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SEED VIABILITY STUDIES IN *NEPENTHES KHASIANA* HOOK.F.: COMPARISON OF *IN VIVO* AND *IN VITRO* SEED GERMINATION

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ABSTRACT

Studies on the seed viability of *Nepenthes khasiana* Hook.f. was conducted. Seeds were collected from different natural habitats and stored both at low temperature (5°C) and at ambient room temperature (25±2°C). Seed viability study was carried out by germinating seeds both *in vivo* and *in vitro*. For *in vivo* study seeds were sown on a leaf mold-based mixture in Petri dishes, whereas *in vitro*, the seeds were inoculated onto half-strength Murashige and Skoog (1962) medium. Within 2 week of culture over 50% of seeds germinated *in vitro*, whereas in vivo seed germination reached 50% after 8 week. Light and dark conditions were also used to test their effect on germination. Addition of Gibberelic acid did not show any effect on the germination of seeds. The study has shown high seed viability as shown from the high rate of germination, both *in vivo* and *in vitro*. For optimum seed germination, high humidity (80 - 90%) and a warm temperature of around 25°C-30°C are essential. The study has also shown that seeds stored at ambient room temperature condition lose its viability rapidly within a year but seeds stored at low temperature (5°C) remain viable for over 5 years.

INTRODUCTION

In India, *N. khasiana* is the only pitcher plant species found, and endemic to Meghalaya State. Several workers had carried out studies on different aspects of *N. khasiana*, such as habitat (Rao & al., 1969), morphology and histology (Bordoloi, 1977), status (Jain & Baishya, 1977), ornamental (Mukherjee & al., 1984), taxonomy (Joseph & Joseph, 1986), tissue culture (Redwood & Bowling, 1990; Rathore & al., 1991; Latha & Seeni, 1994; Mao & al., unpublished), and distribution and status in natural habitat (Mao & Kharbuli, 2002).

However, so far no detailed study on seed viability has been carried out on *N. khasiana*. The first author has been studying *N. khasiana* for the last ten years on micropropagation, distribution and status in natural habitats and has observed that in natural habitats the germination of seed was very low as compared to the number of seeds produced by a single inflorescence. A single infructescence bears 80 to 100 fruits and each fruit may contain an average of 475 seeds. Therefore, a single infructescence produced thousands of seeds every year. A close examination of seedlings in natural habitats shows that only a few seedlings growing along with the adult plants or sometimes not even a single seedling being found indicating very poor germination of seed. This has prompted the

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authors to investigate reason for the low germination of seed in natural condition. Successful seed germination is a must for a plant species survival in nature.

The aim of the present investigation was therefore, to carry out seed viability study by germinating them both *in vivo* and *in vitro*, and also, to assess the optimum conditions required for seed germination and the viability of short-term seed storage at low temperature.

MATERIAL AND METHOD

N. khasiana flowers in the months of June to August and fruit mature in about 3 months time. For the study seeds were collected during the months of November to January from the Botanical Survey of India, Experimental Botanic Garden, Barapani, Mawsynram and Lawh Bah, East Khasi Hills and Jarain in Jaintia Hills. The seeds were used for germination within a week of collection or stored at ambient room temperature (18-25°C) for 12 months or in sealed bottle with silica gel at 5°C in refrigerator for up to 6 years or more. A few hundred seeds from the seeds stored were taken out every year and tested for its viability by germinating *in vitro* and *in vivo*. The accumulative

Table 1 : *In vivo* Seed Germination

Treatment	No. of seeds	Accumulative % of seed germination			
		4 week	6 week	8 week	10 week
T_0	100	56	70.4	71	71
T_1	100	0	1	4	6
T_2	100	12	12	22	90
T_3	100	16	74	74	76
T_4	100	8	9	49	71
T_5	100	11	55	62	64
T_6	100	12	35	56	58

Table 2 : *In vitro* Seed Germination

Treatment	No. of seeds	Accumulative % of seed germination			
		2 week	4 week	6 week	8 week
T_0	100	10	60	72	72
T_1	100	0	4	4	8
T_2	100	86	90	90	90
T_3	100	50	68	68	68
T_4	100	82	82	82	86
T_5	100	40	52	56	56
T_6	100	51	53	53	53

ABBREVIATIONS: T_0 = 2-month stored seeds at ambient room temperature condition, T_1 = 1 year old seeds stored at room temperature, T_2 = 1 year, T_3 = 2 year, T_4 = 3 year, T_5 = 4 year and T_6 = 5 year old seeds stored at 5°C.

percentage of seed germination results are presented in Table 1, 2. Effect of GA_3 at four different concentrations (0.1,1.0,10.0,100 μM) on seed germination was also tested.

IN VIVO GERMINATION: Two sets of experiments were set up as follows: (1) The seeds were spread evenly on the surface and (2) The seeds sown ca 1 cm deep in leaf-mold based compost mixture in seed trays or Petri dishes (90 mm × 20 mm) with the same compost mixture. Trays were placed under diffuse natural daylight in glasshouse unit where high humidity (70-85%) and the Petri dish with its lid on in room condition (18-25°C) were maintained. Watering was done regularly to maintain the surface of the compost moist at all time. For each experiment seeds were sown in four replications. Each replication contained 100 seeds. The germination of these seeds recorded at intervals of 4, 5, 6, 7, 8 and 10 week after sowing of seeds.

IN VITRO GERMINATION: Seeds were sterilized by soaking in 10% solution of Sodium Hypochlorite (minimum 8% available chlorine) with 1-2 drops of Tween 20, for 15 minutes. After three washes with sterile distilled water under aseptic conditions, 20 seeds each were inculated onto basal medium supplemented with or without activated charcoal. Basal medium consisted of half-strength Murashige and Skoog (1962) (MS) salt plus 2% (wt/vol) sucrose that was adjusted to pH 5.8, supplemented with 0.8% (wt/vol) Difco Bacto agar and autoclaved for 20 min at 121°C. About 20 ml of the medium was disposed aseptically into each 90 mm Petri dish.

The experiment was laid out in a randomized block design with five replications. Each Petri dish contained 20 seeds. All Petri dishes were sealed with Nescofilm and placed in a growth room where the photosynthetic photon flux density (PPFD) and temperature were 70 $\mu mol.m^{-2}s^{-1}$ and 25±2°C, respectively. An equal number of replications were placed under dark was well as under light conditions. "Dark" signifies permanent darkness during the entire culture period except during the time of taking observation. "Light" means a light/dark period of 12 h/12 h with light supplied by warm fluorescent tubes. However, the initial results of the study shows no significance different between light and dark condition, hence, all the subsequent experiments were carried out in the light condition. The weekly progress of germination was recorded up to 8 week and cumulative percentage of germination calculated.

RESULTS AND DISCUSSION

IN VIVO GERMINATION : Seed germination started within 3 week where the seeds were sown on the surface of the compost mixture. Germination spread over 10 week after which no subsequent germination occurred (Table-1). The seed germination was high in Petri dishes but very low (5-10%) in seed trays. The differences could be that the Petri dishes with its lids on maintained uniformly higher humidity (80-95%) and temperature (25±2°C). However, no seed germination occurred on the treatment where the seeds were sown ca 1 cm deep in soil even after 20 week or more.

There were 90%, 76%, 71%, 64% & 58% seed germination from the experiments where the seeds were stored for 1 yr., 2 yr., 4 yr., and 5 yr. respectively at 5°C after harvest (Table-1). Freshly harvested seeds gave very low germination (not shown in the table) but when seeds were stored for 2-3 months there was over 71% germination. However, when the seeds were stored at ambient room temperature for about a year, germination of seeds became very low (6%).

IN VITRO GERMINATION : The seeds began to swell within a week of inoculation and the first germination was apparent by 12 day and within 4 week 50-90% seed germination occurred on the different treatments with the exception of T_1 (Table-2). In few of the treatments sporadic seed germination continues up to 8 week after which no more germination occurred. Similar to in vivo result, very low seed germination occurred from the seeds stored at ambient room temperature for over a year (8%).

The seed germinated faster in vitro than *in vivo* by week and reached the peak within 4 week whereas *in vivo* the peak was reached after 8 week from the date of sowing after which the seeds in most cases do not germinate anymore. A possible explanation for the early seed germination in vitro could be due to the control condition of the culture. *In vivo* the seed should be sown thinly on surface of the soil and not deep in the soil. Seeds sown deep (ca 1 cm) in soil failed to germinate in spite of providing right conditions for its germination. Otherwise, apart from the fast seed germination *in vitro*, there was no significant difference in the percentage of accumulative seed germination between *in vitro* and *in vivo*. The seedlings can be transplanted into small individual pots when they have developed two to three leaves which normally take bout three months after its germination.

Low germination of seed was observed on freshly harvested seeds but after storing for 2-3 months at room temperature condition, high percentage of seed germination occurred (T_0) indicating that the seeds undergo a period of dormancy. Also, as shown by the study for short term germplasm storage of seed it required storage at low temperature to maintain its viability otherwise the seeds start losing its viability if stored at room temperature condition for about a year (T_1).

GA_3 has been reported to be effective for promoting *in vitro* seed germination in *N. khasiana* (Rathore & al., 1991), however, in the present experiment the addition of GA_3 did not improve germination percentage (Table-3). Similarly, dark conditions did not improve the germination percentage, although there are reports in literature that the presence of light acts as negative photoblastism in the germination of excised embryos in other species (Hisajima & al., 1991, Pancholi & al., 1995). The study has shown that *N. khasiana* seeds germinated freely without any difficulties. The seeds can be germinated freely in light or in dark. However, high humidity with warm temperature of about 25 to 30°C is a must for its successful germination.

Table 3 : Effect of GA_3 on the *in vitro* Seed Germination

GA_3 (μM)	No. of seeds*	Accumulative % of seed germination			
		2 week	4 week	6 week	8 week
0.0	100	80	85	88	90
0.1	100	78	84	88	88
1.0	100	86	90	90	90
10.0	100	75	80	86	86
100	100	82	82	82	86

* 1 year old seeds stored at 5°C used for the experiment.

Also, the results of the experiments suggest that *N. khasiana* seeds can be stored at low temperature conditions for many years without losing its viability. The finding, thus, is in agreement with reports that many 'orthodox' species of plants seeds can be stored at low temperature conditions for over many years without losing its viability (Withers, 1991).

It may be concluded that to combat the hostile conditions in nature *N. khasiana* produces thousands of seeds and only few that falls on suitable conditions germinate. The low germination of seed in natural habitats could be due to various reasons such as forest fire that burns the seeds during the dry season or lack of proper moisture and humidity. The plant may be compared to orchids which also produce thousands of seeds in a single pod but only few seeds germinate when it is infected by fungi.

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नेपेन्थेस खसियाना हुक. एफ में बीज अंकुरण क्षमता - पात्रे एवं पर-सेते बीज अंकुरण में तुलनात्मक अध्ययन

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सार संक्षेप

नेपेन्थेस खासियाना हुक.एफ. में बीज अंकुरण क्षमता का अध्ययन किया गया। बीजों का संग्रहण विभिन्न प्राकृतवासों से किया गया एवं भंडारण 5°C के न्यूनतम तापमान और सामान्य तापमान (25±2°C) पर किया गया। पर-सेते अध्ययन के लिए बीज पत्ती खाद पर अंकुरित किए गए तथा पात्रे अध्ययन हेतु *मूराशीग एवं स्कूग* (1962) माध्यम का प्रयोग किया गया। पात्रे परीक्षण में दो सप्ताह के भीतर 50 प्रतिशत अंकुरण प्राप्त हुआ जबकि पर-सेते प्रयोग में में यह चार सप्ताह के पश्चात हो पाया। प्रकाश एवं अंधेरे का प्रभाव भी बीजों के अंकुरण पर जांचा गया। इष्टतम बीज अंकुरण के लिए उच्च आर्द्रता (80-90 प्रतिशत) एवं 25-30°C तापमान जरुरी है। अध्ययन द्वारा यह भी ज्ञात हुआ कि सामान्य तापमान पर बीज एक वर्ष में अंकुरण क्षमता रोक देते हैं जबकि न्यूनतम तापमान (5°C) पर भंडारण करने से अंकुरण क्षमता 5 वर्ष तक कायम रहती है।