Conservation of *Mantisia spathulata* Schult. and *Mantisia wengeri* Fischer, Two Critically Endangered and Endemic Zingibers of Northeast India

Sudipta S. Das Bhowmik, Suman Kumaria* and Pramod Tandon

ABSTRACT

***Mantisia spathulata* Schult and *M. wengeri* Fischer are critically endangered and endemic ornamental zingibers restricted to a few pockets of Mizoram, Northeast India. Plants are decreasing at an alarming rate due to natural calamities and few countable representatives of both species exist in their wild habitats. To develop a system for rapid mass recovery and sustainability of the genetic diversity of the few existing plants, seeds of both species were germinated under *in vivo* and *in vitro* conditions. *In vivo* germination of seeds was very low (20% and 24% for *M. spathulata* and *M. wengeri*, respectively). However, germination *in vitro* was significantly enhanced. A maximum of 90.0% and 96.6% germination was recorded for *M. spathulata* and *M. wengeri* within 2 wk in MS medium supplemented with 4.3 µM and 7.2 µM GA_3, respectively. Seedlings of both species were transferred to an experimental garden and maintained as a field gene bank that could be utilized for studying genetic variability.**

EXPERIMENTAL TECHNIQUES

Mantisia spathulata Schult. and *M. wengeri* Fischer are two critically-endangered ornamental zingibers that are found only in restricted pockets of Mizoram, Northeast India. The two species belong to the smallest genera of the family Zingiberaceae and are highly significant for understanding the molecular phylogenetic classification of the family. It has been strongly suggested that an extensive field survey of all the species of *Mantisia* in their wild habitats should be made (Williams et al., 2004). However, plants of both species have been significantly reduced in natural habitats due to natural calamities and human intervention (Ganeshaiah, 2005; Tandon et al., 2007). We previously reported the conservation of *Mantisia* spp. through *in vitro* clonal propagation and *ex situ* reintroduction in a garden planting (Bhowmik et al., 2009).

Tissue culture has been extensively used for rapid multiplication of many important rare zingibers (Prathanturarug et al., 2004; Tefera and Wannakrairo, 2004; Tandon et al., 2007). These *in vitro* raised plantlets represent the true-to-type clones of their germplasm. However, for effective conservation of rare and endangered plants it is crucial to sustain the genetic diversity among plants. The possibility of sustaining the genetic diversity of rare and endangered plants becomes possible only through seed propagation because of their

Plant Biotechnology Laboratory, Centre for Advanced Studies in Botany, North Eastern Hill University, Shillong - 793 022, India; *Corresponding author (E-mail: sumankhatrikumaria@hotmail.com).
Received 28 August 2009.

heterogeneity (Pence, 1999; Tandon and Kumaria, 2005). However, natural propagation through seeds in many rare and endangered plants is limited due to many factors such as seed dormancy (Rathore et al., 1991), poor seed viability (Sudha and Seeni, 1996) and little or no seed production (Agrawal, 1991; McKently and Adams, 1994). In some cases, seeds have particular requirements for germination such as sandfood [*Pholisma sonorae* (Torr. ex Gray) Yatsk.], an endangered parasitic plant that requires association with the root tissues of the host plant for germination (Pence, 1999). Similarly, mycorrhizal association with particular fungal species is necessary for orchids because less than 5% of the orchid seeds germinate in nature (Rao, 1977). These barriers have been mostly overcome by germinating the seeds under *in vitro* conditions which allow immediate large-scale propagation and conservation of the genetic diversity, especially for rare and endangered plants. Genetic variations under natural conditions in most of the zingibers are sluggish due to clonal propagation through rhizomes (Nadgauda et al., 1978) and lack of seed propagation (Sajina et al., 1997; Miceli et al., 2008). The objective of this study was to evaluate *in vitro* seed germination of *M. spathulata* and *M. wengeri* for maintaining the genetic diversity of these rare and endemic plants and compare it with *in vivo* seed germination so as to assess the natural mode of propagation.

Collection of seeds

The fruiting capsules enclosing seeds were collected from wild plants of *M. spathulata* and *M. wengeri* from Lunglei in Mizoram, India (23°52′30″ N latitude, 92°45′30″ E longitude) during the early monsoon season of 2007 and stored in a refrigerator (4 °C) until the experiments were initiated.

***In vivo* seed germination**

To study *in vivo* seed germination, soil was collected from natural habitats of both species and ground to fine particles. The pH of the soil was determined using a pH meter (Eutech Instruments, UK). Seeds (~1.0–2.0 mm in size) of both species were germinated in soil placed over water-soaked filter paper in petri dishes (12.5 × 12.5 cm) inside a 25 ± 2 °C plant growth chamber (Narrang Scientific Works Pvt. Ltd., New Delhi, India) under a 12 h photoperiod [photosynthetic photon flux density (PPFD) of 40.5 μmoles $m^{-2} \cdot s^{-1}$]. The soil was regularly sprinkled with water at 3 d intervals to maintain optimum humidity (70–80% RH). Experiments were replicated 3 times with 120 seeds per experiment and percent seed germination (emergence of plumule) was recorded 4 wk after sowing.

***In vitro* seed germination**

To study *in vitro* seed germination, 1 wk old immature capsules of *M. spathulata* and *M. wengeri* were treated with 0.2% cetramide (Shalaks Pharm Industrial Ltd., New Delhi, India) for 10 min, surface sterilized with 0.075% mercuric chloride for 5 min and rinsed several times with sterile pure water. Ten seeds dissected from the aseptic capsules were inoculated into a 150 mL conical flask containing 50 mL of sterilized MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and gibberellic acid (GA_3) at six concentrations (0.0, 1.4, 4.3,

7.2, 14.2 and 28.9 μM). The medium was solidified with 0.8% extra pure agar (Hi-media, India). The pH of the medium was adjusted to 5.8 prior to autoclaving at 1.06 $kg \cdot cm^{-2}$ (121 °C) for 15 min. Cultures were incubated at 25 ± 2 °C under 12 h photoperiod with a PPFD of 40.5 μmoles $m^{-2} \cdot s^{-1}$ provided by cool white fluorescent lamps. Seed germination (emergence of plumule and radicle) was recorded after 2 wk of culture. Experiments were repeated 3 times with 10 replicates (10 seeds for each treatment) per experiment. Statistical analysis was an analysis of variance (ANOVA) and means were compared using Tukey's test ($p = 0.05$) (Origin 7.0, Northampton, MA, USA).

Hardening and field transfer of plantlets

Two-month-old seedlings (2.5–3.0 cm in size) that were raised *in vitro* were removed from culture vessels and washed with water to remove adhering agar. Seedlings were potted in paper cups containing mixtures of soil and compost (1:1). Cups were covered with perforated polybags and sprinkled with water alternately at 2 d intervals for the initial 2 wk, and acclimatized at 30 ± 2 °C and 70 ± 5% RH under glass-house conditions. The established hardened plantlets were removed from paper cups and transferred to the experimental garden after 1 year.

RESULTS AND DISCUSSION

Plants of *M. spathulata* and *M. wengeri* were found blooming in their natural habitats during May 2007 on the rocky hills along the roadside of Lunglei, Mizoram, India at an elevation of about 1395 msl. It was observed that the habitats of the plants had severely eroded at different places due to slope failures and landslides as a result of heavy rainfall. Inflorescences bearing numerous splendid flowers of both *M. spathulata* (pale violet with yellow lip) and *M. wengeri* (yellow) appeared before the onset of vegetative shoots. A single flower of *Mantisia* spp. resembles a 'dancing girl', an exclusive rare ornamental characteristic among flowering plants. Capsules of both species were collected from the wild plants. It was noticed that the seed-bearing capsules dehisce immediately after seed formation. Since no new young seedlings were seen growing around the areas where the capsules dispersed, it could be assumed that seed germination in nature is not common. This assumption was confirmed in the *in vivo* seed germination experiments in soil where only 20% and 24% germination was recorded within four weeks for *M. spathulata* and *M. wengeri*, respectively. The pH of the soil from natural habitats was acidic (6.7 and 6.8 for *M. spathulata* and *M. wengeri*, respectively).

Although seeds showed very poor germination in soil under *in vivo* conditions, germination was significantly enhanced under *in vitro* conditions in MS medium incorporated with GA_3 at various concentrations. A maximum of 90% seed germination occurred for *M. spathulata* within 4 wk in MS media supplemented with 4.3 μM of GA_3, which was significantly higher than the control (Table 1). Seed germination was reduced with either an increase or decrease in the concentrations of GA_3 beyond this optimum level. Similarly, maximum seed germination of 97% occurred for *M. wengeri* under *in vitro*

Table 1. Mean *in vitro* seed germination percentage (± SE) of *Mantisia spathulata* and *M. wengeri* after four weeks in MS medium supplemented with GA_3

GA_3 (μM)	*M. spathulata*	*M. wengeri*
0.0	16.7 ± 3.3 a†	13.3 ± 3.3 a
1.4	53.3 ± 3.3 b	30.0 ± 5.7 a
4.3	90.0 ± 5.7 b	56.6 ± 3.3 b
7.2	83.3 ± 6.7 b	96.6 ± 3.3 b
14.2	63.3 ± 3.3 b	73.3 ± 3.3 b
28.9	36.7 ± 3.3 a	46.6 ± 6.6 b

†Germination values within a column followed by the same letter were not significantly different based on Tukey's test (p = 0.05).

conditions (MS with 7.2 μM GA_3) within 4 wk, which was significantly higher than the control (Table 1).

The regulation of seed germination with GA_3 has also been reported in devil's claw [*Physoplexis comosa* (L.) Schur.] and *Primula glaucescens* Moretti (Cerabolini et al., 2004). The growth regulator GA_3 has been widely-studied to break seed dormancy in various plants (Nicolas et al., 1996; Rehman and Park, 2000). However, reports on seed germination of zingibers are very limited. A maximum of only 35% seed germination of pink ginger [*Alpinia purpurata* (Vieill.) K. Schum.] and 25% germination in large cardamom (*Amomum subulatum* Roxb.) were recorded in MS medium without growth regulators (Brain and Richard, 1993; Sajina et al., 1997). Similarly, only 20% seed germination was recorded in *Renealmia mexicana* Klotzsch ex. Petersen (Miceli et al., 2008). It has also been reported that endogenous GA_3 facilitates germination in tomato (*Solanum lycopersicon* L.) seeds by reducing the mechanical restraint of endosperm cells to permit radicle protrusion (Groot and Karssen, 1987). Thus, it can be assumed that seeds of both the species studied may have some physiological restraint in the mobilization of nutrients from endosperm cells leading to poor germination under *in vivo* conditions.

In vitro-raised seedlings measuring 1–2 cm in size were transferred to MS medium without GA_3 and produced well-developed plantlets with healthy roots and shoots within 3 wk of subculture. Seedlings obtained under both *in vitro* and *in vivo* conditions were removed from the glass-house and transferred to the experimental garden. More than 95% of the seedlings survived in the garden for a year and are being maintained as separate lineages for studying their genetic diversity.

ACKNOWLEDGMENT

Financial support received from the Department of Biotechnology, Government of India vide grant no. BT/PR-7055/BCE/08/437/2006 is gratefully acknowledged. The authors are grateful to Mr. K.M. Lalhmachlwana, Forest Department, Mizoram and Botanical Survey of India, Shillong for their generous help.

REFERENCES

Agrawal, D.C., S.S. Pawar., G.C. Morwal and A.F. Mascarenhas. 1991. *In vitro* micropropagation of *Delphinum malabaricum* (Huth) Munz — a rare species. Ann. Bot. 68(3):243–245.

Bhowmik, S.S.D., S. Kumaria., S.R. Rao and P. Tandon. 2009. High frequency plantlet regeneration from rhizomatous buds in *Mantisia spathulata* Schult. and *Mantisia wengeri* Fischer and analysis of genetic uniformity using RAPD markers. Ind. J. Exp. Biol. 47(2):140–146.

Brain, K.W. and A.C. Richard. 1993. Clonal propagation of pink ginger *in vitro*. HortScience 28:1203–1205.

Cerabolini, B., R. De Andreis., R.M. Ceriani., S. Pierce and B. Raimondi. 2004. Seed germination and conservation of endangered species from the Italian Alps: *Physoplexis comosa* and *Primula glaucescens.* Biol. Conserv. 117:351–356.

Ganeshaiah, K. N. 2005. Recovery of endangered and threatened species: Developing a national priority list of plants and insects. Curr. Sci. 89: 599–600.

Groot, S.P.C. and C.M. Karssen. 1987. Gibberellins regulate seed germination in tomato by endosperm weakening: a study with gibberellin-deficient mutants. Planta 171: 525–531.

McKently A.H. and J.B. Adams. 1994. *In vitro* propagation of *Paronychia chartacea*. HortScience 29(8):921–930.

Miceli, F.A.G., A.D. Estudillo, M.A. Archila, T.R.A. Talavera and L. Dendooven. 2008. Optimization of *Renealmia mexicana* (Klotzsch ex. Petersen) cultivation *in vitro*. In Vitro Cell Dev. Biol. Plant 44:33–39.

Murashige, T. and F.A. Skoog. 1962. Revised medium for rapid growth and bioassays for tobacco tissue cultures. Physiol. Plant. 15: 473–497.

Nadgauda, R.S., A.F. Mascarenhas, R.R. Hendre and V. Jagannathan. 1978. Rapid multiplication of turmeric (*Curcuma longa* Linn.) plants by tissue culture. Ind. J. Exp. Biol. 16:20–25.

Nicolas, C., G. Nicolas and D. Rodriguez. 1996. Antagonistic effects on abscisic acid and gibberellic acid on the breaking of dormancy of *Fagus sylvatica* seeds. Physiol. Plant. 96:244–250.

Pence, V.C. 1999. The application of biotechnology for the conservation of endangered plants. p. 227–250. *In* Plant conservation Biotechnology. E.E. Benson (ed.), TJ International Ltd. Padstow, UK.

Prathanturarug, S., D. Angsumalee, N. Pongsiri, S. Suwacharangoon and T. Jenjittikul. 2004. *In vitro* propagation of *Zingiber petiolatum* (Holttum) I. Theilade, a rare zingiberaceous plant from Thailand. In Vitro Cell Dev. Biol. Plant. 40:317–320.

Rao, A. N. 1977. Tissue culture in the orchid industry. p. 44–69. *In* Applied and Fundamental aspects of Plant Cell, Tissue and Organ Culture. J. Reinert and Y.P.S. Bajaj (eds.), Springer-Verlag, Berlin.

Rathore, T.S., P. Tandon and N.S. Shekhawat. 1991. *In vitro* regeneration of pitcher plant (*Nepenthes khasiana* Hook. f.) — a rare insectivorous plant of India. J. Plant Physiol. 139:246–248.

Rehman S. and I.H. Park. 2000. Effect of scarification, GA and chilling on the germination of golden rain-tree (*Koelreuteria paniculata* Laxm.) seeds. Sci. Hort. 85:319–324.

Sajina, A., P.M. Mini, C.Z. John, K.N. Babu, P.N. Ravindran and K.V. Peter. 1997. Micropropagation of large cardamom (*Amomum subulatum* Roxb.). J. Spice Arom. Crops. 6:145–149.

Sudha, C. G. and S. Seeni. 1996. *In vitro* propagation of *Rauwolfia micrantha*, a rare medicinal plant. Plant Cell Tiss. Org. Cult. 44(3):243–248.

Tandon, P., S.S.D. Bhowmik, A.A. Mao and S. Kumaria. 2007. Rapid *in vitro* clonal propagation of *Mantisia spathulata* Schult, a rare and endemic plant of Northeastern India for recovery. Biotechnology 6(1):68–71.

Tandon P. and S. Kumaria. 2005. Prospects of Plant Conservation Biotechnology in India with special reference to Northeastern region. p. 79–92. *In* Biodiversity status and prospects. P. Tandon., M. Sharma and R. Swarup (eds.), Narosa Publishing House, New Delhi.

Tefera, W. and S. Wannakrairo. 2004. Micropropagation of Krawan (*Amomum krervanh* Pierre ex Gagnep). Sci. Asia. 30:9–15.

Williams, K.J., W.J. Kress and P.S. Manos. 2004. The phylogeny, evolution, and classification of the genus *Globba* and tribe Globbeae (Zingiberaceae): appendages do matter. Am. J. Bot. 91(1):100–114.