Spectrum of Chromosome Associations in Synaptic Variants of *Mantisia wengeri* (Zingiberaceae)—An Endemic, Critically-Endangered and Probable Inter-Specific Hybrid

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Summary Male meiotic studies have been conducted in a synaptic variant of *Mantisia wengeri*, a critically—endangered and endemic rhizomatous herb of north-east India. Studies revealed synaptic variation of the chromosomes and the only 10% of pollen mother cells (PMCs) showed the normal meiotic pattern, while 90% of PMCs demonstrated abnormal meiotic behavior by showing a spectrum of chromosome associations. A total of 747 bivalents along with a good number (506) of univalents were recorded with moderate chiasma frequency (14.94 ± 9.20) with the terminalization coefficient of 0.82 only. About 40% of PMCs showed anomalous distribution patterns of chromosomes, including unequal distribution and/or presence of laggards in the form of univalents/bivalents resulting in low pollen-stainability (57.33 ± 8.40). A comparative analysis of synaptic behavior of chromosomes during meiosis in *M. wengeri* and *M. spathulata* was carried out. The role of genomes *vis-à-vis* the chromosome behavior of *M. spathulata* and *M. radicalis* in the development of the crossbreed *M. wengeri* has been discussed. Unbalanced chromosome associations with partial synaptic failure are considered as causative factors for sustained diversity in critically-endangered and endemic genus *Mantisia*.

Key words Univalents, Synaptic variants, Inter-specific Hybrid, Laggards, Rare, Endangered species.

The genus *Mantisia* Sims., which belongs to the family Zingiberaceae, is endemic to hilly areas of the north-eastern India, Myanmar and Bangladesh (Dam *et al.* 1997, Rahman and Yusuf 2002, Tandon *et al.* 2007, Bhowmik *et al.* 2009, 2011). Comprising only 4 medicinal and horticultural important species, namely *Mantisia wengeri*, *M. radicalis*, *M. spathulata* and *M. wardii*, it represents the smallest genus of family Zingiberaceae. Rahman and Yusuf (2002) have recently added a new species, specifically *M. salarkhanii* from Bangladesh.

Mantisia wengeri is a highly fascinating zingiber, commonly known as ‘dancing girl’. It is endemic to a single population in Lunglei, Mizoram, North-East India (Bhowmik *et al.* 2011). It is suspected to be a hybrid of *M. spathulata* and *M. radicalis* (Dam *et al.* 1997). The rhizome of this plant is used by local people as a remedy for bone fractures and gastrointestinal ailments. Due to natural calamities and anthropogenic activities, the rarity of *M. wengeri* has reached a critical level, so that only a few (60–70) plants are now available in their natural habitat (Bhowmik *et al.* 2009, 2010a, b, 2011). *M. wengeri* has been listed in the Red Data sheet of rare and endangered Indian plants and therefore has been included in the national priority list for species recovery by the Department of Biotechnology, New Delhi, India (Ganeshaiah 2005).

From a perusal of published literature, it is amply clear that the genus *Mantisia* has not re-

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ceived much attention from scientists worldwide, which may be due to the rarity and endemism of the genus. Few reports are available for the phylogenetic analysis related to *Mantisia* and its allied genus *Globba* (Takano and Okada 2001, Kress *et al.* 2001, 2002, Williams *et al.* 2004). Combining the molecular data from ITS and trnK-matK sequences, Williams *et al.* (2004) found *Mantisia* merged with *Globba*. Such observations were also depicted in numerous cytogenetical studies conducted on *Globba* and *Mantisia* (Larsen 1964, Larsen *et al.* 1998, Lim 1972a, b, c, 1973a, b, Newman and Jong 1986, Takano 2001). Williams *et al.* (2004) added that $x=8$ is the basic number for *Globba* and $x=10$ for *Mantisia* and proposed a revised classification for the tribe Globbeae. Attempts for conservation through rapid *in vitro* clonal propagation as well as seed germination were made by our research group (Tandon *et al.* 2007, Bhowmik *et al.* 2009, Bhowmik *et al.* 2010a, b). *In vitro* raised plantlets of both the species have, after ensuring genetic fidelity, been re-introduced into their natural habitat for conservation under the species recovery program (Bhowmik *et al.* 2009). Male meiotic analysis in synaptic variants of *Mantisia spathulata* and genetic variation analysis using single primer amplification reaction (SPAR) methods in both *Mantisia spathulata* and *M. wengeri* have also been reported recently by our research group (Sharma *et al.* 2011a,b).

Despite having deduced the rationale for the rarity of the genus *Mantisia*, the cyto-taxonomy including chromosome counts, karyo-morphology, meiotic details and genetic control of chromosome behavior of *M. wengeri* still remain a matter of utmost interest and importance. In line with our earlier report on the genus *Mantisia*, male meiotic studies have been conducted with the sole objectives of comparing and comprehending chromosomal associations, chiasma frequency as well as terminalization coefficient, and chromosome distribution at anaphase I/II in both *M. wengeri* and its allied species *M. spathulata*. The data generated by meiotic analyses revealed a variety of chromosome behavior exhibiting a deviant pattern in chromosome associations and rearrangements leading to abnormal meiotic blueprints. The present paper deals with the meiosis of this partially desynaptic variant of *M. wengeri vis-à-vis M. spathulata* (Sharma *et al.* 2011a), which has some bearing on chromosomal behavior with a focus on synaptic events during various stages of prophase I. To the best of our knowledge, this is the first report on occurrence of natural synaptic variants of 2 species of a genus.

Materials and methods

Plant samples of *M. wengeri* were collected randomly from the naturalized population of the experimental garden located at North-Eastern Hill University, Shillong (Meghalaya). Flower buds of appropriate size were collected during the flowering season, *i.e.* May–June. Details regarding collection of flower buds, staining, chromosome preparation, micro-photography and pollen-stainability have been described elsewhere (Sharma *et al.* 2011a).

Results

Detailed male meiotic analyses of a natural synaptic variants of *M. spathulata* have already been published elsewhere (Sharma *et al.* 2011a).

The present cytological investigations revealed the diploid chromosome number of *M. wengeri* to be $2n=20$ with the observation of 10 bivalents at diplotene/diakinesis/metaphase I during meiotic analyses. Detailed analysis of PMCs showed a spectrum of chromosomal association and rearrangements in most of the cells analyzed presently and the data are illustrated in Figs. 1–15 and summarized in Tables 1–4.

For detailed meiotic analysis, more than 500 pollen mother cells (PMCs) were observed, of which 100 randomly selected PMCs revealed that only 10% of PMCs had shown the normal meiotic pattern of bivalents and had 10 bivalents (Figs. 1,2), whereas 90% of PMCs demonstrated ab-

Figs. 1–15. Male meiosis in *M. wengeri*: **Fig. 1** Diplotene, 10II; **Fig. 2** Diplotene, 9II+2I; **Fig. 3** Diplotene, 8 II+4I; **Fig. 4** Diplotene, 8 II+4I; **Fig. 5** Diplotene, 7II+6I; **Fig. 6** Diplotene, 6 II+8I; **Fig. 7** Diplotene, 5II+10I; **Fig. 8** Diplotene, 4II+12I; **Fig. 9** Diplotene, 3II+14I; **Fig. 10** Diakinesis 2II+14I; **Fig. 11** Diakinesis 1II+18I; **Fig. 12** Diakinesis 20I; **Figs. 13–15** Anaphase I; **Fig. 13** early Anaphase I with unequal distribution of chromosomes; **Fig. 14** Anaphase I with equal distribution of chromosomes (10:10); **Fig. 15** Anaphase I with unequal distribution of chromosomes and laggards (9:3U:8). Arrowheads showing the nucleolar nature of some bivalents/univalents; Scale bar=10 μ m.

Table 1. Associations at diplotene/diakinesis in *Mantisia wengeri*.

Associations	Total	20I	1II+18I	2II+16I	3II+14I	4II+12I	5II+10I	6II+8I	7II+6I	8II+4I	9II+2I	10II
Number of cells	100	1	2	6	6	8	25	14	12	8	8	10
Percentage	100	1	2	6	6	8	25	14	12	8	8	10

normal meiotic behavior as characterized by the presence of univalents ranging from 0 to 20 in number. The most common association observed was 5II+10I (25%) followed by 6II+8I (14%), 7II+6I (12%), 10II (10%), 4II+12I, 8II+4I and 9II+2I (8% each), 2II+16I, 3II+14I (6% each), and 20I (1%) in the PMCs analyzed (Figs. 3–12, Table 1).

Table 2. Mean number of associations and chiasmata per cell at diplotene/diakinesis in *Mantisia wengeri*.

Source (number of Cells)	Bivalents						Univalents						Chiasmata			
	Ring			Rod			Mean			Total			Mean		Terminalized	
	Mean ± SD	Range	No.	Mean ± SD	Range	No.	Mean ± SD	Range	No.	Mean ± SD	Range	No.	Mean ± SD	Range	± SD	Un-terminalized Terminalization coefficient
100	7.47±4.37	0-10	452	4.52±2.93	0-10	295	2.95±1.85	0-8	506	5.06±3.97	0-20	1494	14.94±9.20	0-22	12.40±7.88	2.54±1.32
																0.82

A total of 747 bivalents were observed in 100 PMCs with a mean of 7.47 ± 4.37 , ranging from 0–10 in number, out of which 452 were ring and 295 were rod bivalents with a mean value of 4.52 ± 2.93 and 2.95 ± 1.85 and their numbers ranging from 0–10 and 0–8, respectively (Table 2). A quite good number of univalents (506) were recorded with an average of 5.06 ± 3.97 , ranging from 0 to 20 in number. The total number of chiasmata observed was 1494 with a mean value of 14.94 ± 9.20 , with their numbers ranging from 0 to 22, of which 1240 chiasmata were terminalized with a mean value of 12.40 ± 7.88 , whereas 254 chiasmata were unterminalized with an average of 2.54 ± 1.32 . The terminalization coefficient was recorded as 0.82 (Table 2). Besides these differences, some of the PMCs were characterized with the presence of 0–5 supernumerary nucleoli. Only 2% of the PMCs analyzed had shown the anaphase I distribution of bivalents. Out of which 10 clear and non-ambiguous PMCs were selected for further analysis of distribution of bivalents at different poles. Six PMCs (60%) showed the normal (10:10) distribution of chromosomes at anaphase I (Figs. 13, 14), while the rest (40%) PMCs had shown anomalous distribution of chromosomes (Fig. 15), including 10: 2U (univalents) 8, 9:2U:9, 8: 2B (bivalents): 8, and 12:8 (10% respectively) distribution (Table 3). The pollen stainability was recorded ranging from 44.66 to 70% with an average of $57.33\% \pm 8.40$ (Table 3).

Another interesting observation made during the analysis of the PMCs was the position and orientation of univalents determined by the number of bivalents (Table 4). When the number of univalents was high, most of the PMCs had shown the non-polar and/or random distribution of univalents. The association of 1II+18I did show polar distribution with a complete lack of non-polar behavior. However, 2II+16I comparatively showed the most non-polar distribution of univalents. Noticeably, as the number of bivalents increased in such associations, the univalents tended to be non-polar in distribution and aligned with the bivalents in most of the PMCs (Table 4).

A salient and contrasting observation, re-

Table 3. Anaphase I (AI) distribution and pollen stainability in *Mantisia wengeri*.

	AI (10:10)	AI (10:2U:8)	AI (10:4U:6)	AI (9:2U:9)	AI (9:3U:8)	AI (8:2B:8)	AI (13:7)	AI (12:8)	AI (11:9)	Percentage pollen stainability		
										Min.	Max.	Average±SD
Number of cells	6	1	–	1	–	1	–	1	–	44.66	70.00	57.33±8.40
Percentage	60.00	10.00	–	10.00	–	10.00	–	10.00	–			

Table 4. Orientation of univalents in relation to number of bivalents in *Mantisia wengeri*.

No. of bivalents	No. of cells	Univalents			
		Pole I	Pole II	Non polar	Random
1	2	4	2		12
2	2			16	
2	2				16
2	2		2	14	
3	4				14
3	2			14	
4	5			12	
4	3				12
5	10			10	
5	7				10
5	7		2	8	
5	1		1	9	
6	7			8	
6	5				8
6	2		1	7	
7	5			6	
7	3		5	1	
7	2				6
7	2		4	2	
7	1	5		1	
8	4			4	
8	2				4
8	1		3	1	
9	6			2	
9	2				2

corded in *M. wengeri* when compared to *M. spathulata*, is that most of the PMCs showed the un-failing attachment of bivalent(s)/univalent(s) with nucleolus in varying numbers thus showed nucleolar nature of the chromosomes.

Discussion

The north-eastern region of India is the biogeographical gateway to India’s richest biodiversity zones and important for the genetic resources the world over. Ironically, this is also one of the world’s most threatened areas and recent estimates indicate that all this diversity may disappear in the next 12–15 years. Climate change may pose as an added threat to the rich biodiversity of India. The development of a conservation strategy for rare and endemic plant species requires the maximum available information on the level of genetic variation and population subdivision among these species. All measures in the present study are aimed at the reintroduction and recovery of the species for sustainable restoration of the allelic composition/heterozygosity in the genetic constitu-

tion of each species /population of genus *Mantisia*.

Synaptic variation (asynapsis/desynapsis/partial asynapsis/disturbance of synapsis) is genetically controlled phenomenon and their anxious expression levels may lead to any aspect of meiotic abnormality. Rare and endangered plants are, however, naturally more susceptible to such abnormalities. Biologists have long been fascinated with rare and endemic species to ascertain various factors for their scant populations in nature. In recent years, this fascination has turned to urgency, as even more species dwindled towards extinction (Gitzendanner and Soltis 2000). In the present investigation, most of the PMCs analyzed at either diplotene or diakinesis/metaphase I did not reveal the expected chromosome associations of 10II. Univalents were observed most consistently in the majority of the PMCs. The number and range of univalents were quite high. This was probably the result of the partial/complete absence of synapsis/pairing and chiasmata formation. Our observations are supported by the earlier published work of Sharma *et al.* (2011a), who reported imbalanced meiotic deeds with variant synaptic behavior of bivalents in *M. spathulata* and considered them a cause for loss of genetic variation leading to rarity and endangeredness. Our present observations are in agreement with those of Pandit and Babu (2003), that rarity in species with a small population size may not only be a result of environmental factors but may be associated with the demographic stochasticity driven by synaptic mutations resulting in its rarity. Such observations of low genetic diversity at DNA level using SPAR methods (Sharma *et al.* 2011c,d) in both *Mantisia spathulata* and *M. wengeri* were also emphasized and considered as plausible reasons for the endemism and endangeredness of genus *Mantisia* (Sharma *et al.* 2011b). The present findings of synaptic variation in a natural population of *M. wengeri* add one more example to a small pool of perennial rhizomatous zingibers.

It is worthwhile to note that *M. wengeri* did reveal a significantly low degree of synaptic failure as compared to *M. spathulata*, which is evident from the fewer number of cells (23%) with more univalents rather than the expected bivalent associations. On the other hand, *M. spathulata* revealed a contrasting pattern of chromosome associations at diplotene/diakinesis by having PMCs with a greater number of univalents in respect of regular bivalents. It is further interesting to note that *M. wengeri* is believed to be an inter-specific hybrid, and *M. spathulata* is suspected to be one of the putative parents. Our present observations compel us to opine that the genome with a high degree of synaptic failure, a salient feature of *M. spathulata*, might have contributed to the abnormal synaptic events observed in *M. wengeri*. The role of *M. radicalis*, the other suspected putative parent species in influencing the synaptic behavior of chromosomes, needs to be ascertained with substantial data coming from cellular and molecular cytogenetical investigations.

In the present investigation, very low pollen stainability, as 57.33%, was recorded. Recently, Bhowmik *et al.* (2010a) reported comparatively low *in vivo* germination of seeds for *M. wengeri* (24%). Various reports revealed that natural propagation through seeds in many rare and endangered plants such as *M. wengeri* is limited due to seed factors such as seed dormancy (Rathore *et al.* 1991), poor seed viability (Sudha and Seenii 1996) and little or no seed production (Agrawal *et al.* 1991, McKently and Adams 1994). However, we would like to add that deviant synaptic behavior of chromosomes at Prophase I of meiosis also contributes to reduced seed fertility with low pollen stainability.

The loss of genetic diversity results in the disturbance of gene frequency in endangered species and thereby reduces evolutionary potential to adapt to changing environments (Tamaki *et al.* 2008). Other factors, like squat cross-pollination, entomophily (Bhowmik 2009), low karyomorphological variation, presence or absence of either heteromorphic pair or satellite chromosomes in root tip mitotic complements (Sharma *et al.* 2012), as well as by and large imbalanced meiotic deeds with total asynaptic/desynaptic behavior of bivalents, also confirm the rarity and endangered status of both the species of genus *Mantisia*. Such challenges can be attempted by polyploidization of synaptic variants to increase bivalent associations.

One of the goals of many conservation programs, in addition to habitat preservation, is to maintain existing levels of genetic variation within species that are rare, endangered and endemic. Further *in situ* conservation strategies must include the introduction of plants at low altitude, low landslide-prone and more rainfall areas, in addition to the establishment of protected fields, biosphere reserves, as well as the *ex situ* conservation of *Mantisia* species (Sharma *et al.* 2011b), *M. wengeri*, in particular, is extremely vital for conservation and to circumvent the risk of extinction of the genus *Mantisia*.

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