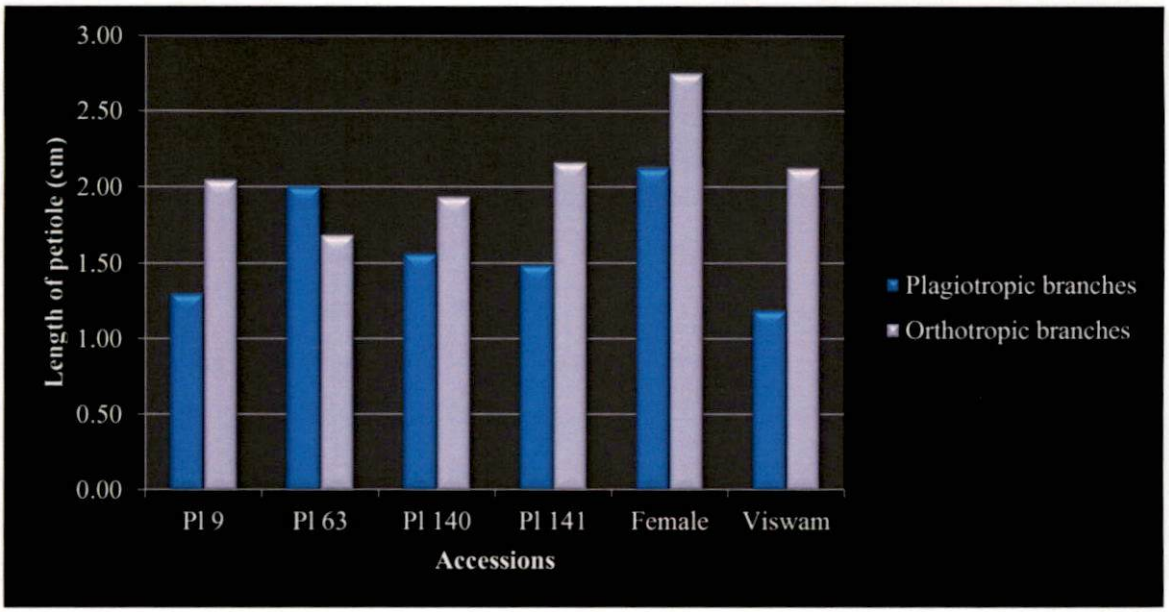


Fig 19. Length of petiole in orthotropic and plagiotropic shoots of *Piper longum* accessions



Correlation studies revealed that number of primary branches, number laterals, number of spikes per lateral branch, spike length and fresh spike yield had significant influence on dry spike yield per plant. This is in agreement with the findings of Manuel (1994) who reported that number of stems per hill, number of spike bearing branches per plant, number of spikes per spike bearing branch, and green spike yield had positive correlation with dry spike yield.

Highest yield was observed in Pl 9 followed by Pl 63 (fig 21). Pl 9 produced 8.11 fold more dry spike yield than Viswam. Similarly, Pl 63 showed 5.57 fold increase in yield over Viswam. However, the experiment is to be repeated for confirmatory results. Spikes are produced on laterals in *Piper longum*. In present study, lateral production was also more in these two hybrids compared to other accessions. This may be the reason for higher yield in Pl 9 and Pl 63. Correlation analysis revealed that, number of laterals per plant (number of spike bearing branches per plant) has positive correlation with yield. This is in agreement with the report of Manuel (1994), that number of spike bearing branches per stem is an important character which influence dry spike yield.

High variability with respect to quality characters such as oleoresin and piperine content was observed during present study. Variability in *Piper longum* accessions for these characters were reported by Manuel (1994), Jaleel (2006), Joseph (2008), Chandran (2012) and Sujatha (2016). Essential oil content was uniform (0.8 per cent) in all accessions except Pl 140 (0.83 per cent). Uniformity in essential oil content (0.8 per cent) among *Piper longum* accessions was reported by Sujatha (2016). Incidence of leaf spot caused by *Colletotrichum gloeosporoides* was observed in field planted *Piper longum* accessions. Chandran (2012) also reported incidence of *Colletotrichum gloeosporoides* in field planted *Piper longum* accessions. Incidence of papaya mealy bug was observed in field. This was also reported by Chandran (2012).

Fig 20. Spike characters of *Piper longum* genotypes in field

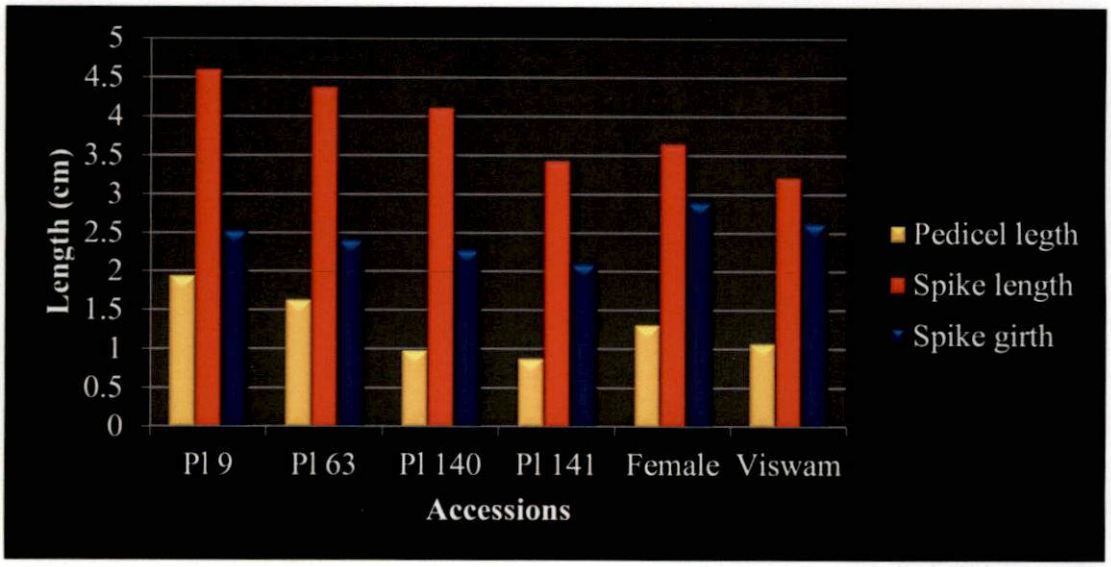
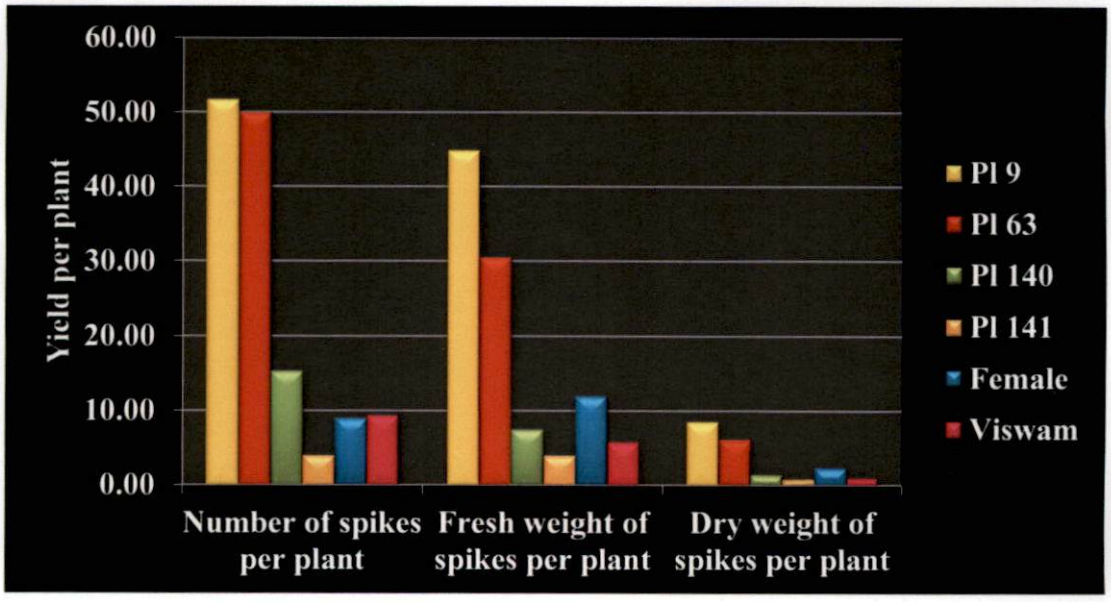


Fig 21. Yield per plant in *Piper longum* genotypes in field



5.3 Molecular characterisation of promising hybrids and parents

In this experiment, four promising hybrids along with their parents and released variety 'Viswam' were characterised using molecular markers.

Out of thirty primers screened, ten primers gave good amplification with 6 or more bands for each primer. These ten RAPD primers *viz.*, OPAW-02, OPAU-04, OPX-01, OPX-20, OPC-05, OPA-16, OPA-11, OPBA-3, OPM-15 and OPAU-02 were used in the present study, gave good amplification of the genomic DNA of *Piper longum*, producing 6 – 9 amplicons for each sample. With all the primers the two male parents showed monomorphic bands except in OPX-20. In OPX-20, at 1250 bp a specific band was found in male parent bisexual type II. All the ten primers showed polymorphism between the male and female parents though the level of polymorphism was low.

RAPD is one of the widely used techniques owing to its simplicity and rapidity. Quantity of DNA required is very less in RAPD compared to other markers and they are able to generate numerous polymorphisms. However, concerns exist about the reproducibility of RAPD-PCR reactions. RAPD markers are dominant, i.e. it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). RAPD is not specific in amplifying the sequence like other markers *viz.*, SSR, RFLP, AFLP etc.

In the present study, among the hybrids, hybrid Pl 140 was found to be more distinct from the rest of the hybrids, but it belonged to the major cluster in which the male and female parents were included. In OPC-05, Pl 140 showed polymorphism with all other genotypes with respect to absence of bands of two sizes *viz.*, 500 bp and 1200 bp. At 1300 bp, genotype specific band was present in Pl 140. For the remaining hybrids also polymorphism was found for all the primers expressed as presence or absence of bands.

Among the four promising hybrids evaluated, Pl 9 followed by Pl 63 were found to be more promising as they were significantly higher yielders compared to Pl 140, Pl 141, female parent and released variety 'Viswam'. The molecular marker analysis also showed that these two hybrids belong to the same cluster and are different from the rest of the genotypes. These hybrids could be further evaluated in multi-location trials to explore the possibility of releasing them as high yielding hybrids. Molecular characterization is essential to protect breeders rights. It is expected that the molecular characterisation of promising hybrids will be highly useful while releasing them as promising hybrids.

However, in future, genotypes could be characterised using SSR markers for better understanding of the extent of relationship between hybrids and parents.

Summary

6. SUMMARY

The study entitled 'Evaluation and characterisation of promising hybrids of long pepper (*Piper longum* L.) was done with the objective of evaluating four promising hybrids in pots under different shade levels, evaluation of their performance in field condition and characterisation of hybrids along with the parents using molecular markers. The experiments on evaluation of the hybrids were laid out at Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara and characterisation was conducted at the Department of Plant Biotechnology, College of Agriculture, Padannakkad. The findings of the investigation are summerised below.

Variations were observed in vegetative and reproductive characters of *Piper longum* genotypes grown in pots under different shade levels. Spike orientation was erect in all the accessions studied.

Significant variation was observed among hybrids at different shade levels for vegetative characters like plant height, number of primary branches per plant and time taken for production of first lateral. Variation was also observed in yield attributes like number of spikes per plant and fresh and dry weight of spikes per plant.

Flowering and fruiting were higher at zero per cent and 25 per cent shade compared to 50 per cent shade level. At zero per cent shade, Pl 9, Pl 63 and Pl 140 were on par with respect to number of spikes per plant and fresh spike yield (g) per plant. For dry spike yield, Pl 9 (7.37 g) and Pl 63 (8.14 g) were on par.

At 25 per cent shade, significant difference was observed among hybrids in number of spikes per plant. Pl 140 and Pl 63 were on par with respect to number of spikes per plant. Maximum fresh yield of spikes was for Pl 140 (12.06 g) followed by Pl 9 (5.79 g). For dry spike yield, Pl 9 (0.95 g), Pl 63 (1.05 g) and Pl 140 (2.01 g) were on par. However, at 50 per cent shade, yield was maximum in Pl 9 (1.10 g dry

spike yield). Yield was higher at lower shade level (zero per cent) compared to higher (25 per cent and 50 per cent).

At zero per cent shade, Pl 9 showed maximum oleoresin (15.2 per cent) and piperine (3.47 per cent). Essential oil was uniform (0.8 per cent) in all genotypes except Pl 140 (0.83 per cent). At 25 per cent shade Pl 140 had maximum essential oil (0.86 per cent) and oleoresin (9.23 per cent).

Significant variations were observed among field planted *P. longum* genotypes in number of primary branches, internodal length of orthotropic as well as plagiotropic shoots and leaf area. Maximum vine length was observed in female parent (221.75 cm). Viswam produced maximum number of primary branches per plant (31.67). Hybrids Pl 9 and Pl 63 produced maximum number of laterals per plant (45) compared to all other genotypes studied. Characters like pedicel length, spike length, spike girth and yield in terms of number of spikes per plant, fresh weight per plant and dry spike yield per plant also differed significantly. Hybrid Pl 9 produced maximum yield (44.87 g fresh spike per plant) followed by Pl 63 (30.57 g fresh spike per plant). Hybrids Pl 9 and Pl 63 were statistically on par with respect to number of spikes per plant and dry spike yield. Number of laterals and number of spikes per lateral branch had highly significant correlation with yield. Correlation analysis revealed that number of primary branches per plant and spike length also had significant influence on yield.

Among the four promising hybrids evaluated, Pl 9 followed by Pl 63 were found to be more promising as they were significantly higher yielders compared to other hybrids, female parent and Viswam.

In field, high variability with respect to quality characters such as oleoresin and piperine content was observed. Essential oil content was uniform (0.8 per cent) in all accessions except Pl 140 (0.83 per cent). Maximum oleoresin and piperine content

were recorded in Pl 9. Pest like papaya mealy bug and diseases like leaf spot and fusarium wilt were observed in field. However, the attack was not severe.

Characterisation of the four selected accessions of *Piper longum* along with parents and Viswam was done using RAPD markers. Among thirty decamer primer screened, ten primers showed difference between genotypes. Polymorphism between male and female parents was shown by six primers *viz.*, OPAW-02, OPAU-04, OPX-01, OPX-20, OPC-05 and OPAU-02. Pl 140 was most distinct from all other hybrids whereas it was found to be closer to the parents than others.

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*Originals not seen

Appendices

Appendix I

Table. 1 Monthly observations on plant height (cm) at different shade levels

Hybrids	July				August				September			
	Shade levels			Mean	Shade levels			Mean	Shade levels			Mean
	0 %	25 %	50 %		0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	26.75 (5.26)	20.75 (4.53)	27.75 (5.24)	25.08 (5.01)	39.25 (6.26)	30.75 (5.53)	28.25 (5.32)	32.75 (5.70)	55.50 (7.25)	41.50 (6.35)	36.25 (6.05)	44.42 (6.55)
Pl 63	22.25 (4.79)	21.00 (4.64)	30.25 (5.56)	24.50 (5.00)	35.00 (5.93)	24.00 (4.97)	32.00 (5.72)	30.33 (5.54)	65.25 (7.90)	30.25 (5.54)	36.50 (6.11)	44.00 (6.51)
Pl 140	20.50 (4.63)	21.50 (4.59)	38.75 (6.25)	26.92 (5.16)	28.25 (5.40)	30.25 (5.49)	39.50 (6.30)	32.67 (5.73)	40.25 (6.37)	39.25 (6.25)	41.50 (6.46)	40.33 (6.36)
Pl 141	35.75 (5.86)	21.75 (4.70)	30.50 (5.45)	29.33 (5.34)	40.75 (6.32)	34.25 (5.83)	30.75 (5.50)	35.25 (5.88)	52.75 (7.20)	44.00 (6.63)	32.75 (5.69)	43.17 (6.51)
Female	23.50 (4.91)	48.75 (6.78)	46.75 (6.70)	39.67 (6.13)	43.50 (6.49)	42.50 (6.52)	60.50 (7.76)	48.83 (6.92)	78.00 (8.51)	48.00 (6.96)	69.25 (8.28)	65.08 (7.92)
Viswam	21.25 (4.64)	18.75 (4.36)	19.00 (4.39)	19.67 (4.47)	32.00 (5.56)	23.25 (4.86)	20.50 (4.60)	25.25 (5.01)	43.00 (6.45)	35.00 (5.95)	34.75 (5.80)	37.58 (6.07)
Mean	25.00 (5.02)	25.42 (4.93)	32.17 (5.60)		36.46 (5.99)	30.83 (5.53)	35.25 (5.87)		55.79 (7.28)	39.67 (6.28)	41.83 (6.40)	
	C.D.(0.05)				C.D.(0.05)				C.D.(0.05)			
Shade	NS				NS				NS			
Hybrid	0.99				0.97				NS			
Shade × Hybrid	NS				NS				NS			

* Note: values given in parenthesis are square root transformed

Table. 2 Monthly observations on plant height (cm) at different shade levels

Hybrids	October				November				December			
	Shade levels			Mean	Shade levels			Mean	Shade levels			Mean
	0 %	25 %	50 %		0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	65.25 (7.82)	56.00 (7.48)	41.75 (6.50)	54.33 (7.27)	79.25 (8.60)	63.75 (8.00)	47.00 (6.91)	63.33 (7.84)	88.00 (9.17)	80.75 (8.96)	55.50 (7.50)	74.75 (8.54)
Pl 63	89.00 (9.20)	34.25 (5.90)	46.00 (6.75)	56.42 (7.28)	106.75 (10.07)	51.75 (7.23)	59.25 (7.68)	72.58 (8.33)	121.50 (10.64)	91.00 (9.56)	89.25 (9.39)	100.58 (9.86)
Pl 140	42.75 (6.56)	46.25 (6.80)	44.50 (6.69)	44.50 (6.68)	51.25 (7.19)	52.75 (7.30)	51.00 (7.15)	51.67 (7.21)	63.75 (8.01)	78.50 (8.83)	55.50 (7.46)	65.92 (8.10)
Pl 141	64.75 (7.97)	61.50 (7.87)	33.75 (5.78)	53.33 (7.21)	88.00 (9.17)	100.00 (9.75)	37.25 (6.09)	75.08 (8.34)	108.50 (10.10)	149.25 (11.76)	44.75 (6.69)	100.83 (9.52)
Female	107.25 (10.06)	53.25 (7.32)	75.00 (8.58)	78.50 (8.65)	159.00 (12.06)	75.00 (8.46)	80.00 (8.83)	104.67 (9.78)	218.50 (14.35)	104.75 (9.99)	85.00 (9.14)	136.08 (11.16)
Viswam	51.00 (7.00)	40.25 (6.36)	60.00 (7.16)	50.42 (6.84)	56.25 (7.32)	45.00 (6.69)	67.75 (7.76)	56.33 (7.26)	71.75 (8.27)	49.00 (6.94)	72.75 (8.05)	64.50 (7.75)
Mean	70.00 (8.10)	48.58 (6.96)	50.17 (6.91)		90.08 (9.07)	64.71 (7.90)	57.04 (7.40)		112.00 (10.09)	92.21 (9.34)	67.13 (8.04)	
	C.D.(0.05)				**C.D.(0.05)**				**C.D.(0.05)**			
Shade	1.06				1.27				1.41			
Hybrid	NS				NS				1.99			
Shade × Hybrid	NS				NS				NS			

* Note: values given in parenthesis are square root transformed

Table. 3 Monthly observations on plant height (cm) at different shade levels

Hybrids	January				February				March			
	Shade levels			Mean	Shade levels			Mean	Shade levels			Mean
	0 %	25 %	50 %		0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	93.75 (9.51)	88.00 (9.32)	62.25 (7.93)	81.33 (8.92)	95.25 (9.59)	89.25 (9.38)	66.75 (8.19)	83.75 (9.05)	97.50 (9.71)	93.75 (9.62)	84.75 (9.02)	92.00 (9.45)
Pl 63	142.75 (11.67)	108.75 (10.43)	99.25 (9.87)	116.92 (10.65)	144.50 (11.74)	123.00 (11.02)	103.25 (10.05)	123.58 (10.94)	146.75 (11.83)	144.25 (11.88)	106.25 (10.20)	132.42 (11.30)
Pl 140	67.00 (8.21)	118.25 (10.81)	64.75 (8.00)	83.33 (9.01)	70.00 (8.41)	146.25 (12.00)	69.00 (8.24)	95.08 (9.55)	73.25 (8.61)	170.50 (12.98)	74.00 (8.52)	105.92 (10.04)
Pl 141	115.75 (10.44)	154.50 (12.00)	48.00 (6.92)	106.08 (9.79)	127.50 (10.96)	158.75 (12.19)	50.50 (7.12)	112.25 (10.09)	143.00 (11.57)	167.00 (12.59)	58.75 (7.65)	122.92 (10.60)
Female	252.75 (15.40)	134.00 (11.14)	89.75 (9.41)	158.83 (11.98)	279.75 (16.08)	176.75 (12.87)	94.50 (9.67)	183.67 (12.88)	307.50 (16.69)	215.00 (14.27)	102.00 (10.09)	208.17 (13.68)
Viswam	79.25 (8.71)	50.75 (7.08)	78.00 (8.32)	69.33 (8.04)	84.50 (9.03)	53.50 (7.27)	81.00 (8.48)	73.00 (8.26)	89.25 (9.32)	57.00 (7.50)	86.25 (8.72)	77.50 (8.51)
Mean	125.21 (10.66)	109.04 (10.13)	73.67 (8.41)		133.58 (10.97)	124.58 (10.79)	77.50 (8.62)		142.88 (11.29)	141.25 (11.48)	85.33 (9.03)	
	C.D.(0.05)				**C.D.(0.05)**				**C.D.(0.05)**			
Shade	1.49				1.57				1.67			
Hybrid	2.11				2.22				2.36			
Shade × Hybrid	NS				NS				NS			

* Note: values given in parenthesis are square root transformed

Table. 4 Monthly observations on plant height (cm) at different shade levels

	April				May			
Hybrids	**Shade levels**			**Mean**	**Shade levels**			**Mean**
	0 %	**25 %**	**50 %**		**0 %**	**25 %**	**50 %**	
Pl 9	99.50 (9.82)	122.75 (10.87)	91.00 (9.28)	104.42 (9.99)	101.50 (9.93)	147.25 (11.96)	92.00 (9.35)	113.58 (10.41)
Pl 63	149.00 (11.93)	164.00 (12.59)	109.00 (10.34)	140.67 (11.62)	151.50 (12.03)	175.50 (12.95)	110.75 (10.43)	145.92 (11.81)
Pl 140	77.50 (8.85)	239.25 (15.12)	76.75 (8.67)	131.17 (10.88)	79.75 (8.98)	273.50 (16.31)	79.75 (8.82)	144.33 (11.37)
Pl 141	165.50 (12.40)	173.75 (12.86)	64.75 (8.00)	134.67 (11.09)	178.75 (12.86)	185.75 (13.39)	69.75 (8.30)	144.75 (11.52)
Female	375.00 (17.96)	301.75 (16.94)	106.00 (10.29)	260.92 (15.06)	379.00 (18.08)	367.50 (18.68)	110.50 (10.50)	285.67 (15.75)
Viswam	91.75 (9.46)	58.75 (7.62)	101.75 (9.31)	84.08 (8.80)	97.50 (9.80)	60.75 (7.75)	122.50 (10.00)	93.58 (9.18)
Mean	159.71 (11.74)	176.71 (12.67)	91.54 (9.32)		164.67 (11.95)	201.71 (13.51)	97.54 (9.57)	
	C.D.(0.05)				**C.D.(0.05)**			
Shade	2.01				2.06			
Hybrid	2.85				2.92			
Shade × Hybrid	NS				NS			

* Note: values given in parenthesis are square root transformed

Table 5. Rate of growth (%) of plant at different shade level

Hybrids	August				September				October			
	Shade levels			Mean	Shade levels			Mean	Shade levels			Mean
	0 %	25 %	50 %		0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	54.90	64.10	8.50	42.50	31.90	25.30	25.30	27.50	25.10	44.00	14.20	27.80
Pl 63	51.90	9.00	13.20	24.70	55.00	18.30	10.60	28.00	31.30	11.10	22.00	21.50
Pl 140	52.30	121.00	3.80	59.10	74.30	23.00	4.40	33.90	31.80	12.60	11.30	18.50
Pl 141	31.60	34.30	2.90	22.90	55.60	35.10	8.70	33.10	33.00	57.30	3.20	31.20
Female	50.40	35.00	127.70	71.10	67.50	21.70	17.40	35.60	51.50	11.30	6.20	23.00
Viswam	45.90	34.10	28.60	36.20	33.50	89.70	84.40	69.20	15.10	10.70	47.00	24.30
Mean	47.90	49.60	30.80		53.00	35.50	25.10		31.30	24.50	17.30	
	C.D (0.05)				C.D (0.05)				C.D (0.05)			
Shade	NS				NS				NS			
Hybrid	NS				NS				NS			
Shade × Hybrid	NS				NS				NS			

Table 6. Rate of growth (%) of plant at different shade level

Hybrids	November				December				January			
	Shade levels			Mean	Shade levels			Mean	Shade levels			Mean
	0 %	25 %	50 %		0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	19.20	23.50	31.60	24.80	13.80	27.20	38.60	26.50	8.20	12.00	16.50	12.20
Pl 63	20.40	51.20	23.40	31.60	6.80	53.50	47.40	35.90	29.80	17.20	10.60	19.20
Pl 140	15.50	11.10	15.70	14.10	20.80	38.80	11.00	23.50	5.10	58.10	15.50	26.20
Pl 141	49.80	60.50	6.40	38.90	22.80	58.30	14.40	31.80	11.50	7.90	3.10	7.50
Female	37.40	8.20	5.40	17.00	43.20	51.00	8.20	34.10	19.10	27.80	7.60	18.10
Viswam	6.20	11.60	31.10	16.30	31.20	7.70	5.60	14.80	8.50	4.00	8.70	7.00
Mean	24.80	27.70	18.90		23.10	39.40	20.90		13.70	21.20	10.30	
	C.D (0.05)				**C.D (0.05)**				**C.D (0.05)**			
Shade	NS				NS				NS			
Hybrid	NS				NS				NS			
Shade × Hybrid	NS				NS				24.35			

Table 7. Rate of growth (%) of plant at different shade level

Hybrids	February				March				April			
	Shade levels			Mean	Shade levels			Mean	Shade levels			Mean
	0 %	25 %	50 %		0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	2.80 (9.20)	10.80 (13.60)	8.80 (14.28)	12.40	3.40 (10.10)	15.20 (19.80)	27.50 (27.20)	19.00	2.30	38.90	7.10	16.10
Pl 63	1.60 (7.10)	8.40 (13.90)	4.20 (10.86)	10.60	2.20 (8.30)	13.60 (18.60)	3.20 (10.10)	12.40	2.60	6.80	2.80	4.10
Pl 140	5.50 (12.30)	26.40 (29.80)	6.90 (14.69)	18.90	4.70 (11.10)	15.10 (22.60)	6.70 (14.50)	16.10	5.60	32.10	3.10	13.60
Pl 141	10.70 (18.10)	6.50 (13.50)	6.60 (11.94)	14.50	16.90 (23.80)	9.80 (13.50)	17.80 (21.20)	19.50	12.50	10.30	9.00	10.60
Female	3.50 (10.20)	42.30 (40.50)	7.60 (15.29)	22.00	1.20 (5.20)	33.80 (34.00)	13.80 (18.40)	19.20	8.70	49.90	4.80	21.10
Viswam	5.80 (13.50)	5.90 (13.60)	2.90 (8.34)	11.80	1.80 (7.60)	12.50 (19.60)	4.80 (12.30)	13.20	3.80	3.30	11.60	6.20
Mean	(11.70)	(20.80)	(12.50)		(11.00)	(21.40)	(17.30)		5.90	23.50	6.40	
	C.D (0.05)				C.D (0.05)				C.D (0.05)			
Shade	5.30				7.60				9.30			
Hybrid	7.50				NS				NS			
Shade × Hybrid	13.0				NS				22.90			

Note : * Values in parenthesis are angular transformed

Table 8. Rate of growth (%) of plant at different shade level

May				
Hybrid	**Shade levels**			**Mean**
	0%	**25%**	**50%**	
Pl 9	4.30 (10.90)	31.00 (31.40)	3.00 (8.20)	16.80
Pl 63	2.30 (7.50)	2.50 (7.60)	2.90 (9.20)	8.10
Pl 140	2.70 (8.90)	8.00 (12.70)	5.10 (12.60)	11.40
Pl 141	6.40 (13.30)	8.30 (14.50)	11.20 (18.50)	15.40
Female	1.90 (7.30)	21.40 (25.20)	11.10 (17.60)	16.70
Viswam	7.30 (14.20)	3.40 (10.60)	13.50 (20.60)	15.10
Mean	10.40	17.00	14.40	
	C.D (0.05)			
Shade	NS			
Hybrid	NS			
Shade × Hybrid	NS			

Note : * Values in parenthesis are angular transformed

Table 9. Number of primary branches per plant at different shade level

Hybrids	July				August				September			
	Shade levels			Mean	Shade levels			Mean	Shade levels			Mean
	0 %	25 %	50 %		0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	1.20 (1.50)	1.00 (1.40)	0.20 (1.10)	0.80 (1.30)	1.70 (1.60)	0.50 (1.20)	0 (1.00)	0.70 (1.30)	3.20 (2.00)	2.70 (1.90)	0.20 (1.10)	2.00 (1.70)
Pl 63	0.20 (1.10)	1.00 (1.40)	0.50 (1.20)	0.50 (1.20)	0.20 (1.10)	0.50 (1.20)	0.20 (1.10)	0.30 (1.10)	0.70 (1.30)	0.20 (1.10)	0.70 (1.30)	0.50 (1.20)
Pl 140	0 (1.00)	0 (1.00)	0.50 (1.20)	0.10 (1.10)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0.20 (1.10)	0.20 (1.10)	0 (1.00)	0.10 (1.10)
Pl 141	0.20 (1.10)	0.20 (1.10)	0.20 (1.10)	0.20 (1.10)	0 (1.00)	0.20 (1.10)	0.20 (1.10)	0.10 (1.10)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
Female	0 (1.00)	0.70 (1.30)	0.70 (1.30)	0.40 (1.20)	0.50 (1.20)	0 (1.00)	0 (1.00)	0.20 (1.10)	0.70 (1.30)	0.20 (1.10)	0 (1.00)	0.30 (1.10)
Viswam	1.00 (1.40)	0.70 (1.30)	0.70 (1.30)	0.80 (1.30)	2.50 (1.90)	2.20 (1.70)	3.20 (2.00)	2.60 (1.90)	3.70 (2.10)	4.50 (2.30)	2.20 (1.80)	3.50 (2.10)
Mean	0.40 (1.20)	0.60 (1.30)	0.50 (1.20)		0.80 (1.30)	0.60 (1.20)	0.60 (1.20)		1.40 (1.50)	1.30 (1.40)	0.50 (1.20)	
	C.D (0.05)				C.D (0.05)				C.D (0.05)			
Shade	NS				NS				0.16			
Hybrid	0.16				0.23				0.22			
Shade × Hybrid	0.28				NS				0.38			

Note : * Values in parenthesis are square root transformed

Table 10. Number of primary branches per plant at different shade level

Hybrids	October			Mean	November			Mean	December			Mean
	Shade levels				Shade levels				Shade levels			
	0 %	25 %	50 %		0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	4.75 (2.30)	3.75 (2.10)	0.50 (1.20)	3.00 (1.90)	11.00 (3.40)	6.00 (2.60)	1.50 (1.60)	6.20 (2.50)	17.50 (4.20)	7.25 (2.90)	2.25 (1.80)	9.00 (2.90)
Pl 63	4.00 (2.10)	0.75 (1.30)	1.00 (1.40)	1.90 (1.60)	10.25 (3.30)	3.25 (2.10)	2.25 (1.80)	5.30 (2.40)	16.75 (4.10)	6.75 (2.70)	2.75 (1.90)	8.80 (2.90)
Pl 140	0.50 (1.20)	0.50 (1.20)	0.25 (1.10)	0.40 (1.20)	1.25 (1.40)	1.50 (1.60)	1.00 (1.40)	1.25 (1.50)	2.25 (1.80)	1.75 (1.70)	1.75 (1.60)	1.90 (1.70)
Pl 141	0 (1.00)	0 (1.00)	0.75 (1.30)	0.30 (1.10)	1.00 (1.40)	0.25 (1.10)	1.00 (1.40)	0.80 (1.30)	3.25 (2.00)	1.75 (1.60)	1.00 (1.40)	2.00 (1.70)
Female	1.00 (1.40)	0.50 (1.20)	0.25 (1.10)	0.60 (1.20)	3.75 (2.20)	1.00 (1.40)	0.25 (1.10)	1.70 (1.50)	7.00 (2.80)	1.75 (1.60)	0.25 (1.10)	3.00 (1.80)
Viswam	5.50 (2.50)	5.50 (2.50)	3.50 (2.10)	4.80 (2.40)	10.25 (3.30)	7.50 (2.90)	5.25 (2.40)	7.70 (2.90)	11.75 (3.60)	8.50 (3.00)	3.00 (2.00)	7.80 (2.90)
Mean	2.60 (1.70)	1.80 (1.60)	1.00 (1.40)		6.30 (2.50)	2.10 (1.90)	1.90 (1.60)		9.80 (3.10)	4.60 (2.30)	1.80 (1.60)	
	C.D (0.05)				**C.D (0.05)**				**C.D (0.05)**			
Shade	0.24				0.26				0.31			
Hybrid	0.34				0.36				0.44			
Shade × Hybrid	NS				0.63				0.77			

Note : * Values in parenthesis are square root transformed

Table 11. Number of primary branches per plant at different shade level

Hybrids	January			Mean	February			Mean	March			Mean
	Shade levels				Shade levels				Shade levels			
	0 %	25 %	50 %		0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	22.00 (4.50)	12.00 (3.60)	2.75 (1.90)	12.25 (3.40)	17.75 (4.10)	15.50 (4.00)	4.75 (2.40)	12.70 (3.50)	21.00 (4.40)	27.00 (5.20)	4.75 (2.40)	17.60 (4.00)
Pl 63	17.50 (4.20)	12.00 (3.60)	4.00 (2.20)	11.20 (3.30)	18.75 (4.30)	21.25 (4.70)	6.00 (2.60)	15.30 (3.90)	27.25 (4.90)	32.50 (5.80)	6.25 (2.60)	22.00 (4.40)
Pl 140	2.75 (1.90)	3.25 (2.00)	2.25 (1.80)	2.80 (1.90)	3.25 (2.10)	6.00 (2.60)	3.25 (2.00)	4.20 (2.20)	3.25 (2.10)	6.50 (2.70)	2.75 (1.90)	4.20 (2.20)
Pl 141	4.50 (2.30)	2.75 (1.90)	1.00 (1.40)	2.80 (1.90)	5.00 (2.40)	3.00 (1.90)	2.50 (1.90)	3.50 (2.10)	6.00 (2.60)	3.75 (2.10)	2.25 (1.80)	4.00 (2.20)
Female	7.50 (2.90)	4.00 (2.20)	1.00 (1.40)	4.20 (2.20)	9.50 (3.20)	5.50 (2.50)	2.25 (1.80)	5.80 (2.50)	9.25 (3.20)	4.75 (2.30)	1.75 (1.60)	5.30 (2.40)
Viswam	26.25 (5.10)	13.00 (3.70)	5.75 (2.60)	15.00 (3.80)	20.50 (4.60)	12.50 (3.70)	6.50 (2.70)	13.20 (3.60)	27.75 (5.30)	15.75 (4.00)	8.00 (2.90)	17.20 (4.10)
Mean	13.40 (3.50)	7.80 (2.80)	2.80 (1.90)		12.50 (3.40)	10.60 (3.20)	4.20 (2.20)		15.80 (3.70)	15.00 (3.70)	4.30 (2.20)	
	C.D (0.05)				**C.D (0.05)**				**C.D (0.05)**			
Shade	0.38				0.37				0.52			
Hybrid	0.54				0.53				0.74			
Shade × Hybrid	0.94				0.91				1.28			

Note : * Values in parenthesis are square root transformed

Table 12. Number of primary branches per plant at different shade level

Hybrids	April				May			
	Shade levels			Mean	Shade levels			Mean
	0 %	25 %	50 %		0 %	25 %	50 %	
Pl 9	27.75 (5.10)	21.50 (4.60)	5.25 (2.50)	18.20 (4.10)	24.00 (4.80)	23.25 (4.90)	6.00 (2.60)	17.80 (4.10)
Pl 63	22.00 (4.70)	30.75 (5.60)	6.75 (2.70)	19.80 (4.40)	33.75 (5.90)	36.00 (6.10)	6.00 (2.60)	25.30 (4.80)
Pl 140	5.50 (2.50)	7.25 (2.90)	4.50 (2.30)	5.80 (2.60)	7.00 (2.80)	7.00 (2.80)	4.50 (2.30)	6.20 (2.60)
Pl 141	7.75 (2.90)	5.75 (2.60)	3.50 (2.10)	5.70 (2.50)	11.00 (3.40)	5.00 (2.40)	4.00 (2.20)	6.70 (2.70)
Female	11.50 (3.50)	7.00 (2.80)	3.75 (2.20)	7.40 (2.80)	12.25 (3.60)	8.50 (3.00)	4.50 (2.30)	8.40 (3.00)
Viswam	23.00 (4.80)	24.00 (4.90)	8.75 (3.10)	18.60 (4.30)	14.00 (3.80)	22.50 (4.80)	9.25 (3.10)	15.30 (3.90)
Mean	16.30 (3.90)	16.00 (3.90)	5.40 (2.50)		17.00 (4.10)	17.00 (4.00)	5.70 (2.50)	
	C.D (0.05)				C.D (0.05)			
Shade	0.45				0.37			
Hybrid	0.64				0.52			
Shade × Hybrid	1.11				0.91			

Note: * Values in parenthesis are square root transformed

Appendix II

Table 1. Monthly observations on plant height (cm) in field

Hybrid	June	July	August	September	October	November	December	January	February	March	April	May
Pl 9	24.79 (4.96)	29.13 (5.38)	38.79 (6.21)	46.13 (6.78)	57.88 (7.59)	63.50 (7.94)	70.08 (8.30)	74.00 (8.51)	75.33 (8.59)	77.17 (8.70)	78.92 (8.80)	80.75 (8.90)
Pl 63	31.47 (5.60)	36.94 (6.04)	52.52 (7.22)	70.15 (8.35)	85.77 (9.25)	90.54 (9.50)	99.63 (9.94)	100.02 (9.95)	102.03 (10.05)	104.43 (10.17)	107.19 (10.30)	109.18 (10.40)
Pl 140	24.73 (4.95)	27.58 (5.23)	38.36 (6.17)	55.38 (7.41)	61.07 (7.77)	67.23 (8.14)	73.10 (8.45)	75.92 (8.61)	77.53 (8.69)	79.05 (8.78)	80.61 (8.87)	84.13 (9.06)
Pl 141	25.46 (5.02)	26.08 (5.09)	34.29 (5.84)	44.71 (6.67)	52.17 (7.17)	58.83 (7.60)	64.46 (7.94)	67.38 (8.10)	68.96 (8.20)	73.73 (8.47)	77.92 (8.69)	92.46 (9.39)
Female	43.46 (6.59)	55.38 (7.42)	91.29 (9.54)	117.08 (10.80)	156.63 (12.40)	182.58 (13.26)	202.04 (13.90)	212.13 (14.22)	216.00 (14.33)	217.83 (14.39)	219.96 (14.48)	221.75 (14.54)
Viswam	20.21 (4.49)	18.83 (4.34)	29.58 (5.42)	52.77 (7.26)	68.25 (8.26)	79.35 (8.90)	84.42 (9.18)	86.52 (9.30)	88.23 (9.39)	89.73 (9.47)	90.96 (9.53)	92.56 (9.62)
C.D (0.05)	0.62	0.83	0.86	0.93	1.53	2.17	2.63	2.83	2.89	2.91	2.93	3.07

* Note: values given in parenthesis are square root transformed

Table 2. Monthly observations on primary branches per plant in field

Hybrid	June	July	August	September	October	November	December	January	February	March	April	May
Pl 9	0.75 (1.10)	1.30 (1.14)	1.67 (1.26)	2.63 (1.58)	4.13 (1.99)	5.33 (2.25)	6.53 (2.43)	7.54 (2.55)	6.51 (2.47)	7.08 (2.56)	9.29 (2.95)	11.83 (3.31)
Pl 63	0.50 (0.97)	1.29 (1.13)	2.13 (1.45)	3.37 (1.79)	4.21 (2.02)	4.56 (2.07)	7.69 (2.67)	7.04 (2.51)	7.00 (2.58)	7.63 (2.73)	9.96 (3.15)	11.67 (3.42)
Pl 140	0.00 (0.71)	1.00 (1.00)	1.33 (1.14)	1.33 (1.14)	1.92 (1.36)	2.13 (1.45)	2.25 (1.49)	2.18 (1.44)	2.46 (1.57)	4.00 (1.98)	5.00 (2.21)	6.50 (2.52)
Pl 141	0.25 (0.84)	1.00 (1.00)	1.33 (1.15)	1.89 (1.37)	1.77 (1.30)	1.92 (1.38)	2.68 (1.74)	3.23 (1.78)	2.57 (1.58)	3.75 (1.90)	4.75 (2.16)	6.50 (2.52)
Female	0.50 (0.97)	1.25 (1.11)	2.08 (1.44)	2.20 (1.46)	3.43 (1.83)	4.50 (2.06)	5.94 (2.33)	6.97 (2.58)	7.88 (2.76)	8.33 (2.84)	11.71 (3.37)	13.54 (3.61)
Viswam	2.07 (1.59)	2.83 (1.66)	5.13 (2.18)	6.44 (2.40)	7.56 (2.69)	8.40 (2.79)	10.54 (2.84)	13.38 (3.56)	12.58 (3.36)	19.92 (4.38)	25.75 (5.06)	31.67 (5.61)
C.D (0.05)	0.36	0.25	0.55	0.754	0.65	0.84	NS	1.20	1.09	0.97	0.83	0.95

* Note: values given in parenthesis are square root transformed

Table 3. Monthly observations on laterals in field

Hybrid	July	August	September	October	November	December	January	February	March	April	May
Pl 9	4.25 (2.03)	7.25 (2.63)	9.25 (2.94)	14.00 (3.59)	17.50 (4.02)	35.00 (5.50)	40.50 (5.80)	41.00 (5.86)	41.75 (5.92)	42.75 (6.02)	45.00 (6.29)
Pl 63	1.25 (1.10)	6.00 (2.30)	9.25 (2.84)	17.50 (4.07)	18.00 (4.19)	36.75 (5.77)	38.75 (5.95)	39.50 (6.01)	40.50 (6.10)	41.75 (6.20)	45.00 (6.52)
Pl 140	1.25 (1.10)	2.75 (1.52)	4.00 (1.83)	8.75 (2.78)	10.25 (3.08)	11.25 (3.22)	14.25 (3.68)	14.75 (3.76)	15.25 (3.82)	16.25 (3.96)	19.50 (4.32)
Pl 141	2.00 (1.35)	2.75 (1.60)	3.25 (1.72)	4.25 (2.03)	5.25 (2.28)	8.00 (2.77)	8.00 (2.77)	8.25 (2.81)	8.50 (2.84)	9.00 (2.93)	9.00 (2.93)
Female	1.00 (1.00)	2.75 (1.56)	4.50 (2.02)	7.75 (2.72)	9.00 (2.91)	13.00 (3.49)	15.75 (3.79)	18.75 (4.16)	20.50 (4.37)	21.25 (4.48)	24.00 (4.82)
Viswam	1.50 (1.18)	3.00 (1.65)	7.00 (2.30)	9.50 (2.82)	11.25 (3.01)	12.25 (3.21)	12.25 (3.20)	13.25 (3.35)	13.50 (3.43)	13.75 (3.46)	14.00 (3.49)
C.D (0.05)	0.49	NS	NS	NS	NS	NS	NS	NS	NS	NS	2.41

* Note: values given in parenthesis are square root transformed

EVALUATION AND CHARACTERISATION OF PROMISING HYBRIDS OF LONG PEPPER (*Piper longum* L.)

By

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ABSTRACT OF THE THESIS

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140

ABSTRACT

Piper longum L., commonly known as long pepper, belongs to family Piperaceae. The species has originated in South Asia. *Piper longum* is an important medicinal plant used in more than 300 ayurvedic preparations. Inspite of the importance of the species, 'Viswam' is the only variety released so far. As a part of a KSCSTE funded project, hybridization studies were carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara to develop high yielding types in *Piper longum*. In the preliminary evaluation trials, four hybrids were found promising. The present study entitled 'evaluation and characterisation of promising hybrids of long pepper (*Piper longum* L.)' was conducted with the objective of evaluating these promising hybrids at different shade levels for growth, yield and quality and also to characterise them using molecular markers. The research was conducted in three experiments viz., evaluation of hybrids in pots at different shade levels, field evaluation of selected hybrids and molecular characterisation of promising hybrids and parents.

Morphological characterisation of the accessions was done using IPGRI descriptor for *Piper nigrum* with necessary modifications. Variations were observed among accessions in shape of leaf, immature and mature spike color as well as shape of spike. Characters like plant height, number of primary branches per plant and time taken for production of first lateral were significantly different among hybrids. Flowering and fruit set were higher at zero per cent and 25 per cent shade compared to 50 per cent shade level.

Field planted genotypes of *P. longum* showed significant difference in number of primary branches, internodal length of orthotropic as well as plagiotropic shoots and leaf area. Characters like pedicel length, spike length, spike girth and yield in terms of number of spikes per plant, fresh weight of spikes per plant and dry spike yield per plant also differed significantly. Among the hybrids evaluated in the field, Pl 9 followed by Pl 63 were found to be promising. They were significantly higher yielders compared to other hybrids, female parent and Viswam. Essential oil content was found to be uniform (0.8 per cent) in all the accessions except Pl 141 (0.83 per cent). Pl 9 showed maximum oleoresin (15.2 per cent) and piperine (3.47 per cent) content than other genotypes.

For molecular characterisation using RAPD, 30 decamer primers were screened. From these ten best primers were selected. Six primers showed polymorphism between the male and female parents. The hybrids Pl 9 and Pl 63 were closely related with 92 per cent similarity. Pl 140 was found different from the rest of the three hybrids and it was grouped along with the parents. Among the accessions studied, Viswam showed highest variability from others.

Among the hybrids evaluated, Pl 9 and Pl 63 were found to be promising in terms of yield. Pl 9 was superior in quality. These hybrids could be further evaluated in multi-location trials to explore the possibility of releasing as high yielding hybrids in future.