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ORIGINAL ARTICLE

Comparative karyo-morphology of the two endemic and critically-endangered species of *Mantisia* (Zingiberaceae)

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Abstract Karyo-morphological studies have been carried out in two critically rare, endangered and endemic rhizomatous, horticulturally important species of genus *Mantisia* (Zingiberaceae) from North-East India. The somatic chromosome number (2n=20) has been recorded in both *M. spathulata* and *M. wengeri* with distinct inter-specific variation in the arm ratio of homologous pairs in the complements. All the chromosomes were sub-metacentric in nature (20 L) and more or less symmetrical karyotypes in both the species indicating stabilized genomic pattern. In *M. wengeri* two chromosome pairs exhibited heteromorphy. Nucleolar organizing region (NOR) and meta-, telo-, and sub-telocentric chromosomes were absolutely lacking. Chromosome class based on total length of each chromosome as well as its designation through centromeric position was determined. This karyo-morpological study provides valuable baseline genetic data for clearly distinguishing *Mantisia* (x=10) from its phenotypically very similar allied genus *Globba* L. (x=8). Thus, present investigation provides valuable insight on karyotypic variation, speciation and evolution of the genus *Mantisia* and also addresses the conservation concerns.

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Abbreviations

NOR	Nucleolar organizing region
FISH	Fluorescent in situ hybridization
McFISH	Multi-color FISH
SPAR	Single primer amplification reaction

Introduction

The genus *Mantisia* Sims. [26], is endemic to hilly areas of the northeastern India, Myanmar and Bangladesh [1, 4, 19, 30]. It comprises only five species i.e. *Mantisia wengeri*, *M. radicalis*, *M. spathulata M. wardii* and *M. salarkhanii*. Commonly known as '*dancing girl*', *Mantisia* species are annual/perennial herbs [1]. The aerial shoots of these species appear after the development of inflorescence with numerous splendid flowers [19]. *M. spathulata* and *M. wengeri* are two critically-endangered species endemic to Mizoram, a northeastern state of India [1]. Due to natural calamities and anthropogenic activities, *M. spathulata* and *M. wengeri* have become critically-endangered in the natural habitat and are listed in the Red Data sheet [1–3, 6, 23, 24, 30]. The rarity of *Mantisia* species has reached a critical level and has been included in the national priority list for their recovery by the Department of Biotechnology, New Delhi, India [1, 30].

Karyo-morphological analysis is a useful method for characterizing plant chromosomes and genome organization. The structure and morphology of the chromosome is of vital importance when studying the origin, evolution and classification of taxa [33]. From the perusal of published literature, it is amply clear that while the genus *Globba* of Zingiberaceae has received overwhelming attention of cytogeneticists, its

beleaguered cousin *Mantisia* has not received due consideration from scientists either from Asian region or abroad, which may be due to the rarity of occurrence and endemism of the genus. The main differences separating *Globba* and *Mantisia* have traditionally been inflorescence position and lateral staminode position [26]. The complex nomenclatural history of *Mantisia* is summarized by Dam et al. [4]. Williams et al. [31] found *Mantisia* to be nested with *Globba* using molecular data, and it is considered along with *Globba* under the same tribe Globbeae but in different sections *Mantisia* and *Globba*, respectively. Cytogenetical studies conducted on *Globba* and *Mantisia* [9–15, 17] are limited confined to just enumeration of basic/somatic chromosome numbers. These reports suggested that $x=8$ is the base number for *Globba* and $x=10$ for *Mantisia* and proposed a revised classification of the tribe Globbeae and even the family Zingiberaceae. Except these reports, no other information is available for its genome constitution and organization dealing with the karyo-morphological and meiotic details. Only a few reports on phylogenetic analysis of *Mantisia* and its closely related genus *Globba* [7, 8, 28, 29, 31] are available till date. Genetic variation analysis using single primer amplification reaction (SPAR) methods besides attempts for conservation through rapid in vitro clonal propagation, seed germination have been made by our research group earlier [1–3, 23, 25, 30].

Rarity of the genus *Mantisia* has reached to such a level that only few representatives are available. Thus, there is an urgent need for thorough exploration and characterization of the available genetic resources of both *M. spathulata* and *M. wengeri*, so as to accomplish their sustainable conservation and utilization. It is also necessary to investigate plausible cytogenetical mechanisms including karyo-morphological studies leading to rarity and endangeredness of endemic *Mantisia*.

Materials and methods

The plant materials of *Mantisia spathulata* and *M. wengeri* were collected from their natural habitat from Lunglei (Mizoram) and potted in glass house at North-Eastern Hill University, Shillong (Meghalaya). For obtaining actively growing root tips, plants were raised in earthen pots and the root tips 0.5–1.0 cm long were excised and pretreated with saturated solution of ρ-dichlorobenzene for 3 h at room temperature followed by fixation in freshly prepared Carnoy's fluid for 24 h. Root tips were hydrolyzed with 5 N HCl for 30 min at room temperature and stained in 1 % leucobasic fuchsin. The stained tips were squashed in 1 % aceto-carmine and the micro-photographs were taken using *Jenoptik* CCD camera (Germany) attached to *Labomed* LX 400 fluorescent microscope. For each species, a minimum of 50 metaphase plates were analyzed out of which, at least five clear preparations of chromosome complements of each species were selected for karyotyping. Karyotypes were prepared from photomicrographs by cutting out individual chromosomes, arranging them in descending order of their total length and matching on the basis of morphology. The measurement of chromosome arms were made using Micro-Measure V. 3.3 software and centromeric position was deduced from the generated data. Mean of long to short arm ratio (L/S) of each chromosome pair along with ratio of longest to shortest chromosome length in the karyotype was also determined. The standard method of chromosome classification was used for comparison. The degree of symmetry was estimated as per the scheme proposed by Stebbins [27].

Results

Both *M. spathulata* and *M. wengeri* had shown the occurrence of $2n=20$ chromosomes in all the root tip cells analyzed which were clearly resolved into 10 pairs of homologous chromosomes, forming a series from the longest to shortest pair within the complement (Table 1, Fig. 1a–d). One notable feature of the present study was lack of distinct nucleolar chromosomes in the complements in either of the two species presently investigated. In *M. spathulata*, the longest chromosome in the complement was more than two and half times longer than the smallest one. However, in *M. wengeri*, the longest chromosome was only two times longer than the smallest one in the chromosome complements (Table 1). Both *M. spathulata* and *M. wengeri*, characteristically had ten homologous pairs comprising of only sub-metacentric chromosomes. Variation was recorded with respect to presence or absence of heteromorphic pairs in the chromosome complements in both the species of *Mantisia* (Table 1). Two out of ten, chromosome pairs viz. II and X were found to be heteromorhic in *M. wengeri*, whereas not a single pair of the chromosome was found to be heteromorphic in nature in case of *M. spathulata* (Table 1, Fig. 1a–d). The karyotype formula was 20 L for both *Mantisia* species (Table 1). Recently, our group [25] has also carried out the meiotic analysis in *M. spathulata*, which collectively revealed the synaptic variation but there was no deviation for chromosome number.

Discussion

Present observations support the view expressed by Williams et al. (2004) confirming $2n=20$ as the somatic chromosome number reported for the genus *Mantisia*. It also tends us to speculate $x=10$ as the basic chromosome number for the genus *Mantisia*. A closely related genus *Globba* has been found to exhibit $x=8$ in its entire native species. Thus, the authors support the phylogenetic studies of *Mantisia* which is different from the genus *Globba*, with $x=10$ as its true basic

Table 1 Karyo-morphology and arm ratio in *Mantisia* species

Taxa	2n	Mean arm ratio (L/S) in chromosome pairs										Karyo-typic formula	Chromosome length (µm)		X/Y	Category of symmetry	Chromosome class and designation
		I	II	III	IV	V	VI	VII	VIII	IX	X		Longest (X)	Shortest (Y)			
M. spathulata	20	1.81 L	1.30 L	1.43 L	1.11 L	1.98 L	1.83 L	1.20 L	1.21 L	1.23 L	1.32 L	20 L	4.24	1.71	2.48	2B	2smA+6smB+9smC+3smD
M. wengeri	20	1.26 L	1.15 L	2.14 L	1.59 L	1.28 L	1.48 L	1.27 L	2.13 L	1.33 L	1.20 L	20 L	3.57	1.68	2.12	2B	3smB+15smC+2smD

Heteromorphic pairs are marked with *underlined* value(s)

Chromosome class based on total length of chromosome: A. more than 4 µm–5 µm, B. more than 3 µm–4 µm, C. more than 2 µm–3 µm, D. more than 1 µm–2 µm

Chromosome designation based on centromere position determined through standard method

number. This karyo-morpological study would provide valuable baseline genetic data for clearly distinguishing *Mantisia* (x=10) from its phenotypically very similar allied genus *Globba*.

The symmetry of karyotypes in *Mantisia spathulata* and *M. wengeri* were resolved into 2B category and are indicative of stabilized genome. Genetic variation was recorded in terms of ratio of longest to shortest chromosome length, presence or absence as well as number of heteromorphic pair(s) in both the species. Such variation in chromosome length and heteromorphism could be due to minor chromosome structural changes, but not leading to gross genomic alterations/modifications. These minor genetic modifications occurring in *M. wengeri* apparently did not influence the stability of genome but has resulted in asymmetrical karyotype. Moreover, analysis of karyotype characteristics has contributed valuable data for inferring evolutionary trends within particular plant groups. Karyology may also be utilized to study several genetic traits such as changes in chromosome numbers, length, and symmetry etc. [18]. Such observations are in line of our view that more or less stability of genome as inferred by karyo-morphological details might be the plausible reasons for rarity and endemism of the genus *Mantisia*. Such low variation was also observed at DNA level through molecular characterization using SPAR methods which collectively revealed low genetic diversity in both *M. spathulata* and *M. wengeri* [23]. Similarly, meiotic analysis in *Mantisia* species [25] revealed the fact that most of the pollen mother cells (PMCs) analyzed were either at

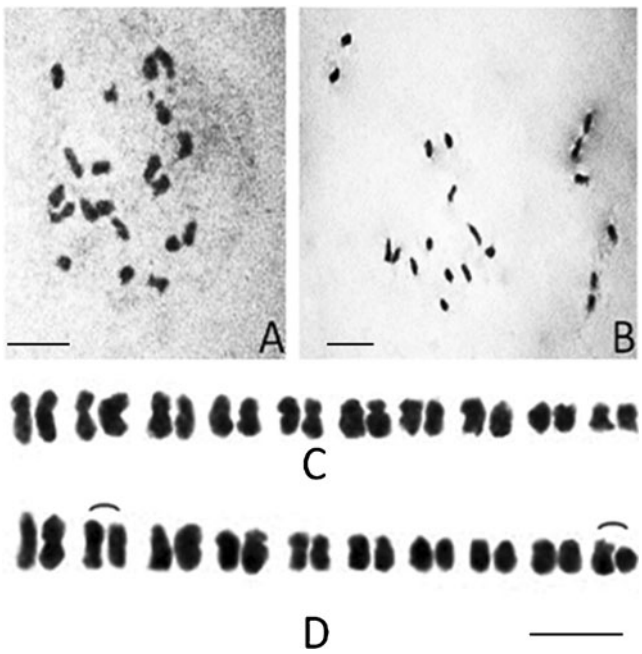

Fig. 1 Mitotic complements and karyotypes of *Mantisia* species: **a** *M. spathulata*, 2n=20; **b** *M. wengeri*, 2n=20; **c** photo-idiogram of *M. spathulata*; **d** photo-idiogram of *M. wengeri*. Heteromorphic pair marked above the short arm, scale bar=10 µm

diplotene or diakinesis/metaphase I and did not exhibit the expected chromosome associations of 10II, which is indicative of synaptic variation. The imbalanced meiotic events characterized by anomalous pattern of chromosome distribution including unequal division and/or presence of laggards in the form of univalents/bivalents resulting in low pollen stainability may be responsible for loss of genetic variation in *Mantisia* species and considered as a substantial cytogenetical basis leading to the rarity and endangeredness of the species [25]. Other factors like, low seed germination [2], squat cross pollination, insect dependent pollination [1], low karyomorphological variation, presence or absence of either heteromorphic pair in complements as well as imbalanced meiotic events with variant synaptic behavior of bivalents [25] also depict the confirmation of rarity and endangered status of genus *Mantisia*. A large and significantly excess number of endangered species and populations used to have low level of genetic variation [5]. Rare and endangered species are susceptible to loss of genetic variation through genetic drift in small populations [32] including inbreeding depression as well as out-breeding complicacies, accumulation of deleterious mutations, genetic adaptation to domestication. All these factors contribute to rarity, endangeredness and endemism, ultimately leading to extinction of the plant species.

Modern molecular cytogenetical techniques i.e. fluorescent in situ hybridization (FISH) and multi-color FISH (McFISH) could be more appropriate for detection of satellite chromosome with a use of ribosomal DNA specific probes as reported in various plant species and can reveal more details of chromosome structure, behavior and evolution [16, 20–22]. However, the present investgation provides a significant insight into karyotypes *vis-à-vis* speciation and evolution of the genus *Mantisia* confirming the distinct phylogenetic status as determined by cytogenetic approaches for *Mantisia-Globba* complex in the family Zingiberaceae. It also addresses the conservation concerns about extinction risk of this horticulturally important rhizomatous herb.

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