Morphology of the Pachytene Chromosomes of *Impatiens balsamina* L. var. *bicolor*

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Pachytene analysis has been proved to be one of the ground works in cytogenetic and evolutionary studies in species and species hybrids after the cytological studies of McClintock (1929) in *Zea mays* and now have been extended to many other species of plants. Venkateswarlu (1962) has fully recognised the usefulness of such a study especially in the problem of chromosome aberrations and in the proper understanding of the degree of chromosome homology. Accurate study of structure of chromosomes at pachytene gives details of the linear differentiation of chromosomes, namely chromomeric pattern, position of centromere, location of nucleolus and the distribution of the heterochromatin. As the homologous chromosomes are paired even a small structural difference can be studied.

The present paper is concerned with the pachytene morphology of *Impatiens balsamina* L. (var. *bicolor*) chromosome complement which has 2B chromosomes. Particular emphasis is placed on identification of the characteristics of each chromosome which is useful in tracing the origin of accessory chromosomes in *Impatiens balsamina*.

Material and methods

In *Impatiens balsamina* the pachytene chromosomes present much difficulty in preparation of well spread and well stained slides, so it was necessary to find a technique for suitable staining of the pachytene chromosomes. The following technique proved suitable for study of the pachytene chromosomes in the present material—anthers from young flower buds were fixed randomly in 1 : 3 acetic alcohol (Carnoy's fluid) fortified with iron and then the anthers were kept in the fixative from 58 to 72 hours at room temperature, black colour of the anthers was good indication of proper mordanting. After fixation the anthers were transferred to 70% ethyl alcohol and stored at low temperature in a refrigerator.

The anthers were squashed in 2% acetocarmine. Alternate heating and squashing aided in the differentiation and flattening of chromosomes. Temporary slides were sealed with wax for study. In a single PMC it was difficult to trace from end to end all the pachytene bivalents. PMCs with excellent spreading and differentiation of pachytene bivalents at mid pachytene stage were selected for study, as this was the best stage for accurate identification of morphological details and measurement of chromosomes.

Drawings were made with the help of prism type camera lucida at bench level using $10\times$ eye piece and $100\times$ oil immersion objective.

The length of chromosomes, position of centromere, the number, size and distribution of chromomeres were drawn and measured in microns. An idiogram was drawn with long arms on the lower side and short arms on upper side. The chromosomes were numbered as was done by Barton (1950) who had followed the method of numbering adopted by McClintock (1929) in *Zea mays*. According to this the longest chromosome was numbered as chromosome one and the successive ones 2-3 etc. Length and arm ratios (length of short arm/long arm) was measured for individual chromosomes separately. Identification of individual chromosomes was made as follows—median arm ratio 1.00 to 0.80, submedian arm ratio 0.79 to 0.40 and subterminal arm ratio 0.39-0.10.

Observations

The pachytene bivalents displayed the poorly differentiated type where the chromosomes stained uniformly throughout their length and exhibited a certain degree of clumping. However, differentiated and well spread pachytene configurations were observed occasionally in the PMCs which were selected for this pachytene analysis. The homologous chromosome pairing was found to be complete and apparently normal. The darkly stained regions flanking the centromere are the chromatic regions and the distal regions are the achromatic regions.

The data of total length (including centromere), length of long and short arm, relative length, arm ratio, chromatic and achromatic lengths obtained for each of the eight bivalents (7A+1B) are given in Table 1. The photomicrographs along with the line drawings of 8 bivalents (7A and 1B) are given in figures 1A to 8A and 1B to 8B respectively and an idiogram of these chromosomes is given in Fig. 9.

The characters of 7A bivalents and the accessory bivalent at pachytene stage are described as follows:—

Chromosome. 1.—Longest of the complement with an average length of $56.41\ \mu\text{m}$ has a median centromere and an arm ratio of 0.95 : 1. In the long arm there are 3 chromomeres while in the short arm there are 6 chromomeres adjacent to the centromere followed by achromatic segments.

Chromosome. 2.—Average length $51.28\ \mu\text{m}$ with an arm ratio of 0.78, the centromere is submedian, relative length 90.90. In both the arms there are 3-3 chromomeres flanking the centromere and the rest of the portion consists of achromatic regions.

Chromosome. 3.—Average length $43.59\ \mu\text{m}$ with an arm ratio of 0.74. This chromosome has the highest amount of chromatic segment than any other chromosome of the complement, relative length 77.27, centromere is submedian, seven chromomeres are present in the short arm and 9 in the long arm.

Chromosome. 4.—Submedian centromere with an average length of $41.66\ \mu\text{m}$, arm ratio 0.71, relative length 73.87. Three chromomeres are present in the short arm and two in the long arm surrounding the centromere and rest of the part of the arms is achromatic.

Chromosome. 5—This chromosome is distinguished from the other chromosomes by the attachment of its long arm to the nucleolus by a deeply staining nucleolar organizing body. Nucleolar organizing body is terminal to the long arm situated at a little distance from the nucleolus. The chromosome is nearly median

Figs. 1-8. Eight pachytene bivalents in B chromosome carrier cells of *Impatiens balsamina*. 1A-8A, microphotographs of pachytene bivalents arranged in decreasing order of length. 1B-8B, line drawings of the same.

with an average length of $36.54 \mu\text{m}$, arm ratio is 0.58 and relative length is 64.77. In the short arm there are four chromomeres and in long arm there are 2 chromomeres.

Chromosome. 6—This is the only subterminal chromosome with an arm ratio

of 0.39, average length is $33.97 \mu\text{m}$ and relative length is 60.22. Two chromomeres are present in the short arm and five in the long arm adjacent to the centromere.

Chromosome. 7—Average length $33.33 \mu\text{m}$, centromere submedian, arm ratio 0.73 and relative length is 59.08. Two chromomeres are present in short arm and

Table 1. Average values from each of the 8 chromosomes (7A+1B) of *Impatiens balsamina* L. (All lengths are given in microns)

Chromosome number	Total chromosome length (including centromere)	Long arm			Short arm			Relative length	Arm ratio short arm/long arm	Centromere type
		Total length	Chromatic segment	Achromatic segment	Total length	Chromatic segment	Achromatic segment			
1	56.41 ± 0.5229	28.85	5.13	23.72	27.56	10.26	17.30	100.00	0.95	M
2	51.28 ± 0.5220	28.85	5.13	23.72	22.43	4.48	17.95	90.90	0.78	SM
3	43.59 ± 0.9050	25.00	12.18	12.85	18.59	10.26	8.33	77.27	0.74	SM
4	41.67 ± 0.8640	24.36	4.49	19.87	17.31	3.20	14.10	73.87	0.71	SM
5*	36.54 ± 0.9260	23.08	2.56	20.51	13.46	5.13	8.33	64.77	0.58	SM
6	33.97 ± 0.7400	24.36	7.05	17.31	9.61	1.92	7.69	60.22	0.39	ST
7	33.33 ± 0.4530	19.23	2.56	16.67	14.10	3.85	10.25	59.08	0.73	SM
8	30.13 ± 0.5500	16.67	7.69	8.97	13.46	3.20	10.26	53.41	0.80	M
(B)										(nearly)

* Chromosome 5 is the nucleolus organising chromosome, M: Median, SM: Submedian, ST: Subterminal.

FIG. 9

Fig. 9. Idiogram of pachytene chromosome complement of *Impatiens balsamina* L.

two in long arm adjacent to the centromere followed by achromatic segments.

Chromosome. 8 (B chromosome)—This is the smallest chromosome of the complement and is well distinguished from the smallest chromosome of the A complement. It has a nearly median centromere with a relative length of 53.41 and an

average length of 30.13 μm . Flanking the centromere two chromomeres are present in the short arm and 5 in long arm and rest portion consists of achromatic regions.

For more accurate study of the pachytene chromosomes length of achromatic and chromatic segments was also studied. One chromosome had subterminal centromere, one had median, five had submedian and the B chromosome had a nearly median centromere.

Discussion

Few plants have well defined chromosomes like those of *Zea mays* (McClintock 1929). Many plants have an average degree of pachytene differentiation as observed by Lima-de-Faria (1952) in *Secale*. While plants such as *Lycopersicon* (Barton 1950) and *Sorghum* (Magoon and Shambulingappa 1960) do not have pachytene chromosomes which are readily resolved into distinct chromomere patterns and chromatid structure.

The size of pachytene chromosomes varies widely in the plant kingdom. Shastry *et al.* (1960) reported *Oryza* pachytene chromosomes to vary from 20 to 80 μm in length. Magoon and Shambulingappa (1960) observed *Sorghum* chromosomes to vary from 28 to 79 μm in length. The pachytene chromosomes of *Impatiens balsamina* L. var. *bicolor* varied from 30.12 μm to 56.41 μm in length.

The genome of *Impatiens balsamina* consists of five submedian (chromosome numbers 2, 3, 4, 5, and 7), one median (chromosome number 1), one subterminal (chromosome number 6) and a nearly submedian B chromosome.

Centromeres can be made out clearly as a gap in the chromatic regions. Critical study revealed that each pachytene bivalent has a distinct structural pattern by which it could be identified from the others in the complement such as i) total length of the chromosomes, ii) arm ratio and iii) the relative length of the chromatic and achromatic segments.

In most cases B chromosomes are smaller than the chromosomes of the normal complement but in *Impatiens balsamina* the B chromosome is not easily distinguished from A chromosome complement except at pachytene stage. At metaphase and later stages of meiosis the extra B bivalent is indistinguishable from the normal complement.

Although B chromosomes have been found in many plants and animal species (Battaglia 1964, Jones 1975) but their origin is unknown. Different modes have been suggested for the origin of B chromosomes in different species. Since long it was thought that they are ancient in origin. However, they may arise as by-product by fragmentation or by structural rearrangement of the chromosomes, within the normal complement. These structural alternations may involve translocations and inversions, such as Lewis (1951) has postulated that unequal distribution in translocations of heterozygotes is probable mechanism in the origin of accessory chromosomes in *Clarkia elegans*. Swanson (1943) assumed the presence of overlapping inversions in *Tradescantia*, consequent crossing over in the area of the overlap could result in the origin of accessory chromosomes.

Wu and Pi (1975, 1978) postulated that the accessory chromosome of *Sorghum*

nitidum is similar in structure to the short arm of the nucleolus associated chromosome which is fully chromatic thus it appears to be an isochromosome. At the pachytene stage it does not pair with any of the A chromosomes but it folds back to pair with itself and looks like a telocentric chromosome which seems to have a terminal knob distal to the centromere.

B chromosomes of *Impatiens balsamina* can be distinguished from rest of the chromosomes at pachytene as it has the shortest length and a nearly median centromere. Comparative analysis of B chromosomes and A chromosomes at pachytene appear to indicate some relationship with chromosome number six on the basis of chromomeric patterns. It may be possible to visualise the origin of B chromosomes from this chromosome by deletion and translocation. However, it must be mentioned that at present there does not appear to be any homology between B chromosomes and any A chromosome, because during meiosis Bs do not pair with any other chromosome. But the fact remains that whenever present in a PMC the Bs are two in number and are homologous thus always having a pairing partner. Under these circumstances even if the Bs have some homology still left with chromosome number six probably B homologous may pair with each other without giving any opportunity for pairing with chromosome number six. One can as well speculate that it is possible that this pairing relationship may be under some sort of genetic control.

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Summary

Detailed pachytene morphology of chromosomes of *Impatiens balsamina* L. var. *bicolor* with two accessory chromosomes ($2n=14+2B$) has been analysed. The two B chromosomes always pair to form a bivalent which behaves normally during meiosis. All the chromosomes were analysed individually on the basis of total length of chromosomes, length of chromatic and achromatic segments, arm ratio and an idiogram was drawn, chromosome 5 has the nucleolar organiser in its long arm, chromosomes 2, 3, 4, 5 and 7 have submedian centromere, chromosome one has a median centromere and chromosome 6 has a subterminal centromere. The B chromosome has a nearly median centromere, the short arm has two chromomeres and the long arm has five chromomeres and may have originated from chromosome number six as a result of deletion and translocation. However these Bs do not pair with any of the A chromosomes during meiosis.

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