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ORIGINAL ARTICLE

Karyomorphological analysis of five species of *Murdannia* Royle (Commelinaceae), including two endemics to India

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Abstract

The study examines the karyotype and somatic chromosome numbers of five species of *Murdannia* Royle (Commelinaceae). The karyotype details of the two species namely *M. blumei* and *M. lanceolata* endemic to India are reported for the first time. The karyotype formula of the examined species are: *M. blumei* - 2*n* (36) = 1M+12m+5sm; *M. lanceolata* - 2*n* (20) = 2m+8sm; *M. crocea* subsp. *ochracea* - 2*n* (36) =7m+8sm+3st; *M. spirata* - 2*n* (40) = 9m+8sm+3st and *M. triquetra* - 2*n* (40) = 16m+4sm. Further details on karyomorphology including estimates of asymmetry indices, total form percent, ratio of mean length of short arms to long arms, intrachromosomal / interchromosomal asymmetry indices, and centromeric indices are provided. The karyomorphological parameters thus analysed suggest that *M. blumei* and *M. lanceolata* fit into the 2A category while *M. crocea* subsp. *ochracea, M. spirata* and *M. triquetra* belonged to the 2B category of Stebbins' classification.

Keywords *Murdannia* · Chromosome · Ideogram · Karyotype · Mitosis

Introduction

Murdannia Royle is one of the largest genera of family Commelinaceae consisting of about 60 species worldwide [13, 32]. It is one of the only six genera of the family that has native species in both the Old World and the New World [11], with its centre of diversity in India [2, 32] representing 52% of the global diversity. Twenty four species of *Murdannia* have been studied for chromosome number till date [39].

Previous reports indicated the basic chromosome numbers, x = 6, 9, 10, 11, 12 [6, 18, 23, 41, 35, 24, 33, 34] for *Murdannia*. Different authors reported various numbers: 2*n* = 18 [18, 42], 40 [15] and 42 [33] for *M. edulis* (Stokes) Faden, 2*n* = 20 for *M. assamica* Nampy & Ancy [29], *M. dimorpha* (Dalzell) G.Brückn. [44, 18], *M. esculenta* (Wall. ex C.B.Clarke) R.S.Rao & Kammathy [18], *M. hookeri* (C.B.Clarke) G.Brückn. [19], *M. nudiflora* (L.) Brenan [1, 5, 38, 17, 25] and *M. striatipetala* Faden [29], 2*n* = 36 for *M. crocea* subsp. *ochracea* (Dalzell) Faden [35, 36]. The smallest chromosome number, *n* = 6 is reported for *M. semiteres* (Dalzell) Santapau [19, 44] while *n* = 12 also reported for the same species as well as *M. juncoides* (Wight) R.S.Rao & Kammathy [19]. An uncommon number, 2*n* = 22 was reported for *M. gigantea* (Vahl) G.Brückn. by Rao et al. [36] and Panigrahi and Kammathy [30]. Rao et al. [36] described 2*n* = 64 and 42 for *M. japonica* (Thunb.) Faden. Most species investigated previously have *n* = 10 or its multiples [36]. According to Lewis [23], the basic number for *Murdannia* is x = 5, which occurs as a dominant line of x = 10, and basic numbers like x = 9 and 11 may have been formed subsequently from the dominant line, x = 10. *M. zeylanica* exhibits a bimodal complement with an essentially equal number of chromosomes of two sizes [10].

During our study we found that karyomorphological data of some species of *Muradannia* are not yet reported. In this study, the karyomorphological analysis of *M. blumei* (Hassk.) Brenan, *M. lanceolata* (Wight) Kammathy, *M. crocea* subsp. *ochracea, M. spirata* (L.) G.Brückn. and *M. triquetra* is done, of which, *M. blumei* and *M. lanceolata* are studied for the first time.

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Materials and methods

The samples were collected from different parts of India and grown in the Calicut University Botanical Garden (details provided in Table 1). The voucher specimens were deposited in the Calicut University Herbarium (CALI).

Karyomorphological studies were done using mitotic squash preparation. Five metaphase cells were counted for finalizing the chromosome number for each species. Root tips of 5–8 mm length were pre-treated in saturated solution of *para*-dichloro benzene (PDB) mixed with 1% saponin (1:0.01) at 12–16°C for 3 h, followed by washing in distilled water and fixed in modified Carnoy's fluid (4 chloroform: 3 ethanol: 1 glacial acetic acid) at room temperature for about 3 h. Root tips were hydrolysed in 1.2 M HCl for 15–20 min at room temperature. They were then stained with saturated solution of aceto-orcein. Micro slides were prepared in 45% glacial acetic acid and photographs were taken with a DMC 4500 camera attached to DM 2000 compound microscope (Leica, Switzerland). Chromosomes were identified and classified based on the length of chromosome, and centromeric indices according to Levan et al. [22]. Number of chromosomes was finalized by counting five somatic cells and karyotype analyses were based on three mitotic metaphase preparations of each species. Karyotypic formula was based on position of centromere and number of chromosomes [22]. Total form percentage (TF%) [16], dispersion index (DI) [21], karyotype asymmetry index (As K%) [3], syi index [14], rec index [46] intrachromosomal asymmetry index (A1), interchromosomal asymmetry index (A2) [48] and asymmetry index (AI) [31] were calculated.

Results

Murdannia blumei is easily recognized by its axillary lilac flowers, hardly exerted from the sheath, and biseriate arrangement of seeds in each capsule locule (Fig. 1a). It has a somatic chromosome number of $2n = 36$ (Fig. 1b). The shortest chromosome was 1.03 µm and the longest 1.97 µm. The average chromosome length (ACL) was 1.50 µm and the total haploid chromosome length 27.09 µm. *M. blumei* had 26 metacentric (M and m) and 10 submetacentric (sm) chromosomes with a karyotype formula of 2M+24m+10sm. The karyogram is shown in Fig. 2a.

Murdannia lanceolata is distinguished by its quite larger flowers and numerous small seeds arranged biseriately in each capsule locule (Fig. 1c). It showed $2n = 20$ as somatic

Table 1 Voucher information of the species investigated

Name of species	Locality	Coordinates	Altitude in m	Voucher
M. blumei	North Lakhimpur, Assam	27°14'14.77" N, 94°10'48.85" E	61.6	CALI 167953
M. crocea* subsp. *ochracea	Madayippara, Kannur district, Kerala	11°58'34.1" N, 75°18'78.3" E	43	CALI 158929
M. lanceolata	Thiruchirappilly, Tamil Nadu	10°40'8.19" N, 78°44'6.2" E	73.4	CALI 167974
M. spirata	Calicut University Botanical Garden, Kerala	11°8'15.2" N 75°53'24.2" E,	75	CALI 158901
M. triquetra	Boichagarumaria, Assam	27°13'45.05" N, 94°8'57.47" E	90.2	CALI 167951

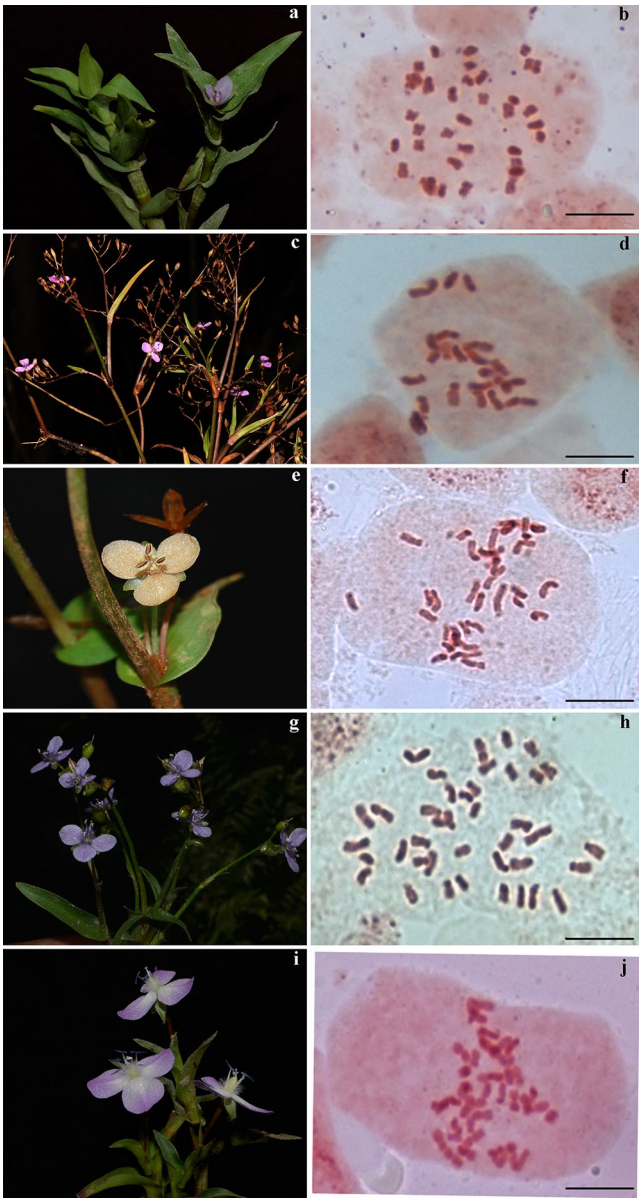

Fig. 1 Habit and somatic metaphase chromosome of: a and b *Murdannia blumei*, c and d *M. lanceolata*, e and f *M. crocea* subsp. *ochracea*, g and h *M. spirata*, i and j *M. triquetra* (scale bar = 10 µm)

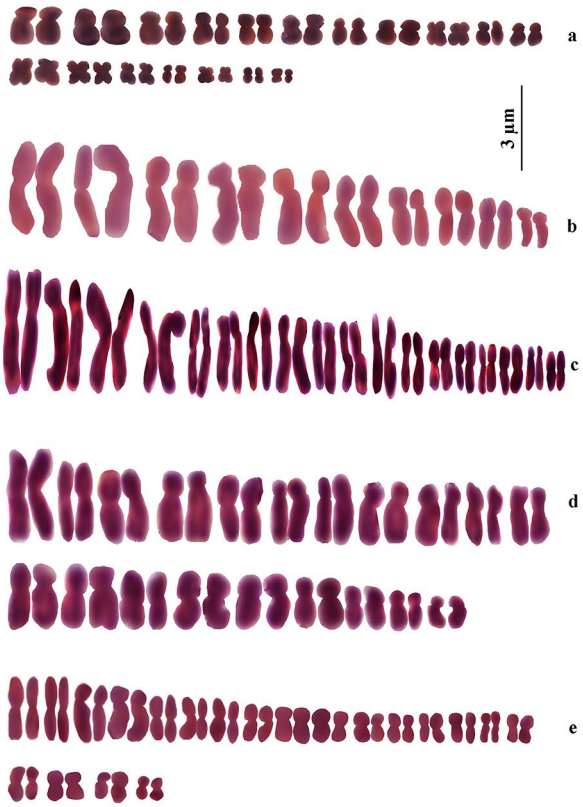

Fig. 2 Karyograms of: a *Murdannia blumei*, b *M. lanceolata*, c *M. crocea* subsp. *ochracea*, d *M. spirata*, e *M. triquetra*

chromosome number (Fig. 1d). The range of chromosome length was 1.79–3.15 µm. The average chromosome length (ACL) was 2.3 µm and the total haploid chromosome length of 23 µm. It had 4 metacentric (m) and 16 submetacentric (sm) chromosomes with a karyotype formula of 4m+16sm. The karyogram is given in Fig. 2b.

Murdannia crocea subsp. *ochracea* is endemic to South India and readily identified by its ochre flowers, obovate–orbicular petals and biseriately arranged seeds in the capsule locule (Fig. 1e). It had $2n = 36$, and a haploid number, $n = 18$ (Fig. 1f). The range of chromosome length lay between 1.17 and 4.56 µm while the average chromosome length was 2.72 µm and the total haploid chromosome length 49.06. Median (m), sub-median (sm) and sub-terminal (st) centromeric chromosomes are with a karyotype formula 14m+16sm+6st. The karyogram is given in Fig. 2c.

Murdannia spirata is a widespread species in India, easily recognized by its axillary or terminal thyrse inflorescence with 1–3 alternate cincinni having lilac to lavender flowers (Fig. 1g) while *M. triquetra* is distributed from Assam to China and Indo-China. The latter is readily identified by its white–lavender petals, uniseriate arrangement of seeds in the capsule locule and a recurved fruiting pedicel (Fig. 1i). Both the species showed $2n = 40$ with a basic chromosome number x = 10 and haploid number $n = 20$ (Fig. 1 h and 1j). The range of chromosome length of *M. spirata* was between 1.74 and 3.58 µm and the average chromosome length 2.30 µm while the total haploid chromosome length 46.16. Median (m), sub-median (sm) and sub-terminal (st) centromeric chromosomes are found with a karyotype formula 18m+16sm+6st. *M. triquetra* has chromosome length ranging from 0.73 to 2.58 µm with an average of 1.63 µm and the total haploid chromosome length 32.64. Median (m) and sub-median (sm) centromeric chromosomes were found with a karyotype formula 32m+8sm. Their karyograms are given in Fig. 2d and e, respectively.

Considering Stebbins [45] definition of symmetry, the karyotypes of *M. blumei* and *M. lanceolata* fit into the 2A category while *M. crocea* subsp. *ochracea, M. spirata* and *M. triquetra* belonged to the 2B category as the relation between the longest and shortest arms was lower than 2:1.

Discussion

Chromosome numbers of 24 species of *Murdannia* have been reported so far [39]. The size of chromosomes in this genus is particularly small and the numbers vary from $n = 6$ to 30 [10]. Most species studied previously showed x = 10 or its multiple, though other number such as x = 9, 11, 12 had also been recorded [6, 18, 35, 23, 24, 26, 39].

In this work, we reported the chromosome numbers of five species of *Murdannia* from the root tips: $2n = 36$ for *M. blumei* and *M. crocea* subsp. *ochracea*, $2n = 20$ for *M. lanceolata* and $2n = 40$ for *M. spirata* and *M. triquetra*. Based on our study, in an evolutionary line there are eight species with axillary fascicled inflorescence reported from India [2, 8, 28]. Among them, *M. blumei, M. crocea* subsp. *ochracea*, *M. versicolor* and *M. pauciflora* have somatic chromosome numbers $n = 18$, 18, 9 and 9, respectively with basic chromosome number x = 9. Whereas, *M. triquetra, M. lanuginosa* and *M. keisak* showed, $n = 20$, 10 and 10, respectively, with a basic chromosome number x = 10. *M. sanjappae* is another axillary flowered species whose chromosome number is not available. From an evolutional conception it is surmised that low basic numbers had given rise to higher ones, and the taxa with variable chromosome number are considered young and still in evolutionary flux [40, 47]. In another evolutionary line *M. lanceolata* and *M. spirata* with terminal or axillary thyrse having one or two opposite cincinni grouped with *M. dimorpha* and *M. striatipetala* [2]. The chromosome number for *M. lanceolata* is reported for the first time ($2n = 20$). Different chromosome counts were reported for *M. spirata*: $2n = 18$ [35]; $2n = 20$, 40 [37]; $2n =$

40 [18] and $2n = 24$ [20] while our results corroborate with Kammathy and Rao [18]. For *M. dimorpha* and *M. striatipetala*, the chromosome number is $2n = 20$ [18, 29, 44]. Thus, basic chromosome number is concluded as x=10 for this group, with two exceptions $n = 9$ and 12 for *M. spirata*.

The karyotype analysis revealed that all the species have small sized chromosomes. Among them the largest chromosome size was observed in *M. crocea* subsp. *ochracea* (2.72 µm) and the smallest in *M. blumei* (1.50 µm). Karyotypes of three species possess predominantly median chromosomes and remaining possesses submedian chromosomes. *M. crocea* subsp. *ochracea* and *M. spirata* showed subtelocentric chromosomes. The primitiveness of karyotype is expressed by the presence of symmetrical karyotype, having longer chromosomes, median centromere with chromosome arms of equal size and low basic chromosome number, while more advanced karyotype depicts asymmetrical karyotype having, shorter chromosomes, submedian or other centromere, unequal length of chromosome arms and higher basic chromosome numbers [45, 47].

The karyotype asymmetry was assessed based on fourteen parameters given in Table 2. In which TF%, Syi, A1, A2 and AI have been formulated to evaluate the variation in centromere position in a chromosome complement. The total chromosome length (TCL) varied from 23 to 49.06 µm and the highest and the lowest values are present in *M. lanceolata* and *M. crocea* subsp. *ochracea* respectively. The A1 index was used to estimate karyotype asymmetry for the relationships between the chromosomal arms, with values ranging from zero to one. The A1 index is unaffected by the number of chromosomes or their size. The A1 index obtained for *M. blumei* is 0.29, for *M. lanceolata* 0.47, *M. crocea* subsp. *ochracea* 0.43, *M. spirata* 0.42 and *M. triquetra* 0.26. The A2 index is also represented as 0.17, 0.20, 0.30, 0.22 and 0.30, respectively. The lowest value for A1 and A2 indicates there is a small difference in length of chromosome arms and least variation in chromosome length, whereas highest value of A1 and A2 indicates greatest difference in length of chromosome arm and large variation in chromosome length. Low values of TF%, Syi and Rec indices and increased value of AsK%, A1, A2, AI indicates asymmetry of chromosomes. *M. crocea* subsp. *ochracea* has lower TF% and higher AsK% (34.49, 65.50 respectively), indicating an asymmetrical karyotype than the others (Table 2). This information is also supported by high A1, A2 and AI values indicating the existence of asymmetrical karyotypes. *M. blumei* which showed high value for TF%, Syi index, Rec index and low value for A1, A2, AI and DI indicated the symmetry of chromosomes than the rest. Dispersion index which facilitates quantitative gradation between the closely related karyotypes, that fall under the same class of karyotype asymmetry and its higher value indicate higher level of karyotype specialization [21]. *M. crocea* subsp. *ochracea, M. spirata* and *M. triquetra* falls under 2B category of asymmetry. *M. triquetra* showed highest DI value than *M. crocea* subsp. *ochracea* and *M. spirata*. Based on the DI value, *M. triquetra* and *M. crocea* subsp. *ochracea* are highly specialized in their karyotype. *M. spirata* also showed asymmetry in their karyotype formula, due to the presence of subterminal chromosomes and this information is supported by all the morphometric analysis. The information generated from the study of karyotype constitution, chromosome number when combined with morpho-taxomonic features could help differentiate and elucidate species affinities, for which data on more species is desirable to arrive at a meaningful inferences [21, 47].

Table 2 A comparison of karyotype and asymmetry indices of the species studied

	Karyotype & asymmetry indices	*M. blumei*	*M. lanceolata*	*M. crocea* subsp. *ochracea*	*M. spirata*	*M. triquetra*
1.	Chromosome number $2n$	36	20	36	40	40
2.	Karyotype formula	1M+12m+5sm	2m+8sm	7m+8sm+3st	9m+8sm+3st	16m+4sm
3.	Range of chromosome length (RCL µm)	1.03–1.97	1.79–3.15	1.17–4.56	1.74–3.58	0.73–2.58
4.	Total chromosome length (TCL µm)	27.09	23	49.06	46.16	32.64
5.	Average chromosome length (ACL µm)	1.50	2.3	2.72	2.30	1.63
6.	TF value (%)	40.55	34.66	34.49	35.77	41.79
7.	Karyotype asymmetry index (As K%)	59.44	65.33	65.50	64.22	58.20
8.	Intrachromosomal asymmetry index (A1)	0.29	0.47	0.43	0.42	0.26
9.	Interchromosomal asymmetry index (A2)	0.17	0.20	0.30	0.22	0.30
10.	Asymmetry index (AI)	2.45	3.06	7.31	4.92	3.41
11.	Stebbins type	2A	2A	2B	2B	2B
12.	Sci Index	67.41	52.66	52.24	55.40	69.38
13.	Rec index	38.19	36.50	29.88	32.23	31.62
14.	Dispersion Index (DI)	6.68	6.58	10.75	7.35	12.10

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Declarations

Conflict of interest Authors declare that there is no conflict of interest.

Consent for publication: Authors approve of publication.

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