Karyotype Analysis of *Chlorophytum kolhapurense* Sardesai, S. P. Gaikwad & S. R. Yadav: A Rare Endemic from Northern Western Ghats

Manoj Lekhak*, Avinash Asraji Adsul and Shrirang Ramchandra Yadav

Department of Botany, Shivaji University, Kolhapur-416 004, Maharashtra, India

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Summary The genus *Chlorophytum* Ker-Gawl., with about 216 species, is distributed mainly in the Old World tropics especially in Africa and India. In India 17 species represent the genus. This genus has attracted the interest of scientific community owing to the presence of pharmacologically important saponins. Recently described species, *C. kolhapurense* Sardesai, S. P. Gaikwad & S. R. Yadav was studied cytologically, the chromosome number was $2n=16$ and $n=8$ and the karyotype was symmetrical.

Key words *Chlorophytum kolhapurense*, Karyotype, Meiosis, Mitosis.

The genus *Chlorophytum* Ker-Gawl., is one of the main genera of the family Anthericaceae (Conran 1998). It comprises of about 215 species, distributed in the Old World tropics especially in Africa and India (Mabberley 2000, Govaerts 2006). Recently Malpure and Yadav (2009) added new taxa to the genus viz., *C. gothanense* Malpure & S. R. Yadav making a final count of 216. In India, the genus is represented by 17 species (modified after Karthikeyan *et al.* 1989) of which Western Ghats alone harbors 15 species.

The genus has been studied in detail from cytotaxonomic and cytogeographic points of view by Pahuja and Kumar (1969), Baldwin and Speese (1951), Sheriff (1967, 1971), Sheriff and Cheenaveeraiah (1975), Mukherjee (1975), Naik (1977a) and others (Table 1). These studies have revealed that there are 2 basic chromosome numbers in this genus, $x=7$, $x=8$ and polyploidy has certainly played active role in speciation. However, from the cytological studies of *C. laxum* (Naik 1976), it is evident that these numbers are secondary, mostly probably derived from the hypothetical original base number $x=4$, by interspecific hybridization and subsequent polyploidy. It has also been conclusively shown that $x=7$ has been derived from $x=8$ by deletion (Naik 1977b).

Chlorophytum kolhapurense Sardesai, S. P. Gaikwad & S. R. Yadav (Figs. 1a–f), is a rare endemic of northern Western Ghats. It grows in sandy soils on granite gneiss rocks in scrub forests in open ground around trees and shrubs with an altitude of about 600 m from MSL (Sardesai *et al.* 2006). *C. kolhapurense* is an unexploited plant of potential economic value. Like other medicinally important members of the same genus such as *C. arundinaceum* and *C. borivilianum*, the fasciculated horizontal roots (Fig. 1e) of *C. kolhapurense* can serve as a major source of pharmacologically important saponins. In light of above fact the present paper aims studies on chromosome number and karyotype analysis of the species.

Materials and methods

The materials, fascicled roots of *C. kolhapurense* were collected from the wild resources of northern Western Ghats. The voucher specimens are deposited in the Herbarium of Department of

* Corresponding author, e-mail: mlekhak@gmail.com

Table 1. *Chlorophytum* species and their somatic chromosome counts

S. No.	Species	2n	Authors
1	<i>Chlorophytum acaule</i> Baker	16	Sheriff and Cheenaveeraiah (1972)
2	* <i>Chlorophytum amaniense</i> Engl.	14	Jones and Smith (1967)
3	<i>Chlorophytum arudinaceum</i> Baker	28, 40, 42	Kumar and Pahuja (1967), Malla <i>et al.</i> (1978), Malla <i>et al.</i> (1979), Pahuja and Kumar (1969), Zaman and Khatun (1975)
4	<i>Chlorophytum attenuatum</i> Baker	14, 42, 84	Boraiah (1967), Sheriff and Cheenaveeraiah (1975)
5	<i>Chlorophytum bharuchae</i> Ansari, Raghavan & Hemadri	16	Naik (1974, 1977b, 1979), Raghavan <i>et al.</i> (1977)
6	<i>Chlorophytum borivilianum</i> Santapau & R. R. Fern.	16	Raghavan <i>et al.</i> (1977)
7	* <i>Chlorophytum comosum</i> (Thunb.) Jacques	28	Naik (1980)
8	<i>Chlorophytum glaucoides</i> Blatt.	42	Naik (1977b), Raghavan <i>et al.</i> (1977)
9	<i>Chlorophytum glaucum</i> Dalzell	16, 42	Raghavan <i>et al.</i> (1977), Sheriff and Cheenaveeraiah (1972)
10	<i>Chlorophytum gothanense</i> Malpure & S. R. Yadav	28	Malpure and Yadav (2009)
11	<i>Chlorophytum heynei</i> Rottler ex Baker	14, 28, 56	Mathew and Thomas (1974), Sheriff and Cheenaveeraiah (1972), Thomas, K. (1960), Thomas, S. (1960)
12	<i>Chlorophytum khasianum</i> Hook. f.	22, 56, 60	Naik (1977b), Sharma and Sarkar (1967, 1968)
13	<i>Chlorophytum kolhapurense</i> Sardesai, S. P. Gaikwad & S. R. Yadav	16	Present communication
14	<i>Chlorophytum laxum</i> R. Br.	14, 16, 32	Baldwin and Speese (1951), Boraiah (1966), Mathew and Thomas (1974), Naik (1976), Raghavan <i>et al.</i> (1977), Sheriff and Nagaraj (1971), Sheriff and Rao (1974)
15	<i>Chlorophytum malabaricum</i> Baker	42	Jones and Smith (1967), Mathew and Thomas (1974), Naik (1977b)
16	<i>Chlorophytum nepalensis</i> (Lindl.) Baker	28, 42, 50, 54, 56, 63	Basu and Jha (2008)
17	<i>Chlorophytum nimmonii</i> Dalzell	42	Raghavan <i>et al.</i> (1977)
18	<i>Chlorophytum tuberosum</i> (Roxb.) Baker	16	Dynansagar and Chowta (1969), Kumar and Rao (1958), Naik (1977b), Pahuja and Kumar (1969), Raghavan <i>et al.</i> (1977), Sheriff and Cheenaveeraiah (1972)

* Denotes species which are used as ornamentals in India.

Botany, Shivaji University, Kolhapur (SUK). For mitotic studies the root tips obtained from tubers were used after pretreatment with aqueous saturated solution of para-dichlorobenzene at 8–10°C for 4 to 5 h. Root tips were hydrolyzed in 1 N HCl and stained and squashed with 2% propionic-orcein. Suitable somatic plates were photographed with a Nikon Coolpix 4500 digital camera. Ten well-separated somatic chromosome plates were selected for karyotype analysis by adopting the method of Levan *et al.* (1964). For analysis and comparison of the karyotype, the chromosomes of *C. kolhapurense* were categorized on the basis of their length and centromeric position. The degree of karyotype asymmetry has been determined as per the categories of Stebbins (1971).

Meiotic studies were done on the pollen mother cells (PMCs). Flower buds were fixed in Carnoy's fluid (acetic acid: alcohol) (1 : 3) during morning hours. Propionic-orcein technique was used for staining the chromosomes.

Fig. 1. *Chlorophytum kolhapurens* Sardesai, S. P. Gaikwad & S. R. Yadav. (a) inflorescence, (b) flower close-up, (c) stamen, (d) gynoecium, (e) fascicled horizontal roots, (f) capsules, (g) somatic chromosome number $2n=16$, (h) PMC's showing haploid chromosome number $n=8$, (i) and (j) diakinesis showing 8 bivalents, (k) metaphase-I, (l) idiograph.

Results

Somatic chromosome number of *C. kolhapurens* is $2n=16$ (Fig. 1g). Four different types of chromosomes could be identified in the species examined karyologically (Table 3). These ranged from $6.18\ \mu\text{m}$ to $10.79\ \mu\text{m}$ in length and centromere positions varied from submedian to median. Mean chromosome length was found to be $8.19\ \mu\text{m}$. The data for karyotypic analysis are presented

Table 2. Karyotype analysis of *C. kolhapurens*

Chromosome pairs	Long arm (l) (μm)	Short arm (s) (μm)	Total length c=l+s (μm)	'd' value l-s (μm)	'r' value l/s	'i' value s/c $\times 100$	Centromere position	Type
I	7.25 $\pm$ 1.20	3.54 $\pm$ 0.43	10.79 $\pm$ 1.32	3.71	2.05	32.78	Sm	A
II	6.76 $\pm$ 0.87	3.15 $\pm$ 0.57	9.90 $\pm$ 0.96	3.61	2.15	31.76	Sm	B
III	6.24 $\pm$ 0.53	3.00 $\pm$ 0.73	9.24 $\pm$ 0.94	3.23	2.08	32.51	Sm	B
IV	5.65 $\pm$ 0.99	2.72 $\pm$ 0.32	8.37 $\pm$ 1.00	2.92	2.07	32.54	Sm	B
V	4.56 $\pm$ 0.52	2.98 $\pm$ 0.61	7.54 $\pm$ 0.88	1.58	1.53	39.55	m	C
VI	4.14 $\pm$ 0.37	2.80 $\pm$ 0.43	6.94 $\pm$ 0.59	1.35	1.48	40.30	m	C
VII	3.85 $\pm$ 0.51	2.71 $\pm$ 0.29	6.56 $\pm$ 0.65	1.14	1.42	41.31	m	C
VII	3.81 $\pm$ 0.43	2.37 $\pm$ 0.38	6.18 $\pm$ 0.48	1.44	1.61	38.32	m	D

Table 3. Types of chromosomes in *C. kolhapurens*

Types of Chromosomes	Description
Type A (Chromosome I):	A pair of long chromosomes (10.79 μm) with submedian Centromere (sm). This is the longest pair.
Type B (Chromosomes II, III and IV):	3 pairs of long chromosomes (8.37–9.90 μm) with submedian Centromere (sm).
Type C (Chromosomes V, VI and VII):	3 pairs of short chromosomes (6.56–7.54 μm) with median Centromere (m).
Type D (Chromosomes VIII):	A pair of short chromosomes (6.18 μm) with median Centromere (m).

in Table 2 and is idiographically represented in Fig. 11.

The karyotype analysis of *C. kolhapurens* can be summarized as follows: Haploid chromosome no.: $n=8$, Diploid chromosome no.: $2n=16$, Total chromosome length (TCL) μm : 6.18–10.79, Total chromosome length of haploid (TCLH): 65.52, Range of total chromosome length (TCL) %: 9.43–16.47, Karyotype formula: $2A^{\text{sm}}+6B^{\text{sm}}+6C^{\text{m}}+2D^{\text{m}}$, Karyotype symmetry: 4a.

Discussion

In India there are 6 species (including *C. kolhapurens*) which have been reported to have $2n=16$ as their somatic chromosome number (Table 1). After comparing the data on difference between longest and shortest chromosome (L–S), the ratio of longest to shortest chromosome (L/S) and TCLH value (Patil *et al.* 1987) amongst these species it can be said that *C. kolhapurens* and *C. tuberosum* come near to each other than to any other species.

The karyotype was found to be symmetrical type of 4a as per Stebbins's (1971) classification which seems to be comparatively more advanced to the 2a grade of *C. tuberosum*. Similarly, *C. attenuatum*, *C. comosum* and *C. laxum* exhibit 2a grade. The other grades common in *Chlorophytum* species are 2b (*C. glaucum* and *C. orchidastrum*=*C. nimmonii*, Patil *et al.* 1987) and 3b (*C. arundinaceum*, Pahuja and Kumar 1969). The detailed karyotype analysis of *C. kolhapurens* showed that, out of the 8 pairs of chromosomes 4 pairs have submedian centromere while other 4 pairs possess median centromere. Subterminal chromosomes are relatively more in *C. nimmoni*, *C. glaucum*, *C. glaucoides*, *C. khasianum*, *C. nepalense* and *C. comosum* (Patil *et al.* 1987, Naik 1977a). In case of *C. malabaricum* and *C. tuberosum* only 1 pair has subterminal centromere while in *C. bharuchae* all chromosomes have submedian centromere (Naik 1977b).

Apart from this, as proposed by Naik (1977) species with $x=8$ are adapted to arid or semi-arid conditions, it can be said that *C. kolhapurens* is not an exception to it, which occurs on hilly tracts of some parts of Maharashtra and Karnataka.

However, Naik (1976), reported following abnormalities in the genus *Chlorophytum* are: 1) multivalent association, 2) unoriented bivalent at Metaphase I and II and Anaphase I and II, 3) non-disjunction of the entire complement while in case of *C. tuberosum* occasionally unoriented bivalents were observed. Meiosis was found to be normal as PMCs showed (Figs. 1h–k) 8 bivalents with very few anomalies.

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