EXPLOITATION OF BISEXUAL VARIANT IN DEVELOPING HIGH YIELDING TYPES OF *Piper longum* L.

By

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(2007-12-102)

THESIS

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DECLARATION

I, hereby declare that the thesis entitled **"Exploitation of bisexual variant in developing high yielding types of *Piper longum* L."** is a bonafide record of research work done by me during the course of research and that it has not been previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that the thesis entitled "**Exploitation of bisexual variant in developing high yielding types of *Piper longum* L.** " is a bonafide record of research work done independently by **Miss. K. Kanimozhi** under my guidance and supervision and that it has not formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of **Miss. K. Kanimozhi (2007-12-102)** a candidate for the degree of **Master of Science in Horticulture,** agree that the thesis entitled **"Exploitation of bisexual variant in developing high yielding types of *Piper longum* L."** may be submitted by Miss. K. Kanimozhi, in partial fulfillment of the requirements for the degree.

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Introduction

INTRODUCTION

'Piper longum' L. is also known as Indian long pepper. *Piper* is the most important genus in the family Piperaceae which includes many economically important species like *Piper nigrum* (black pepper), *Piper longum* (thippali) and *Piper betle* (betel vine). The genus name *Piper* was derived from the Sanskrit word pippali for long pepper.

Piper longum is a very important and most extensively used medicinal plant in the ayurvedic system of medicine. It is used in over 320 classical medicinal formulations.

Long pepper is indigenous to South Asian countries including India. It was well known in ancient India and was used as a spice in the early days. Dried female spike which consists of minute fruit embedded in a fleshy rachis is the officinal part in *Piper longum* which is being used in many ayurvedic preparations.

Piper longum can very well be grown as an intercrop in coconut and arecanut gardens. The price realised for the raw drug is also very attractive. In spite of these advantages, cultivation of *Piper longum* is not fast spreading. One of the reasons for this may be the low return from the crop and small length of female spike.

Kerala Agricultural University had released the first improved variety in *Piper longum* in 1996. The yield potential was reported as 472kg/ha. The variety was named Viswam after its late breeder. Till date Viswam is the only improved variety released.

Piper longum was reported to be dioecious. Sujatha and Nybe, (2007) reported a new sex form (bisexual) in *Piper longum*. The bisexual spikes are as long as male spikes which are three to four times of the female spikes. The present study has been proposed to develop stable high yielding bisexual type with long spikes, so that the problem of low yield of the officinal part in cultivation could be overcome.

If a high yielding variety could be developed with spikes as long as the male ones, it would be a breakthrough in thippali cultivation. Identification of a bisexual variant is a ray of hope in this direction. In the related species, *Piper nigrum* (black pepper), bisexual types are highly productive and all the improved varieties have over 90 per cent bisexual flowers.

With this background, the present study on "Exploitation of bisexual variant in developing high yielding types of *Piper longum* L." has been proposed with the following objectives.

1. To study the reproductive biology of *Piper longum* L.
2. To develop long spiked high yielding varieties of *Piper longum* L.

Based on the above study, hybrid / selfed progeny involving bisexual variant will be developed and evaluated for yield and other economic traits. The study in the long run may solve the constraints in the cultivation of the important medicinal species *viz*., *Piper longum*.

Review of Literature

2. REVIEW OF LITERATURE

Studies on different aspects of *Piper longum*, including phylogenetic and systematic studies, characterization, cytogenetics, crop improvement, crop management, crop protection etc are reviewed in this chapter. Use of growth regulators in regulation of sex expression, fruit set, yield, preventing fruit drop etc in crop plants are also included in the chapter.

*Piper longum.*L popularly known as long pepper is used as condiment and also in ayurvedic medicine (Mathew, 1958). This species is found both wild and cultivated in the tropical parts of India, Ceylon and Malaya.

Samuel *et al*. (1980) reported that the two species *Piper nigrum* L. and *Piper longum* L. show distinct foliar, floral and anatomical differences. The shape of leaf, type of venation, presence of trichomes, length and nature of spike, sex composition, shape of bract, length of guard cells and stomatal index are the major distinguishing features of these two species. In *Piper longum*, the spikes are very much short compared to that of *Piper nigrum*. The bracts in *P.longum* are circular in shape.

Joshi (1944) observed that the flowers were arranged almost perpendicularly on the axis of the inflorescence and ovules were orthotropous. Serial transverse sections of the spikes were found to yield perfect longitudinal sections of the gynoecium and the ovule. Martin and Gregory (1962) reported that the anther dehiscence is controlled to a great extent by temperature and relative humidity in Piperaceae. Dewarrd and Zevan (1969) observed that in Sarawak, the flower opening usually takes place between 12.00 and 14.00 h on days where relative humidity of 60 percent is attained and at a temperature of 32^0C, combined with bright sunshine.

Work done in *P.longum* L.

2.1 Phylogenetic and systematic studies in *Piper*

The earliest record of the description of *Piper* of Indian sub continent was by Rheede (1678) in his *Hortus Indicus Malabaricus*, wherein he described five types of wild *Piper* including black pepper and long pepper. Gamble (1925) in his 'Flora of the Presidency of Madras' described 13 species viz., *P. argyrophyllum, P. attenuatum, P. galeatum, P. trichostachyon, P. longum, P. hapinum, P. brachystachyum, P. schmidtii, P. hookeri, P. hymenophyllum, P. barberi, P. nigrum and P. wightii* from South India.

Hooker (1886) had included *P. argyrophyllum* and *P. attenuatum* under the section Eupiper along with *P. nigrum*. *P. galeatum* was included in section Muldera along with *P. trichostachyon. P. longum, P. chaba, P. betle and P. hapinum* were members of section Chavica.

2.2 Morphology

Rahiman (1981) observed the morphological variations among *Piper* spp as follows. All the species were climbers except *P. longum*, which was a creeper. The leaf shape varied from broadly ovate in *P. longum* and *P. betle* to lanceolate or ovate lanceolate in *P. argyrophyllum*. Most of the *Piper* species were unisexual. Inflorescences were cylindrical and erect in *P. longum* and *P. hapnium* (Rahiman, 1981).

2.3 Biometrics

Based on D^2 analysis carried out in both male and female plants of eight species, Rahiman and Bhagavan (1985) found that *P. attenuatum* and *P. argrophyllum* formed one cluster. *P. trichostachyon* was clustering with *P. galeatum*. *P. nigrum* and *P. longum* retained their identity and formed separate clusters indicating that these species were distinct from all other species. Ravindran *et al*. (1992) carried out centroid cluster analysis in 11 species of *Piper* based on observation of 30 characters. This study led to six clusters of *Piper* genus. *P. longum* stood alone, forming a separate cluster.

2.4 Anatomy

2.4.1 Leaf

Datta and Dasgupta (1977 a) reported varying number of layers of cells in hypodermis in *P. nigrum, P. betle* and *P. longum*. According to him, adaxial and abaxial hypodermis were unilayered in *P. longum*. Leaf epidermal studies by Samuel *et al*. (1984) showed that epidermal cells of *P. betle, P. longum* and *P. argyrophyllum* were much larger than that of *P. nigrum* and *P. attenuatum*.

2.4.2 Root

Datta and Dasgupta (1977 b) based on root anatomical studies, proposed the sequential advancement among *Piper* species as *P. longum*, *P. nigrum* and *P. betle*.

2.5 Cytogenetics

2.5.1 Chromosome number

The cytological investigations by various workers showed wide variations in chromosome number of the same species in the genus *Piper*. The chromosome number reported in *P. longum* were 2n = 24 (Tjio, 1948); 2n = 44 (Sampathkumar and Navaneethan, 1981); 2n = 48 (Dasgupta and Datta, 1976); 2n = 52 (Mathew, 1958; Jose and Sharma, 1983, 1984; Rahiman and Nair, 1986; Samuel and Morawetz, 1989); 2n = 96 (Sharma and Bhattacharya, 1959); 2n = 32 (Anand *et al*. 2000).

2.5.2 Karyomorphology

According to Sharma and Bhattacharya (1959), choromosome sizes ranged from 0.6 - 1.7 µ in *P. longum* with 2n = 48 and 0.6 - 2.2 µ for another type with n = 24. The karyotypes of *Piper* species showed a gross uniformity in size with 0.56 - 2.41µ in *P. betle*, 0.56 - 2.05 µ in *P. nigrum*, 0.56 - 1.48 µ in *P. chaba*, 0.74 - 1.9 µ in *P. attenuatum* and 0.74-1.85 µ in *P. longum*.

2.5.3 Species affinities and evolutionary pattern

Sharma and Bhattacharya (1959) had reported that polyploidy might have played a distinct role in the origin of *P. longum, P. nigrum* and *P. betle*. Chromosome numbers were multiples of 12 and 16 in *P. longum* (2n = 24 and 96).

2.6 Chemotaxonomy

The flavonoid analysis carried out by Rahiman and Subbaiah (1984) had revealed that *P. nigrum* and *P. longum* were very distinct among eight species studied. A comparative study of flavonoid profile by Ravindran (1990) and Ravindran and Babu (1994) indicated *P. longum* and *P. mullesua* with 69 per cent affinity and *P. longum* and *P. silentvalleyensis* with 35 per cent affinity.

2.7 Isoenzyme analysis

Isozyme studies (Sebastian *et al*., 1996) showed three groups of closely related species *P. longum* and *P. betle* were distinct from all others.

2.8 Variation and characterization

Banerjee, *et al.* (1999) analysed polymorphism in the genomic DNA of 25 female and six males 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 *P. longum*. This is the first report of genetic or chromosomal basis of dioecy in *Piper longum.*

Philip, *et al.* (2000) reported genotypic and morphogenetic differences among three female varieties of *P. 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 random oligonucleotide primers, revealed that these varieties were genetically different. Compared to the Assam variety, Viswam and Calicut varieties were genetically closer (95% similarity) among themselves. Morphogenetic potential of node, internode and leaf explants from all the three varieties were compared. Leaf explants from different varieties exhibited maximum regeneration potential. Among the types tested, Viswam variety exhibited best morphogenetic response, followed by the varieties from Calicut and Assam. 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 (OPA 10_{825} and $OPA15_{144}$) and one male associated SCAR marker. They sequenced male sex-associated RAPD bands for the development of male-associated SCAR markers in *P. longum*. 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 (per cent) of the female population of *P. 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 *P. longum.*

Ramakrishnappa, *et al.* (2006) using RAPD markers evaluated relatedness of six species of *Piper*. The study also aimed at estimation of the genetic relatedness of these six morpho-agronomically contrasting genotypes. The genetic distance between these six species was calculated based on the RAPD data set as per squared Euclidean distances. Based on the number of bands, all the six species were grouped into three clusters, and the dendrogram revealed maximum similarity between *P. betle* and *P. longum*.

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

Chikkaswamy, *et al.* (2007) studied six species of *Piper,* using RAPD. Based on the number of bands all the species were grouped into 3 clusters and the dendrogram revealed maximum similarity between *P. betle* and *P. longum* and also between *P. nigrum* and *P. mullesua* species, altogether forming one cluster.

Jaleel (2007) carried out characterisation of long pepper (*P. longum* L.*)* 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, whereas reproductive branches had lanceolate leaves with rudimentary petiole. The l/b ratio of leaves of reproductive branches was higher compared to leaves of vegetative branch. The spikes of females were short, bold and greenish black on maturity whereas it was long, slender and yellow in the case of males. At early stages of spike growth, female spikes were creamy yellow and male, green in colour.

The stem, leaf and root anatomy of the male and female accessions showed almost similar features. The difference was noticed in the number of medullary and cortical bundles and the presence or absence of mucilage canals. A mucilage canal was present only in the female accessions, Assam, NL-84-68 and Viswam. The male and female spikes differed in their anatomical features. The physiological parameters revealed higher photosynthetic rate

for the female accession, Viswam. The male accession had lower photosynthetic rate compared to female accession.

RAPD analysis done on eight accessions using five selected primers revealed 15 to 49% variability among the accessions at the molecular level and the primer OPF5 produced male specific bands.

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

2.9 *In vitro* culture

Bhat and Chandel (1992) reported that the competent callus was initiated around the nodal ring of *in vitro* grown shoot explants using MS medium supplemented with NAA 1.0 mg $^{-1}$ and benzyladenine 0.2 mg $^{-1}$. Optimum growth regulator concentrations for shoot induction and shoot elongation were 0.5 mg IAA with 1.5 mg benzyladenine, and 0.1 mg IAA with 0.2 mg benzyladenine/l respectively. Elongated shoots were rooted on half-strength MS medium with 0.1 mg IAA. The rooted plants successfully established in soil.

Parani, *et al.* (1997) reported that multiple shoots were induced from nodal segments of mature plants of *Piper longum* in MS medium supplemented with 0.1-2.0 mg BA [benzyladenine] and 0.1-0.5 mg kinetin/liter. Maximum shoot proliferation without callus formation was observed in MS medium supplemented with 0.5 mg BA and 0.5 mg kinetin/litre. Shoots were cut into segments and rooted on half-strength MS medium. Comparison of RAPD fingerprints of 20 micropropagated plants and the mother plant with 10 decamer primers indicated polymorphism in 37 out of 65 products, with only one primer producing all monomorphic fragments.

2.10 Crop management

Bhuse, *et al.* (2002) reported that long pepper cuttings planted during June exhibited approximately 70% rooting, while those planted in February recorded a rooting percentage of almost zero. Cuttings with leaves recorded significantly higher values for rooting percentage

(78.33%), number of roots (11.70), root length (13.59 cm) and vine length (22.47 cm) than cuttings without leaves. Among the plant growth regulators (IBA and NAA at 100, 200 and 300 ppm), IBA at 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 treatment interactions, cuttings with leaves treated with IBA at 100 ppm exhibited the highest rooting percentage (96.66%) and number of roots (15.44).

Etampawala, *et al.* (2002) studied performance of vegetative stem cuttings comprising the two uppermost nodes and the cuttings obtained from vertically growing reproductive parts of the plant which showed that they were the appropriate types for propagation. *P. longum* plants grown under 50 per cent shade (maximum instantaneous light intensity 850 micro mol $m^{-2}s^{-1}$) performed well, compared to plants grown under 25 and 75 per cent shade, respectively. Planting medium comprising sand, top soil and farmyard manure in the ratio of 1:1:1 was found as the best substratum for the growth of *P. longum* plants. Plants raised from vertically grown branches produced fruits earlier compared to those from horizontally grown branches. However, nearly 50 per cent of fruits were shed from the mother plant in about 22 days after their emergence.

Krishnan (2003) reported that integrated nutrient management system involving incorporation of vermicompost @ 6.25 t ha $^{-1}$ yr $^{-1}$ 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.

Attempts were made to develop suitable cultural practices for obtaining higher yields in three locally available selections (Selections 1, 2 and 3) of *Piper longum*. (Pathiratna, *et al.,* 2005). The effects of plant pruning and training methods, shade and the type of cutting on the production of reproductive branches and spikes were studied. Pruning of runners and/or training methods were important for the increased production of reproductive branches and spikes. Pruning of runners in Selection 1 produced more reproductive branches and spikes. Restriction of the 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 with selection 3. A shade level of around 50 per

cent under field conditions, gave good growth and highest spike yields 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.

Vegetative, reproductive and biochemical 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 for nine characters like number of vegetative branches per stem, number of spike bearing branches per stem, total number of leaves per hill, number of spikes per spike bearing branch, length of spike, girth of spike, fresh weight of spike, dry weight of spike and fresh yield per plant.

Comparative evaluation of five selected types of *Piper longum* L. namely Cheematippali, Panniyur, Mala, Pattambi and Kaanjur was carried out at the Department of Agricultural Botany, College of Horticulture, Vellanikkara during the year 1990-92 (Manuel, 1994). The study revealed that the five types of *Piper longum* differed for eleven vegetative characters namely, length of the longest stem, number of leaves per hill, number of stems per hill, number of vegetative branches per stem, length of main stem, number of spike bearing branches per stem and angle of insertion of spike bearing branch and for three productive characters namely, number of spikes per spike bearing branch, yield of green spike and yield of dry spike at one or all of the stages for which observations were recorded. Correlation studies and path analysis revealed that angle of insertion of spike bearing branch, number of stems per hill, number of spikes per spike bearing branch, number of spike bearing branches per stem and yield of green spike were the most important characters influencing dry spike yield. Cheemathippali showed consistently superior performance for all the important characters at all stages.

Ayisha (1997) reported that growth and yield characteristics of *Piper longum* increased with an application of 20t ha^{-1} organic manure and 30:30:60 NPK kg ha^{-1} .The optimum spacing was found to be 60 × 60 cm. The growth and yield of the crop was poor in dry months and the peak yield was obtained at 17 MAP. After that there was a general decline. The two peak bearing stages were identified during July – August and October-

November months. The NPK uptake was higher in plots receiving 20 t ha^{-1} organic manure and 30: 30: 60 NPK kg ha^{-1} , under a spacing of 60 × 60 cm.

Economic analysis of thippali cultivation revealed that it could be a profitable intercrop in coconut gardens if planted under a spacing of 60 × 60 cm with an application of organic manure @ 20 t ha^{-1} and fertilizer @ 30:30:60 NPK kg $ha^{-1.}$

Sujatha and Nybe (2007) reported a bisexual variant in *Piper longum* which had spikes as long as male type. Anthesis in bisexual variant reported was between 9.00 am to 11.00 am which was extended in cloudy weather, even though there was profuse production pollen, viability could not be attained in different growing media.

2.11 Crop protection

Chourasia and Roy (1989) studied the effect of RH (33-96%) on fungal association and aflatoxin production in stored *P. longum* fruits. Fungi were divided into 4 groups: group I (*Aspergillus flavus, A. niger, Alternaria alternata, Fusarium oxysporum* and *Rhizopus stolonifer*) were recorded at 33-96% RH; group II (*Aspergillus candidus, A. nidulans, Curvularia lunata* [*Cochliobolus lunatus*] and *Penicillium* sp.) were recorded at 75-96 % RH; group III (*A. ochraceus* and *Fusarium moniliforme* [*Gibberella fujikuroi*]) were recorded at 33-55% RH; and group IV (*Trichoderma viride*) had rare occurrence. Production of aflatoxin B_1 was high at 75-96 % RH. The higher the RH, the higher the fungal incidence and the greater the level of aflatoxin production in stored samples.

Roy and Chourasia (1990) studied the effect of temperature on aflatoxin B_1 (Afl-B_1) production by *Aspergillus flavus* on *P. longum* fruits. The highest level of Afl-B_1 (1.25 micro g/g) production was at 30 ^{0}C after 3 weeks of incubation. At 20, 25, 35 and 40 ^{0}C aflatoxin levels ranged from 0.22-1.00 microg/g. However, at 15^0 C aflatoxin production was much lower (0.12-0.24 microg/g).

Lee (2001) reported that the fungicidal activity of *Piper longum* fruit-derived materials towards six phytopathogenic fungi, *Pyricularia oryzae*, *Rhizectonia solani*, *Botrytis cinerea*, *Phytophthora infestans*, *Puccinia recondita*, and *Erysiphe graminis*, was tested using a whole plant method *in vivo*. It was compared with synthetic fungicides (chlorothalonil, dichlofluanid and mancozeb) and four commercially available compounds (eugenol, piperine, piperlongumine, and piperettine) derived from *P. longum*. The response varied with the plant pathogen tested. At 1 mg ml^{-1}, the hexane extract of *P. longum* showed fungicidal activities

against *P. oryzae*, *B. cineria*, *P. infestans*, and *P. recondita* with the control values of 33, 15, 40, and 100 per cent, respectively. A piperidine alkaloid, pipernonaline, was isolated from the hexane fraction using chromatographical techniques and showed a potent fungicidal activity against *P. recondita* with 91 and 80% control values at the concentration of 0.5 and 0.25 mg ml^{-1}, respectively. Structural elucidation of pipernonaline was by means of MS, ^{1}H-NMR and ^{13}C-NMR. In the test with commercially available components derived from *P. longum*, piperettine exhibited weak activity against *E. graminis*, but no activity was observed from treatments with eugenol, piperine, and piperlongumine. In comparison, potent fungicidal activity was registered with chlorothalonil against *P. infestans* at 50 micro g ml^{-1}, dichlofluanid against *B. cinerea* at 50 micro g ml^{-1} and mancozeb against *P. recondita* at 5 micro g ml^{-1}. These results may be an indication of at least one of the fungicidal actions of pipernonaline, derived from *P. longum* fruit.

Bhat, *et al*., (2004) observed a virus causing mosaic mottling in Indian long pepper (*Piper longum*) which was isolated and identified as an isolate of cucumber mosaic virus (CMV) based on biological, physicochemical and serological properties. The virus isolate was easily sap transmissible to *Chenopodium album*, *Nicotiana benthamiana*, *N. glutinosa*, *N. tabacum* cv. White Burley, *Physalis floridana* indicator hosts and a few other cultivated plant species including black pepper. The virus was purified from tobacco and negatively stained purified preparations contained isometric particles of about 28 nm in diameter. The molecular weight of the viral coat protein subunit was 25.6 kDa. The leaf extract from diseased *Piper longum* leaves and purified preparations showed positive reaction with polyclonal antiserum of CMV both in direct antigen-coated enzyme linked immunosorbent assay (DAC-ELISA) and electro-blot immunoassay (EBIA). Particle morphology, antigenic relationships and molecular weight of coat protein suggest that the virus associated with Indian long pepper is an isolate of CMV.

Alam and Deshmukh (2006) investigated the biochemical changes in *P. longum* due to *C. gloeosporioides* [*Glomerella cingulata*] infection. Potato dextrose agar media was found to support the maximum growth of the test isolate. Among the amino acid-containing media, the SB agar was the best. The sugar, total phenol, chlorophyll and protein contents of the *C. gloeosporoides* - infected parts decreased.

The species of root knot nematode attacking thippali was identified as *Meloidogyne arenaria* (Neal, 1889, Chitwood, 1949). 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 bioagents viz. *B. subtilis, T. viride, P. fluoresences*, and AMF improved the growth of thippali with maximum vine length, number of leaves, number of branches, root length, shoot and root weight and minimum root knot index, gall formation and nematode population in root and soil. Early spike formation and also an increase in number of spikes were observed in plants treated with *B. subtilis* and *P. fluorescens* respectively. The control of root knot nematode achieved as a result of application of biocontrol agents was superior to that due to carbofuran application. The study clearly indicated that the root knot nematode population in *P. longum* can be effectively managed using the bioagents and is a better alternative to nematicide application.

Poornima (2007) reported that the cross inoculation studies conducted using *C. gloeosporides* isolate of thippali, black pepper and betel vine – showed that the thippali isolate was highly host specific. The colony and conidial morphology of the three isolates also showed considerable differences. *C. 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 *C. gloeosporioides, T. viride* and *A. terreus* were found to be most effective under *in vitro* condition. Among the different resistance inducers tested *in vitro* SA (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 (T_2T_5 - *A. terreus* + neem cake) was found to be best in disease suppression at 45 DAT.

2.12 Insecticidal properties

Lee *et al.* (2002) reported that the toxicities of two piperidine alkaloids, pipernonaline and piperoctadecalidine, isolated from *Piper longum* L. were determined against five species of arthropod pests. The most potent insecticidal activities of both alkaloids, pipernonaline (LD50 = 125 mg/l) and piperoctadecalidine (LD50 = 95.5 mg/l), were against *Spodoptera litura* F. (Lepidoptera: Noctuidae). Both alkaloids also showed insecticidal activities towards *Myzus persicae* Sulzer (Hemiptera: Sternorrhynche: Aphididae). Piperoctadecalidine (LD50 = 246 mg/l) but not pipernonaline showed acaricidal activity against *Tetranychus*

urticae Koch (Acari: Tetranychidae). Neither compound showed insecticidal effects on *Nilaparvata lugens* Stal (Hemiptera: Fulgoromorpha: Delphacidae) or *Plutella xylostella* L. (Lepidopetera: Yponomeutoidae).

2.13 Use of growth regulators in sex expression and sex regulation in horticultural crops

Auxin (Naphthalene Acetic Acid) (NAA)

Laibach and Kribben (1950) reported that the auxin application resulted in the development of female flowers instead of male flowers in cucurbitaceous plants. The sex ratio was calculated as per method suggested by Singh (1954). Mitchell and Wittwer (1962) also reported that auxins promoted femaleness in sweet lime. Subhadrabandhu *et al.* (1997) reported that in pawpaw (papaya), seedlings grown in plastic bags were sprayed until runoff with 100 ppm NAA, 500ppm GA_3 or distilled water at 30 and 60 days after sowing. Among the treatments, NAA (100 ppm) treatment increased female plants (113/109 and sex ratio 0.34/0.33) as compared to control. In bottle gourd, application of NAA 20 ppm, GA (5 ppm) and triacontanol (4 ppm) were most effective for early induction of female flowers and high sex ratio (14.10 to 19.05per vine) (Kore *et al*., 2003).

Shekhargouda and Kukanoor (2005) reported that application of NAA 50 ppm, at 2^{nd} and 4^{th} leaf stage, flower initiation stage and 15 days after flower initiation increased female flowering in bitter gourd. Hilli *et al.* (2008) reported that application of NAA 50 ppm took minimum number of days to first pistillate flower appearance (37.98 and 31.59) and 50% flowering (59.76 and 50.25) compared to other growth regulators, (GA 50 ppm, Ethrel 500 ppm) during both summer and kharif season, in bitter gourd.

Ethrel (2-chloroethane phosphoneic acid)

Rudich *et al.* (1969) observed that application of ethrel, to monoecious cultivars of cucumber and squash and andromonecious cultivars of muskmelon caused a shift towards femaleness in all the three species. Studies conducted by Robinson *et al.,* (1970) showed that application of ethrel at 250 ppm promoted pistillate flower formation in cucurbits. Arora and Partap, (1988) opined that application of ethephon at 250mg recorded high female flowers in pumpkin. Ethrel at 50 and 100 ppm increased the sex ratio (female: male 1:1 to1: 0.33) in pointed gourd, *Trichosanthes dioica* Roxb. (Sarkar *et al*., 1989). Studies conducted by Damodhar *et al.* (2004) revealed that application of ethrel at 200 ppm increased female flower production and considerably reduced male flowers in bitter gourd.

Gibberllic acid (GA_3)

Ursula and Fuchigami (1990) observed that the effect of GA_3 (100 mgl^{-1}) on flowering is dependent on the stage of flower bud development in coffee. Damodhar *et al.* (2004) reported that sex ratio was significantly higher with the application of GA_3 at 25 ppm and 50ppm in bitter gourd (*Momordia charantia* L.) cv. Hirkani.

Cytokinin

The cytokinin 6 benzylamino - 9 - (2 tetrahydropyranyl) purine BTP induced hermaphrodite flowers in staminate plants of *Vitis vinifera* (Negi and Olmo, 1966) and *Vitis thumbegii* (Iizuka, 1967). Cytokinins can alter sex ratio in species with imperfect flowers. Cytokinins generally increased the ratio of female flowers to male flowers which has implications for fruit production (Halmann, 1990). In bitter gourd (*Momordia chanratia L.*), foliar spray of boron 4 ppm increased the number of pistillate flowers (31.2) per plant (Verma *et al.,* 1984).

2.14 Use of plant growth regulators in fruit set, fruit development and yield

Naphthalene acetic acid (NAA)

Stimulatory effect of auxin on the development of the orchid embryo sac has been observed by (Heslop-Harrison, 1957). He found that auxin introduced to the orchid ovary by pollination, triggers the entire development of embryo sac. Thus it was concluded that until and unless the flower is pollinated or supplied with an external source of auxin, the orchid embryo sac failed to develop beyond the single cell stage. The profound positive influence of auxins on the maturation of the onion anther sac was demonstrated by Vasil (1957).

Application of planofix (90,120 and150 ppm) enhanced the berry size in black pepper (Pillai *et al.,* 1977). Number of berries per unit length of spike, berry volume and berry weight were increased and spike shedding decreased by 52.2 percent with the application of 50 ppm planofix (Geetha and Nair, 1990) She also observed increased oleoresin content of berries per spike and five times higher yield in pepper with application of Planofix (20,40 and 60 ppm). Application of NAA was also found to reduce the harvesting duration in pepper (Salvi and Desai, 1989). It took minimum number of days (eight) from first picking to last picking as against 15days, in the control. The green berry weight and dry recovery

percentage of berries also increased with the application of NAA (10 ppm and 100 ppm respectively).

In mango, application of NAA was reported to result in enhanced fruit set, fruit retention, fruit weight, fruit length, fruit diameter, fruit volume and quality (Singh and Ram, 1983).The highest retention of fruits was recorded in coffee plants with 10 ppm NAA (Raghuramulu *et al*., 1990). In grape, application of 25 ppm NAA resulted in highest bunch weight, number of berries per bunch, bunch width and berry diameter (Farooq and Hulamani, 2000). In mandarins, 300 ppm NAA decreased the fruit number and increased the fruit size without affecting the total yield (Greenberg *et al*., 2000).

Gommase *et al.* (2004) reported that, NAA 300 ppm was the best for increasing fruit set (27.96%), fruit retention (22.13%) per shoot, number of fruit and weight of fruit per plant (1868.33fruits and 117.03kg) at pea size fruit stage in sapota. Kanthaswamy (2006) reported that NAA 20 ppm recorded highest number of fruits per tree, both in summer (171.6) and rainy season (150.3) in PKM 2 moringa. 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 Brix) in Mango cv. Langra.

Ethrel

Ethephon at 250 mg/l gave maximum fruit yield (29.76 tonnes / ha) in long melon. (Arora *et al.,* 1994).

Gibberllic acid (GA_3)

Williams and Stanly (1969) observed that exogenous application of both cytokinines and gibberellins on delicious type of apple had enhanced fruit growth and well developed calyx lob. Reddy and Srinivasan (1979) reported that GA_3 treatment improved the fruit set in Arabica coffee. Singh *et al*. (1985) noted that application of GA_3 at 25 and 50 ppm improved the fruit set and fruit yield (72.13g) in phalsa.

Ram and Rao (1997) suggested that application of GA_3 at 50 ppm was more effective in papaya by increasing the fruit set (53.06%), fruit size (20.65cm x 17.57cm), flesh thickness (3.70 cm) and fruit yield (540.259 kg/ha). The effect of plant growth regulators in fruit set of tomato under high temperature were examined by Hidekazu *et al.* (2005) in a controlled

environment and in field under rain shelter. The fruit set ratio observed was about 88 percent in the treatment with the application of GA over 54 per cent in the control.

Benzyladenine (BA)

Jones (1965) reported that application benzyl adenine is useful for increasing fruit set in musk melon. Takeno *et al.* (1989) reported that when treated with BA an increase in cell enlargement and size of pericarp tissue of enlarging cucumber fruits were accompanied with an increase in the levels of endogenous auxin. The effect of BA applied at 100mg/l, two weeks after full bloom improved fruit size in two varieties of 'Pear' viz., 'Spadona' and 'Coscia' (Stern and Flaishman, 2003). In gladiolous, foliar spray of 6-BA 50 ppm, at 40 and 60 days after planting improved the corms and cormels per plant (Tawar *et al*., 2007).

Boron

Increased fruit set by application of boron was reported in 'Reblush' grapefruit (Maure and Davies, 1993). Maurer and Taylor (1999) suggested that foliar spray of boron (250,750 and 1000 ppm) promoted fruit set and yield in Navel oranges.

2.15 Use of plant growth regulators in preventing fruit drop

Auxin

In sapota, application of NAA 300 ppm was found to reduce fruit drop (32.91%) at pea size fruit (Gomase, *et al.,* 2004). Auxin content in mango fruit during first 2-3 weeks after pollination was low and the ability of fruit to mobilize food material was poor due to low auxin level which resulted in fruit drop (Chacko *et al.,* 1972).

Vijayaraghavan *et al,* (1989), studied the effect of six growth regulators in 35-year-old East Coast Tall palms 30 days after the spathes opened (fertilization completed in all buttons). Button shedding was reduced most by NAA at 20 ppm followed by 2, 4-D at 10 ppm, which gave mean setting of 60.7 and 45.7 percent, respectively. The nut characteristics were not affected by the treatments.

Maximum reduction in fruit drop was observed in foliar application of NAA 10 ppm (34.80%) followed by NAA 20 ppm (36.17%) in ber (Yadav *et al.*, 2004). Sahoo *et al.* (2006) suggested that, 2, 4-D alone or in combination with coconut water was useful in minimizing shedding of buttons and premature nuts after four and six months of bunch opening in coconut palms.

Gibberllic acid (GA_3)

ChangPin (1999) reported that fruits of 12-year-old orange (cv. Skaggs Bonanza) trees on *Poncirus trifoliata* rootstock were coated or sprayed with various combinations of BA24 powder + GA53 [benzyl adenine + gibberellins] in solution. Spray treatments increased fruit set without affecting fruit let development or fruit appearance. Coating fruits also increased fruit set.

2.16 Use of growth regulators in seed set and germination of seeds

Naphthalene Acetic Acid (NAA)

In pigeon pea, Shinde and Jadev (1994) observed that application of 40 ppm NAA at 60 and 90 DAS increased the number of pods per plant from 80.66 to 110.00. Shinde and Jadhav (1994) suggested that, foliar spray of NAA at 20 ppm increased the grain yield in Niger. The yield increase includes seeds/capsule (52%), 1000 seed weight (2%), capsule per plant (11%) and harvest index (6%). Rashmi (2003) noticed improvement in seed germination, growth and seed yield in bottle gourd by application of NAA 100 ppm.

Gibberllic acid (GA_3)

Tanimoto (1989) opined that GA_3 application at 300 ppm had a significant effect in promotion of pollen fertility and seed yield in Chinese arrowhead. Application of GA_3 at 50 ppm increased vegetative growth and seed yield in coriander (Piyush and Sen, 2006; Sen and Malhotra, 2006).

In coriander, the effect of growth regulators on yield characters was studied by Panda *et al*. during 2007 who found that application of GA_3 100 ppm was best over the control, regarding yield characters i.e., days to maturity 106.33(132.33), seed yield/ plant 23.75g (16.06), seed yield 17.93q/ha (12.66 q /ha), number of umbels 61.83 (24.6) and number of seeds/umbel 31.12 (23.17).

Boron

Molgaard and Hardman (1980) noted higher number of branches and pods in fenugreek, with increase in boron concentration from 0 to 0.33 mg /l. Foliar application of boron increased yield in French bean, *Phaseolus vulgaris.* It was achieved by the pod retention due to the effect of boron in pollen tube growth (Weaver *et al.,* 1985). Schon and Belvins (1990) stated that the multiple foliar application of boron increased the number of pods on branches and seed yield in soybean. Dordas (2006) reported that application of boron 400mg/l increased the number of pods (52 per cent) and seed yield (37 per cent).

Materials and Methods

3. MATERIALS AND METHODS

The study on the "Exploitation of bisexual variant in developing high yielding types of *Piper longum* L." was carried out at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during 2007-2009.

3.1 Experimental materials

The experimental material included the following sex forms of *Piper longum* L.

1. Female
2. Male
3. Acc. P25, the bisexual variant reported by Sujatha and Nybe (2007)

Plants of all the three sex forms were maintained in sufficient number in pots. Plants were also raised in beds in the open field.

3. 2 Details of experiments

3. 2. 1 Experiment: I

Study of reproductive biology

Reproductive biology of *Piper longum* L. was studied in detail utilizing the plants maintained for the purpose.

The following observations were made

3. 2. 1. 1 Season of flowering and fruit set

Monthly observations were taken on the number of flowers produced per plant. Average of ten plants were recorded.

3. 2. 1. 2 Spike characters

Visual observations were made on the spike characters, such as orientation, shape and colour. Length of 10 fully developed spikes was measured and the average length expressed in centimeter.

3. 2. 1. 2. 1 *Number of spikes per plant*

Observations were taken at monthly intervals on the number of spikes per plant. Average of five plants were worked out in each month.

3. 2. 1. 2. 2 ***Type of hermaphroditism in spike***

Inflorescences were observed under a dissection microscope and number of pistillate, staminate and bisexual flowers in a spike were observed. Ten spikes were examined at monthly intervals and variation in sex ratio was recorded.

3. 2.1.3 Floral characters

Observations were made on the following floral characters.

3. 2. 1.3.1 ***Number of stamens per flower***

100 flowers each were examined in the male and bisexual types of inflorescences to record the number of anthers per flower.

3. 2. 1. 3. 2 ***Number of stigmatic lobes***

Observations were made on 100 flowers each, in the female and bisexual types of inflorescences.

3. 2. 1. 4 Time of anthesis

Anthesis was recorded at hourly intervals during the 24 h cycle of the day to find out the time of anthesis. Detailed observations on anthesis were made during the time of anthesis. Number of flowers opened at hourly intervals was recorded in ten spikes each in all the sex forms and average worked out.

3. 2. 1.5 Time of anther dehiscence

As in the case of anthesis, anther dehiscence was also observed at hourly intervals. The colour and appearance of anthers were examined with hand lens. Detailed observations were made during the dehiscence period to find out the nature and peak of anther dehiscence. As in the case anthesis, anther dehiscence was also observed in ten spikes each in male and bisexual types and average worked out.

3. 2. 1. 6 Time of stigma receptivity

The stigmatic surface was observed for any change in colour or appearance at hourly intervals to find out the stigma receptivity. Flowers of five spikes were examined to find out period of stigma receptivity.

3. 2. 1.7 Pollen fertility

Pollen fertility was studied using one per cent acetocarmine. Ten microscopic fields were examined to find out the number of pollen grains that have taken stain. The fertility percentage was calculated using the formula

$$\frac{\text{Number of well stained pollen grains}}{\text{Total number of pollen grains in the field}} \times 100$$

3. 2. 1. 8 Pollen viability

Viability of pollen was tested in the following media

a. Sucrose solutions - 2, 4, 6, 8, 10, 20, 40 and 50 per cent.

b. Medium suggested by Ravindran (1979)

c. Brewbaker and Kwacks medium (1963)

d. ME_3 medium (Leduc *et al,* 1990)

e. Medium for liquid culture of pollen (Sunderland and Robert's, 1977)

f. Water (control)

Observations were made on viable / germinating pollen, if any.

3. 2. 2 Experiment: II

Developing stable bisexual type

Different techniques were tried to induce or improve femaleness and seed set in the bisexual type.

3. 2. 2. 1 Inducing female and bisexual flowers using growth regulators

To improve femaleness in the bisexual type, following growth regulator treatments were given at monthly intervals. Five single plant replications were maintained in each treatment.

Treatments

T1 - NAA 25 mg l^{-1}

T2 - NAA 50 mg l^{-1}

T3 - NAA 100 mg l^{-1}

T4 - Ethrel 100 mg l^{-1}

T5 - Ethrel 150 mg l^{-1}

T6 - Ethrel 250 mg l^{-1}

T7 - GA_3 5 mg l^{-1}

T8 - GA_3 10 mg l^{-1}

T9 - GA_3 50 mg l^{-1}

T10- BA 100 mg l^{-1}

T11- BA 500 mg l^{-1}

T12- Boron 3 mg l^{-1}

T13- Control (water)

Observations were recorded on number of spikes produced and sex ratio of inflorescences.

3. 2. 2. 2 Standardisation of pollination techniques

Pollen was collected from male parent at the time of anther dehiscence and applied to the stigmatic lobes in female by using any of the following techniques. As many inflorescences available were pollinated in each method.

3. 2. 2. 2. 1 *Dry method*

Pollen was collected with a camel hair brush and the dry pollen was dusted on the stigma of female flowers using the brush.

3. 2. 2. 2. 2 *Wet method*

Pollen was collected from mature anther and suspended the medium. Pollen suspension was applied by using a dropper on the inflorescence of the female parent.

The following media were used

a. Water

b. Boric acid (100 mg l^{-1})

c. Sucrose (10 per cent)

Pollinations were repeated till all the flowers in the inflorescence were pollinated (5 to 7 days)

3. 2. 2. 3 Self pollination in bisexual flowers

By collecting pollen from the same inflorescence, bisexual flowers were pollinated.

3. 2. 2. 4 Cutting inflorescences and selfing

Since abscission of inflorescence in the bisexual type before full development of fruit was a problem, attempt was made to reduce the size of inflorescence by cutting half the length of spike and pollinating them.

3. 2. 2. 5 Reciprocal crosses using bisexual type as male and female parent

Crosses were done using

a. Bisexual type as female parent and male type as male parent

b. Female type as female parent and bisexual type as male parent

Observations were made on the fruit set and development and seed set.

3. 2. 2. 6 Recovery of seeds

The number of seeds set per spike was recorded. Well developed seeds were collected from mature ripe spike resulting from controlled crosses. Seeds were washed free of pulp and sown in pure sand medium. Observations were made on number of days taken for germination, opening of cotyledons and development of first true leaf.

Results

4. Results

In order to develop high yielding types in *Piper longum* L. utilising a bisexual variant (Acc. P25), the present study was undertaken at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara from 2007-2009. The result are presented below:

4.1 EXPERIMENT: 1

Study on reproductive biology of *Piper longum* L.

The reproductive biology of *Piper longum* L. was studied in detail. For this, the plants of different sex forms raised in pots and field were utilised. (Plate1).

4.1.1 Seasonal variation in flowering and fruit set

Monthly variation in spike production and sex form in *Piper longum* L. (Acc.P25) was studied in detail to find out the season (month) of maximum flowering and to observe whether there is seasonal variability in the production of bisexual and male flowers in *Piper longum* Acc.P25 (Table.1).

Table1. Monthly variation in spike production and sex form in *Piper longum* L. (Acc. P25)

Month	Number of spikes per plant	Bisexual flowers per spike	Pistillate flowers per spike	Staminate flowers per spike
January	2.2	3.8	0	401.5
February	2.4	4.5	0	432
March	5.0	0	0	348
April	5.4	0.5	0	372
May	10.8	3.9	0	414.8
June	12.6	0.4	0	319.8
July	9.8	0	0	353.7
August	3.0	0	0	364.2
September	2.0	0	0	369.1
October	3.8	1.2	0	379
November	3.6	0	0	275.3
December	0	0	0	0
Mean	5.1	1.2	0	335.8
SD	3.9	1.8	0	113.6

Plate1. Experimental plot

As can be seen from table, number of spikes varied from zero (December) to 12.6 (June). Maximum flowering was observed from May to July which was significantly higher in number compared to all other months. However, maximum number of bisexual flowers were produced during February (4.5) followed by May (3.9) and January (3.8). There was predominance of male flowers in the accession during March, July to September, November and December. The number of bisexual flowers recorded during these months was zero. Pistillate flower production was not noticed in the accession. Staminate flowers were produced in large number throughout the year (275.3 - 432).

4.1.2 Spike orientation, shape and colour

The spikes were cylindrical and erect in all the sex forms. However, the colour of female spike was different from the male and bisexual types. In female type, the spikes were creamy white until fruit set, when 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.

4.1.3 Number of spikes / lateral branch

The number of spikes/ lateral branch varied from 1 to 3 in male and female types. In bisexual type, the number ranged from 1 to 4 (Table 2). As can be seen from the table, spike production / lateral branch was maximum in the bisexual type (Acc. P25) followed by male and female types.

Table 2. Number of spikes per lateral branch in *Piper longum* L.

Sl. No	Bisexual type (Acc. P25)	Male type	Female type
Range	1 to 4	1 to 3	1 to 3
Mean	2.7	1.9	1.7

(Average of 5 plants)

4.1.4 Rate of growth of spike in *Piper longum*

In order to find out the rate of growth of spike and the number of days taken for complete development of spike, daily observations were taken on the length of spike in the 3 different sex forms (Table.3). As can be seen from the table, there was an increment of 1 to 2 mm/day

in the male and bisexual types, whereas the height increment was 0.4 to 1.4 cm per day in the female type. The female spike attained full length (2.31 cm) in around 22 days, whereas male type attained full length in 43 days (7.76 cm) and bisexual type in 46 days (6.35 cm). As evident from Fig.1, growth of spike in all the sex forms showed linear pattern.

Table 3. Rate of growth of spike in *Piper longum* L.

Days	Male (cm)	Bisexual (cm)	Female (cm)	Days cont.	Male (cm)	Bisexual (cm)	Female (cm)
1	0.1	0.1	0.15	25	4.1	3.31	Nil
2	0.2	0.2	0.24	26	4.32	3.46	-
3	0.3	0.3	0.31	27	4.52	3.63	-
4	0.4	0.4	0.4	28	4.75	3.76	-
5	0.5	0.6	0.46	29	4.77	3.91	-
6	0.5	0.6	0.56	30	5.15	4.05	-
7	0.8	0.7	0.66	31	5.32	4.21	-
8	0.8	0.85	0.75	32	5.51	4.31	-
9	1.05	0.96	0.86	33	5.68	4.45	-
10	1.24	1.1	0.96	34	5.84	4.6	-
11	1.4	1.25	1.06	35	6.0	4.75	-
12	1.5	1.38	1.2	36	6.18	4.88	-
13	1.7	1.5	1.32	37	6.37	5.01	-
14	2.07	1.6	1.44	38	6.76	5.2	-
15	2.25	1.8	1.54	39	6.96	5.33	-
16	2.45	1.95	1.64	40	7.16	5.48	-
17	2.62	2.06	1.74	41	7.38	5.6	-
18	2.84	2.25	1.84	42	7.58	5.8	-
19	3.02	2.38	1.98	43	7.76	5.96	-
20	3.2	2.56	2.09	44	7.76	6.08	-
21	3.38	2.75	2.2	45	7.76	6.2	-
22	3.5	2.9	2.31	46		6.35	-
23	3.7	3.01	2.31	47		6.35	-
24	3.9	3.18	2.31	48		6.35	-

4.1.5 Flower characters

The flowers were represented by ovary and stigmatic lobes 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 (Plate 2). Data furnished in table 4 shows that, the number of anthers in male and bisexual flowers were varying. Male flowers possessed only two anthers, whereas in bisexual flowers, three anthers could be seen surrounding the ovary. (Plate.3)

Number of stigmatic lobes varied from 2 to 6 in bisexual flowers. Ninety per cent flowers showed bilobed stigma. Three and four lobed stigma were present in 5 per cent in each of flowers. In the female type, predominant number of stigmatic lobes was four (74 per cent) (Plate.4). Twenty per cent of the flower possessed 3 stigmatic lobes. Six (4 per cent) and five (2 per cent) stigmatic lobes were relatively less frequent. Two stigmatic lobes were not observed in the female type. (Table.5)

Table 4 . Number of anthers in male and bisexual flowers of *Piper longum* L.

Sl.No	No. of anthers	Percentage in bisexual type	Percentage in male type
1 2	3 2	100 0	0 100
Total		100	100

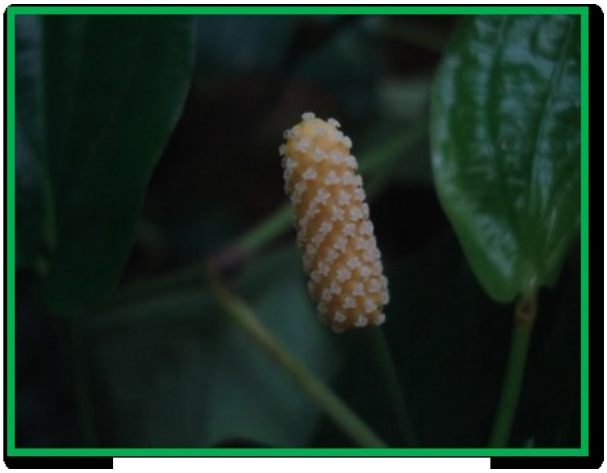
Table 5. Number of stigmatic lobes in female and bisexual types of *Piper longum* L.

Sl. No	No. of stigmatic lobes	Percentage in female type	Percentage in bisexual type
1	2	0	90
2	3	20	5
3	4	74	5
4	5	2	0
5	6	4	0
Total		100	100

4.1.6 Anthesis in *Piper longum* L.

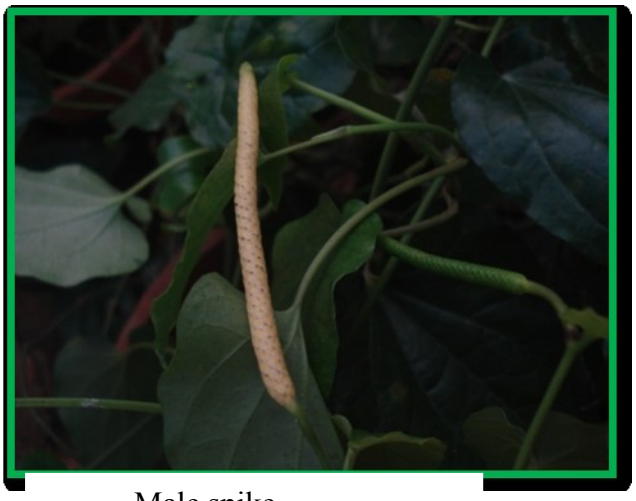
4.1.6.1 Time of anthesis

Data on time of anthesis in male, female and bisexual types are given in table 6. Anthesis started from 7.30 am and continued upto 4.30 pm, in all the sex forms. Peak time of anthesis was 10.30 am to 12.30 pm in male, female and bisexual types.



Female spike

Magnified view (x 40)



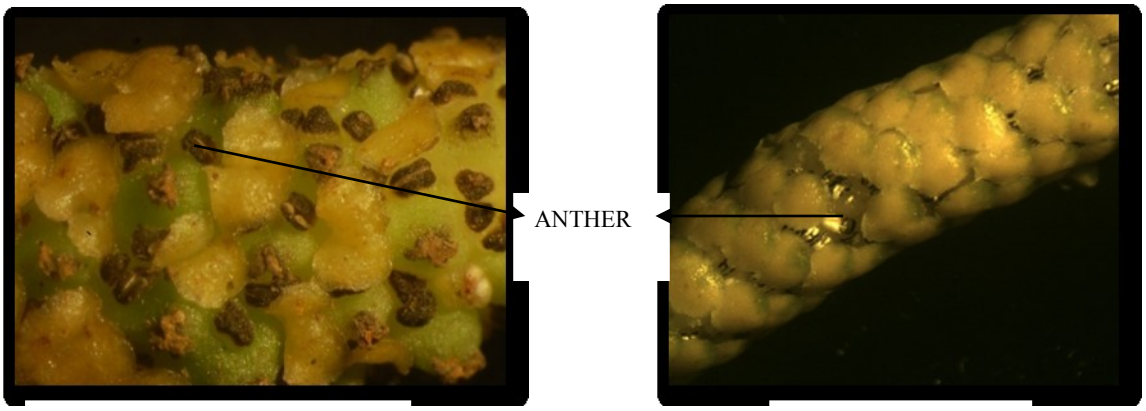
Male spike

Magnified view(x 40)

Bisexual spike

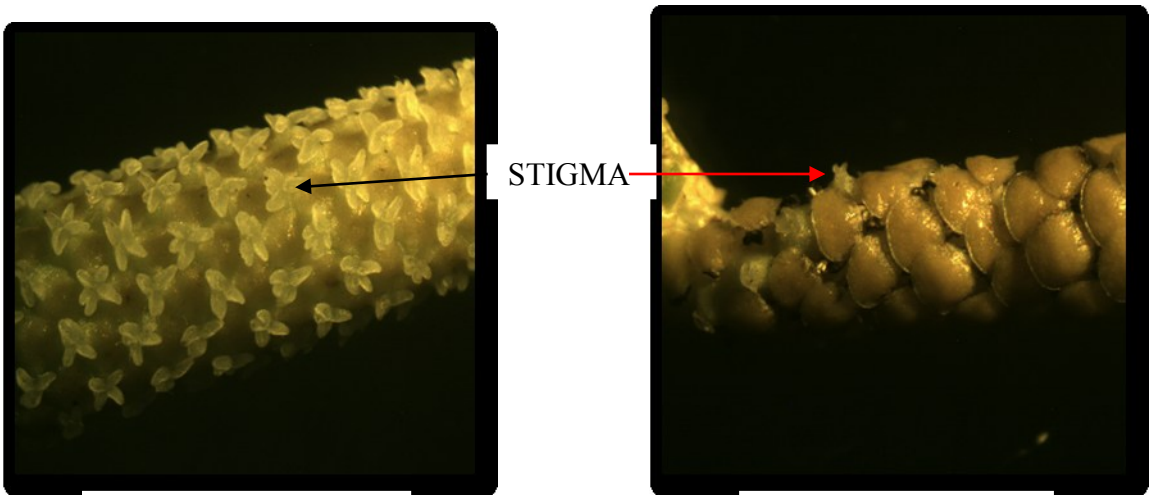
Magnified view(x 40)

Plate 2. Inflorescence and flowers of male, female and bisexual types



Bisexual type(x 40)

Male type(x 40)



Female type(x 40)

Bisexual type(x 40)

Plate 3. Anther and stigmatic lobes in male, female and bisexual types of *Piper longum* L.

Table 6. Time of anthesis in male, female and bisexual types of *Piper longum* L.

Time	Male		Female		Bisexual	
	Mean/spike	SD	Mean/spike	SD	Mean/spike	SD
Before 7.30 am	0	0	0	0	0	0
7.30 am-8.30 am	73.83	11.02	0.6	1.89	16.67	3.08
8.30 am-10.30 am	120.7	18.55	39.7	7.10	123.83	10.38
10.30 am-12.30 pm	128.2	14.81	43	9.12	205.67	19.96
12.30 pm-2.30 pm	95.5	10.76	35.1	4.93	161.83	14.53
2.30 pm-4.30 pm	56.67	3.66	18.4	4.01	62.17	3.31
After 4.30 pm	0	0	0	0	0	0
Total	474.9	58.8	136.8	27.05	570.17	51.26

4.1.6.2 Time taken for complete opening of flowers in a spike

Number of days taken for complete opening of flowers in a spike was seven days in male, female and bisexual types (Table 7). Maximum flower opening was on the 3rd and 4th day in the male type. In female and bisexual types, profuse opening of flowers was noticed from second to sixth day.

Table7. Number of days taken for complete opening of flowers in male, female and bisexual types of *Piper longum* L.

Days	Number of flowers opened					
	Male		Female		Bisexual	
	Mean/spike	SD	Mean/spike	SD	Mean/spike	SD
1	58.66	4.37	16.1	6.69	43.33	12.94
2	83.00	13.48	21.2	6.40	88.66	6.88
3	93.16	3.87	20.8	5.70	94.16	1.94
4	93.83	10.43	20.1	6.66	89.83	11.39
5	73.66	11.34	19.1	5.65	92.5	3.14
6	50.16	15.68	21.7	7.95	101.5	14.63
7	32.33	14.1	11.1	2.61	65.16	24.86
Total	484.8	73.27	130.1	41.66	575.14	75.78

4.1.7 Stigma receptivity

Stigma remained white for two days from anthesis. The colour of stigmatic lobes changed to brown on the 3rd day around noon indicating end of receptivity.

4.1.8 Anther dehiscence

As in the case of anthesis, dehiscence of anthers also started from 7.30 am and continued upto 4.30 pm. The peak time of anther dehiscence was from 8.30 am to 12.30 pm in male and 10.30 pm to 2.30 pm in bisexual types (Table 8).

Table 8. Time of anther dehiscence in male and bisexual types of *Piper longum* L.

Time	Male		Bisexual	
	Mean/spikes	SD	Mean/spikes	SD
Before 7.30 am	0	0	0	0
7.30 am - 8.30 am	71.17	4.58	14.5	5.24
8.30 am - 10.30 am	97.16	4.36	63	8.15
10.30 am - 12.30 pm	100.33	5.92	98.1	12.92
12.30 pm - 2.30 pm	64.5	8.17	96.33	8.43
2.30 pm - 4.30 pm	35.33	5.24	73.5	10.60
After 4.30 pm	0	0	0	0
Total	368.49	28.27	345.43	45.34

The number of days taken for complete dehiscence of anthers was seven in male and bisexual types. Peak dehiscence of anthers was from second to fourth day in both male and bisexual types (Table.9)

Table 9. Number of days taken for complete dehiscence of anthers in male and bisexual types of *Piper longum* L.(average of five spike)

Days	Male		Bisexual	
	Mean/spike	SD	Mean/spike	SD
1	34.16	14.76	30.16	16.75
2	71.33	5.65	73.66	6.91
3	84.16	3.13	71.33	9.03
4	85.16	8.86	87.16	6.65
5	57.16	9.15	59.5	11.74
6	19.16	3.92	11.83	5.38
7	12.83	4.88	13.16	5.74
Total	363.96	50.35	346.8	62.2

4. 1. 9 Pollen fertility

Pollen was collected from dehisced anthers at hourly intervals from 8.30 am to 11.0 am. The collected pollen was stained using one per cent acetocarmine. The pollen collected was placed on the microscopic slide, stained using one percent acetocarmine and examined under research microscope. As can be seen from table 10, the percentage pollen fertility ranged from 17.91 at 8.30 am to 42.54 at 9.30 am. Plate.4 shows pollen stained using one per cent acetocarmine.

Table 10. Percentage pollen fertility in bisexual type (Acc.P25) of *Piper longum* L. (average of 10 fields)

Time	Fertile pollen	Sterile pollen	Percentage fertility
8.30 am	3.5	17.4	17.91
9.30 am	4.5	6.3	42.54
10.30 am	3.1	8.4	27.76
11.00 am	2.8	6.1	31.50

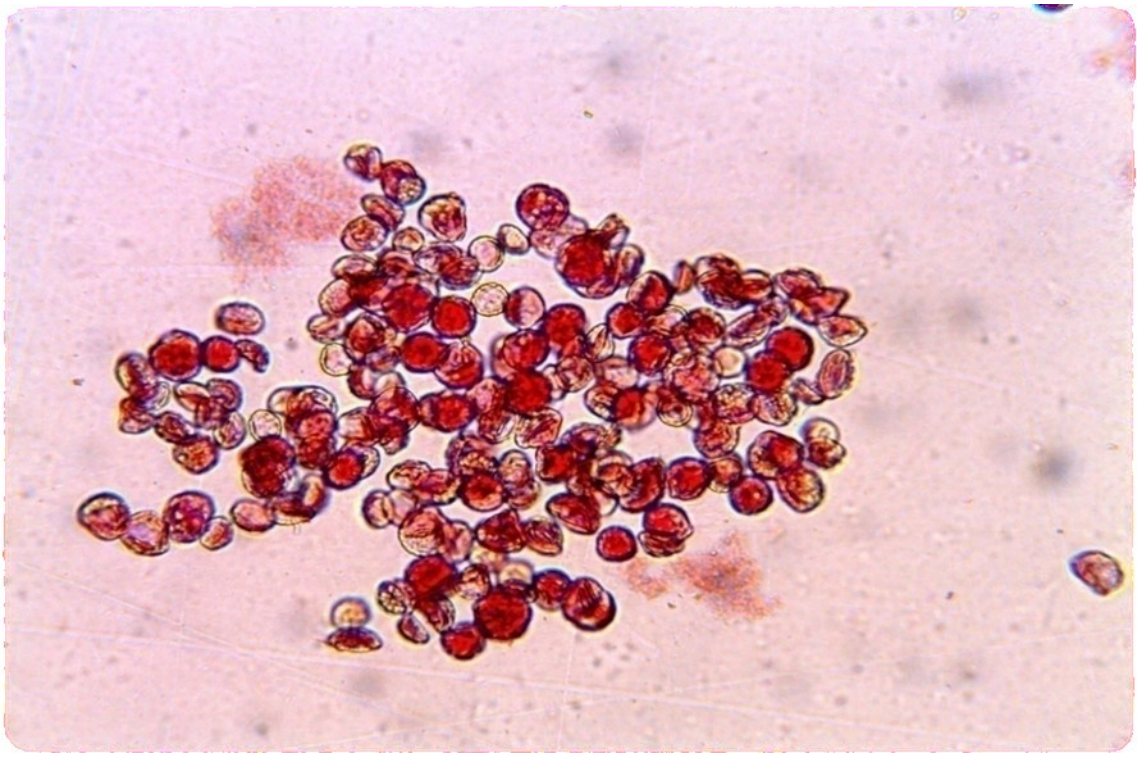
4.1.10 Pollen viability

Pollen viability of Acc.P25 was studied in six different media and eight different concentrations of sucrose. Fresh pollen collected was cultured in the media placed on a microscopic slide. Germination of pollen if any was examined under the microscope 24 hours after culturing. In none of the media, pollen germination could be achieved (Table 11)

Table 11. Pollen viability in *Piper longum* L. Acc. P25

Sl. No	Medium	Pollen viability (24h.)
1	Sucrose	
	2%	Nil
	4%	-
	6%	-
	8%	-
	10%	-
	20%	-
	40%	-
	50%	-
2	Medium suggested by Ravindran (1979)	-
3	Brew Baker and Kwacks (1963)	-
4	ME_3 medium (Leduc *et al*, 1990)	-
5	Medium for liquid culture of pollen (Sunderland and Robert's, 1977)	-
6	Water	-

Plate 4. Pollen fertility in one percent acetocarmine (x 1600)



4.2 Experiment: II

Developing bisexual types

4.2.1 Inducing female /bisexual flowers using growth regulator /nutrient suppliments

Effect of four different growth regulators at a total of 11concentrations, Boron 3 mg l^{-1} and water as control as suggested in materials and methods were assessed in the study (Table 12). Growth regulators were sprayed at monthly intervals for one year, starting from August 2008. Spraying was continued upto July 2009. Observations were recorded at monthly intervals starting from one month after the first spray on the spike production and number of male and bisexual flowers produced. (Table 12). There was high plant to plant variation in most of the treatments, in terms of spike production. Mean number of spikes produced (over 12 month) was maximum in T7 (GA_3 5 mg l^{-1}) followed by T13 (control) and T9 (GA_3 50 mg l^{-1}) and T8 (GA_3 10 mg l^{-1}). Spike production was adversely affected in treatments T1, T2, T3, T4, T5, T6 and T11. In the NAA treated plants, vegetative growth was adversely affected at higher concentration (T3) and spraying had to be stopped in between.

Table 12. Variation in spike production in different growth regulator/nutrient suppliment treated plants

Month	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
Sep.08	30	10	16	13	5	10	29	10	17	36	21	10	10
Oct.08	25	14	20	19	18	25	27	16	25	42	27	16	15
Nov.08	17	18	17	13	12	15	11	14	19	16	17	20	18
Dec.08	0	0	0	0	0	0	0	0	0	0	0	0	0
Jan.09	0	0	0	0	0	0	28	16	1	5	12	5	11
Feb.09	0	0	0	0	0	0	26	15	12	14	9	9	12
Mar.09	0	0	0	0	0	0	51	20	21	13	0	16	25
Apr.09	0	0	0	0	0	0	47	13	35	30	0	17	27
May.09	0	0	0	0	0	0	67	31	58	0	0	44	54
June.09	52	0	0	0	0	0	77	54	55	36	0	45	63
July.09	62	3	0	0	0	0	25	35	18	33	0	34	49
Aug.09	14	4	0	0	0	0	10	20	19	14	0	5	15
Mean	16.6	4.08	4.42	3.75	2.92	4.17	33.2	20.3	23.3	19.9	7.67	18.4	24.9
SD	21.79	6.35	8.03	6.94	5.96	8.21	23.16	13.95	18.15	14.85	9.85	15.01	19.83

Friedman two way analysis of variance by ranks (Hollander and Wolfe, 1973) was conducted to study influence of growth regulators /nutrient elements on bisexual flower production over 12

Table 13. Variation in male and bisexual flowers in growth regulator /suppliments treated plants

Month	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	T13
	M/B	M/B	M/B	M/B	M/B	M/B	M/B	M/B	M/B	M/B	M/B	M/B	M/B
Sep.08	383.7/0.5	0	384/ 10	365.5/ 2.3	0	0	381.8/ 0.5	368.7/ 1.3	0	386.3/ 8.3	395/ 4.3	389.2/0	369.14/0
Oct.08	371.75/2.5	398.8/ 5.2	396/0	406.3/0	400.2/5	390.2/ 0.4	428.2/ 1.4	388.6/ 0.8	394.8/ 2.8	396/12	393.6/ 1.6	398.8/0	379/1.2
Nov.08	277.3/12	358.3/5.7	295/0	390/0	386.5/0	370.2/0.83	361.4/ 2.8	350/0	359/1	327.0/26	397.3/ 0.8	368.3/0	275.3/0
Dec.08	0	0	0	0	0	0	0	0	0	0	0	0	0
Jan.09	0	0	0	0	0	0	370.3/ 15.8	310.7/25	327/20	357.8/47.4	329/10.3	400/12.6	401.5/3.75
Feb.09	0	0	0	0	0	0	399.6/ 10.6	384.3/13.6	0	0	0	406/7	432/4.5
Mar.09	0	0	0	0	0	0	325/2.1	354.1/1.6	387.8/ 1.7	322.4/1.0	0	345.1/0.5	348.3/0
April.09	0	0	0	0	0	0	363.3/1	360.4/1.4	367.2/ 0.5	382.3/6.3	0	358.3/0.3	372.2/0.5
May.09	0	0	0	0	0	0	385.7/ 7.7	370.7/8.3	391/6.3	0	0	405/2	414.8/3.87
June.09	382.5/ 11.7	0	0	0	0	0	354.9/ 4.2	345.4/5.8	374.0/ 4.9	352.6/9.7	0	375.2/0	319.7/0.44
July.09	349.8/ 34.8	0	0	0	0	0	335.1/ 3.5	340.1/17.7	335.5/ 7.5	311.6/5.0	0	351.8/0.3	353.7/0
Aug.09	350.6/1.8	342.7/0.8	0	0	0	0	366.8/ 1.3	361/2.8	351.4/ 1.6	355.2/1.8	0	346.8/0.8	364.2/0
Mean rank	6.3/7.8	4.9/ 5.8	4.0/4.8	4.5/ 4.6	5.1/ 5.3	4.9/ 4.6	11.2/ 9.8	8.4/10.0	7.7/8.5	9.0/10.4	5.9/6.0	9.9/6.9	9.3/6.5

Plate 5. Parthenocarpic development of bisexual spikes sprayed with growth regulators

months (Table13). The ranking showed that treatment T10 (BA100 mg l^{-1}) followed by T8 (GA_3 10 mg l^{-1}) and T7 (GA_3 5 mg l^{-1}) were more stable in producing bisexual flowers over the months. However, fully bisexual inflorescences were induced in less number of sprays in T1 (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}. The fully bisexual flowers produced were retained on the plant to study further development. It was noticed that, the spikes turned green and were retained on the plant for 40- 50 days compared to 7 to 10 days in unsprayed spikes. However, no seed set was noticed. (Plate 5).

4.2.2 Developing bisexual type through controlled pollination

4.2.2.1 Fruit set in crosses with bisexual type as female parent

Using bisexual type as female parent, selfing and crossing were attempted. For selfing, pollen was collected from other inflorescences on the same plant and dusted on the receptive stigma of spikes having more bisexual flowers. The inflorescences were also topped and selfing was done. As can be seen from table14, a single spike setting was noticed in selfing attempted. This spike dropped 15 days after pollination. Using bisexual type as the female parent and pure male as the male parent, crosses were done. Out of the 25 spikes pollinated, three (12 per cent) were set. However, none of the spikes was carried to maturity.

Table 14. Fruit set in Acc. P25 under controlled pollination

Crossing/ selfing	Number of spikes pollinated	Number set	Number of spikes matured
Selfing	10	1	0
Cutting inflorescences and selfing	25	0	0
Crossing with male	25	3	0

4.2.2.2 Fruit set in crosses with bisexual type as male parent

Using bisexual type as pollen parent and female type as the female parent, crosses were done to get hybrid seeds (Table 15). Pollinations were started from February and continued upto September. Three methods of pollinations were done viz., dry method, suspending pollen in water and in boric acid (100 mg l^{-1}).

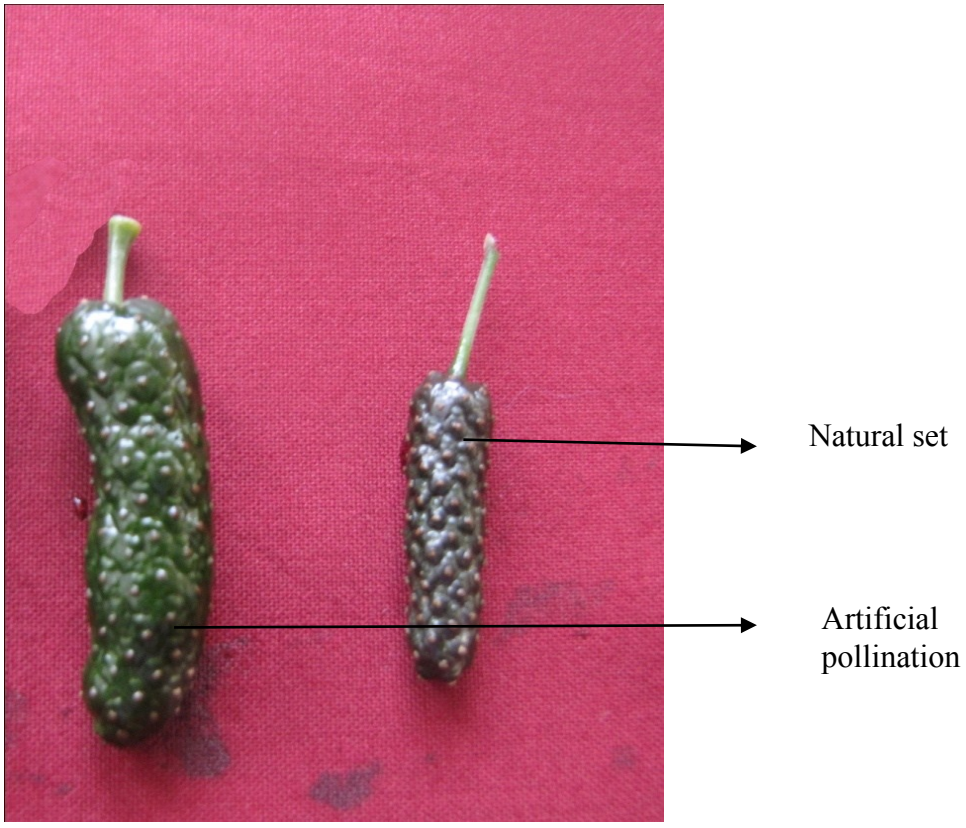
In dry method, pollen was collected using camel hair brush and dusted on the receptive stigma of the female spike. In water and boric acid methods, pollen collected was suspended in the media and pollen suspension was dropped over the inflorescences using an ink dropper. Pollinations were repeated for seven days starting from first day of anthesis, until all the flowers in an inflorescences were pollinated. As can be seen from table, fruit set was poor from February to May. Eleven fruits were set in boric acid method during June. However, these fruits were dropped before attaining maturity. Fruit set and fruit maturity were improved from July to September. Maximum fruit set was observed in dry method in September followed by boric acid in August and dry method and boric acid methods in September. Number of spikes matured and harvested was maximum under dry pollination in September (33). Percentage spike set was maximum in boric acid method (75.0) followed by dry method in July (50), boric acid method in July (37.5) and water method in September (34.1).

Table 15. Fruit set in crosses with Acc. P25 as male parent

Pollination methods	Number of spikes pollinated			Number of spikes set			Number of mature spikes harvested			Percent spike set			Percentage of mature fruits harvested		
Month	Boric acid	Water	Dry	Boric acid	Water	Dry	Boric acid	Water	Dry	Boric acid	Water	Dry	Boric acid	Water	Dry
Feb.	10	11	7	0	0	1	0	0	0	0	0	14.2	0	0	0
Mar.	18	5	9	0	0	0	0	0	0	0	0	0	0	0	0
April	5	14	16	0	0	0	0	0	0	0	0	0	0	0	0
May	6	9	6	0	0	0	0	0	0	0	0	0	0	0	0
June	18	16	10	11	0	0	0	0	0	61.1	0	0	0	0	0
July	16	7	6	6	0	3	1	0	1	37.5	0	50	6.3	0	16.7
Aug.	91	50	5	25	11	1	5	0	0	27.5	22	20	5.5	0	0
Sep.	32	45	90	24	14	40	6	4	33	75.0	34.1	44.4	18.8	8.8	36.7
Total	196	157	149	66	25	45	12	4	34	33.7	15.9	30.2	6.12	2.55	22.82

Mature fruit could be harvested only from the pollinations during July, August and September. Maximum percentage of fruits were harvested from dry method of pollination (36.7 per cent) followed by boric acid method (18.8 per cent) in September and dry method in July. The results of the present study indicated that dry method of pollination was most effective in *Piper longum* and September was the best month for pollination.

Plate 6. Variation in characters between naturally set and artificially pollinated spike in *Piper longum* L.



4.2.3 Seed set in different methods of pollination

Table.16 shows the number of seeds set under different methods of pollination in different months. Maximum number of seeds were set under dry method of pollination in September (554). In boric acid (100 mg l^{-1}) method, a few seeds were obtained. Here again, September was the best month for pollination followed by August and July. In water method, seeds were obtained only in crosses made during September. As can be seen from table 16, 573 seeds were obtained in the present study. Table 17 and plate 6 show that the spike developed as a result of artificial pollination were longer, plumper and heavier compared to natural spikes set. The well developed seeds were bigger in size with hard seed coat. Undeveloped seeds were seen as black specks. Magnified view of the undeveloped and fully developed seeds are given in plate 7 and 8.

Table 16. Seed set under different methods of pollination

Pollination methods	Month	Number of mature fruits	Total number of seeds
Water	July	0	0
	August	0	0
	September	4	4
Dry	July	1	2
	August	0	0
	September	33	554
Boric acid 100 mg l^{-1}	July	1	2
	August	4	5
	September	2	6
Total		45	573

Table 17. Variation in characters between naturally set and artificially pollinated spike in *Piper longum* L.

Spikes characters	Artificial pollinated	Natural set
Length(cm)	4.9	3.6
Pedicel length(cm)	1.7	1.2
Girth(cm)	3	2.2
Diameter(cm)	0.8	0.5
Weight(g)	1.59	0.41

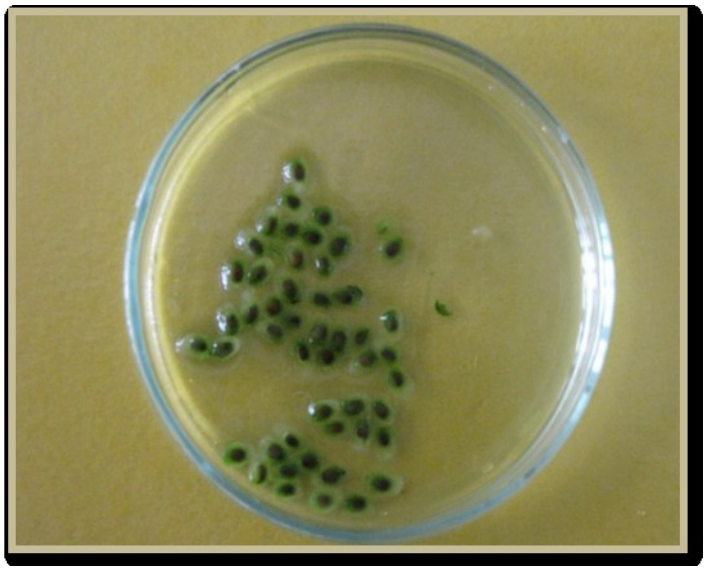
4. 2. 4 Seed germination

The fully mature, ripe fruits were collected from the plant, pulped and washed in water to remove the pulp and sown immediately after extraction. Seeds were sown in polythene bag filled with river sand. Seeds germinated in 21 to 69 days. The growth of seedlings were very slow. Cotyledonary leaves opened in 2 to31 days. The first true leaves opened in 2 to 41 days. The percentage of germination was 23.73 (Table 18).

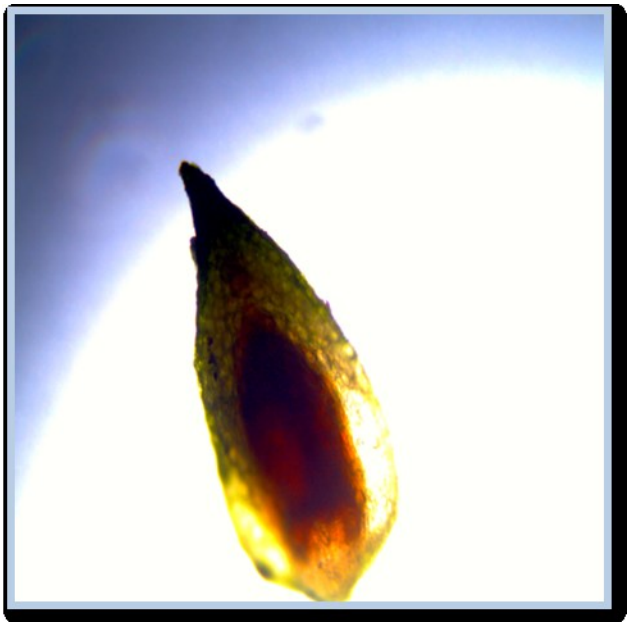
Table18. Seedling of *Piper longum* L.

Sl. no	Number of seeds sown	Number germinated	Percentage germination	Number of days taken for germination	Number of days taken for opening of cotyledons	Number of days taken for opening of first true leaf
1	573	136	23.73	21- 69	2- 31	2- 41

Plate 7. Seeds of *Piper longum* L.



Mature seeds



Undeveloped seed (x 160)

Developed seeds(x 40)

Plate 8. Seedlings of *Piper longum* L.

Single plant

seeds sown in sand

Seedlings at different stages of growth

Discussion

5. DISCUSSION

Piper longum L. (thippali) is an important medicinal plant used in many ayurvedic preparations. However, the crop is not being cultivated on a commercial scale and is mainly collected from the wild. To overcome the bottlenecks in cultivation, an attempt was made to develop high yielding types utilising the bisexual variant, (Acc. P25) reported by Sujatha and Nybe (2007). As a prelude, reproductive biology of *Piper longum* was studied in detail to facilitate breeding programmes. The results of the investigations are discussed in this chapter.

5.1 Study of reproductive biology in *Piper longum* L.

Detailed study on reproductive biology of *Piper longum* L. with special reference to bisexual variant was carried out. It was observed that there was seasonal variation in spike production and sex ratio in the bisexual type. Male and bisexual spikes were longer (6-7 cm) than female spikes (2-3 cm) in *Piper longum*. Longer male spikes compared to female spikes are seen in other dioecious species of *Piper* like *P. argyrophyllum, P. attenuatum, P. sugandhi, P. wightii and P. mullesua.* Only in *Piper hymenophyllum,* female spikes are reported to be slightly longer than male ones. It took only 22 days for attaining full length of spikes in the case of female types whereas more number of days ie., 43 and 46 were required in male and bisexual types respectively. This shows that in *Piper longum,* more time was required for attaining full length in the longer spike of male and bisexual types compared to shorter spike of female type.

5.2 Flower characters

Number of anthers in male flowers was two whereas in bisexual flowers it was three. Stamens were reported to be 3 - 4 in *P. longum* (Ravindran, 2000). The accessions analysed in the present study may be having variable number of anthers compared to the one already reported. However, Ravindran (2000) describes the genus to possess 2- 4 stamen and the number reported in the present study is within the number of stamens reported for the genus.

The number of stigmatic lobes in *Piper longum* varied from two to six. Two was the predominant number in the bisexual type (90 per cent) whereas two lobed stigma was absent in the female type. In the female type, four stigmatic lobes were predominantly seen (74 per cent) whereas it was only 5 per cent in the bisexual type. Twenty per cent flowers possessed three lobed stigma in the female type. Five and six lobed stigmas were present, even though less frequently in female type. Number of stigmatic lobes in the female flowers of *P. longum*

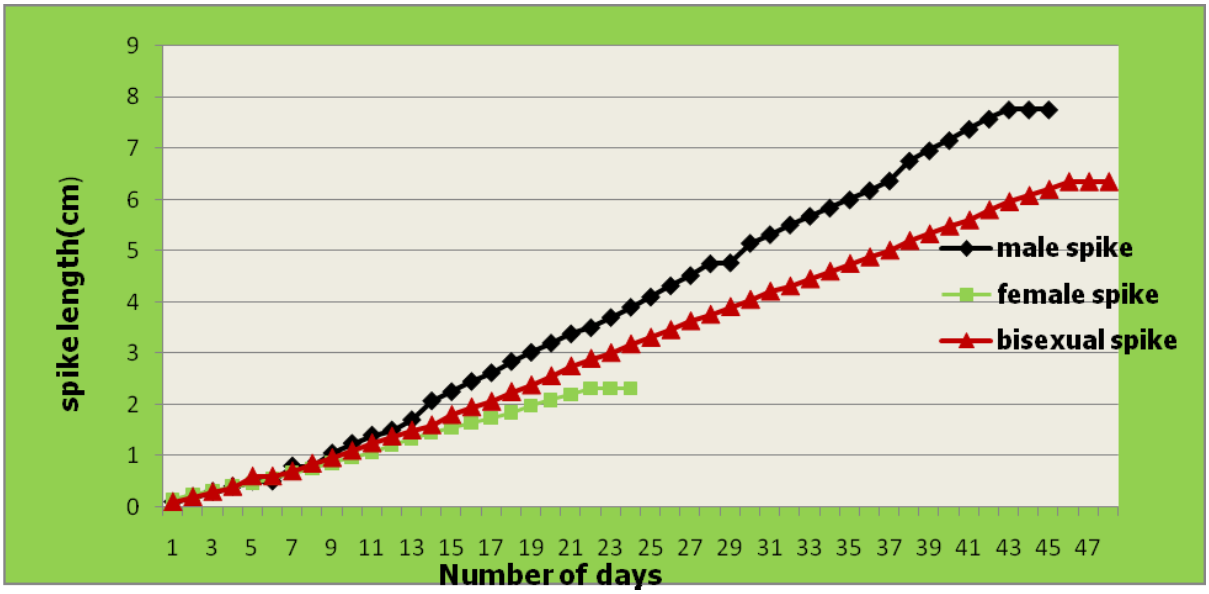


Fig 1. Growth pattern of spikes in three sex forms of *Piper longum* L.

was reported to be 3 - 4 (Ravindran, 2000). The results of the present study is in agreement with the report of Ravindran (2000) in this regard. However, the results of the present study show that structure of the flower in the bisexual type was different from its male and female counterparts in *P. longum.*

5.3 Anthesis and anther dehiscence

The time of anthesis was uniform in all the three sex types of *Piper longum* starting from 7.30 am and continuing upto 4.30 pm. Time taken for complete opening of flowers in an inflorescence was also uniformly one week in all the sex types (figure 2 and 3). There are no earlier reports on time of anthesis in *P. longum*.

Anther dehiscence, as in the case of anthesis was between 7.30 am and 4.30 pm in male and bisexual type. 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. The report was a preliminary observation in the bisexual variant which coincides with the peak period of anther dehiscence in male and bisexual types in the current study (figure 3 and 4). In *P. nigrum*, anther dehiscence was reported to take place around 4 pm (Ravindran, 2000).

Pollen fertility in one per cent acetocarmine was observed as 42.5 per cent at 9.30 am. However, pollen viability could not be achieved in the six different media and eight concentrations of sucrose tested. Sujatha and Nybe (2007) also could not obtain pollen viability in the bisexual variant. There may be some plant factor, essential for pollen germination which is limiting in the different media tried. A detailed study on the effect of stigmatic fluids on pollen germination may throw some light into this.

5.4 Developing bisexual types

5. 4. 1 Inducing female / bisexual flowers using growth regulators / nutrient suppliments

Four different growth regulators at a total of eleven concentrations, boron 3 mg l^{-1} and water as control were used to induce bisexual / female flower in the variant sex form. Growth regulators and their concentrations were carefully selected based on the earlier reports on their ability to induce femaleness. All the treatments except ethrel at three concentrations were able to induce fully bisexual inflorescences. The fully bisexual inflorescences which turned green, were retained on the plant for 40 to 50 days compared to 7 to 10 days in the unsprayed control. However, no seed set could be observed, indicative of parthenocarpic

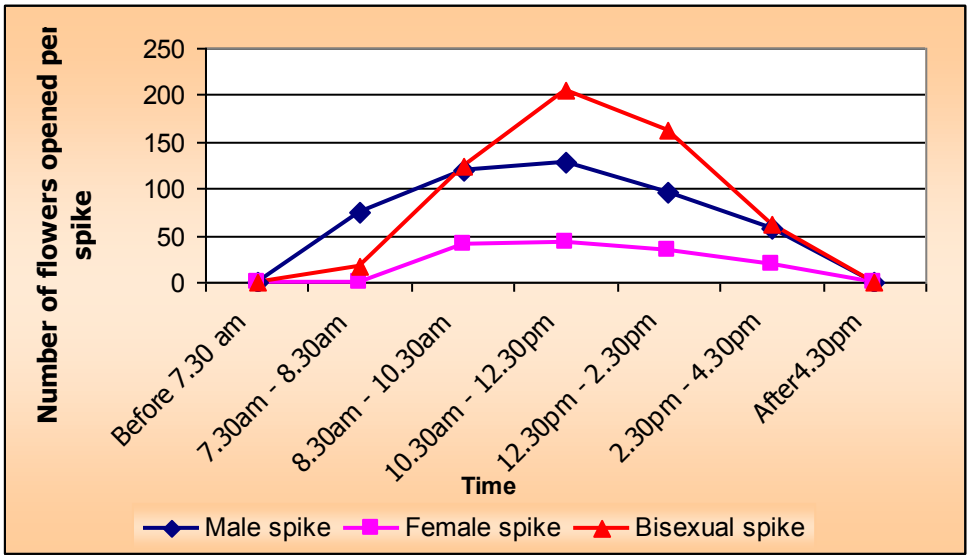


Fig 2. Time of anthesis in male, female and bisexual types of *Piper longum L.*

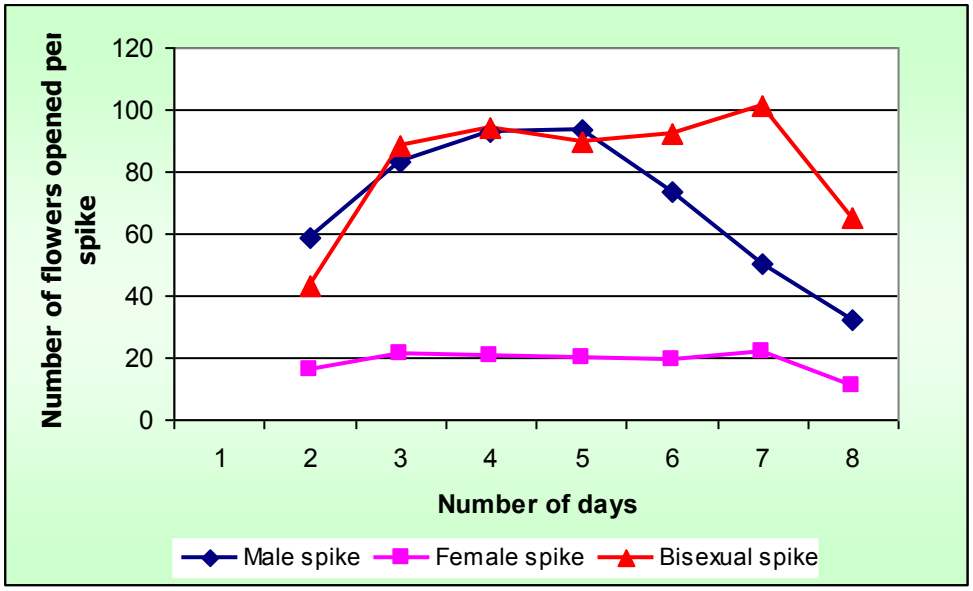


Fig 3. Number of days taken for complete opening of flowers in male, female and bisexual types of *Piper longum* L.

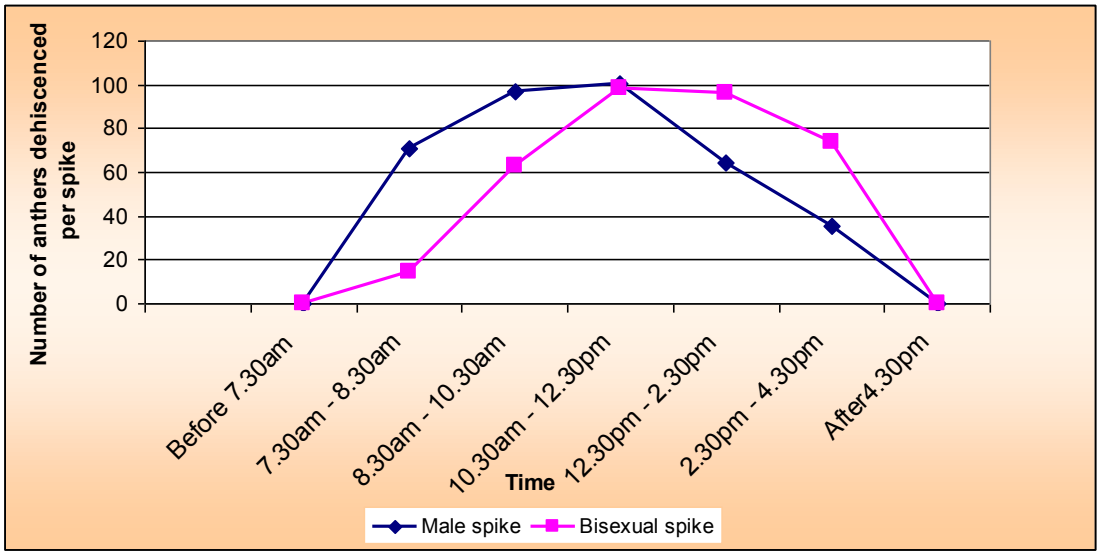


Fig 4. Time of anther dehiscence in male and bisexual types of *Piper longum* L.

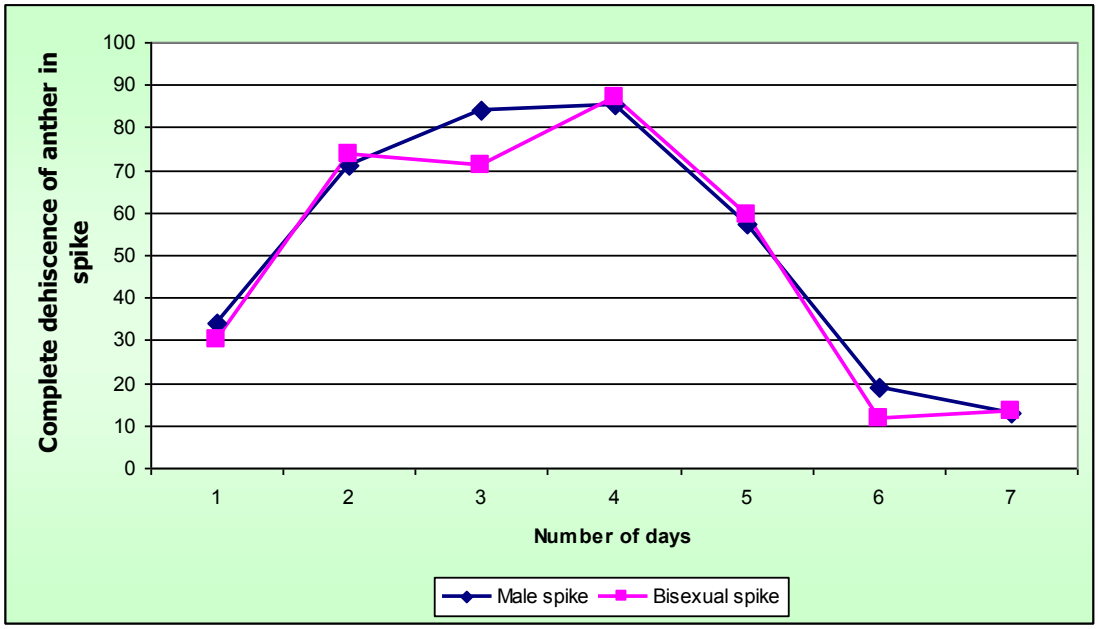


Fig 5. Number of days taken for complete dehiscence of anthers in male and bisexual types of *Piper longum* L.

development of fruit. The ability of different growth regulators to induce fully bisexual inflorescences in the bisexual type of *P. longum* is very promising. Further detailed studies on the effect of artificial pollination on the growth regulator induced bisexual inflorescence may give seed set in crosses / selfing involving bisexual type as female parent.

5. 4. 2 Developing bisexual type through controlled pollination

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 with male parent were attempted. In 35 spikes selfed, set could be achieved only in one spike. In 25 crosses attempted, three 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 spike to promote fruit development and fruit maturity. Etampawala, *et al.* (2002) reported 50 per cent fruit shedding even in female type of *Piper longum.* A more detailed study involving artificially induced fully bisexual inflorescences as female parent may be useful in achieving seed set and fruit maturity in the bisexual type.

5. 4. 3 Using bisexual type as male parent

Using bisexual type as pollen parent and female type as female parent, crosses were done to get hybrid seeds. Three methods of pollinations were done, namely dry, water and boric acid (100 mg l^{-1}). Pollinations done from February to September indicated that maximum percentage of spike set was in boric acid method in September followed by June and dry method in September. However, mature spikes harvested and seed set were maximum in dry method during September. The seeds were sown in sand filled bags and germination recorded.

It took 21 to 69 days for complete germination. This is the first report of seed set in *Piper longum.* The species has been earlier reported to be apomictic, where seeds develop without external stimulus of pollination. However, the observations in the present study disprove this theory and suggest that normal spike set in female *Piper longum* is through parthenocarpy. No viable seeds were present in naturally set fruit of female type of *Piper longum.* Seed set could be obtained under artificial pollination in naturally parthenocarpic fruits like banana and pineapple. Ability to produce true seeds which are viable could be a break through in breeding programme of *Piper longum.* Only variety released so far in the species is Viswam, which was developed through selection from the existing genotypes.

In the present study, a systematic effort was made to study the reproductive biology of *Piper longum.* The information on the time of anthesis, anther dehiscence and stigma receptivity could be effectively utilised in the selfing / crossing programmes in *P. longum*. Dry as well as wet methods of pollination were effective in inducing seed set in *Piper longum*. During wet months, dry method of pollination was more effective. Growth regulators could be effectively used to induce fully bisexual inflorescences in the bisexual variant of *Piper longum*. These observations will be useful in the future breeding programmes of *Piper longum*.

The hormone induced fully bisexual inflorescences could be used as female parent in selfing and crossing programmes. Also more number of pollinations are to be attempted during August- September season which was found more favourable for seed set in *P. longum.*

Summary

6. Summary

Piper longum is an important medicinal plant used in more than 320 classical preparations. In order to get economic returns from the crop, high yielding long spiked genotypes are required. With this view in mind a study on "Exploitation of bisexual variant in developing high yielding types of *Piper longum* L." was conducted at the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara utilising the bisexual variant (Acc.P25) reported by Sujatha and Nybe (2007) during the period from 2007 to 2009. The major objectives of the investigations were

1. To study the reproductive biology of *Piper longum* L.
2. To develop long spiked high yielding varieties of *Piper longum* L.

The results are summarised below:

Detailed studies on reproductive biology showed that the spikes were cylindrical, erect and creamy white in female. In male and bisexual types, immature spikes were green, changing to dull yellow on maturity.

Number of anthers in male flowers was two. In bisexual flowers, it was three. The number of stigmatic lobes in *Piper longum* varied from two to six. Two was predominant number in the bisexual type (90 per cent). In the female type, four stigmatic lobes were common (74 per cent) followed by three lobes (20 per cent).

Observations on time of anthesis had shown that it was uniform in all the three sex types of *Piper longum*. Anthesis started from 7.30 am and continued upto 4.30 pm. Peak time of anthesis was 10.30 am to 12.30 pm in male, female and bisexual types.

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 the 3rd and 4th day in the male type. In female and bisexual types, profuse flower opening was noticed from second to sixth day.

Anther dehiscence in male and bisexual types of *P. longum* was between 7.30 am to 4.30 pm. The peak time of anther dehiscence was from 10.30 am to 12.30 pm in male and bisexual types. The number of days taken for complete dehiscence of anthers was seven in male and

bisexual types. Peak dehiscence of anthers was from second to fourth day in both male and bisexual types.

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.

Effect of growth regulators in developing fully bisexual inflorescences in Acc. P25 indicated that NAA 25 mg l^{-1}, GA_3 5, 10 and 50 mg l^{-1}, BA 100 mg l^{-1}, 500 mg l^{-1}, and Boron 3 mg l^{-1} were effective in inducing fully bisexual spikes. The fully bisexual inflorescences turned green and were retained on the plant for 40 to 50 days compared to 7 to 10 days in unsprayed control. However, no seed set was obtained indicating that the development of spike was parthenocarpic.

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 with male parent were attempted. In 35 spikes selfed, only one was set. In 25 crosses attempted, three 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 spike to promote fruit development and fruit maturity.

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, plumper and heavier compared to natural spike set. The well developed seeds were bigger in size with hard seed coat. Undeveloped seeds were seen as black specks. In the present study 573 seeds were obtained from different crosses. Seeds obtained from crosses were sown in polybags filled with river sand. Seeds germinated in 21- 69 days. Percentage of germinated seedlings was 23.73. Cotyledonary leaves opened in 2 to 31 days and first true leaf appeared in 22 - 41 days.

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*Originals not seen

EXPLOITATION OF BISEXUAL VARIANT IN DEVELOPING HIGH YIELDING TYPES OF *Piper longum* L.

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Piper longum L. is a dioecious medicinal species. Mature female spike is the officinal part. However, female spikes are small (2.0 - 3.0 cm) compared to male (6.0 - 7.5 cm). A bisexual variant (Acc.P25) was identified in *P. longum* (Sujatha and Nybe, 2007) with spikes as long as male spikes.

The present investigation was undertaken with the objective of studying the reproductive biology of *Piper longum* L. and for developing long spiked high yielding varieties of *Piper longum* L.

Reproductive biology

Spikes 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. Anthesis and anther dehiscence were between 7.30 am and 4.30 pm with a peak between 10.30 am to 12.30 pm.

Pollen fertility was maximum at 9.30 am (42.54 per cent). Complete opening of flowers in an inflorescence took seven days in male, female and bisexual types. Complete dehiscence of anthers also took one week in male and bisexual inflorescences.

Developing bisexual types

Effect of growth regulators in developing fully bisexual inflorescences in Acc.P25 indicated that GA_3 (5 ppm, 10 ppm and 50 ppm), BA (100 ppm and 500 ppm) and Boron 3 ppm could induce fully bisexual spikes.

Different methods of pollination showed that dry method was the most effective for getting seed set in *P. longum* L. In selfing and crossing experiments, seed set could be obtained only in crosses involving female type as female parent and bisexual type as male parent. Maximum seed set was obtained during September, under dry method of pollination. Seeds germinated in 21- 69 days. Cotyledonary leaves opened in 2 to31 days. The first true leaves opened in 2 to 41 days.