EVOLVING SUPERIOR TYPES IN THIPPALI (*Piper longum* L.) UTILISING BISEXUAL VARIANTS

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KERALA, INDIA

2012

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(2010-12-110)

THESIS

Submitted in partial fulfilment of the

requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR - 680656

KERALA, INDIA

2012

DECLARATION

I, hereby declare that this thesis entitled **"Evolving superior types in thippali (*Piper longum* L.) utilising bisexual variants"** 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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Certified that this thesis, entitled **"Evolving superior types in thippali (*Piper longum* L.) utilising bisexual variants"** is a record of research work done independently by **Ms. Anjana Chandran** 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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CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Anjana Chandran (2010-12-110)** a candidate for the degree of **Master of Science in Horticulture** with major field in **Plantation Crops and Spices** agree that this thesis entitled **"Evolving superior types in thippali (*Piper longum* L.) utilising bisexual variants"** may be submitted by **Ms. Anjana Chandran,** in partial fulfillment of the requirement for the degree.

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ACKNOWLEDGEMENT

First and foremost I humbly bow my head before the ***Almighty God,*** *who blessed me with will power and courage to complete this endeavour successfully.*

It is great respect and devotion, I place on record my deep sense of gratitude and unforgettable indebtedness to my major advisor ***Dr. V. S. Sujatha,*** *Professor, Department of Plantation Crops and Spices, Chairperson of my Advisory Committee, for her sustained and valuable guidance, constructive suggestions, unfailing patience, constant support and encouragement during the conduct of my research work and preparation of thesis. I gratefully remember her knowledge and wisdom, which nurtured this research project in right direction without which this could have been a futile exercise. I really consider it my fortune in having her guidance for the thesis work.*

It is my privilege to oblige ***Dr. E.V. Nybe,*** *Professor and Head, Department of Plantation Crops and Spices and member of my Advisory Committee, for his ardent interest, valuable suggestions, critical scrutiny of the manuscript and ever willing help which has helped a lot for the refinement of this work.*

No words can truely represent my profound gratitude and indebtedness to ***Dr. M. Asha Sankar****, Professor, Department of Plantation Crops and Spices and member of my Advisory Committee for her expert counsel, invaluable guidance, untiring interest, patient hearing, constructive suggestions, esteemed advice and immense help rendered throughout the course of this investigation.*

I place a deep sense of obligation to ***Dr. R. Sujatha,*** *Associate Professor, Dept. of Plant Bio-technology, College of Agriculture, Padannakad and member of my Advisory Committee for her ever willing help, valuable suggestions, warm and friendly nature in rendering help during the study period.*

I specially thank ***Sri. S. Krishnan****, Associate Professor and Head, Department of Agricultural Statistics for his keen interest, valuable suggestions and immense help rendered in statistical analysis of the data.*

I also express my sincere thanks to ***Dr. N Mini Raj****, Professor,* ***Dr. Alice Kurian****, Professor,* ***Dr. P.V. Nalini****, Professor,* ***Dr. P.C. Rajendran****, Professor,* ***Dr. B. Suma****, Associate Professor, Department of Plantation Crops and Spices for their support during the period of study.*

I am greatly thankful to ***Dr. Betty Bastin****, Professor, STCR, Department of Soil Science and Agricultural Chemistry for providing facilities for the conduct of the study.*

I also express my sincere thanks to ***Dr. Haseena Bhaskar,*** *Associate Professor, Department of Agricultural Entomology,* ***Dr. Minimol J.S.****, Assistant Professor, KAU Cadbury Cocoa Research Project, Dr.* ***Madhu Subrahmanian,*** *Assistant Professor, Department of Agricultural Entomology,* ***Dr. Dijee Bastin,*** *Associate Professor, Department of Plant Breeding and Genetics, for their timely help and cooperation for the entire period of study.*

I am highly indebted to the Farm officers ***Mr. George****,* ***Mrs. Sulaja O. R.,*** ***Mr. Sunil A. Nair*** *and* ***Mr. Vineesh Kumar K.V.*** *and Research Associates* ***Amrutha****,* ***Divya****,* ***Deepa****,* ***Daya****,* ***Sindhu*** *and* ***Hima****. A special note of thanks is also to the labourers of Department of Plantation Crops and Spices for their help at various phases of my study.*

I am thankful to ***Dr. A. T. Francis****, Librarian, College of Horticulture,* ***Mrs. V. S. Swapna,*** *Technical Assistant,* ***Mr. E. M. Sineesh,*** ***Mrs. K.V. Ambika*** *and* ***Mrs. Jayanthi*** *and all other supporting staffs in the library, for their cooperation throughout the period of investigation.*

I wish to express my sincere gratitude to ***Mr. K. S. Aravind,*** *Administrator, Computer club, College of Horticulture, Vellanikkara for his help during the study.*

Words cannot really express the true friendship that I realized from ***Fahi****,* ***Binisha, Meera, Jimcy, Priya*** *and* ***Adheena,*** *for the heartfelt help, timely suggestions and back up which gave me enough strength to get through all mind numbing circumstances.*

I wish to place on record the help rendered to me by all my M.Sc. classmates especially ***Syama, Savitha, Smitha, Deepthi, Roshin, Jeethu, Athul, Negi, Swati, Pawan, Pramod, Yamuna*** *and* ***Punya.***

I also express my sincere thanks to all my dear juniors especially ***Tess, Devu, Anu, Shelly, Roshna, Airina, Uma, Asna, Chandhini*** *and* ***Tuttu.***

My heart never forget my dear seniors ***Anisu, Haju, Anulakshmi, Chandana, Amrutha, Jyothi, Seeshma, Jyothi Sara*** *and* ***Resmi Paul,*** *whose helping hands, love and affection helped me in my difficult times.*

Words fall short as I place on record my indebtedness to my friend ***Mr. Najeeb Naduthodi,*** *for his prompt help and cooperation for the entire period of study.*

I am extremely thankful to my juniors from ***Avalon '08, Kallisto '09, Aristaeus '10*** *and* ***Nakshathra '11*** *for their moral support and encouragement.*

The award of ***KAU Junior fellowship*** *is thankfully acknowledged.*

Above all, I am forever behold to ***my parents*** *and* ***brothers*** *to their love, affection, personal sacrifice, incessant inspiration and constant prayers which helped me to complete this venture successfully.*

A word of apology to those I have not mentioned in person and a note of thanks to one and all who helped in the successful completion of this endeavour.

Anjana Chandran

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Introduction

1. INTRODUCTION

Piper longum L. (Indian long pepper or thippali) is the third most important species of genus *Piper* after black pepper (*Piper nigrum*) and betel vine (*Piper betle*). Long pepper is indigenous to South Asia including India, Sri Lanka, Bhutan and Nepal. *Piper longum* L. is a dioecious plant species. Mature female spikes are highly valued for their use in the treatment of respiratory diseases in traditional and ayurvedic medicines in India. It is used in over 320 classical ayurvedic formulations like pippaliasavam, pippalichurnam, pippalimodaka, amritarishta, chandanasava etc. *Piper longum* is also used in many modern herbal formulations. Further, the use of the plant as a bioavailability enhancer has immensely increased its importance in the field of ayurveda.

Both male and female plants remain indistinguishable until the flowering stage, when male plants can be identified by their long spikes and female plants by their short spikes. Being a shade loving crop, thippali can be cultivated as intercrop in coconut and arecanut gardens and even in rubber plantations. However, due to low yield of spikes, the cultivation of *Piper longum* has not gained much popularity.

The only improved variety released is Viswam, which was evolved through selection, from a few collections available under AICRP on Medicinal and Aromatic plants in Kerala Agricultural University. After the release of Viswam in 1996, no new varieties could be evolved in the species.

A variant sex form (bisexual) of *Piper longum* has been reported by Sujatha and Nybe (2007). The variant type had spikes as long as male spikes, which were three to four times that of female spikes. The variant sex form

was utilized in hybridization programme under a KSCSTE funded project "Exploitation of bisexual variant in developing high yielding types of *Piper longum* L." during 2008-2011. The main objective of the programme was to develop high yielding types through hybridization.

As a part of the aforesaid project an M. Sc. research programme was taken up (Kanimozhi, 2010) in which, the floral biology of *Piper longum*, including the different sex forms was studied in detail. Technique of pollination was standardized and viable seeds were produced in *Piper longum* for the first time. Out of the 573 seeds obtained, 136 seeds germinated and seedlings were raised in pots and evaluated for growth characters.

The present study entitled "Evolving superior types in thippali (*Piper longum* L.) utilising bisexual variants" was taken up as a follow up of the earlier work with the objective of developing high yielding types in *Piper longum* and evaluation of seedling progeny for growth, yield and quality.

Review of Literature

2. REVIEW OF LITERATURE

Piper longum L. is an important medicinal plant belonging to the family Piperaceae, commonly known as "long pepper". Long pepper or thippali is the most extensively used medicinal plant in the ayurvedic system of medicine. It is used in over 320 classical compound medicinal formulations and in many modern herbal formulations (Ravindran and Balachandran, 2005). Economic part of long pepper is the dried mature spikes of female plant. Besides fruits, the roots and thicker parts of stem of male plant (Pipalamool) are cut and dried and used as an important drug in the Ayurvedic and Unani systems (Ali *et al.*, 2007).

Studies on different aspects of *Piper longum*, including origin and distribution, cytogenetics, morphological and reproductive characters, use of growth regulators in regulation of sex expression, crop improvement, crop management and crop protection, quality aspects and medicinal properties are reviewed in the chapter.

2.1 Origin and distribution

Piper longum is indigenous to South Asian countries such as India, Sri Lanka, Bhutan and Nepal. It also occurs in Malaysia, Indonesia and Philippines. Kirtikar and Basu (1935) reported the distribution of *Piper longum* in hotter provinces of India, Ceylon and Malay Islands.

Krishnamurthy (1969) described the occurrence of *Piper longum* in the hotter parts of India, from Central Himalayas to Assam, Khasi and Mikir hills, lower hills of Bengal and evergreen forests of Western Ghats from Konkon to Travancore and has been recorded from Nicobar Islands.

In India, it is distributed widely in the low altitude evergreen forests occurring in sub Himalayan hills, Assam, Khasi regions, Eastern Ghats and Western Ghats and in the low elevation forest lands. It is reported that from India, it has

reached the rest of Asia and Mediterranean through the "spice route" and it was used as a spice in all these regions (Ravindran and Balachandran, 2005).

Long pepper is cultivated for its fruits and roots in the tribal belts of Andhra Pradesh, parts of Orissa, North West Bengal, Assam and in North Eastern states. In ancient India, it was mainly used as a source of pungency, used in flavouring various beverages and dishes. The cultivation of long pepper in Kerala was reported by Ravindran and Balachandran (2005).

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

2.2 **Cytology**

Investigations on cytological aspects of *Piper longum* done by various workers showed wide variations in chromosome number. Somatic chromosome number reported were 2n= 24 (Tjio, 1948); 2n=44 (Sampathkumar and Navaneethan, 1981); 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 Bhattacharaya, 1959).

Cyto- morphological investigations in *Piper* species were done by Anand (1997). The procedure for mitotic studies in *Piper* spp. was standardized. The somatic chromosome number of *Piper longum* was observed as 2n=32, which is a new count in this crop not reported by earlier workers.

2.3 Variation and characterization

Polymorphism in the genomic DNA of 25 female and six male plants of *Piper longum* was analyzed by Banerjee *et al*. (1999), by RAPD, using 40 decamer random oligonucleotide primers. Two RAPD bands consistently appeared only in the plants showing male genotype, suggesting thereby the male-associated nature of

these DNA markers in dioecious *Piper longum*. This was the first report of genetic or chromosomal basis of dioecy in long pepper.

The study utilized Random Amplified Polymorphic DNA for the evaluation of relatedness among 6 *Piper* species. Based on the number of bands, all the species were grouped into 3 clusters and the dendrogram revealed maximum similarity between *P. betel* and *P. longum* and also in between *P. nigrum* and *P. mullesua* species, altogether forming one cluster (Chikkaswamy *et al.*, 2007).

Sen *et al.* (2010) conducting studies using Random Amplified Polymorphic DNA, found high genetic variations among different *Piper* species. The results of the study helped in germplasm identification, management and conservation.

Philip *et al.* (2000) reported genotypic and morpho-genetic differences among three male varieties of *Piper longum*, one variety each from Assam and Calicut and one released variety Viswam, which were investigated for the development of a common and efficient method of plant regeneration. RAPD analysis, using oligo nucleotide primers revealed that these varieties were genetically different. Compared to the Assam variety, Viswam and Calicut varieties are genetically closer (95 % similarity) among themselves. Morpho genetic potential of node, internode and leaf explants from all the three varieties were compared. Leaf explants from different varieties exhibited maximum regeneration potential. An efficient protocol was developed for regeneration from leaf calli of all the three genotypically different varieties. Callus regenerated plants from leaf explants were subsequently rooted, hardened and established on soil under natural conditions of growth.

Manoj *et al.* (2005) described two male associated RAPD markers in *Piper longum*. RAPD profiles of the male samples were distinctly different from those of the females because of the presence of prominent male-associated bands $OPA10_{827}$ and $OPA15_{744}$.They sequenced male sex-associated RAPD bands for the development of male sex-associated SCAR markers. They converted RAPD marker

$OPA10_{827}$ into a SCAR marker. The SCAR developed based on the DNA sequence of the other RAPD marker, was found to be present in some 20 percent of the female population of *Piper longum*. Based on this, and Southern hybridization experiments, it was understood that there was considerable homology between the chromosomes of male and female genotypes from which RAPD markers were derived. The sequences showed detectable differences between the male and female plants of dioecious *Piper longum*.

Jaleel (2006) carried out characterization of long pepper using morphological, anatomical and molecular markers in six female and two male accessions. The accessions used in the study showed variation in the morphology of vegetative and reproductive branches, in leaf size, leaf shape and l/b ratio. Vegetative branches had large cordate leaves with petioles. The l/b ratios of leaves of reproductive branches were higher compared to leaves of vegetative branch.

The spikes of females were short, bold and greenish black on maturity whereas it was long, slender and yellow in the case of males. At early stages of spike growth, female spikes were creamy yellow and male, green in colour. Anatomical differences were found in the case of number of medullary and cortical bundles and the presence or absence of mucilage canals. The physiological parameters revealed higher photosynthetic rate for the female accessions, Viswam. RAPD analysis done in eight accessions revealed 15-40% variability among the accessions at the molecular level and the primer OPF5 produced male specific bands.

Keshavachandran *et al.* (2007) reported finger printing of *Piper longum* cultivars. Eleven land races and one advanced cultivar of long pepper was used in the study. Of these, ten primers that yielded clear and dominant band patterns were selected for the final analysis of 11 accessions. Cultivar specific single bands were obtained for a few land races and accessions of *Piper longum*.

2.4 Isoenzyme analysis

Isozyme studies (Sebastian, 1995) revealed the distinctness of *Piper longum* and *Piper betle* from rest of the *Piper* species and they stood individually having only low similarity index with others. Pooled analysis of similarity indices have shown that out of 11 species studied, nine could be grouped into three groups. *Piper nigrum*, *P. pesudonigrum*, *P. bababudani* and *P. galeatum* formed first group. *P. argyrophyllum* and *P. attenuatum* formed secong group and third group with *P. chaba, P. hapnium* and *P. colubrinum*. The selection of ideal parts for analysis was also done based on observations of isoenzyme banding pattern of stem, root and leaf.

2.5 Morphological chatacters

2.5.1 Stem characters

Ravindran and Balachandran (2005) reported that long pepper produced distinct dimorphic branches, those main branches creeping on the ground were called orthotropes and those axillary branches which grew erect were called plagiotropes. The former shoots were vegetative and grew by the activity of the terminal bud, while the short axillary branches are fruiting branches, produces spikes opposite to the leaves and the growth is sympodial.

Kumar (1998) reported that stems of long pepper were numerous, ascending, cylindrical or globose. Stem is swollen and irregularly knotty with each piece quarter inch long, irregularly thick, hard and of a brownish colour. Branches were erect, prostrate or creeping, soft and grooved when dry. The whole stem was finely pubescent.

Manuel (1994) conducted a comparative evaluation of selected types of *Piper longum* in coconut plantation and reported significant variability in stem characters like the length of longest stem, number of stem/ hill, number of vegetative branches / stem and these characters influenced the dry spike yield in *Piper longum*. Inter nodal length of main stem, number of spike bearing branches per stem and angle of

insertion of spike bearing branches significantly varied in different accessions in *Piper longum*.

Jaleel (2006) studied eight accessions of *Piper longum viz.*, Assam, Kanjur, Maharashtra, NL- 84-68, Viswam, Pattambi, Nilambur, Odakkali and reported that length of spike bearing branch and internodal length of different accessions differed significantly. At the same time, angle of insertion of reproductive branches of different accessions did not differ significantly.

2.5.2 Leaf characters

Leaves of *Piper longum* were numerous ranging in size from 6.0-9.0 cm, lower leaves are broadly ovate and vary with cordate with broad rounded lobes at base (Kirtikar and Basu, 1935). Upper leaves are oblong-oval, cordate at base. All leaves are sub-acute, entire, glabrous, thin, bullate with reticulate venation, sunk above and raised below, dark green and shiny above and dull and pale beneath, petiole of lower leaves range in length from 5-7.5 cm and are stout, the petiole of upper leaves are very short or without petioles, stipules are mebraneous, lanceolate, obtuse, soon falling, having a size of about 1.3 cm.

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

Kumar (1998) observed that leaves in long pepper were numerous, simple, stipulate and petiolate or sessile according to their position on the plant. Leaf blade varies in shape in the same plant. Upper leaves are generally sessile, amplexicaul or stem creeping, ovate or ovate oblong, acute and most often unequally sided or unequally cordate at base. It was also reported that leaves were 6.5 -9 cm long and 3-5 cm wide. Lower leaves were broadly ovate, pale dull beneath; cordate at base. Petioles of lower leaves were 0.5-7.5 cm long and stout but that of upper one was

very short and absent. Stipules were about 1-3 cm, membranous, lanceolate, obtuse and falling soon.

2.5.3 Inflorescence, flowers and spikes

Pursglove *et al.* (1981) reported that in black pepper, the pendant spikes were borne opposite to the leaves on plagiotropic branches and was 3-5 cm long, bearing 5-150 minute flowers, borne in the axils of ovate fleshy bracts. The flowers may be unisexual with monoecious or dioecious or hermaphrodite but most of the cultivated types were bisexual. It is reported that the cultivars exhibited great variability in the percentage of bisexual or productive flowers on their spikes.

Higher the percentage of bisexual flowers, greater will be the productivity and most of the high yielding and popular cultivars produced as much as 70-98 % bisexual flowers. He also added that under intense shade conditions the bisexual types produced more female flowers and less hermaphrodite flowers.

Kumar (1998) reported that *Piper longum* produced spikate inflorescence, whose flowers were sessile, usually unbranched, elongated, simple and intermediate. Flowers were without perianth, very densely packed and male and female parts were on separate plants. Spikes were 5 cm long, cylindrical, solitary, pendunculate and upright. Flowers were unisexual, minute and sessile. Male spikes were large, narrow and slender, bracts narrow, 1-3 inch long, peltate, stamens two in number. Female spikes were 1.3-2.5 cm long and 4-5 mm in diameter, bracts circulate, flat and peltate, stigma 3-4, very short and persisting. Flowering season was July-August.

The male and female plants are morphologically very similar till the formation of spikes (Manoj *et al.*, 2004).

Ravindran and Balachandran (2005) found that long pepper plant was dioecious, male and female plants were separate. Flowers were arranged on a short cylindrical spike, male spikes were very much longer than the female spikes. Female

flowers were short, stout, flowers fused laterally. Female flowers consist of one ovary only that arises from the axil of the bract.

Kumar (1998) reported that fruits in *Piper longum* were short, consists of multitude of minute buccate fruits closely packed among a common axis, whole forming a spike, one and half inch length and quarter inch thickness, fruits are ovoid, crowned with stigma and arranged with small peltate bracts beneath each. When ripe, fruits were grayish green and nearly blackish and particularly sunk in fleshy axils. Fruiting season was November to March. Seeds were globose, testa thin with in the hardened periphery.

Ravindran and Balachandran (2005) reported that fruits of *Piper longum* were small, closely packed. Fruiting was apomictic and fruits were produced without pollination. Hence male plants were not required for fruit production. They also reported that fruits of *Piper chaba* were larger, more conical and not cylindrical as in *Piper longum*, become orange red on ripening and were more pungent with less flavor. Spikes of *Piper peepuloids* looked similar to *Piper longum*.

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

Female spikes of *Piper longum* were very small (2.0-3.0 cm) compared to male (6.0- 7.5 cm). A bisexual variant (Acc. P 25) was identified with spikes as long as male spikes (Sujatha and Nybe, 2007).

The spikes were cylindrical and erect in all the three sex forms. However, the colour of female spike was different from that of the male and bisexual types. In female type, the spikes were creamy white until fruit set, then colour changed to green. In male and bisexual types, immature spikes were dark green which turned to light yellow and further to dull yellow on maturity (Kanimozhi, 2010).The female spike attained full length (2.31 cm) in around 22 days, where as male type attained full length in 43 days (7.76 cm) and bisexual type in 46 days (6.35 cm).

The flowers were represented by ovary and stigmatic lobe subtended by bract in female. The male flowers were represented by anthers covered by peltate bract. Male, female and bisexual types did not possess perianth parts. The number of stigmatic lobes varied from 2 to 6 in bisexual flowers, two was the prominent number in the bisexual type (90 %). In the female type, four stigmatic lobes were common (74 %) followed by three lobes (20 %)(Kanimozhi, 2010).

2.6 Reproductive biology of *Piper longum*

Sujatha and Nybe (2007) reported anther dehiscence in the bisexual variant to be between 9.00 am to 11.00 am which extended further in cloudy weather. Pollen fertility was maximum at 9.30 am (42.54 per cent).

Spikes of *Piper longum* were cylindrical and creamy white in female. In male and bisexual types, immature spikes were green, changing to dull yellow on maturity. Time taken for attaining full length of spike was 22 days in female, 43 days in male and 46 days in bisexual types. Time taken for complete opening of flowers in an inflorescence was uniformly one week in all sex types (Kanimozhi, 2010).

Anthesis and anther dehiscence were between 7.30 am and 4.30 pm with a peak between 10.30 am to 12.30 pm (Kanimozhi, 2010). Number of days taken for complete opening of flowers in a spike was seven days in male, female and bisexual types. Maximum flower opening was on 3rd and 4th day in the male type. In female and bisexual types, opening of flowers was noticed from second and sixth day. Complete opening of flowers in an inflorescence took seven days in male, female and bisexual types. Complete dehiscence of anthers took one week in male and bisexual inflorescences.

2.7 Pollen fertility

Pollen fertility was observed in one percent acetocarmine. Stained pollen grains were maximum at 9.30 am (42.54 per cent) pollen viability was studied in six

different media and eight concentrations of sucrose. Viability could not be obtained in the different media tried (Kanimozhi, 2010).

2.8 Hybridization

In long pepper, Sujatha and Nybe (2007) reported a new sex form (bisexual) having spikes as long as male spikes. Controlled pollinations were attempted using bisexual type as female parent as well as male parent. Using the bisexual type as female parent, both selfing and crossing were attempted. In crosses, only a few spikes were set but none was carried to maturity. This could be either due to lack of seed set in the bisexual type or may be due to lack of sufficient number of bisexual flowers in the spikes to promote fruit development and fruit maturity (Kanimozhi, 2010).

Using bisexual type as male parent and female type as female parent crosses were done. Three methods of pollination were tried *viz*., dry, water and boric acid methods. The pollination done from February to September indicated that maximum spike set was in boric acid method in September followed by June and dry method in September. Artificially pollinated fruits were longer, plumpy and heavier as compared to natural spike set. However, maximum number of seeds were obtained under dry method of pollination in September (554). In boric acid (100 mg l^{-1}) method, eventhough the spike set was better, only 13 seeds were obtained. In wet method, four seeds were obtained in crosses made during September. The well developed seeds were bigger in size with hard seed coat. Undeveloped seeds were seen as black specks (Kanimozhi, 2010).

2.9 Seed germination studies in *Piper* spp.

The chemical composition of seeds is genetically defined, although it may, to some extent, be influenced by environmental conditions to which the plants were submitted (Fait *et al*., 2006).

Effects of temperature, light and different concentrations of plant growth regulators on germination of *Piper nigrum* L. seeds was studied by Li *et al.* (2010). The seeds generally germinated within six or seven days. There was no difference in germination percentage between dark and light treatments, but the development of radical length was significantly influenced by both light and temperature. Germination was highest at 30°C, but seeds also germinated at 25 and 35°C. The results are consistent with the *Piper nigrum* L.

In another work, Dousseau *et al.* (2011) attempted to characterize the germination process of *Piper aduncum* L., as well as to verify the influence of the interaction between presence and absence of light and temperature.

Piper longum seeds took 21 to 69 days for germination in sand. The growth of seedlings was very slow. Cotyledonary leaves opened in 2 to 31 days. The first true leaf opened in 2 to 41 days. The germination percentage was also low in *Piper longum* (Kanimozhi, 2010).

2.10 Use of growth regulators in sex expression and sex regulation

Number of berries / unit length of spike, berry volume and berry weight were increased and spike shedding decreased by 52.2% with the application of 50 ppm planofix in black pepper (Geetha and Nair, 1990). They also observed increased oleoresin content of berries /spike and five times higher yield in pepper with application of planofix (20, 40 and 60 ppm).

Karuna *et al.* (2007) reported that NAA at 50 ppm resulted in increased fruit set per panicle (54.83) and fruits with higher TSS (22.46) in mango cv. Langra.

In musk melon, application of benzyl adenine was useful for increasing fruit set and size of pericarp tissue enlarging cucumber fruits were accompanied with an increase in the levels of endogenous auxin (Jones, 1965). The effect of BA applied at 100 mg/l, two week after full bloom improved fruit size in two varieties of pear viz., Spadona and Coscia (Stern and Flaishman, 2003).

Pan and Xu (2011) conducted a study to determine the effects of 6-benzyladenine (BA) on floral development and floral sex determination of *Jatropha curcas*. Exogenous application of BA significantly increased the total number of flowers per inflorescence, reaching a 3.6 fold increase (from 215 to 784) at 160 mg/l of BA. BA treatments induced bisexual flowers, consequently a 4.5 fold increase in fruit number and a 3.3 fold increase in final seed yield were observed in inflorescences treated with 160 mg/l of BA, which resulted from the greater number of female flowers and the newly induced bisexual flowers in BA-treated inflorescences.

Effect of growth regulators in developing fully bisexual inflorescence in bisexual type I indicated that NAA 25 mg l^{-1}, GA 5, 10 and 50 mg l^{-1}, BA 100 mg l^{-1}, 500 mg l^{-1} and B 3 mg l^{-1} were more stable in inducing fully bisexual spikes. The fully bisexual inflorescence turned green and was retained on the plant for 40 to 50 days as compared to 7 to 10 days in unsprayed control. However, parthenocarpic development of spikes were induced in less number of sprays in NAA 25 mg l^{-1} followed by GA_3 50 mg l^{-1} and BA 500 mg l^{-1} and 100 mg l^{-1} and Boron 3 mg l^{-1}(Kanimozhi, 2010).

In bisexual type II, stable bisexual flowers were produced by the treatments Boron 3 mg l^{-1} followed by NAA 25 mg l^{-1}. But parthenocarpic development of spikes were induced in NAA 25 mg l^{-1}, NAA 50 mg l^{-1}, NAA 100 mg l^{-1} and BA 500 mg l^{-1} (Sujatha, 2011).

2.11 Cultivation practices

2.11.1 Nursery management

P. longum is usually propagated by stem cuttings comprising 3-4 nodes and cuttings planted during June exhibited approximately 70% rooting, while those planted in February recorded a rooting percentage of nearly zero. Cuttings with leaves recorded significantly higher values for rooting percentage (78.33%), number

of roots (11.70), root length (13.59) and vine length(22.47 cm) than cuttings without leaves (Bhuse *et al.*, 2002). Among the plant growth regulators (IBA and NAA at 100, 200 and 300 ppm), IBA 100 ppm recorded the highest rooting percentage (88.33%), number of roots (13.60), root length (15.05 cm) and vine length (24.38 cm). Based on the treatment interactions, cuttings with leaves treated with IBA at 100 ppm exhibited the highest rooting percentage (96.66%) and number of roots (15.44).

The performance of vegetative stem cuttings was studied by Etampawala *et al.* (2002) comprising the two uppermost nodes and cuttings obtained from vertically growing reproductive parts of *Piper longum*, which showed the appropriate types for propagation. Long pepper plants grown under 50 per cent shade performed well, compared to plants grown under 25 and 75 per cent shade, respectively. Planting medium comprising sand, top soil and farm yard manure in the ratio of 1:1:1 was found as the best substratum for the growth of long pepper. Early fruit production was observed in vertically grown reproductive branches compared to that in horizontally grown vegetative branches of *P. longum*. However, nearly 50 per cent of fruits were shed from the mother plant in about 22 days after their emergence.

Reghuvaran *et al.* (2010) conducted a study on coir pith biodegradation with white rot fungus and nitrogen fixing bacteria for cultivation of medicinal plants (*Phyllanthus amaraus, Andrographis paniculata, Bacopa monneiri, Piper longum*). Proportion (25% garden soil and75% compost) yielded an effective growth of all medicinal plants. Thus, composted coir pith with nitrogen fixing bacteria is an effective potting medium for cultivation of medicinal plants.

The effect of inoculation of different AM fungi on growth performance of *Piper longum* plants was evaluated by Gogoi and Singh (2011). It showed a positive effect of AM inoculation on different growth parameters viz. root and shoot length, fresh and dry weight of root and shoot, total biomass, chlorophyll content and

percent root colonization of *Piper longum* plants. In most cases, increase in shoot length and biomass was significant due to the inoculation of *Glomus fasciculatum* followed by other 5 inoculants viz, *G. versiforme ,Glomus sp., G. mosseae, G. geosporum* and *G. etunicatum*, respectively.

2.11.2 Field planting

According to Viswanathan (1993), about 25-50% shade is required for growing long pepper. Field experiments revealed that a spacing of 60 x 60 cm is ideal for growing long pepper in coconut gardens.

Vegetative, reproductive and bio chemical characters were compared with the characters of the released variety, Viswam and the accessions, which performed on par with Viswam, were identified (Joseph, 2008). Correlations of the various vegetative and reproductive characters were worked out with dry spike yield and significant positive correlations were observed in nine characters like number of vegetative branches /stem, number of spike bearing branches/ stem, total number of leaves per hill, number of spikes per spike bearing branch, length of spike, girth of spike, fresh weight of spike, dry weight of spike and fresh yield per plant.

Etampawala *et al.* (2002) studied the performance of Piper *longum* under different shade levels. Long pepper plants grown under 50 percent shade (maximum instantaneous light intensity 850µmol/m/s) performed well, compared to plants grown under 25 and 75 per cent shade, respectively.

2.11.3 Pruning and training

Attempts were made to develop suitable cultural practices for obtaining higher yields in three locally available selections (Selections 1, 2, and 3) in *Piper longum* (Pathiratna *et al.*, 2005). The effects of plant to pruning and training methods, shade and type of cutting on the production of reproductive branches and

spikes were studied. Pruning of runners and training methods were found important for the increased production of reproductive branches and spikes.

Pruning of runners in Selection 1 produced more reproductive branches and spikes. Restriction of growth of runners by pruning them at a distance of 40 cm from the base of the mother plant induced the formation of more number of reproductive branches and spikes in Selection 2. Training of runners to erect supports to encourage the production of reproductive branches was very successful in Selection 3. A shade level of around 50 per cent under field conditions, gave good growth and highest spike yield in all three selections. Cuttings from reproductive branches of Selection 3 kept on producing only reproductive branches during a period of one year under observation.

2.11.4 Nutrient management

Thippali needs heavy manuring for luxurious growth. In soils with low fertility, growth of the plant is very poor. Since the crop will give economic yield for three years, manuring has to be done each year. During the first year, organic manure can be applied in pits at the time of field planting. In subsequent years, manuring is done by spreading it in beds and covering with soil. Application of organic manure increases the water holding capacity of the beds. Viswanathan (1995) suggested the need for application of 15-25 tones of FYM for growing long pepper.

After analyzing growth and yield performance of *Piper longum* during initial 11/2 years in coconut garden, Sheela (1996) reported that 20 t/ ha of organic manure is optimum.

Increased yield of *Piper longum* through supplementation by urea was reported by Pande *et al.* (1995). Growth and yield of long pepper during first year of growth when grown as an inter crop in coconut garden was found to be higher with application of fertilizer @ 30:30:60 NPK kg/ha (Sheela, 1996).

Ayisha (1997) reported that growth and yield characteristics of *Piper longum* increased with the application of 20 t ha^{-1} organic manure and 30:30:60 NPK kg ha^{-1}. The optimum spacing was found to be 60 x 60 cm. The growth and yield of the crop was poor in dry months and peak yield was obtained at 17 MAP. After that there was a general decline. Two peak bearing stages were identified as July-August and October –November months. The NPK uptake was higher in plots which received 20 t ha^{-1} organic manure and 30:30:60 NPK kg ha^{-1}, under a spacing of 60 x 60 cm. Economic analysis of thippali cultivation also revealed that, it could be a profitable intercrop in coconut gardens with the above recommendations.

2.11.5 Intergrated nutrient management

Krishnan (2003) reported that integrated nutrient management system involving incorporation of vermi-compost @ 6.26 t/ ha/yr and combined application of bio inoculants viz, *Azospirillum,* fluorescent pseudomonas and AMF was found favorable for enhancing both total fresh and dry spike yield and total alkaloid production in long pepper under partial shade.

Field experiments conducted by Anilkumar *et al.* (2009) observed that integrated nutrient management system involving incorporation of vermicompost @ 6.25 t ha^{-1} $year^{-1}$, addition of NPK @ 30:30:60 kg ha^{-1} $year^{-1}$ and combined inoculation of bioinoculants, viz, Azospirillum (AZ) + Fluorescent pseudomonas (FP)+Arbuscular Mycorrhizal Fungi (AMF) was found favourable for enhancing both total fresh and dry spike yield and total alkaloid production for intercropped long pepper in coconut garden

From the integrated nutrient management studies carried out by Rao *et al.* (2010) in thippali, it was revealed that dry spike yields significantly increased due to integrated management of FYM and fertilizers. Application of 40 t/ha FYM and 125: 50: 160 Kg N, P_2O_5 and K_2O per ha gave significantly higher spike yield (2412 kg/ ha) and in turn increased the piperine yield (32.3 kg/ha). The higher total dry matter

with 40 t/ha FYM was positively correlated with higher plant height, more number of branches and maximum plant spread of 2560 cm^2. Further growth, yield and quality attributes were also significantly higher with this combination.

Studies conducted by Ilyas (1976) showed that yield of *Piper longum* increased from 560 kg in first year to 1680 kg in third year. After this vine should be replaced. If it is grown for its roots, first harvest could be done 18 months after planting.

Jaleel (2006) conducted a study to characterize the morpho- physiological attributes that favour the productivity of *Piper longum*. There was a slight decline in the yield of oil, when spikes were harvested after 75 days. The piperine content of all the accessions was maximum when spikes were harvested at full maturity *ie.,* 60-70 days of their formation. Assam clones recorded maximum piperine content of 1.97 % when it was harvested after 25-30 days followed by Viswam (1.09%). After 75 days, the piperine content of the spikes declined slightly, but the decline showed the same trend as that of spikes at maturity.

The essential oil of *Piper longum* spikes was found to be greenish yellow liquid with an yield of 0.6 %. Caryophyllene was found as the major constituent of the oil. A sesquiterpene hydrocarbon has been also isolated (Nigan and Radhakrishnan, 1986).

2.11.6 Irrigation

Not much irrigation is required for long pepper, but at the commencement of hot season the roots should be carefully covered with straw. According to Viswanathan (1995), *Piper longum* could be irrigated once or twice a week during the hotter parts of year in Kerala starting from January. Under intercropping situation, the main crop is irrigated, no additional irrigation is necessary. Mulching should be done in un-irrigated crops.

2.11.7 Plant protection

Leaf spot in long pepper was first identified at RARS, Pilicode. The causal organism was *Colletotrichum gloeosporioides*. Symptoms were mainly confined to the leaves. Infection on the leaves was generally manifested as discoloured areas at the tip or occasionally near the margin. In severe cases of infection, the leaves were shrivelled and dried (Sathyarajan and Naseema, 1985).

Chourasia and Roy (1989) reported the influence of relative humidity on fungal association and aflatoxin production in *Piper longum* fruits. They found that the level of aflatoxin B1 production was higher when the relative humidity was between 75 - 76%. Higher the fungal incidence, greater will be the level of aflatoxin production in stored samples.

Roy and Chourasia (1990) studied the effect of tempertature on aflatoxin B1 production by *Aspergillus flavus* on *P. longum* fruits. The highest level of Afl-B1 (1.25 µg/g) production was at 30 ^{0}C after 3 weeks of incubation. At 20, 25, 35 and 40 ^{0}C, aflatoxin levels ranged from 0.22 to1.00 µg/g. However, at 15 ^{0}C aflatoxin production was much lower (0.12-0.24 µg/g).

Occurrence of *Helopeltis theovora* as a pest of *Piper longum* was reported by Abraharm (1991). He found that spraying 2% neem kernel suspension reduced damage by 70%.

Bhat *et al.* (2004) observed a virus causing mosaic mottling in Indian long pepper, which was isolated and identified as an isolate of cucumber mosaic virus (CMV) based on morphological, physio-chemical and serological properties. The virus was purified from tobacco and negatively stained purified preparations contained isometric particles of about 28 nm in diameter. Particle morphology, antigenic relationships and molecular weight of coat protein suggested that the virus is associated with Indian long pepper.

Seena (2006) first reported *Meloidogyne arenaria* of thippali from Kerala. Among the various treatments studied for the management of root knot nematode,

the application of bio agents viz. *Bacillus subtilis, Trichoderma viridae, Pseudomonas fluorescens* and AMF improved the growth of thippali with maximum vine length, number of leaves, number of branches, root length, root and shoot weight, gall formation and nematode population in root and soil. Early spike formation and also an increase in no. of spike were observed in plants treated with *Bacillus subtilis* and *Pseudomonas fluorescens* respectively. Study clearly indicated that root knot population could be effectively managed using bioagents and was a better alternative to nematicide application.

Poornima (2007) reported that the cross inoculation studies conducted using *Colletotrichum gloeosporioides* isolates of thippali, black pepper and betel vine-showed that thippali isolate was highly host specific. The colony and conidial morphology of the three isolates also showed considerable differences. *Colletotrichum gloeosporioides* of thippali had very small conidia and the culture was also found to be shy sprouting. Of the eleven fungal and four bacterial isolates tested against *Colletotrichum gloeosporioides*, *Trichoderma viridae* and *A. terreus* were found to be most effective under *in vitro* condition. Among the different resistance inducers tested *in vitro*, salicylic acid (1g/l) was selected for field evaluation, as it had no action on the pathogen. Of the two plant based chemicals tested, Ovis reported the higher suppression of the pathogen. Among the different eco friendly materials tested in the field, *A. terrus* + neemcake was found to be best in disease suppression at 45 DAT.

Efficacy of fungicides and bioagents on *Colletotrichum gloeosporioides* causing blight in *Piper longum* were studied by Wathore *et al*. (2010). Among the chemicals, mancozeb + carbendazim was found most effective to inhibit 96.26 per cent growth followed by carbendazim, mancozeb and copper oxy chloride. Amongst bioagents, *Trichoderma viride* was found effective to inhibit the growth of pathogen upto 70.42 per cent. In a field experiment, spraying of mancozeb + carbendazim (@ 0.2%) was found effective in reducing disease intensity (18.70%) with 33.38 per

cent disease control followed by carbendazim (@ 0.1%), copper oxychloride (@ 0.3%) and *Trichoderma viride* with 20.08 % disease intensity having 30.95, 30.10 and 28.46 per cent disease control.

2.11.8 Harvesting and yield

Trials conducted by Davies (1992) showed that the vegetatively propagated crop established well within six months. The first harvest could be made eight months, after planting. During second year, two harvests could be taken. The crop grown in irrigated coconut gardens showed excellent performance, yielding 500 kg dried spikes/ ha during the first year, 750 kg during second year and 1000 kg during third year.

Piper longum vines started bearing spikes six months after planting. Three to four pickings were made as and when spikes attained maturity for harvest. The yield of dry spikes during first year was around 400 kg / ha and increased upto 1000 kg in third year, after which the vines become less productive and had to be replanted (Viswanathan, 1995).

2.11.9 Drying

Drying is one of the most critical and fundamental unit operations in the post harvest processing of medicinal plants. Generally, drying at a temperature of 50º C was found optimum, since quality reduction due to discolouration occurs at higher temperature (Muller and Heindl, 2006).

In herbs of lemon balm (*Melissa officinalis*), fresh herbs had the highest essential oil content followed by shade dried, oven dried and sun dried herbs respectively. Drying methods had no effect on the number of chemical components of the essential oil (Khalid *et al.*, 2008).

2.12 Medicinal properties

In *Ayurvedic* medicine, the herb is said to be a good rejuvenator and helps to improve vitality; and is employed as a tonic to stimulate appetite. It is also being used against tumors, indigestion, epilepsy, flatulence, gout, laryngitis etc. This herb has nerve depressant and antagonistic effects on muscular spasms; and when applied topically, it soothes and relieves muscular pains and inflammation. The oil extracted has antibacterial and anthelminthic properties.

Indian long pepper is an exceptionally important medicinal plant with anti- HIV constituents (Hareesh *et al*., 2006). The roots and fruits of the plant are used as an antidote to snake bite, scorpion stings, chronic bronchitis, cough and cold. The ripe fruits are used as an alternative to tonic (Chahal *et al.,* 2011).

Trikatu is the most popular formulation containing *Piper longum* which is used to mitigate the diseases due to kapha dosha, to digest amino acids and also it increases the bioavailability of the drug, when it is used as a complementary medicine. The extract of trikatu churna shows highest activity which is almost equal to the effects shown by standard ampicillin solution (Malvankar and Abhyankar, 2012).

Piperlonguminine from *Piper longum* is a potential novel therapeutic agent for Alzheimer's disease. Extracellular deposits of Aβ in senile plaques of the cerebral cortex are known to be hallmarks of Alzheimer's disease. In India, thippali is used mainly in the preparation of pickles and health stimulants like '*chawanprash*' and also for imparting flavour and pungency to beverages.

2.13 Quality parameters

Chopra *et al*. (1999) reported that *Piper longum* contains essential oil consisting of long chain hydrocarbon, mono and sesquiterpenes, caryophyllene being the main product.

The phytoconstitutes of *P. longum* fruits include volatile oil, other minor alkaloids such as piplartin, piperlogumine, piperidine, starch, resin and pungent alkaloid piperin (Khare, 2006). Piperine is the main therapeutically active constituent of this plant.

Long pepper on steam distillation yield 0.7- 1.5 % light green viscous essential oil with spicy odour (Ravindran and Balachandran, 2005). Among various chromatographic methods including HPTLC (Suthar *et al*., 2003), HPLC was reported to be best for the quantification of piperine.

The volatile constituents and microbiological studies on *Kaempheria galanga, Hibiscus abelmoschus*, and *Piper longum* revealed the presence of over 15 components which were further identified by GC-MS of the volatile oil of *Piper longum*. The variations in the piperine content with maturity were also monitored. Daniel (1991) reported the presence of piperine on the TLC plate, when methanol extract of finely powdered black pepper was run in solvent system toluene-ethyl acetate (70:30). The developed chromatograms were seen in UV 360 nm and the major spot was fluorescing blue with Rf value 0.4 and reported the same as piperine. Two more spots were observed below piperine which is dipiperine and piperettine.

Chemo taxonomical study of South Indian taxa of *Piper* was carried out by Ravindran and Babu (1994). Fourteen taxa were analyzed for their flavanoids and based on the presence or absence of these compounds, percentage similarity indices were calculated. A chemical dichotomy was evident between the two sub genera Pipali (having erect spikes) and Muricha (having pendant spikes), thereby supporting the validity of the sectional classification.

Manuel (1994) studied the total alkaloid content of five types of *Piper longum* and recorded maximum percentage of alkaloid for Panniyur (2.91%)

followed by Cheemathippali (2.87%), Pattambi (2.85%) and Kaanjur(2.85%). The minimum alkaloid content of 2.80 % was recorded in Mala.

Forty-five compounds were separated from *Piper longum* L. and identified. The main components were b-caryophyllene (33.44%), 3-carene (7.58%), eugenol (7.39%), D-limonene (6.70%), zingiberene (6.68%) and cubenol (3.64%). Piperine is the major and active constituent and piperine content is 3-5% (on dry weight basis) in *P. longum* (Zaveri *et al.*, 2010).

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

The chemical composition of the essential oil of *Piper chaba* was analyzed by the GC-MS. Fifty four compounds representing 95.4% of the total oil were identified, of which caryophyllene oxide, veridiflorol, globulol , selinene, linalool , 3-pentanol, tricyclene and p-cymene were the major compounds. The oil and organic extracts revealed a great potential with anti-listerial effect (Rahman *et al.*, 2011).

Piperine, a characterizing compound present in fruits of *Piper nigrum* and *Piper longum* is used as bioavailability enhancer. An ingredient of antioxidant, anti-inflammatory activity has been extracted using soxhlet and supercritical fluid extraction technique. It was isolated using column chromatography. Characterization of compound was done by spectroscopic technique (Hamrapurkar *et al.*, 2011).

Materials and Methods

3. MATERIALS AND METHODS

The study on "Evolving superior types in thippali (*Piper longum* L.) utilising bisexual variants" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period 2010 to 2012.

3.1 EXPERIMENTAL MATERIALS

The experimental materials included various sex forms of thippali (*Piper longum* L.) and hybrids produced using bisexual types. They were

1. Female types
2. Male types
3. Bisexual types I and II identified and maintained at the Department of Plantation Crops & Spices, College of Horticulture, Vellanikkara
4. Seventy three hybrids from crosses made by Kanimozhi (2010) and Sujatha (2011)

Plants of first three sex forms were maintained in sufficient number in pots. Hybrid seedlings produced by Kanimozhi (2010) and Sujatha (2011) were maintained in pots and further multiplied by rooting of cuttings.

Production of hybrids was continued during the present study, which were also evaluated for growth and reproductive characters.

3.2 DETAILS OF EXPERIMENT

3.2.1 Experiment I - Developing hybrids and back crosses

3.2.1.1 Inducing bisexual flowers using growth regulators

For induction of bisexual flowers in bisexual type I, the growth regulators *viz.*, NAA 50 mg l^{-1}, NAA 100 mg l^{-1} and BA 500 mg l^{-1} (Kanimozhi, 2010) were applied at monthly intervals for three months consecutively.

In the first month, three potted plants were treated with each growth regulator concentration. In the subsequent month, in addition to the first set of plants, another set of plants were also sprayed with various growth regulator concentrations specified. This was repeated in third month as well. Growth regulators in each treatment were sprayed on plants using small hand sprayers. Thus, three plants each were treated with growth regulators once, twice or thrice at monthly intervals. Inflorescence production as well as ratio of male to bisexual flowers was recorded in all the treatments following growth regulator application

3.2.1.2 Reciprocal crosses using bisexual types as male and female parents

Crosses were made using bisexual type I and II as either male or female parents.

3.2.1.2.1 Bisexual types as female parent

Crosses were made using male types as male parent and bisexual types as female parent. Pollen grains from male types were taken in a petridish and pollinated using a camel hair brush on flowers of bisexual types. Either dry or wet method of pollination was resorted to. In wet method, distilled water was used as the medium.

3.2.1.2.2 Bisexual types as male parent

In this experiment, bisexual type I and II were used as male parents and pure female type as female parent. Crosses were made using pollen grains from bisexual types and pollination was done on pure female type. In dry method of pollination, pollen grains were dusted on female spikes by using a camel hair brush and in wet method, pollen suspension with distilled water was prepared and poured on female spikes with the help of an ink dropper.

3.2.1.3 Back crossing predominantly bisexual hybrids with female and bisexual parents

In the earlier study conducted at the Department of Plantation Crops and Spices (Kanimozhi, 2010), two hybrids (Acc. no. 53 and 97) produced bisexual flowers in higher proportion. These predominantly bisexual types were utilized in the study.

3.2.1.3.1 Back crossing predominantly bisexual hybrids as male parents

Crosses were made by using pure female types as female parents and predominantly bisexual hybrids as male parents.

3.2.1.3.2 Back crossing predominantly bisexual hybrids as female parents

Predominantly bisexual types were back crossed with bisexual types I and II as male parents. Here also, dry method of pollination was followed.

The following observations were made

1. Number of spikes pollinated
2. Number of spikes set
3. Number of seeds / spike
4. Time taken from pollination to harvest
5. Seasonal variation in spike set and fruit set
6. Number of seeds germinated
7. Time taken for germination of seeds
8. Percentage germination

3.2.2 Experiment II

Evaluation of hybrids and back cross progeny

Hybrids used in this experiment were divided into two categories

1. Hybrids produced as a part of KSCSTE funded project (2008-2011) and as a part of M Sc. thesis project of Kanimozhi (2010)

Hybrids were maintained in pots and evaluated for growth, yield and quality (Plate 1). The genotypes will be ranked based on major desirable traits like sex form, length of spike, yield etc.

2. Hybrids produced during 2011 under Experiment I of the present study

The seeds obtained from Experiment I were sown in sand. At five to six leaf stages, they were transplanted to mud pots.

The following observations were recorded

1. Time taken for germination
2. Rate of growth of plant
3. Time taken for production of first lateral branch
4. Season of flowering and fruit set
5. Spike orientation
6. Spike shape
7. Spike colour
8. Spike length
9. Type of hermaphroditism
10. Number of spikes / lateral branch
11. Per cent bisexual flowers
12. Per cent pistillate flowers
13. Per cent staminate flowers
14. Fresh yield / plant
15. Driage

3.2.3 Experiment III

Preliminary evaluation of selected female and bisexual types in the field

In this experiment, ten hybrids either female or bisexual types, were selected and planted in field. In addition to these accessions, the female type used in crosses and the released variety Viswam were used as checks. The statistical design followed was CRD.

Accessions of *Piper longum* selected for field planting

Sl. No.	1	2	3	4	5	6	7	8	9	10
Acc. No.	2	9	16	32	33	48	53	77	95	97

The experiment was laid out in a coconut garden with partially shaded conditions (Plate 2). Land was thoroughly ploughed and beds of suitable size (1.2 m ×1.2 m) were formed. Two to three node cuttings of hybrids were raised in the nursery. After two months, rooted cuttings were planted in beds. Four plants were planted in a bed, at a spacing of 60cm × 60cm. Gap filling was carried out occasionally after planting in main field. Weeding and earthing up were done at regular intervals. Plants were irrigated twice a week during summer months. Integrated nutrient management was done for *Piper longum* accessions.

The following observations were recorded

1. Number of main branches
2. Number of spike bearing branches / stem
3. Length of spike
4. Fresh yield / plant
5. Driage
6. Quality parameters

Plate 1. Seedling progenies in pots

Plate 2. View of experimental field

a) Essential oil - The oil content in the spikes was estimated employing solvent extraction by Clevenger apparatus [AOAC, 1980] and expressed in percentage.
b) Oleoresin - The oleoresin content in the spikes of *Piper longum* was estimated employing solvent extraction by Soxhlet apparatus [AOAC, 1980] and expressed in percentage.
c) Piperine - Piperine content in the dried spikes was estimated using spectrophotometer by the method suggested by Soubhagya et *al.* (1990).

Freshly powdered samples of dried spikes was taken in a volumetric flask and extracted with 100 ml acetone. The flask was kept at room temperature and shaken well for 2 hours. 0.25 ml of clear solution from flask was taken in a cuvette and made upto 5 ml with 4.75 ml acetone. The solution was shaken well and absorbance of the solution was read at 337 nm in a UV spectro photometer. Acetone was used as blank.

Preparation of the standard curve

Standard piperine solutions of concentrations *viz.*, 0.4, 0.8, 1.2, 1.6 and 2 mg l^{-1} were prepared and their absorbance values at 337nm were found out. The values were plotted on a graph and from the graph the concentration corresponding to the absorbance of the sample was found out and piperine content in the samples were worked out.

7. Incidence of pests and diseases - Incidence of pests and diseases in experimental plants were scored and expressed in percentage.

3.3 STATISTICAL ANALYSIS OF DATA

Data were statistically analysed using the MSTAT.C package. Treatment means were compared using DMRT (Freed, 1986).

Results

4. RESULTS

The present study was under taken at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period 2010 to 2012, with the objective of developing superior types in thippali (*Piper longum* L.) utilising bisexual variants.

The results obtained are presented below:-

4.1 DEVELOPING HYBRIDS AND BACK CROSSES

4.1.1 Inducing bisexual flowers using growth regulators

For induction of bisexual flowers, growth regulators *viz*., NAA 50 mg l^{-1}, NAA 100 mg l^{-1} and BA 500 mg l^{-1} were applied at monthly intervals for three months. Three plants each were treated with growth regulators, once, twice and thrice at monthly intervals. Inflorescence production was recorded in all the treatments following growth regulator applications.

4.1.1.1 Effect of growth regulators on inflorescence production

Table 1 shows the effect of growth regulator spray on inflorescence production at monthly intervals. Spraying was commenced in October 2011 and continued during November and December 2011. Observations on effect of growth regulators were recorded till March 2012. Vegetative growth as well as spike production were adversely affected by both concentrations of NAA. Flaccidity and downward curling of leaves were noticed on spraying with NAA. This was followed by yellowing and dropping of leaves (Plate 3). There was no spike production in plants treated with NAA 50 mg l^{-1} or 100 mg l^{-1} single, two and three applications at monthly intervals during the period under observation.

Table 1. Monthly variations in inflorescence production in growth regulator treated plants

Sl. No.	Growth regulator & concentration (mgl $^{-1}$)	Production of inflorescence/ month						Mean
		0ctober 2011	November 2011	December 2011	January 2012	February 2012	March 2012	
1	NAA 50 mgl $^{-1}$ single application	0	0	0	0	0	0	0
2	NAA 50 mgl $^{-1}$ two applications	0	0	0	0	0	0	0
3	NAA 50 mgl $^{-1}$ three applications	0	0	0	0	0	0	0
4	NAA 100 mgl $^{-1}$ single application	0	0	0	0	0	0	0
5	NAA 100 mgl $^{-1}$ two applications	0	0	0	0	0	0	0
6	NAA 100 mgl $^{-1}$ three applications	0	0	0	0	0	0	0
7	BA 500 mgl $^{-1}$ single application	13	12	13	10	8	8	10.67
8	BA 500 mgl $^{-1}$ two applications	-	16	12	12	9	12	12.2
9	BA 500 mgl $^{-1}$ three applications	-	-	10	14	13	12	12.25
	Control plants	16	9	12	13	7	9	11

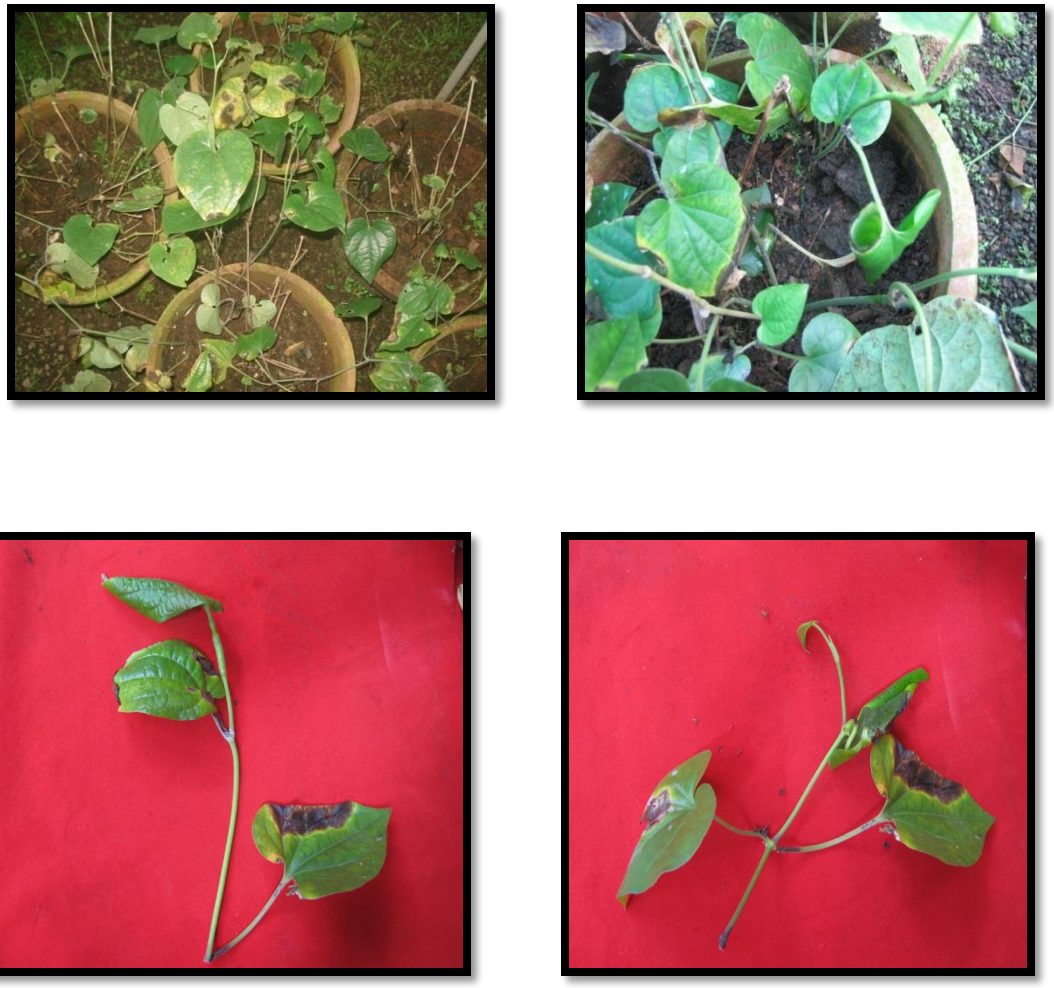


Plate 3. Phytotoxic symptoms on NAA application

It can be seen from Table 1 that in BA 500 mg l^{-1} single application, number of spikes produced from October to March varied from 8 to 13 with a mean of 10.67. In BA 500 mg l^{-1} applied twice, number of spikes produced varied from 9 to 16 with a mean of 12.2. The mean spike production at three applications of BA was 12.25 with a range of 10 to 14. Control plants that received no growth regulator treatment showed a monthly production of 7 to 16 spikes with a mean of 11 during the six month period under observation.

4.1.1.2 Effect of growth regulators on change in sex-ratio

In addition to the total number of inflorescences produced, the effect of three growth regulators on ratio of male to bisexual flowers was also observed. Since there was no inflorescence production in plants treated with both concentrations of NAA, change in sex ratio could be analyzed only in BA 500 mg l^{-1} treated plants.

Table 2. Male to bisexual flower ratio in BA 500 mg l^{-1} application

Sl. No.	Growth regulator and concentration	Male to bisexual flower number	Ratio
1	BA 500 mgl $^{-1}$ single application	362.37/5.7	63.6:1
2	BA 500 mgl $^{-1}$ two applications	350.23/9.61	36.4:1
3	BA 500 mgl $^{-1}$ three applications	351.8/13.8	25.5:1
4	Control plants	306.02/3.87	79.1:1

From Table 2, it is clear that bisexual flower production was increased with each application of BA 500 mg l^{-1}. In BA 500 mg l^{-1} single application, male to bisexual ratio was 63.6:1 which changed to 36.4:1 in two applications. After the third application, the ratio was 25.5:1. The ratio of male and bisexual flowers in control plants without growth regulator application was 79.1:1.

4.1.2 Reciprocal crosses using bisexual types as male and female parents

To develop hybrids, crosses were made using bisexual type either as male or as female parent.

4.1.2.1 Bisexual types as female parent

As can be seen from Table 3, crosses were made during June to October 2011, in which bisexual types I and II were used as female parents and pure male as male parent. Dry method of pollination was resorted to, since spike setting during rainy weather was found better in this method (Kanimozhi, 2010). It can be seen from Table 3 that, no spike set was obtained when either of the bisexual type was used as female parent.

Table 3. Spike set in crosses with bisexual type as female parent

Sl. No.	Month	Female parent	No. of spikes pollinated	No. of spikes set
1	June	Type I	3	0
2		Type II	4	0
3	July	Type I	2	0
4		Type II	1	0
5	August	Type I	2	0
6		Type II	3	0
7	September	Type I	2	0
8		Type II	3	0
9	October	Type I	1	0
10		Type II	1	0

4.1.2.2 Bisexual types as male parents

Studies were also conducted using bisexual types as male parent and pure female as female parent. As can be seen from Table 4, pollinations were done from June 2011 to February 2012. Dry method of pollination was carried out from June to October 2011 and wet method was followed during December, January and February. Methods of pollination used were dry or wet based on climatic conditions as per the observations of Kanimozhi (2010).

4.1.2.2.1 Bisexual type I as male parent

Table 4 shows the data on number of spikes pollinated, number of spikes set and number of seeds obtained in each month from June 2011 to February 2012. The percentage of spike set was maximum during October (82.92%) followed by November (78.2%) and December (74.07%) and minimum during July (44.46%). Number of seeds obtained was maximum during July 2011 (75) followed by September 2011 (67). No seed set could be obtained during November to February, even though the percentage of spike set was good and method of pollination used was wet method instead of dry. From Table 4, it is evident that July and September were the best months to get maximum seeds, when bisexual type I was used as male parent.

4.1.2.2.2 Bisexual type II as male parent

Pollinations were done from July 2011 to February 2012, using bisexual type II as male and pure female as female parent. Maximum spike set was observed during December 2011(71.42%) in wet method, followed by October 2011(61.53%) under dry method of pollination. Spike set was minimum during the month January 2012 (25%). As can be seen from table 4, there was good spike set using bisexual type II as male parent, during August to October and in December (57.14 - 71.42 %). However, seed set was obtained only in September (16 numbers) and October (one number) pollinations. During other months of the year, no seed set could be observed.

Table 4 . Monthly variations in spike set and seed set using bisexual type as male parent

Sl. No.	Month	Method of pollination	Bisexual type I as male parent				Bisexual type II as male parent			
			No. of spikes pollinated	No. of spikes set	% spike set	No. of seeds obtained	No. of spikes pollinated	No. of spikes set	% spike set	No. of seeds obtained
1	June 2011	Dry	34	24	70.58	7	0	-	-	-
2	July 2011	Dry	27	12	44.44	75	2	0	0	0
3	August 2011	Dry	29	21	72.41	1	14	8	57.14	0
4	September 2011	Dry	23	16	69.56	67	21	12	57.14	16
5	October 2011	Dry	41	34	82.92	2	13	8	61.53	1
6	November 2011	Dry	23	18	78.26	0	10	3	30	0
7	December 2011	Wet	27	20	74.07	0	21	15	71.42	0
8	January 2012	Wet	22	16	72.72	0	8	2	25	0
9	February 2012	Wet	18	10	55.56	0	11	4	36.36	0
	Total		244	171	70.08	152	100	52	52	17

4.1.2.2.3 Germination of seeds

A total of 169 seeds were obtained under different methods of artificial pollination carried out. Out of this 152 seeds were produced using bisexual type I as male parent and 17 seeds were obtained, when bisexual type II was used as male parent. Number of seeds germinated under different conditions of pollinations is given in Table 5. As can be seen from Table 5, out of a total of 169 seeds obtained from different crosses, only 32 seeds germinated (18.9%). These were the seeds obtained using bisexual type I as male parent, which showed 21.05% germination. Seeds produced using bisexual type II as male parent, did not germinate.

Table 5. Seed set and seed germination under different methods of pollination

Method of pollination		No. of spikes pollinated	No. of spikes set	No. of seeds sown	No. of seeds germinated	Germination %
Dry	Type I	177	125	152	32	21.05
	Type II	60	31	17	0	0
Wet	Type I	67	46	0	0	0
	Type II	41	21	0	0	0
Total		345	223	169	32	18.9

4.1.3 Back crossing hybrids having high percentage of bisexual flowers with female and bisexual parents

In an earlier study conducted in the Department of Plantation Crops and Spices (Kanimozhi, 2010), two hybrids (Acc. no. 53 and 97) produced inflorescences with more number of bisexual flowers (predominantly bisexual). In the present study, these accessions were back crossed with female and bisexual parents for developing back cross progenies.

4.1.3.1 Back crosses using predominantly bisexual hybrids as male parent

As can be seen from Table 6, spike set was observed in the pollinations done during June, July, September and October, when acc. no. 97 was used as male parent. However, no seed set was observed in any of the spikes. Using acc. no. 53 as male, spike set could be obtained during September and October 2011.

4.1.3.2 Back crosses using predominantly bisexual types as female parents

Table 7 & 8 show crosses involving predominantly bisexual types as female parent. As is evident from the Tables, spikes dropped in 5-18 days after pollination and no mature spikes could be harvested when acc. no. 97 was used as female parent. When acc. no. 53 was used as female parent, spikes dropped at more immature stage (3-7 days) and no mature spikes could be harvested.

Table 6. Spike and seed set in crosses with predominantly bisexual hybrids (Acc. No. 53 and 97) as male parent

Sl. No.	Month	Method of pollination	Acc. No. 97 as male parent				Acc. No. 53 as male parent			
			No. of spikes pollinated	No. of spikes set	% spike set	No. of seeds obtained	No. of spikes pollinated	No. of spike set	% spike set	No. of seeds obtained
1	June 2011	Dry	4	2	50	0	4	0	0	0
2	July 2011	Dry	5	1	20	0	5	0	0	0
3	August 2011	Dry	4	0	0	0	4	0	0	0
4	September 2011	Dry	4	3	75	0	4	1	25	0
5	October 2011	Dry	5	2	40	0	5	1	20	0
	Total		22	8	36.36	0	22	2	9.09	0

Table 7. Spike set in crosses with predominantly bisexual type as female parent (Acc. No. 97)

Month	Male parent	Method of pollination	No. of spikes pollinated	No. of days from pollination to flower drop	No. of mature spikes harvested
June 2011	Type I	Dry	9	6-18	0
July	Type I	Dry	6	7-12	0
August	Type I	Dry	8	6-9	0
September	Type I	Dry	6	5-17	0
October	Type I	Dry	4	6	0
November	Type I	Dry	5	5-8	0
December	Type I	Wet	4	6-7	0

Table 8. Spike set in crosses with predominantly bisexual type as female parent (Acc. No. 53)

Month	Male parent	Method of pollination	No. of spikes pollinated	No. of days from pollination to flower drop	No. of mature spikes harvested
June 2011	Type I	Dry	8	5	0
July	Type I	Dry	9	4-6	0
August	Type I	Dry	3	3-5	0
September	Type I	Dry	4	4-7	0
October	Type I	Dry	5	5	0

4.2 EVALUATION OF HYBRIDS AND BACK CROSS PROGENIES

Hybrids used in Experiment II fall under two categories

1. Hybrids produced as a part of a KSCSTE funded project and as a part of an M Sc. study (Kanimozhi, 2010) during 2009-2010
2. Hybrids produced during 2011under Experiment I of the present study

In the first set of plants, observations were concentrated on a few vegetative characters like leaf characters and emphasis was given on reproductive characters. Observations on early growth phases were completed in the earlier projects.

In the second set of seedlings, observations were taken on number of days to germination, days to opening of cotyledonary leaf, number of days to opening of first true leaf, days to lateral branch production, increment in height, number of leaves produced, leaf characters, colour of leaf petiole, node and inter node.

4.2.1 Observations on seedling progenies produced during 2009-2010

Table 9 and 10, show the observations on vegetative and reproductive characters of hybrids produced during 2009-10. Altogether, 73 hybrid seedlings were evaluated.

4.2.1.1 Qualitative characters of accessions in pots

Vegetative characters especially leaf characters were recorded for all surviving accessions. Leaf lamina was mostly cordate while in some accessions they were ovate-elliptic (Acc.no. 14, 40, 73, 89, 103, 113, 122, 139) and in a few others elliptic lanceolate (Acc.no. 8, 13, 28, 42, 59, 60, 90, 110) (Plate 4).Variation in shape of leaf base was observed, most of them being cordate and some oblique (Acc. no. 97, 110) or acute (Acc. no.14) (Plate 5). Leaf margins were wavy in most of the accessions and entire in a few accessions (Acc. no. 3,

11, 21, 43, 52, 55, 63, 83, 85, 96, and 116) (Plate 6). Venation was acrodromous in all accessions under study. Colour of leaves was green, light green or purple. Leaf petiole colour also ranged from light green to green (Table 9).

4.2.1.2 Reproductive characters of hybrids in pots

Reproductive characters like sex form and spike characters of all accessions which flowered are described in Table10 (Plate 7). All the accessions showed high amount of variation, in most of the characters. Number of spike bearing branches in the accessions was also recorded. As can be seen from Table 10, only two accessions *viz.*, acc. no. 53 (20.6%) and acc. no. 97 (28.75%) produced bisexual flowers. Number of pistillate flowers per spike varied from 32.54 (Acc. no. 48) to 245.8 (Acc. no.33) and number of staminate flowers per spike varied from 182.75 (Acc. no.97) to 328.7 (Acc. no.3).

Spikes were erect in all the accessions / seedlings (Plate 8). Shape of spikes varied from filiform (Acc. no. 2, 3, 15, 31, 53, 55, 63, 97, 104, 115, 137), cylindrical (Acc. no. 9, 16, 28, 32, 33, 77, 83, 140) and globular (Acc. no. 48, 91, 95) (Plate 9). Colour of immature spikes was green, light green or yellow. Mature spike colour also ranged from light green, green, and dark green and greenish yellow to yellow.

Spikes were as small, ranging from 1.32 cm (Acc. no. 48) to 6.20 cm (Acc. no. 115) in length. Fresh weight of a spike ranged from 0.09 g (Acc. no. 48) to 1.43g (Acc. no. 16). Dry weight of spikes ranged from 0.05 g (Acc. no. 48) to 0.24 g (Acc. no. 9). Fresh yield per plant varied from 1.29 g (Acc. no.2) to 121.90 g (Acc. no. 9). Driage or per cent dry weight of spike varied from 13.12 per cent (Acc. no. 115) to 18.13 (Acc. no. 9).

Table 9. Leaf characteristics of seedling progenies (2009-'10)

Sl. No.	Acc. No.	Leaf lamina shape	Leaf base shape	Leaf margin	Leaf texture	Type of venation	Leaf lamina colour		Leaf petiole colour
							Immature	Mature	
1	1	Ovate elliptic	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
2	2	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
3	3	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Green	Dark green	Green
4	4	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
5	5	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
6	6	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
7	7	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
8	8	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Green	Dark green	Green
9	9	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
10	11	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
11	12	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
12	13	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green

Table 9. Leaf characteristics of seedling progenies (2009-'10) *contd…*

Sl. No.	Acc. No.	Leaf lamina shape	Leaf base shape	Leaf margin	Leaf texture	Type of venation	Leaf lamina colour		Leaf petiole colour
							Immature	Mature	
13	14	Ovate elliptic	Oblique	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
14	15	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
15	16	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
16	17	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
17	19	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Green	Dark green	Green
18	21	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
9	26	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Green
20	28	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
21	29	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
22	30	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Green	Dark green	Light green
23	31	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Green	Dark green	Green
24	32	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green

Table 9. Leaf characteristics of seedling progenies (2009-'10) *contd…*

Sl. No.	Acc. No.	Leaf lamina shape	Leaf base shape	Leaf margin	Leaf texture	Type of venation	Leaf lamina colour		Leaf petiole colour
							Immature	Mature	
25	33	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Green
26	35	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
27	36	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
28	39	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
29	40	Ovate elliptic	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green with purple tinch
30	41	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Green
31	42	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Green	Dark green	Green
32	43	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
33	46	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
34	48	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
35	50	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Green
36	52	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Green	Dark green	Light green

Table 9. Leaf characteristics of seedling progenies (2009-'10) *contd…*

Sl. No.	Acc. No.	Leaf lamina shape	Leaf base shape	Leaf margin	Leaf texture	Type of venation	Leaf colour		Leaf petiole colour
							Immature	Mature	
37	53	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
38	55	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
39	59	Ovate- lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Green
40	60	Ovate- lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
41	62	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
42	63	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
43	64	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
44	69	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
45	73	Ovate elliptic	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Green	Green	Light green
46	74	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
47	75	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Green
48	83	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Light green	Green	Light green

Table 9. Leaf characteristics of seedling progenies (2009-'10) *contd…*

Sl. No.	Acc. No.	Leaf lamina shape	Leaf base shape	Leaf margin	Leaf texture	Type of venation	Leaf lamina colour		Leaf petiole colour
							Immature	Mature	Light green
49	84	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
50	85	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
51	88	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
52	89	Ovate elliptic	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
53	90	Ovate lanceolate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
54	91	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
55	94	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
56	95	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
57	96	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Light green	Green	Green
58	97	Elliptic lanceolate	oblique	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
59	98	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
60	103	Ovate elliptic	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Green	Green	Light green

Table 9. Leaf characteristics of seedling progenies (2009-'10) *contd…*

Sl. No.	Acc. No.	Leaf lamina shape	Leaf base shape	Leaf margin	Leaf texture	Type of venation	Leaf lamina colour		Leaf petiole colour
							Immature	Mature	
61	106	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
62	110	Elliptic lanceolate	Oblique	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
63	113	Ovate elliptic	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
64	115	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Green
65	116	Cordate	Cordate	Entire	Glabrous coriaceous	Acrodromous	Green	Dark green	Green
66	121	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
67	122	Ovate elliptic	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Green
68	130	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
69	133	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
70	138	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Light green
71	139	Ovate elliptic	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green
72	140	Cordate	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Green	Green
73	141	Ovate elliptic	Cordate	Wavy	Glabrous coriaceous	Acrodromous	Light green	Dark green	Light green

Table10. Spike characters of seedling progenies (2009-'10)

Sl. No.	Acc. No.	Sex form	Spike orientation	Spike shape	Immature spike colour	Mature spike colour	Number of bisexual flowers/spike	Number of pistillate flowers/spike	Number of staminate flowers/spike
1	2	Androecious	Erect	Filiform	Light green	Dark green	-	-	264.2 (100)
2	3	Androecious	Erect	Filiform	Light green	Yellow	-	-	328.7 (100)
3	9	Gynoecious	Erect	Cylindrical	Yellow	Dark green	-	218.2 (100)	-
4	15	Androecious	Erect	Filiform	Light green	Yellow	-	-	298.7 (100)
5	16	Gynoecious	Erect	Cylindrical	Yellow	Green	-	82.4 (100)	-
6	28	Gynoecious	Erect	Cylindrical	Yellow	Green		214.2 (100)	-
7	31	Androecious	Erect	Filiform	Green	Yellow	-	-	232.2 (100)
8	32	Gynoecious	Erect	Cylindrical	Yellow	Light green	-	218.4 (100)	-
9	33	Gynoecious	Erect	Cylindrical	Yellow	Green	-	254.8(100)	-
10	48	Gynoecious	Erect	Globular	Light green	Green	-	32.54 (100)	-
11	53	Andro monoecious	Erect	Filiform	Green	Greenish yellow	40.4 (20.6)	-	167(78.2)
	55	Androecious	Erect	Filiform	Green	Yellow	-	-	192.4 (100)
13	63	Androecious	Erect	Filiform	Light green	Dark green	-	-	213.8 (100)
14	77	Gynoecious	Erect	Cylindrical	Light green	Green	-	-	-
15	83	Gynoecious	Erect	Cylindrical	Yellow	Dark green	-	49.62(100)	-
16	88	Androecious	Erect	Filiform	Light green	Yellow	-	-	201.3(100)
17	91	Gynoecious	Erect	Globular	Yellow	Green	-	-	-
18	95	Gynoecious	Erect	Globular	Yellow	Light green	-	98.31 (100)	-
19	97	Andro monoecious	Erect	Filiform	Green	Greenish yellow	56.8(28.75)	-	186.7(71.27)
20	104	Androecious	Erect	Filiform	Light green	Yellow	-	-	-
21	115	Androecious	Erect	Filiform	Light green	Green	-	-	243.12(100)
22	137	Androecious	Erect	Filiform	Light green	Yellow	-	-	-
23	140	Gynoecious	Erect	Cylindrical	Yellow	Dark green	-	176.46(100)	-

* NS: Values in paranthesis denotes the percentage of total number of flowers produced

Table 10. Spike characters of seedling progenies (2009-'10) *contd…..*

Sl. No.	Acc. No.	Spikes/lateral branch	Spike length (cm)	Fresh weight of a spike (g)	Dry weight of a spike (g)	Fresh yield of plant (g)	Driage (%)
1	2	1.5[a]	4.13[g]	0.29[i]	0.05[fg]	1.29	16.02[c]
2	3	1.2[d]	5.27[d]	0.97[d]	0.14[c]	-	14.71[e]
3	9	1.5[a]	5.64[c]	1.31[b]	0.24[a]	121.90	18.13[a]
4	15	1.1[ef]	4.86[e]	0.77[e]	0.11[d]	18.14	14.03[f]
5	16	1.3[bc]	3.25[i]	1.43[a]	0.22[ab]	54.10	15.41[d]
6	28	1.2[d]	3.76[h]	0.74[ef]	0.12[c]	8.38	16.67[b]
7	31	1.1[ef]	4.53[f]	0.37[h]	0.05[fg]	-	13.92[f]
8	32	1.1[ef]	4.12[g]	0.78[e]	0.12[c]	-	15.61[c]
9	48	1.2[d]	1.32[k]	0.09[j]	-	1.09	-
10	53	1.4[b]	4.01[g]	0.30[i]	0.05[fg]	14.53	15.01[d]
11	63	1.3[bc]	4.78[e]	0.62[g]	-	-	-
12	83	1.3[bc]	3.12[j]	1.24[c]	0.18[cd]	7.12	15.12[d]
13	95	1.2[d]	3.7[h]	0.68f	0.10[d]	-	14.65[e]
14	97	1.5[a]	5.81[b]	0.84[de]	0.13[cd]	7.67	15.97[c]
15	115	1.1[ef]	6.20[a]	0.62[d]	0.08[e]	-	13.12[g]
16	140	1.5[a]	3.82[h]	-	-	-	-

Cordate

Ovate elliptic

Elliptic lanceolate

Plate 4. Variation in shape of leaf

Cordate **Oblique**

Plate 5. Variation in leaf base

Entire **Wavy**

Plate 6. Variation in leaf margin

Androecious

Gynoecious

Andromonoecious

Plate 7. Different sex forms of *Piper longum*


Erect

Plate 8. Orientation of spike


Cylindrical **Conical**


Filiform **Globular**

Plate 9. Variation in shape of spikes

4.2.2 Observations on seedling progenies produced during 2011

Out of 169 seeds sown, 32 seeds germinated (18.9%). Out of these 32 seedlings, 15 were lost due to seedling rot (11 numbers) and albinism (four numbers). Observations on germination and growth characteristics of survived seedlings are given in Table 11.

Number of days taken for germination of seeds varied from 33(Acc. no. 153) to 71(Acc. no. 149) days. Number of days taken from germination to opening of cotyledonary leaf also varied from 4 (Acc. no. 158) to 21(Acc. no. 153). First true leaf appeared in 10(Acc. no. 158) to 33(Acc. no. 151) days.

The rate of height increment varied from 0.56 cm (Acc. no. 149) to 11.81 cm (Acc. no. 146) in a month (Plate 10). Similarly, number of leaves produced ranged from 5 (Acc. no. 148) to 35 (Acc. no.155). Number of branches produced during the period ranged from 1 (Acc. no.144, 145, 148, 149 and 150) to 5 (Acc. no.155). Lateral branches were produced in eight accessions only. Time taken for lateral branch production varied from 109 (Acc. no. 156) to 260 (Acc. no. 143) days in these accessions. Colour of emerging shoot tip ranged from light green to green.

Leaf lamina was mostly cordate and ovate-elliptic (Acc. no.144, 147,151and 156) and leaf base was cordate in all accessions. Margins were wavy in most of the accessions and entire in Acc. no. 153. Venation was acrodromous in all accessions under study. Colour of immature leaves was light green to green and that of mature leaf was green to dark green (Acc. no. 143, 145, 147 etc.). Vegetative nodes were mostly purple or sometimes green (Acc. no. 144, 148, 150 and 151). Inter nodes were green or purple (Table 12).

Table 11. Vegetative characters of seedling progenies (2011)

Sl. No.	Acc. No.	Days to germination	Days to opening of cotyledonary leaf	Days for first true leaf emergence	Days to first lateral branch production	Mean monthly increment in length (cm)	Length 4 month after germination (cm)	Emerging shoot tip colour	No. of branches	No. of leaves
1	142	52	9	30	208	2.33	15.1	Light green	4	15
2	143	39	12	21	260	2.77	17.3	Green	2	12
3	144	57	13	22	-	0.85	5.1	Light green	1	5
4	145	61	6	17	-	1.44	7.8	Light green	1	5
5	146	57	15	21	161	11.81	71.0	Green	3	34
6	147	44	10	19	-	2.31	14.6	Green	3	6
7	148	45	10	19	-	0.89	5.2	Green	1	5
8	149	71	8	19	-	0.56	4.2	Light green	1	6
9	150	47	10	22	-	1.75	11.0	Light green	1	6
10	151	51	13	33	170	2.65	17.0	Light green	2	8
11	153	33	21	27	120	4.76	29.5	Light green	3	19
12	154	33	11	21	134	2.28	14.1	Light green	2	12
13	155	54	10	24	115	5.30	32.5	Light green	5	35
14	156	63	10	15	109	1.30	8.4	Green	2	12
15	157	68	5	11	-	-	-	Green	1	3
16	158	69	4	10	-	-	-	Light green	1	3
17	159	40	6	15	-	-	-	Green	1	3

Table 12. Leaf characters of seedling progenies (2011)

Sl. No.	Acc. No.	Leaf lamina shape	Leaf base shape	Leaf margin	Type of venation	Colour of lamina		Colour of petiole	Colour	
						Immature	Mature		Node	Internode
1	142	Cordate	Cordate	Wavy	Acrodromous	Light green	Green	Light green	Purple	Green
2	143	Cordate	Cordate	Wavy	Acrodromous	Light green	Dark green	Green	Purple	Purple stripes
3	144	Ovate elliptic	Cordate	Wavy	Acrodromous	Green	Green	Green	Green	Green
4	145	Cordate	Cordate	Wavy	Acrodromous	Light green	Dark green	Green	Purple	Purple stripes
5	146	Cordate	Cordate	Wavy	Acrodromous	Light green	Green	Green	Purple	Purple stripes
6	147	Ovate elliptic	Cordate	Wavy	Acrodromous	Light green	Dark green	Green	Purple	Purple stripes
7	148	Cordate	Cordate	Wavy	Acrodromous	Light green	Green	Light green	Green	Purple stripes
8	149	Cordate	Cordate	Wavy	Acrodromous	Green	Dark green	Light green	Purple	Green
9	150	Cordate	Cordate	Wavy	Acrodromous	Light green	Dark green	Green	Green	Purple stripes
10	151	Ovate elliptic	Cordate	Wavy	Acrodromous	Light green	Dark green	Green	Green	Purple stripes
11	153	Cordate	Cordate	Entire	Acrodromous	Light green	Dark green	Green	Purple	Purple stripes
12	154	Cordate	Cordate	Wavy	Acrodromous	Light green	Dark green	Light green	Purple	Purple stripes
13	155	Cordate	Cordate	Wavy	Acrodromous	Light green	Green	Light green	Purple	Green
14	156	Ovate elliptic	Cordate	Wavy	Acrodromous	Light green	Dark green	Light green	Purple	Purple stripes
15	157	Cordate	Cordate	Wavy	Acrodromous	Light green	Green	Light green	Purple	Green
16	158	Cordate	Cordate	Wavy	Acrodromous	Light green	Dark green	Light green	Purple	Green
17	159	Cordate	Cordate	Wavy	Acrodromous	Light green	Green	Light green	Purple	Purple stripes

Plate 10. Seedlings of *Piper longum* at different growth stages

4.3 PRELIMINARY EVALUATION OF FEMALE AND BISEXUAL TYPES IN THE FIELD

Preliminary evaluation of female and bisexual types was carried out in the field. From the hybrids produced during previous study, ten accessions listed below were selected and planted in field (Table 14). In addition to these accessions, the female type used in crosses (pure female) and the released variety Viswam were used as checks and the statistical design adopted was CRD.

Table 13. Accessions of *Piper longum* selected for field planting

Sl. No.	1	2	3	4	5	6	7	8	9	10
Acc. No.	2	9	16	32	33	48	53	77	95	97

4.3.1 Morphological description of selected accessions

The ten accessions selected along with checks were planted in field in beds of size 1.2m x 1.2m. Four rooted cuttings were planted in each bed. The accessions were described as per IPGRI descriptor for *Piper* species for vegetative and reproductive characters. Additional observations were recorded, wherever necessary.

4.3.1.1 Vegetative characters

All the accessions of *Piper longum* showed a creeping growth habit (Table 14). Branching pattern was dimorphic. Young orthotropic shoot tips were light green in most of the accessions and greenish yellow in Acc. no. 16 and 97. *Piper longum* accessions produced a few runner shoots. Stem was minutely pubescent. The lateral branches produced were erect. The accessions showed high variability in the number of main branches produced, which ranged from 0.78 (Acc. no. 77) to 9.01 (Viswam). Pure female type produced 5.65 branches and Acc. no.9 produced 5.32 branche

Table 14. Vegetative characters of field planted *Piper longum* accessions

Sl. No.	Acc. No.	Plant growth habit	Branching type	Young orthotropic shoot tip colour	Runner shoot production	Pubescence on stem	Lateral branch habit	No. of main branches	Length of longest stem(cm)
1	2	Creeper	Dimorphic	Light green	Few	Present	Erect	1.15^{de}	39.18^{d}
2	9	Creeper	Dimorphic	Light green	Few	Present	Erect	5.32^{b}	61.10^{b}
3	16	Creeper	Dimorphic	Greenish yellow	Few	Present	Erect	1.98^{d}	23.68^{h}
4	32	Creeper	Dimorphic	Light green	Few	Present	Erect	1.38^{de}	27.14^{f}
5	33	Creeper	Dimorphic	Light green	Few	Present	Erect	0.88^{de}	22.71^{i}
6	48	Creeper	Dimorphic	Light green	Few	Present	Erect	1.60^{de}	20.46^{k}
7	53	Creeper	Dimorphic	Light green	Few	Present	Erect	1.78^{de}	21.80^{j}
8	77	Creeper	Dimorphic	Light green	Few	Present	Erect	0.78^{e}	15.90^{k}
9	95	Creeper	Dimorphic	Light green	Few	Present	Erect	3.10^{c}	24.52^{g}
10	97	Creeper	Dimorphic	Greenish yellow	Few	Present	Erect	1.25^{de}	35.12^{e}
11	Pure female	Creeper	Dimorphic	Light green	Few	Present	Erect	5.65^{b}	57.20^{c}
12	Viswam	Creeper	Dimorphic	Light green	Few	Present	Erect	9.0167^{a}	88.75^{a}

As can be seen from Table 15, length of the longest stem ranged from 15.90 cm (Acc. no. 77) to 88.75cm (Viswam) at seven months after planting. Acc. no.9 had the longest stem length as 61.1cm and pure female type, 57.20 cm.

4.3.1.2 Leaf characters

Piper longum accessions used in the study showed variability in leaf characters also. Length of leaf varied from 4.2 cm (Acc. no.48) to 8.3 cm (Acc. no. 9). Leaf width ranged from 3.1 cm (Acc. no. 32 and 48) to 7.5cm (Acc. no.9). Large amount of variation was noticed in length of leaf petiole which ranged from 1.7cm (Acc. no. 48) to 6.4 (Acc. no. 9). Total number of leaves produced by plants during seventh month after planting ranged from 16.3 (Acc. no. 77) to 176.4 (Viswam) (Table 15).

Shape of leaf lamina varied from cordate to ovate-lanceolate or elliptic-lanceolate. Leaf base was mostly cordate. It was oblique in Acc. no 9 and 97. Leaf margin was wavy and venation, acrodromous. Leaves were coriaceous and hairs were present along the veins on the under surface of leaves (Table 15).

Table 15. Leaf characters of field planted *Piper longum* accessions

Sl. no.	Acc. No.	Leaf length (cm)	Leaf width (cm)	Leaf petiole length (cm)	Number of leaves produced	Leaf lamina shape
1	2	4.3^{h}	4.1^{f}	2.3^{g}	38.2^{d}	Cordate
2	9	8.3^{a}	7.5^{a}	6.4^{a}	159.4^{b}	Elliptic lanceolate
3	16	4.8^{f}	5.3^{c}	2.9^{e}	32.1^{e}	Cordate
4	32	5.6^{e}	3.1^{h}	1.8^{h}	29.1^{f}	Cordate
5	33	4.9^{f}	4.5^{de}	1.8^{h}	21.1^{h}	Elliptic lanceolate
6	48	4.2^{h}	3.1^{h}	1.7^{h}	28.2^{f}	Cordate
7	53	4.5h	4.6^{d}	2.8^{e}	23.3^{g}	Cordate
8	77	5.6^{e}	3.6^{g}	3.2^{d}	16.3^{i}	Ovate-lanceolate
9	95	4.7^{fg}	4.3^{ef}	2.5^{f}	20.2^{h}	Cordate
10	97	6.8^{d}	3.5^{g}	2.6^{f}	28.2^{f}	Elliptic lanceloate
11	Pure female	7.6^{c}	5.3^{c}	4.1^{b}	138.2^{c}	Cordate
12	Viswam	7.8^{b}	5.9b	3.6^{c}	176.4^{a}	Cordate

Table 15. Leaf characters of field planted *Piper longum* accessions *contd…*

Sl. no.	Acc. No.	Leaf base shape	Leaf margin	Type of veining	Leaf texture	Leaf hairiness
1	2	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
2	9	Oblique	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
3	16	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
4	32	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
5	33	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
6	48	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
7	53	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
8	77	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
9	95	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
10	97	Oblique	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
11	Pure female	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins
12	Viswam	Cordate	Wavy	Acrodromous	Glabrous coriaceous	Mainly along the veins

4.3.1.3 Reproductive characters

All the accessions varied in reproductive characters like number of spike bearing branches per stem [0.018 (Acc. no. 33) to 2.83 (Viswam)]. Acc. no. 77 did not flower during the period under study. Spike orientation was erect in all the accessions. Spikes were either filiform (Acc. no. 2, 53 and 97), cylindrical (Acc. no. 32, 33 and 95) or globular (Acc. no. 16 and 48) in shape (Table 16). Colour of immature spike was green, yellow or greenish yellow turning green, dark green or yellowish green on maturity. Spike length ranged from 1.3 cm (Acc. no. 48) to 5.27 cm (Acc. no. 97). Pedicel length also showed variation among accessions (Plate 11). Minimum length was 0.84 cm (Acc. no. 48) and maximum, 2.31cm (Acc. no.9). Spikes of all the accessions were mildly fragrant.

Flowers were arranged on spike and were fused laterally. They were hirtellus in texture and had peltate orbicular bracts. Spikes were bitter and pungent in all the accessions (Table 16). Weight of a fresh spike varied from 0.27 g (Acc. no. 48) to 1.404 g (Viswam). Dry weight of a spike varied from 0.085 g (Acc. no. 2) to 0.257 g (Viswam).

Of the twelve accessions planted in the field, seven including checks flowered. Yield per plant was recorded in seven accessions including checks which gave recordable yield. Five accessions did not flower in the field during the period under report. There was considerable variation in yield of the accessions varying from 2.76g in Acc. no. 95 to 87.39g in Viswam. Per cent dry weight varied from 13.0 (Acc. no. 95) to 18.07 (Viswam) (Table 17).

4.3.1.4 Quality characters

Essential oil, oleoresin and piperine were estimated only for three accessions, which recorded good flowering. As can be seen from Table 18, Viswam recorded maximum oil content (1.25 %) followed by Acc. no. 9 (0.75%) and pure

female (0.60 %). Oleoresin content of the accessions are given in table 18. Acc. no. 9 recorded maximum oleoresin content (11.75%) followed by Viswam (11.4%) and pure female check (9.8%). Maximum piperine content was recorded in Viswam (4.1) followed by acc. no. 9 (2.0) and pure female (1.2).

4.3.2 Incidence of pests and diseases

Pests and diseases incidence was recorded in the accessions planted in the field for preliminary evaluation trial. The major disease noticed was leaf spot caused by *Colletoteichum gloeosporioides* (Plate 12). Disease was severe during rainy season. As can be seen from Table 19, incidence of disease was 41.67 % in acc. no. 53 and acc. no. 77 and 8.33% in acc. no. 9, Viswam and pure female.

Leaf spot caused by *Myrothecium* sp. was also observed. Attack of two tailed mealy bug and scales was observed in the accessions maintained in pots, but they were not severe. Spike eating caterpillars were observed on pure female types, under potted condition. Minor attack of papaya mealy bug was observed under potted conditions in shade house (Plate 13).

Table 16. Spike characters of field planted *Piper longum* accessions

Sl. no.	Acc. No.	No. of spike bearing branches per stem	Spike orientation	Spike shape	Immature Spike colour	Colour change on ripening
1	2	0.04^{efg}	Erect	Filiform	Green	Green
2	9	2.26^{b}	Erect	Cylindrical	Yellow colour	Dark green
3	16	0.56^{d}	Erect	Globular	Greenish yellow	Dark green
4	32	0.22^{ef}	Erect	Cylindrical	Greenish yellow	Green
5	33	0.017^{fg}	Erect	Cylindrical	Greenish yellow	Green
6	48	0.25^{e}	Erect	Globular	Greenish yellow	Green
7	53	0.10^{efg}	Erect	Filiform	Green	Yellowish green
8	77	0	-	-	-	-
9	95	0.63^{d}	Erect	Cylindrical	Greenish yellow	Green
10	97	0.16^{efg}	Erect	Filiform	Green	Yellowish green
11	Pure female	2.003^{c}	Erect	Cylindrical	Greenish yellow	Dark green
12	Viswam	2.83^{a}	Erect	Cylindrical	Greenish yellow	Dark green

Table 16. Spike characters of field planted *Piper longum* accessions *contd…*

Sl. No.	Acc. No.	Spike fragrance	Type of hermaphroditism	Flower arrangement on spike	Spike texture	Bract type	Spike taste
1	2	Present	Predominantly staminate flowers	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
2	9	Present	Pistillate flowers only	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
3	16	Present	Pistillate flowers only	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
4	32	Present	Pistillate flowers only	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
5	33	Present	Pistillate flowers only	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
6	48	Present	Pistillate flowers only	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
7	53	Present	Andromonoecious	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
8	77	-	-	-	-	-	-
9	95	Present	Pistillate flowers only	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
10	97	Present	Andromonoecious	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
11	Pure female	Present	Pistillate flowers only	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter
12	Viswam	Present	Pistillate flowers only	Fused laterally	Hirtellus	Peltate orbicular	Pungent & bitter

Table 16. Spike characters of field planted *Piper longum* accessions *contd…*

Sl. No.	Acc. No.	Spike length (cm)	Pedicel length (cm)	Fresh weight of a spike(g)	Dry weight of a spike (g)
1	2	4.95^{c}	1.27^{d}	0.573^{f}	0.085^{f}
2	9	5.1^{b}	2.31^{a}	1.325^{b}	0.232^{b}
3	16	3.3^{h}	0.91^{e}	1.238^{c}	0.194^{c}
4	32*	4.1^{f}	1.29^{d}	0.776^{e}	0.121^{d}
5	33*	4.2^{f}	1.78^{bc}	0.604^{f}	0.0935^{f}
6	48*	1.3^{i}	0.84^{c}	0.270^{h}	Negligible
7	53*	4.34^{e}	1.65^{c}	0.324^{g}	0.043^{g}
8	77	-	-	-	-
9	95	3.76^{g}	1.4^{d}	0.894^{d}	0.116^{e}
10	97	5.27^{a}	1.32^{d}	1.246^{c}	0.187^{c}
11	Pure female	4.76^{d}	1.84^{b}	0.907^{d}	0.145^{d}
12	Viswam	5.15^{b}	1.67^{bc}	1.404^{a}	0.257^{a}

* Flowering stage not attained in field, spike observations recorded from plants in pots

Table 17. Yield characters of field planted *Piper longum* accessions

Sl. No.	Acc. No.	Yield / plant (g)	Driage (%)
1	2	4.842	14.98[e]
2	9	51.29	17.56[b]
3	16	3.982	15.59d
4	32	*	15.51[d]
5	33	*	15.49[d]
6	48	*	-
7	53	*	13.27[f]
8	77	*	0
9	95	2.76	13.00[g]
10	97	3.217	14.99[e]
11	Pure female	23.10	16.04[c]
12	Viswam	87.39	18.07[a]

Table 18. Quality characters of field planted *Piper longum* accessions

Sl. No.	Acc. No.	Essential oil (%)	Oleoresin (%)	Piperine (%)
1	9	0.75	11.75	2
2	Pure female	0.60	9.8	1.2
3	Viswam	1.25	11.4	4.1

Table 19. Incidence of leaf spot diseases in field planted *Piper longum* accessions

Sl. No.	Acc. No.	Incidence of leaf spot(%)
1	2	33.33^{b}
2	9	8.33^{e}
3	16	16.67^{d}
4	32	25.00^{c}
5	33	16.67^{d}
6	48	33.33^{b}
7	53	41.67^{a}
8	77	41.67^{a}
9	95	25.00^{c}
10	97	33.33^{b}
11	Pure female	8.33^{e}
12	Viswam	8.33^{e}

Viswam

Pure female

Acc. No. 97

Acc. No. 6

Acc. No. 9

Acc. No. 95

Plate 11. Spike characters of field planted *Piper longum* accessions

Leaf spot
Colletotrichum gloeosporioides

Leaf spot
Myriothecium sp.

Plate 12. Incidence of leaf spot diseases in *Piper longum*

Leaf scale
Lepidosaphaes piperis

Papaya mealy bug
Paracoccus marginatus

Plate 13. Incidence of pests in *Piper longum*


Discussion

5. DISCUSSION

Piper longum L. (long pepper or thippali) is an important medicinal plant, used in many ayurvedic preparations. The species is reported to be dioecious. Female spikes are the officinal part. Even though the species is well suited for cultivation in coconut and arecanut gardens and even in rubber plantations, cultivation is not fast spreading due to low return from the crop. Spikes are generally collected from wild. One of the reasons for low returns is short length of female spikes. To improve cultivation of *Piper longum*, an attempt was made to evolve superior types by utilising 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 (2011). Present study was taken up as a continuation of these works. In the present study, production of hybrids and back crosses were continued. The hybrids produced were initially evaluated in pots and the promising ones were further multiplied and planted in the field for a preliminary evaluation. The results of the investigations are discussed in this chapter.

5.1 DEVELOPING HYBRIDS AND BACK CROSSES

5.1.1 Inducing bisexual flowers using growth regulators

For inducing more bisexual flowers in the bisexual variant, the growth regulators *viz*., NAA 50 mg l^{-1}, NAA 100 mg l^{-1} and BA 500 mg l^{-1} were applied at monthly intervals for three months. Growth regulators and their concentrations were carefully selected based on the earlier reports on their ability to induce femaleness (Kanimozhi, 2010). From the results it is clear that, of the three growth regulators tried inflorescence production was obtained in BA 500 mg l^{-1} treated plants only. When the plants were sprayed with two specified concentrations of NAA, deformation of vegetative characters like flaccidity, down ward curling and

dropping of leaves followed by stunting and in severe cases death of the plants were noticed. Some plants rejuvenated after a few months from the harmful effects of growth regulator treatments. Similar effects on treatment with NAA were reported by Minima (1938) in cucurbits and Kanimozhi (2010) in *Piper longum*.

As can be seen from table 2, there was only a marginal increase in bisexual flowers in BA 500 mg l^{-1} treated plants. Even though, Kanimozhi (2010) got 34.8 bisexual flowers in a spike on growth regulator application, in the present study, number of bisexual flowers in an inflorescence ranged from 3.9 to 13.8 only. The results of this study indicated that induction of bisexual flowers using growth regulators is neither reproducible nor it is a viable method to improve spike setting in *Piper longum*.

5.1.2 Reciprocal crosses using bisexual types as male and female parents

Crosses were done using bisexual types either as male or as female parents

5.1.2.1 Bisexual type as female parents

Pure male types were selected as male parent. Crosses were made during June 2011 to October 2011. Method of pollination was dry method, because spike setting during rainy weather was found to be better in this method (Kanimozhi, 2010). When bisexual type was used as female parent, spike setting was not observed in any of the crosses. This could be either due to lack of seed set in the bisexual type or may be due to lack of sufficient number of bisexual flowers in the spike to promote fruit development and fruit maturity. Another reason may be that the gynoecium present in the bisexual type is rudimentary or defective. Etampawala *et al.* (2002) reported about 50 per cent fruit shedding even in female types of *Piper longum*.

5.1.2.2 Bisexual types as male parents

Using bisexual type as pollen parent and pure female type as female parent, crosses were made to get hybrid seeds. Two methods of pollination were done, namely dry during wet months and wet (using distilled water) during dry months. Pollinations were done from June 2011 to February 2012.

5.1.2.2.1 Bisexual type I as male parent

Results of the study indicated that maximum per cent of spike set was obtained during October and November using wet method of pollination (Fig1). However, the seed set was maximum during July followed by September under dry method of pollination (Fig 2).

5.1.2.2.2 Bisexual type II as male parent

When bisexual type II was used as male parent, per cent of spike set was maximum during December followed by October, September and August (Fig 3). However, seed setting was maximum during September (16 numbers) followed by October (one) (Fig 4). Maximum spike set was observed in wet method, followed by dry method.

Kanimozhi (2010) also got high per cent of seed set during September, under dry method of pollination. However, Kanimozhi got poor seed set during July in both wet and dry methods of pollination, unlike in the present study.

Seed set was found to be better when bisexual type I was used as male parent as compared to bisexual type II. The reason for the same could be difference in the pollen viability or any other genetic factor between the two bisexual types. Further

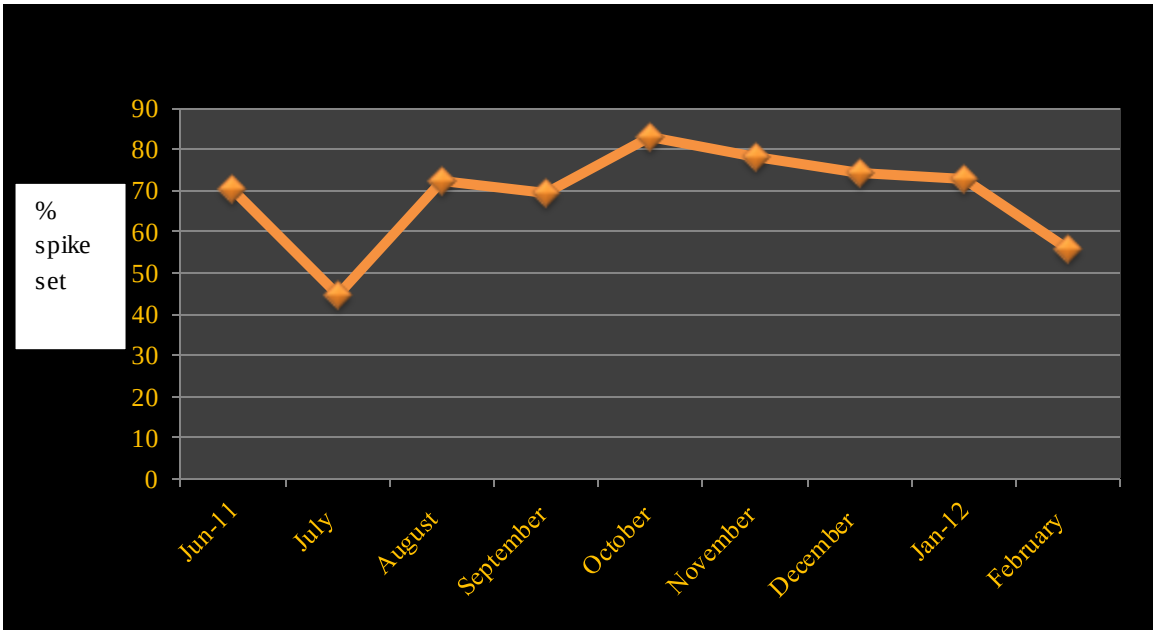


Fig 1. Monthly variations in spike set when bisexual type I as male parent

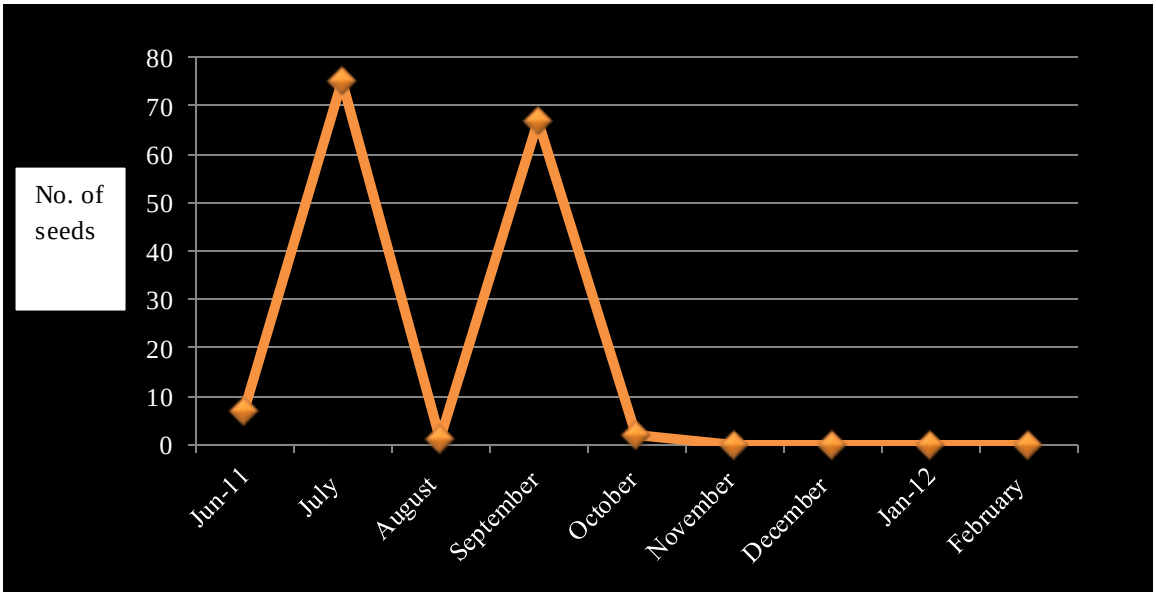


Fig 2. Monthly variations in seed set when bisexual type I as male parent

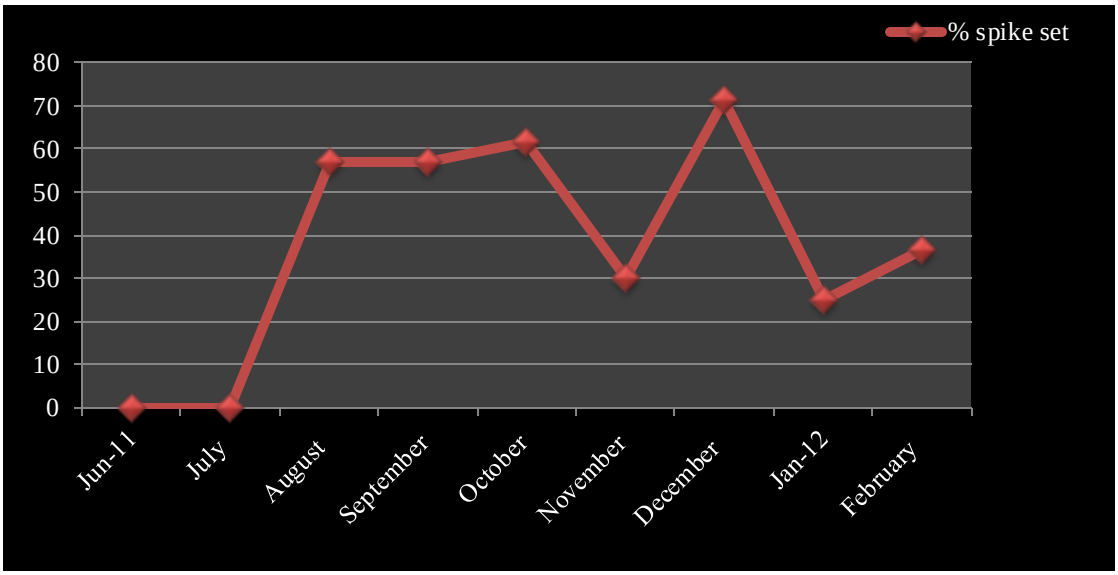


Fig 3. Monthly variations in spike set when bisexual type II as male parent

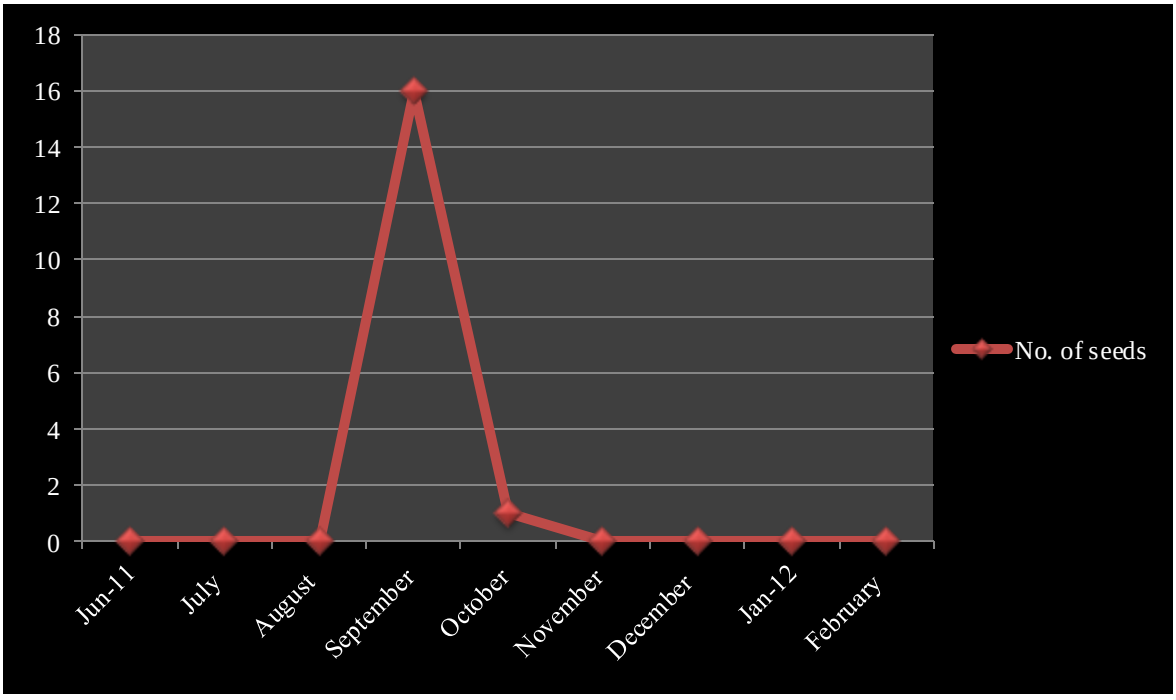


Fig 4. Monthly variations in seed set when bisexual type II as male parent

detailed studies are required to throw light in to the difference in performance of two bisexual types as pollen parent.

5.1.3 Back crossing predominantly bisexual hybrids with female and bisexual parents

Back crossing was attempted using pure female type as female parent and predominantly bisexual hybrids as male parent and also predominantly bisexual hybrids as female and bisexual type I and II reported as male parent. Even though spike set was obtained, when predominantly bisexual hybrids were used as male parent and pure female as female parent, no seed set was obtained (Fig 5). Further detailed study is warranted in this regard to improve seed set when either pure female or bisexual types are used as female parents.

5.2 EVALUATION OF HYBRIDS AND BACK CROSS PROGENIES

In this experiment, hybrids produced during 2009-2010 (73 numbers) and those produced in the present study (17 numbers) were evaluated in pots. Vegetative and reproductive characters of hybrids were evaluated. The hybrids showed high variability for growth and reproductive characters. Even though production of back cross was proposed and attempts were made to produce back cross progeny using predominantly bisexual hybrid with either male or female parent, the results were not encouraging. There was no spike set in such back crosses.

5.2.1 Evaluation of hybrids produced during 2009-2010

These hybrids were produced under earlier project carried out during 2008-2011 (Kanimozhi, 2010; Sujatha, 2011). Initial growth characters of hybrids produced were already recorded under a KSCSTE funded project.

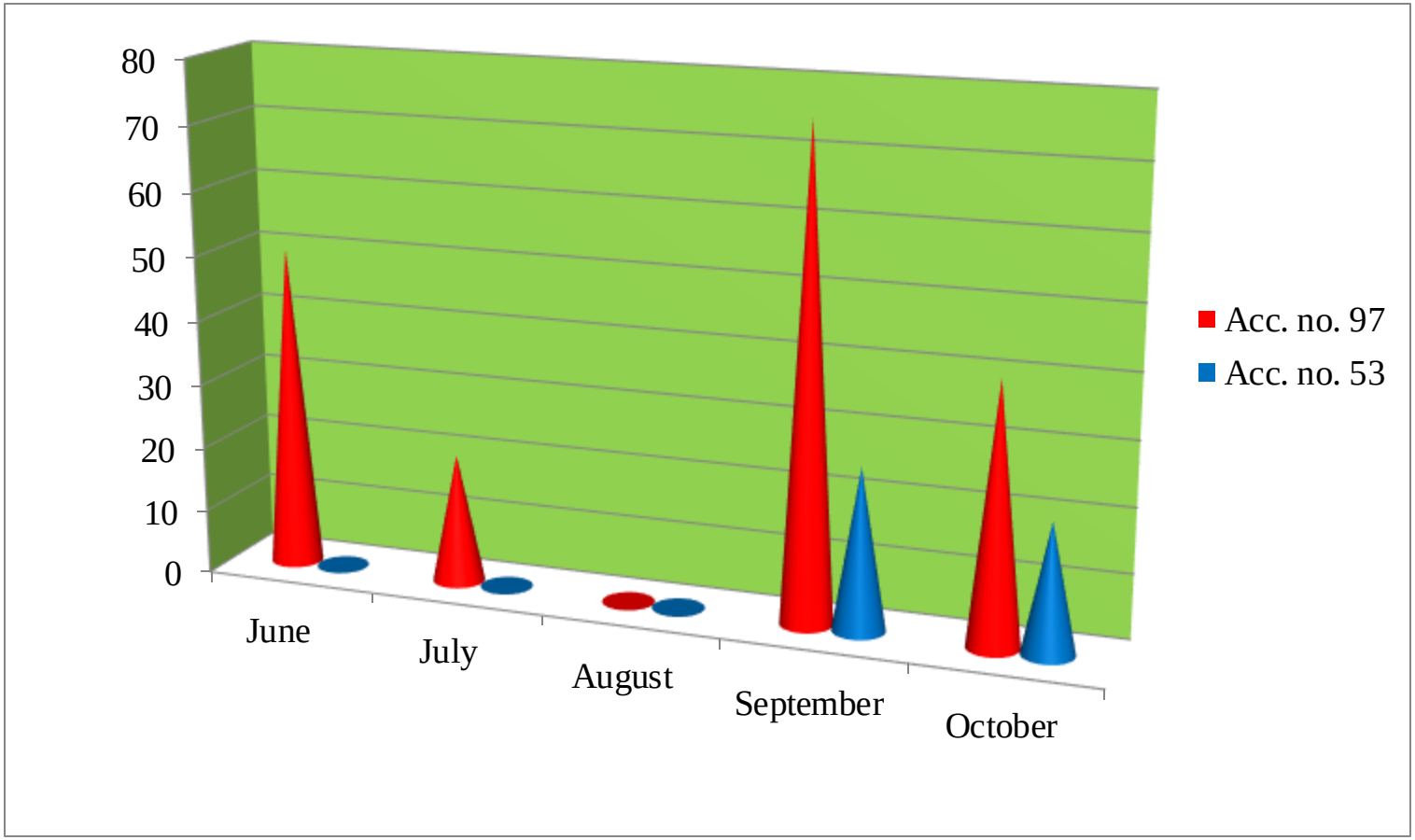


Fig 5. Monthly variation in spike set when predominantly bisexual types (Acc No. 53 and 97) used as male parent

5.2.1.1 Vegetative characters of accessions in pots

In this, detailed analysis of vegetative characters like leaf lamina shape, leaf base shape, leaf margin, leaf texture, venation, leaf colour (both immature and mature), colour of leaf petiole etc were made.

Shape of leaf lamina was mostly codrate, some of them were ovate-elliptic and a few were elliptic-lanceolate. Variation was also observed for shape of leaf base like cordate and oblique. Margins of leaves were either wavy or entire. Venation was acrodromous in all accessions/ hybrids, which is typical of *Piper longum*. However, colour of immature leaves varied from light green to green and mature leaves from green to dark green.

5.2.1.2 Variability in reproductive characters of accessions in pots

Hybrids showed high amount of variability for reproductive characters like sex form, spike characters like orientation, spike shape, size, colour and per cent of bisexual flowers in spikes.

Androecious, gynoecious and andromonoecious (with high percentage of bisexual flowers) sex forms were present in the hybrid population. The number of bisexual flowers produced per spike also varied. Variability in characters like length, fresh weight, dry weight and driage of spikes were also found to be varying. Variability in germplasm collection of *Piper longum* was reported earlier by Jaleel (2006) and Joseph (2008). Sujatha (2011) reported very high amount of variability for vegetative and reproductive characters in the seedling population of *Piper longum* .

5.2.2 Hybrids produced during 2011

A total of 169 seeds were obtained by crossing bisexual type as male parent and pure female as female parent. Out of these 32 seeds germinated. Seeds were

found to germinate in 33 to 71 days. Days taken for opening of cotyledonary leaf varied from 4 to 21. First true leaf emergence was in 10 to 33 days time. In studies conducted by Kanimozhi (2010) and Sujatha (2011) results obtained were similar. High amount of variability was reported for days taken for germination, opening of cotyledonary leaf and emergence of first true leaf.

Mean monthly increment in height showed wide variation ranging from 0.56 cm to 11.8cm. Variation in number of leaves produced and branching were also observed. There was high amount of variability in the seedling progeny of *Piper longum* in all the character studied and results of present study is in agreement with finding of Sujatha (2011).

5.3 PRELIMINARY EVALUATION OF FEMALE AND BISEXUAL TYPES IN THE FIELD

In this experiment, ten accessions which were either female or bisexual were selected from hybrids produced and laid out in field in completely randomized design with four replications. Two types namely, released variety Viswam and accessions used as female parent in crosses were also planted in the experiment as check varieties.

All the accessions were evaluated as per IPGRI descriptor for *Piper* species. There was no variation in characters like plant growth habit, branching type, runner shoot production, pubescence on stem, lateral branch habit, leaf margin, venation, leaf texture, leaf hairiness, spike orientation, spike fragrance, flower arrangement on spike, spike texture, bract type and taste of spikes.

Some of the characters like colour of shoot tip, leaf lamina shape, spike shape, immature spike colour, colour of ripened spike and type of hermaphroditism showed some amount of variation among different accessions. However, variation was very high for quantitative characters like number of main branches, length of

longest stem, length and width of leaves, length of petiole, number of spike bearing branches per stem, spike and pedicel length, fresh and dry weight of spike, yield and driage.

Even though, the key morphological characters for *Piper* species were same for all the accessions typical for *Piper longum*, high amount of variability was observed for other characters. The observations in the present study are in agreement with the earlier reports of Sujatha (2011) that there was very high amount of variability in seedling population of *Piper longum*.

Significant differences were observed among different accessions for the characters such as number of main branches, length of longest stem, length and width of leaves, length of leaf petiole and number of leaves produced by the plants. Variability in these characters were studied by Manuel (1994), Jaleel (2006) and Joseph (2008) in *Piper longum*.

Manuel (1994) conducted a comparative evaluation of five types of *Piper longum* in coconut gardens and significant variability in characters like number of stems/ hill, number of vegetative branches, number of spike bearing branches/stem, angle of insertion of spike bearing branch, length of leaf and petiole and number of leaves/ hill.

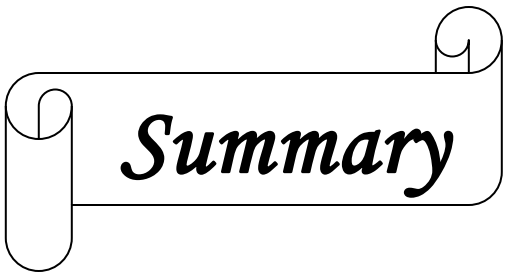
Reproductive characters like number of spike bearing branches, spike characters, length of spike, fresh weight as well as dry weight of spikes, yield per plant and driage were also noted. These are characters which significantly influenced the yield of *Piper longum*. This was agreement with the findings of Manuel (1994), Jaleel (2006) and Joseph (2008).

Number of spikes/ spike bearing branches is an important parameter suggested by Ibrahim *et al.* (1985) and Sujatha and Namboodiri (1995) to assess the yield in black pepper.

Present study also indicated that there is a high variability with respect to quality characters such as essential oil, oleoresin and piperine content. Variability in *Piper longum* accessions for these characters were reported by Manuel (1994), Jaleel (2006) and Joseph (2008).

Preliminary evaluation for growth and yield of selected hybrids indicated that Acc. no. 9 had yield and quality on par with Viswam. This could be further confirmed in comparative varietal trials. Further production of hybrids and their evaluation could be continued so that the huge genetic potential available in seedling progeny of *Piper longum* could be exploited to develop high yielding types.

In the present study, seed set in pollination and germination percentage of seeds obtained were very low. Improvement in seed set and germination could be achieved by refining pollination techniques and *in vitro* method of seed germination. The hybridity of seedlings should be verified and confirmed using molecular markers. Evaluation for yield and quality of promising types and their performance under different shade levels should also be checked, so that the varieties developed could be released for cultivation as an intercrop in coconut and arecanut gardens or rubber plantations.


Summary

6. SUMMARY

Piper longum L. (thippali) is an important medicinal plant used in more than 320 classical preparations. Cultivation of *Piper longum* is not fast spreading due to low spike yield and small length of spikes. In order to get economic returns from the crop, high yielding long spiked genotypes are required. To overcome the bottlenecks in thippali cultivation, study on "Evolving superior types in thippali (*Piper longum* L.) utilising bisexual variants" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period 2010 to 2012. The major objectives of the investigations were production of hybrids and back crosses of *Piper longum* utilising bisexual variants and their evaluation for growth, yield and quality

The results of the study are summarized below:

Effect of growth regulators in developing fully bisexual inflorescence in bisexual type I indicated that only BA 500 mg l^{-1} was effective in inducing bisexual inflorescence and there was a marginal increase in bisexual flowers in BA 500 mg l^{-1} treated plants. When the plants were sprayed with the two specified concentrations of NAA, flaccidity, down ward curling and dropping of leaves followed by stunting was observed.

Hybridisations were carried out using bisexual type as male parent and pure female as female parent. Crosses were also made using bisexual type as female parent and pure male as male parent. Crosses involving bisexual type as female parent were not successful. The percentage of spike set was maximum during October (82.92%) followed by November (78.2%) and December (74.07%) and minimum during July (44.46%) when bisexual type I used as male parent. Number of seeds were maximum during July (75) followed by September (67).

When bisexual type II was used as male parent, maximum spike set was observed during December (71.42%), followed by October (61.53%) and number of seeds obtained was maximum during September (16 numbers) and October (one number).

A total of 169 seeds were obtained under artificial pollination. Out of this 152 seeds were obtained when bisexual type I was used as male parent and 17 seeds when bisexual type II was used as male parent. Seed set was obtained only under dry method of pollination. Back crossing two predominantly bisexual hybrids (Acc. no. 53 and 97) with female and male parents were not successful.

Observations on germination and early growth characteristics of seedlings which were produced during the present study showed that number of days taken for germination of seeds varied from 33 to 71 and that from germination to opening of cotyledonary leaf varied from 4 to 21. The first true leaf appearance was after 10 to 33 days after opening of cotyledonary leaf. Evaluation of hybrids produced in the earlier studies conducted at the department showed high amount of variability for vegetative and reproductive characters, this offers good scope for selection and further improvement.

Preliminary evaluation of ten selected female and bisexual types from the hybrids produced was carried out in the field. Pure female type and released variety Viswam were used as checks. High amount of variability was observed among accessions for number of main branches, length of longest stem, length and width of leaves, length of petiole, number of spike bearing branches per stem, spike and pedicel length, fresh and dry weight of spike, yield and driage. Per plant yield was highest for Viswam followed by Acc. no. 9. Spike characters of Acc. no. 9 were comparable to Viswam. Oleoresin content of Acc. no. 9 was higher even though oil and piperine content were lower. Main disease noticed in seedling population was leaf spot caused by *Colletotrichum gloeosporioides* and pest attack was not severe.

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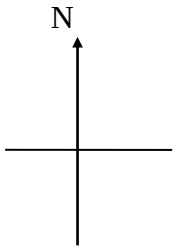
* Originals not seen

Appendices

Appendix I

Layout plan



R3

Acc. No.95	Pure ♀	Acc. No.33	Acc. No.97	Viswam	Acc. no.16	Acc. No.32	Acc. No.9	Acc. No.77	Acc. No.2	Acc. No.48	Acc. No.53

Acc. No.48	Viswam	Acc. No.33	Acc. No.16	Acc. No.53	Pure ♀	Acc. No.9	Acc. No.2	Acc. No.77	Acc. No.97	Acc. No.95	Acc. No.32

R2

R1

Acc. No.33	Acc. no.48	Acc. No.32	Acc. No.2	Acc. No.9	Acc. no.77	Pure ♀	Viswam	Acc. No.53	Acc. No.95	Acc. No.16	Acc. No.97

COCONUT GARDEN ROAD

Appendix II

Genetic cataloguing of long pepper

1. Vegetative characters

1. Growing habit - Climbing / Trailing / Erect
2. Branching habit - Dimorphic / Polymorphic
3. Runner shoot production - Few / Many
4. Pubescence on stem - Absent / Present
5. Lateral branch habit - Erect / Horizontal / Hanging
6. Leaf lamina shape – Ovate / Ovate elliptic / Ovate lanceolate / Elliptic lanceolate / Cordate
7. Leaf base shape - Round / Cordate / Acute / Oblique
8. Leaf margin - Even / Wavy
9. Types of venation – Acrodomous / Camphylodromous / Eucamptodromous
10. Leaf texture - Glabrous coriaceous / Glabrous mamberaneous / Glabrous sarcous / Downy membraneous / Downy along the veins
11. Leaf scales- Absent / Present

2. Inflorescence and fruit characters

1. Spike orientation - Erect / Prostrate
2. Spike shape - Filiform / Cylindrical / Globular / Conical
3. Immature spike colour - Green / Greenish yellow / Light yellow / Light purple / Others
4. Colour change while fruit ripening- Green to black / Green to yellow or orange and then to red
5. Spike fragrance - Not Fragrant / Fragrant

6. Flower arrangement on spike- Free / Fused laterally

7. Spike texture - Glabrous / Hirtellus

8. Bract type - Sessile oblong and adnate to the rachis / Cupular with decurrent base / fleshy, Connate, transformed in to a cup / Deeply cupular with decurrent base / Others

9. Flower nature -Sessile / Shortly stipitate / Pedicellate

10. Fruit shape - Round /Ovate /Oblong /Others

11. Fruit taste - Bitter / Pungent / Spicy

EVOLVING SUPERIOR TYPES IN THIPPALI (*Piper longum* L.) UTILISING BISEXUAL VARIANTS

By

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(2010-12-110)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the

requirement for the degree of

Master of Science in Horticulture

Faculty of Agriculture

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DEPARTMENT OF PLANTATION CROPS AND SPICES

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2012

ABSTRACT

The study on "Evolving superior types in thippali (*Piper longum* L.) utilising bisexual variants" was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period 2010 to 2012. The objective of the programme was production of *Piper longum* hybrids and back crosses and their evaluation for growth, yield and quality. The investigations consisted of three experiments *viz.*, developing hybrids and back crosses, evaluation of hybrids in pots and preliminary evaluation of selected female and bisexual types in the field.

To induce bisexual flowers using growth regulators, NAA 50 mg l^{-1}, NAA 100 mg l^{-1} and BA 500 mg l^{-1} were applied. Vegetative growth and spike production were adversely affected by both concentrations of NAA and there was no inflorescence production in NAA treated plants. In BA 500 mg l^{-1} treated plants, there was a marginal increase in bisexual flowers.

Utilising bisexual type as one of the parents, spike set and seed set could be obtained only in crosses involving bisexual types as male parent and pure female type as female parent. Maximum per cent of spike set was obtained during October followed by November and maximum seed set during July followed by September when bisexual type I was used as male parent. When bisexual type II was used as male parent, per cent spike set was maximum during December followed by October. However, seeds obtained were maximum during September followed by October.

Back crossing was attempted using predominantly bisexual hybrids either as male parent or as female parent. Even though spike set was obtained, when predominantly bisexual hybrids were used as male parent and pure female as female parent, no seed set could be obtained.

Detailed observations on vegetative characters were recorded in the hybrids produced under earlier projects (Kanimozhi, 2010; Sujatha, 2011). Leaf lamina was codrate, ovate-elliptic or elliptic-lanceolate. Variation was also observed for shape of leaf base like cordate or oblique. Margins of leaves were either wavy or entire. Venation was acrodromous in all hybrids, which is typical of *Piper longum*. Colour of immature leaves varied from light green to green and mature leaves from green to dark green. Hybrids showed high amount of variability for reproductive characters like sex form, spike shape, size, colour and per cent of flowers of different sex forms in a spike. Shape of spikes varied from filiform, cylindrical or globular. Colour of immature spikes were green, light green or yellow. Mature spike colour also ranged from light green, green, and dark green or yellow.

During the present study, 169 seeds were produced, of which 32 seeds germinated. Observations on germination and early growth characteristics of seedlings were taken. Number of days taken for germination of seeds varied from 33 to 71 days. Number of days taken from germination to opening of cotyledonary leaf varied from 4 to 21. The first true leaf appeared in 10 to 33 days.

Ten hybrids, either female or bisexual were selected for preliminary evaluation in the field. Two check varieties, pure female type and the variety Viswam were also planted. The design adopted was CRD with four replications. Even though, 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, length and width of leaves, length of petiole, number of spike bearing branches per stem, spike and pedicel length, fresh and dry weight of spike, yield and driage.

Per plant yield was highest for Viswam followed by Acc. no. 9. Spike characters of Acc. no. 9 were comparable to Viswam. Oleoresin content of Acc. no.

9 was superior to Viswam but oil and piperine contents were lower. Pests and diseases scoring were also done. The main disease noticed was leaf spot caused by *Colletotrichum gloeosporioides* and pests were not severe.

Further detailed studies involving more promising hybrids are required to evolve superior varieties with high yield and quality.