CYTO - MORPHOLOGICAL INVESTIGATIONS IN *Piper* spp.

BY

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THESIS

Submitted in partial fulfilment of the
requirement for the degree of

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Kerala Agricultural University

DEPARTMENT OF PLANTATION CROPS AND SPICES
COLLEGE OF HORTICULTURE
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DECLARATION

I hereby declare that the thesis entitled "**Cyto-morphological investigations in *Piper* spp.**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship, associateship or other similar title, of any other university or society.

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Certified that the thesis entitled "**Cyto-morphological investigations in *Piper* spp.**" is a record of research work done independently by **Miss.Ambili Anand**, 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 **Miss.Ambili Anand**, a candidate for the degree of **Master of Science in Horticulture**, with major in **Plantation Crops and Spices**, agree that the thesis entitled **"Cyto-morphological investigations in *Piper* spp."** may be submitted by Miss.Ambili Anand, in partial fulfilment of the requirement, for the degree.

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EXTERNAL EXAMINER

I express my sincere gratitude to Dr.R.Vikraman Nair, Professor, CCRP for his good will in rendering me all help during the period of investigation.

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I lovingly thank my parents for their constant prayers and blessings at every juncture and my sister who has been a source of encouragement and moral support at times of despair.

Above all I bow my head before the ALMIGHTY whose unmerited blessings enabled me to undertake this venture successfully.

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AMBILI ANAND

To my parents

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Introduction

INTRODUCTION

The genus *Piper* L. belonging to the family Piperaceae is very large, comprising of more than 3000 species. The species of the genus are distributed mainly in South America, Malaysia, Indonesia and India. In India the genus has a disjunct distribution concentrated mainly in the Eastern Himalayas and in the Western Ghats.

Many economically important species such as *P. nigrum* (black pepper), *P. betle* (betel vine), *P. longum* (thippalli), *P. chaba* (chaba thippalli), *P. cubeba* (tailed pepper) etc. are included in the genus, *Piper*. Of these species, *P. nigrum*, black pepper is the most important spice crop of Kerala and an important foreign exchange earner. The crop, however is faced with many serious threats like pests and diseases, especially foot rot. As resistance to foot rot is not reported within the species, introgression of genes from wild relatives is the probable solution to the problem. For any scientific crop improvement programme, studies on phylogeny and evolution are essential pre-requisites.

Hooker (1886) has described the genus *Piper* as the most difficult to classify due to widely varying vegetative characters, closely packed spikate inflorescence and extremely small floral parts. In a relatively recent report Bornstein (1989) mentions *Piper* as the largest taxa devoid of a natural classification.

The importance of cytological study in tracing the systematic position, the inter-relationship of different species and in understanding their evolutionary trends has been demonstrated in many plant species. Despite the great economic importance and the wide distribution of a large number of species and varieties of *Piper*, only very little is

known regarding their cytology. Cytological studies in the genus *Piper* is still in its infancy and the available reports are highly controversial. This may be probably due to the large number of extremely small chromosomes in *Piper* species.

Under these circumstances cyto-morphological investigations in *Piper* species was undertaken with the following objectives:

1) to prepare a morphological descriptor for the eight species of *Piper* collected from different geographical areas.
2) cytological investigations to find out the chromosome number of these species.

The present investigations form a part of a major project on studying morphological, cytological and biochemical relationship in the genus *Piper* and working out the species relationship in the same.

Review of Literature

REVIEW OF LITERATURE

2.1 Taxonomic history of Indian *Piper*

The earliest known reference of plants belonging to *Piper* was of Bauhin (1563). His *Pinax Theatri Botanici* included black pepper (*Piper rotundum nigrum*) and long pepper (*Piper longum orientale*). In *Hortus Indicus Malabaricus* Rheede (1678) described five types of wild pepper, four of which with illustrations. Later, Casparus (1696) published the *Flora Malabarica sive Horti Malarici Catalogue* and in that he also described five species. Linnaeus (1753) included seventeen species from India in his *Species Plantarum*. Roxburgh (1832) described eighteen species of which seven were from Indian peninsula. Miquel (1843, 1845, 1846a, 1846b) described more than 600 species and of these only seven were from India. Wight (1853) in his *Icones Plantarum Indiae Orientalis* illustrated sixteen species, fifteen of which were from Indian peninsula. Of the 640 species described by De Candolle (1869) 52 were from the Indian peninsula.

The first major study on Indian *Piper* was by Hooker (1886). In his 'Flora of British India' he had pointed out the problems encountering the floristic study of *Piper*. He described 45 species, of which 29 were assigned to Indian peninsula. Hooker divided the genus *Piper* into six sections which are as follows:

Section I: Muldera: This section included five species. *P. galeatum* and *P. trichostachyon* were included in this section.

Section II: Cubeba: Six species were included in this.

Section III: Chavica: Sixteen species were included in this section. *P. longum, P. betle, P. chaba* and *P. hapnium* were members of this section.

Section IV: Pseudochavica: Six species were included in this section.

Section V: Eupiper: Eleven species were included in this section like *P. nigrum, P. argyrophyllum, P. attenuatum* etc.

Section VI: Heckeria: Included *P. subpeltatum* which is now under a seperate genus *Heckeria*.

In his Flora, Hooker had also given a long list of undeterminable or doubtful species.

Brandis (1906) described eight species under the genus *Piper*.

Apart from these, several Indian taxonomists described *Piper* species in their regional publications. Prain (1903) described eight species in his 'Flora of Bengal'. Duthie (1903) in the 'Flora of the Upper Gangetic Plains and the adjacent Siwalik and Sub-Himalayan tracts' included *P. longum, P. betle, P. mullesua, P. napalense* and *P. nigrum*. Cooke (1903) in his 'Flora of the Presidency of Bombay' reported *P. trichostachyon, P. nigrum, P. hookeri* and *P. longum* in addition to the cultivated *P. betle*.

Rao (1914) in his treatise 'Flowering Plants of Travancore' listed the following species from Western Ghats: *P. galeatum, P. trichostachyon, P. longum, P. brachystachyum, P. hookeri, P. nigrum, P. sylvestre, P. hymenophyllum, P. argyrophyllum, P. wightii, P. subpeltatum* and *P. longicaule*. Fisher (1921) described

six species from Annamalai hills. Burkill (1924) included thirteen species in his 'Flora of Abor Hills of North Eastern India'.

Hains (1924) in his 'Botany of Bihar and Orissa' reported *P. longum, P. peepuloides, P. chaba, P. attenuatum, P. nigrum* and the cultivated species of *P. betle*. The most authoritative floristic study of the Western Ghats was that of Gamble (1925) who in his 'Flora of the Presidency of Madras' included the following species along with keys: *P. galeatum, P. trichostachyon, P. longum, P. hapnium, P. brachystachyum, P. hookeri, P. hymenophyllum, P. attenuatum, P. argyrophyllum, P. schmidtii, P. wightii* and *P. barberi*. Fyson (1932) in his 'Flora of Nilgiris and Pulney hill tops' reported *P. brachystachyum, P. schmidtii, P. nigrum* and *P. wightii*. Kanjilal *et al.* (1940) in their 'Flora of Assam Region' reported three species of *Piper*.

Rahiman (1981) identified a new species, *P. bababudani* from the Bababudan hills of Karnataka but that species was not validly published. Ravindran *et al.* (1987) reported two new taxa from the Silent Valley forests of Kerala, namely *P. silentvalleyensis* and *P. nigrum* var. *hirtellosum*. Velayudhan and Amalraj (1992) reported a new species from Western Ghats namely *P. pseudonigrum*. Babu *et al.* (1993) reported two new taxa of Piper, *P. sugandhi* and *P. sugandhi* var. *brevipilis* from Sugandhagiri project area of Western Ghats and they were related to *P. nigrum, P. galeatum* and *P. trichostachyon*.

The other floristic enumerations of *Piper* species were those of Santapau (1960), Parker (1924), Sharma and Tiagi (1979), Saldanha and Nicholson (1976), Rao and Razi (1981), Rahiman *et al.* (1981) and Rahiman and Nair (1987).

2.2 Morphology

2.2.1 Vegetative morphology

Plants belonging to genus *Piper* were shrubs, rarely herbs or trees, erect or scandent, often glandular and aromatic. Branches were with swollen nodes. Leaves alternate, simple, entire, often unequal sided and range from very thin and membranous to thick and coriaceous in texture. Leaf surface vary from smooth to rugose, some entirely glabrous, others pubescent and few had surface scales. Majority of the species had small but conspicuous glandular dots frequently on leaves but sometimes on other tender and fleshy parts. Petiole varied in length among different species and often within the same species. Leaves were rarely sessile. Majority of the species had five to nine principal nerves on leaves which were multiple-palmately or rarely pinnately nerved. Stipules were none to two, connate or adnate to the petiole (Hooker, 1886; Gamble, 1925; Trelease and Yuncker, 1950).

P. argyrophyllum Miq.

P. argyrophyllum was a dioecious woody climber with stem thickness intermediate between those of *P. nigrum* and *P. longum*. The leaves were somewhat thick and covered with silvery scales (Rahiman *et al.*, 1979). The leaves were lanceolate to ovate-lanceolate in shape (Samuel *et al.*, 1983).

P. attenuatum Buch. Ham.

Rahiman *et al.* (1979) reported *P. attenuatum* as a dioecious woody climber with stem thickness intermediate between those of *P. nigrum* and *P. longum*. The leaves

were membranous and glabrous. Samuel *et al.* (1983) reported that the leaves were orbicular in shape.

P. bababudani

Rahiman (1981) described *P. bababudani* as a thick stemmed dioecious vine. The leaves were alternate, rounded-ovate to cordate, coriaceous with deciduous stipule adnate to petiole.

P. barberi Gamble

Subramanyam and Henry (1970) described *P. barberi* as scandent under shrubs. Leaves petiolate, linear-lanceate, elliptic-lanceate or lanceate, membranous, with acuminate apex and acute base. Babu *et al.* (1992) described *P. barberi* as a dioecious climber. The plant produced three different type of shoots *viz.*, the juvenile shoots, orthotropic shoots and lateral plagiotropic shoots. From juvenile shoot, orthotropic shoots arouse which produced small lanceolate leaves with slightly unequal base and acuminate tip. From orthotropic shoot, the lateral fruiting branches arouse which bore unequal leaves with acuminate tip and acute base. There were boat shaped prophylls at each node which were persistant for sometime and fell off later.

P. betle Linn.

The plant was dioecious. The leaves were broadly or narrowly ovate with acuminate apex and oblique or slightly rounded base and were thinly coriaceous (Samuel *et al.*, 1983). Balasubramanyam and Rawat (1990) conducted studies on morphology and chemistry of *P. betle*. The morphology of the five distingushing cultivars Bangla,

Desawari, Kapoori, Meetha and Sanchi were described. While the cultivar Bangla was characterised by roundish to cordate leaf lamina cultivar Desawari had short acuminate curved tip with cordate leaf base. Kapoori leaves were recognized by their narrow ovate shape, characteristic venation and yellowish green colour. Metha leaves were distingushed from others by the short acute apex and yellowish spots on lamina and those of Sanchi by broadly ovate leaves with attenuate apex and shortly channelled petiole.

P. brachystachyum Wall. Cat.

The plant was a dioecious woody climber. The leaves were somewhat thick and glabrous (Rahiman *et al.*, 1979).

P. chuvya Roxb.

The plant was dioecious. The leaves were broadly or narrowly ovate with acuminate apex and oblique or slightly rounded base and were thinly coriaceous (Samuel *et al.*, 1983).

P. galeatum Cas. DC.

P. galeatum was a dioecious woody climber with very thick stem. The leaves were coriaceous and glabrous (Rahiman *et al.*, 1979).

P. hapnium Miq.

Hooker (1886) and Rahiman (1981) described *P. hapnium* as a dioecious climber with stout branches. The leaves on runners were broad cordate but the leaves on

floriferous branches were elliptic or oblong-lanceolate. *P. hapnium* had close resemblance with *P. longum* in a number of morphological characters.

P. hymenophyllum Miq.

The plant was a dioecious woody climber with stem thickness intermediate between those of *P. nigrum* and *P. longum*. The leaves were somewhat thick and densely pubescent (Rahiman *et al.*, 1979).

P. longum Linn.

P. longum was a dioecious creeper with comparatively very thin stem. The leaves were membranous (Rahiman *et al.*, 1979). The leaves of *P. longum* were broadly or narrowly ovate with acuminate apex and deeply cordate base and were membranous (Samuel *et al.*, 1983).

P. nigrum Linn.

Rahiman *et al.* (1979) described *P. nigrum* as a bisexual woody climber with comparatively thicker stem. However, the wild selections of *P. nigrum* were reported as dioecious (Samuel *et al.*, 1983). The leaves were coriaceous and glabrous. Samuel *et al.* (1983) described the cultivated varieties of *P. nigrum* as bisexual. The leaves were broadly or narrowly ovate with acuminate apex and oblique or slightly rounded base. The leaves were highly coriaceous. Vegetative morphology of *P. nigrum* had been described by Ravindran and Babu (1994).

P. pseudonigrum Velayudhan and Amalraj

Velayudhan and Amalraj (1992) described *P. pseudonigrum* - a new species from Western Ghats. The plant was a shrubby climber with alternate leaves which were petiolate, very broadly ovate, coriaceous and glabrous on both sides, dorsally shiny and ventrally glaucous, tip acuminate, base rounded to equal.

P. sugandhi Ravindran, Babu and Naik and *P. sugandhi* var. *brevipilis* Ravindran, Babu and Naik

Babu *et al.* (1993) described two new taxa of *Piper* from Western Ghats *viz.*, *P. sugandhi* and *P. sugandhi* var. *brevipilis*. *P. sugandhi* was a dioecious climber with purple shoot tip, alternate leaves which were glabrous, coriaceous, ovate to lanceolate, tip acuminate, base rounded to acute and often oblique. Petiole was grooved and the margins modified as caducous sheaths. *P. sugandhi* var. *brevipilis* had similar vegetative morphology.

P. thawaitseii Cas. DC.

The plant was dioecious. The leaves were broadly or narrowly ovate with acuminate apex and oblique or slightly rounded base and were highly coriaceous (Samuel *et al.*, 1983).

P. trichostachyon Cas. DC.

The plant was a dioecious woody climber with very thick stem. The leaves were coriaceous (Rahiman *et al.*, 1979).

P. trineuron Miq.

Samuel *et al.* (1983) described *P. trineuron* as a dioecious plant. The leaves were elliptic and membranous.

P. trioicum Roxb.

Subramanyam and Henry (1970) described *P. trioicium* as a slender climber with elliptic - lanceate to orbicular - ovate membranous leaves with acuminate apex and rounded or obliquely cordate base.

P. zeylanicum Miq.

The plant was dioecious. The leaves were broadly or narrowly ovate with acuminate apex and oblique or slightly rounded base and were highly coriaceous (Samuel *et al.*, 1983).

Central American *Piper* spp.

Burger (1972) surveyed the gross morphology of the Central American species of *Piper*. The cap like structure covering the shoot tip in many species was interpreted as a modified prophyll. A ligulate development and the inclusion of the inflorescence within the sheathing leaf base in a few species were discussed.

Four species of *Piper viz., P. amplilimbum, P. haenkeanum, P. protractum* and *P. ridley* were described by Chew (1972).

2.2.2. Floral morphology

Spikes were always opposite to the leaves, predominantly filiform, sometimes cylindric and rarely sub-globose or globose. Peduncle was glabrous, hirtellous or puberulous and vary in length in different species. In some species stamens were two in number occupying either side of the ovary. In others, three of which the third was posterior, rarely more than three, filaments short, anther lobes two, rarely one, each lobe with two pollen sacs which after dehiscence became one due to confluence. Ovary was single, sessile, subglobose or flask shaped, one ovuled, stigma usually sessile, orthotropous (Le Mount and Decaine, 1876; Hooker, 1886; Gamble, 1925).

P. argyrophyllum

Inflorescences of *P. argyrophyllum* were filiform and drooping. Plants flowered profusely in May-July. Flowering was observed in the very next season of planting (Rahiman *et al.*, 1979). The spikes were slender with prominant individual flowers arranged spirally on the inflorescence axis. The bracts were broadly oval with raised margin. The number of stamens varied from three to four (Samuel *et al.*, 1983).

P. attenuatum

In *P. attenuatum* inflorescences were filiform and drooping. Plants flowered profusely in May-July. Flowering was observed in the very next season of planting (Rahiman *et al.*, 1979). The spikes were slender with prominant individual flowers arranged spirally on the inflorescence axis. The bracts were oblong and adnate to the inflorescence axis (Samuel *et al.*, 1983).

P. bababudani

Spikes were narrow, filiform and pendulous with flowers arranged in spirals. Bracts in both male and female were linearly oblong with decurrent base and hood-like apex (Rahiman, 1981).

P. barberi

Spikes of *P. barberi* were terminal with peltate orbicular bracts. Male flowers had two stamens and female flowers had three stigmas (Subramanyam and Henry, 1970). In *P. barberi* the lateral plageotropic shoots produced spikes which were opposite to the leaves. Spikes were slender, long and borne on dangling stalks with peltate orbicular bracts. Male flowers had two anthers and female flowers had a single ovary with three stigmatic lobes (Babu *et al.*, 1992).

P. betle

Samuel *et al.* (1983) reported that the inflorescences of *P. betle* were cylindrical and pendulous spikes. Flowers were spirally arranged and sunken in the inflorescence axis. Bracts were circular, peltate with free and membranous margin. Stigma was four to five lobed. Balasubramanyam and Rawat (1990) reported that in *P. betle* the spikes were subpendulous with peltate bracts. The male flowers had two to three stamens and the female flowers had five to eight stigmatic lobes.

P. brachystachyum

Inflorescences were globose and erect in *P. brachystachyum*. Flowering was observed profusely in May-July (Rahiman *et al.*, 1979).

P. chuvya

Samuel *et al.* (1983) described the inflorescences of *P. chuvya* as long, pendulous spikes. Flowers were spirally arranged and sunken in the inflorescence axis. Bracts were circular, peltate with free and membranous margin. Stigma was three to four lobed.

P. galeatum

Inflorescences were filiform and drooping. Flowering was observed profusely in May-July. Cuttings took three years for the initiation of flowering (Rahiman *et al.*, 1979).

P. hapnium

Female spikes were short, cylindric, erect, shining white with puberulous peduncle. Bracts were peltate orbicular and pedicelled. Stigma three to four lobed. Flowering was observed during June-July (Hooker, 1886; Rahiman, 1981).

P. hymenophyllum

Rahiman *et al.* (1979) described the inflorescences of *P. hymenophyllum* as filiform and drooping. Plants flowered profusely in May-July.

P. longum

Inflorescences of *P. longum* were cylindrical and erect. Flowering was observed profusely in May-July (Rahiman *et al.*, 1979). Samuel *et al.* (1983) described the inflorescences of *P. longum* as short, thick and erect spikes. Flowers were minute, spirally arranged and sunken in inflorescence axis. Bracts were circular with free and membranous margin. Stigma was three to four lobed.

P. nigrum

Inflorescences of *P. nigrum* were filiform and drooping. Cuttings took three years for the initiation of flowering (Rahiman *et al.*, 1979). Samuel *et al.* (1983) described the inflorescences of *P. nigrum* as long, thick and pendulous spikes. Flowers were bisexual or unisexual, spirally arranged on the inflorescence axis. Bracts were oblong and adnate to the inflorescence axis. Flowers had three to four lobed stigma and two stamens. *P. nigrum* inflorescence was a filiform pendant spike borne opposite to the leaves and had ovate, fleshy, cupular bracts. Bisexual flowers had two anthers and three to five lobed stigma.

P. pseudonigrum

Male and female spikes of *P. pseudonigrum* were purple to greenish purple, drooping, with cupular bracts. Female spikes were glabrous but male spikes had very minute hairs. Male flowers had two stamens and female flowers had sessile three to four lobed stigma (Velayudhan and Amalraj, 1992).

P. sugandhi and *P. sugandhi* var. *brevipilis*

Male and female spikes of *P. sugandhi* were filiform and pendant with cupular bracts. Flowering time was April-May. Male flowers had two stamens and female flowers had three lobed stigma. *P. sugandhi* var. *brevipilis* was very similar to *P. sugandhi* but differed from that in having pubescent bracts (Babu *et al.*, 1993).

P. thawaitseii

Samuel *et al.* (1983) described the inflorescences of *P. thawaitseii* as short, stout and erect spikes. Flowers had three to four lobed stigma and were spirally arranged on inflorescence axis. Bracts were circular with free and membranous margin.

P. trichostachyon

Inflorescences were filiform and drooping. Plants flowered profusely in May-July. Cuttings took three years for the initiation of flowering (Rahiman *et al.*, 1979).

P. trineuron

Samuel *et al.* (1983) described the inflorescences of *P. trineuron* as short, slender and pendulous spikes. Flowers were spirally arranged on inflorescence axis and had two stamens. Bracts were oblong and adnate to inflorescence axis.

P. trioicum

P. trioicum had spikes upto fifteen centimetre long and slender (Subramanyam and Henry, 1970).

P. zeylanicum

Samuel *et al.* (1983) described the inflorescences of *P. zeylanicum* as short, thick and erect spikes. Flowers were spirally arranged on inflorescence axis. Bracts were circular with free and membranous margin. Female flowers had three to four lobed stigma and male flowers had two stamens.

Central American *Piper* spp.

In the Central American species *P. hispidum* and its close allies spikes were usually slender and erect with tightly congested parts (The stamens, style and stigmas were rarely exerted above the level of the floral bracts). In the other group of Central American species *P. obliqum* and its allies spikes were long, pendulous and the floral parts were not closely compacted (Burger, 1972).

Floral morphology of four *Piper* spp. *viz.*, *P. amplilimbum*, *P. haenkeanum*, *P. protractum* and *P. ridley* were described by Chew (1972).

2.2.3. Fruit

Fruit was a small indehiscent berry, testa thin, cartilaginous, albumen floury, embryo antitropous, occupied a cavity in the albumen at the top of the fruit. Cotyledons minute or absolent, radicle thick and superior (Le Mount and Decaine, 1876; Hooker, 1886; Gamble, 1925).

P. argyrophyllum

Rahiman *et al.* (1979) reported that the developing berries were oval in *P. argyrophyllum*. However when mature they were spherical in shape and were slightly bitter in taste. Samuel *et al.* (1983) reported that the berries were ovoid, less pungent and never turned red when mature.

P. attenuatum

Rahiman *et al.* (1978) reported that though the developing berries of *P. attenuatum* were oblong, the mature berries were spherical in shape. The berries were slightly bitter in taste. The berries of *P. attenuatum* were ovoid, less pungent and dark green when mature (Samuel *et al.*, 1983).

P. bababudani

Rahiman (1981) reported that the berries of *P. bababudani* were globose and 0.75 centimetre in diameter.

P. barberi

Berries of *P. barberi* were obovoid or subcylindric and reddish when ripe (Subramanyam and Henry, 1970). Babu *et al.* (1992) reported that in *P. barberi* fruits were usually very few, fleshy drupe, round when mature and deep red when full ripe. Seed was ovoid to round and slightly pungent.

P. betle

Samuel *et al.* (1983) reported that the whole spike became the fruit when mature and did not turn red in colour.

P. brachystachyum

Rahiman *et al.* (1979) reported that the berries of *P. brachystachyum* were highly pungent and gave a burning sensation when chewed.

P. chuvya

Samuel *et al.* (1983) reported that in *P. chuvya* the whole spike became the fruit when mature.

P. galeatum

Rahiman *et al.* (1979) reported that the shape of developing berries were spherical to oval in *P. galeatum* and the berries were pungent.

P. hapnium

Fruit was a small berry, obovate and very pungent. Fruits matured in December-January period (Rahiman, 1981).

P. hymenophyllum

Rahiman *et al.* (1979) reported that though the developing berries of *P. hymenophyllum* were oval the mature berries were spherical in shape. The green berries on ripening directly turned black and were slightly bitter in taste.

P. longum

Rahiman *et al.* (1979) reported that the developing berries of *P. longum* were more or less obconical and pungent. Samuel *et al.* (1983) reported that in *P. longum* the whole spike became the fruit when mature.

P. nigrum

In *P. nigrum* the developing and mature berries were spherical in shape and were pungent. The berries were green when young, turned yellow, then orange and finally red (Rahiman *et al.*, 1979). Samuel *et al.* (1983) reported that the berries of *P. nigrum* were globose, large and highly pungent. Ravindran and Babu (1994) observed that the fruit was a sessile, globose drupe in *P. nigrum*. The green unripe fruit turned red upon ripening and turned black after drying. The size and number of drupes per spike varied with different cultivars.

Rahiman *et al.* (1979) reported that the berries of wild *P. nigrum* showed considerable variations in pungency.

P. pseudonigrum

Velayudhan and Amalraj (1992) reported that fruits of *P. pseudonigrum* ripened through yellow to red and were less pungent than that of *P. nigrum*.

P. sugandhi

Fruits of *P. sugandhi* were oblong, bold and pungent as in black pepper, turned yellow and then to red on ripening. Fruits took seven to eight months to mature (Babu *et al.*, 1993).

P. thawaitseii

Samuel *et al.* (1983) reported that the berries of *P. thawaitseii* were globose in appearance and turned red when mature.

P. trichostachyon

Rahiman *et al.* (1979) reported that the berries of *P. trichostachyon* were spherical or oval and were pungent.

P. trioicum

Berries were globose and sessile in *P. trioicum* (Subramanyam and Henry, 1970).

P. zeylanicum

Samuel *et al.* (1983) reported that the berries of *P. zeylanicum* were large and globose. Berries were less pungent and turned red when mature.

Fruits of four *Piper* spp. *viz.*, *P. amplilimbum, P. haenkeanum, P. protractum* and *P. ridleyi* were described by Chew (1972).

2.3 Cytology

2.3.1 Chromosome number

Cytology of *Piper* has been studied by various workers and most of the studies were confined to determination of chromosome numbers.

2.3.1.1 Somatic chromosome number

P. nigrum

The chromosome number of *P. nigrum* was reported as 2n=52 (Mathew, 1958; Martin and Gregory, 1962; Mathew, 1972; Samuel and Bavappa, 1981; Samuel, 1981; Mathew and Mathew, 1982; Jose and Sharma, 1983; Jose and Sharma, 1984; Rahiman and Nair, 1986; Nair *et al.*, 1993). Janakiammal (1945) reported the chromosome number of *P. nigrum* as 2n=128. Sharma and Bhattacharya (1959) reported the chromosome number of *P. nigrum* as 2n=48. Dasgupta and Datta (1976) conducted cytological studies in Piperaceae. They reported the chromosome number of *P. nigrum* from Agarthala as 2n=36 and *P. nigrum* from South India as 2n=60. Chromosome number of *P. nigrum* was reported as 2n=54 by Sampathkumar and Navaneethan (1981). In cultivated varieties of *P. nigrum* the somatic chromosome number was reported as 2n=104 by Samuel (1981) and Jose and Sharma (1984).

In wild varieties of *P. nigrum* chromosome numbers of 2n=52 and 104 were reported by Mathew (1958) and Mathew (1972).

Polyploidy in *P. nigrum*

Nair and Ravindran (1992) induced polyploidy in hybrid cultivar of black pepper Panniyur-1. Seeds were treated with 0.05% colchicine and plants raised. One tetraploid of Panniyur-I with 2n=104 chromosomes in somatic cells was recovered from

the treated plants. Nair *et al.* (1993) identified polyploidy in a cultivar of *P. nigrum*. The plant was a triploid with chromosome number 2n=78 and the progenies showed a range of variation from 2n=52 to 2n=104.

P. betle

As in *P. nigrum* somatic chromosome number in *P. betle* was also varyingly reported by various workers. Johnson (1910) and Janakiammal (1945) reported 2n as 32. Somatic chromosome number was reported as 2n=78 (Mathew, 1958; Okada, 1986). Varying chromosome numbers of 78, 42, 58 and 195 were reported by Jose and Sharma (1983, 1985). Sharma and Bhattacharya (1959) and Dasgupta and Datta (1976) reported the chromosome number as 2n=64. Samuel and Morawetz (1989) reported 2n=52 and 65 as the chromosome numbers in *P. betle*.

Somatic chromosome number reports of other species of the genus *Piper* are given in Table 1.

Table 1. Somatic chromosome number reported in *Piper* spp.

Species	Somatic chromosome number (2n)	Reference
1	2	3
P. argyrophyllum	36, 39	Samuel and Bavappa (1981)
	52	Rahiman and Nair (1986)
	52	IISR unpublished (Ravindran and Babu, 1994)
P. attenuatum	26, 39	Samuel and Bavappa (1981)
	36	Bai and Subramanian (1985)
	52	Jose (1981)
	52	Jose and Sharma (1983, 1984)
	52	Rahiman and Nair (1986)
	52, 104	IISR unpublished (Ravindran and Babu, 1994)
P. bababudani	52	Rahiman (1981)
P. barberi	52	Babu *et al.* (1992)
	52	Mathew and Mathew (1992)
P. boehmeriafolium Wall.	52	Jose and Sharma (1984)
P. brachystachyum	132	Bai and Subramanian (1985)
	132	IISR unpublished (Ravindran and Babu, 1994)
P. chaba Hunter	24	Janakiammal (1945)
	104	Jose and Sharma (1984)
P. chuvya	52	Samuel and Bavappa (1981)

Contd.

Table 1. Continued

1	2	3
P. colubrinum Lamk.	26	IISR unpublished (Ravindran and Babu, 1994)
P. cubeba Linn.	24	Janakiammal (1955)
	24	Dasgupta and Datta (1976)
	24	Jose and Sharma (1983, 1984)
P. futokazura Sub. *et* Zuce.	24	Yoshida (1960)
P. galeatum	40	Bai and Subramanian (1985)
	52	Rahiman and Nair (1986)
	52	IISR unpublished (Ravindran and Babu, 1994)
P. geniculatum Sw.	28	Maugini (1951)
P. gibbilimbum	130	Lebot *et al.* (1991)
P. grissico-argenta Yunck.	22	Smith (1966)
P. hapnium	52	Rahiman (1981)
P. hookeri Miq.	60	Bai and Subramanian (1985)
	104	Rahiman and Nair (1986)
P. hymenophyllum	104	IISR unpublished (Ravindran and Babu, 1994)
P. longum	24	Tjio (1948)
	44	Sampathkumar and Navaneethan (1981)
	48	Dasgupta and Datta (1976)
	52	Mathew (1958)
	52	Jose and Sharma (1983, 1984)
	52	Rahiman and Nair (1986)
	53	Samuel and Morawetz (1989)
	96	Sharma and Bhattacharya (1959)
P. magnificum Trel.	24	Dasgupta and Datta (1976)
	26	Smith (1966)

Contd.

Table 1. Continued

1	2	3
P. medium Jacq.	28	Maugini (1953)
P. methysticum	130	Lebot *et al.* (1991)
P. mullesua Ham.	52	Samuel (1981)
	104	Rahiman and Nair (1986)
P. obliqum	52	Samuel (1981)
P. ornatum N.E.Br.	52	Samuel (1981)
	80	Sharma and Bhattacharya (1959)
P. peepuloides Roxb.	156	Jose and Sharma (1984)
P. posteltanum	26	Ono (1975)
P. schmidtii Hook.f.	96	Bai and Subramanian (1985)
P. subpeltatum	24	Johansen (1930)
P. sugandhi	52	IISR unpublished (Ravindran and Babu, 1994)
P. sylvestre Lamk.	26, 39	Samuel and Bavappa (1981)
P. thawaitseii	39,65	Samuel and Bavappa (1981)
P. trichostachyon	52	Rahiman and Nair (1986)
	52	IISR unpublished (Ravindran and Babu, 1994)
P. trineuron	26	Samuel and Bavappa (1981)
P. umbellatum	28	Gadella (1972)
P. ungiculatum Ruiz and Pav.	26	Bedi *et al.* (1981)
	28	Maugini (1951)

Contd.

Table 1. Continued

1	2	3
P. wichmanii	130	Lebot *et al.*(1991)
P. wightii Miq.	52	IISR unpublished (Ravindran and Babu, 1994)
P. zeylanicum	39	Samuel and Bavappa (1981)
Piper spp. (undetermined)	104	Mathew (1958)

2.3.1.2 Haploid chromosome number

Haploid chromosome number reported in different *Piper* spp. are given in Table 2.

Table 2. Haploid chromosome number reported in *Piper* spp.

Species	Haploid chromosome number (n)	Reference
P. hymenophyllum	65	Love (1984)
P. schmidtii	72	Love (1984)
P. subpeltatum	12	Johansen (1930)
P. wightii	24	Bai and Subramanian (1985)

2.3.1.3 Basic chromosome number

Basic chromosome number of *Piper* was reported as x = 12 (Sharma and Bhattacharya, 1959; Dasgupta and Datta, 1976; Bai and Subramanian, 1985; Rahiman and Nair, 1986). Basic chromosome number of x = 13 was also suggested in the genus

Piper (Mathew, 1958; Samuel and Bavappa, 1981; Samuel, 1981; Rahiman and Nair, 1986).

Rahiman and Nair (1986) suggested that mostly multiples of twelve chromosomes had been reported from North India and multiples of thirteen from South India. It was assumed that the species from these two centres of distribution probably had different evolutionary pathway starting from basic numbers of six and seven.

2.3.2 Karyomorphology

Based on the positions of constrictions and lengths Sharma and Bhattacharya (1959) classified the chromosomes in the genus *Piper* into Type A, B, C, D, E, F, G, H and I. Chromosomes were characterized in having mostly median and a few submedian constrictions. The chromosomes of *Piper* were classified into Type A, B, C, D, E and F (Dasgupta and Datta, 1976), Type A, B, C, D and E (Jose and Sharma, 1985), Type A, B, C, D, E, F, G, H, I, J, K, L, M and N (Bai and Subramanian, 1985).

Samuel (1981) reported that the chromosomes were very small ranging from 1.0 to 2.8 μ in diploid species of *Piper* and were even smaller ranging from 0.6 to 1.2 μ in tetraploid species. The morphology of chromosomes in all the species of *Piper* showed a homogeneity in the extremely short size which ranged between 0.56 to 2.41 μ (Jose and Sharma, 1985). Rahiman and Nair (1986) observed that the chromosomes in *Piper* spp. were very small in size and varied from 0.7 to 2.5 μ. Interspecific size variation of chromosomes in *Piper* was observed by Samuel and Morawetz (1989).

P. nigrum

Mathew (1958) reported that in all the five cultivated varieties of *P. nigrum* studied the chromosomes were very small in size ranging from 1 to 2.7 μ in length. The root tip cells showed 52 chromosomes consisting of apparently two pairs of medium sized, six pairs of short and eighteen pairs of shorter chromosomes. The chromosomes were very much condensed and the positions of centromeres were not clear.

Dasgupta and Datta (1976) observed the length of chromosomes as 0.77 to 1.9 μ in *P. nigrum* from Agarthala and 0.77 to 2.3 μ in *P. nigrum* from South India. Karyotype was represented as 2A, 4B, 26E and 4F in *P. nigrum* from Agarthala and 8B, 44E and 8F in *P. nigrum* from South India.

In hybrid pepper variety Panniyur-1 root tip cells showed 52 small sized chromosomes which could be grouped into three size classes such as four pairs of relatively long and rod shaped (1.6 to 1.8 μ), ten pairs of medium sized (1.1 to 1.0 μ) and twelve pairs of very small sized (0.8 to 1.0 μ) chromosomes. The parental varieties with 2n = 52 also showed closely similar karyotypic features (Mathew and Mathew, 1982).

In *P. nigrum* varieties the primary constrictions ranged from nearly median to nearly submedian in position. The chromosome size ranged between 0.74 to 2.2 μ (Jose and Sharma, 1983).

Mathew (1958) reported that in one wild *P. nigrum* variety with 2n = 104, there were four pairs of medium sized, twelve pairs of short and 36 pairs of shorter chromosomes. From this it was seen probable that this was an auto-tetraploid derived from one of the 52 chromosomed wild varieties.

Karyotypic comparison of the different cultivated and wild varieties of *P. nigrum* based on absolute chromosome size indicated a positive correlation between spike length and chromatin content in general (Mathew, 1972). The variety Karimunda which had the smallest absolute chromosome size produced the shortest spikes and variety Aripadappan which showed relatively higher value of absolute size of chromosomes produced longest spikes among cultivated varieties. The wild diploid varieties which produced longer spikes showed relatively larger absolute chromosome size. The same correlation between spike length and absolute chromosome size was repeated in the wild tetraploid varieties as well.

P. betle

P. betle (2n = 78) had 6 pairs of medium sized, 8 pairs of short and 25 pairs of shorter chromosomes ranging from 1 to 2.7 μ in length (Mathew, 1958). Dasgupta and Datta (1976) observed the length of chromosomes as 0.77 to 1.5 μ in *P. betle* var. jhalpan and 0.77 to 1.2 μ in *P. betle* var. mithapan. Karyotype was represented as 4B, 56E and 4F in *P. betle* var. jhalpan and 6B, 36E and 22F in *P. betle* var. mithapan. In *P. betle* the chromosomes were in general medium to small sized with nearly median to nearly submedian primary constrictions (Jose and Sharma, 1983). The size range of chromosomes of *P. betle* was observed as 0.6 to 2.1 μ (Bai and Subramanian, 1985).

P. longum

P. longum male and female plants with 2n = 52 had two pairs of medium sized, six pairs of short and eighteen pairs of shorter chromosomes ranging from 1 to 3 μ in length (Mathew, 1958). Dasgupta and Datta (1976) reported the length of chromosomes as 0.7 to 1.5 μ in *P. longum*. In *P. longum* the chromosome size ranged

from 0.74 to 1.8 μ with nearly median to nearly submedian primary constrictions (Jose and Sharma, 1983). Bai and Subramanian (1985) reported the size range of chromosome in *P. longum* as 1.0 to 4.0 μ.

P. attenuatum

In *P. attenuatum* with 2n = 52, there were clearly two pairs with secondary constrictions, one pair with satellite and one pair slightly slender which were the sex chromosomes. All the other 22 pairs progressively diminished in size and were thick and stout. These chromosomes ranged from 0.75 to 1.9 μ in length (Jose, 1981). In *P. attenuatum* chromosome size ranged between 0.75 to 1.9 μ (Jose and Sharma, 1983). Bai and Subramanian (1985) observed the size range of chromosomes in *P. attenuatum* as 1.0 to 4.0 μ.

P. barberi

Chromosomes of *P. barberi* - a rare species endemic to Western Ghats ranged from 0.5 to 1.5 μ in length (Mathew and Mathew, 1992). The number, morphology and size of chromosomes of this species showed similarity with karyological features of most of the other *Piper* species of Western Ghats. Karyomorphological studies on *P. barberi* revealed that the chromosome compliment had thirteen metacentric, ten submetacentric and three acrocentric pairs (Babu *et al.*, 1992). The chromosome length ranged from 0.74 to 1.85 μ. Based on Stebbins (1971) the karyotype asymmetry was classified as 2B.

P. brachystachyum

Bai and Subramanian (1985) recorded the size range of chromosomes in *P. brachystachyum* between 0.8 to 2.0 μ.

P. cubeba

Dasgupta and Datta (1976) observed the length of chromosomes in *P. cubeba* as 0.77 to 2.5 μ. In *P. cubeba* there had been an over-all increase in the chromosome size which ranged from 1.48 to 3.33 μ. Nearly median to nearly submedian primary constrictions were observed (Jose and Sharma, 1983).

P. galeatum

Bai and Subramanian (1985) observed the size range of chromosome in *P. galeatum* as 1.0 to 3.0 μ.

P. hookeri

The size range of chromosomes in *P. hookeri* was recorded as 1.0 to 2.0 μ (Bai and Subramanian, 1985).

P. magnificum

The length of chromosomes in *P. magnificum* was recorded as 1.2 to 3.7 μ (Dasgupta and Datta, 1976).

P. schmidtii

The size range of chromosomes was observed as 0.8 to 2.8 μ in *P. schmidtii* (Bai and Subramanian, 1985).

P. subpeltatum

Johansen (1930) observed that the chromosomes of *P. subpeltatum* were of an extremely small size.

Piper spp. (undetermined)

The undetermined species of *Piper* (2n = 104) pocessed four pairs of medium sized, sixteen pairs of short and 32 pairs of shorter chromosomes (Mathew, 1958).

Materials and Methods

MATERIALS AND METHODS

The present study was conducted in the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period October 1994 to December 1996.

3.1 Materials

Materials included eight species of *Piper* available in the germplasm collection maintained in the Department of Plantation Crops and Spices. The list of selected species along with their accession number are given in Table 3.

Table 3. *Piper* spp. included in the study

Sl.No.	Name	Accession number
1	*P. argyrophyllum* Miq. (male)	P-48
2	*P. argyrophyllum* Miq. (female)	P-52
3	*P. attenuatum* Buch. Ham. (male)	P-80
4	*P. bababudani* Rahiman	P-89
5	*P. betle* Linn. (var. Kasoori)	P-87
6	*P. chaba* Hunter	P-86
7	*P. colubrinum* Lamk.	P-85
8	*P. longum* Linn. (male)	P-92
9	*P. longum* Linn. (female)	P-33
10	*P. nigrum* Linn. (Panniyur-1)	Pn-134
11	*P. nigrum* Linn. (wild male)	P-45

3.2 Methods

3.2.1 Morphological studies

To prepare a morphological descriptor for the eight species studied, detailed morphological observations were recorded. Morphological scoring was done as per the descriptor given below.

Details of morphological scoring

A. Back ground information

1. Origin/place of collection
2. Elevation/Altitude
3. Soil type
4. Planting material used

B. Descriptor

I. Young leaf

1. Colour
1.a. Upper surface
1.b. Lower surface
2. Shape of leaf
3. Leaf tip
4. Leaf base
5. Average length of ten leaves
6. Average width of ten leaves
7. Length-width ratio of leaf blade
8. Stipules
8.a. Present
8.b. Absent

If present

8.a.1. Colour
8.a.2. Shape

8.a.3. Position

8.a.4. Texture

8.a.5. Hairyness

9. Prophylls

9.a. Present

9.b. Absent

If present

9.a.1. Colour

9.a.2. Shape

9.a.3. Position

9.a.4. Texture

9.a.5. Hairyness

II. Mature leaves

1. Petiole

1.a. Length (average of ten petioles)

1.b. Hairyness

1.c. Shape

2. Leaf blade

2.a. Shape

2.b. Leaf tip

2.c. Leaf base

2.d. Average length of ten leaves

2.e. Average width of ten leaves

2.f. Length-width ratio of leaf blade

2.g. Texture

2.h. Colour

2.h.1. Upper surface

2.h.2. Lower surface

2.i. Venation

2.j. Hairyness

2.j.1. Upper surface

2.j.2. Lower surface

2.k. Position of leaf/phyllotaxy

III. Stem

1. Shape

2. Colour

2.a. Young stem

2.b. Mature stem

3. Internodal length (average of ten nodes)

4. Branching

5. Position of branch

6. Direction of growth of laterals

7. Nodal characters

8. Adventitious roots

8.a. Present

8.b. Absent

If present - amount

8.a.1. Low

8.a.2. Medium

8.a.3. High

9. Clinging ability

*IV. Flower

1. No. of spikes/fruiting branch
2. Length of spike (average of ten spikes)
3. Time of flowering/season
4. Sex form
5. Spike orientation

5.a. Pendulous

5.b. Erect

6. Spike texture

6.a. Glabrous

6.b. Hairy

7. Bract type
8. Stamen number
9. Stigmatic lobes number

*V. Fruit

1. Shape
2. Colour of young berry
3. Colour of mature berry
4. Time taken from flowering to fruit maturity
5. No. of berries/spike

* Observations on flower and fruit were recorded wherever possible.

3.2.2 Cytological studies

To find out the somatic chromosome number of the different *Piper* spp., mitotic studies were carried out using root tip squash method.

3.2.2.1 Standardisation of material

In all the eight species studied, two node cuttings were planted in polybags filled with 1:1 mixture of sand and cowdung. Before planting, the leaves were removed keeping the petiole intact. The cuttings were dipped in 1000 ppm solution of Indole-3-butyric acid for 45 seconds prior to planting. Roots were collected from four weeks old cuttings. The rooted cuttings were taken out without disturbing the roots and the roots were thoroughly washed in running water to remove the adhering dirt and soil particles. One centimetre long root tips were cut from the roots which were creamy white in colour with yellow tips.

In species like *P. nigrum* (cultivated and wild), *P. bababudani, P. argyrophyllum* and *P. attenuatum,* root tips were also procured from climbing roots and analysed for comparison.

The material giving maximum number of dividing cells was recorded and used for further studies.

3.2.2.2 Standardisation of time of collection of roots

Actively growing roots were collected at two hours interval throughout the full day cycle of 24 hours and fixed after pre-treatment. The fixed roots were

stained after hydrolysis. The time at which maximum number of dividing cells were observed was fixed as the best time for collection of samples.

3.2.2.3 Standardisation of pre-treatment chemicals, fixatives and stains

The collected roots were taken in a tea strainer and thoroughly washed in running cold water to remove the last traces of dirt or soil adhering to it. The roots were blotted between folds of filter paper and transferred to pre-treatment chemical in a glass vial. The vials were kept under low temperature (4°C) in a refrigerator for two to three hours. The pre-treatment chemicals used in the present study along with their composition and preparation are given in Table 4.

Table 4. Preparation and composition of the pre-treatment chemicals used for mitotic studies in *Piper* spp.

Pre-treatment chemicals	Components	Preparation
1. 8-hydroxy-quinoline (0.03%)	8-hydroxy-quinoline : 0.03 g Distilled water : 100 ml	0.03 g of 8-hydroxyquinoline was dissolved in a small volume of water taken in a 100 ml volumetric flask and the volume was made upto 100 ml
2. para-dichloro benzene (saturated aqueous solution)	para-dichloro benzene : 150 mg Distilled water : 50 ml	150 mg of para-dichlorobenzene was added to 50 ml of distilled water taken in a 125 ml flask. The flask was corked and incubated overnight at 60°C and cooled. The solution was shaken vigorously before use.
3. α-bromo-naphthalene (saturated aqueous solution)	α-bromo naphthalene : 1 drop Distilled water : 1 ml	One drop of α-bromonaphthalene was added to one ml of distilled water taken in a vial and shaken vigorously, so that the α-bromonaphthalene particles were well dispersed in the water

The pre-treated roots were taken in the tea strainer again and washed thoroughly in running water so that all the traces of pre-treatment chemical was removed. After pre-treatment, the tips of roots were white in colour. The roots were blotted between folds of filter paper and fixed in a fixative. The roots were kept in the fixative for one or two days for better fixation. Keeping quality of the fixed material could be improved by storing in a refrigerator. The fixatives tried along with their composition are given in Table 5.

Table 5. The composition of fixatives used for mitotic studies in *Piper* spp.

Fixatives	Composition
1. Carnoy's A	1 part acetic acid + 3 parts ethyl alcohol
2. Carnoy's B	1 part acetic acid + 3 parts chloroform + 6 parts ethyl alcohol

After one or two days, the fixed roots were taken in the tea strainer and washed thoroughly in running water to remove traces of fixative. After washing, the roots were blotted between folds of filter paper. This was then hydrolysed in 1N hydrochloric acid taken in a glass vial. The vial was suspended in a water bath maintained at a temperature of 60°C for fifteen minutes. Touching of the glass vial on the sides of the water bath was avoided to prevent direct heating of the material. After fifteen minutes, the glass vial was taken out and the contents were transferred to a petridish containing water. Since the roots were cooked by that time washing in a strainer can cause damage to the tips. So to wash out hydrochloric acid the roots were washed in three to four changes of water in a petridish. After washing the roots were blotted between folds of tissue paper and stained. The preparation of stains used along with their composition are given in Table 6.

Table 6. Preparation of stains used for mitotic studies in *Piper* spp.

Stain	Components	Preparation
Acetocarmine 2%	Carmine powder : 2 g 45% acetic acid : 100 ml	For preparing 100 ml of 45% acetic acid, 55 ml of distilled water was added to 45 ml of glacial acetic acid. The mixture was boiled in a conical flask on gas flame. When it started boiling, the flame was lowered and 2 g carmine powder was added. To avoid splashing, a funnel was placed on the top of the conical flask. It was then kept on low flame and allowed to boil for about ten minutes. It was then removed from the gas flame and allowed to cool. On cooling, it was filtered using Whatmann No.42 filter paper for slow filtration and collected in a glass stoppered bottle.
Acetoorcein 0.5%	Orcein powder : 0.5 g 45% acetic acid : 100 ml	The procedure for preparation of acetoorcein was same as that of acetocarmine except that in place of carmine powder orcein powder was used. Extreme care was taken while adding orcein powder since it splashes vigorously. The flame was lowered and orcein powder was added little by little to avoid splashing out and danger.

Staining was done in a glass vial. A liberal quantity of stain, i.e., about two to three times as much as needed to cover the material was used for getting good penetration. After 45 minutes of staining slide preparations were made.

The pre-treatment methods, fixatives and stains were used in different combinations (Table 7) and the best combination was used for further studies.

Table 7. The pre-treatments, fixatives and stains used for mitotic studies in *Piper* spp.

Pre-treatments	Fixatives	Stains
1. 8-hydroxyquinoline (0.03%)	Carnoy's A	Acetocarmine Acetoorcein
	Carnoy's B	Acetocarmine Acetoorcein
2. α-bromonaphthalene (saturated aqueous solution)	Carnoy's A	Acetocarmine Acetoorcein
	Carnoy's B	Acetocarmine Acetoorcein
3. para-dichlorobenzene (saturated aqueous solution)	Carnoy's A	Acetocarmine Acetoorcein
	Carnoy's B	Acetocarmine Acetoorcein

3.2.2.4 Cytological techniques

After staining, the root tips were taken out from the stain and put on a slide along with a drop of stain. The tip portion which had high meristematic activity could be seen as a dark portion. That portion which was about one millimetre at the tip was collected and the remaining portion was discarded. A coverslip was placed carefully over the root tip avoiding air bubbles. Gentle tapping was done over the cover slip using the blunt end of a glass rod. Then it was put between folds of filter paper and hard pressed using index finger. After pressing the slide was warmed slightly over a spirit lamp and allowed to cool. Alternate warming

and cooling was repeated three to four times. Again the slide was placed between thick folds of filter paper and hard pressed. The slides thus prepared were sealed with nail polish to avoid entry of air bubbles and mitotic chromosome numbers were examined.

Another technique which doesn't involve hydrolysis was also tried. The roots were washed thoroughly after fixation and blotted between folds of filter paper. Root tips of about two millimetre from the tip were put in a drop or two of stain on a slide. The drop containing the root tip was heated slowly over a spirit lamp upto boiling. The stain should not boil and spurt. The 'cooked' root tips were rinsed in another drop of stain and then transferred to another slide into a drop of stain. The root tips were then squashed completely with the flat edge of a glass rod. All the visible debris from the squash were removed and a cover slip was put over the stain which had good quantity of free cells. The slide was warmed gently over the flame of a spirit lamp, put between folds of filter paper and hard pressed using index finger. The slides were sealed with nail polish and screened for mitotic chromosome variations.

Microphotographs were taken using Leitz BIOMED microscope, with automatic photo control unit, WILD MPS 28. Good mitotic preparations were made permanent.

3.2.2.5 Method for preparing permanent slides

Slides which were sealed with nail polish were first put in water for two to three minutes so that the nail polish was easily removed. It was then put in 1:1 solution of acetic acid and butyl alcohol till the coverslip got detached from the

slide. The slide and coverslip were then put in butyl alcohol alone. After two to three minutes the slide and coverslip were taken out and allowed to dry in air. A fresh slide was taken and a drop of DPX mountant was put in its centre. The coverslip was placed over the drop of DPX mountant with the side containing cells upside down. On the slide over the cells a drop of DPX mountant was put and covered with a fresh coverslip. The slides were placed like that in open without disturbing for one or two days and then stored in a slide box.

Results and Discussion

RESULTS AND DISCUSSION

The results of the present study are presented under two main heads as follows:

a. Morphological studies in *Piper* spp.

b. Cytological studies in *Piper* spp.

4.1 Morphological studies in *Piper* spp.

4.1.1 Morphological scoring of *Piper* spp.

Observations on detailed morphological scoring of *Piper* spp. are given in Table 8.

4.1.2 Descriptions of the *Piper* spp.

Based on the salient observations on morphology, brief descriptions of the eight *Piper* spp. studied are given below:

P. argyrophyllum: A tall dioecious climber; stem dark green, branches dimorphic; leaves on orthotrops cordate, on laterals cordate to elliptic-ovate, two lateral stipules sheathing petiole turn black and fall early, leaves coriaceous, hairs present on the petiole and lower surface of the leaves, venation acrodromous (Fig.1a); prophylls axillary, coriaceous; inflorescence pendulous, bracts sessile, adnate to rachis, stamens two to three, stigma three to four lobed; berries conical, globose when mature, turn dark green to black in colour on ripening (Plates 1, 2 and 3).

Table 8. Details of morphological scoring

A. Background information

Sl. No.	Species	1. Origin/place of collection	*2. Elevation/ altitude	*3. Soil type	4. Planting material used
1	*P. argyrophyllum*	Western Ghats	22.25 m above MSL	Laterite	Cuttings from orthotrops
2	*P. attenuatum*	Western Ghats	"	"	"
3	*P. bababudani*	Western Ghats	"	"	"
4	*P. betle* (var. Kasoori)	Western Ghats	"	"	"
5	*P. chaba*	North East India	"	"	Cuttings from runners
6	*P. colubrinum*	South America	"	"	Cuttings from orthotrops
7	*P. longum*	Western Ghats	"	"	Cuttings from runners
8	*P. nigrum* (Panniyur-1)	PRS, Panniyur	"	"	"
9	*P. nigrum* (wild)	Western Ghats	"	"	Cuttings from orthotrops

*Since the observations were made on plants grown at Vellanikkara, elevation and soil type of that place is recorded

Table 8. Continued

Sl.No.	Species	B. Descriptor							
		I. Young leaf							
		1. Colour		2. Shape of leaf	3. Leaf tip	4. Leaf base	5. Average length of ten leaves (cm)	6. Average width of ten leaves (cm)	7. Length-width ratio of leaf blade
		(1.a) Upper surface	(1.b) Lower surface						
1	*P. argyrophyllum*	Light green	Pale green	Cordate to ovate-lanceolate	Acuminate	Cordate to oblique	3.92	1.98	1.98
2	*P. attenuatum*	Light green	Pale green	Elliptic-lanceolate	Acuminate	Rounded	4.8	1.95	2.46
3	*P. bababudani*	Light green	Pale green	Cordate	Acuminate	Cordate to rounded	3.78	1.96	1.93
4	*P. betle* (var. Kasoori)	Light green	Pale green	Cordate	Acute	Cordate	2.91	1.88	1.5
5	*P. chaba*	Light green	Pale green	Elliptic-lanceolate	Acuminate	Oblique	4.4	1.9	2.3
6	*P. colubrinum*	Light green	Pale green	Elliptic-ovate	Acute	Oblique	11.31	6.12	2.2
7	*P. longum*	Light green	Pale green	Cordate to elliptic-lanceolate	Acuminate	Cordate to oblique	5.52	1.88	2.93
8	*P. nigrum* (Panniyur-1)	Light green	Pale green	Cordate	Acuminate	Cordate to rounded	7	4.6	1.52
9	*P. nigrum* (wild)	Light green	Pale green	Cordate	Acuminate	Cordate to rounded	3.32	1.63	2.04

Table 8. Continued

Sl. No.	Species	B. Descriptor						
		I. Young leaf						
		8. Stipules		If present				
		(8.a) Present	(8.b) Absent	(8.a.1) Colour	(8.a.2) Shape	(8.a.3) Position	(8.a.4) Texture	(8.a.5) Hairyness
1	*P. argyrophyllum*	Present	-	Creamy green	Oblong	Adnate upto leaf base	Membranous	Absent
2	*P. attenuatum*	Present	-	Creamy green	Oblong	"	"	"
3	*P. bababudani*	Present	-	Purple	Oblong	"	"	"
4	*P. betle* (var. Kasoori)	Present	-	Creamy green	Oblong	Adnate upto half the length of petiole	"	"
5	*P. chaba*	-	Absent	-	-	-	-	-
6	*P. colubrinum*	-	Absent	-	-	-	-	-
7	*P. longum*	Present	-	Creamy green	Oblong	Adnate upto one third the length of petiole	Membranous	Absent
8	*P. nirgum* (Panniyur-1)	Present	-	Light green	Oblong	Adnate upto leaf base	"	"
9	*P. nigrum* (wild)	Present	-	Purple	Oblong	"	"	"

Table 8. Continued

Sl. No.	Species	B. Descriptor						
		I. Young leaf						
		9. Prophylls		If present				
		(9.a) Present	(9.b) Absent	(9.a.1) Colour	(9.a.2) Shape	(9.a.3) Position	(9.a.4) Texture	(9.a.5) Hairyness
1	*P. argyrophyllum*	Present	-	Light green	Elliptic-lanceolate	Axillary	Coriaceous	Absent
2	*P. attenuatum*	Present	-	Light green	"	"	"	"
*3	*P. bababudani*	-	-	-	-	-	-	-
*4	*P. betle* (var. Kasoori)	-	-	-	-	-	-	-
5	*P. chaba*	Present	-	Light green	Elliptic-lanceolate	Axillary	Coriaceous	Absent
6	*P. colubrinum*	Present	-	"	"	Interpetiolar	Membranous	"
7	*P. longum*	Present	-	"	"	Axillary	"	"
8	*P. nirgum* (Panniyur-1)	Present	-	"	"	"	Coriaceous	"
9	*P. nigrum* (wild)	Present	-	"	"	"	"	"

* Flowering not observed under Vellanikkara conditions and hence observations on prophylls not made

Table 8. continued

Sl. No.	Species	B. Descriptor									
		II. Mature leaves									
		1. Petiole			2. Leaf blade						
		(1.a) Length (cm) (average of ten petioles)	(1.b) Hairyness	(1.c) Shape	(2.a) Shape	(2.b) Leaf tip	(2.c) Leaf base	(2.d) Average length of ten leaves (cm)	(2.e) Average width of ten leaves (cm)	(2.f) Length-width ratio of leaf blade	(2.g) Texture
1.	*P. argyrophyllum*	1.53	Present	Grooved	Cordate to elliptic-ovate	Acuminate	Cordate to oblique	9.72	5.70	1.70	Coriaceous
2.	*P. attenuatum*	1.01	Absent	"	"	"	"	9.02	4.13	2.18	"
3.	*P. bababudani*	1.91	"	"	Cordate to ovate	"	Cordate to rounded	9.20	5.62	2.00	"
4.	*P. betle* (var. Kasoori)	2.8	Present (very small hairs)	"	Cordate	Acute	Cordate	6.12	4.39	1.40	"
5.	*P. chaba*	0.72	Absent	"	Lanceolate	Acuminate	Oblique	13.13	4.68	2.80	"
6.	*P. colubrinum*	2.10	Absent	"	Elliptic-ovate	Acute	Rounded	13.30	8.30	1.60	"
7.	*P. longum*	3.26	Present (very small hairs)	"	Cordate to elliptic-lanceolate	Acuminate	Cordate to oblique	7.80	5.00	1.56	"
8.	*P. nigrum* (Panniyur-1)	2.80	Absent	"	Cordate to ovate	Acuminate	Cordate to rounded	14.80	10.50	1.40	"
9.	*P. nigrum* (wild)	2.52	"	"	"	"	"	9.39	6.46	1.45	"

Table 8. Continued

B. Descriptor							
II. Mature leaves							
Sl. No.	Species	2. Leaf blade					
		(2.h) Colour		(2.i) Venation	(2.j) Hairyness		(2.k) Position of leaf/ phyllotaxy
		(2.h.1) Upper surface	(2.h.2) Lower surface		(2.j.1) Upper surface	(2.j.2) Lower surface	
1	*P. argyrophyllum*	Dark green	Pale green	Acrodromous	Absent	Present	Alternate distichous
2	*P. attenuatum*	"	"	"	"	Absent	"
3	*P. bababudani*	"	"	Campylodromous	"	"	"
4	*P. betle* (var. Kasoori)	"	"	"	"	"	"
5	*P. chaba*	"	"	Eucamptodromous	"	"	"
6	*P. colubrinum*	"	"	"	"	"	"
7	*P. longum*	"	"	Acrodromous	"	"	"
8	*P. nigrum* (Panniyur-1)	"	"	Campylodromous	"	"	"
9	*P. nigrum* (wild)	"	"	"	"	"	"

Table 8. Continued

B. Descriptor								
		III Stem						
Sl. No.	Species	1. Shape	2. Colour		3. Internodal length (cm) (average of ten nodes)	4. Branching	5. Position of branch	6. Direction of growth of laterals
			(2.a) Young stem	(2.b) Mature stem				
1	*P. argyrophyllum*	Cylindrical	Dark green	Grey	9.30	Alternate	Axillary	Semierect/horizontal
2	*P. attenuatum*	"	"	"	9.73	"	"	"
3	*P. bababudani*	"	"	"	3.90	"	"	"
4	*P. betle* (var. Kasoori)	"	Purple	"	4.34	"	"	"
5	*P. chaba*	"	Dark green	"	6.05	"	"	"
6	*P. colubrinum*	"	Dark green with white patches	"	8.20	"	"	"
7	*P. longum*	"	Dark green	"	5.10	"	"	"
8	*P. nigrum* (Panniyur-1)	"	Dark green	"	6.70	"	"	"
9	*P. nigrum* (wild)	"	Purple green	"	6.36	"	"	"

Table 8. Continued

Sl. No.	Species	B. Descriptor						
		III Stem						
		7. Nodal characters	8. Adventitious roots		If present - amount			9. Clinging ability
			(8.a) Present	(8.b) Absent	(8.a.1) Low	(8.a.2) Medium	(8.a.3) High	
1	*P. argyrophyllum*	Swollen node	Present	-	-	_	High	Cling
2	*P. attenuatum*	"	"	-	-	-	"	"
3	*P. bababudani*	"	"	-	-	-	"	"
4	*P. betle* (var. Kasoori)	"	"	-	-	Medium	-	"
5	*P. chaba*	"	"	-	-	-	High	"
6	*P. colubrinum*	"	"	-	Low	-	-	Nil
7	*P. longum*	"	"	-	Low	-	-	"
8	*P. nigrum* (Panniyur-1)	"	"	-	-	-	High	Cling
9	*P. nigrum* (wild)	"	"	-	-	-	High	"

Table 8. Continued

Sl. No.	Species	B. Descriptor								
		IV. Flower								
		1. No. of spikes/ fruiting branch	2. Length of spike (cm)			3. Time of flowering/season			4. Sex form	5. Spike orientation	
			Male	Female	Bisexual	Male	Female	Bisexual		(5.a) Pendulous	(5.b) Erect
1	*P. argyrophyllum*	2-3	13.6	14.5	-	Throughout the year	Throughout the year		Dioecious	Pendulous	-
2	*P. attenuatum*	1-2	11.6	-	-	"	-	-	"	"	-
* 3	*P. bababudani*	-	-	-	-	-	-	-	-	-	-
* 4	*P. betle* (var. Kasoori)	-	-	-	-	-	-	-	-	-	-
5	*P. chaba*	1-2	-	4.08	-	-	Throughout the year	-	Dioecious	-	Erect
6	*P. colubrinum*	5-6	-	-	4.5	-	-	Throughout the year	Bisexual	Pendulous	-
7	*P. longum*	2-3	6.68	2.40	-	Throughout the year	May September		Dioecious	-	Erect
8	*P. nigrum* (Panniyur-1)	1-3	-	-	14.9	-	-	May-June	Bisexual	Pendulous	-
9	*P. nigrum* (wild)	1-2	5.6	-	-	May-June	-	-	Dioecious	Pendulous	-

*Flowering not observed under Vellanikkara conditions

Table 8. Continued

B. Descriptor						
IV. Flower						
Sl. No.	Species	6. Spike texture		7. Bract type	8. Stamen number	9. Stigmatic lobes number
		(6.a) Glabrous	(6.b) Hairy			
1	*P. argyrophyllum*	Glabrous	-	Sessile and adnate to rachis	2-3	3-4
2	*P. attenuatum*	"	-	"	2-3	NA
*3	*P. bababudani*	-	-	-	-	-
*4	*P. betle* (var. Kasoori)	-	-	-	-	-
5	*P. chaba*	Glabrous	-	Peltate	NA	3
6	*P. colubrinum*	"	-	"	2	3-4
7	*P. longum*	"	-	"	3	3-4
8	*P. nigrum* (Panniyur-1)	"	-	Sessile and adnate to rachis	2	3-5
9	*P. nigrum* (wild)	"	-	"	2	NA

* Flowering not observed under Vellanikkara conditions
NA - Male or female flowers could not be observed

Table 8. Continued

B. Descriptor						
		V. Fruit				
Sl. No.	Species	1. Shape	2. Colour of young berry	3. Colour of mature berry	4. Time taken from flowering to fruit maturity	5. No. of berries/spike
1	*P. argyrophyllum*	Conical (young) globose (mature)	Light green	Dark green to black	4½-5 months	65-95
* 2	*P. attenuatum*	-	-	-	-	-
* 3	*P. bababudani*	-	-	-	-	-
* 4	*P. betle* (var. Kasoori)	-	-	-	-	-
5	*P. chaba*	Conical	Light green	Red	2½ months	166-234
* 6	*P. colubrinum*	-	-	-	-	-
7	*P. longum*	Cylindrical	Light green	Dark green to black	1½-2 months	120-150
8	*P. nigrum* (Panniyur-1)	Globose	Light green	Red	5½-6 months	92-122
* 9	*P. nigrum* (wild)	-	-	-	-	-

* Fruitset not observed under Vellanikkara conditions

1a. Acrodromous

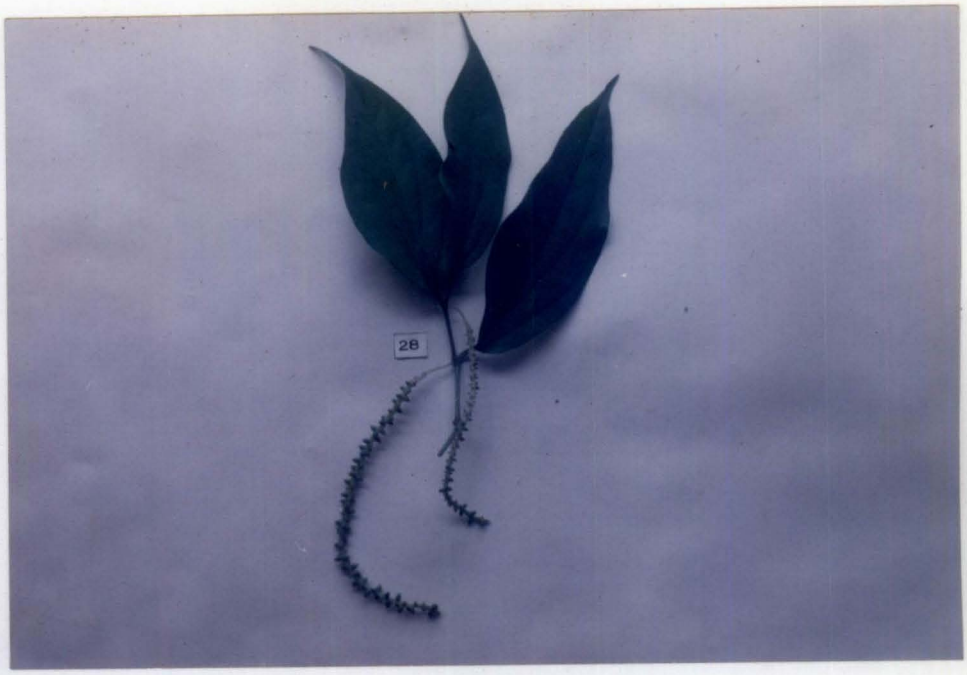
1b. Campylodromous

1c. Eucamptodromous

Fig. 1. Types of venation in *Piper*

Plate 1. Lateral branch of *P. argyrophyllum* with developing berries

Plate 2. Immature conical berries of *P. argyrophyllum* (x 200)


28

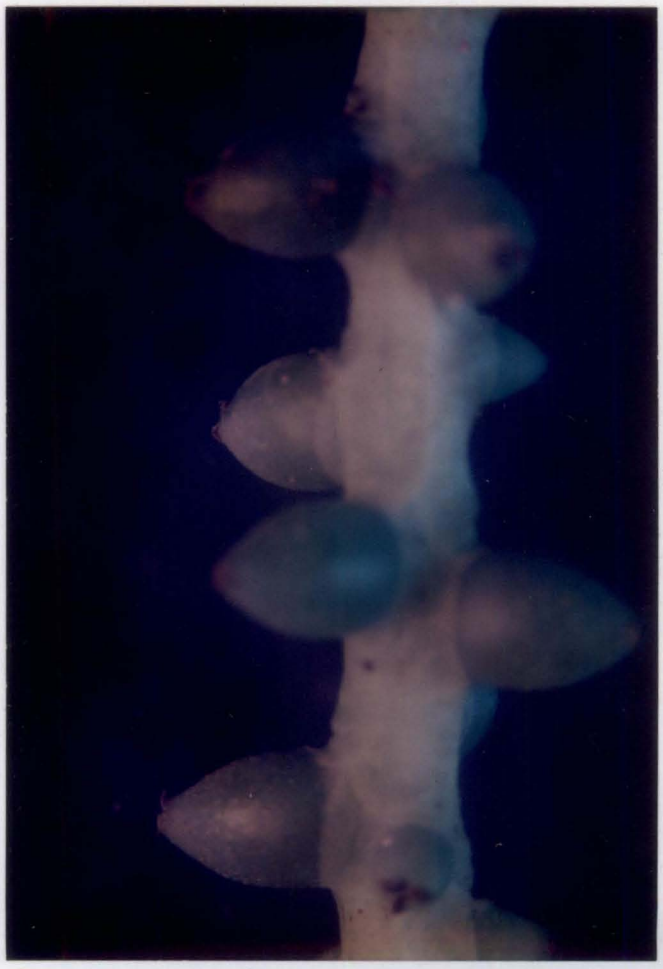


Plate 3. Mature globose berries of *P. argyrophyllum*


cms.
camlin

P. attenuatum: Very similar to *P. argyrophyllum* morphologically except that hairs on petiole and undersurface of leaves were absent. This was found to be the distingushing character between the two species in the present study (Plates 4 and 5).

P. bababudani: A tall dioecious climber; stem dark green, branches dimorphic; leaves on orthotrops cordate, on laterals cordate to ovate, stipulate, coriaceous, glabrous, venation campylodromous (Fig.1b); inflorescence pendulous, spikes very fleshy, bracts hooded at tip, arranged in clear spirals; berries bigger than that of *P. nigrum* and turn orange in colour on ripening (Plate 6).

The plant similar to *P. nigrum* except in certain floral and fruit characters. (Since the plant doesn't flower under Vellanikkara conditions, distingushing floral and fruit observations were recorded based on herbarium specimens).

P. betle (var. Kasoori): A dioecious climber; stem purple, branches dimorphic; leaves on orthotrops and laterals cordate, stipulate upto half the length of petiole, coriaceous, very small hairs present on petiole, upper and lower surfaces of leaves glabrous, venation campylodromous (Plate 7).

(Floral observations could not be recorded since flowering was not observed under Vellanikkara conditions).

P. chaba: A dioecious climber; stem dark green, branches dimorphic; leaves on orthotrops and laterals lanceolate, exstipulate, coriaceous, glabrous, venation eucamptodromous (Fig.1c), leaves on runners cordate, acrodromous; prophylls axillary,

Plate 4. Lateral branch of *P. attenuatum* (male) with inflorescence

Plate 5. Inflorescence of *P. attenuatum* (male) (x 200)

Plate 6. Lateral branch of *P. bababudani*

Plate 7. Shoot of *P. betle* (var. Kasoori)

coriaceous; inflorescence erect, bracts peltate, stigma three lobed; whole spike turn to fruit, turn bright attractive red on ripening (Plates 8 and 9).

(Only female vine was available in the germplasm collection at the College of Horticulture, Vellanikkara).

P. colubrinum: A tall shrub; stem dark green with prominent white patches, stilt roots present at the base to support the stem, branches dimorphic; leaves on orthotrops and laterals elliptic-ovate, exstipulate, coriaceous, glabrous, venation eucamptodromous; prophylls axillary later becoming interpetiolar, membranous; inflorescence pendulous, bracts peltate, flowers bisexual, stamens two and stigma three to four lobed (Plates 10 and 11). (Fruitset was not observed under Vellanikkara conditions).

P. longum: A dioecious creeper; stem thin, dark green, branches dimorphic; leaves on orthotrops cordate, on laterals elliptic lanceolate, stipulate upto one-third the length of petiole, coriaceous, glabrous, very small hairs present on petiole, venation acrodromous; prophylls axillary, membranous; inflorescence erect, bracts peltate, stamens three, stigma three to four lobed; fruit cylindrical, whole spike turn to fruit, dark green to black in colour on ripening (Plates 12 and 13).

P. nigrum (Panniyur-1): A tall climber; stem dark green, branches dimorphic; leaves on orthotrops cordate, on laterals cordate to ovate, stipulate, coriaceous, glabrous, venation campylodromous; prophylls axillary, coriaceous; inflorescence pendulous, bracts sessile and adnate to rachis, flowers bisexual, stamens two, stigma three to five lobed; berries globose, turning red on ripening (Plate 14).

Plate 8. *P. chaba* showing (a) dimorphic shoots and (b) berries at different stages of development

Plate 9. Inflorescence of *P. chaba* showing peltate bracts (x 200)


P. chaba

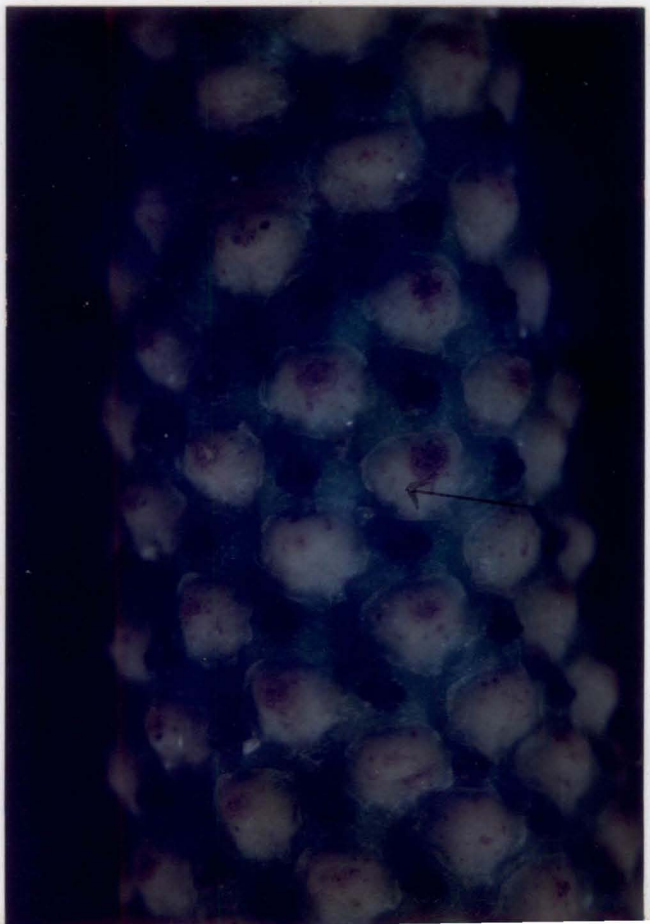


Plate 10. *P. colubrinum* showing stilt roots

Plate 11. Branch of *P. colubrinum* showing inflorescence and prophyll

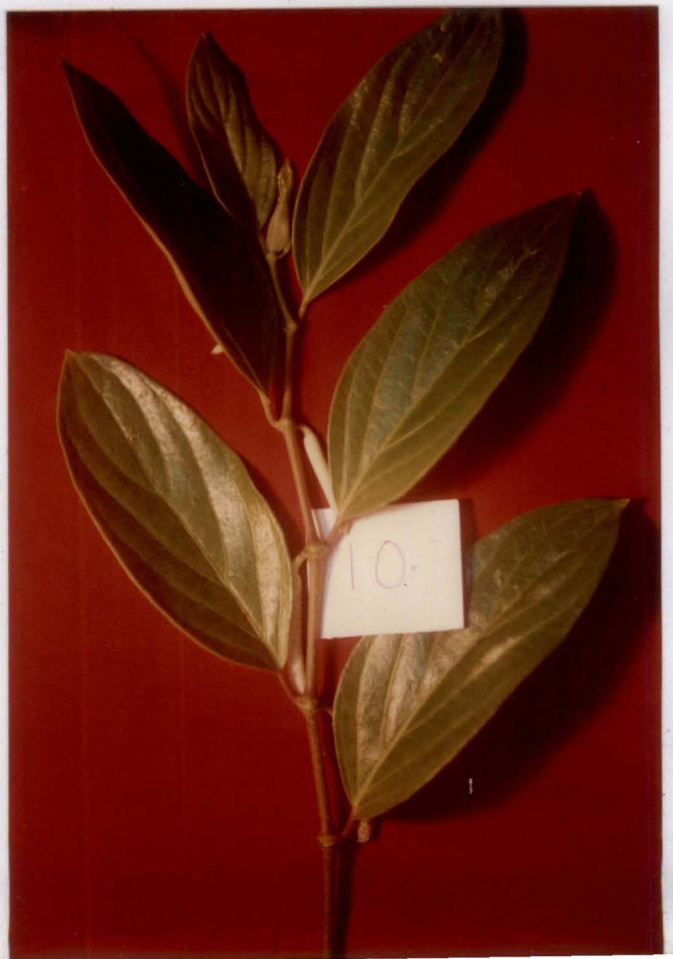

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Plate 12. Lateral branch of *P. longum* (male) with inflorescence

Plate 13. Lateral branch of *P. longum* with immature fruit

Plate 14. Lateral branch of *P. nigrum* with developing inflorescence


11

P. nigrum (wild): Very similar to cultivated *P. nigrum* morphologically except that wild types were dioecious (Plate 15).

4.1.3 Comparative study of the morphological characters of *Piper* spp.

The eight species of *Piper* studied were analysed for the similarities and differences in their morphological characters in order to find out the relationship among the species. The observations are discussed below:

Habit

Except *P. longum* and *P. colubrinum* all other species were climbers. *P. longum* was a creeper and *P. colubrinum* a shrub with stilt roots.

Young leaf

The colour of young leaf was light green on upper surface and pale green on lower surface in all the eight species studied except in *P. nigrum* (wild) where the colour on lower surface was purple green. Shape of leaf was cordate in *P. nigrum* (cultivated and wild), *P. bababudani* and *P. betle*; cordate to ovate-lanceolate in *P. argyrophyllum*, cordate to elliptic-lanceolate in *P. longum* and *P. attenuatum*, elliptic-lanceolate in *P. chaba* and elliptic-ovate in *P. colubrinum*. Leaf base was cordate to rounded in *P. nigrum* (cultivated and wild), *P. bababudani* and *P. attenuatum*, cordate in *P. betle*, cordate to oblique in *P. argyrophyllum* and *P. longum* and oblique in *P. colubrinum* and *P. chaba*. The leaf tip was acuminate in most of the species except in *P. colubrinum* and *P. betle* where it was acute. Young leaves were exstipulate in *P. colubrinum* and *P. chaba* and stipulate in rest of the species. The different species also showed variation in the length of stipules. In *P. nigrum* (cultivated and wild), *P. bababudani*,

Plate 15. Lateral branch of wild *P. nigrum* (male) with inflorescence

P. argyrophyllum and *P. attenuatum* stipules were adnate upto leaf base. In *P. betle*, stipules were adnate upto half the length of petiole and in *P. longum* upto one-third the length of petiole. The stipules were oblong, membranous and without hairs in all the species. The colour of stipules were creamy green in *P. betle, P. argyrophyllum, P. attenuatum* and *P. longum*; purple in *P. bababudani* and *P. nigrum* (wild) and light green in *P. nigrum* (cultivated). Prophylls which covered lateral flowering shoot tips before emergence were present in all the species. But in *P. betle* and *P. bababudani* the presence of prophylls could not be determined due to lack of flowering under Vellanikkara conditions. Prophylls were light green in colour and elliptic-lanceolate in shape. Position of prophylls were generally axillary. In *P. colubrinum*, eventhough axillary initially, prophylls occupied an interpetiolar position after leaf emergence. In all the species prophylls were glabrous. Texture was membranous in *P. colubrinum* and *P. longum* and coriaceous in rest of the species.

Mature leaves

Petiole was grooved in all the species. Hairs were present on the petiole in *P. argyrophyllum, P. betle* and *P. longum*. In *P. betle* and *P. longum* hairs were very small. Mature leaves were cordate to ovate in shape in *P. nigrum* (cultivated and wild) and *P. bababudani*, cordate in *P. betle*, cordate to elliptic-lanceolate in *P. longum*, cordate to elliptic-ovate in *P. argyrophyllum* and *P. attenuatum*, ellitpic-ovate in *P. colubrinum* and lanceolate in *P. chaba*. Leaf base was cordate to rounded in *P. nigrum* (cultivated and wild) and *P. bababudani*, rounded to oblique in *P. argyrophyllum, P. attenuatum* and *P. longum*, cordate in *P. betle*, rounded in *P. colubrinum* and oblique in *P. chaba*. The leaf tip was acuminate in all the species except *P. colubrinum* and *P. betle* where it was acute. In all the species mature leaves were coriaceous in texture.

Colour of the upper surface of leaves were dark green and lower surface pale green in all the species. Venation of *P. nigrum* (cultivated and wild), *P. betle* and *P. bababudani* were campylodromous, *P. colubrinum* and *P. chaba* eucamptodromous and *P. argyrophyllum*, *P. attenuatum* and *P. longum* acrodromous. Hairs were absent on the upper surface of leaf blade in all the species. Hairs were present on the lower surface of leaf blade of *P. argyrophyllum* and absent in rest of the species. Ravindran (1990) observed that *P. argyrophyllum* and *P. attenuatum* could be distingushed by the venation of leaves - the leaves of *P. argyrophyllum* were five ribbed at the base and those of *P. attenuatum* seven ribbed. This difference could not be observed in the present study as both the species had leaves with five to seven ribs.

Stem

The stem was cylindrical in all the species. The colour of young stem was dark green in *P. nigrum* (cultivated), *P. chaba*, *P. bababudani*, *P. argyrophyllum*, *P. attenuatum* and *P. longum*, purple in *P. betle*, purple green in *P. nigrum* (wild) and dark green with white patches in *P. colubrinum*. In all the species, the stem turned grey when mature. Branching was alternate and direction of growth of laterals were semi erect/horizontal. All the species had swollen nodes and adventitious roots. The amount of roots varied from low in *P. colubrinum* and *P. longum*, medium in *P. betle* to high in rest of the species. Except *P. colubrinum* and *P. longum* all the species showed clinging ability.

Flower

Time of flowering was observed as May to June in *P. nigrum* (cultivated and wild), May to September in *P. longum* (female) and throughout the year in rest of the species including *P. longum* (male). Rahiman (1981) observed that flowering was

during May-July in *P. argyrophyllum* and *P. attenuatum*. He also observed that off season flowering was common in those species. In the present study, flowering was observed throughout the year in these two species and this was in confirmation with Rahiman's (1981) observations. Rahiman (1981) reported that time of flowering in *P. longum* was May to August. Eventhough this held true in the case of female *P. longum* male types used in the current study were found to flower throughout the year. In *P. betle, P. bababudani, P. nigrum* (wild female) and *P. attenuatum* (female) flowering was not observed under Vellanikkara conditions and floral observations could not be taken. The species were dioecious in *P. nigrum* (wild), *P. chaba, P. argyrophyllum, P. attenuatum* and *P. longum* and bisexual in *P. nigrum* (cultivated) and *P. colubrinum*. The spike orientation was erect in *P. chaba* and *P. longum* and pendulous in rest of the species. Spikes were glabrous in all the species. Bracts were sessile and adnate to rachis in *P. nigrum* (cultivated and wild), *P. argyrophyllum* and *P. attenuatum*, hooded at the tip in *P. bababudani* and peltate in *P. colubrinum, P. chaba* and *P. longum*. The number of stamens varied from two to three and the number of stigmatic lobes varied from three to five in the different species.

Fruits

The fruit was conical in shape in *P. chaba* and cylindrical in *P. longum*. The berries were globose in *P. nigrum*. The young berries were conical and the mature berries were globose in *P. argyrophyllum*. The colour of young berries were light green in all the species. Colour of ripe berries were red in *P. nigrum* (cultivated) and *P. chaba*, dark green to black in *P. argyrophyllum* and *P. longum* and orange in *P. bababudani*. Time taken from flowering to fruit maturity was 5½-6 months in *P. nigrum*, 2½ months in *P. chaba*, 4½-5 months in *P. argyrophyllum* and 1½-2 months in

P. longum. Maximum number of berries per spike was observed in *P. chaba* (166-234), followed by *P. longum* (120-150) and *P. nigrum* (92-122). The species *P. argyrophyllum* had the minimum number of berries (65-95). In *P. nigrum* (wild), *P. betle, P. colubrinum, P. bababudani* and *P. attenuatum* fruit set could not be observed under Vellanikkara conditions.

4.1.4 Key for identification of species

Based on morphological comparisons, a key for the identification of species has been proposed as follows:

A. Plants erect shrubs with stilt roots, stem dark green with white patches, leaves elliptic ovate, venation eucamptodromous, inflorescence pendulous, bracts peltate.

P. colubrinum.

AA. Plants creepers, stipules adnate upto one-third the length of petiole, venation acrodromous, inflorescence erect, bracts peltate, fruits cylindrical.

P. longum.

AAA. Plants climbers with medium to high amount of adventitious roots with clinging ability.

B. Leaves cordate, stipules adnate upto half the length of petiole, venation campylodromous.

P. betle.

BB. Leaves lanceolate, exstipulate, venation eucamptodromous, inflorescence erect, bracts peltate, fruits conical, distinct dimorphism between orthotrops and runners.

P. chaba

BBB. Leaves cordate to elliptic ovate, venation acrodromous, inflorescence pendulous, bracts sessile and adnate to rachis, berries conical, turn globose when mature, turning to dark green to black on ripening.

C. petiole and undersurface of leaves hairy. *P. argyrophyllum*

CC. petiole and both surfaces of leaves glabrous. *P. attenuatum*

BBBB. Leaves cordate to ovate, venation campylodromous, inflorescence pendulous

C. bracts sessile and adnate to rachis, berries globose, turning to red on ripening.

P. nigrum

CC. spikes fleshy, bracts hooded at the tip, arranged in clear spirals, bigger berries, turning to orange on ripening.

P. bababudani

4.2 Cytological studies in *Piper* spp.

To find out the somatic chromosome number of different *Piper* spp., mitotic studies were carried out using root tip squash method. The results are presented below:

4.2.1 Standardisation of material

Two noded cuttings produced fresh actively growing roots in four weeks time and gave maximum number of dividing cells. Similar results were obtained when these cuttings after collection of roots were replanted. Prior to replanting, the roots were removed completely and the sprouts on the cuttings were retained as such. Then the cuttings were planted as in the case of fresh cuttings. These sprouted cuttings produced large number of actively growing roots in two weeks time. Thus by reusing the cuttings a gain of two weeks could be obtained.

During standardisation of cytological techniques in *Piper*, there was a need for large amount of root material at the correct stage of growth. This technique developed to reuse the cuttings to get profuse rooting in a shorter period of two weeks was highly advantageous for the standardisation work. This also helped in saving the planting material of rare species. Bulk supply of planting material was difficult in many species studied.

Root material from climbing roots were also examined for dividing cells. However, practically no dividing cells could be observed.

Climbing roots showed stunted growth when compared to the roots growing in soil. Their tips were not as meristematic as the soil roots. This could be the reason for lack of dividing cells from climbing root tips. So the material for mitotic studies were collected by rooting of cuttings.

4.2.2 Standardisation of time of collection of roots

Maximum number of dividing cells was observed between 11 a.m. and 1 p.m. Almost all the cells were found to be dividing during this time of the day. For all the eight *Piper* spp. studied, maximum number of cells were in the early metaphase between 11.15 a.m. and 12.15 p.m. Hence the roots for mitotic studies were collected between 11.15 a.m. and 12.15 p.m.

Rahiman and Nair (1986) collected root tips between 10.00 and 14.00 hours for mitotic studies of *Piper* spp. For cytological analysis in *P. nigrum,* actively growing root tips were collected at 11 a.m. by Nair and Ravindran (1992) and between 11.00 a.m. and 12 noon by Nair *et al.* (1993). The results of the present study do agree with the results obtained by the previous workers.

However, a few dividing cells were observed when the roots were collected between 2 p.m. and 4 p.m. during November-February period. This may be due to the fact that November-February months are winter months. During these months the atmosphere was cooler when compared to other parts of the year. So all the cells may not divide before noon and some cells may lag behind.

4.2.3 Standardisation of pre-treatment chemicals, fixatives and stains

4.2.3.1 Pre-treatment chemical

Three pre-treatment chemicals viz. 8-hydroxyquinoline, para-dichlorobenzene and α-bromonapthalene were used. Pre-treatment for the study of chromosomes is generally performed for specific reasons. It may be carried out for (a) clearing the cytoplasm, (b) separation of the middle lamella causing softening of the

tissue, or (c) bringing about scattering of chromosomes with clarification of constriction regions. Pre-treatment may also be needed to achieve rapid penetration of the fixative by removing undesirable deposits on the tissue (La Cour, 1935). The underlying principle of pre-treatment is the viscosity change in the cytoplasm. As spindle formation is dependent on the viscosity balance between cytoplasmic and spindle constituents, a change in cytoplasmic viscosity brings about a destruction of the spindle mechanism with the chromosomes remaining free or, more precisely, not attached to any binding force within the cell (Sharma and Sharma, 1980b).

As pre-treatment agents, all the three chemicals were equally good giving desirable results. However, α-bromonaphthalene had a peculiar smell and cleaning of glass vials was difficult when compared to the other two. Preparation of para-dichlorobenzene involved overnight heating and every time before use the solution had to be warmed. Preparation and use of 8-hydroxyquinoline was comparatively easy and hence it was used as pre-treatment agent for mitotic studies in *Piper* spp.

4.2.3.2 Fixatives

Carnoy's A and Carnoy's B were the fixatives used in the present study. Fixation may be defined as the process by which tissues or their components are fixed selectively at a particular stage to a desired extent. The purpose of fixation is to kill the tissue without causing any distortion of the components to be studied (Sharma and Sharma, 1980a).

Both Carnoy's A and Carnoy's B were equally good as fixatives. According to availability of components, either could be used as fixative for cytological studies in *Piper*.

4.2.3.3 Stains

Acetocarmine and acetoorcein were the two different stains used. With acetocarmine, chromosomes were not deeply stained, but there was no cytoplasmic staining. Whereas, with acetoorcein the chromosomes were deeply stained, but the cytoplasm also took stain slightly. However, better contrast was obtained with acetoorcein compared to staining with acetocarmine and hence acetoorcein was used for staining the chromosomes in mitotic studies of *Piper* spp.

4.2.4 Standardisation of cytological technique

Among the two cytological techniques attempted as described in materials and methods, the method involving hydrolysis was found to be better. Squashing was very easy and the cell spread was very good when compared to the other method. So, for further analysis that technique was employed.

The results of the standardisation experiments are concluded as follows:

The roots were collected between 11.15 a.m and 12.15 p.m and pre-treated in 8-hydroxyquinoline for two to three hours at 4°C. The pre-treated roots were fixed in Carnoy's A or Carnoy's B for one or two days. The material was then hydrolysed in 1N hydrochloric acid in a water bath maintained at a temperature of 60°C for fifteen minutes. The roots were then stained in 0.5% acetoorcein for 45 minutes. These roots were squashed and slides prepared.

4.2.5 Chromosome number

Somatic chromosome number of the eight different *Piper* spp. studied are given in Table 9.

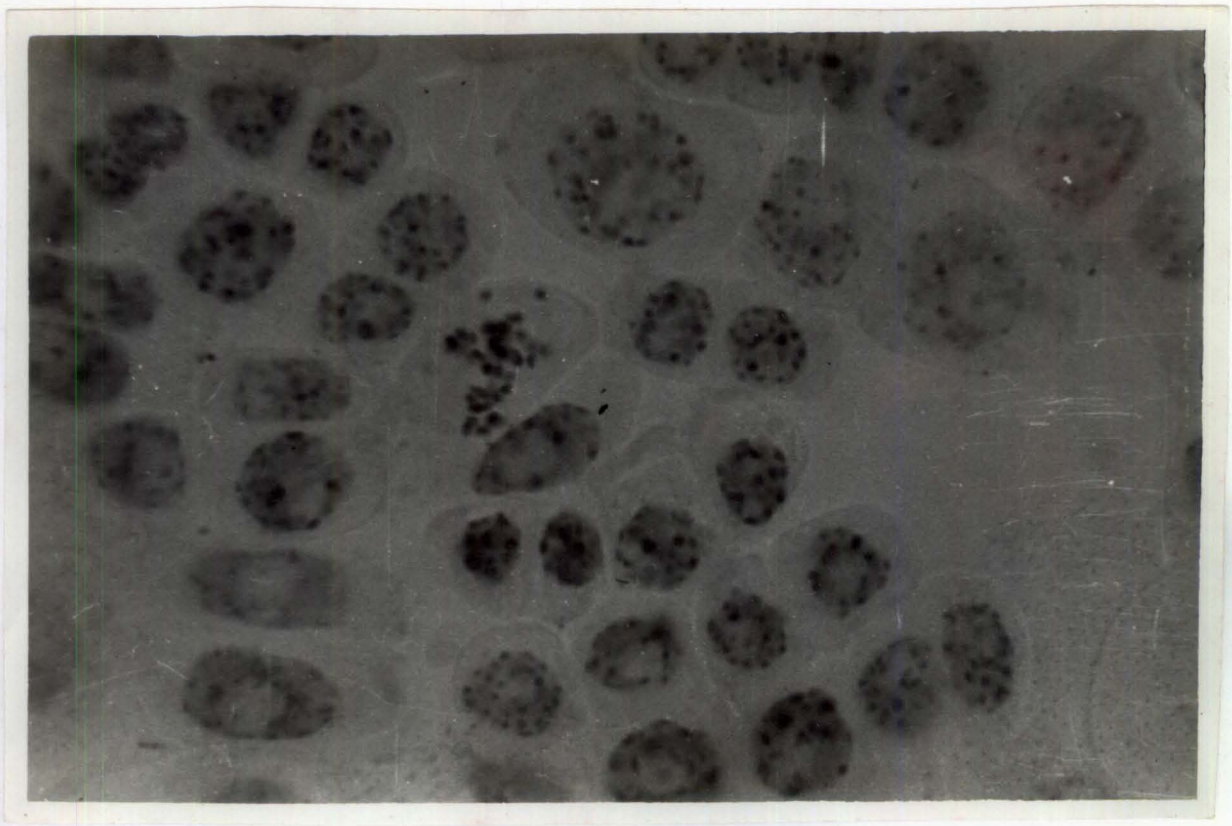
Table 9. Somatic chromosome number in *Piper* spp. studied

Species	Chromosome number (2n)	Plate No.
P. argyrophyllum	36	16
P. attenuatum	52	17
P. bababudani	32	18
P. betle	32	19
P. chaba	24	20
P. colubrinum	26	21
P. longum	32	22
P. nigrum (Panniyur-1)	52	23
P. nigrum (wild)	52	24

Chromosome number of *P. argyrophyllum* was reported as 2n=36, 39 (Samuel and Bavappa, 1981) and 2n=52 (Rahiman and Nair, 1986 and IISR unpublished). The results of the present investigation agree with the result obtained by Samuel and Bavappa (1981) as 2n=36.

Plate 16. Somatic chromosomes of *P. argyrophyllum* (x 4000)

Plate 17. Somatic chromosomes of *P. attenuatum* (x 4000)



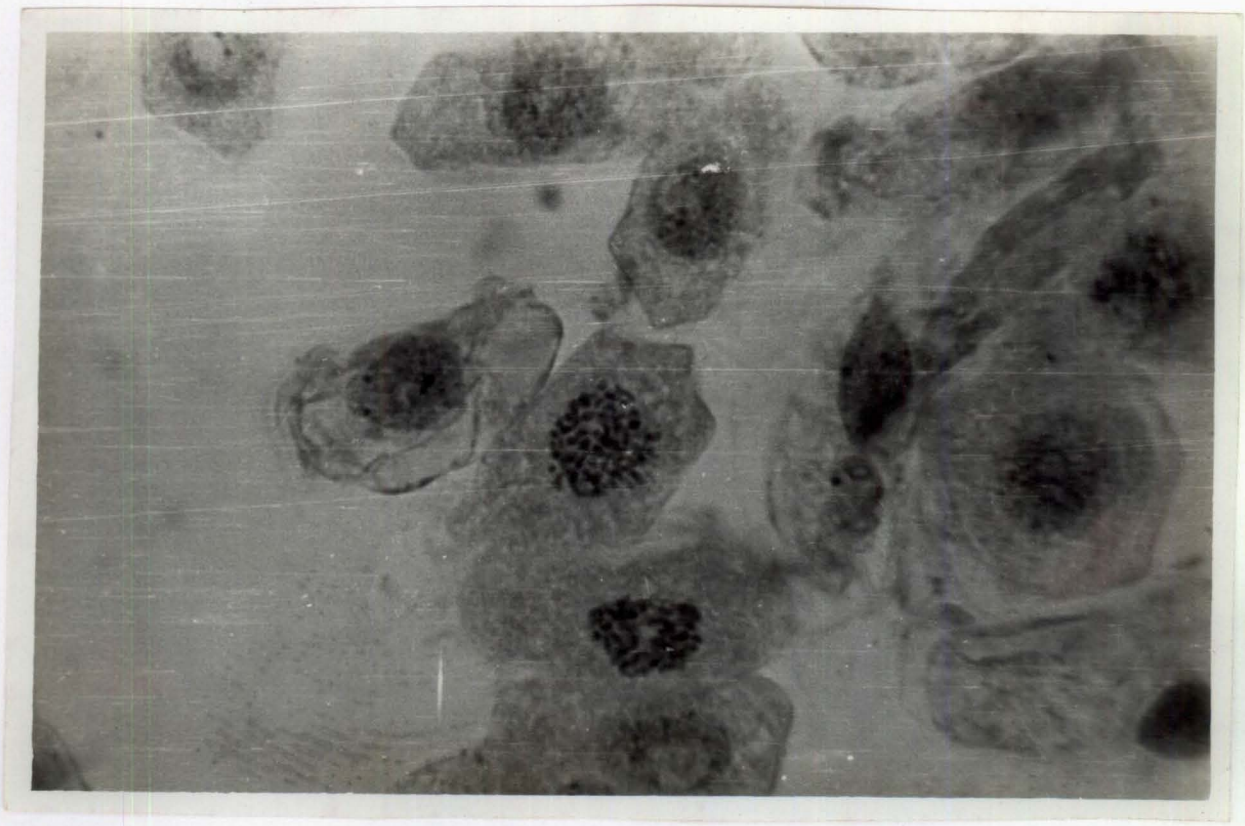
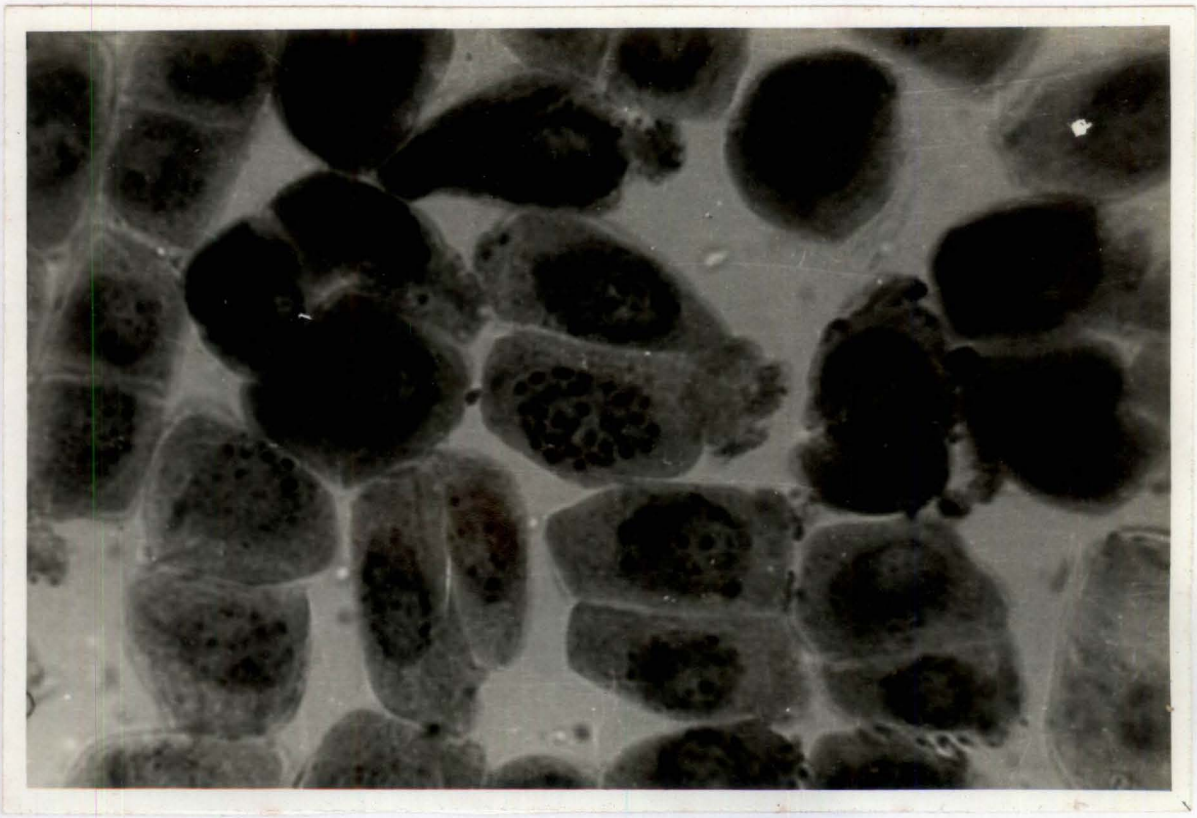


Plate 18. Somatic chromosomes of *P. bababudani* (x 4000)

Plate 19. Somatic chromosomes of *P. betle* (x 4000)



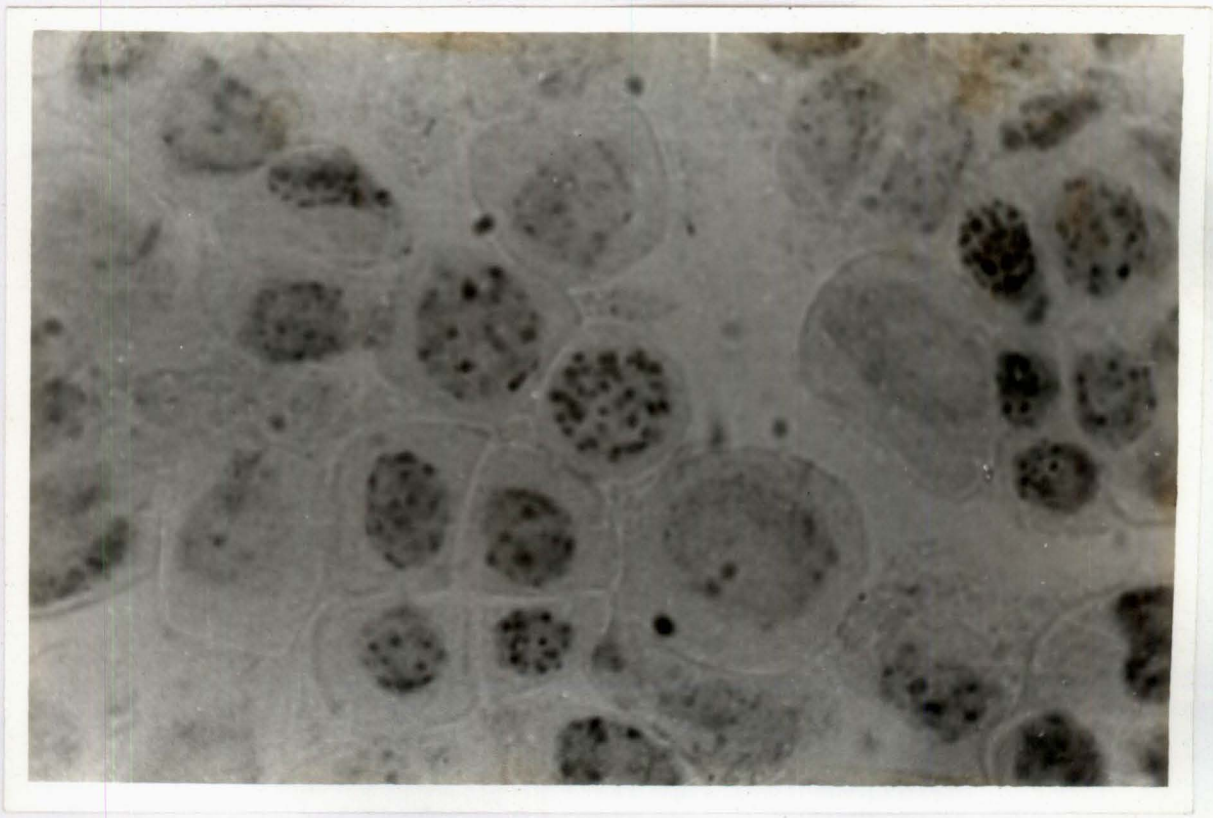
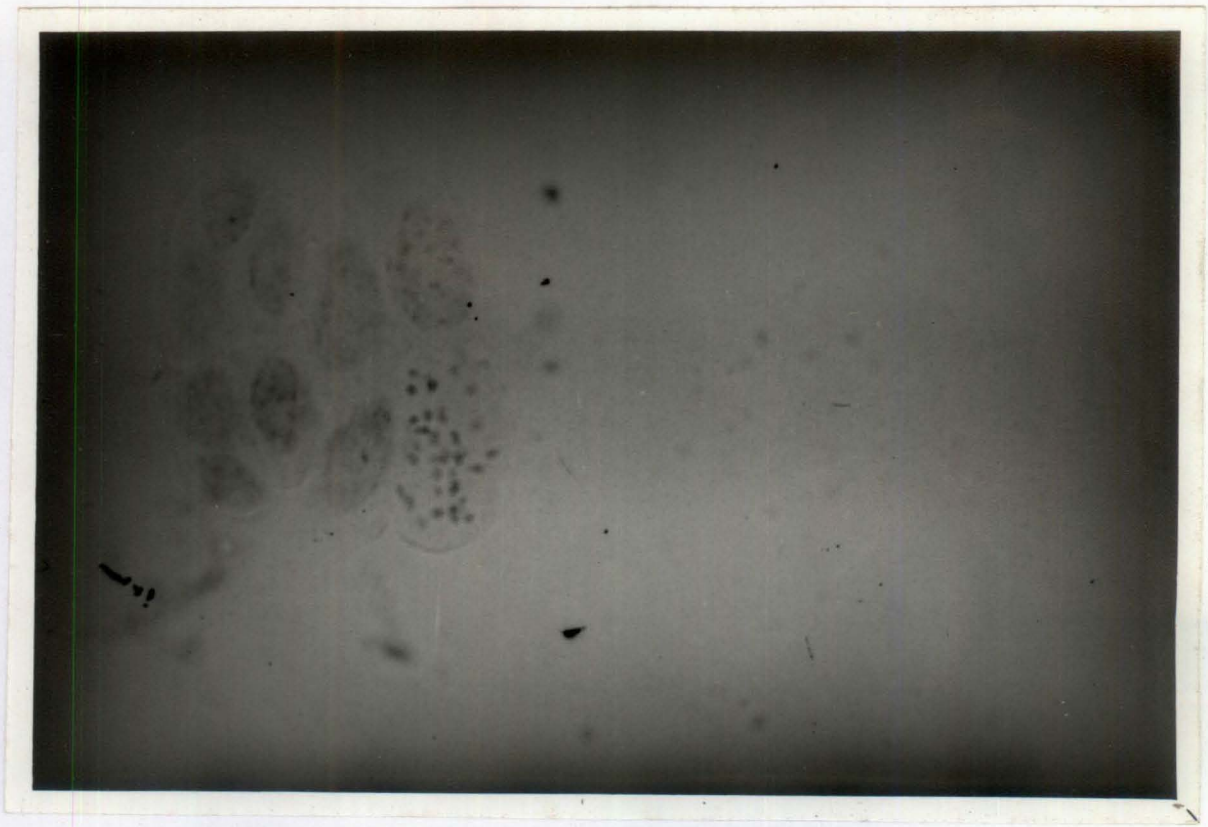


Plate 20. Somatic chromosomes of *P. chaba* (x 4000)

Plate 21. Somatic chromosomes of *P. colubrinum* (x 4000)



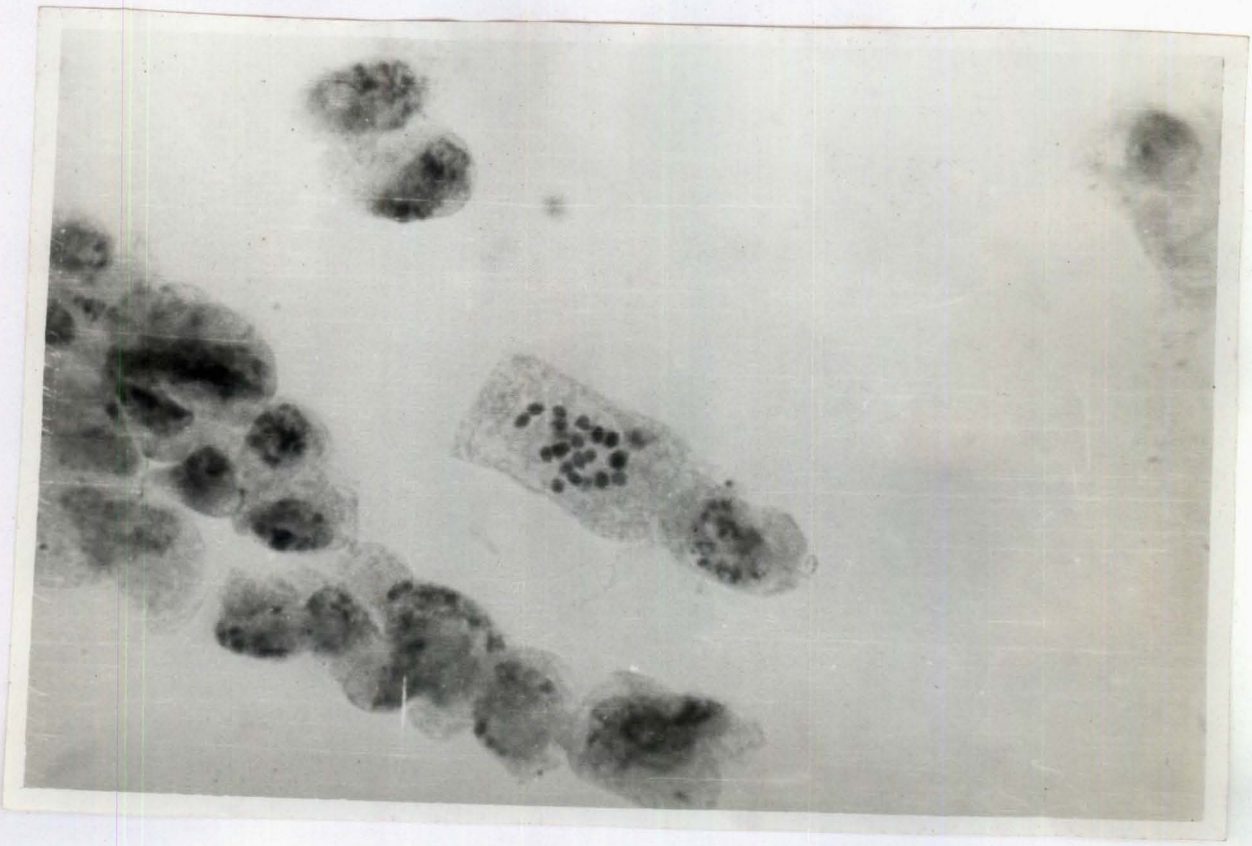
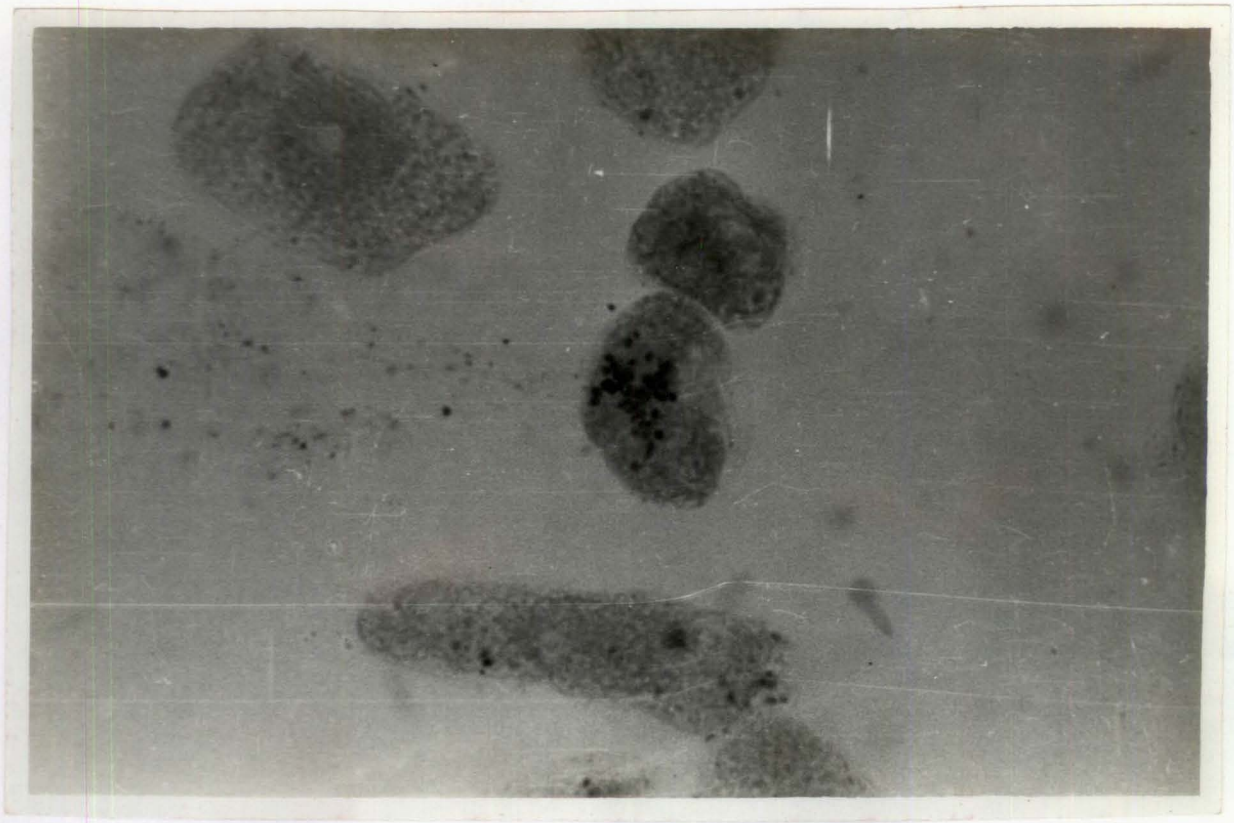


Plate 22. Somatic chromosomes of *P. longum* (x 4000)

Plate 23. Somatic chromosomes of *P. nigrum* (Panniyur-1) (x 4000)



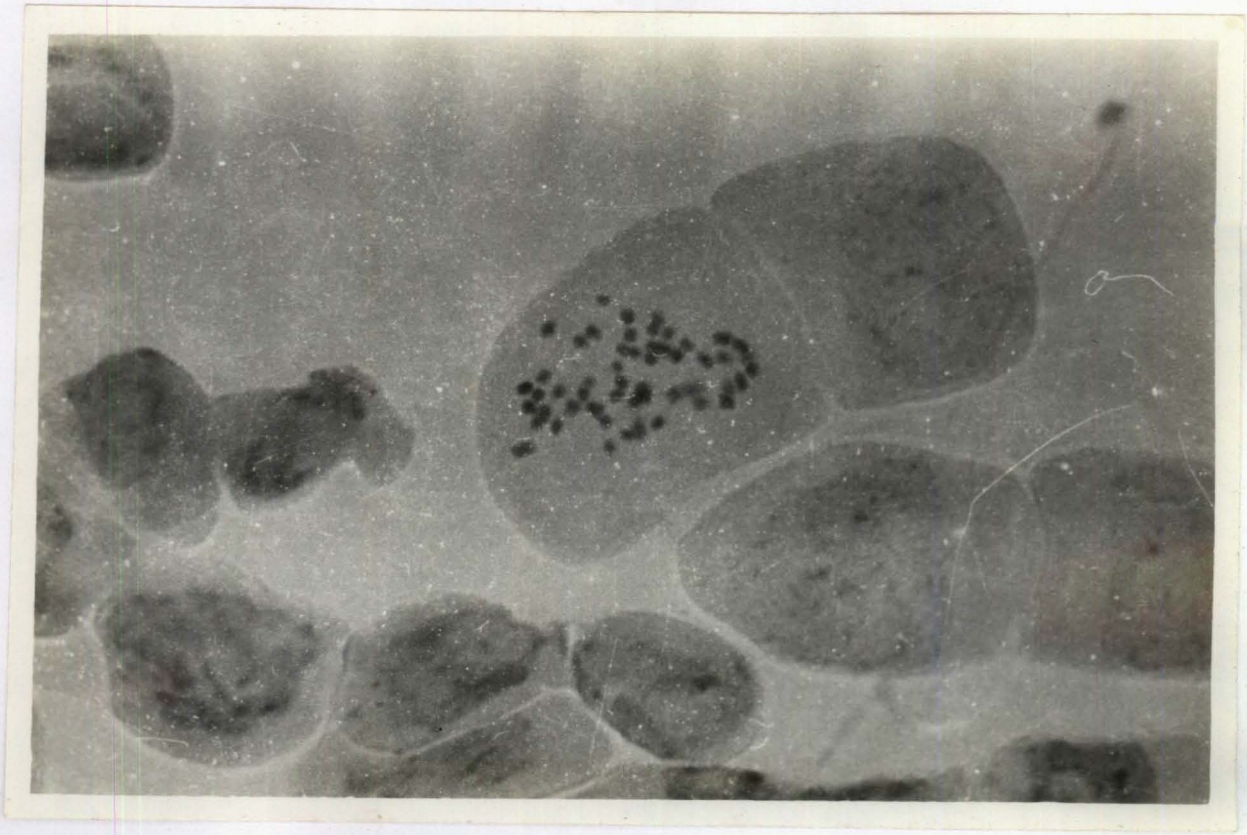
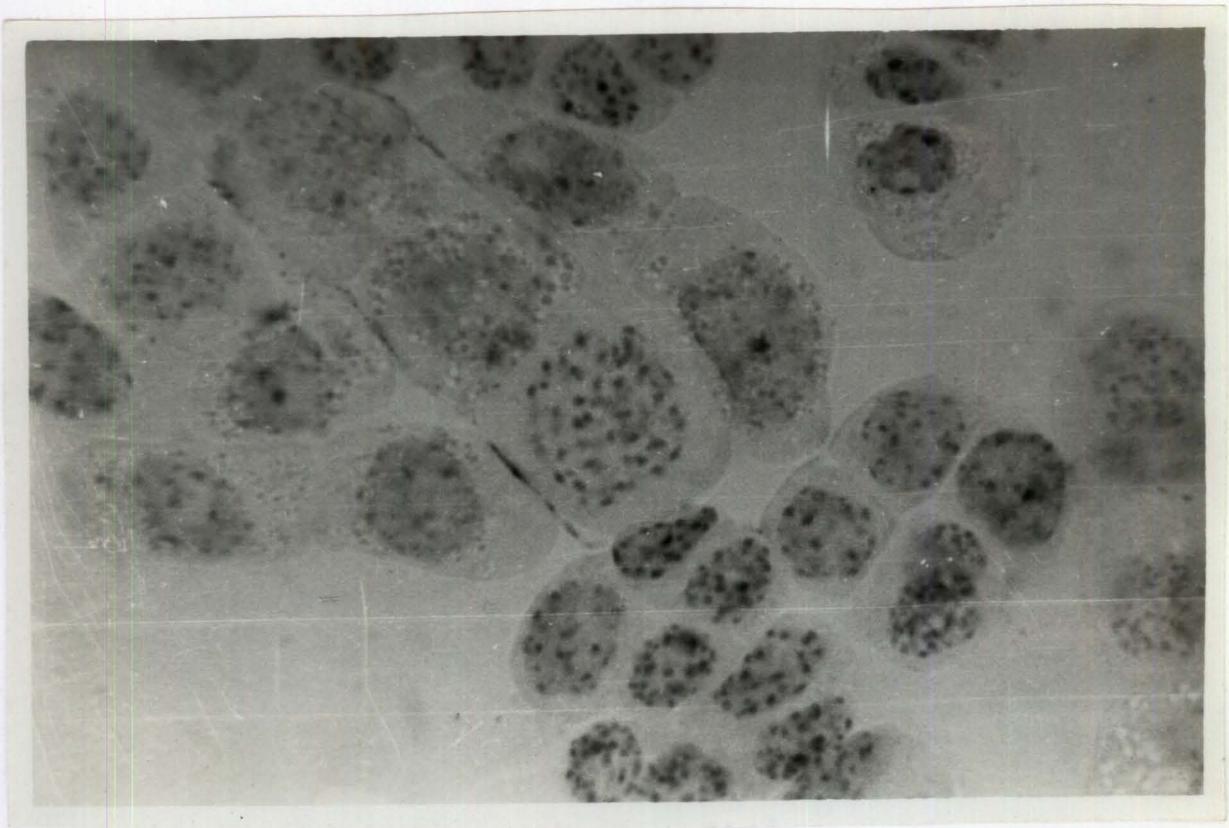


Plate 24. Somatic chromosomes of *P. nigrum* (wild) (x 4000)



Somatic chromosome number of *P. attenuatum* had been reported variously by different workers. 2n=26 and 39 (Samuel and Bavappa, 1981), 2n=36 (Bai and Subramanian, 1985) and 2n=104 (IISR unpublished). However, chromosome number of 2n=52 was also reported by various workers like Jose (1981), Jose and Sharma (1983, 1984), Rahiman and Nair (1986) and IISR unpublished. The result of the present study is in agreement with their results.

Chromosome number of *P. bababudani* was observed to be 2n=32. This was contradictory to the report of Rahiman (1981) as 2n=52.

Somatic chromosome number of *P. betle* was also varyingly reported by different workers as 2n=78 (Mathew, 1958; Okada, 1986), 2n=78, 42, 58 and 195 (Jose and Sharma, 1985), 2n=64 (Sharma and Bhattacharya, 1959; Dasgupta and Datta, 1976) and 2n=52 and 65 (Samuel and Morawetz, 1989). The result of the present study is in agreement with the early findings of Johnson (1910) and Janakiammal (1945) who reported the 2n number as 32.

Chromosome number of *P. chaba* was reported as 2n=104 by Jose and Sharma (1984). Janakiammal (1945) observed the chromosome number as 2n=24 and the result of the present study is in confirmation with her result.

In *P. colubrinum,* there is no published report on chromosome number. However, there was one unpublished report from IISR where the 2n number was reported as 26 and the result of the present study do agree with their findings.

Somatic chromosome number of *P. attenuatum* had been reported variously by different workers. 2n=26 and 39 (Samuel and Bavappa, 1981), 2n=36 (Bai and Subramanian, 1985) and 2n=104 (IISR unpublished). However, chromosome number of 2n=52 was also reported by various workers like Jose (1981), Jose and Sharma (1983, 1984), Rahiman and Nair (1986) and IISR unpublished. The result of the present study is in agreement with their results.

Chromosome number of *P. bababudani* was observed to be 2n=32. This was contradictory to the report of Rahiman (1981) as 2n=52.

Somatic chromosome number of *P. betle* was also varyingly reported by different workers as 2n=78 (Mathew, 1958; Okada, 1986), 2n=78, 42, 58 and 195 (Jose and Sharma, 1985), 2n=64 (Sharma and Bhattacharya, 1959; Dasgupta and Datta, 1976) and 2n=52 and 65 (Samuel and Morawetz, 1989). The result of the present study is in agreement with the early findings of Johnson (1910) and Janakiammal (1945) who reported the 2n number as 32.

Chromosome number of *P. chaba* was reported as 2n=104 by Jose and Sharma (1984). Janakiammal (1945) observed the chromosome number as 2n=24 and the result of the present study is in confirmation with her result.

In *P. colubrinum,* there is no published report on chromosome number. However, there was one unpublished report from IISR where the 2n number was reported as 26 and the result of the present study do agree with their findings.

In *P. longum*, chromosome number was reported varyingly by different workers as 2n=24 (Tjio, 1948), 2n=52 (Mathew, 1958; Jose and Sharma, 1983, 1984; Rahiman and Nair, 1986), 2n=96 (Sharma and Bhattacharya, 1959), 2n=48 (Dasgupta and Datta, 1976), 2n=44 (Sampathkumar and Navaneethan, 1981) and 2n=53 (Samuel and Morawetz, 1989). In the current study, chromosome number was observed as 2n=32 and this is a new count in *P. longum*, not reported by earlier workers.

Somatic chromosome number of *P. nigrum* had been reported variously by different workers in the past. 2n=128 (Janakiammal, 1945), 2n=48 (Sharma and Bhattacharya, 1959), 2n=36 and 60 (Dasgupta and Datta, 1976), 2n=54 (Sampathkumar and Navaneethan, 1981) and 2n=104 (Jose and Sharma, 1984) were the numbers thus reported. However, most of the workers agreed with the number 2n=52 (Mathew, 1958; Martin and Gregory, 1962; Mathew, 1972; Samuel and Bavappa, 1981; Samuel, 1981; Mathew and Mathew, 1982; Jose and Sharma, 1983, 1984; Rahiman and Nair, 1986 and Nair *et al.*, 1993). The results of the present study is in agreement with the majority's view that in cultivated *P. nigrum* 2n number is 52.

Two diploid chromosome numbers has been reported in wild accessions of *P. nigrum* by Mathew (1958, 1972). They were 2n=52 and 104 suggesting a role of polypolidy in the evolution of *Piper*. The accessions used in the current study showed 2n number as 52 which was a number earlier reported by Mathew in wild *P. nigrum*.

Lack of uniformity in the reports of chromosome number by different workers indicate the existence of many cytotypes in *P. nigrum* and other species of *Piper* as suggested by Ravindran and Babu (1994). Occurrence of different cytotypes in *P. nigrum* and other species of the genus suggests a probable role of polyploidy in the evolution of species of the genus. These cytotypes are being maintained in the population by predominant vegetative propagation.

Morphological, numerical taxonomical and biochemical studies including secondary metabolites and isoenzyme analysis had shown a close relationship between *P. argyrophyllum* and *P. attenuatum* (Rahiman, 1981; Ravindran, 1990 and Sebastian, 1995). However, the results of the cytological studies do not agree with these morphological and biochemical observations. There could be high level of cytological variation within species in *Piper* in general and these two species in particular. Detailed examination of the taxa for cyto-morphological variation is therefore warrented.

In the present study, chromosome numbers of 2n=24, 26, 32, 36 and 52 have been observed in different *Piper* spp. The numbers do not follow a clear arithmetic progression and as such it is difficult to draw any definite conclusions regarding the basic chromosome number. However, apart from the South American species all other species studied processed chromosome numbers which were multiples of four. Eventhough the present study suggests '4' as the basic chromosome number of Indian *Piper* the study was not exhaustive to come into conclusions regarding basic chromosome number.

Summary

SUMMARY

The present study was conducted in the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period from October 1994 to December 1996. The major objectives were to prepare a morphological descriptor for the eight *Piper* spp. collected from different geographical areas and to conduct cytological investigations in these species. To prepare a morphological descriptor, detailed morphological observations were recorded. Cytological studies included standardisation of plant part, time of collection, pre-treatment chemicals, fixatives, stains, cytological technique and count of chromosome number.

The results of morphological studies are summarised below:

Observations on vegetative characters (young leaf, mature leaf and stem) and reproductive characters (flower and fruit) were recorded. Based on the salient observations on morphology, brief descriptions with distinguishing characters of each of the eight *Piper* spp. studied were made. *P. argyrophyllum* had acrodromous venation, hairs were present on petiole and under surface of leaves, berries were conical when young and globose when mature and the berries turned dark green to black on ripening. *P. attenuatum* had acrodromous venation, petiole and under surface of leaves were glabrous, berries were conical when young and globose when mature and the berries turned dark green to black on ripening. *P. bababudani* had fleshy spikes, bracts were hooded at the tip and arranged in clear spirals, berries turned orange on ripening. *P. betle* had purple stem and cordate leaves on orthotrops and laterals. *P. chaba* showed distinct dimorphism between runners and orthotrops. Leaves on orthotrops and laterals were lanceolate with eucamptodromous venation while the leaves on runners were

cordate with acrodromous venation. *P. colubrinum* was a tall shrub with stilt roots and the plant never climbed. *P. longum* was a creeper with cylindrical male and female spikes. *P. nigrum* had campylodromous venation, bracts were sessile and adnate to rachis and the fruits turned red on ripening. *P. nigrum* (wild) plant was dioecious.

The eight species of *Piper* studied were analysed for the similarities and differences in their morphological characters inorder to find out the relationship among the species. *P. argyrophyllum* and *P. attenuatum* were similar morphologically except for the hairyness on petiole and under surface of leaves. *P. bababudani* and *P. nigrum* were very similar in vegetative morphology. The two species differed in bract characters and size and colour of fruits. Except *P. longum* and *P. colubrinum* all species were climbers. *P. longum* was a creeper and *P. colubrinum* was a shrub with stilt roots. Among the species, only *P. betle* had purple stem colour. *P. chaba* showed distinct dimorphism between orthotrops and runners in leaf shape and venation whereas this distinction was not shown by rest of the species.

Based on morphological comparisons, a key for identification of species has been proposed.

To find out the somatic chromosome number of the different *Piper* spp., mitotic studies were carried out using root tip squash method. The results are presented under the following heads:

Standardisation of material: Two node cuttings produced fresh actively growing roots in four weeks and gave maximum number of dividing cells. These cuttings after collection of roots were replanted which helped in harvesting fresh root tips in two weeks time. Thus by re-using the cuttings a gain of two weeks could be obtained. Climbing roots

contained no dividing cells. So the root material for mitotic studies were collected by rooting of cuttings alone.

Standardisation of time of collection of roots: Roots were collected at different time intervals and examined for the presence of dividing cells. Maximum number of dividing cells were observed between 11 a.m and 1 p.m. During November-February period, dividing cells were also observed between 2 p.m. and 4 p.m., but the number of dividing cells were very few. For all the eight *Piper* spp. studied, maximum number of cells were in the early metaphase between 11.15 a.m. and 12.15 p.m. and hence the roots for mitotic studies were collected between 11.15 a.m. and 12.15 p.m.

Standardisation of pre-treatment chemicals, fixatives and stains

Three pre-treatment chemicals *viz.*, 8-hydroxyquinoline, para-dichlorobenzene and α-bromonaphthalene were attempted. As pre-treatment agents, all the three chemicals were equally good giving desirable results. But the preparation and use of 8-hydroxyquinoline was comparatively easy and hence it was used as pre-treatment agent for mitotic studies.

Carnoy's A and Carnoy's B were the fixatives used in the present study and both were equally good as fixatives. According to availability of components either could be used as fixative for cytological studies in *Piper*.

Staining was studied with acetocarmine and acetoorcein. With acetocarmine chromosomes were not deeply stained, but there was no cytoplasmic staining whereas with acetoorcein the chromosomes were deeply stained, but the cytoplasm also took stain

slightly. However, better contrast was obtained with acetoorcein. Hence orcein was used for staining of mitotic preparations in *Piper* spp.

Cytological technique

Among the two cytological techniques tried, one involved hydrolysis and the other did not involve hydrolysis. The method involving hydrolysis was found to be better because squashing was very easy and the spread of cells were very good and hence employed for further analysis.

Chromosome number in *Piper* spp.

Somatic chromosome number of the eight different *Piper* spp. studied were counted. 2n number of 36, 52, 32, 32, 24, 26, 32, 52 and 52 were observed in *P. argyrophyllum, P. attenuatum, P. bababudani, P. betle, P. chaba, P. colubrinum, P. longum, P. nigrum* (Panniyur-1) and *P. nigrum* (wild) respectively. Microphotographs were taken and good mitotic preparations were made permanent.

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CYTO - MORPHOLOGICAL INVESTIGATIONS IN *Piper* spp.

BY

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ABSTRACT OF A THESIS

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ABSTRACT

The study 'Cyto-morphological investigations in *Piper* spp.' was conducted in the Department of Plantation Crops and Spices, College of Horticulture, Vellanikkara during the period from October 1994 to December 1996. The major objectives were to prepare morphological descriptors for the eight *Piper* spp. collected from different geographical areas and to conduct cytological investigations in these species.

To prepare morphological descriptors for the eight species studied detailed morphological observations were recorded. Based on the salient observations on morphology brief descriptions of the eight *Piper* spp. were made. The eight species of *Piper* studied were analysed for the similarities and differences in their morphological characters to find out the relationship among the species. Based on morphological comparisons, a key for identification of species has been proposed.

The procedure for mitotic studies in *Piper* spp. was standardised. The roots were collected between 11.15 a.m. and 12.15 p.m. and pre-treated in 8-hydroxyquinoline for two to three hours at 4°C. The pre-treated roots were fixed in Carnoy's A or Carnoy's B for one or two days. The material was then hydrolysed in 1N hydrochloric acid in a water bath maintained at a temperature of 60°C for fifteen minutes. The roots were then stained in 0.5% acetoorcein for 45 minutes. After that

slide preparations were made and examined for the presence of mitotic chromosomes. Microphotographs were taken and good slides were made permanent.

Chromosome number of 36, 52, 32, 32, 24, 26, 32, 52 and 52 were observed in *P. argyrophyllum, P. attenuatum, P. bababudani, P. betle, P. chaba, P. colubrinum, P. longum, P. nigrum* (Panniyur-1) and *P. nigrum* (wild) respectively. Except for the South American species, *P. colubrinum*, all the species studied possessed chromosomes in multiples of four suggesting a basic number of four for the Indian *Piper*.