

**MICROPROPAGATION AND CONSERVATION OF CRITICALLY
ENDANGERED *MANTISIA SPATHULATA* SCHULT. AND *MANTISIA
WENGERI* FISCHER (ZINGIBERACEAE) OF NORTH-EAST INDIA**

(ABSTRACT)

By

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Abstract

A large number of plants belonging to Zingiberaceae family are concentrated in the North-eastern region of India. The largest family Zingiberaceae comprises 53 genera and over 1200 species. Out of these, around 22 genera with about 200 species are found in India. Among the 22 genera of family Zingiberaceae, *Mantisia*, is the smallest genus with only four species viz. *M. wengeri*, *M. spathulata*, *M. radicalis* and *M. wardii*. Excluding *M. wardii* which is endemic to Myanmar, the rest of the three species are found only in restricted pockets of North-eastern India. Locally known as 'Aiting', these plants have been used in the past for curing gastro-intestinal ailments and bone injury. In addition to their medicinal properties, species of *Mantisia* possess splendid flowers which are commonly known as 'dancing girl' and can be utilized for commercial purposes in future. The vegetative and floral characteristics between the species of genus *Globba* and *Mantisia* are almost similar. Hence, the possibility of identifying the species of *Mantisia* among other *Globba* species in wild habitats during the blooming period depends largely on the floral characteristics. A characteristic difference between the two genera is the of position inflorescence. Unlike all the species of *Globba* which bear flowers at the terminal end of the vegetative shoot itself, separate floral spikes bearing numerous splendid flowers of *M. spathulata* (pale violet with yellow lip) and *M. wengeri* (yellow colour) appear during early monsoon before the onset of vegetative shoots. During the monsoon season the connectivity in the remote areas often becomes difficult due to excessive rainfall and landslides. Hence, these species remained unrecorded in their natural habitat for the past two decades. The plants have become endangered in their natural habitat and are listed in the Red Data Sheet of Indian plants (www.envfor.nic.in/bsi/research.html).

The rarity of *M. wengeri* has reached a critical level and has been included in the national priority list for its recovery (Department of Biotechnology, New Delhi) (Ganeshiah, 2005). Due to its endemism and rarity, the Government of Mizoram, India has declared the genus *Mantisia* as the 'State Flower'.

Breeding of zingibers including the species of *Mantisia* is seriously handicapped by poor flowering and seed set. The vegetative multiplication under *in situ* conditions through underground clonal multiplication of rhizomes is usually sluggish. Moreover, the common diseases such as soft rot and yellow rot caused by *Pythium aphanidermatum* and *Fusarium oxysporum* and bacterial wilt caused by *Pseudomonas solanaccrum* that are widely prevalent in most of the zingibers can also hamper the vegetative multiplication of the species of *Mantisia* in their wild habitat. To overcome the difficulties of conventional propagation, *in vitro* techniques of conservation have been used in a number of cases.

Due to the severe decline in the number of both *M. spathulata* and *M. wengeri* in their natural habitats, experiments were conducted for immediate large-scale propagation and conservation of these species with the following objectives in mind:

1. Micropropagation of *Mantisia spathulata* and *Mantisia wengeri*.
2. Establishment of the hardened plants in experimental garden and natural habitats.
3. Conservation of these species using seed germination and cryopreservation.

These objectives were fulfilled by using the following methods:

1. Survey was conducted in different parts of Mizoram, North-east India for the collection of the two species from their wild habitats. Collected plants were then maintained in the glasshouse.

2. Due to limited availability of plant materials, efforts were given to initiate sufficient aseptic primary cultures for conducting micropropagation experiments. Different explants viz., juvenile leaves, roots and rhizomatous buds after appropriate surface sterilization were inoculated in different nutrient medium viz., Murashige and Skoog (1962), Gamborg's *et al.* (1968), Mitra *et al.* (1976) and Knudson (1946) supplemented with various plant growth regulators for obtaining aseptic shoots.
3. Experiments were conducted to formulate an efficient micropropagation protocol for the *in vitro* multiplication of both the species. The effect of different physio-chemical factors viz., growth regulators, photoperiod, light intensity, temperature, etc. was studied for optimizing the regeneration, growth and development of the plantlets.
4. Various substrata and growth conditions were tested for hardening and acclimatization of *in vitro*-raised plants.
5. As cryptic variations might arise under *in vitro* conditions due to culture stress and mode of regeneration of the plantlets, experiments were conducted to assess the genetic stability of the *in vitro* plantlets with their mother stocks using Random Amplified Polymorphic DNA (RAPD).
6. *In vitro*-raised hardened plantlets were reintroduced under *ex situ* (Experimental Garden of the Department of Botany, NEHU, Shillong) and *in situ* (natural habitats of Lunglei, Mizoram, North-East India).
7. For conserving the genetic diversity of the species, seeds germination under *in vitro* (using different nutrient media supplemented with growth regulators) and *in vivo* (using soil from natural habitats) conditions were carried out.

8. Long-term conservation of the plants was carried out through cryopreservation of the seeds.

Main outcomes of the present study:

The important outcomes of the present research work are summarized below:

- An effective survey at different parts of the North-east India during the monsoon was conducted. There is no report on the survey of these plants for the past 20 years. Around 60 - 70 plants of *M. spathulata* and 40 - 50 plants of *M. wengeri* were found growing on the rocky hills along the road sides of two villages of Lunglei, viz. Lunglawn and Sethlun respectively at an altitude of around 1100 - 1300m above mean sea level. It was observed that the number of plants of the two species has severely declined in their natural habitats due to heavy landslides and rainfall. Therefore, experiments were carried out for multiplication of these two species using limited plant materials available from the nature.
- Out of the various explants used viz., juvenile leaves, roots etc., rhizomatous shoot buds explants were found to be only responsive for initiating aseptic cultures. This could probably be due to the presence of active cambial meristems in these tissues. After appropriate surface sterilization, the explants formed around 2 - 3 aseptic shoot buds within 3 - 4 weeks from the nodal portion of rhizomatous bud in MS medium incorporated with a combination of 8.8 μ M BA and 2.7 μ M NAA (*M. spathulata*) and 4.4 μ M BA and 2.7 μ M NAA (*M. wengeri*). The initial primary cultures thus obtained for both the species were further multiplied in their respective initiation medium to obtain sufficient explants.
- Finally, experiments were carried out to study the effect of different physio-chemical factors viz., growth regulators, photoperiod, light, temperature etc:

required for optimizing the shoot multiplication, growth and development of the *in vitro* plantlets.

- In the present study, it was found that explants of *M. spathulata*, regenerated with a highest of 6.1 ± 0.55 BFC in MS medium supplemented with $10 \mu\text{M}$ BA and $2.5 \mu\text{M}$ of NAA. Similarly, 7.82 ± 0.73 BFC was achieved for *M. wengeri* in MS medium containing $5 \mu\text{M}$ BA and $2.5 \mu\text{M}$ of NAA. The parameters used for measuring the growth and development of the *in vitro* plantlets of both the species were also found to be optimum at these treatments. The efficacy of shoot multiplication, growth and development of *in vitro* plantlets in medium containing other growth regulators showed varying responses. Incubating the cultures at a temperature of $24 \pm 2^\circ\text{C}$ under 12h daily illuminations with white fluorescent light of $40.5 \mu\text{moles m}^{-2}\text{s}^{-1}$ was found to optimize the shoot multiplication, growth and development of the *in vitro* plantlets of both the species.
- More than 5000 plantlets of *M. spathulata* and *M. wengeri* were regenerated *in vitro* in multiplication medium under optimum growth conditions.
- The transfer of plantlets from the culture vessels to the glasshouse conditions requires a careful and stepwise procedure. Successful transplantation also depends on suitable size of the plantlets and their state of growth *in vitro*. Healthy plantlets showing vigorous growth in the culture vessels were transferred to the pots. Out of the different substrata used for hardening and acclimatization of both *M. spathulata* and *M. wengeri* in this investigation, it was found that equal ratios (1:1) of soil and compost to be most suitable for higher survivability of transferred plants. Around 90.7% plantlets of *M. spathulata* and 84% of *M. wengeri* got acclimatized in this compost. Exposing the plantlets gradually to a relatively lower

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humidity, higher temperature and higher light intensity also maximized the survivability of the *in vitro* plantlets during acclimatization.

• In the present study, the plantlets were found to be morphologically similar, however tissue culture-raised plantlets are very often associated with somaclonal variations as reported in many cases. Any cryptic variants arising out of cultures might not help in the conservation of rare and endangered plants as these variants may become lethal for their survivability later. Therefore, investigation was carried out to assess the genetic fidelity of *in vitro*-raised hardened plantlets of *M. spathulata* and *M. wengeri* before their transfer to the field conditions. RAPD markers were used to evaluate the genetic stability of *in vitro*-raised hardened plantlets as it can detect single base change in genomic DNA. Similarity coefficient among the regenerated plants ranged between 0.85 - 0.98 for *M. spathulata* and 0.83 - 0.98 for *M. wengeri*. A maximum of 88% and 90% genetic similarity were obtained between *in vitro* raised hardened plantlets and mother stock of *M. spathulata* and *M. wengeri* respectively. There are many reports of 80 - 90% similarities between *in vitro* and mother plants which are considered to be normal. Thus, in the present study, the plants of both the species showing these similarities could be categorized as normal plants and equivalent to mother stock with regard to their genetic make up.

Did we discover the base pair from to change

• The success of any conservation measures depends upon the rate at which the plants survive in the natural habitat after reintroduction. As reported in other species maximum loss occurs during the phase when plants get acclimatized in the natural habitats due to several biotic as well as abiotic factors. A trial on the reintroduction of the plant species was carried out by transferring the hardened

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plantlets in the natural habitats for their recovery. Around 52% and 45% of *M. spathulata* and *M. wengeri* plants survived respectively under natural climatic conditions of Lunglei, Mizoram after reintroduction. As a measure of *ex situ* conservation the hardened plantlets were also introduced in the Experimental Garden of the Plant Biotechnology laboratory, Department of Botany, North Eastern Hill University, Shillong. More than 90% plantlets of both the species introduced in the Experimental Garden survived without noticeable morphological variations. It is assumed from the study that the most of the ecological factors like precipitation, temperature, humidity, light intensity etc. prevailing in the natural habitats must be similar in the reintroduced area of Shillong, which allowed the higher success rate of reintroduction of the two species in Shillong.

- In the present study, it was observed that new young seedlings were not seen to grow in and around the areas where the seeds from the capsules dispersed. Hence, it could be assumed that the seeds fail to germinate in nature either due to abiotic or biotic factors. The low percentage of seed germination of around 19.7% and 24.2% under *in vivo* condition for *M. spathulata* and *M. wengeri* respectively in the soil in the present study proves it.
- For the conservation of these species it was also desirable to propagate plants through seeds so as to maintain the heterogeneity which would lead to the production of diversity in the germplasm of seedlings. Therefore, the seeds collected from the nature were germinated under *in vitro* conditions in culture medium supplemented with growth regulators. Germination of the seeds was observed to be significantly enhanced to 90% and 96.7% for *M. spathulata* and *M. wengeri* in MS medium supplemented with 4.3 μ M and 7.2 μ M of GA₃

Why not RAPD
seeds? seeds

respectively. To propensate the diversity of these rare species, the seed derived plantlets of *M. spathulata* and *M. wengeri* obtained under *in vitro* conditions were finally transferred in the experimental garden.

- Long-term conservation of *M. spathulata* and *M. wengeri* has been successfully achieved through cryopreservation of the seeds. Cryopreservation provides an important practical approach for germplasm conservation as compared to *in vitro* cultures. Although different tissues such as shoot tips, protocorm-like bodies (PLBs), meristems etc. have been used, seeds of many species were found to be most suitable due to their inherent low moisture content and therefore, been easily cryopreserved under LN in most of the cases. Seeds of *Mantisia* species after pretreatment with higher osmoticum were dehydrated under sterile air of clean bench. With the fall of moisture level to 26% in the seeds after 2h of dehydration, around 40% seeds of *M. spathulata* were recovered in the regrowth medium after cryopreservation. Similarly, when the seeds of *M. wengeri* were dehydrated to 16.2% moisture level after 4h, around 36% recovery was possible in regrowth medium after cryostoring in LN for 1h.

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Conclusion and Future works

The conservation and recovery of these two rare and endemic zingibers has opened up the possibilities of conducting various significant studies in future. The rhizome of these plant species has been used as a remedy for bone fracture and gastrointestinal ailments in the past by local people. Therefore, serious efforts must be paid to explore the phyto-chemical constituents of these rare zingibers.

Studies on the developmental biology of the species of *Mantisia* are very essential due to their unique flowering characteristics. Flowers appear before the onset

of vegetative shoots during monsoon. The vegetative cycle is only for six months and the rhizomes remain dormant in the later part of the year. From our preliminary studies on the plants introduced in the experimental garden, it was observed that the dormant rhizomes are destined to produce a single inflorescence for the successive year after pre-monsoonal showers. However, not all the rhizomes are able to form floral inflorescences and instead form vegetative shoots.

The pollination studies for these plants are very urgent as most of the flowers produce capsules bearing seeds. To our observations insect pollinators, mostly bees, are observed to cross the flowers. Therefore, detailed study on the pollination of these ornamental flowers should be carried out to understand interesting phenomenon of plant-animal interaction for these rare zingibers. It was also observed that before getting detached from the floral stock the seed-bearing capsules burst and disperse the seeds in the soil which are eaten by ants. It was observed that these seeds are preyed by ants. New seedlings were not found to emerge in the soil during the same or successive year. Therefore, extensive studies must be carried out for finding the reasons for the non-germinability of the seeds in the soil.

As the species adapted rapidly under the climatic conditions of Shillong, further reintroduction of these species in other parts should be attempted using Niche Ecological Modelling Tools.

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