Indian J.Sci.Res. 7(2): 223-227, 2017 ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

CYTOLOGICAL STUDIES OF SOME SPECIES OF *Cheilanthoid ferns* COLLECTED FROM INDO-NEPAL BORDER ADJACENT TO BIHAR

SUSHMA[a1] AND R.B. SRIVASTVA[b]

[ab]Department of Botany, M.S. College, Motihari, B.R. Ambedkar Bihar University, Muzaffarpur, Bihar, India

ABSTRACT

Cytological studies of four species of *Cheilanthes* that were, *Cheilanthes tenuifolia*, *Cheilanthes albomarginata*, *Cheilanthes farinosa* and *Cheilanthes argentea*, collected from Indo-Nepal border adjacent to Bihar state were performed. The mitotic chromosomes were measured and chromosomes with double constriction and single constrictions were noted. In case of *C. tenuifolia*, chromosomes having double or single constriction were noted. The number of double constrictions was 1+5, while single constriction containing chromosomes were 54 pairs. Similarly, the length of double constriction containing chromosomes ranged between 3.2 µM to 4.7µM, while single constriction containing chromosome length ranged between 2.8 µM to 4.7µM. In case of *C. albomarginata* only chromosomes with single constrictions were noted and the length ranged between 3.0µM to 7.1µM. In case of *C. farinosa* both double and single constrictions were found and the length ranged between 3.1µM to 5.5µM. In the case of *C. argentea* also chromosomes having single constrictions and double constrictions were found. Here the length of chromosomes ranged between 3.1µM to 5.9µM. In meiotic cell divisions in *C. tenuifolia* the mean of rod bivalents was 18.5, mean of ring bivalents was 11.5, total bivalents were 30, and mean of chiasma = 41.5. In case of *C. albomarginata*, the mean of rod bivalents was 18, ring bivalents 12.5. Total bivalents = 30.5, mean of chiasma 44.5, in the case of *C. farinosa*, mean of rod bivalents = 18.5, mean of ring bivalents = 11.5, total bivalents = 30, chiasma = 41.5, in the case of *C. aregentea*, mean of rod bivalents = 70, ring bivalents = 11.0, total bivalents = 81, mean of chiasma = 45.

KEYWORDS: *Cheilantehs*, Double Constriction, Mitotic Chromosomes, Rod Bivalent, Ring Bivalent, Chiasma

Among the pteridophytes, ferns are much more predominant, considering the richness of genera and species in a wide range of distribution. More than 12000 species of pteridophytes are estimated to be distributed in various regions of India (Chandra, 2000). The increasing number of chromosomal data on the ferns has played a significant role in the identity and phylogenetic affinities of several species and genera of ferns (Jin Mei *et al*; 2006). The cytological changes like hybridity, polyploidy and aneuploidy have played an important role in evolution (Bhavanandan, 1981). Evolution in many ferns involves changes in ploidy level and reproductive system (Manton, 1950). The grade of poluyploidy and the percentage of polyploids are indicators of the rate of evolution of ferns and these considerations suggest that the rate of evolution is faster in the tropics than the temperate latitude (Manton, 1953). Cytological studies of different ferns growing in different regions of India have been done by different workers like, Manton (1953), Mehra and Singh (1957); Mehra and Bir (1958); Mehra and Khare (1959); Mehra and Neema (1960); Verma & Loyl (1960); Roy and Sinha (1961); Pal and Pal (1961); Roy and Pandey (1963); Ghatak, J. (1964); Bir (1965); Verma and Khullar (1965); Mehra and Loyl (1965); Sinha and Pandey (1969); Bir (1971); Roy *et al*; (1971); Roy and Singh (1975); Khare and Roy (1977); Khuller and Gupta (1978); Khare (1980); Bhavanandan (1981); Srivastva (1984); Gibby (1985); Thakur and Srivastva (1985); Khare and Kaur (1987); Singh and Roy (1988); Ammal and Bhavanandan (1990); Ammal and Bhavanandan (1991a); Ammal and Bhavanandan (1991b); Ammal and Bhavanandan (1991c). Bir and Irudaya raj (2001). All of them have studied the mitotic and meiotic chromosomes in different species of ferns collected from South India to North India, respectively. Keeping these ideas in mind the present was conducted to observe the mitotic and meiotic chromosomes in four species of *Cheilanthes*.

MATERIALS AND METHODS

Roots were collected from the site where new *Cheilanthes* plants were growing during rainy seasons. After washing carefully, these roots were fixed in Caronoy's fluid (Absolute alcohol + chloroform+ glacial acetic acid in the ratio of 6:3:1). Within a week these root tips were used for cytological studies.

Young sori were collected early in the morning between 6.30 and 7:30 AM and fixed Carnoy's fluid. Meiotic studies were based on the usual aceto carmine squash method. (Manton1950). The chromosome count was made in the spore mother cell at first meiotic division. The chromosome numbers were verified and confirmed with the help of previous flora, including "cytology of ferns of the western ghats, South India" (Manickam and Irudraj, 1988). In the case of meiotic study fixation of young sporangia for 6-8 hours gave

---

[1]Corresponding Author

better result. After squashing properly the cover slip on the slide was sealed with paraffin wax, cells were focused and microscopic field, first 10S, 40X, then 10X100X, 15X100X. Here oil drops were poured for better resolutions. For the mitotic chromosomes, primary and secondary constrictions were noted. These chromosomes were measured with help ocular micrometer. The microscopic constant was calculated with the help of Ocular micrometer. The microscopic constant was calculated with the help of stage micrometer. In this value of one division of ocular was calculated. Based on this mitotic chromosome were measures. All the experiments were done in triplicate and the data was utilized for discussion.

Similarly, for meiotic chromosome number of rod bivalents, ring bivalents total bivalents and the chiasma frequency was determined. The mean of the data was placed in table-1.

RESULTS AND DISCUSSION

Mitotic chromosomes in the slide prepared form the root tips of all the four species of *Cheilanthes* were studied. The features of the chromosome of all the four species such as, C. *tenuifolia*(Burn f)., *C. albomarginata* (clarke), *C. farinosa* (Forsk)kauf, *C. argentea* (Gmel) kunze have been described below.

***C. tenuifolia*(Burn f).**

Chromosome No. 2n = 120

Length varies – 2.7 – 4.7µM

Features of chromosome

Constriction	No. of Pairs	Range of length µM
Double		
Primary median	1	4.0
Primary Sub median	5	3.2-4.7
Primary Sub terminal	-	-
Single		
Median	26	2.8-4.7
Sub Median	18	2.8-4.0
Sub terminal	10	3.5-4.5

This species is abundantly distributed in foot hills and plains. Fertile period is between July-October. In somatic cells 2n = 120 chromosomes. The length varies between 2.7-4.7µM. Details of chromosomes have been described above.

Manton and Sledge (1954) reported a tetraploid species with n = 56 from Ceylon. Mehra and Verma (1961) reported an apogamous diploid form with n = 56, in the specimen collected from Darjeeling. Roy and Pandey (1963) observed n = 60 in specimen collected from Parasnath hills. Pandey and Srivastva (1978) also observed n = 60. In the present study 60 bivalents at late diakinesis in spore mother cell has been observed. Somatic studies reveals 2n = 120, the length of the chromosome varies between 2.7 – 4.7µM. Six pairs of chromosomes with double constrictions have been found.

C. *albomarginata* (clarke)

Root tip squash shoots 2n = 60. Roy and Pandey (963) also recorded 2n = 60 in the specimen collected from Parasnath Hills. The chromosome length varies 2.7-4.7µM. The centromeres were distinct. Chromosome with secondary constrictions could not be traced. The details of somatic observations are as follows:

Chromosomes:

Constrictions	No. of pairs	Range of length in µM
Single		
Median	17	3.0 – 6.5
Sub median	8	4.1-7.1
Sub terminal	5	3.5-6.5

Mehra and Verma (1961) reported n = 30, among the specimen collected form Darjeeling and Masoorie Hills. Roy and Pandey (1963) observed 30 bivalents at the late diakinesis in the materials collected form Parasnath Hills. Pandey and Srivastva (1973) observed 30 bivalents at late diakinesis in the specimen from Indo-Nepal border zone. Present study is in conformity with previous findings.

C. farinosa (Forsk) kaulf

Here also root tip squash revealed 2n = 60. 10 pairs of chromosomes had both primary and secondary constriction and rest possess only primary constriction. The details of somatic observations are as follows:

Constriction	No. of Pairs	Range of length µM
Double		
Primary median	1	4.3
Sub median	9	3.5-5.5
Sub terminal	-	-
Single		
Median	5	3.1-5.1
Sub Median	15	3.2-5.5
Sub terminal	-	-

The length of the chromosomes ranged between 3.2 – 5.5µM. Irene Manton (1959) had reported a tetrapoloid fern with n = 60 and apogamous triploid fern with n = 2n = 90. Verma and Loyal (1960) reported a diploid with n =29. Above finding was confirmed by Mehra and Verma (1961). Roy and Sakya (1963) have reported 2n = 60 in specimen from, Nepal. Roy and Pandey (1963) observed n = 30, among the specimen collected form Parasnath Hills. Pandey and Srivastva (1978), observed 30 bivalents at late diakinesis.

In the present study 30 bivalents at late diakinesis have been observed in each spore mother cell. Pairing of chromosomes was quite regular. All stages were normal.

C. *argentea* (Gmel) kunze

Root tip squash of the above species shown 2n = 87. Chromosomes revealed both primary and secondary constrictions. The details of somatic chromosomes are as follows:

2n = 87 with irregular meiotic cell division.

Constriction	No. of Pairs	Range of length µM
Double		
Primary median	5	3.5
Sub median	15	3.5-5.6
Sub terminal	-	-
Single		
Median	10	3.1-5.9
Sub Median	14	3.2-5.8
Sub terminal	-	-

The present study reveals n =30 at late diakinesis. Meiotic divisions irregular.

MEIOTIC STUDIES

Slides prepared form the sporangium of young stage were studied for, rod bivalents, ring bivalents, tetrad bivalents, total chiasmata and half chiasmata per chromosome. The data were presented in table-1.

From the table, it was noted that in C. farinosa, the rod bivalents ranged between 16-21 and the mean was 18.5, where as the rod bivalents ranged between 9-14 and the mean was 11.50. Thus total bivalents were 301. Similarly, the chiasma ranged between 39-44 and the mean was 41.50, while ½ Chiasma was 0.69 per chromosome. In case of *C. tanuifolia*, the rod bivalents ranged between 35-45 and the mean was 40.0. While the ring bivalents ranged between 15-25 and the mean were 20.0. Here total bivalents were 60.0. The chiasma ranged between 78-86 and mean was 82.0. While half chiasmata per chromosome was 0.68. In case of C. *albomarginata* the rod bivalents ranged between 15-21 and the mean was 18.0. The ring bivalents ranged between 10-15 and the mean value was 12.5. Therefore, total bivalents were 30.5. The chiasma ranged between 42-47 and the mean was 44.5, while half chiasmata per chromosome were 0.44. In case of *C. aregentea* rod bivalents ranged between 50-90 and the mean was 70.0. The ring bivalents ranged between 8-14 and the mean was 11.0. So the total bivalents were 81.0. The chiasma ranged between 42-48 and mean was 45, while the half chiasma per chromosome was 0.68.

Table 1: Chromosome pairing and chiasma frequency in species of *Cheilanthes*

Species	No. of Cells	Rod bivalent		Ring bivalent		Total bivalent	Total chiasmata			½ chiasmata per chromosome
		R	M	R	M		R	M	SE	
C. farinosa	10	16-21	18.50	9-14	11.50	30.0	39-44	41.50	0.69	0.69
C. tenuifolia	10	35-45	40.0	15.25	20.0	60.0	78-86	82.0	0.79	0.68
C. *albomarginata*	10	15.21	18.0	10.15	12.5	30.5	42-47	44.5	0.44	0.44
C. aregentea	10	50-90	70.0	8.14	11.0	81.0	42-48	45.0	0.68	0.68
R = Range, M = Mean, SE= Standard Error										

DISCUSSION

Here we got discrepancy in the rod and ring bivalents and total chiasma. This may be correlated with the length and chromosome numbers. Roy and Pandey (1963), Srivastva (1984) have reported that in *C. farinosa*, *C. tenuifolia*, *C. albomarginata* the 2n=60, While *C. aregentea* 2n=3X=81. In addition, other workers have reported the chromosomal numbers and meiotic behaviour of the cells. Reported related with the cytological studies of ferns have been presented by Thakur and Srivastva (1985), Bhavanandan KV (1981); Ammal and Bhavanandan (1991); Ammal and

Bhavanandan (1991c). Present findings are in agreement with the findings of the above workers. Crossing over causes formation of rod bivalents or ring bivalents which are formed due to single or double cross over.

CONCLUSION

Cheilanthes species revealed different chromosomal structural and behavioural features. We get variations in mitotic as well as in meiotic chromosomes. All these are related with evolutionary and ecological adaptations. The chromosome number, size in mitotic and meiotic counts are important parameters in determining cytological evolution. The karyological analysis is a basic factor which is considered useful in fern, classification on cytological basis. Full reliable information concerning ancestral base number may provide clues to the affinities existing between families and their classification into higher categories. In this way the present findings may bad to the existing knowledge of the cytotaxonomists.

ACKNOWLEDGEMENT

The authors are grateful to the Principal, M.S. College, Motihari, B.R. Ambedkar Bihar University, Muzaffarpur, for providing Laboratory and Library facilities for this work.

REFERENCES

Ammal L.S. and Bhavanandan K.V., 1991a. Cytological studies of some members of Polypodiaceae. Indian Fern J., **8**: 154-158.

Ammal L.S. and Bhavanandan K.V., 1991b. Cytological studies on the genus *Asplenium* Linn. Indian Fern J., **8**: 69-73.

Ammal L.S. and Bhavanandan K.V., 1991c. Cytological studies on some members of *Pteridaceae*, from South India. Indian Fern J., **8**: 87-92.

Ammal L.S. and Bhavanandan K.V., 1990. A note on the cytology of a tetraploid cytotype of *Diplagium* polyploids from Idukhi (Kerala), South India. Indian Fern. J., **7**: 131-132.

Bhavanandan K.V., 1971. Supernumerary cell division during meiosis in *Rumohra artista*. Cytologia, **36**: 575-578.

Bhavanandan K.V., 1981. Studies on the cytology of South Indian *Aspidiaceae*, Cytologia, **46**: 195-207.

Bir S.S., 1965. Chromosome numbers in some ferns from Kodaikanal, South India. Cytologia, **18**: 107-115.

Bir S.S., 1971. Cytological studies in Himalayan species of *Diplazium swartz*. Caryologia, **24**: 269-281.

Bir S.S. and Irudayaraj, 2001. Cytology of some ferns from the Nilgiris. South India. Fern Gazette, **16**: 177-190.

Chandra S., 2000. The ferns of India (Enumeration, Synonyms & distribution). Dehradun, India. International Book Distributors, pp. 459.

Ghatak J., 1964. A preliminary note on the peculiar chromosome pairing in two species of finely ferns from South India. Nucleus, **7**: 29-36.

Gibby M., 1985. Cytological observations on Indian subcontinent and Chinese *Dryopteris* and *Polystichum*. Bull. Br. Bot. Ser., **14**: 1-42.

Jin-Mei L., De-Zhu L. and Ding W.U., 2006. Chromosome numbers of four genera in the *Dryopteridaceae*. Acta Phytotaxonomica Sinica, **44**: 516-522.

Khare P.B. and Kaur S., 1987. Genetic load in tetraploid cytotype of *Pteris vittata* L. Indian Fern J., **4**: 82-85.

Khare P.B. and Roy S.K., 1977. Genetic aspect of capacity of colonization of *Cheilanthes tenuifolia* (Burm). Genetica, **47**: 183-185.

Khare P.B., 1980. A note on the chromosome number of the fern flora of Amarkantak Hills. Sci. Cult., **46**: 138-139.

Khuller S.P. and Gupta S.C., 1978. Cytotaxonomy of the genus *Polystichum* in the western Himalayas. Plant Syst. Evo., **129**: 269-275.

Manickam V.S. and Irudayaraj V., 1988. Cytology of ferns of Western ghats, South India, New Delhi. Today and Tomorrow's Printers and Publishers, pp-81.

Manton I., 1953. The cytological evolution of the fern flora of Ceylon. Evolution, **7**: 174-185.

Manton I., 1950. Problems of cytology and evolution in the Pteridophyta *Cambridge*. Pp. 316 (Cross. Ref.).

Mehra P.N. and Singh G., 1957. Cytology of *Hymenophyllaceae*. J. Genet., **55**: 379-393.

Mehra P.N. and Khanna K.K., 1959. Cytology of some Himalayan ferns. J. Genet., **56**: 1-14.

Mehra P.N. and Loyal D.S., 1965. Cytological investigation in the Himalayan *Dryopteris* a*danson*. Caryologia, **18**: 461-498.

Mehra P.N. and Bir S.S., 1958. Cytology of some *Blechnoid* ferns together with the note on the affinities of *Stenochlaena palustris*. Proc. Nat. Acad. Sci. India Plant, pp. 47-58.

Mehra P.N. and Verma S.C., 1960. Cytotaxonomic observations on some rest Himalayan *Pteridaceae*. Caryologia, **13**: 619-650.

Pal S. and Pal N., 1961. Studies on cytology of two species of *Phymatodes*. Cytology, **26**: 460-467.

Roy R.P. and Singh B.M.B., 1961. Meiotic studies in the genus *Adiantum*. Caryologia, **14**: 413-428.

Roy R.P. and Singh J.B., 1973. Cytology and ecological notes on the ferns of Panchmarhi. Plant Sci., **5**: 1-16.

Roy R.P., Singh B.M.B. and Sakya A.R., 1971. Cytology of some ferns of Kathmandu Valley. British Fern Gazett., **10**: 193-199.

Roy R.P. and Singh J.B., 1975. A note on the chromosome numbers in some ferns from Panchmarhi Hills. Sci. Cult., **41**: 181-183.

Roy R.P. and Pandey S.N., 1963. Cytotaxonomy of ferns of Nepal. Ph.D. Thesis, Patna University.

Roy R.P. and Pandey S.N., 1963. Cytotaxonomy of ferns of Parasnath Hills. Ph.D. Thesis, Patna University.

Singh V.P. and Roy S.K., 1988. Cytology of forty four species of ferns form Sikkim, Himalaya. Indian Fern. J., **5**: 162-169.

Sinha B.M.B. and Pandey S.N., 1969. Cytotaxonomic studies in some epiphytic ferns of Parasnath Hills. J. Cytol. Genet., **4**: 97-104.

Srivastva R.B., 1984. Observation on ferns of aquatic environment from Champaran. In Symp. Vol. towards understanding the biology of Pteridophytes. Ed. S.C. Verma, PP- 33-34.

Thakur S. and Srivastva R.B., 1985. Cytotaxonomical investigation of ferns of Valmiki Nagar, West Champaran. Ph.D. Thesis, Bihar University, Muzaffarpur.

Verma S.C. and Khullar S.P., 1965. Cytotaxonomy and cytogenetics of *Onychium contiguum* complex in W. Himalaya. Caryologia, **18**: 555-556.

Verma S.C. and Loyal D.S., 1960a. Chromosome counts in some ferns from Nainital. Curr. Sci., **29**: 69-70.