

Propagation of Threatened *Nepenthes khasiana*: Methods and Precautions

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Abstract

Ex-situ conservation is an important key in the management of rare, endangered and threatened (RET) plant species and its effectiveness depends on several factors. Maintenance of viable germplasm and its subsequent propagation plays an important role in long term conservation of many RET species. *Nepenthes khasiana* is a rare and gravely threatened species in the wild due to over-collection and other threats. The species needs urgent *in-situ* and *ex-situ* conservation. Development of easy to propagate techniques would pave faster multiplication for its use of educational, medicinal and horticultural purpose. In the present paper, successful propagation technique of *Nepenthes khasiana* through seeds is demonstrated along with detailed information on precautions to be taken during the adoption of the techniques.

Keywords: carnivorous plants, conservation, germination, pitcher plant, propagation, RET species, seeds

Introduction

Nepenthes khasiana Hook. f. is the only pitcher plant species found in India. It is the only representative of the genus *Nepenthes* in India and belongs to monotypic family Nepentheaceae. This dioecious carnivorous plant is endemic to Meghalaya state. Locally it is known as 'Ksete-phare' (in Jaintias), 'Memangkoksi' (in Garo), 'Tiew-rakot' (in Khasi). Various uses of *Nepenthes khasiana* by the tribals and local people are well known. Khasi and Garo tribes used the fluid of unopened pitcher as eye drops to cure cataract and night blindness, as well as in treating stomach troubles, diabetes and gynecological problems (Singh and Mudgal, 1999; Ghosh and Ghosh, 2012). They also used paste of pitcher and its contents to cure leprosy (Ghosh and Ghosh, 2012). In recent times, habitat destruction, acid mine drainage associated with coal mining and over-collection for medicinal and ornamental uses have threatened this species (Bordoloi, 1977; Prasad and Jeeva, 2009; Tandon *et al.*, 2009; Singh *et al.*, 2011; Ghosh and Ghosh, 2012; Verma *et al.*, 2014).

Nepenthes khasiana is protected by the government of India under Schedule VI (Section 2) of The Indian Wildlife Protection Act from 1972, as amended in 1993. This rare and threatened species is under *ex-situ* conservation in Himalayan region which includes Botanical Survey of India, Shillong; North Eastern Hill University, Shillong; Forest Research Station at Umian (Barapani) under Meghalaya State Forest Department (Silviculture); the germplasm collection centre in the Garden of Medicinal Plants of the University of North Bengal, West Bengal; State Forest Research Institute, Itanagar; Guahati University, Guwahati and Experimental Garden of Botanical Survey of India in Yercaud (Mandal and Mukherjee, 2011; Ghosh and Ghosh, 2012). Growing them

outside the Himalayan region has been a challenging task since the species thrive in humid and cooler region of evergreen tropical forest.

In vitro seed germination and *in vitro* vegetative propagation were deployed to multiply the species (Rathore *et al.*, 1991; Bahadur *et al.*, 2008; Nongrum *et al.*, 2009). *In vitro* techniques require high financial investment, infrastructures, specialized experts and space for carrying out the propagation, which are beyond the possibility of the common plant enthusiasts.

RET Species Propagation Centre at CSIR-NBRI Botanic Garden, Lucknow, formulated an easy growing technique which will support wider cultivation and propagation of pitcher plants.

In the present paper, methods of propagating and precautions involved for multiplication of *Nepenthes khasiana* were demonstrated.



Fig. 1. A) *Nepenthes khasiana* Hook. f. in East Khasi Hills, Meghalaya; B) a young plant in *ex-situ* conservation at CSIR-NBRI Botanic Garden

Materials and Methods

Freshly collected seeds of *Nepenthes khasiana* were used for the study. The small seeds were soaked in distilled water for 24 hours before placing on sterilized germinating medium. Following three types of medium, 8 × 8 cm size plastic pots were used for the study as follows:

Medium I- Chopped sphagnum moss, coir or coco peat, and sand (1:3:3),

Medium II- Chopped sphagnum moss and Coir or Coco peat (1:5),

Medium III- Coir or Coco peat.

For Medium I, the pots were first filled with a mixture of sand and coir followed by a layer of sphagnum moss respectively. Medium II had a layer of chopped sphagnum over coir. The medium were watered with nutrient free distilled water before placing the soaked seeds on their upper surface. Fifty seeds were placed in each medium. Two sets of each medium were prepared. One set of each medium was kept inside of 14 × 20 cm zip loc transparent plastic bags and kept closed inside further analysis. Other set was kept open without the plastic bags. All the plastic pots were kept inside a temperature regulated mist house with temperature ranging from 25-28 °C and humidity 70-80%. The medium were daily sprayed with distilled water to make the medium moist and the pots were kept in semi shade area. At night they were put under fluorescent lights with temperature ranging from 23-25 °C.

Results

Seeds in all tested mediums kept inside the plastic bags started germinating in 4-5 weeks. None of the seeds kept in the medium without plastic bags germinated and their colour changed from brown to dark black. 100 percent germination was observed in Medium III, while Medium I and II had 85-90% germination. In the fourth week, two small leaves started protruding from the seeds

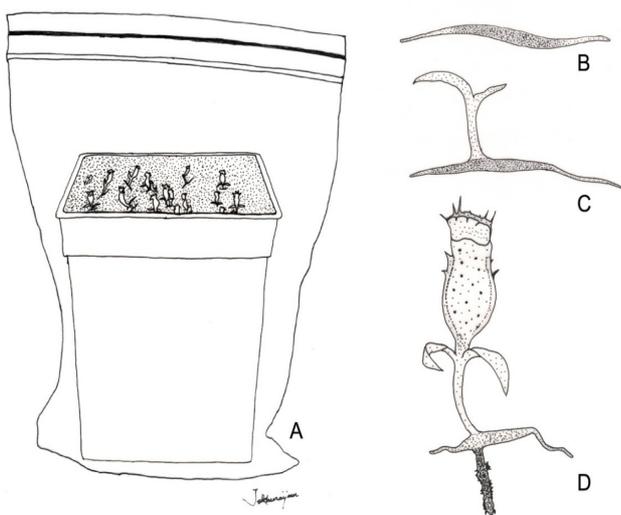


Fig. 2. A) Small seedlings of *Nepenthes khasiana* in closed zip loc plastic bag; B) Seed; C) Germinated seed; D) Seedling with pitcher and root



Fig. 3. A) Seed; B) Germinated seed; C) Seedling with pitcher and root; D) Two months old healthy seedlings. Photos by Little AC

(Fig. 2C). When they attained the height of 2-4 mm within the six-seventh weeks, they started developing roots and subsequently started producing small pitchers. The light green coloured pitchers had small soft wings and transparent spikes protruding out of the lid (Fig. 2D). In the eightth week, another pitcher developed opposite to the older one.

Precautions

The growing medium should be nutrient free and well drained. *Nepenthes khasiana* grows in nutrient deficient soil in natural habitats and they depend on insects trapped in pitchers for nitrogen and other nutrients. The best medium is pure coir. Regular spraying of water (distilled or tap water) is necessary to keep the medium and seeds moist. Keeping the pots in zip loc plastic bags helped not only in maintaining the seeds and mediums' moist, but also protected from fungal spores from the air. In the case of any fungal growth, a pinch of fungicide powder diluted in water can be sprayed on the infected area. Blowing of air by mouth inside the plastic bags once in a week after seed germination help in supply of carbon-dioxide for the growing seedlings. Temperature should be strictly maintained between 25-30 °C. The pitcher plants can be feed with small insects or small pieces of insects. However, it is not necessary as pitcher plants will start trapping themselves once they grow up. The over-crowded seedlings can be transplanted to other pots. Exposing to direct sunlight should be avoided and the seedlings should be always kept moist in semi-shade place.

Conclusions

The above propagation method is an easy to follow technique which does not require sophisticated equipment or expertise. However, it requires certain precautions that need to be carefully followed for the proper plant development. It is important in the case of endangered species to have effortless and uncomplicated proceedings for germination and cultivation of plantlets. Maintenance of viable germplasm and its subsequent propagation plays an important role in long term conservation of many RET species, including *Nepenthes khasiana*.

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