Cytotaxonomical Studies of South Indian Piperaceae

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Cytological studies in the family Piperaceae were poor and the literature concerning many taxa has been accumulated from time to time. According to Das Gupta and Datta (1976) the chromosome numbers of only 64 species of Piperaceae have been cytologically investigated so far, even though there are nearly 2000 species in this family. Gamble (1957) has recorded thirteen wild species of *Piper* and *P. betle*, cultivated one, *Heckeria subpeltata* Kunth, and seven wild species of *Peperomia* from Tamilnadu. Most of these species have not been cytologically studied and therefore, the present investigation has been undertaken to fill up this gap. The present text deals with the cytological findings of eight species of *Piper* and thirteen species of *Peperomia* and their implications in species evolution.

Materials and methods

The wild species of *Piper* were collected from Ooty and Kodaikanal of Western Ghats and Tropical Plants Research Station, Trivandrum. *Piper betle* is locally cultivated. The cultivated species of *Peperomia* studied were obtained from Lalbagh Garden, Bangalore, Government Botanic Garden, Pondicherry and Botanic Garden, Annamalai University. *P. pellucida* is a locally available wild species.

For comparison of karyotypes of all the species, root tips were pretreated in saturated solution of 0.02% hydroxyquinoline kept at 4°C for 3 hours. Fixative used was acetic ethanol (1:3) for 3 hours. Root tip squashes were made following the schedule of iron alum haematoxylin squash technique described by Marimuthu and Subramaniam (1960). For meiotic study, flower buds were fixed in acetic ethanol (1:3) and staining was done with 1% aceto orcein. Camera lucida drawing was done at a magnification of $\times 1250$ and some of the mitotic stages were photographed.

Observations

In *Piper wightii* there are 24 bivalents in most of the pollen mother cells (Fig. 1), but there are rarely cells with 28 bivalents at metaphase I (Fig. 2). In *P. schmidtii* there is persistent nucleolus (Fig. 3). Instead of the usual occurrences of 49 bivalents, 32 bivalents and 11 bivalents have been also rarely observed in this species (Figs. 4 and 5). Anaphasic bridges have also been rarely observed in this species (Fig. 6). In *P. longum*, 30 bivalents have been observed in most of the pollen mother cells (Fig. 10) and rarely 12 bivalents noted in this species (Fig. 11). In *P. betle* per-

sistent nucleolus has been observed upto metaphase I. In this species there are cells (21%) with various combinations of multivalents and quadrivalents together with bivalents (Figs. 13 to 15). In *Peperomia pellucida*, 22 bivalents have been observed in most of the cells (Fig. 18). There are rarely cells with 11 bivalents (Fig. 19) at metaphase I and 34 chromosomes in each at metaphase II. There are rarely cells with anaphasic laggards (Fig. 21). Together with normal occurrences of tetrads having spherical pollens (Fig. 22), polyads have also been formed rarely with tailed pollens (Fig. 23). In *P. scandens* there are 12 bivalents commonly observed in pollen mother cells. Besides, there are rarely cells with one or two quadrivalents together with bivalents (Figs. 25 and 26). There are direct divisions of the nuclei in some of the pollen mother cells into 2, 3 or 7 nuclear bits (Figs. 30 to 32). After the diad formations, the chromatin connections are present between the nuclei of the two cells (Fig. 34). In *P. reflexa* there are rarely cells with various combinations of multivalents and quadrivalents together with bivalents at metaphase I (Figs. 37 to 40). In *P. magnoliaefolia*, 19 bivalents have been observed in most of the pollen mother cells. Besides, there are rarely cells with quadrivalents along with bivalents in various combinations at metaphase I (Figs. 43 and 44). There are unequal anaphases and anaphasic laggards and bridges observed in this species. In *P. obtusifolia* there are rarely ring formations at metaphase I, by the combinations of all bivalents in a circular configuration (Fig. 49). There are also anaphasic bridges at anaphase I and II and telophase I and II (Figs. 50 to 52). Polyads have been also observed rarely in this species (Figs. 53 to 56). The usual haploid chro-

Figs. 1 to 68: Meiosis. 1 and 2, *Piper wightii* prometaphases with $n=24$ and $n=28$ respectively. 3 to 9, *P. schmidtii*. 3 to 5, prometaphases with 48, 32 and 16 bivalents respectively. 6, anaphasic bridge. 7, monad. 8 and 9, tetrads with one or two sterile pollens. 10 and 11, *P. longum*. 10, prometaphase I with 30 bivalents. 11, metaphase I with 15 bivalents. 12 to 17, *P. betle*: 12, prometaphase with 32 bivalents. 13, prometaphase with 16 bivalents. 14 and 15, various combinations of multivalents and quadrivalents along with bivalents. 16 and 17, tetrads with one and three sterile pollens. 18 to 23, *Peperomia pellucida*: 18 and 19, metaphase I with 11 bivalents and 22 bivalents respectively. 20, metaphase II with 34 chromosomes. 21, II anaphasic laggard in one of the cells. 22, tetrad. 23, polyads with tailed pollens. 24 to 36, *P. scandens*. 24, metaphase with 12 bivalents. 25 and 26, presence of quadrivalents together with bivalents. 27, metaphase II. 28 and 29, constricted and unconstricted pollens with two nuclei. 30 and 31, pollens with 2 and 3 nuclei. 32 and 33, triads and polyads. 34, chromatin connections between two nuclear of the PMC. 35 and 36, monads. 37 to 41, *P. reflexa*. 37, metaphase with persistent nucleolus and 20 bivalents. 38 to 41, metaphases with various combinations of quadrivalents and multivalents together with bivalents. 42 to 44, *P. magnoliaefolia*. 42, metaphase with 19 bivalents. 43, metaphase with 15 bivalents. 44, combinations of multivalents and quadrivalents together with bivalents. 45 to 56, *P. obtusifolia*. 45, metaphase with 11 bivalents. 46, associations of trivalents and univalents with bivalents. 47 and 48, irregular anaphases. 49, ring formation by bivalents. 50 to 52, I and II anaphasic bridges. 53 to 56, multinuclei formations. 57 and 58, *P. marmorata*, I metaphases with 16 and 32 bivalents respectively. 59 to 62, *P. argyreia*. 59, metaphase with 10 bivalents. 60 to 62, triad and polyads. 63 to 68, *P. caperata*. 63, metaphase with 12 bivalents and one bivalent attached with nucleolus. 64, metaphase II with 12 chromosomes at each pole. 65, isobilateral tetrad with one sterile pollen. 66, tetrahedral tetrad. 67 and 68, isobilateral tetrads with one two or even three sterile pollen. Figs. 69 to 77: Mitosis. 69, *Piper attenuatum* prometaphase, $2n=36$. 70, *P. longum* metaphase $2n=40$. 71, *P. betle* prometaphase, $2n=64$. 72, *Peperomia pellucida*, $2n=44$. 73, *P. pellucida*, $2n=33$. 74, *P. hederifolia*, $2n=26$. 75, *P. hederifolia* prophase. 76, *P. magnoliaefolia*, $2n=38$. 77, *P. obtusifolia*, $2n=22$.

MEIOSIS

Table 1.

S. no.	Species studied	Haploid no.	Diploid no.	Previous records	Authors and year
1	<i>Piper attenuatum</i>	—	2n=36	—	—
2	<i>P. galeatum</i>	—	2n=40	—	—
3	<i>P. schmidtii</i>	n=48	2n=96	—	—
4	<i>P. longum</i>	n=30	2n=60	2n=24 2n=24, 48, 96	Jjio, 1948 Sharma, A. K. Bhattacharrya, N. K. 1958. 48, 96 Sharma, A. K. Bhattacharrya, N. K. 1958. 52 Mathew, P. M. 1958
5	<i>P. betle</i>	n=32	2n=64	2n=32 64 78	Jonson, D. S. 1910 Janaki Ammal 1945 Sharma, A. K. Bhattacharrya, N. K. 1858 Mathew, P. M. 1958
6	<i>P. wightii</i>	n=24	—	—	—
7	<i>P. brachystachyum</i>	—	2n=132	—	—
8	<i>P. hookeri</i>	—	2n=60	—	—
9	<i>Peperomia pellucida</i>	n=22	2n=44	2n=20, 24, 22 22, 46 44 46	Brown, W. H. 1908 Sobti, Singh 1961 Sharma, A. K. Bhattacharrya, N. K. 1958 Mathew, P. M. 1958 Harvey 1966 Smith J. B. 1966 Sharma, A. K. Bhattacharrya, N. K. 1958
10	<i>P. scandens</i>	n=12	2n=24	2n=60	Blot, 1960
11	<i>P. hederifolia</i>	—	2n=26	2n=24 22	Blot, 1960 Mathew, P. M. 1958
12	<i>P. sandersii</i>	—	2n=24	24	Sugiura, 1936 a, b.
13	<i>P. magnoliaefolia</i>	n=19	2n=38	2n=24	Hauser, 1916
14	<i>P. obtusifolia</i>	n=11	2n=22	2n=22 24	Smith, J. B. 1966 Blot, 1960
15	<i>P. rubella</i>	—	2n=32	—	—
16	<i>P. marmorata</i>	n=16	2n=32	—	—
17	<i>P. verschaffeltii</i>	—	2n=26	2n=24	Abele, 1923
18	<i>P. incana</i>	—	2n=22	2n=22	Abele, 1923, 1924 Smith, J. B. 1966.
19	<i>P. argyreia</i>	n=10	2n=20	2n=22	Sharma, A. K. and Bhattacharrya, N. K. 1958 Smith, J. B. 1966
20	<i>P. reflexa</i>	n=20	2n=40	—	Blot, 1960
21	<i>P. caperata</i>	n=12	—	2n=33	Smith, J. B. 1966.

mosome number is $n=16$ in *P. marmorata* (Fig. 57). Besides, there are rarely tetraploid PMCs with 32 bivalents (Fig. 58). Together with tetrad formations, there are rarely formations of triads and polyads in this species (Figs. 60 to 62). In *P. caperata*, the haploid chromosome number is 12 (Fig. 63). During metaphase, one bivalent has been found attached with nucleolus which is persistent (Fig. 63). The

particulars about the present reports of the somatic and meiotic chromosome numbers and the previous reports, authors and years are furnished in Table 1.

In *Piper longum* there are chromosomal variabilities such as $2n=40$, 48 and 60, but $2n=60$ is the actual diploid chromosome number of this species, as revealed by the present investigation (98%). In *Peperomia pellucida*, there are $2n=44$ chromosomes. Besides, there are rarely cells with $2n=20$ (6%) and $2n=60$ (8%) chro-

mosomes. In *P. scandens* together with common occurrences of $2n=24$ chromosomes (94%), there are rarely cells with $2n=40$ chromosomes (6%). In *P. sandersii* the actual diploid chromosome number is $2n=24$. Besides, there are rarely cells with $2n=22$ chromosomes in this species (5%). In *P. magnoliaefolia*, the diploid chromosome number is $2n=38$. There are also rare occurrences of cells with various somatic chromosome numbers such as 18 (6%), 20 (4%), 22 (5%)

Table 2. Classification of chromosomes

Taxa studies	Long			Medium			Short				Shorter			Shortest
	A	B	C	D	E	F	G	H	I	J	K	L	M	
<i>Piper attenuatum</i>		2			4	2			4				20	
<i>P. galeatum</i>					2			6			10	6	16	
<i>P. schmidtii</i>									8		12	22	22	34
<i>P. longum</i>	2				2	2		6	8	14			24	
<i>P. betle</i>							6		2		26	18	2	10
<i>P. hookeri</i>								4	6		12	18	20	
<i>P. brachystachyum</i>							2		2		32	38	34	24
<i>Peperomia pellucida</i>								2	2		14	16	2	8
<i>P. scandens</i>					2		2	4			4	8	4	
<i>P. hederifolia</i>					2		2	2		4		6	2	
<i>P. sandersii</i>							2	8	2			4	8	
<i>P. magnoliaefolia</i>	2				6	2		6	10	2	2		6	
<i>P. obtusifolia</i>						2		2	2		2	8	6	
<i>P. rubella</i>					2			2	8		4	2	4	
<i>P. marmorata</i>	2				2			10	12				6	
<i>P. verchaffeltii</i>					2	14	2	2	4	4				
<i>P. incana</i>	2				8	4			8					
<i>P. argyreia</i>							2	6		2	2	6	2	

See the text p. 589.

Table 3. Summarised karyomorphological features

Taxa studied	1	2	3	4	5	6	7	8
<i>Piper attenuatum</i>	36	1.0 to 4.0	—	6	6	20	59.4	1.65
<i>P. galeatum</i>	40	1.0 to 3.0	2	16	6	16	61.0	1.53
<i>P. schmidtii</i>	96	0.8 to 2.8	—	12	30	54	117.0	1.22
<i>P. longum</i>	60	1.0 to 4.0	2	8	12	38	116.4	1.94
<i>P. betle</i>	64	0.6 to 2.1	6	28	20	10	87.6	1.36
<i>P. hookeri</i>	60	1.0 to 2.0	—	16	24	20	85.2	1.42
<i>P. brachystachyum</i>	132	0.8 to 2.0	2	32	40	58	152.2	1.15
<i>Peperomia pellucida</i>	44	0.8 to 2.3	—	16	18	10	52.2	1.18
<i>P. scandens</i>	24	1.0 to 3.4	4	8	8	4	45.6	1.90
<i>P. hederifolia</i>	26	1.2 to 3.0	4	2	14	6	51.6	1.98
<i>P. sandersii</i>	24	1.3 to 2.8	2	8	6	8	44.4	1.85
<i>P. magnoliaefolia</i>	38	1.1 to 5.2	2	14	12	10	93.2	2.45
<i>P. obtusifolia</i>	22	1.0 to 3.6	—	4	12	6	34.0	1.54
<i>P. rubella</i>	22	1.2 to 3.2	—	8	10	4	41.6	1.89
<i>P. marmorata</i>	32	1.0 to 4.2	2	12	12	8	70.0	2.18
<i>P. verchaffeltii</i>	26	2.1 to 3.0	—	4	18	4	73.0	2.80
<i>P. incana</i>	22	2.0 to 4.0	2	8	12	—	66.4	3.01
<i>P. argyreia</i>	20	1.2 to 2.4	2	8	6	4	37.4	1.87

The descriptions of various columns of Table 3 are as follows:

1. Chromosome number (2n). 2. Size range in millimicrons. 3. Number of chromosomes with primary centromere and secondary constriction. 4. Number of chromosomes with submedian centromeres. 5. Number of chromosomes with median centromeres. 6. Number of chromosomes with subterminal centromeres. 7. Absolute chromosome length. 8. Average chromosome length.

and 24 (7%). In *P. argyreia*, the actual diploid chromosome number is $2n=20$ (88%). But there are also occurrences of cells with 22 chromosomes (12%).

The number of secondary constricted chromosomes has been found to vary from species to species. The chromosomes of *Piper* species studied are mostly shorter and the shortest and those of species of *Peperomia* are medium and short (Table 2). Table 2 shows the frequency distribution of different types of chromosomes of species of *Piper* and *Peperomia* studied herein and Table 3 shows the summarised karyomorphological features with special reference to chromosome number ($2n$), size range in millimicrons, number of chromosomes with primary centromere and secondary constriction, submedian centromeres, median centromeres and sub-terminal centromeres and absolute and average chromosome lengths.

Fig. 78. Histogram showing the absolute chromosome lengths of the various species of Piperaceae studied herein.

The chromosomes are classified into the following types:

Long (more than $3.9 \mu\text{m}$)

Type A Chromosome with submedian primary centromere and subterminal secondary constrictions.

Type B Chromosome with submedian centromere.

Type C Chromosome with median centromere.

Medium (3.0 to $3.8 \mu\text{m}$)

Type D Chromosome with submedian primary centromere and subterminal secondary constriction.

Type E Chromosome with submedian centromere.

Type F Chromosome with median centromere.

Short (2.0 to $2.9 \mu\text{m}$)

Type G Chromosome with submedian primary centromere and subterminal secondary constriction.

Type H Chromosome with submedian centromere.

Type I	Chromosome with median centromere.
Type J	Chromosome with subterminal centromere. Shorter (1.0 to 1.9 μm)
Type K	Chromosome with submedian centromere.
Type L	Chromosome with median centromere.
Type M	Chromosome with subterminal centromere. Shortest (Less than 1.0 μm)
Type N	Chromosome with subterminal centromere.

Discussion

In 21 species of *Piper* and *Peperomia*, mitotic studies have been made of which, first record of chromosome number has been made in *Piper attenuatum*, *P. galeatum*, *P. schmidtii*, *P. wightii*, *P. brachystachyum*, *P. hookeri*, *Peperomia marmorata* and *P. rubella* (Table 1).

The chromosome numbers occur as multiples of 8, 11, 12, 13 and 14 of which multiples of 11 and 12 are of the most frequent. *Piper* contains multiples of 12 and *Peperomia* multiples of 11 in most of the species studied. According to Mathew (1958) and Sharma and Bhattacharrya (1958), 11 may be the basic number for *Peperomia*. On the other hand, there may be more than one basic number in this genus according to Smith (1966). From this primary basic number 11, secondary basic numbers of the genus can evolve. The basic numbers 12 and 14 may arise by additions and 8 by loss of certain chromosomes through aneuploidy. Such reduction of chromosome number from the original diploid number has been explained in *Crepis* by Togby (1943), Babcock (1937) and Sherman (1946), in Cichorieae by Stebbins *et al.* (1953). By means of unequal segmental interchange, *Crepis neglecta* ($n=4$) gave rise to *C. fuliginosa* ($n=3$) (Togby 1943, Babcock 1937). Similarly, *C. foetida* ($n=5$) should have given rise to *C. kotschyana* ($n=4$) (Sherman 1946). Some such processes might have been in operation in the evolutionary derivation of the 8 basic numbered species of *Peperomia* from the 11 basic numbered species of *Peperomia*.

P. pellucida is a tetraploid with 44 chromosomes and *P. obtusifolia*, *P. rubella* and *P. incana* are diploids with 22 chromosomes. Other species of *Peperomia* studied are aneuploids. Therefore more frequently aneuploidy and rarely polyploidy play important roles in speciation in *Peperomia*. The rare occurrences of aneuploid and euploid cells along with diploid cells in some of the species like *P. pellucida*, and *P. marmorata* provide further evidences for the above conclusion.

Sharma and Bhattacharrya (1958) suggested $n=12$ as the basic number in *Piper*. Mathew (1958) and Smith (1966), on the contrary recorded $n=13$ as the basic number for the genus. Based on the chromosome numbers of the various species, Das Gupta and Datta (1976) explained that nearly 47.3% of the species of *Piper* have $n=12$ and only 19.04% have $n=13$ and concluded that the actual diploid chromosome number of *Piper* is 12 and other basic numbers would have evolved from it.

The present study reveals that *Piper attenuatum*, *P. longum*, *P. schmidtii*, *P.*

wightii, *P. brachystachyum* and *P. hookeri* are higher polyploids, having the chromosome numbers in multiples of 12 (Table 1), whereas *P. galeatum* and *P. betle* are higher polyploids, not having the chromosome numbers in multiples of 12. Therefore, the former species are euploids and the latter aneuploids. It is very interesting to note that no diploid species with 24 chromosomes are available among the species of *Piper* studied.

Eventhough the basic chromosome number of *Peperomia* has been considered to be 11, only 3 species namely *P. obtusifolia*, *P. rubella* and *P. incana* have such numbers. The other species have a range of chromosome numbers from 24 to 40. *P. pellucida*, on the other hand has 44 somatic chromosomes. Besides, there are also chromosomal biotypes in this species having 20, 22, 24, 44 and 46 chromosomes respectively (Sharma and Bhattacharrya 1958, Mathew 1958, Smith 1966). Therefore, this species is a highly variable one completely differing from other species of *Peperomia* studied. Numerical changes in chromosomes may arise from aneusomatic cells (Sharma 1956, Sharma and Datta 1960) or mitotic aneuploidy or parthenogenesis. Excepting this species, all the other species of *Peperomia* studied are aneuploids with an addition of one or a few haploid chromosome numbers with the original haploid number, that is 11.

In general the species of *Piper* are characterised by more number of chromosomes and more absolute chromosome length and those of *Peperomia* by lower number of chromosomes and lesser absolute chromosome length (Table 2).

Anapahsic bridges and laggards have been observed in the meiosis of *Piper schmidtii*, *Peperomia pellucida*, *P. scandens*, *P. magnoliaefolia* and *P. obtusifolia*. It is an established fact that the bridges and fragments at anaphase I and II are indicative of inversion heterozygosity (Upcott 1936). The frequency of bridge formation in any species depends on three factors namely chiasma frequency, number of inversions and size of inversions. Out of 12 species meiotically studied here, bridges and laggards have been observed in 5 species. This shows that inversion heterozygosity may be, to some extent, responsible for the origin and evolution of species, particularly in *Peperomia*. The inversion heterozygosity, is known to cause univalency in various species of *Citrus* (Raghuvanshi 1962a and b), as the presence of inversion prevents the regular pairing and chiasma formation. The univalents formed are lagged behind as anaphasic laggards.

In most of the species of *Peperomia* studied multivalents and quadrivalents occur along with bivalents during metaphase. The formations of multivalents and quadrivalents may be attributed to polyploidy or structural alteration in the chromosomes. *Peperomia* is mostly an aneuploid. Therefore, the presence of multivalents and quadrivalents in the species of *Peperomia* may be due to the aneuploid nature of the species.

Summary

Cytotaxonomical investigation has been made in 21 species of *Piper* and *Peperomia*, of which first records of chromosome numbers have been made in *Piper attenuatum*, *P. galeatum*, *P. schmidtii*, *P. wightii*, *P. brachystachum*, *P. hookeri*, *Peper-*

omia marmorata and *P. rubella*.

11 is the basic number for *Peperomia* and 12 for *Piper* as evidenced by the present investigations. The species of *Piper* are higher polyploids and aneuploids and the species of *Peperomia* are mostly aneuploids. The karyotype analyses show that both genera are characterised by the presence of mostly submetacentric and telocentric chromosomes showing thereby that the two genera are highly evolved but in different lines, eventhough, they might have been originated from a common ancestor.

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