Cytological Characterization of a Rare Medicinal Herb 'Safed Musli' (*Chlorophytum borivillianum* Sant. et Fernand.)

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Summary Karyomorphological studies were carried out in seven accessions of *Chlorophytum borivillianum* collected from the states of Rajasthan and Madhya Pradesh. All the accessions were found to be tetraploid with $2n=28$ chromosomes which were resolved into 7 groups, each comprising 7 homologous chromosomes. All the accessions had submetacentric chromosomes in high proportion while metacentrics, telocentrics and subtelocentrics recorded were very few in number. Nucleolar chromosomes were recorded in 5 (PBL-1, PBL-2, PBL-4, PBL-5 and PBL-7) out of the 7 accessions studied. The karyotypes of all the accessions were more or less symmetrical. The role of karyotypic variation in speciation and evolution of the genus *Chlorophytum* has been discussed in detail.

Key words *Chlorophytum*, Karyotype analysis, Medicinal herb, Nucleolar chromosome, Symmetry.

Chlorophytum borivillianum (Family: Liliaceae) is an important threatened medicinal herb designated as 'Rare' in Red Data Book of Indian plants. It grows wild during rainy season on the slopy fertile land of forest in tropical and subtropical regions of the world (Li *et al.* 1990, Kativu and Nordal 1993). In India, it is mainly distributed in southern Rajasthan, north Gujarat and western Madhya Pradesh (Geetha and Maiti 2002). It is valued for the fasciculated storage roots which are reputed to have aphrodisiac properties and form an important ingredient of herbal tonics prescribed in the Ayurvedic system of medicine in India (Kirtikar and Basu 1975). The plant is of great economic importance as its dried roots are sold in the Indian market at a price of Rs. 1000/- (US \$ 20) per kilogram and are exported in large quantities to the middle east.

The natural regeneration of this herb is through tuberous roots that have become scarce because of its indiscriminate collection from wild populations. The seed germination is also very poor (Jat and Bordia 1990). For efficient conservation and management of medicinal plant diversity, the genetic composition of species collected from different phytogeographical regions needs to be assessed. There have been only a few efforts on characterization of *C. borivillianum* accessions based on morphological and biochemical traits (Geetha and Maiti 2002, Bhagat and Jadeja 2003). The data on chromosome numbers and comparative karyology is fundamental to overall understanding of genome in different species or in morphologically diverse populations within a species (Stace 2000). Although the somatic chromosome number of *C. borivillianum* has been reported as $2n=16$ (Raghavan *et al.* 1977), there is no worthwhile information available on the karyological details of this species. Therefore, the present investigation was undertaken to characterize 7 accessions of *C. borivillianum* from cytological point of view.

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Materials and methods

Field surveys in different areas of Rajasthan, Gujarat and Madhya Pradesh were undertaken for the collection of 'Safed Musli' germplasm. In the month of August, 2001 well grown plants along with the root tubers were removed from their natural habitats along with the soil, and they were kept in polybags and brought to Udaipur for their establishment in the field gene bank. During ensuing season the plants were allowed to remain in polybags till maturity. Germplasm collected from each location was given an accession number. In the following rainy season collected accessions of *C. borivilianum* were transferred in the Field Gene Bank established in the nursery of M.L. Sukhadia University at Udaipur.

Root tips were collected from the actively growing roots of the plants grown in the field gene bank. For this purpose, soil near the root zone was carefully removed without damaging the delicate root system. The root tips (0.5–1.0 cm long) were excised and pretreated with 0.025% colchicine (Himedia, India) for 3 h followed by fixation in freshly prepared carnoy's fluid (3 parts of ethanol+1 part of glacial acetic acid) for 24 h. Root tips were hydrolyzed with 1 N HCl for 8–10 min at 60°C and stained in 0.5% leuco-basic fuchsin. The stained tips were squashed in 1% acetocarmine. At least 5 clear preparations of chromosome complements of each accession were analyzed for the karyotypes. Idiograms were prepared from photomicrographs by cutting out individual chromosomes, arranging them in descending order of their length and matching on the basis of morphology. Battaglia's (1955) classification of median (V), submedian (L), subtelocentric (J) and telocentric (I) based on the arm ratio of 1:1; >1:1<1:3; >1:3<1:0; and 1:0 respectively was used for comparison. The degree of symmetry was estimated as per the scheme proposed by Stebbins (1971).

Observations

Chromosomal complements

The cytological studies in 7 accessions of *C. borivilianum* have been conducted in detail for somatic chromosome number. All the accessions collected from various locations showed $2n=28$ chromosomes (Fig. 1) which were resolved into 7 distinct groups, each comprising 4 homologous chromosomes (Fig. 2). These groups formed a discrete series from the longest to shortest pair within complement. The accessionwise details are as follows:

PBL-1 This accession was cytologically characterized into 7 groups of chromosomes, each comprising 4 chromosomes. Barring 2 chromosomes belonging to 6th group, all the chromosomes in the complement were observed as submetacentrics. The 5th group of chromosomes was found to be nucleolar in nature. The karyotype was resolved into $2V+24L+2Ln$. The largest chromosome in the complement was about 1.98 times larger than the shortest chromosome in the complement.

PBL-2 The unique feature of this accession was that all the 28 chromosomes were submetacentric and the 5th group was nucleolar in nature. The longest chromosome was 1.9 times longer than the shortest in the complement. The karyotypic formula was resolved into $26L+2Ln$.

PBL-3 In this accession all the chromosomes belonging to 5 groups were submetacentric in nature. However, in the remaining 2 groups 2 chromosomes were submetacentric while the remaining were either subtelocentric (3rd group) or metacentric (6th group). There were no nucleolar chromosomes in this accession. Longest chromosome was 2 times larger than the smallest in the complement. The karyotype was resolved into $2V+24L+2J$.

PBL-4 All the 28 chromosomes belonging to 7 groups of this accession were submetacentric in nature and as in case of PBL-1 and PBL-2, the 5th group comprised of a pair of nucleolar chromosomes. The largest chromosome was 1.90 times larger than the smallest chromosome in the complement. The karyotypic formula was resolved into $26L+2Ln$.

Fig. 1. Mitotic complements of seven accessions of *C. borivilianum* ($2n=28$).

Fig. 2. Photo-idiograms of seven accessions of *C. borivilianum*. Nucleolar chromosomes are marked above the short arm.

PBL-5 Four out of 7 groups of chromosome complement of this accession were submetacentric in nature. The remaining of 3 groups had either telocentric (2^{nd} group) or metacentric (3^{rd} and 6^{th} groups) chromosomes in pairs. The largest chromosome was more than 2.3 times larger than the smallest chromosome in the complement. The karyotypic formula was resolved into $4V+20L+2Ln+2I$.

PBL-6 This accession was characterized by the presence of submetacentric chromosomes only. Any other morphological type or marker chromosomes (nucleolar) were not observed. The largest chromosome was roughly 2.9 times larger than the smallest one. Karyotypic formula was simply recorded as $28L$.

PBL-7 This accession had karyotypic resemblance with that of PBL-5 in having metacentric and submetacentric chromosomes in the complement. Five out of 7 groups were submetacentric, one group (3^{rd}) was characteristically telocentric (I) in nature, 2 chromosomes of 5^{th} group were metacentric in nature and also possessed a secondary constriction showing the nucleolar nature. The length of longest chromosome in the complement was 2 times that of shortest chromosome. The karyotypic formula was resolved into $2Vn+22L+4I$.

Karyological details

A unique feature observed in various accessions of *C. borivilianum* is that all the accessions had submetacentric chromosomes (ranging from 22–28) in overwhelming proportion, while metacentric, telocentric and subtelocentrics recorded were very few in numbers (Table 1). PBL-2, PBL-4

Table 2. Category of chromosome symmetry of various accessions of *C. borivillianum*

S. No.	Accession No.	$2n$	No. of chromosomes with arm ratio $>2:1$	Ratio of longest to shortest chromosomes	Category of symmetry
1.	PBL-1	28	13	1.98	2A
2.	PBL-2	28	13	1.90	2A
3.	PBL-3	28	9	2.01	2A
4.	PBL-4	28	14	1.90	2A
5.	PBL-5	28	12	2.30	2B
6.	PBL-6	28	8	2.90	2B
7.	PBL-7	28	6	2.00	2B

and PBL-6 were characteristically devoid of any other morphological types, while the others had metacentrics (ranging from 2–4), submetacentrics (0–2) and telocentrics (0–4) per accession. The chromosome morphology with regard to particular group in the karyotypes had shown some variation only in II, III, V and VI while remaining groups in all accessions were submetacentric in nature.

Symmetry

The karyotypes of 7 accessions of *C. borivillianum* were more or less symmetrical. They were resolved into either 2A (PBL-1 to PBL-4) or 2B type (PBL-5 to PBL-7) (Table 2).

Nucleolar Chromosomes

Nucleolar chromosomes were observed in 5 out of the 7 accessions studied (Fig. 2). The satellite chromosomes revealing their nucleolar nature were found in PBL-1, PBL-2, PBL-4, PBL-5, PBL-7 leaving PBL-3 and PBL-6 with no apparent satellite chromosomes/secondary constriction in the complement. An interesting aspect associated with nucleolar chromosomes is that wherever they were recorded, nucleolar chromosomes belonged to 5th group except in case of PBL-5 where they were traced to Group 2. In all those cases where nucleolar chromosomes were recorded, predominant secondary constrictions were found.

Discussion

The somatic chromosome number of *C. borivillianum* was earlier reported as $2n=16$ (Raghavan *et al.* 1977) suggesting that $n=8$ is the basic chromosome number of the genus *Chlorophytum*. However, our present investigations showed 28 chromosomes in all the somatic cells analysed. This observation indicated that *C. borivillianum* is a tetraploid species with $2n=28$. Similar opinion was also expressed earlier (Anonymous, 2002). Baldwin and Speese (1951) and many other workers like Boraiah (1967), Mathew and Thomas (1974), Kanchan and Geeta (1977), Thomas (1960), Naik (1977a,b) and Sarkar *et al.* (1973) have also reported the occurrence of tetraploidy in the genus *Chlorophytum*, majority of them based on $x=7$ giving a series of polyploids with $2n=14, 28, 56$ etc. in various species. Our studies are in agreement with these reports. The present data together with available details indicated that the genus *Chlorophytum* is characterized by at least 2 basic chromosome numbers *i.e.* $x=7$ and 8. Polyploids of both $x=7$ (28, 42, 56) and $x=8$ (24, 32) are known in the genus. Therefore, the above view is in complete agreement with the idea of existence of 2 chromosome numbers $x=7$ and 8 in the genus *Chlorophytum*.

From the above observations it is also amply clear that polyploidy has played an important role in speciation of the genus *Chlorophytum*. The observation of 14 bivalents at diakinesis during mei-

otic cell division (Anonymous 2002), though tends us to believe that *C. borivilianum* is an allotetraploid, but in our present observations 28 chromosomes could be resolved into 7 distinct groups, thereby indicating the nature of autotetraploidy rather than allotetraploidy. We also derive support for our observations on autotetraploid nature of *C. borivilianum* from the earlier reports of Baldwin and Speese (1951) who reported occurrence of univalents and trivalents, a characteristic feature of autotetraploids, during meiotic analysis of some related species like *C. orchidastrum* and *C. viviparum*. *C. borivilianum* mostly reproduce by predominant vegetative propagation. Although seed set is known to occur in *C. borivilianum*, the most reliable method of reproduction is through vegetative root tubers. Such an obligate vegetative means of propagation may also be responsible for the occurrence and prevalence of polyploidy in *C. borivilianum* (Anonymous 2002). A genetic mechanism which possibly favors the formation of polyploidy complexes with predominant asexual reproduction by vegetative means can be considered the reason for high occurrence of polyploidy in the genus *Chlorophytum*. Autotetraploids are generally characterized by the presence of multivalent and resultant univalents. Therefore, the autotetraploid nature of *C. borivilianum* is strongly advocated.

It appears that there is an overall symmetry in majority of the chromosomes since submetacentric chromosomes have outnumbered all the types. Subtelocentric and telocentric chromosomes were negligible in proportion.

Structural changes through fusion and fission of chromosome segments involving metacentric and submetacentric chromosomes are generally believed to be the causative factors for the genesis of subtelocentric and telocentric chromosomes. Ironically, in the present investigation although submetacentrics were highest in number however, they did not generate subtelocentrics and/or telocentrics through structural alterations involving fusion and fission of chromosome segments. This tends us to believe that structural alteration of chromosome has a little role to play in speciation and evolution of the genus *Chlorophytum*.

The absence of any deviant chromosome numbers other than $2n=28$ and overall symmetry of chromosomes suggest that the diversification in the genus *Chlorophytum* at intra-specific level has occurred without any chromosome structural changes. This means mutation at gene levels including alterations of DNA sequences in the genome might have helped the evolutionary process of the genus *Chlorophytum*, which comprises of about 175 species. Therefore, it is essential to adopt modern molecular techniques like *In-situ* Hybridisation (FISH, GISH etc.) to reveal such intricate mechanisms that are operative in the evolutionary process.

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