

SYSTEMATIC STUDIES IN THE GENUS *PIPER*

M. R. A. SAMUEL, K. V. A. BAVAPPA* and
S. BALASUBRAMANIAM**

UNDP/FAO Project

Department of Minor Export Crops, Matale, Sri Lanka

ABSTRACT

A survey was conducted in Sri Lanka to collect and conserve different species of *Piper*. Among 69 accessions collected and established at Matale Research Station, 24 were used for a detailed study of morphological, abaxial leaf epidermal features, chromosome numbers and phenolic constituents. On the basis of morphological characters, 24 accessions were identified as *Piper nigrum* L., *P. betle* L., *P. longum* L., *P. argyrophyllum* Miq., *P. sylvestre* Lam., *P. attenuatum* Buk. Ham., *P. chuvya*, *P. thwaitseii* C. Dc., *P. trineuron* Miq. and *P. zeylanicum* Miq. Of these *P. attenuatum* is reported for the first time from Sri Lanka. The data available from the study of chromosome number, phenolic constituents and abaxial leaf epidermal characteristics suggest that *P. sylvestre* and *P. argyrophyllum* are conspecific and a specific status is assigned to *P. chuvya* which was earlier considered as a variety of *P. betle*.

Variation in chromosome number was observed among the different species ranging from $2n = 26$ to 65. Stomatal index and phenolic constituents are found to be specific for some species.

INTRODUCTION

The genus *Piper* is the most important among the eight genera (Hooker, 1885; Cooke, 1908) of the family Piperaceae. Different authors have recognised varying number of species in the genus *Piper*, the number ranging from 500 to 1200 (Hooker, 1885; Trimen, 1895; Ridley, 1924). Of these, *Piper nigrum*, *P. longum*, *P. betle*, *P. thwaitseii*, *P. zeylanicum*, *P. trineuron*, *P. argyrophyllum*, *P. sylvestre* and *P. subpeltatum* have been reported to be present in Sri Lanka (Trimen, 1895).

The existing classification of the genus *Piper* (Hooker, 1885; Ridley 1924; Gamble, 1956) has been entirely on the basis of foliar, floral and inflorescence characteristics. Phytochemical, anatomical and cytological data have been widely used in recent years as aids to taxonomic investigations (Stebbins, 1950; Senanayake, 1969; Kubitzki, 1977; Gupta, 1978, Bain and Denford, 1979) at different hierarchical levels. From an islandwide collection of 69 accessions of cultivated and wild species of the genus *Piper*, 24 were studied in

Present address : * Director, Central Plantation Crops Research Institute, Kasaragod 670 124, Kerala, India

** University of Sri Lanka, Peradeniya, Sri Lanka

detail for their morphological characters, leaf epidermal pattern, chromosome number and phenolic constituents. Classification of these 24 accessions are reported in this paper.

MATERIALS AND METHODS

A survey was carried out in the districts of Matale, Matara, Kandy, Ratnapura and Badulla (Fig. 1). Collections were made from these areas and accession numbers (Acc. No.) were given for each one of them. Rooted cuttings of 42 accessions were planted under uniform field condition, in three sets, adopting a randomised block design for each. Of this, 24 accessions were used to study length and breadth of leaf, petiole length, colour, shape and nature of the lamina and type of venation, leaf epidermal pattern, chromosome numbers and phenolic constituents of leaf. Length of spikes and peduncle, floral characteristics and percentage of fruit set were studied with samples collected from plants in their natural habitat.

Epidermal peels were obtained from the 5th and 6th leaf below the growing tip of the plagiotrophic branches using razor blade. The peels were stained with cotton blue in lactophenol. The number of epidermal cells and stomata (complete) in 30 microscopic fields were counted at 10×40 magnification and stomatal index calculated following the method of Salisbury (1928). The length and breadth of epidermal cells and the length of guard cells were measured at a magnification of 10×15 using a micrometer eye piece.

Methanolic extract of the leaf was used for chromatographic separation of

the phenolic constituents. Twenty five μ l of the extract was applied near the left hand corner of the 30×30 cm square sheet of (Whatman) paper. The paper was first developed with saturated solution of butan-2-ol and in the second direction using 2% acetic acid. Two chromatograms were prepared for each accession. One of the chromatograms was first viewed under the UV light and the colour of the fluorescent spots were recorded. The same chromatogram was sprayed with $AlCl_3$ (2% in ethanol) and the colour of the UV active spots marked. The second chromatogram was sprayed with sulphanic acid and the colour and position of the spots were marked.

Somatic chromosome number of each accession was determined from root tips following aceto orcein squash method.

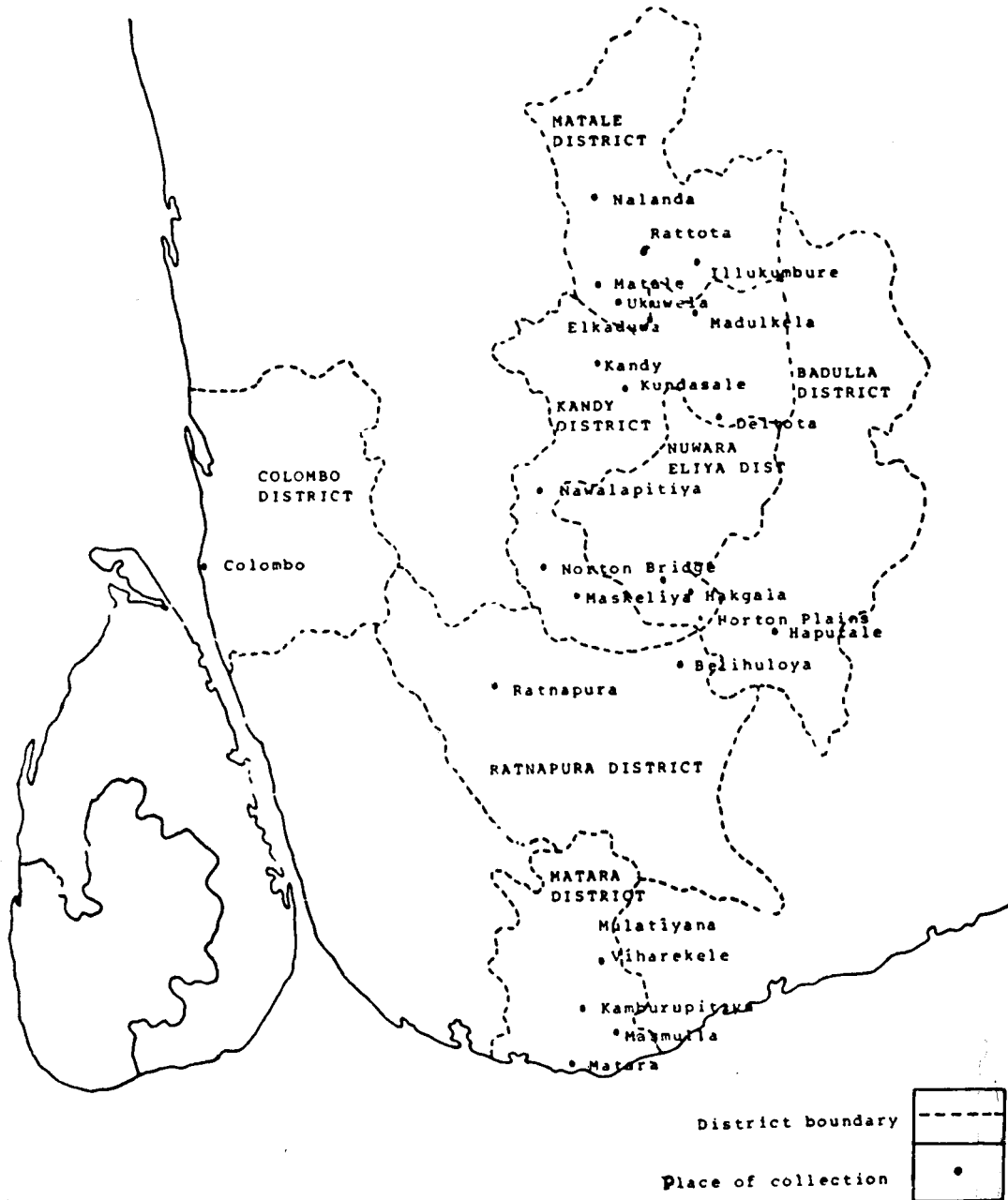
RESULTS

Observations recorded on growth, habit, foliar characteristics and floral characters, viz., sex ratios, length of spike, shape of bract, number of stamens, stigmatic lobe, shape of berries and percentage of fruit set for each of the species are described below:

Piper attenuatum (Fig. 2)

A tall glabrous climber much thickened at the nodes; leaves orbicular, apex acuminate, base obtuse and symmetrical. The length of lamina varies from 9 to 11 cm and breadth 6 to 8 cms, glabrous on both surfaces, having 7 main veins arising from the base with no prominent secondaries,

FIG. 1. AREA SURVEYED FOR GERmplasm OF PEPPER



Vine dioecious having long (10-12 cm) slender and pendulous spikes arising from the node opposite to the leaf, flowers spirally arranged on inflorescence axis and stigma 3-4 lobed (Fig. 14). Berries ovoid, small and less pungent, mature spikes dark green in colour with 50% fruit set.

Piper nigrum (Figs. 3-5)

Tall, profusely branched glabrous vine, nodes much thickened leaves broadly or narrowly ovate, apex acuminate, base obtuse or slightly rounded and asymmetrical, lamina coriaceous, glabrous, having a length of 11-14 cms and breadth varies from 8 to 10 cms; 3 or 5 main veins arise from the base and a pair of prominent secondaries arise alternately from the mid vein. The vines are dioecious or polygamodioecious. Spikes long, thick and pendulous arising from the node opposite the leaf, flowers bisexual, (Fig. 15) or unisexual (Figs. 16 and 17) spirally arranged on the inflorescence axis, each subtended by an oblong bract. Stigma 3-4 lobed. Stamens 2, found on either side of the ovary in the case of hermaphrodite flowers, berries globose, large and highly pungent. Mature spikes dark green in colour and turns red.

Piper longum (Fig. 6)

The plant 60-90 cm high, ascending or prostrate but not climbing, highly branched and nodes less thickened. There are two types of leaves. Of these the lower leaves are broadly ovate, apex acuminate, base deeply cordate and symmetrical. The length of lamina varies from 8 to 10 cm and breadth 5-7 cm, membranous, slightly pubescent

on the abaxial surface and glossy on the adaxial surface with fairly long petioles. Upper leaves are oblong, ovate, lanceolate, apex acuminate, base slightly cordate and asymmetrical. In both cases, there are 5 or 7 main veins arising from the base with no prominent secondaries. Plant dioecious having short (2 cm) thick and erect spikes, flowers minute, spirally arranged and sunken in inflorescence axis. Each flower has a circular bract (Fig. 18) with free and membranous margin. Stigma 3-4 lobed and the whole spike become the "fruit" when mature.

Piper trineuron (Fig. 7)

Tall, profusely branched vine, having nodes which are less thickened. Leaves narrowly ovate, elliptic, apex acuminate, base acute and symmetrical, lamina membranous, and pubescent on the abaxial side. The length of the lamina varies from 8-10 cm and breadth 3-5 cms; 3 main veins arise from the base and a number of prominent laterals arise from the mid vein. Vine dioecious, having short (5-6 cm) slender and pendulous spikes arising from the node opposite to leaf; flowers spirally arranged on inflorescence axis, each subtended by an oblong bract (Fig. 19) adnate to inflorescence axis, stamens 2, and the mature spikes are yellow in colour.

Piper zeylanicum (Fig. 8)

Tall, sparsely branched vine, nodes very much thickened and internodes very short (.5 - 2.0 cm). Leaves broadly or narrowly ovate, apex acuminate, base obtuse, slightly rounded and more or less symmetrical; lamina coriaceous.

pubescent on abaxial surface, having a length of 5-6 cm and breadth 3-4 cms; 5 (or 3) main veins arise from the base with no prominent secondaries. Vine dioecious having short (2-3 cm) thick, erect spikes, arising from the node opposite to leaf, flowers spirally arranged on inflorescence axis, bracts circular with free and membranous margin (Fig. 20); stigma 3-4 lobed (♀ vine) and stamens 2 (♂ vine). Berries globose, large, less pungent, becomes red when mature and the spikes show 5-10% fruit set.

Piper sylvestre (Fig. 9)

Tall, glabrous less branched vine, having fairly thick nodes; leaves broadly or narrowly ovate, apex acuminate, base obtuse or slightly rounded and more or less symmetrical. Lamina, membranous, glabrous, bullate on adaxial surface, length of lamina varies from 10-12 cm and breadth 5 to 8 cms; 5 or 7 main veins arise from the base with no prominent secondaries, vine dioecious, having long (6-10 cm) pendulous spikes arising from the node opposite to leaf. Flowers spirally arranged on a inflorescence axis, subtended by broadly oval bract with free and membranous margin, 3-5 stamens in ♂ vine (Fig. 21). Berries ovoid, small less pungent and do not become red in colour when mature, the spike show 50-60% fruit set.

Piper argyrophyllum (Fig. 10)

Tall, profusely branched vine, nodes not much thickened, leaves narrowly ovate, lanceolate, ovate lanceolate, elliptic, apex acuminate, base obtuse, oblique, acute and symmetrical; lamina membranous, pubescent on the abaxial

surface and having a length of 6-10 cm and breadth varying from 4-6 cms; 5 main veins arise from the base with no prominent secondaries. Vine dioecious, having long (5-6 cm) slender, pendulous spikes arising from the node opposite the leaf; flowers spirally arranged on inflorescence axis, each subtended by broadly oval bract with free and membranous margin, stamens 3 to 4 (♂ vine) and stigma 3 and 4 lobed (Fig. 22).

In male vine the spikes turn yellow in colour when mature, female vines berries are ovoid, less pungent and becomes green in colour when mature, showing a fruit set of 70-80%. The berries never become red in colour.

Piper thwaitseii (Fig. 11)

Tall, woody climber, nodes much thickened and internodes fairly long; leaves broadly or narrowly ovate, apex acuminate, base obtuse and slightly rounded and more or less symmetrical; lamina coriaceous, pubescent on abaxial surface, length of lamina 8-10 cm and breadth 5 to 7 cms; 5 main veins arise from the base with no prominent secondaries; vine dioecious, having short (4.5 cm) stout and erect spikes, arising from the node opposite of leaf, flowers spirally arranged on inflorescence axis, subtended by circular bracts (Fig. 23) with free and membranous margin, stigma 3-4 lobed. Berries globose and turns red when mature with 25-50% fruit set.

Piper betle (Fig. 12)

Tall, less branched vine, nodes not much thickened; leaves broadly ovate

with acuminate apex, base slightly cordate and asymmetrical, thinly coriaceous, pubescent on abaxial and glossy adaxial surface; lamina length 8–10 cm and breadth 3–8 cm; 5 main veins arise from the base, a pair of prominent secondary veins arise from mid vein, almost alternately. Vine dioecious, having long (6–10 cm) fleshy, cylindrical, pendulous spikes arising from the node opposite to leaf; flowers spirally arranged and sunken in inflorescence axis, bracts circular, peltate with free and membranous margin (Fig. 24) stigma 4–5 lobed, when mature, the whole spike becomes the “fruit” which is dark green in colour.

Piper chuvya (Fig. 13)

Large stout less branched vine, nodes much thickened, internodes 6–7 cm; leaves broadly ovate, acuminate apex, base oblique and asymmetrical, lamina coriaceous, pubescent and slightly bullate on adaxial surface and 15 cm × 9 cm, petiole 2.0 cm long, 5 main veins arise from the base and a pair of prominent secondary arise from mid vein almost alternately; vines dioecious, having long (10 cm) fleshy, pendulous spikes arising from the node opposite the leaf; flowers spirally arranged and sunken in inflorescence axis, bracts circular, peltate with free and membranous margin, stigma 3–4 lobed (Fig. 25), when mature the whole spike becomes the “fruit”.

Data gathered on leaf epidermal pattern, chromosome numbers and phenolic constituents are given in Table I which show that there are

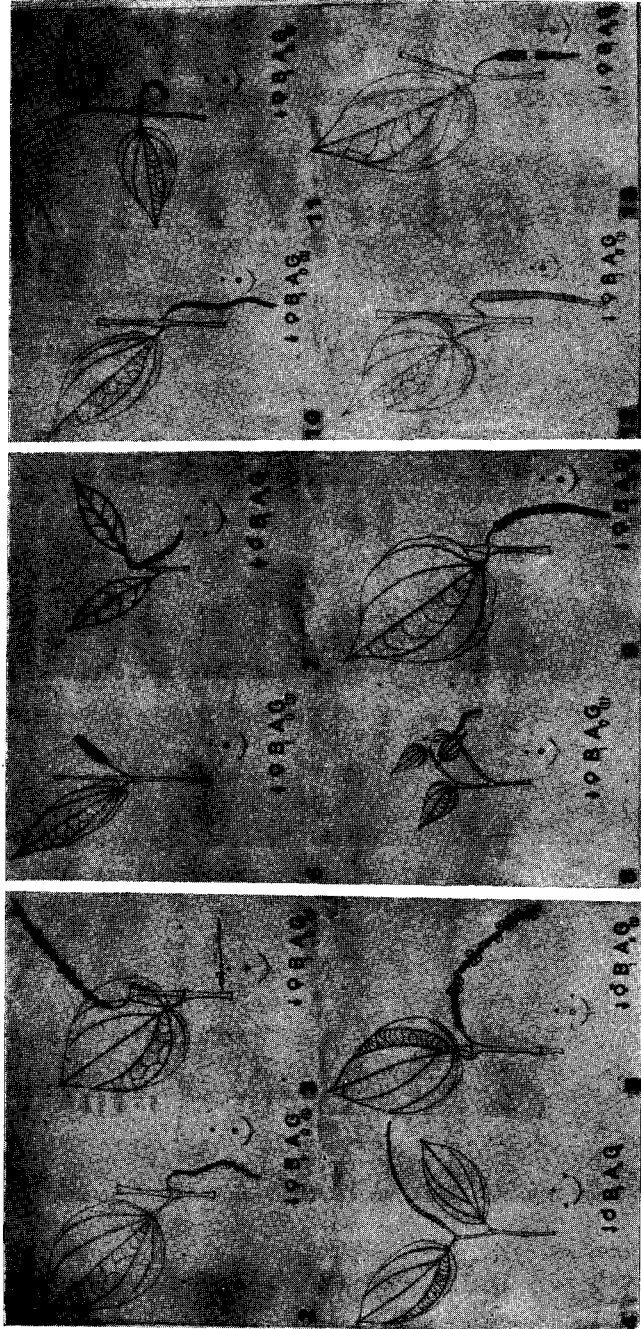
considerable variations between different accessions for various characters studied.

Of the different foliar characteristics observed in this study, the leaf shape, size and venation pattern have been found to be highly variable among the members of this genus. The leaves of *P. longum*, *P. betle*, *P. chuvya*, *P. thwaitseii*, *P. zeylanicum* and *P. nigrum* are broadly or narrowly ovate with acuminate apex and base oblique or slightly rounded except in *P. longum* where the base is deeply cordate. The leaves of *P. zeylanicum* and *P. thwaitseii* are however much smaller in size compared to the others. Marked variation was found in the leaf shape of *P. trineuron*, *P. attenuatum* and *P. argyrophyllum* in that the leaves were elliptic in *P. trineuron*, orbicular in *P. attenuatum* and lanceolate to ovate lanceolate in *P. argyrophyllum*.

The number of main veins arising from the base, varied from 3 to 7 in this genus. *P. trineuron* has 3 main veins and a number of prominent laterals arising from the mid vein. In *P. nigrum* *P. betle* and *P. chuvya* there are 3 or 5 main veins and a pair of prominent secondaries arising from the mid vein almost alternately. In the case of other species, there are 5 or 7 main veins with no prominent secondaries.

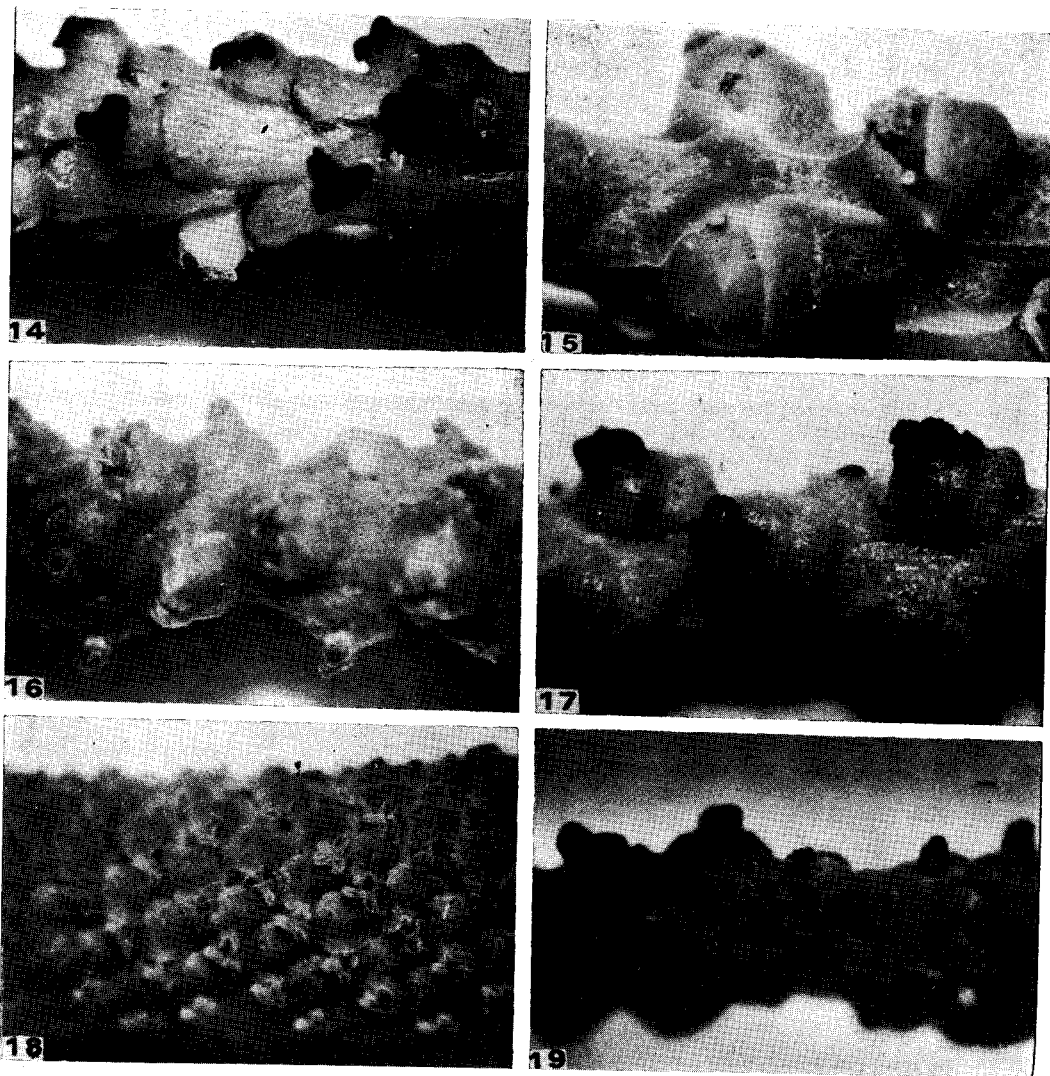
The leaves of *P. nigrum*, *P. zeylanicum* and *P. thwaitseii* are highly coriaceous whereas that of *P. chuvya* and *P. betle* are thinly coriaceous, and membranous in others. The cultivated varieties Panniyur 1, Kuching and local selection of *P. nigrum* are polygamodioecious, while the wild selections of

FIGS. 2-13. FOLIAR AND FLORAL CHARACTERISTICS OF THE DIFFERENT SPECIES OF THE GENUS *PIPER*

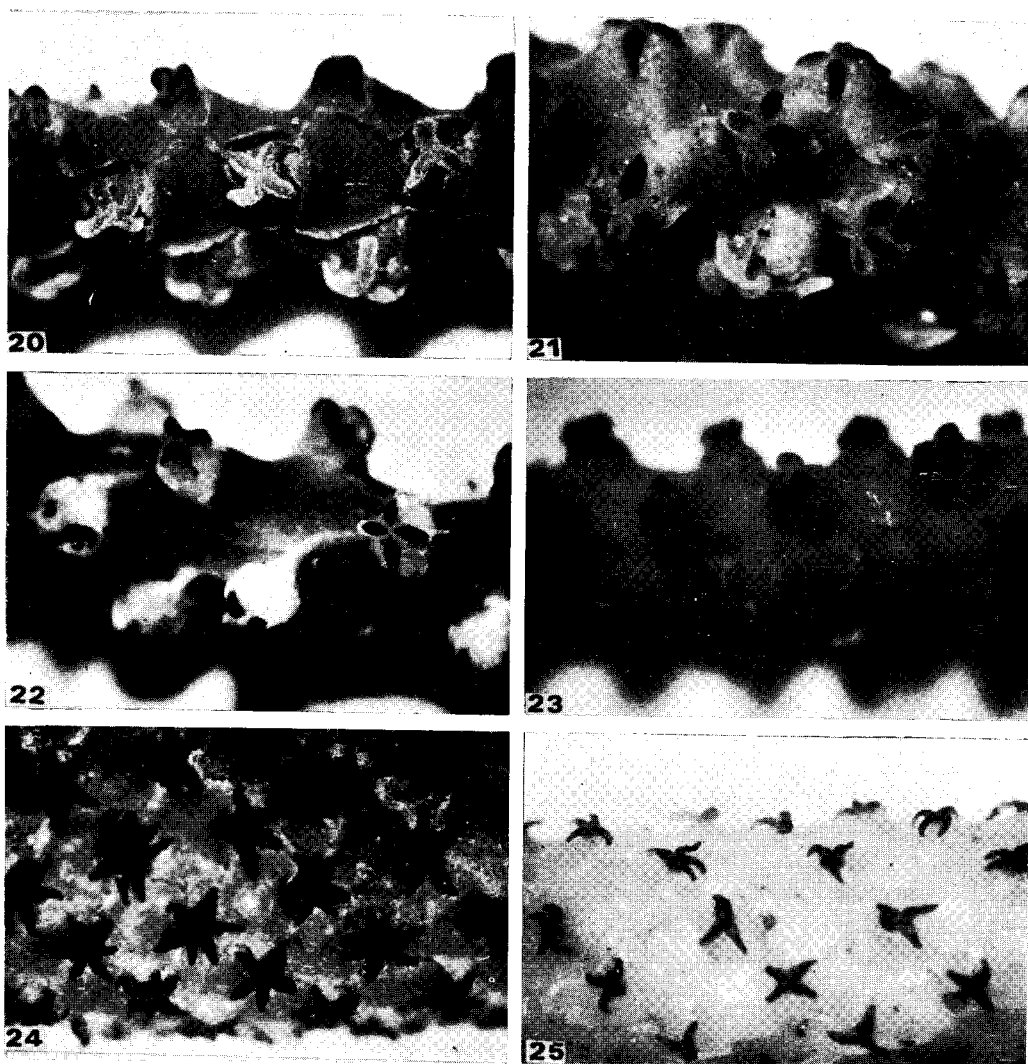


- 2. *Piper attenuatum* (♀)
- 3. *Piper nigrum* (♀)
- 4. *Piper nigrum* (♂)
- 5. *Piper nigrum* (♀)
- 6. *Piper longum* (♀)
- 7. *Piper trineuron* (♂)
- 8. *Piper zeylanicum* (♀)
- 9. *Piper sylvestre* (♀)
- 10. *Piper argyrophyllum* (♀)
- 11. *Piper thwaitsetii* (♀)
- 12. *Piper betle* (♀)
- 13. *Piper churuya* (♀)

FIGS. 14—19. SPIKE CHARACTERISTICS OF THE DIFFERENT SPECIES ($\times 6$)



14. *P. attenuatum* (♀) 15. *P. nigrum* (♀) 16. *P. nigrum* (♂)
17. *P. nigrum* (♀) 18. *P. longum* (♀) 19. *P. trineuron* (♂)

FIGS. 20—25. SPIKE CHARACTERISTICS OF THE DIFFERENT SPECIES ($\times 6$)

20. *P. zeylanicum* (♀) 21. *P. sylvestre* (♀) 22. *P. argyrophyllum* (♀)
 23. *P. tl. waitsei* (♀) 24. *P. betle* (♀) 25. *P. chuyva* (♀)

Table I. *Species of Piper and their related characteristics*

Species	Accession nos	Epidermal cell length (μ)	Guard cell length (μ)	Stomatal index	Chromosome numbers 2n	Phenolic spots
<i>P. nigrum</i>	65,66,67 (cultivated)	20.26	24.26	3.6-3.9	52	2,7,11,17
	39, (wild)	26.00	28.14	3.9	52	2,7,11
	25, (wild)	32.85	30.61	4.9	52 (78)	2,7,11
	16, (wild)	30.83	30.16	4.4	65	2,7,11
<i>P. zeylanicum</i>	34	26.55	34.64	3.8	39	4,7,9
<i>P. thwaitseii</i>	9	31.68	32.47	3.5	39	7
	15	28.30	29.34	4.3	65	3,7
<i>P. trineuron</i>	57	41.89	27.93	5.8	26	3,5,7
<i>P. betle</i>	6	45.89	28.12	10.3	52	2,4,7,10
<i>P. chuvya</i>	24	35.59	33.31	6.2	52	2,4,7,11
<i>P. longum</i>	3	44.06	27.64	9.1	26	4,5,7,11
<i>P. argyrophyllum</i>	4	46.89	27.04	6.2	26	5,7,8
	21	45.62	26.71	6.7	25	1,5,7
	26	47.07	25.41	7.0	26	7
	54	46.19	24.20	6.0	39	5,7,8,14
<i>P. sylvestre</i>	7	42.88	28.76	7.6	39	5,7,14
	13	32.09	22.11	6.7	26	3,5,7,14
	29	44.55	28.64	8.2	39 (26)	3,5,7
	42	44.72	25.99	7.2	26	5,7,8
	58	44.28	26.64	6.8	26 (39)	5,7
<i>P. attenuatum</i>	1	28.03	27.64	4.1	39	3,5,7
	32	32.23	26.54	4.7	26	3,7,8

P. nigrum and other species of this genus are dioecious. *P. longum*, *P. betle* and *P. chuvya* have stout, fleshy inflorescence axis in which minute flowers are sunken.

However, the spikes of *P. longum* is short and erect compared to the other two. In other species, spikes are slender with prominent individual flowers arranged spirally on the inflorescence axis. The bracts were oblong and adnate to inflorescence axis in *P. nigrum*, *P. trineuron* and *P. attenuatum*, broadly oval with raised margin in *P. sylvestre* and *P. argyrophyllum* and circular in other species. The number of stamens varied from 3-4 in *P. sylvestre* and *P. argyrophyllum* but in *P. nigrum*, *P. trineuron* and *P. zeylanicum*

there were only 2 stamens. No male plants were located in *P. attenuatum*, *P. betle* and *P. longum*.

DISCUSSION

The genus *Piper* is a habitant of the tropics with limited distribution confined mostly to the forest belts of India, Burma, Sri Lanka and Malaysia. The cultivated as well as the wild species of this genus exhibit considerable variation in their morphology. In the present study, it has been possible to delimit the 24 accessions into 10 different species on the basis of foliar and floral characteristics and on comparing this determination with the earlier identification of Hooker (1885) Trimen (1895) and unpublished information

supplied by Prof. Huber (personal communication). Of these, *P. attenuatum* is reported for the first time from Sri Lanka. However in the present study, no significant difference was observed between some of the quantitative parameters recorded from samples collected in their natural habitat as well as from material grown at the Research Station and the not too wide variation in the climatic conditions appear to be the underlying cause for this relative stability.

A critical examination of the abaxial leaf epidermal characters, phenolic constituents and chromosome number of the 10 species revealed that *P. sylvestre* and *P. argyrophyllum* are identical in respect of all the above characters (Table I).

Trimen (1895) has pointed out the difficulty of distinguishing these two species and has remarked that the two could be amalgamated. The data available on leaf epidermal features such as epidermal cell length, guard cell length, stomatal index together with chromosome number ($2n=26, 39$) and phenolic constituents of these two species strongly support this view of Trimen. As such *P. sylvestre* and *P. argyrophyllum* could be considered to be conspecific. Since *P. sylvestre* was the species first described (1791) according to international rules of botanical nomenclature the name of this species will be henceforth *P. sylvestre*.

Hooker (1885) and Trimen (1895) have described an introduction from the Malay Archipelago and placed it as *P. chuvya* which according to them is a

cultivated form of *P. betle*. Acc. No. 24 which has similar morphological characters as *P. chuvya* but guard cell length, epidermal cell length and stomatal index were different from that of *P. betle* (Acc. No. 6). Further the leaf base of this accession is oblique and very unequal unlike that of *P. betle*. The two accessions differ also in their phenolic constituents, spot No. 10 being specific to *P. betle* (Table I). Taking morphological, histological and phytochemical differences into consideration, the present delimitation of *P. chuvya* (Acc. No. 24) as a cultivated form of *P. betle* does not seem to be justified. As such *P. chuvya* may be given a specific status.

The report on the origin of cultivated pepper indicates that it is a native to the Western Ghats of S. India. The species reported from this region are *P. nigrum*, *P. betle*, *P. chuvya*, *P. sylvestre*, *P. argyrophyllum*, *P. trineuron*, *P. zeylanicum*, *P. thwaitseii* and *P. attenuatum*. The information available on cytomorphological, phytochemical and anatomical characteristics of these species is rather limited. The smallest chromosome number $2n=26$ observed in *P. sylvestre*, *P. trineuron*, *P. longum* and *P. attenuatum* is the first report for these species. Viewed from the occurrence of varying chromosome number from $2n=26$ to 65 accompanied by more or less similar variations in other characteristics probably suggest that Sri Lanka has a great diversity as far as these species are concerned.

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