# *Synaptic variation derived plausible cytogenetical basis of rarity and endangeredness of endemic* Mantisia spathulata *Schult*

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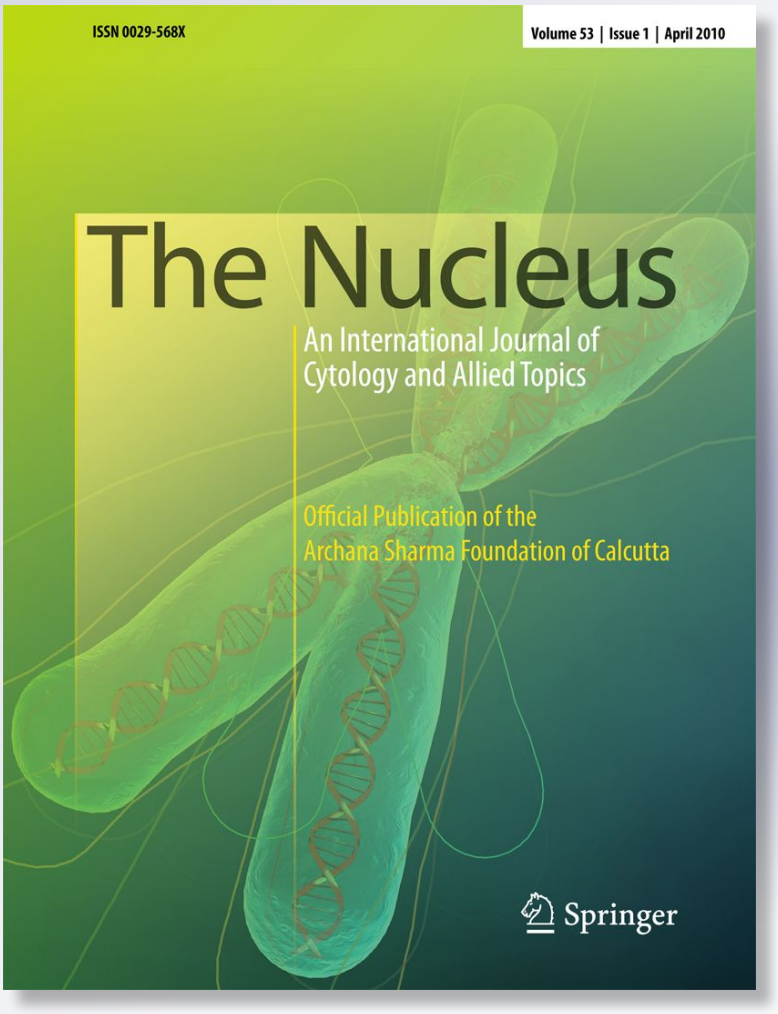


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ORIGINAL PAPER

# Synaptic variation derived plausible cytogenetical basis of rarity and endangeredness of endemic *Mantisia spathulata* Schult

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**Abstract** We studied meiosis in *Mantisia spathulata,* a rare, endangered and endemic rhizomatous horticultural herb showing synaptic variation. Most of pollen mother cells (PMCs) analyzed at either diplotene or diakinesis/metaphase I did not exhibit the expected chromosome associations of 10II, which is indicative of synaptic variation. Seventeen percent of the PMCs have shown normal meiotic pattern while 83% PMCs were with abnormal meiotic behavior of bivalents. A total of 419 bivalents and 1,162 of univalents were recorded with significantly low chiasma frequency (8.38±6.20) and terminalization coefficient of 0.82 only. We recorded in 53.33% PMCs, an anomalous distribution pattern of chromosomes, including unequal distribution and/or presence of laggards in the form of univalents/bivalents resulting in low pollen stainability. We suggest that the imbalanced meiosis with variant synaptic behavior of bivalents recorded in *M. spathulata* may be responsible for loss of genetic variation and considered as substantial cytogenetical factors leading to the rarity and endangeredness of the species.

**Keywords** Synaptic variants · Chiasma frequency · Univalents · Laggards · Rare · Endangered species

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## Introduction

The genus *Mantisia* Sims., belonging to family Zingiberaceae, is endemic to hilly areas of the Northeastern India, Myanmar and Bangladesh [2, 4, 32, 44]. Comprising four medicinal and horticultural important species [1, 2] viz. *Mantisia wengeri*, *M. radicalis*, *M. spathulata and M. wardii,* it represents the smallest genus of the family Zingiberaceae. Rahman and Yusuf [4] have recently added a new species i.e. *M. salarkhanii* from Bangladesh. *Mantisia spathulata* Schult., commonly known as '*dancing girl'*, is critically endangered and rare species which has been rediscovered from Lunglei province of Mizoram (India). Due to natural calamities and anthropogenic activities, *M. spathulata* has become critically rare and endangered in the natural habitat and is listed in the Red Data sheet of Indian plants [1, 2, 9, 44]. The rarity of the genus *Mantisia* has reached to such a level that only countable few representatives are available today and the species has been included in the national priority list for its recovery by Department of Biotechnology, New Delhi, India [2, 44]. The complex nomenclatural history of *Mantisia* is summarized by Dam et al. [4]. Williams et al. [47] using molecular biological data, suggested that *Mantisia* be nested with *Globba.* Finally, the genus *Mantisia* has been classified with *Globba* under the tribe Globbeae but in different sections Mantisia and Globba, respectively. Very few reports are available on phylogenetic

analysis of *Mantisia* and its closely related genus *Globba* [17, 18, 43, 47]. We have successfully attempted conservation and propagation of *M. spathulata* through seed as well as in vitro approaches [1, 2, 44]. There are a few reports on cytogenetical studies of *Mantisia,* reporting basic/somatic chromosome numbers only [19–25, 29, 42, 47]. They suggested the basic chromosome number of $x=10$ for the genus *Mantisia,* and proposed a revised classification of the tribe Globbeae and family Zingiberaceae in which most of the members posses $x=8$.

Meiosis is a highly conserved process in eukaryotes and plays a central role in the life cycle of all sexually reproducing organisms [10]. An important feature of meiosis is an interaction of homologous chromosomes at prophase I, when they are paired, arranged in parallel alignment, facilitated by the synaptonemal complex (SC), and recombine with the help of recombination nodules [50]. Such genetic recombinations occurring at pachytene are cytologically manifested as chiasma at diplotene stage and as terminalized chiasma at diakinesis stage. The terminalized chiasma preserves the bivalents structure through metaphase I (MI) and helps appropriate disjunction of bivalents at anaphase I (AI). Such preservation of terminalized chiasmata until AI is mediated by cohesion of sister chromatid arms, disappearing only before the end of AI. These cytological events are based on molecular processes related to homologous recombinations between DNA strands [31, 40]. Mutations of genes controlling synapsis result in the formation of variable number of univalents at MI, and other abnormalities at subsequent meiotic stages including formation of aneuploid gametes resulting in plant sterility. Alternatively, during desynapsis, homologous chromosomes may fall apart or undergo reduced chiasma formation after normal pairing [35]. The occurrence of synaptic mutation has been reported mostly in crop plants [34, 39] as well as in a few animals [16, 49] including humans [28]. Naturally occurring synaptic mutations in higher plants are rarely reported [30, 34, 35]. Cytological expressions of mutant alleles of synaptic genes in rye have been observed. Several genes responsible for meiotic disturbance in the form of asynapsis (*sy1* and *sy9*), partial asynapsis (*sy3*), heterologous synapsis (*sy2*, *sy6*, *sy7*, *sy8*, and *sy19*), disturbance of synapsis, (*sy18*) hypercompaction (*mei10*), irregular condensation (*mei8*) and fragmentation of chromatins and arrest of meiosis in plants (*mei8-mei10*, *mei5*) were recorded [40]. All such genes and their expression levels may lead to any aspect of meiotic disturbance. Rare and endangered plants are naturally more susceptible to such kinds of abnormalities.

In the present investigation, male meiosis has been studied in *M. spathulata* for comprehensive chromosomal behavior and their association, chiasma formation, frequency as well as terminalization/unterminilazation coefficients, and chromosome distribution at anaphase. The data generated through analysis of PMCs revealed synaptic variation in the behavior of the chromosomes. Possibly, this is the first report of male meiotic analysis in the endemic *M. spathulata*.

## Materials and methods

Plant samples of *M. spathulata* were collected randomly from the naturalized plants from the experimental garden of North-Eastern Hill University, Shillong (Meghalaya), India. Flower buds of appropriate size were collected during May-June and fixed in freshly prepared Carnoy's fluid supplemented with a drop of ferric chloride solution for a minimum of 24 h at room temperature and subsequently stored in 70% ethanol at 10°C. Anthers were squashed in 2% aceto-carmine. For detailed analysis, more than 1,000 PMCs were studied, of which 100 PMCs were randomly selected for further analysis, which clearly revealed the chromosomal associations and chiasma frequency without any ambiguity. 15 cells were also analyzed at AI for the segregation pattern of the chromosomes. For pollen stainability, the pollen grains were stained in 1:1 glycerol:acetocarmine mixture, and on average ten slides from 3 to 5 plants were scored for stainable pollen. Micro-photographs were taken using *Jenoptik* CCD camera (Germany) attached to *Labomed* LX-400 fluorescence microscope.

## Results

Detailed analysis of PMCs showed more or less abnormal chromosomal behavior in 83% of cells characterized by the presence of univalents ranging from 1 to 20 in number and the data are summarized in Tables 1, 2, 3 and 4. Of the 1,000 PMCs analysed, 60%, 23% and 19% PMCs were at diplotene, diakinesis and MI stage respectively, whereas rest of the cells were at AI stage. Only 17% of the PMCs showed the normal meiotic pattern of bivalents and had 10 bivalents (Figs. 1–3), The most common association observed was 1II+18I (17%) followed by 3II+14I (15%), 2II+16I (12%), 20I (11%), 4II+12I (11%), 9II+2I (6%), 5II+10I (5%), 6II+8I (3%), 7II+6I (2%) and 8II+4I (1%) in PMCs analyzed (Figs. 4–27, Table 1, S1).

A total of 419 bivalents were observed in 100 PMCs with a mean 4.19±3.37, the number of which ranged from 0 to 10 per cell. Of these, 340 were ring and 79 were rod types with a mean value of 3.48±3.13 and 0.71±1.20 respectively and their numbers were found to be ranging from 0 to 10 and 0–6, per cell respectively (Table 2, S2). A huge number of univalents (1,162) were recorded with an average of 11.62±6.97, ranging from 0 to 20 in number. Total number of chaismata observed was 838 with a mean

**Table 1** Associations at diplotene/diakinesis in *Mantisia spathulata*

| 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 | 11 | 17 | 12 | 15 | 11 | 5 | 3 | 2 | 1 | 6 | 17 |
| Percentage | 100 | 11 | 17 | 12 | 15 | 11 | 5 | 3 | 2 | 1 | 6 | 17 |

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

| Source (number of cells) | Bivalents | | | | | | | | | Univalents | | | Chiasmata | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ring | | | Rod | | | | | | | | | | | |
| | Total No. | Mean ± SD | Range | No. | Mean ± SD | Range | No. | Mean ± SD | Range | No | Mean ± SD | Range | Total No. | Mean ± SD | Range | Terminalized ± SD | Unterminalized ± SD | Terminalization coefficient |
| 100 | 419 | 4.19±3.37 | 0–10 | 340 | 3.48±3.13 | 0–10 | 79 | 0.71±1.20 | 0–6 | 1162 | 11.62±6.97 | 0–20 | 838 | 8.38±6.20 | 0–22 | 6.88±5.88 | 1.50±1.32 | 0.82 |

**Table 3** Anaphase I distribution (AI) and pollen stain ability in *Mantisia spathulata*

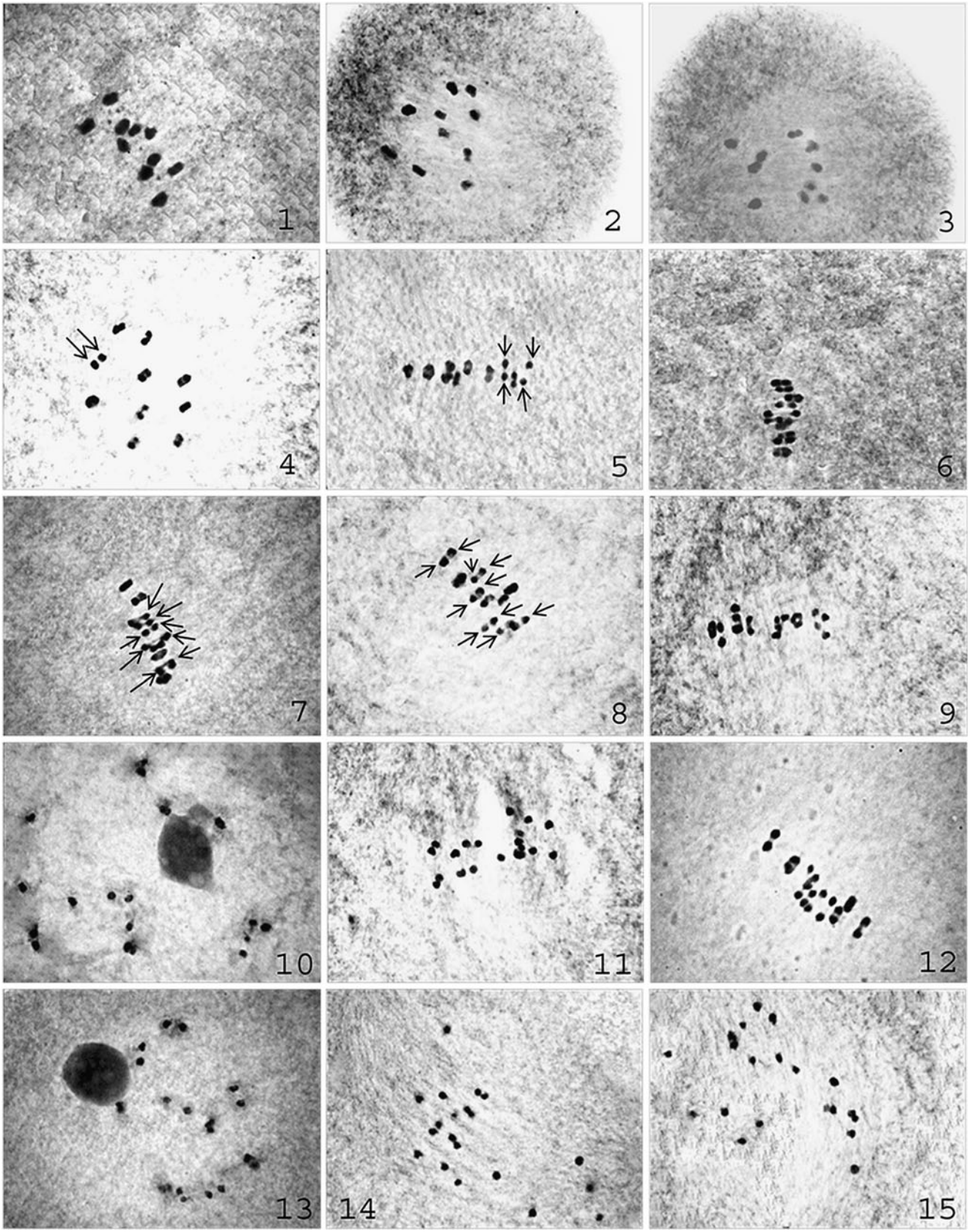
| | AI (10:10) | AI (10:2U:8) | AI (10:4U:6) | AI (9:2U:9) | AI (9:3U:8) | AI (8:2B:8) | AI (13:7) | AI (12:8) | AI (11:9) | Percentage pollen stainability | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | minimum | maximum | Average±SD |
| Number of cells | 7 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 34.56 | 60.00 | 46.92±7.40 |
| Percentage | 46.67 | 6.67 | 6.67 | 6.67 | 6.67 | 6.67 | 6.67 | 6.67 | 6.67 | | | |

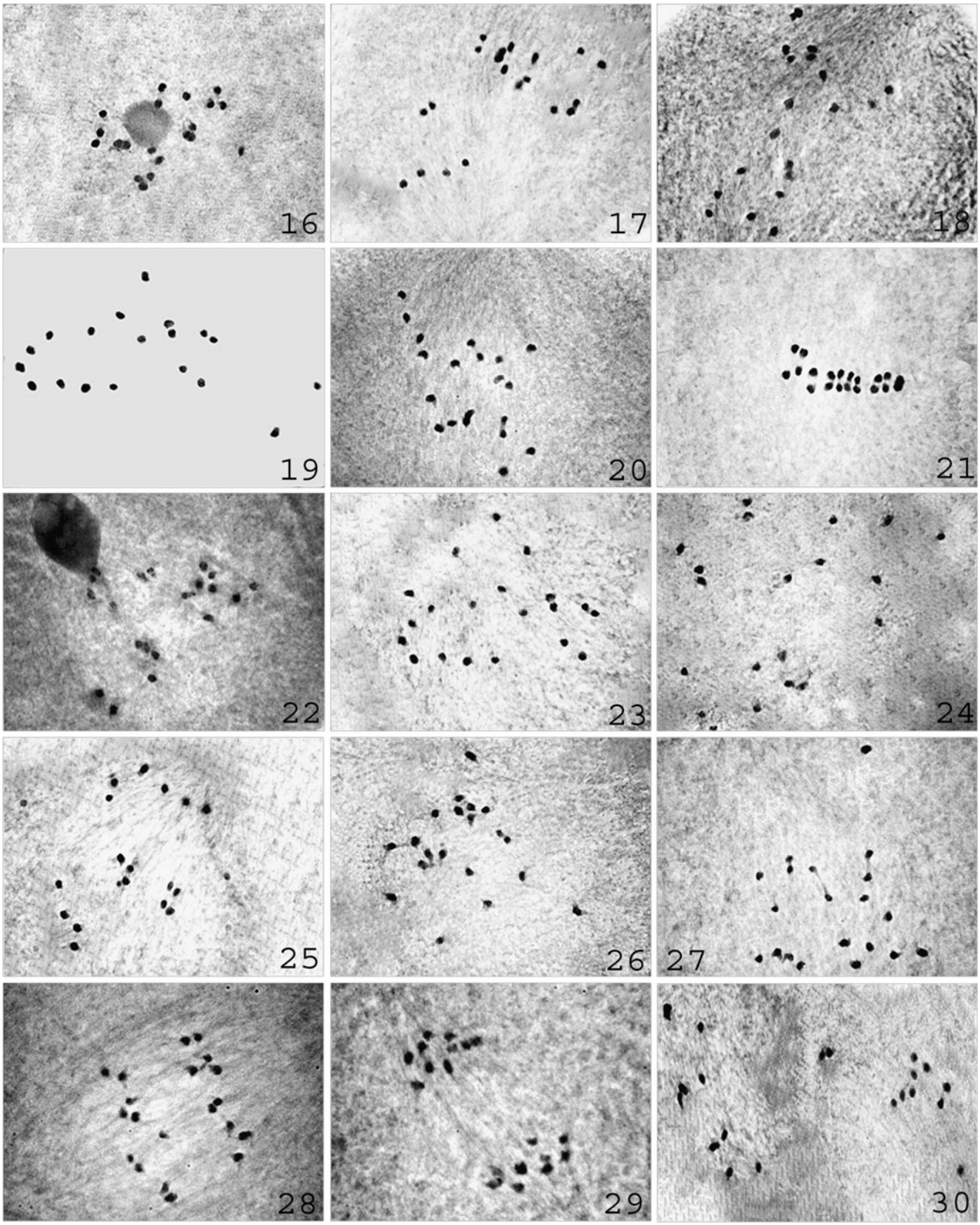
**Table 4** Orientation of univalents in relation to number of bivalents in *Mantisia spathulata*

| No. of bivalents | No. of cells | Univalents | | | |
|---|---|---|---|---|---|
| | | Pole I | Pole II | Non polar | Random |
| 0 | 2 | | | | 20 |
| 0 | | | | 20 | |
| 0 | | 1 | 1 | 18 | |
| 0 | | 4 | | 16 | |
| 0 | | 4 | 2 | 14 | |
| 0 | | 1 | | 19 | |
| 0 | | 10 | 4 | 6 | |
| 0 | | | 7 | 13 | |
| 0 | | 4 | 1 | 15 | |
| 0 | | | 1 | 19 | |
| 1 | 2 | 4 | 2 | | 12 |
| 1 | 4 | | | | 18 |
| 1 | | 4 | 3 | 11 | |
| 1 | | 2 | 1 | | 15 |
| 1 | | 1 | | 17 | |
| 1 | | 13 | 5 | | |
| 1 | | 6 | 2 | 10 | |
| 1 | | 1 | | | 17 |
| 1 | | | 7 | 11 | |
| 1 | 4 | | | 18 | |
| 2 | 6 | | | 16 | |
| 2 | 2 | | | | 16 |
| 2 | 2 | | 2 | 14 | |
| 2 | | 6 | | 10 | |
| 2 | | 4 | | 12 | |
| 3 | 4 | | | | 14 |
| 3 | 6 | | | 14 | |
| 3 | 3 | 4 | 1 | 9 | |
| 3 | | 7 | | 7 | |
| 3 | | 1 | 9 | 4 | |
| 4 | 8 | | | 12 | |
| 4 | 3 | | | | 12 |
| 5 | 5 | | | 10 | |
| 6 | 3 | | | 8 | |
| 7 | 2 | | | 6 | S |
| 8 | 1 | | | 4 | |
| 9 | 6 | | | 2 | |

value of 8.38±6.20 and their number ranging from 0 to 22, of which 688 chiasmata were terminalized with a mean value of 6.88±5.33, whereas 150 chiasmata were unterminalized with an average of 1.50±1.32 (Table 2, S2). The terminalization coefficient was recorded as 0.82 (Table 1). Some of the PMCs were characterized by the presence of 0–5 supernumerary nucleoli. Out of 15 clear and non-ambiguous PMCs, seven (46.67%) showed normal (10:10) distribution of chromosomes at AI (Figs. 28–29, Table 3),

**Figs. 1–30** Male meiosis in *M. spathulata*: **Figs. 1–3** Metaphase I, ▶ 10II; **Fig. 4** Metaphase I, 9II+2I; **Fig. 5** Metaphase I, 8 II+4I; **Fig. 6** Metaphase I, 7II+6I; **Fig. 7** Metaphase I, 6II+8I; **Figs. 8–9** Metaphase I, 5 II+10I; **Fig. 10** Diplotene, 4II+12I; **Figs. 11–12** Metaphase I, 4II+12I; **Fig. 13** Diplotene, 3II+14I; **Figs. 14–15** Diakinesis 3II+14I; **Fig. 16** Diplotene 2II+16I; **Figs. 17–18** Diakinesis 2II+16I; **Figs. 19–21** Metaphase I, 1II+18I; **Fig. 22** Diplotene 20I; **Figs. 23–27** Diakinesis 20I; **Figs. 28–29** Anaphase I with equal distribution (10:10); **Fig. 30** Anaphase I with unequal distribution of chromosomes and laggards (9:2U:9). *Arrow heads* showing presence of univalents






**Figs. 1–30** (continued)

while the rest (53.33%) had shown anomalous distribution of chromosomes (Fig. 30, Table 3), including 13:7, 12:8, 11:9 (6.67% each respectively), 8: 2B (bivalents): 8 (6.67%), 10:2U (univalents): 8 (6.67%); 10:4U:6 (6.67%); 9:2U:9 (6.67%), and 9:3U:8 (6.67%) distribution (Table 3). The percentage pollen stainability was recorded ranged from 39.55 to 57.28 with an average of 47.97±6.50.

The position and orientation of univalents was apparently determined by the number of bivalents (Table 4). When the number of univalents was 20, most of PMCs had shown the non-polar distribution of univalents, however PMCs with 1II+18I, showed most random distribution of univalents. Noticeably, as the number of bivalents increased in such associations, the univalents tends to be non-polar in distribution and aligned with the bivalents in most of the PMCs (Table 4).

## Discussion

Our studies revealed the somatic chromosome number of 2n=20 in *M. spathulata* which derives support from earlier reports of $x=10$ for the genus *Mantisia* [21–25, 47]. *S*tudies of meiotic behavior are essential for understanding the reproductive biology, fertility aspects and genomic evolution of any organism [3]. In the present investigation, most of the PMCs analyzed at either diplotene or diakinesis/MI did not reveal the expected chromosome associations of 10II. Univalents were observed most consistently in majority of the PMCs. The number and range of univalents were quite high. This was probably the result of partial/complete absence of synapsis/pairing and chaismata formation. Conventionally the occurrence of univalents at diplotene, diakinesis and MI is regarded as desynapsis and/or asynapsis, depending on the presence or absence of initial pairing of homologues at pachytene stage [15]. During meiotic prophase I, homologues are typically linked by both segmental exchanges and synaptonemal complex (SC). In most eukaryotes, the SC disassembles at diplotene, leaving the chromosomes joined mostly by terminal chiasma [14].The occurrence of such exchange events between homologues were rare in *Montisia spathulata* against expected 10II associations, with comparatively low chiasmata frequency (8.38±6.20) ranging from 0 to 22 with the terminalization coefficient of 0.82 only. Viera et al. [46] observed both features in *Paratettix meridionalis* and they considered them as a consequence of the nuclear polarization shown by cohesion axes during maturation that, in turn, may drive the subsequent loading of recombinases. It is suggested that restricted distribution of recombination events along the bivalent structure leads to the incomplete pairing and synapsis of homologues leading to synaptic variants as observed in *M. spathulata*.

Crossover derived genetic exchanges are cytologically visible as chiasmata. Chiasma is also responsible, in combination with sister chromatid arm cohesion, for the correct bi-orientation of bivalents at MI and the subsequent segregation of homologues at AI. These events could be seen in abnormal distribution of chromosomes at AI with the presence of univalents/bivalents, laggard and unequal distribution at the opposite poles (Table 3). Variation in synaptic behavior of homo/homeologous chromosomes during zygotene, resulting in total (asynapsis) or partial (desynapsis) failure of chromosome pairing, has been observed in a large number of genera and has been reviewed by several authors from time to time [26, 34, 35]. The induction of synaptic mutations using physical and chemical mutagens is also widely reported [11, 12, 45], but only a handful of reports are available on spontaneous origin of desynaptic variants in natural populations [34, 35, 38]. Conventionally, a large number of causative factors are reported to be responsible for the spontaneous origin of synaptic mutants in natural populations. Drastic temperature fluctuation, ageing, water content and humidity, soil conditions, and more importantly gene mutations [34] are such factors. We suggest that it is the anthropogenic and environmental factors that affect normal meiosis in *M. spathulata*. This plant species has been rediscovered after two decades and in spite of its wide distribution reported in the past, this species is now restricted to narrow pockets of northeast India, mainly because of its inability to withstand climate change, habitat destruction and overexploitation [2]. This plant is also experiencing various environmental fluctuations including low temperature, high pH, humidity and altitude of the habitat, which may collectively cause abnormal behavior of chromosomes during cell division.

Seventy one percent of the PMCs showed abnormal meiosis that comprised of more than 10 univalents with variable numbers of bivalents. The percentage of squat number of univalents along with high number of bivalents was very low (29%) (Fig. S1 and Table 2). John and Lewis [13] demonstrated the random distribution of univalents at MI, which was confirmed by Dhesi et al. [5] in pearl millet and by Singh et al. [37] in mung bean and in *Anogeissus sericea* var. *sericea* by Rao and Kumar [34], and recently Sharma et al. [35] in *Panax sikkimensis*. However, in the present material it was evident that the position of univalents was determined by the number of bivalents present. Most of PMCs with high number of univalents with low bivalent frequency (0–1) had shown non-polar and/or random distribution of univalents. Noticeably, as the number of bivalents decreased in such associations, the univalents assumed polar distribution pattern. These observation draw support from various investigations on *Schistocera gregaria* [12], *Crotalaria juncea* [45] and in *Phlox drummondii* [33], thus confirm the deviant synaptic behavior of *M. spathulata*. Barring a few reports [5, 6, 34, 35] the natural occurrence of synaptic mutants, particularly in natural populations of

higher plants, is a rare phenomenon with little morphological variation to differentiate between the mutant and the normal individual [35]. Synaptic mutations have been reported in natural populations of perennial rhizomatous herbs i.e. *Coptis teeta* [30] and *Panax sikkimensis* [35]. Our present observations are in agreement with those of Pandit and Babu [30] that rarity in species with 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.

In the present investigation, very low pollen stainability i.e. 46.92% ranging from 34.56% to 60% only, is considered as reduced fertility leading to reduced seed set [31]. Various reports are available which suggest that natural propagation through seeds in many rare and endangered plants is limited due to many factors such as seed dormancy and pollen sterility, poor seed viability [41] and little or no seed production [27]. Recently, Bhowmik et al. [1] reported comparatively very low in vivo germination of seeds for *M. spathulata* and *M. wengeri* (20% and 24%, respectively). Other factors like, squat cross pollination, insect dependent pollination [2], low karyo-morphological variation, presence or absence of either heteromorphic pair in root tip mitotic complements (unpublished data) as well as by and large imbalanced meiotic deeds with variant synaptic behavior of bivalents also depict the confirmation of rarity and endangered status of *M. spathulata.*

To comprehend the plausible reasons for rarity, endangeredness and endemism of *M. spathulata* at DNA level, it was analyzed to assess natural genetic variation using three different PCR based DNA markers viz. RAPD, ISSR and DAMD, both individually and cumulatively, which are popularly regarded as single primer amplification reaction (SPAR) methods, which collectively revealed low (15%) genetic variation [36]. Rare and endangered species are susceptible to loss of genetic variation through genetic drift in small populations [48] including inbreeding depression as well as out-breeding complicacies, accumulation of deleterious mutations, loss of genetic variation, genetic adaptation to domestication and its effects on reintroduction success, insect pollination, loss of self-compatibility and taxonomic uncertainties as well as interrogation are some of the factors for rarity, endangerment and endemism, ultimately leading to extinction of the plant species [7]. Species with low genetic variation resulted from non-pairing of homologous chromosomes, low chaismata and recombination frequency in bivalents, high number of univalents with unequal distribution at AI, finally considered as probable asynaptic populations, would be expected to have further reduced the ability to cope up with environmental alterations during evolution, and so have shorter life span [7, 8]. A large and significantly excess number of endangered species and populations tend to have low level of genetic variation [7]. Hence, it can be opined that such abnormal meiotic behavior expressed by most of the PMCs of *M. spathulata* analyzed in the present study leading to loss of genetic variation could be considered as cytogenetical rationale of rarity and endangeredness of the species. The present finding of synaptic variation in a natural population of *M. spathulata* adds one more example to a small pool of perennial rhizomatous zingibers.

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## References

1. Bhowmik SDS, Kumaria S, Tandon P. Conservation of *Mantisia spathulata* Schult. and *Mantisia wengeri* Fischer, two critically endangered and endemic zingibers of Northeast India. Seed Tech Notes. 2010;32:57–62.
2. Bhowmik SSD, Kumaria S, Rao SR, Tandon P. High frequency plantlet regeneration from rhizomatous buds in *Mantisia spathulata* Schult. and *Mantisia wengeri* Fischer and analysis of genetic uniformity using RAPD markers. Indian J Exp Biol. 2009;47:140–6.
3. Cai X, Xu SS. Meiosis-driven genome variation in plants. Curr Genom. 2007;8:151–61.
4. Dam DP, Dam N, Dutta RM. *Mantisia saltatoria* Sims versus *Globba radicalis* Roxb. (Zingiberaceae). Bull Bot Surv India. 1997;34:188–93.
5. Dhesi JS, Gill BS, Sharma HL. Cytological studies of desynaptic stock in pearl millet, *Pennisetum typhoides*. Cytologia. 1973;38: 311–6.
6. Elliott EA, Yu J, Ashley T, Arnheim N, Flavell RA, Liskay RM. Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. Cell. 1995;82:309–19.
7. Frankham R. Conservation genetics. Annu Rev Genet. 1995;29: 305–27.
8. Frankham R. Genetic management of captive population for reintroduction. In: Serana M, editor. Reintroduction biology of Australian and New Zealand fauna. Chipping Norton, NSW, Australia, Surrey Beatty; 1995. p. 31–34
9. Ganeshaiah KN. Recovery of endangered and threatened species: developing a national priority list of plant and insects. Curr Sci. 2005;89:599–600.
10. Hamant O, Ma H, Cande WZ. Genetics of meiotic prophase I in plants. Annu Rev Plant Biol. 2006;57:267–302.
11. Havekes FWJ, De Jong JH, Heyting C, Ramanna MS. Synapsis and chiasma formation in four meiotic mutants of the tomato *Lycopersicon esculentum*. Chromosome Res. 1994;2:215–325.
12. Henderson SA. Temperature and chiasma formation in *Schistocera gregaria* II. Cytological effects at 40°C and the mechanism of heat induced univalance. Chromosoma. 1962;13:437–63.
13. John B, Lewis KR. The meiotic system. *Protoplasmatologia* VI. New York: Springer; 1965.
14. Kemp B, Boumil RM, Stewart MN, Dawson DS. A role for centromere pairing in meiotic chromosome segregation. Gene Dev. 2004;18:1946–51.

15. Kitada K, Omura T. Genetic control of meiosis in rice *Oryza sativa* L. II. Cytogenetical analyses of desynaptic mutants. Jpn J Genet. 1983;58:567–77.
16. Koykul W, Basrur PK. Synaptic anomalies in fetal bovine oocytes. Genome. 1994;37:83–91.
17. Kress WJ, Prince LM, Williams KJ. The phylogeny and a new classification of the gingers (*Zingiberaceae*): evidence from molecular data. Amer J Bot. 2002;89:1682–96.
18. Kress WJL, Prince M, Hahn WJ, Zimmer EA. Unraveling the evolutionary radiation of the families of the zingiberales using morphological and molecular evidence. Syst Biol. 2001;51:926–44.
19. Larsen K. Studies on zingiberaceae IV: *Caulokaempferia*, a new genus. Bot Tidsskr. 1964;60:165–79.
20. Larsen K, Lock JM, Maas H, Maas PJM. Zingiberaceae. In: Kubitzki K, editor. The families and genera of vascular plants. Berlin: Springer; 1998. p. 474–95.
21. Lim SN. Cytogenetics and taxonomy of the genus *Globba* L. (*Zingiberaceae*) in Malaya 2: cytogenetics. Notes Roy Bot Gard. 1972;31:229–41.
22. Lim SN. Cytogenetics and taxonomy of the genus *Globba* L. (*Zingiberaceae*) in Malaya 3: comparative studies of polyploids. Malays J Sci. 1972;1:19–33.
23. Lim SN. Cytogenetics and taxonomy of the genus *Globba* L. (*Zingiberaceae*) in Malaya 4: distribution in relation to polyploidy. Garden's Bull. 1972;26:115–26.
24. Lim SN. Cytogenetics and taxonomy of the genus *Globba* L. (*Zingiberaceae*) in Malaya 5: introgressive hybridization in hexaploids. Bot J Linn Soc. 1973;66:143–56.
25. Lim SN. Meiotic instability in the plant genus *Globba*. In: Wahrman J, Lewis KR, editors. Chromosomes today, 4. New York: Wiley; 1973. p. 321–34.
26. Maguire MP, Riess RW. Synaptic defects of asynaptic homozygotes in maize at the electron microscope level. Genome. 1996;39:1194–8.
27. McKently AH, Adams JB. *In vitro* propagation of *Paronychia chartacea*. Hort Sci. 1994;29:921–30.
28. Navarro J, Templado C, Benet J, Lange R, Rajmil O, Egozcue J. Sperm chromosome studies in an infertile man with partial, complete asynapsis of meiotic bivalents. Hum Reprod. 1990;5:227–9.
29. Newman MF, Jong K. Cytotaxonomic observations on *Mantisia wardii* (*Zingiberaceae*). Notes Roy Bot Gard. 1986;43:493–6.
30. Pandit MK, Babu CR. The effects of loss of sex in clonal populations of an endangered perennial *Coptis teeta* (*Ranunculaceae*). Bot J Linn Soc. 2003;143:47–54.
31. Pazy B, Plitmann U. Asynapsis in cistanche tubulosa (*Orobanchceae). Plant Syst Evol. 1996;201:271–3.*
32. Rahman MA, Yusuf M. *Mantisia Salarkhanii* Rahman & Yusuf (*Zingiberaceae*)—a new species from Bangladesh. Saudi J Biol Sci. 2002;9:105–10.
33. Rao SR. Cytogenetics of *Phlox* and *Vigna*. Unpublished PhD thesis. India: University of Jodhpur. 1990
34. Rao SR, Kumar A. Cytological investigations in a synaptic variant of *Anogeissus sericea* var. *sericea* Brandis (*Combretaceae*), an important hardwood tree of Rajasthan. Bot J Linn Soc. 2003;142:103–9.
35. Sharma SK, Bisht MS, Pandit MK. Synaptic mutation-driven male sterility in *Panax sikkimensis* Ban. (Araliaceae) from Eastern Himalaya, India. Plant Syst Evol. 2010;287:29–36.
36. Sharma SK, Bhowmik SSD, Kumaria S, Tandon P, Rao SR. Low genetic variation as revealed by SPAR methods possibly leads to extinction of two critically-endangered and endemic species of *Mantisia.* Biol Plant. (in press)
37. Singh DP, Sharma BL, Kanadia BA. Induced variability in mung bean following two methods of handling M2 populations. Trop Grain Legumes Bull. 1980;19:30–4.
38. Singh RB, Singh BD, Laxmi V, Singh RM. Meiotic behavior of spontaneous and mutagen induced partial desynaptic plants in pearl millet. Cytologia. 1977;42:41–7.
39. Singh RJ. Plant cytogenetics. London: CRC; 2004.
40. Sosnikhina SP, Mikhailova EI, Tikholiz OA, Priyatkina SN, Smirnov VG, Voilokov AV, et al. Genetic collection of meiotic mutants of rye *Secale cereal* L. Russ J Genet. 2005;41:1071–80.
41. Sudha CG, Seeni S. *In vitro* propagation of *Rauwolfia micrantha*, a rare medicinal plant. Plant Cell Tissue Organ. 1996;44:243–8.
42. Takano A. Cytological analyses of 19 taxa in *Globba* (*Zingiberaceae). Acta Phytaxonomica et Geobotanica. 2001;52:65–74.*
43. Takano A, Okada H. Taxonomy of *Globba* (*Zingiberaceae*) in Sumatra, Indonesia. Syst Bot. 2003;28:524–46.
44. Tandon P, Bhowmik SSD, Mao AA, Kumaria S. Rapid *in vitro* clonal propagation of *Mantisia spathulata* Schult, a rare and endemic plant of Northeastern India for recovery. Biotechnology. 2007;6:68–71.
45. Verma RC, Raina SN. Cytogenetics of *Crotalaria* VI. Chiasma frequency and position, and univalents behavior in a (partially) asynaptic mutant of *C. juncea*. Genetica. 1982;58:65–70.
46. Viera A, Santos JL, Rufas JS. Relationship between incomplete synapsis and chiasma localization. Chromosoma. 2009;118: 377–89.
47. Williams KJ, Kress WJ, Manos PS. The phylogeny, evolution, and classification of the genus *Globba* and tribe Globbeae (*Zingiberaceae): appendages do matter. Am J Bot. 2004;91:100–14.*
48. Xue DW, Ge XJ, Hao G, Zhang CQ. High genetic diversity in a rare, narrowly endemic primrose species: *primula interjacens* by ISSR analysis. Acta Botanica Sinica. 2004;46:1163–9.
49. Zetka M, Rose A. The genetics of meiosis in *Caenorhabditis elegans*. Trends Genet. 1995;11:27–31.
50. Zickler D, Kleckner N. Meiotic chromosomes: integrating structure and function. Annu Rev Genet. 1999;33:603–754.