

Propagation and *ex-situ* conservation of *Endocomia macrocoma* subsp. *prainii* (Myristicaceae) from the Andaman Islands in the Bay of Bengal

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Abstract

Endocomia macrocoma (Miq.) W.J. de Wilde subsp. prainii (King) W. J. de Wilde is a Myristicaceae member with a wide range of geographical distribution in Yunnan, Vietnam, Laos, Cambodia, Thailand, Malay Peninsula, Myanmar, Northeast India, Andaman Islands, West Sumatra, West Java, New Guinea and Philippines. Seed germination experiments of the taxon under controlled conditions has been carried out at JNTBGRI seed bank with the seeds obtained from the JNTBGRI field gene bank accessions introduced as part of the *ex-situ* germplasm conservation of the Andaman-Nicobar floristic components outside the islands. The protocol standardization for seed germination of *Endocomia macrocoma* subsp. *prainii* from the Andaman Islands is documented here for the first time with illustrations.

Keywords: Andaman-Nicobar Islands; *Endocomia macrocoma* subsp. *Prainii*; *Ex-situ* conservation; Seed germination

Introduction

The Andaman and Nicobar Islands, located around 650 nautical miles away from the Coromandel Coast of the Peninsular India in the Bay of Bengal between the latitudes 6° to 14° N and the longitudes 92° to 94° E, is hallmarked with dense coverage of rainforests exhibiting high degree of plant diversity. This archipelago comprises roughly 556 islands, islets and rocks simulating an arc lying in North-South direction stretching over an approximate length of 467 km and maximum width of 57 km [1] with the total coastal length of 1962 km. According to an official estimation, around 80.76% of the total landmass is covered with tropical lowland rainforests [2]. This tropical biogeographic domain demonstrates the transit zone vegetation between the Indian Subcontinent and Malesian region with multi-dimensional affinities to other nearer and far-off geographical regions such as Northeast India, Myanmar, Thailand, Vietnam, Malesia, Sri Lanka, Peninsular India (Western Ghats) and even towards the African Continent to some extent. According to available data, this insular region known to host around 2463 Angiosperm taxa beneath 1018 genera of 182 families within the limited region of 8249 sq km indicates the remarkable degree of plant diversity within the limited region.

Endocomia macrocoma (Miq.) W.J. de Wilde subsp. *prainii* (King) W. J. de Wilde is a component of the 'nutmeg family' (Myristicaceae) with synonyms as *Horsfieldia longipedunculata* H. H. Hu, *H. pandurifolia* H. H. Hu, *H. prainii* (King) Warb. And *Myristica prainii* King (The Plant List - A working list of all plant species http://www. theplantlist.org/tpl1.1/record/kew-2789056 - accessed on 15/06/2016). The insular Myristicaceae of Andaman-Nicobar Islands is a small family with 10 species, one sub species and four verities beneath four genera. *Endocomia* in Andaman-Nicobar Islands is represented with two species and one subspecies. *Endocomia macrocoma* (Miq.) W.J. de Wilde subsp. *prainii* (King) W. J. de Wilde is fairly infrequent among the evergreen forests of the Andaman group of islands; besides, it is never being extended towards the Nicobar group of islands beyond the 10° Channel in this archipelago.

Propagation Studies

Endocomia macrocoma (Miq.) W.J. de Wilde subsp. *prainii* (King) W. J. de Wilde is a monoecious taxon with inflorescences often has mixed male and female flowers. Infructescences about 25 cm long with 03 to 15 fruits. Fruits are broadly ellipsoid to ovoid or rarely obliquely

ovoid having 37.73 ± 1.04 mm x 25.79 ± 1.09 mm size and weigh up to 13.72 ± 1.31 gm. As regards to seed characters, Andaman specimen exhibits much similarity with taxon found to occur in Philippines and New Guinea rather than other Southeast Asian countries. Characters such as entire aril and testa variegated by longitudinal markings of the Andaman specimens validate its affinities towards Philippines and New Guinean entities. Seeds are broadly elliptic to rarely ovoid in shape having 23.71 ± 0.97 mm x 13.09 ± 0.5 mm in size and weigh up to 3.07 ± 0.19 gm. Natural regeneration of the taxon in Andaman Islands is observed as moderate in primary forests. Seed dissemination in Andaman forests is generally through rain water and small rivulets. The seeds prefer to grow in humus rich moist damp places in natural habitats. The accessions under *ex-situ* conservation in JNTBGRI field gene bank exhibits very poor natural regeneration. Seeds with ruminate endosperm and small embryo. Seed coat is covered with deep red fleshy aril.

Materials and Methods

Mature fruits were collected for the germination studies from the field gene bank accessions of concerned taxon at JNTBGRI. The mother plants have been introduced in JNTBGRI field gene bank approximately 22 years ago from the Andaman Islands. Ripened fruits turning yellowish and pericarp splits open by longitudinal slits on maturity. Seeds were extracted from mature ripened fruits by removing the pericarp and fleshy deep reddish aril; then cleaned thoroughly by washing in running tap water. Later morphological characteristics, dimensions etc., have been documented and finally given seed bank accession number of JNTBGRI. Purity of the seeds was determined by floating method and distinguished excellent quality seeds for experimental studies. After proper surface drying initial moisture

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content of the seeds were determined. The initial moisture content of seeds was determined on fresh weight basis [3], followed by viability tests of the seeds were carried out. Germination tests were conducted in wet rolled paper towels placed in a seed germinator without light maintained at $30 \pm 2^{\circ}C/80\%$ RH according to ISTA rules. 20 seeds of five replicates were tested for germination.

Another lot of seeds were then desiccated to different levels of moisture content by kept opened in laboratory conditions $(28 \pm 2^{\circ}C/70\%$ RH) to recognize the desiccation response of seeds at different moisture contents. Seeds having different moisture content were collected and carried out viability tests as in the earlier experiments with fresh seeds. Periodical observations were made for documenting germination data. Duration for initiation and completion of germination were also documented during the experiments.

Results and Discussion

The germination experiments conducted with fresh seeds having 90% purity in floating test and 30% moisture content registered 90% germination within one month of incubation period. Three days of desiccation under the laboratory conditions reduced the moisture content to 25% and after 17 days of continuous desiccation, the moisture content was further went down to 7% with only 49% viability. Experiments in between with seeds having 12% moisture content certified the germination rate of 66% and thereafter complete loss of viability was occurred. Hence the safe moisture content will be around 15% (Table 1, Figures 1-3). According to Wiese and Binning (1987) [4] and Auld *et al.*, (1988) [5], germination of a seed is obviously considered only when the tip of the radicle had grown free of the seed coat. After reaching considerable growth in laboratory conditions, seedlings were then transferred to field gene bank nursery.

Since even 15% loss of moisture content had no lethal effect on viability, these seeds may be are of moderately recalcitrant type, as described with the *Myristica malabarica* seeds which can tolerate 10% moisture content reduction [6]. In *Endocomia macrocoma* subsp. *prainii*, during desiccation study it was observed that as the moisture content was decreased in 15 days, the time required for germination increased in a pattern as reported in *Lophopetalum wightianum* [7] and in *Knema attenuata* [8]. Similarly *Gymnacranthera canarica* seeds also have a critical moisture content around 15%, below which resulted in seed deterioration [9]. Later germinated seeds were transferred to Field Gene Bank nursery.

According to Koslowsri *et al.*, (1991) [10], tree growth is a mutual gathering of the genetic potential of the species and natural environmental conditions. As regards to *ex-situ* conservation of species, the 'environmental condition' denotes the totality of microclimatic specifications of the nursery during the earlier stages as well as the field factors in general during the later phases. The climatological features such as average rainfall and humidity of the Andaman

Period of desiccation (days)	Moisture content (%)	Germination started(days)	Germination completed (days)	Germination percentage
0	30.8 ± 0.66	19 ± 0.7	27 ± 0.9	90 ± 1.5
3	25.2 ± 0.8	23 ± 1.7	33 ± 0.7	81 ± 1.9
7	21.3 ± 1.3	31 ± 0.5	41 ± 1.5	78 ± 1.3
11	16.8 ± 0.9	35 ± 1.89	51 ± 0.7	75 ± 1.3
15	12.6 ± 0.83	37 ± 2.1	49 ± 1.9	66 ± 1.7
17	7.01 ±	41 ± 1.35	57 ± 0.6	49 ± 1.4

Table1: Viability of seeds at different moisture contents.



Islands and the Southern Western Ghats are more or less similar, even though these regions are widely separated by the Bay of Bengal [11]. During our efforts on ex-situ conservation of Andaman plants in the Western Ghats region, we have also been confirmed that the various ecological niches found to occur along these regions are ideal for the establishment and sustainable growth of the insular floristic entities. On considering nursery management of seedlings, an ideal nursery should provide the channel to control light, moisture, physical and chemical soil constituents in order to generate healthy and uniform seedlings for field planting Doran, 1977. Potting medium is one of the most important factors in nursery, since it primarily provides food and physical support to seedlings for sustainable growth in the early stage and secondly, it stores and supplies nutrients, water and air to the root system [12]. As regards to Andaman tree species, JNTBGRI field gene bank has standardized different potting substrate protocols with various ratios of different components.

Germinated seeds were brought from the seed bank and carefully transplanted in the mist chamber rooting beds. Rooting medium was initially fine river sand for 10 to 30 days. Later, the one month old seedlings with two to three leaves were carefully transplanted to a potting medium with river sand, top soil and cow dung in 3:1:1 ratio. The seedlings have been well acclimatised with the environment within a period of 50–60 days in the mist chamber and then transferred to the field gene bank nursery covered with 50% shade nets. Ninety to 100 days old seedlings have been well acclimatized with surrounding environment could be kept outside the nursery in pots with river sand, top soil and cow dung in equal ratio and about 150–170 days old seedlings could be transplanted to field 50 x 50 x 50 cm pits filled with compost and river sand.

Ex-Situ Conservation Scenario

Endocomia macrocoma (Miq.) W.J. de Wilde subsp. *prainii* (King) W. J. de Wilde is a lesser known wild relative of commercial 'nutmeg'. The taxon demonstrates wide range of geographical distribution in Southeast Asia from South China (Yunnan) to Indochina (Vietnam, Laos, Cambodia, Thailand, Malay Peninsula and Myanmar), Northeast India (Assam) and towards Indonesia to Philippines through the corridor of Andaman Islands in the Bay of Bengal [13]. Interestingly, this taxon has not been reported from the Nicobar group of islands in between the Andaman Archipelago and Indonesian group of islands [14,15].

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The insular taxa of Andaman-Nicobar Islands dominated with high degree of competition owing to fairly high species density within a limited fragmented landmass and hence these insular zone holding a very fragile ecological equilibrium. Apart from this, these islands are susceptible to frequent catastrophic events like cyclones, volcanic eruptions, earth quacks, tsunamis etc. It makes several species more vulnerable into the road of endangerment and extinction. In order to overcome this crisis, conservation of insular germplasm outside the islands is very much fundamental and Jawaharlal Nehru Tropical Botanic Garden and Research Institute (INTBGRI) located at the foot hills of Southern Western Ghats has undertaken the concern of germplasm conservation of the Andaman-Nicobar plant species. It is one of the mandates of the field gene bank established in 1994 in JNTBGRI. About 25 acres of the campus has been demarcated for the conservation of insular species of medicinal plants, endangered endemics, economically important timber trees, wild relatives of cultivars, palms, bamboos and other interesting ethno botanical species



Figure 2: (A) Mature fruits in mother plant introduced at JNTBGRI field gene bank (B) Ruminate endosperm (C) Fleshy deep reddish aril covered on seeds (D) Germinated seed under controlled condition (E) Mature seed with 90% purity (F) Mature fruit opened fruit and an obliquely ovoid fruit (G) One month old seedlings (H) Six month old seedling.

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of the islands. There are 125 species from Andaman-Nicobar Islands have so far been well established at the field gene bank of JNTBGRI and it is considered as the largest *ex-situ* conservation of Andaman-Nicobar plant species outside the islands. The present studies are the part of protocol standardization for seed germination of Andaman taxa conserving in JNTBGRI field gene bank. In this context, it also relevant to mention that no attempt has so far been made for tissue culture protocol standardization as well as cryopreservation of this taxon through the samples from the Andaman Islands.

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