



ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating 2017: 3.53
JMPS 2017; 5(2): 175-179
© 2017 JMPS
Received: 22-01-2017
Accepted: 23-02-2017

Sree Latha K
Research Scholar, Dept. of
Microbiology & Biotechnology,
Bangalore University,
Bangalore, Karnataka, India

Vijayalakshmi TN
Research Scholar, Dept. of
Microbiology & Biotechnology,
Bangalore University,
Bangalore, Karnataka, India

Shiva Kameshwari
Assistant Professor, Dept. of
Botany, Bangalore University,
Bangalore, Karnataka, India

Thara Saraswathi KJ
Professor, Dept. of Microbiology
& Biotechnology, Bangalore
University, Bangalore,
Karnataka, India

Correspondence
Sree Latha K
Research Scholar, Dept. of
Microbiology & Biotechnology,
Bangalore University,
Bangalore, Karnataka, India

Morphological and cytogenetic studies in wild species of *Cymbopogon martinii* (Roxb.) Wats from Kodaicanal (T.N.)

Sree Latha K, Vijayalakshmi TN, Shiva Kameshwari and Thara Saraswathi KJ

Abstract

The Genus *Cymbopogon* is a major aromatic grass belonging to the family Poaceae. It produces essential oil of commerce and utilized in aromatic and pharmaceutical industries. It consist of more than 180 species, sub species, varieties and sub varieties worldwide. During the present investigation morphological and cytogenetic investigations in *Cymbopogon martinii* (Roxb.) Wats were carried out. Most of the species of *Cymbopogon* hitherto reported have shown difference in ploidy levels ranging from diploid ($2n=20$ Chromosomes) to hexaploid ($2n=60$ Chromosomes) types. The karyomorphotypic studies showed that the presently studied *C. martinii* is of diploid type. The species also showed significance by the occurrence of B- chromosomes ($2n=20+2$). The present study revealed that the B-chromosomes studied were structurally homologous during pairing depicting the polysomic condition.

Keywords: *Cymbopogon martinii*; Morphology; Karyomorphology; Meiosis; B-chromosomes

1. Introduction

Cymbopogon martinii (Roxb) Wats also called Palmarosa is a tropical grass growing wild throughout India and abundantly in South India (Thara Saraswathi *et al.*, 2014) [32]. It is an industrially important multi harvest crop belonging to Rusae series of the family Poaceae. The plant is indigenous to India and the essential oil of the plant is obtained by hydro distillation method. The oil was collected from wildy growing plants sourced out from Madhya Pradesh, Maharashtra, Andhra Pradesh and Tamil Nadu. In the Indian context, two varieties exist-var. *motia* showing high grade oil with higher geraniol content (75-95%) and var. *sofia* having low content of geraniol (less than 70%) (Guenther E, 1950) [11]. Except for minor morphological differences with respect to leaf size and stem structure, these varieties are morphologically identical. Although, the oil was produced using the grass source from the wild, subsequent bulk production was made by cultivated sources (Smitha GR *et al.*, 2014) [28]. The grass has been cultivated in U.P, Assam, Andhra Pradesh, Karnataka, Tamil Nadu and Kerala (Ram Suresh *et al.*, 2014) [23]. The total world production of Palmarosa oil is approximately 60-70 tonnes and India is the major producer followed by Brazil, Guatemala and Indonesia (Virmani *et al.*, 1987) [34]. The Palmarosa oil of commerce is one of the top ten essential oils in the world. The oil finds extensive application in perfumery, cosmetic, flavour and aroma therapy (Bhasker *et al.*, 2015) [5].

Under wild condition, the varieties of *C. martinii* show different ploidal status where var. *motia* expressed $2n=20$ chromosomes with the presence or absence of B-chromosomes and var. *sofia* with $2n=40$ chromosomes without the B-chromosomes (Guptha, 1965) [12]. A wide variation in morphology with respect to plant height, tiller number, size and height of inflorescence, herb yield, oil content and geraniol content has been reported in different ecotypes of *C. martinii* (Prakasa Rao *et al.*, 1985) [22].

The origin of B-chromosomes has persistently nagged cytologists over the years and remains an enigma even today (Jones, 1975) [16]. These chromosomes are generally considered as altered or unaltered members of the basic complement. Evidently, there are different views for the origin of B-chromosomes (Darlington, 1963; Jagadishchandra, 1975; Jones, 1975) [9, 15, 16].

2. Materials and Methods

2.1 Germplasm maintenance

The *Cymbopogon martinii* collections from the wild, were made from the remote sites of Kodaicanal, (T.N.) and maintained as Germplasm in the Department of Biotechnology, Bangalore University, Bengaluru for further studies.

2.2 Morphological Studies

The morphological characters like length and thickness of the culm, length and breadth of the leaf, texture and color of the leaf, angle of leaf with the culm, length of the ligule, length of the leaf sheath, length of the inflorescence, nature of branching, spatheole length, shape of the bisexual spikelet were studied from the flowering culms were considered. A minimum of ten plants were scored for the determination of morphological characters.

2.3 Cytogenetic Studies

The cytogenetic investigations were carried out through mitotic and meiotic studies respectively. For mitotic studies, the chromosomes from the root tip squashes were prepared. For meiotic studies, anthers from the spikelets at different stages of development were investigated.

For karyomorphological studies, the root tips were collected from the plants between 12.30 A.M. and 2.00 P.M. The plants were watered two hours before collecting the root tips to make the cells turgid. Healthy root tips were scrubbed clean using soft brush and pretreated with 0.002 M solution of 8-hydroxy quinolone for 24 hours at 15 °C. After pretreatment, the root tips were washed under running tap water for 30 min. and were fixed in modified Carnoy's-I fluid (3:1, absolute alcohol: propionic acid) for 24 hours at 15 °C. The root tips were transferred to 70% alcohol and used for further studies (Thara Saraswathi *et al.*, 2014) [32]. For meiotic studies, the spikelets of the inflorescence were fixed in modified Carnoy's-II solution (6:3:1, absolute ethanol: chloroform: propionic acid) between 9.30 A.M. and 10.30 A.M. (Sharma and Sharma 1965) [27]. The florets were kept immersed in ample quantity of fixative for 24 hours at 15 °C. Appropriate sized buds were dissected on the microslide and anthers were squashed using 1% propiono-hematoxylin in using trace of iron alum. The rigid anther walls leftover as debris were removed and slide was warmed over the spirit lamp. During squashing, excess of stain was blotted out and was provided with uniform pressure to flatten the cells for chromosome spreading. The slides were made semi-permanent by sealing the coverslip with molten paraffin wax. The photomicrographs were taken using Carl Zeiss research microscope with apochromatic objective lens system fitted with MF 35 mm Camera.

3. Results and Discussion

3.1 Morphological studies

The habitat of the presently investigated wild *C. martinii* species was studied (Fig. 1a and Fig. 1b). The culm of the plant ranged from 140-175 cm high with 16-21 tillers. The leaves (27-35 X 1.6-1.9 cm) were linear, lanceolate, coarsely scabrid along margin, cordate at base, amplexicaul, making an obtuse angle with the culm, ligule membranochartaceous (3-5 mm long), leaf sheaths shorter than internodes, smooth and nodes exposed. The inflorescence were linear-oblong, false decomposed panicle, upto 35 cm long, moderately dense, spatheole (18-20 mm long), orange-bright red at maturity, elliptic acute, racemes (15-18 mm long), one sub sessile, other pedicelled. Lowest pair of spikelet in the sub sessile raceme

swollen and adnate to rachis. The sessile spikelet in the raceme also swollen and adnate to rachis. The sessile spikelet (3-3.5 mm long), elliptic oblong, exclude wings, lower glume with deep median groove, two-nerved, broadly winged, awn (16-18mm long). The pedicellate spikelet (4-4.5 mm long), elliptic acute, lower glume with 7-9 nerves. The presently studied species belong to 'motia' variety of *C. martinii*, as it possessed erect culm, unbranched tillers and the angle of leaf making obtuse angle with the culm. Earlier, 'motia' variety of *C. martinii* has been described based on the morphological characters (Gupta 1970e) [12]



Fig 1a: Young Plant (Three month old)



Fig 1b: Mature Plant (One year old)

3.2 Cytogenetic studies

The *C. martinii* plants undertaken for present investigation showed $n=10$ chromosomes having diploid nature. The chromosomes showed medium to small size and formed ten regular bivalents.

3.3 Mitotic studies

The karyomorphological studies carried out showed that the root tip cells contained $2n=20$ chromosomes, irrespective of presence or absence of B-chromosomes in their germ line (Fig. 2a). The somatic chromosomes ranged from medium to small size. The karyotype consisted of metacentric and sub-metacentric chromosomes (Fig. 2b). In the metacentric

chromosome, the centromere was situated either at midpoint and termed as 'M' type or situated at the median region termed as 'm' type. The karyotype formula was deduced as $2M+6m+1sm+1sm^S$, wherein 'sm^S' represented the sub metacentric chromosome with satellite. The karyotype and ideogram of somatic chromosomes of *C. martinii* are shown respectively (Fig.2b and 2c)

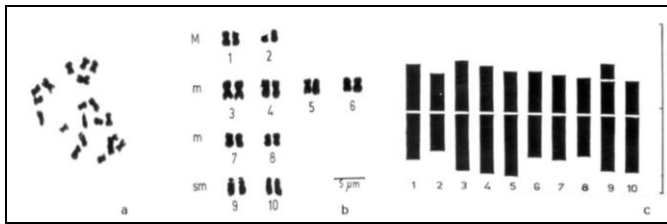


Fig 2: Karyomorphological studies in *C. martinii*, a: Metaphase, b: Karyotype, c: Ideogram

The shortest chromosome in the complement was found to be

Table 1: Karyomorphological data of *C. martinii*

Chromosome pair	Short arm	Long arm	Ratio (la/sa)	TCL	Relative length	Chromosome type
1.	1.38	1.38	1.00	2.76	10.06	M
2.	1.12	1.12	1.00	2.24	8.16	M
3.	1.52	1.68	1.10	3.20	11.66	M
4.	1.36	1.76	1.29	3.12	11.37	M
5.	1.20	1.84	1.53	3.04	11.08	M
6.	1.20	1.28	1.06	2.48	9.04	M
7.	1.14	1.32	1.15	2.46	8.96	M
8.	1.08	1.24	1.14	2.32	8.45	M
9.	0.96+0.40	1.72	1.79	3.08	11.22	Sm
10.	0.98	1.76	1.79	2.74	9.98	sm ^S

Absolute chromatin length: 27.44 µm

Karyotype formula: $2M+6m+1sm+1sm^S$.

Karyotype asymmetry: 1a

2. Meiotic studies

The meiotic studies carried out in *C. martinii* was observed to be normal forming ten bivalents as seen during diakinesis (Fig. 3a and 3b). Occasionally there was formation of additional chromosomes or B-chromosomes as observed during late diakinesis and metaphase-I (Fig. 3c and Fig. 3d). The B-chromosomes were of standard type having the size of normal chromosome of the complement.

The flower bud squashes prepared showed that there was intact association of bivalents with the nucleus during meiotic prophase. The chiasma formation in the bivalents were found to be of either localized or distal type having 1-2 chiasmata per bivalent (Fig. 3a). According to Darlington 1935 [8], the regions which pair first are preferred in chiasma formation and the heterochromatic segments influencing the pairing might also influence chiasma formation. During the study, the mean chiasmata per bivalent was found to be 1.65 and 1.66 and formation of 1-2 chiasmata per bivalent was observed as common feature. It was observed that the B-chromosomes paired strictly inter se (Fig. 3d) and the segregation of B-chromosomes were generally normal (Fig. 3g). However, in about 2-3% of the melocytes, the B-chromosomes exhibited precocious movement (Fig. 3e) or sometimes lagging movement (Fig. 3h) during metaphase-I and anaphase-I respectively. The segregation of B-chromosome was not uniform in 1.6% of the melocytes as observed during late

2.24 µm in size showing 'M' type and the longest chromosome showed 3.2 µm with long 'm' type. The absolute chromatin length of the haploid complement was 27.44 µm. A characteristic sub metacentric (sm) satellite chromosome pair was observed (Fig. 2b). A noteworthy observation was made that one of the chromosome in the satellite pair showed activity as discerned by stretching the chromatin at secondary constriction region.

The nature of the karyotype presently studied can be categorized under the karyotype asymmetry-1a type (Stebbins 1958) [29]. Karyomorphologically, the *C. martinii* presently studied expressed compact type of symmetry consisting of both metacentric and sub metacentric chromosomes. The details of individual chromosome in the complement such as, length of short arm and long arm, long arm/short arm ratio (la/sa), The total Chromosome Length (TCL), relative length and type of the chromosome has been investigated. The details of karyomorphological measurements are shown (Table-1).

anaphase-II (Fig. 3g). The B-chromosomes were seen as distinctly separate entities from the normal chromosomal complement as observed during telophase-II (Fig. 3i). The meiotic division-II was normal with pollen fertility of 86.18%.

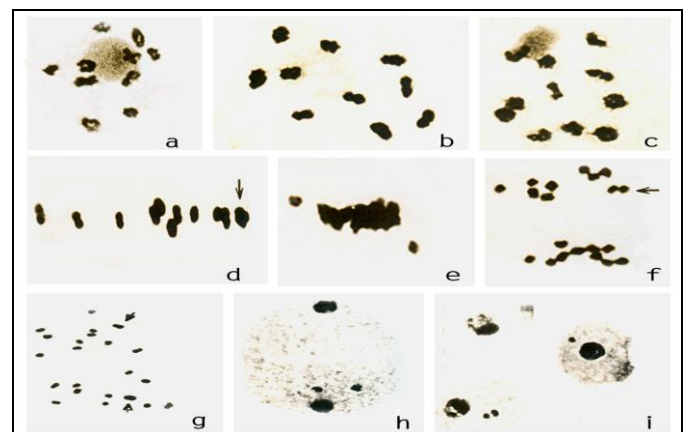


Fig 3: Different stages of meiotic division in *C. martinii*, a. Early Diakinesis, b. Diakinesis, c. Late Diakinesis, d. Metaphase-I, e. Metaphase-I, (precocious movement), f. Anaphase-I, g. Anaphase-II, h. Telophase-I, i. Telophase-II, (Arrow indicates B-chromosome)

Table 2: Data on behavior of B-chromosomes in *C. martinii*

No. of B-chromosome	Metaphase/Anaphase cells observed	Precocious movement	Lagging movement	Elimination
2	360	7 (1.94%)	10 (2.7%)	6 (1.6%)

3. Discussion

The popular view that is repeatedly emphasized upon is the nucleoli organizing or secondary constriction region is the likely site where breakage occur giving rise to B-chromosome. This region is believed to be the weak point in the chromosome complement as it readily get disrupted during squash preparations. The B-chromosomes whether derived from fragmentation of A-chromosomes or otherwise should however have the centromere to be referred as typical or standard type. There are no reports recorded on the loss of B-chromosome after their formation by fragmentation of A-chromosome and hence genetic imbalance is prevented (Wei Huang et. al., 2016) [35].

The *C. martinii* presently studied from wild locations of Kodaicanal, belonged to 'motia' variety according to the characters described (Guptha, 1970e) [12]. The chromosomes showed normal behavior during mitotic and meiotic divisions. There was occurrence of B-chromosomes in Metaphase I, Anaphase I and Anaphase II during meiotic division as also observed earlier (Battaglia, 1964) [4]. When compared to the somatic cells, the B-chromosomes were observed predominantly in the germline cells maintaining their constant number and size, pairing strictly inter perse. The occurrence of B-chromosomes although were recorded in 10-25% of root-tip cells, they were found in 100% of pre-meiotic cells there by indicating the genetic controlled mechanism for their differential distribution. The differential distribution of B-chromosomes in various parts of *Panicum colaratum* has been reported earlier (Swaminathan and Nath, 1956) [31]. The B-chromosomes getting lost in the roots during development of seed and inflorescence has been studied in *Sorghum purpureum sericeum* (Darlington and Thomas 1941) [9]. Remarkable consistency in the B-chromosome number has been reported in different crops (Battaglia 1964) [4]. The present study showed that the B-chromosomes were structurally homologous during pairing depicting the polysomic condition. The B-chromosomes showing formation of bivalents and multivalent have been recorded earlier (Battaglia 1964) [4].

The occurrence of B-chromosomes boosting variability among diploid populations have been reported (Darlington 1965a) [9]. The greater heritable variations existing in the progeny containing B-chromosomes than those progenies without B-chromosomes were studied (Moss 1966) [19]. Studies on the linkage data in maize showed increase in recombination between the markers on chromosomes-3 in the presence of B-chromosomes (Hanson, 1962) [14]. Increase in B-chromosome number contributing to enhanced chiasma frequency has been reported (Ayonoadu and Rees 1968) [3]. In contrary, presence of B-chromosomes reducing the chiasma frequency has also been recorded (Cameron and Rees 1967) [6]. Both enhancement and reduction in the frequency of chiasma formation has been studied in Rye (Jones 1975) [16].

During the present study made in *C. martinii*, there was significant increase in the mean chiasma frequency per bivalent and the increase or decrease in chiasma frequency was associated with fertility of the pollen grain. The B-chromosomes presently studied revealed that they could influence chiasma frequency and distribution among the cells there by affecting the mean chiasma frequency, as also reported earlier (Jones 1975) [16]. The sustenance of B-

chromosomes in the population might seem to depend on the equilibrium between the forces of elimination and accumulation. Various degree of elimination of B-chromosomes have been recorded in Rusae series of *Cymbopogon* and the rate of loss of B-chromosomes are noted to be genetically determined (Jones 1975) [16].

There exist significant correlation between the B-chromosomes and their geographical distribution. In secale, B-chromosomes occur in the primitive strains growing in wild in the centre of origin of the species (Battaglia 1964) [4]. The B-chromosomes were present in highest frequencies in primitive strains of rye (Muntzing 1958) [20]. In Rusae series of *Cymbopogon*, the B-chromosomes were found in all the three species occurring frequently in south India, whereas no report has been made in the same series collected from north India (Jagadish Chandra, 1975a) [15]. The high frequency occurrence of B-chromosomes in south Indian collections indicate the probable origin of this species from south India. (Jagadish Chandra 1975a) [15]. The study revealed that the B-chromosomes could have been designed as devises for controlling recombination by adjusting the chiasma frequency and chiasma distribution.

4. Conflict of Interest

The authors declare they have no conflict of interest.

5. Acknowledgement

The authors are thankful to Prof. K.S. Jagadish Chandra, Retired Professor, Dept. of Studies in Botany, Manasagangotri, University of Mysore, Mysore-570006 for helping in collection of the plant, taxonomical identification and analysis of the present work.

6. References

1. Anand Akhila. Essential oil bearing grasses. 2009.
2. Anonymous. Essential oils and oleoresins. A study of selected producers and Major markets. International Trade Centre, UNCTAD/GATT. Geneva. 1986, 208.
3. Ayonoadu U, Rees H. The regulation of mitosis by B-chromosomes in rye. Expl. Cell Res. 1968; 52:284-290.
4. Battaglia E. Cytogenetics of B-chromosomes. Caryologia. 1964; 17(1):246-299.
5. Bhasker SS, Sathwik Reddy E, Abhishek S, Murthy S, Naga Lakshmi KV. Optimization & Studies on Extraction of Palma Rosa Oil by Steam Distillation. Int. J of Eng. Res. & Reviews. 2015; 3(3):29-34
6. Cameron FM, Rees H. The influence of B-chromosomes on meiosis in Lolium. Heredity. 1967; 22:446-450.
7. Carlson WR. Non-Disjunction and iso-chromosomes formation in the B-chromosomes of maize. Chromosoma Berl. 1970; 30(3):356-36.
8. Darlington CD. The time, place and action of crossing over. J. Genet. 1935; 31:185-212.
9. Darlington CD, Thomas PT. Morbid mitosis and the activity of inert chromosomes. Proc. R. Soc. 1965a; 130:127-150.
10. Darlington CD. Natural populations in Sorghum and the breakdown of classical genetics. Proc. R. Soc. 1965b; 145:350-364
11. Guenther E. The Essential oils. Van Nostrand Co. Inc. New York. 1950; 4:P-752.

12. Gupta R, Gupta PK. B-chromosomes in five species of *Crotalaria* L. *Cytologia*. 1977; (2-3):581-585.
13. Hamed Banu Quraish, Shamim A Faruqi. Cytogenetics of *Cymbopogon* Species from Pakistan. *Int. J of Cytology, Cytosystematics and Cytogenetics*. 2014 0008-7114.
14. Hanson GP. Crossing-over in chromosome-3 as influenced by B-chromosomes, Maize. *Genet. Coop. News Lett*. 1962; 36:34-35.
15. Jagadish Chandra KS. Recent studies on *Cymbopogon* Spreng. (Aromatic grasses) with special reference to Indian taxa; Taxonomy, Cytogenetics, Chemistry and scope. *J. of Plant Crops*. 1975a; 3:43-57.
16. Jones. B-chromosome systems in flowering plants and animal species. *Int. Rev. Cytol*. 1975; 40:1-100.
17. Kumar J, Verma V, Goyal A, Shahi AK, Sparoo R, Sangwan RS *et al*. Genetic diversity analysis *Cymbopogon* species using DNA markers. *Plant Omics J*. 2009; 2(1):20-29.
18. Khanuja SPS. Essential oil constituents and RAPD markers to establish species relationships in Spreng. (Poaceae). *Biochem. And System Ecology*. 2005; 33:171-186
19. Moss JP. The adaptive significance of B-chromosomes in rye. *Chromosome today. Proc. Oxford chromosome Conf. I*. 1966, 15-23.
20. Muntzing A. A new category of chromosomes. *Proc.X Intern. Congr. Genet*. 1958; 1:453-467.
21. Ostergren G. Heterochromatic B-chromosomes in *Anthoxanthum*. *Hereditas*. 1947; 33:261-296.
22. Prakasa Rao EVS, Singh M, Ganesh Rao RS. Effect of plant spacing and application of nitrogen fertilizers on herb and essential oil yield of Palmarosa (*Cymbopogon martinii* Stapf var. *motia*). *J. agri. Sci., Cambridge*. 2014; 104:67-70. 48(6):480-483.
23. Ram Suresh, Sanjay Kumar, Gangwar SP, Hari Shanker, Tomar VKS, Bansal RK *et al*. Economic Analysis of Palmarosa Cultivation in India. *Indian J of Agri. Research*. 2014; 48(6):480-483.
24. Sahoo S, Dutta PK. Selection of high yielding Palmarosa genotypes for the growth of Palmarosa oil industries in the rural sector. *Indian Perfumer*. 1982; 26:41-45.
25. Sahoo S, Dutta PK, Maheshwari ML. Study of the genetic variability and scope of selection in *Cymbopogon martinii* Wats. var. *motia*. *Indian Perfumer*. 1984; s28:167-170.
26. Sethi KL, Sapra RL. Variability in two natural populations of Palmarosa. *Indian J. Genet*. 1983; 43:37-39.
27. Sharma AK, Sharma A. *Chromosome Technique - theory and practice*. Butterworths, London. 1965.
28. Smitha GR, Thania Sara Varghese, Manivels P. *Cultivation of Palmarosa*. 2014.
29. Stebbins GL. Longevity, habitat and release of genetic variability in the higher plants. *Cold Spring Herb*. 1958.
30. Sudarshan MR, Jagadish Chandra KS. Effect of B-chromosomes on free amino acid pattern in *Cymbopogon caesius* (Nees) Stapf. (Poaceae). *Journal of Cytology and genetics*. 1984; 19:15-20.
31. Swaminathan MS, Nath J. B-chromosomes in *Panicum colaratum*. *Curr. Science*. 1956; 25:123-124.
32. Thara Saraswathi KJ, Jayalakshmi NR, Shivakameshwari MN, Jagadish Chandra KS. Karyomorphotypic studies in tetraploid variety of *C. martinii* var. *sofia*. *CIB Tech. J of Biotechnology*. 2014; 3:8-12.
33. Vijeshwarverma, Sobti SN. Cytogenetical studies of Accessory Chromosomes in the Genus *Cymbopogon* Spreng. Regional Research Lab. 1984.
34. Virmani OP, Srivastava GN, Singh DV. Palmarosa and its cultivation of in India. *Farm Bull. CIMAP, Luknow, India*. 1987; 7:14.
35. Wei Huang, Yan Du, Xin Zhao, Weiwei Jin. B chromosome contains active genes and impacts the transcription of a chromosomes in maize (*Zea mays* L.) *BMC Plant Biol*. 2016; 16:88.