J. CYTOL. GENET. VOL. **20** (NS): 29–35 (2019)

RESEARCH ARTICLE

MEIOSIS AND CHIASMA DISTRIBUTION IN *SESBANIA ROSTRATA* BREMEK. & OBERM.

V. RATTAN KUMAR, P. B. S. YADAV* AND M. V. SUBBA RAO
Department of Botany, Andhra University, Visakhapatnam 530 003, India
*For correspondence. Email: dr.pbsyadav@gmail.com

(Received 29 March 2019, revised accepted 24 April 2019)

SUMMARY

In *Sesbania rostrata*, formation of six bivalents in each pollen mother cell was a regular feature at diakinesis and metaphase I. The first, second and the sixth chromosomal pairs could be identified individually during metaphase I. This natural facility has been used in the analysis of interchromosomal distribution of chiasmata in this species. From the analysis it is concluded that chiasma formation in *S. rostrata* is highly regulated and is non-random. This resulted in the bivalents forming chiasmata disproportionate to their lengths.

Keywords: *Sesbania rostrata*, meiosis, chiasma distribution, interchromosomal effects.

INTRODUCTION

The genus *Sesbania* Scop. (Leguminosae), consists of 70 species (Allen & Allen 1981). In South India and Eastern Ghats, 13 nodulated species are available (Pullaiah & Sri Ramamurthy 2001). Of several species of this genus, *S. rostrata* is unique in that it possesses nitrogen-fixing nodules on its stem. In India, it is exotic and cultivated species (Pullaiah & Sri Ramamurthy 2001). It is one of the three angiospermous genera possessing aerial nodules. The other two are, *Aeschenomene* and *Neptunia.* The amount of nitrogen fixed by *S. rostrata* has been estimated to be as high as 250 kg/hectare in a span of 52 d (Joshua et al. 1989). Because of this desirable feature, *S. rostrata* though originally endemic to West Africa, has gained importance as a green manure crop.

Somatic chromosome number in *S. rostrata* was reported to be 12 (Le Coq et al 1985, Joshua & Bhatia 1987). A detailed study of the karyotype revealed it to be asymmetric with marked differences in chromosome lengths and with prominent heterochromatic regions in some of the chromosomes (Radha et al. 1998). This enabled a clear-cut differentiation of the first, second and the sixth pairs (bivalents) even at metaphase I (M I) Hence, a detailed meiotic analysis of the interchromosomal effects on the distribution of chiasmata has been undertaken in *S. rostrata* and the results are presented here.

MATERIAL AND METHODS

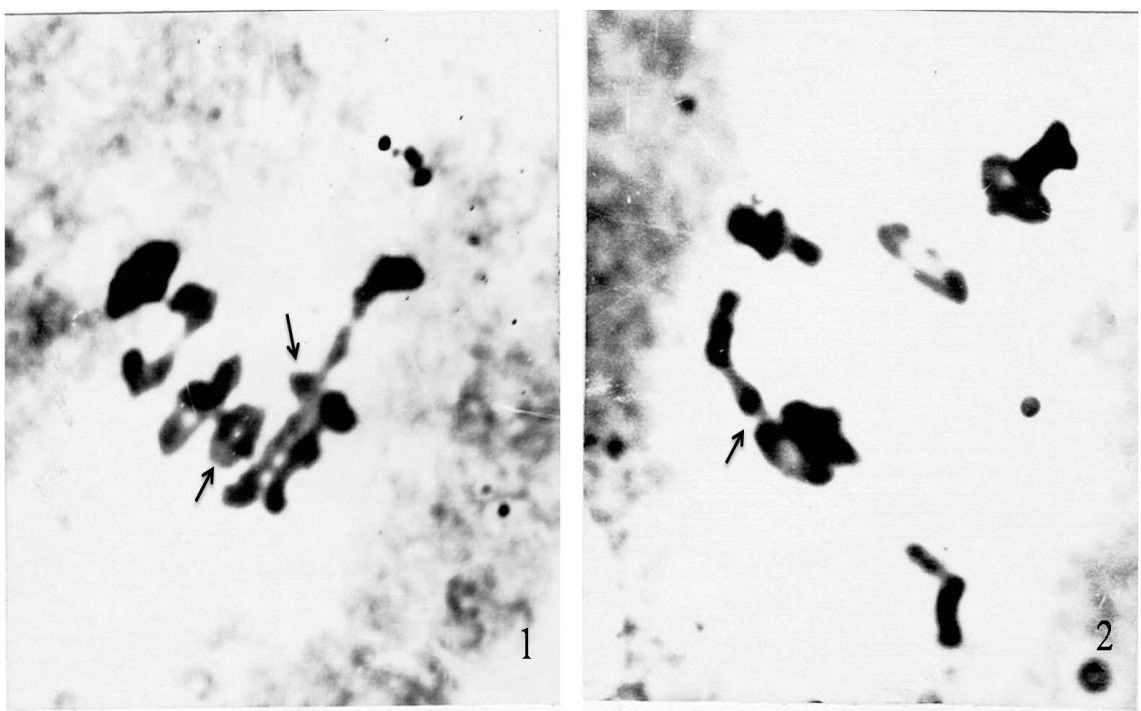
Flower buds were fixed in acetic-alcohol (1:3) and anthers were squashed in 2% acetocarmine. Data were gathered from cells at M I on the number of chiasmata in each of the four groups of bivalents from a minimum of 100 pollen mother cells

(PMCs) in each of the five plants (SR I to SR V).

The analysis of variance as suggested by Mather (1936) has been used. The variance was partitioned into three components: i) between groups of bivalents, ii) between nuclei or internuclear and iii) the inherent variance or mean square ascribable to the deviations of the observed values from the expected ones. This third component represents the variance of the bivalents after allowance has been made for differences between the groups and the nuclei.

OBSERVATIONS

At pachytene, each PMC showed the presence of conspicuous heterochromatic segments in some of the bivalents. The latter appeared to be less condensed and showed a high degree of clumping at diakinesis, thus rendering pachytene, diplotene and early diakinesis stages less amenable for detailed analysis. Metaphase I of meiotic division was the only stage where it was possible to observe chiasma number and positions clearly in the bivalents (Figs 1, 2). Hence, the present observations are mainly confined to cells at M I only. Based on the differences in the sizes of the bivalents together with the heterochromatic segments present in some of the chromosomal pairs, the first, second and sixth could be differentiated from one another and also from the other three pairs (third, fourth and fifth) which could not be differentiated from one another thus enabling the categorization of the total complement into four groups.



Figs 1 & 2: *S. rostrata*. 1. PMC at M I showing longest rod bivalent (arrow) and the smallest ring bivalent (arrow). 2. PMC at M I showing extra chiasma (arrow) in the large ring bivalent.

Data on the frequencies of types of bivalents at metaphase I and on overall pattern of chiasma distribution in the four chromosomal groups along with mean number of chiasmata per nucleus are presented in Tables 1 and 2. The main characteristics of the four groups of bivalents are:

TABLE 1: Frequency of bivalent configurations in the four chromosomal groups at metaphase I in *S. rostrata.*

Plant No.	Group I pair		Group II pair		Group III pair		Group IV pair		Total No. of cells observed	Total No. of ring bivalents	Total No. of rod bivalents
	Ring bivalent	Rod bivalent	Ring bivalent	Rod bivalent	Ring bivalent	Rod bivalent	Ring bivalent	Rod bivalent			
SR I	2	102 (98.08%)	100 (96.15%)	4	101 (97.12%)	3	253 (81.09%)	59	104	456 (73.08%)	168
SR II	0	105 (100%)	101 (96.19%)	4	101 (96.19%)	4	261 (82.86%)	54	105	463 (73.49%)	167
SR III	1	99 (99%)	95 (95%)	5	96 (96%)	4	246 (82%)	54	100	438 (73%)	162
SR IV	0	103 (100%)	99 (96.12%)	4	102 (99.03%)	1	263 (85.11%)	46	103	464 (75.08%)	154
SR V	1	127 (99.22%)	124 (96.88%)	4	125 (97.66%)	3	316 (82.29%)	68	128	566 (73.70%)	202

TABLE 2: Distribution of chiasmata at metaphase I in PMCs of *S. rostrata.*

Plant No.	Chromosomal groups with number of chiasmata																	Total xta	No. of cells observed	Mean xta/cell
	Group I			Total	Group II			Total	Group III			Total	Group IV*							
	1	2	3	xta	1	2	3	xta	1	2	3	xta	3	4	5	6	7			
SR I	99	5	-	109	4	96	4	208	3	100	1	206	--	6	39	50	9	582	104	10.6250 ± 0.0334
SR II	103	2	--	107	4	87	14	220	4	99	2	208	--	6	41	55	3	580	105	10.6190 ± 0.0313
SR III	96	4	--	104	5	86	9	204	3	96	1	198	--	7	36	55	2	552	100	10.5800 ± 0.0344
SR IV	100	3	--	106	4	83	16	218	1	94	8	213	--	7	28	58	10	586	103	10.9029 ± 0.0309
SR V	118	19	--	138	4	105	19	271	3	115	10	263	1	3	45	74	5	719	128	10.8672 ± 0.0249

*The numbers 3 to 7 represent the total number of chiasmata over 3 bivalents in this group.

TABLE 3: Analysis of variance for chiasma distribution (per cell) in five plants of *S. rostrata*.

Source of variation	Df	SS	MSS	F*	P
		SR I			
Between groups	3	1260.08	420.02	1.27	P > 0.05
Internuclear	103	18.59	0.18		
Intranuclear (Inherent)	304	71.16	0.23		
Total	410	1349.83	420.43		
		SR II			
Between groups	3	1225.77	408.59		
Internuclear	104	13.69	0.13	1.35*	P < 0.05
Intranuclear (Inherent)	312	55.47	0.17		
Total	419	1294.93	408.89		
		SR III			
Between groups	3	1164.99	388.33		
Internuclear	99	15.59	0.15	1.04	P > 0.05
Intranuclear (Inherent)	297	49.01	0.16		
Total	399	1229.59	388.64		
		SR IV			
Between groups	3	1283.91	427.97		
Internuclear	102	19.25	0.18	1.15	P > 0.05
Intranuclear (Inherent)	306	66.83	0.21		
Total	411	1369.99	428.36		
		SR V			
Between groups	3	1522.61	507.53		
Internuclear	127	20.68	0.16	1.25*	P < 0.05
Intranuclear (Inherent)	381	74.63	0.19		
Total	511	1616.92	507.88		

*Significant (P < 0.05), Df - Degree of freedom, SS - Sum of sqares, MSS - Mean sum of squares, F - Statistic, P - P-value.

Group I: It is represented by the longest chromosomal pair characterized by the presence of a lump of heterochromatin at one end. This pair remained as a rod or open bivalent with a single chiasma in all the cells in SR II and SR IV.

Group II: The second longest pair was observed to form a ring bivalent in about 96% of the nuclei and the number of chiasmata formed in this pair varied from 1to 3.

Group III: It is constituted by the shortest chromosomal pair (sixth chromosome) which formed a ring bivalent in about 99 % of the cells (SR IV).

Group IV: It is constituted by the remaining there chromosomal pairs (third, fourth and fifth) the percentage of ring bivalents in this group varied from 81 (SR I) to 85 (SR IV, Table 1). The total number of chiasmata in this group varied from 3 to 7 (Table 2).

Among the five plants studied, SR IV, in general, showed high frequency of ring bivalents in the four groups. The bivalents formed by the second as well as the sixth chromosomal pairs showed at least one extra chiasma which was observed to be unterminalized even at M I (Fig. 2). The mean chiasma frequencies per nucleus varied from 10.58 as in SR III to 10.90 in SR IV. In order to test the variation in the interchromosomal effects, the data on mean chiasma frequencies per nucleus in the four groups in the five plants was subjected to Analysis of Variance (ANOVA) (Mather 1936) and the results are presented in Table 3. The ANOVA revealed a greater proportion of intranuclear variation between groups over the internuclear component in the five plants, indicating the negatively correlated chiasma distribution. However, the five plants, exhibited variation in the magnitude of negative correlation. For example, the negative correlation coefficients in SR II and SR V were significant ($P < 0.05$), while they were not significant ($P > 0.05$) in the other three plants. At anaphase I, in general, the distribution of chromosomes to the poles was highly regular (6:6) in all the plants except for a very low frequency of 5:7 segregation observed in SR II. Second meiosis was found to be normal.

DISCUSSION

The single chiasmatic versus two chiasmatic bivalents in *S. rostrata* seems to be of interest when the chromosome lengths are considered. Now, the question arises as to why the longest chromosomal pair is able to form only a single chiasma while, the second pair as well as the shortest pair (sixth) are able to form two chiasmatic associations in considerable frequencies.

The obvious outcome of this consideration will be that the event of chiasma formation in the longest chromosomal pair is somehow regulated. Few such events of highly regulated chiasma formation have been noticed earlier in *Drosophila melanogaster* (Morgan et al.1933), *Vicia faba* (Rowlands 1958), *Delphinium ajacis* (Basak & Jain 1963 Jain & Maherchandani 1961) and *Endymion* (Elliott 1958, Wilson 1959).

The analysis and identification of the factors responsible for such controlled formation or distribution of chiasmata needs the identification of at least some of the chromosomal pairs in the complement individually in all the cells at diakinesis or M I. The above cited examples viz., *Vicia*, *Delphinium* and *Endymion* satisfy this condition. In cases where such natural distinction among the chromosomes is lacking, the analysis could still be done if specialized configurations are formed by at least some chromosomes of the complement as in the case of translocation heterozygotes (Sukhadev 1981) and trisomics (Manga 1976).

S. rostrata has the distinction of possessing highly differentiated chromosome complement which is a natural facility available for the analysis of such controlling factors. The analysis of interchromosomal effects in the present material revealed greater intranuclear variance compared to the internuclear type in all the five plants indicating negative correlation. Such negative correlation was suggested by Mather (1936) to be indicative of competition for chiasma formation among the bivalents.

This was explained by assuming that each chromosomal pair is capable of forming a minimum number of chiasmata (one in the present case) independent of others and that competition sets in only after this minimum requirement has been satisfied; then even the smallest chromosomal pair of the complement can have at least one extra chiasma and as such no strict linear relationship exists between chromosome length and chiasma formation. Hence, chromosomes can acquire chiasmata in numbers disproportionate to their lengths. Similar observations have been made earlier by Lamm (1936) in inbred rye and by Basak & Jain (1963) in *Delphinium*.

The fact that the internuclear component of variance was significantly less than the intranuclear component in two (SR II and SR V) of the five plants and not in the remaining three used in the present study, indicates the existence of natural variation in this species in the magnitude of interchromosomal effects. Nevertheless, the competition for chiasma formation remains to be the same in all the five plants. According to Basak & Jain (1963), such variation might indicate fluctuations in the threshold limits beyond which competition occurs and the saturation point (minimum number of chiasmata per each bivalent) may vary with genetically determined differences or gene combinations. It is not possible to ascertain whether or not such genotypically controlled pairing behavior of chromosomes (Rees 1961) in terms of chiasma distribution is a common feature in *S. rostrata*, since information of this kind is not available in other species of this genus where meiosis was studied (Parihar & Zadoo 1987).

REFERENCES

ALLEN O N & ALLEN E K (1981) The Leguminosae: *A source book of characteristics uses and nodulation.* The University of Wisconsin Press Madison Wisconsin

BASAK S L & JAIN K 1963 Autonomous and interrelated formation of chiasmata in *Delphinium* chromosomes *Chromosoma* (*Berl*) **13** 577−587

ELLIOTT C G 1958 Environmental effects on the distribution of chiasmata among nuclei and bivalents and correlation between bivalents *Heredity* **12** 429−439

JAIN H K & MAHERCHANDANI N 1961 The control of intranuclear distribution of chiasmata in *Delphinium Heredity* **16** 383−392

JOSHUA D C, BHAGWAT A S, GADGIL J D & BHATIA C R 1989 *Sesbania rostrata* Brem A nodulating legume - its potential as green manure crop *Proc Intl Symp Biological Nitrogen fixation associated with rice production* CRRI Cuttack

JOSHUA D C & BHATIA C R 1987 Karyotype analysis of *Sesbania rostrata* Brem *Plant Breeding* **100** 154−156

LAMM R 1936 Cytological studies in inbred rye *Hereditas* **22** 217−240

LE COQ C, GUERVIN C & DUHOUX E 1985 Caryologie du *Sesbania rostrata* Brem I- etude chromosomique *Rev Cytol Biol Végét Bot* **8** 243−252

MANGA V 1976 Chiasma frequencies in primary trisomics of pearl millet *Can J Genet Cytol* **18** 11−15

MATHER K 1936 Competition between bivalents during chiasma formation *Proc Roy Soc* B **120** 208−227

*MORGAN T H, BRIDGES C B & SCHULTZ J 1933 *Constitution of the germinal material in relation to heredity* Carnegie Inst Year book **32** pp 98−302

PARIHAR J R S & ZADOO S N 1987 Cytogenetical studies in the genus *Sesbania* Scop 1 Karyotype *Cytologia* **52** 507−512

PULLAIAH T & SRI RAMAMURTHY K 2001 *Flora of Eastern Ghats* Vol **2** Regency Publications New Delhi

RADHA G, KUSUMA KUMARI P, MANGA V & SUBBA RAO M V 1998 Karyomorphology and heterochromatin patterns in three species of *Sesbania* Scop *Nucleus* **41** 145−151

REES H 1961 Genotypic control of chromosome form and behavior *Bot Rev* **27** 288−318

ROWLANDS D C 1958 The control of chiasma frequency in *Vicia faba* L *Chromosoma* **9** 176−184

SUKHADEV P 1981 *Studies on the cytogenetic effects of gamma rays and some chemical mutagens in pearl millet* (*Pennisetum americanum* (L) Leeke) Ph D Thesis Andhra University

WILSON J Y 1959 Temperature effect on chiasma frequency in the bluebell *Endymion nonscriptus Chromosoma* **10** 337−354

*Not seen in original.