Cytogenetical Studies of Accessory Chromosomes in the Genus *Cymbopogon* Spreng

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Cymbopogon, an important genus of aromatic grasses, consists of nearly 140 species (Verma 1981) of these only a few have been studied cytologically. In India the genus is represented by about 22 species, many of which are cultivated (Jagadish Chandra 1975a, b).

The genus is monobasic with $x=10$ and B-chromosomes have been reported in 7 out of 22 Indian species (Verma 1981). In the present study supernumerary chromosomes were detected in two species, *C. martinii* var. *motia* ($2n=20$) and *C. jwarancusa* ($2n=20$) out of the 9 species investigated. No B-chromosomes were detected in the polyploid species. The B-chromosomes were found both in the root-tip meristematic cells as well as the PMCs. The present paper deals with the nature and behaviour of these supernumerary chromosomes in the above two species, their F_1 hybrids with other *Cymbopogon* species and in the F_2 population raised from one of the partially fertile F_1 hybrid.

Material and methods

The plants were collected from their wild habitat during routine collection and grown in the experimental garden of the laboratory. *C. jwarancusa* was collected from Nanital while *C. martinii* var. *motia* plants were collected from Haldwani. Herbarium sheets of these plants are deposited in the herbarium of the Regional Research Laboratory, Jammu.

Root-tip and male meiosis squashes were made by the standard methods using aceto-orcein and aceto-carmin stains, respectively. Observations and photomicrographs were taken from the temporary preparations which were later made permanent. For denoting the position of centromere in the chromosomes the terminology adopted is reported earlier (Verma and Sobti 1982).

Results

Following types of studies were made:

1. Study of B-chromosomes in two parental species

The two parental species *C. martinii* var. *motia* and *C. jwarancusa* were taken up for the present study after screening 9 species and varieties for the presence of B-chromosomes.

In *C. martinii* var. *motia* ($2n=20$) the frequency of B-chromosomes ranged

Table 1. Total chromatin length (in microns) of A-chromosomes in the root-tip cells with and without B-chromosomes in two *Cymbopogon* species, their F₁ hybrids and F₂ plants of one of the hybrids

Plant	Frequency of B-chromosomes in root-tip cells	Total chromatin length (in microns) of A-chromosomes						
		0B	1B	2B	3B	4B	5B	6B
<i>C. martinii</i> var. <i>motia</i>	0-2	34.45	—	32.50	—	—	—	—
<i>C. jwarancusa</i>	0-6	34.1	—	38.70	36.95	40.95	—	38.3
<i>C. nardus</i> var. <i>confertiflorus</i> × <i>C. jwarancusa</i>	0-2	28.0	—	32.10	—	—	—	—
<i>C. jwarancusa</i> × <i>C. nardus</i> var. <i>confertiflorus</i>	2-4	—	—	36.58	—	37.60	—	—
<i>C. jwarancusa</i> × <i>C. flexuosus</i> (geraniol type)	0-4	36.62	—	38.60	32.05	39.02	—	—
F ₂ plants of (<i>C. nardus</i> var. <i>confertiflorus</i> × <i>C. jwarancusa</i>) hybrid.								
Group no. 1	0-2	39.0	—	36.50	—	—	—	—
Group no. 2	0-2	28.95	—	26.10	—	—	—	—
Group no. 3	0-5	44.8	36.3	—	—	34.4	32.1	—
Group no. 4	0-2	28.35	—	25.9	—	—	—	—

Figs. 1-9. 1, root-tip metaphase in *C. martinii* var. *motia* with $2n=22=20+2B$ -chromosomes. 2, root-tip metaphase in *C. jwarancusa* with $2n=22=20+2B$ -chromosomes. 3, root-tip metaphase in *C. jwarancusa* with $2n=26=20+6B$ -chromosomes. 4, diakinesis in *C. martinii* var. *motia* with $2n=22=10II+1IIB$. 5, diakinesis in *C. martinii* var. *motia* with $2n=26=10II+3IIB$. 6, diakinesis in *C. martinii* var. *motia* with $2n=22=10II+2IB$. 7, late metaphase I in *C. martinii* var. *motia* showing 2IB-chromosomes not included in the metaphase equatorial plate of the PMC. 8, diakinesis in *C. jwarancusa* with $2n=22=10II+2IB$. 9, metaphase I in *C. jwarancusa* with $2n=22=10II+1IIB$. The B-chromosome bivalent is lying outside the equatorial plate of the PMC.

from 0 to 2 in the root-tip meristematic cells (Table 1, Fig. 1). The B-chromosomes were nearly median (nM) with R_1 ratio (SA/LA)=0.8. In the other species *C. jwarancusa* their number ranged from 0 to 6 (Table 1, Figs. 2, 3) and were either median (M) or nearly median (nM) with R_1 ratios (SA/LA)=1.0 and 0.72, respectively.

Figs. 10–14. 10, late metaphase I in *C. jwarancusa* showing 11B not included in the metaphase equatorial plate of PMC. 11, anaphase I in *C. martinii* var. *motia* showing 2B-chromosomes lagging in the centre of the PMC. 12, anaphase I in *C. martinii* var. *motia* with 5B-chromosomes lagging in the centre of the PMC. 13, anaphase I in *C. jwarancusa* with 11B lagging in the centre of the PMC. 14, telophase I in *C. martinii* var. *motia* showing 2B-chromosomes in the one and 1B-chromosome in the other cell of the diad, not included and lying just outside the respective nucleus.

Out of the 40 PMCs studied in *C. martinii* var. *motia* 30 PMCs were found to have B-chromosomes at diakinesis and metaphase I stages. Their frequency ranged from 0 to 6 with an average of 1.75 B-chromosomes/PMC (Table 2). In *C. jwarancusa* 21 PMCs out of the 40 PMCs investigated contained B-chromosomes. Their number ranged from 0 to 3 with an average of 1.07 B-chromosomes/PMC. In both the species the B-chromosomes when present in even numbers mostly formed bivalents but when present singly they remained as univalents (Figs. 4–6, 8, 9). Neither any multivalent formation nor any bivalent formation with A-chromosomes was observed. The average association of B-chromosomes per PMC was $0.25 \text{ I} + 0.75 \text{ II}$ in *C. martinii* var. *motia* and $0.73 \text{ I} + 0.17 \text{ II}$ in *C.*

Table 2. Mean association of B-chromosomes at diakinesis-metaphase I and chiasma frequency of A-chromosomes per PMC in two *Cymbopogon* species, their F₁ hybrids and F₂ plants of one of the hybrids

Plant	Frequency of B-chromosomes in PMCs	Mean association of B-chromosomes/PMC	Mean chiasma frequency/PMC			
			OB		IB	
			Inter-stitial Xta	Terminal Xta	Inter-stitial Xta	Terminal Xta
<i>C. martinii</i> var. <i>motia</i>	0-6 (=1.75)	0.25I+0.75II	0.8	18.0	—	—
<i>C. jwarancusa</i>	0-3 (=1.07)	1.73I+0.17II	2.07	19.27	0.5	20.0
<i>C. nardus</i> var. <i>confertiflorus</i> × <i>C. jwarancusa</i>	0-3 (=1.04)	1.4I + 1.15II	1.30	9.18	5.0	12.71
<i>C. jwarancusa</i> × <i>C. nardus</i>	—	—	—	—	—	—
var. <i>confertiflorus</i> *	—	—	—	—	—	—
<i>C. jwarancusa</i> × <i>C. flexuosus</i> (geraniol type)*	—	—	—	—	—	—
F ₁ plants of (<i>C. nardus</i> var. <i>confertiflorus</i> × <i>C. jwarancusa</i>) hybrid	0-2 (=1.56)	0.51I+0.14II	1.5	9.08	2.6	11.70
Group no. 1	0-2 (=1.05)	0.34I+0.06II	1.45	9.28	2.3	12.48
Group no. 2	0-4 (=1.92)	0.83I+0.4II	2.3	8.96	3.6	12.50
Group no. 3	0-2 (=1.02)	0.51I+0.06II	1.3	8.97	2.6	10.71
Group no. 4						13.31

* B-Chromosomes not recorded in their PMCs.

Table 2. (Continued)

Plant	Mean chiasma frequency/PMC											
	2B			3B			4B			6B		
	Inter- stitial Xta	Ter- minal Xta	Total	Inter- stitial Xta	Ter- minal Xta	Total	Inter- stitial Xta	Ter- minal Xta	Total	Inter- stitial Xta	Ter- minal Xta	Total
<i>C. martinii</i> var. <i>motia</i>	1.85	18.46	20.31	—	—	—	5.5	19.5	25.0	4.0	18.5	22.5
<i>C. jwarancusa</i>	1.72	19.36	21.09	3.5	18.5	22.0	—	—	—	—	—	—
<i>C. nardus</i> var. <i>confertiflorus</i> × <i>C. jwarancusa</i>	4.15	13.79	17.94	4.0	15.0	19.0	—	—	—	—	—	—
<i>C. jwarancusa</i> × <i>C. nardus</i>	—	—	—	—	—	—	—	—	—	—	—	—
var. <i>confertiflorus</i> *	—	—	—	—	—	—	—	—	—	—	—	—
<i>C. jwarancusa</i> × <i>C. flexuosus</i> (geraniol type)*	—	—	—	—	—	—	—	—	—	—	—	—
F ₁ plants of (<i>C. nardus</i> var. <i>confertiflorus</i> × <i>C. jwarancusa</i>) hybrid	4.3	14.5	18.8	—	—	—	—	—	—	—	—	—
Group no. 1	3.9	13.89	17.79	—	—	—	—	—	—	—	—	—
Group no. 2	4.8	14.3	19.1	5.9	16.5	22.4	6.5	18.61	25.11	—	—	—
Group no. 3	3.5	13.8	17.3	—	—	—	—	—	—	—	—	—
Group no. 4	—	—	—	—	—	—	—	—	—	—	—	—

* B-chromosomes not recorded in their PMCs.

jwarancusa. They were mostly seen lying outside the equatorial plate of late metaphase I (Figs. 7, 10), possibly due to their slow movement to orient themselves on the equatorial plate compared to A-chromosomes. Only 4 out of 35 PMCs, studied in *C. martinii* var. *motia* and 6 out of 30 PMCs studied in *C. jwarancusa* showed the normal segregation of the B-chromosomes to the two poles at anaphase I. The remaining PMCs in both species showed either early movement of B-chromosomes or they lagged and were later included in one of the cells of the diad (Figs. 11–14).

The B-chromosomes divided precociously during second meiotic division and failed to congress at the equatorial plate and their movement to the two poles was synchronised with the movement of the A-chromosomes. Sometimes they failed to get included in the nucleus and are observed as deeply staining bodies outside the nucleus (Fig. 14). Their number varied from 0 to 4 in the pollen grains.

Effect of B-chromosomes on the chiasma frequency of A-chromosomes

There is a marked increase in chiasma frequency of A-chromosomes in *C. martinii* var. *motia* (both interstitial and terminal) in presence of 2 or more B-chromosomes (Table 2). The maximum increase in interstitial chiasma frequency in A-chromosomes has been recorded in PMCs with 4 B-chromosomes followed by those with 6 B-chromosomes, and least in PMCs with 2 B-chromosomes. In case of *C. jwarancusa* decrease in total chiasma frequency and interstitial chiasma frequency per PMC in presence of 1 B-chromosome was observed. The frequency of terminal chiasma increased slightly in presence of 2 B-chromosomes. However, the presence of 3 B-chromosomes increased the interstitial as well as total chiasma frequency/PMC in this species (Table 2).

Effect of B-chromosomes on the total chromatin length of A-chromosomes

The presence of B-chromosomes also affected the total chromatin length of A-chromosomes as observed in the root-tip cells (Table 1). The presence of 2 B-chromosomes resulted in slight decrease in the total chromatin length of the A-chromosomes of the complement in case of *C. martinii* var. *motia* and increase in chromatin length of *C. jwarancusa* upto the presence of 4 B-chromosomes. Further increase in the number of B-chromosomes in *C. jwarancusa* resulted in decrease in the chromatin length (Table 1) of A-chromosomes.

2. Study of the B-chromosomes in three F_1 hybrids

The hybridisation involved three *Cymbopogon* species viz. *C. jwarancusa* ($2n=20$), *C. nardus* var. *confertiflorus* ($2n=20$) and *C. flexuosus* (geraniol chemotype, $2n=20$). The other B-chromosomes carrying species *C. martinii* var. *motia* did not cross with any of the *Cymbopogon* species and varieties included in the present investigation. The following three F_1 hybrids carrying B-chromosomes were recorded:

1. *C. nardus* var. *confertiflorus* $\times$ *C. jwarancusa*.
2. *C. jwarancusa* $\times$ *C. nardus* var. *confertiflorus*.
3. *C. jwarancusa* $\times$ *C. flexuosus* (geraniol chemotype).

Out of these, only hybrid no. 1 (*C. nardus* var. *confertiflorus* $\times$ *C. jwarancusa*) carried B-chromosomes both in its root-tip meristem as well as PMCs. In hybrid nos. 2 and 3 the B-chromosomes were present only in their root-tip cells and were

absent in the PMCs (Tables 1, 2).

There was a general increase in the total chromatin length of A-chromosomes complement with the increase in the number of B-chromosomes in the root-tip cells of all the three F_1 hybrids (Table 1). But in hybrid no. 3 the presence of 3 B-chromosomes resulted in the decrease in total chromatin length of the A-chromosomes (Table 1).

The behaviour of B-chromosomes in PMCs could be studied only in the hybrid no. 1 as the B-chromosomes were absent in the PMCs of other two hybrids. The general behaviour of the supernumerary chromosomes in the PMCs of this hybrid was similar to that in its pollen parent *C. jwarancusa*. The pollen fertility of the plant was only 16.34%. The presence of B-chromosomes resulted in the proportional increase in the total chiasma frequency of A-chromosomes (Table 1).

3. *Study of the B-chromosomes in the F_2 plants of the F_1 hybrid, *C. nardus* var. *confertiflorus* $\times$ *C. jwarancusa**

The F_2 plants of the hybrid no. 1 were classified into 4 groups on the basis of their morphology and chemical constitution. The above 4 groups of F_2 plants possessed varying number of B-chromosomes in their root-tip cells as well as PMCs (Tables 1, 2). The plants belonging to groups 1, 2 and 4 had root-tip cells with either 0B or 2B-chromosomes in addition to the normal A-chromosome complement of $2n=20$. The total chromatin length of A-chromosomes in presence of 2B-chromosomes in all the above three groups of plants (groups 1, 2 and 4) was less than that of the cells without B-chromosomes (Table 1). However a single plant included in the group no. 3 had root-tip cells with 0B, 1B-, 4B- and 5B-chromosomes. The total chromatin length of A-chromosomes in the root-tip of this plant decreased proportionately with the increase in the number of B-chromosomes and was least in presence of 5B-chromosomes (Table 1). However the behaviour of B-chromosomes in PMCs was similar to that in the original parent plant (*C. jwarancusa*) and F_1 hybrid no. 1. The pollen fertility in all F_2 plants was reduced in general and the plants included in group no. 3 showed almost complete pollen sterility.

Discussion

The present study of B-chromosomes in the two species of the genus *cymbopogon* i.e. *C. martinii* var *motia* and *C. jwarancusa* shows that the behaviour of these supernumerary chromosomes in the above species is similar to the behaviour of such chromosomes in other grass species. The B-chromosomes in root-tip cells as well as in PMCs has also been reported in *Plantago coronopus* (Palival and Hyde 1959). There are many earlier reports in which the B-chromosomes are stated to be present only in the germline or only in somatic tissue (Darlington and Thomas 1941; Müntzing 1946 a, b, Sudharshan and Jagadish Chandra 1980).

In the present study the B-chromosomes were found to be smaller than the smallest chromosome of the normal complement, the feature which has also been observed earlier in several plants (Raghuvanshi and Joshi 1964, Jones 1975,

Raghuvanshi and Upreti 1977, Gupta and Gupta 1977). The presence of B-chromosomes in above two *Cymbopogon* species does not show any effect on the morphology of the plants. However, the B-chromosomes have been observed to effect the chiasma frequency and genetic recombination of the A-chromosomes (Table 2). The increase in number of B-chromosomes upto 4B-chromosomes per PMC results in the increase in chiasma frequency in A-chromosomes. But further increase in their number does not result in corresponding increase in the chiasma frequency of A-chromosomes (Table 2). Such an effect has also been reported in other plants (Shah 1967, Simchen *et al.* 1971, Zarchi *et al.* 1972, 1974, Müntzing 1974, Rees 1974, Jones 1975, Kaul and Wakhlu 1976, Brown and Jones 1976, Raghuvanshi and Singh 1977).

In the case of *C. jwarancusa* there was an increase in the total chromatin length of A-chromosomes with the increase in the number of B-chromosomes whereas in the case of *C. martinii* var. *motia* increase in their number resulted in the decrease in total chromatin length of A-chromosomes. The present study confirms the earlier finding of Grun (1959), Bosemark (1956), and Frost (1954) that the effect of B-chromosomes on chiasma frequency and total chromatin length differs with different genomes.

In the interspecific hybrids the B-chromosomes were restricted to the root-tip cells of 2 such hybrids (nos. 2 and 3), while in one of the hybrids (no. 1) they were present in root-tip cells as well as PMCs. Their absence from the PMCs of the two hybrids may be due to their elimination prior to sporogenesis because of their having weak centromeres. Bianchi *et al.* (1961) and Carlson (1970) attributed the losses of B-chromosomes to the non-disjunction due to the presence of heterochromatin. The amount of heterochromatin present in a B-chromosome is reported to play a significant role in the non-disjunction and increase in the number of these chromosomes (Roman 1949, Müntzing 1946b, 1954, Bosemark 1957, Lima-de-Faria 1962).

Various postulations have been made to explain the effect of B-chromosomes on the chiasma frequency of A-chromosomes. Ayonoadu and Rees (1968) and Barlow and Vosa (1969, 1970) suggested that the presence of B-chromosomes results in an increase in number of sites of chiasma formation and also results in delay of terminalization of chiasma in A-chromosomes. Barlow and Vosa (1970) suggested that the presence of B-chromosomes reduced the negative interference between the sites of chiasma formation and prolong the time of effective pairing in proportional increase in the total chiasma frequency of A-chromosomes. Subha Rao and Pantulu (1978) showed that it is the balance between euchromatin and heterochromatin that decides the effect of B-chromosomes on the chiasma frequency of A-chromosomes. However it is possible that the presence of B-chromosomes might be effecting more than one factors influencing the chiasma formation in the A-chromosomes.

The effect of B-chromosomes on the total chromatin length and chiasma frequency of A-chromosomes in the F_1 hybrids is similar to that observed in parental species. But these affects due to the presence of B-chromosomes appear to get modified in the F_2 plants raised from the hybrid no. 1. There is a progressive de-

crease in the total chromatin length of A-chromosomes with the increase in number of B-chromosomes in root-tip cells which is particularly significant in plants belonging to group no. 3, where the least chromatin length of 32.1μ has been recorded in presence of 5B-chromosomes.

Thus the present work clearly shows that the presence of B-chromosomes effects the total chromatin length and the chiasma frequency of A-chromosomes. And this effect is variable and depends on the genome type and the number of B-chromosomes present in the plant. However, these chromosomes do not show any significant change in the morphology or chemical composition of the plants carrying them.

Summary

The B-chromosomes were detected in two species (*C. martinii* var. *motia* and *C. jwarancusa*) out of the 9 species of genus *Cymbopogon* investigated. The supernumerary chromosomes were found in both the root-tip meristematic cells as well as the PMCs. Investigations with respect to nature and behaviour of the accessory chromosomes were made in the two parental B-chromosomes carrying species, their F_1 hybrids with other *Cymbopogon* species and in the F_2 population raised from one of the partially fertile F_1 hybrids. The above study clearly shows that the B-chromosomes have a direct effect on the total chromatin length and the chiasma frequency of A-chromosomes. However, no effect on the general morphology or chemical composition of the oil of the plants has been noticed in presence of the accessory chromosomes. The significance of the data on accessory chromosomes is discussed.

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