People Resources and Conservation Foundation

Seed collection and test propagation of Conifer and Magnolia from the Sinh Long Forest Area

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The Ba Be / Na Hang Limestone Forest Complex (BNLFC) holds unique values of high global biodiversity significance, particularly of primates such as the highly threatened Tonkin Snub-nosed Monkey (*Rhinopithecus avunculus*) and the Francois' Langur (*Trachypithecus francoisi*), Conifer and Magnolia species and forms part of the restricted home range of the largely unknown White-eared Night Heron *(Gorsachius magnificus).* The BNLFC is replete with conservation hotspots scattered throughout a landscape, which has undergone degradation of its biodiversity and ecosystem values mainly as a result of shifting cultivation and increasing population density.

The aim of the project is to improve conservation of globally threatened species of primates, the White-eared Night Heron and globally threatened species of conifers and magnolias at sites of high biodiversity interest in the Ba Be / Na Hang Limestone Forest Complex (Tuyen Quang and Bac Kan Provinces).

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1. Introduction

The Ba Be / Na Hang Limestone Forest Complex (BNLFC) holds unique values of high global biodiversity significance, particularly of primates, and Conifer and Magnolia species. Nearly half of all conifer species in Vietnam can be found in the BNLFC, including seven globally threatened species including the internationally significant Vietnamese Golden Cypress (*Xanthocyparis vietnamensis).*

The Center for Plant Conservation (CPC) conducted field surveys in September 2011 of the Sinh Long Forest Area to identify remaining stands of globally threatened tree species in the Sinh Long Forest Area. Results of the survey were valuable in assisting us to identify the significant Magnolia and Conifer species present and are now being used to assist in the planning and development of direct conservation action initiatives during this project and beyond. One significant finding from the survey activities was that there is urgent need to develop both *in-situ* and *ex-situ* actions to recover tree populations before wild populations dwindle to unrecoverable levels.

The aim of the report is to communicate the results of propagation trials conducted by the CPC with four species of Magnolia and two Conifer (of which seed was collected in 2011) prior to implementing at the community-level. The report also includes guidelines for:

(a) propagation measures for each of the species trialled, both in nurseries and thereafter in the field; and
(b) How to engage local communities in propagation activities and outlining perceived benefits and possible constraints.

2. Seed Collection

A seed collection trip was conducted over 11 days, from 26th September to 6th October, 2011. In total, we tried to collect seed from eight (8) species of magnolia and nine (9) species of conifer. However, were only successful in collecting from four (4) Magnolia and two (2) Conifer species and are listed in Table 1 below.

Table 1. Magnolia and Conifer seed collected at the Sinh Long Forest Area

No	Scientific Name	Collecting number	Seed quantity	Seed Qualities
1	*Magnolia liliifera* (L.) Baill. var. *liliifera*	CPC 4501, 4511, 4572, 4521, 4522	300	Mature seeds with orange to brilliant red
2	*Manglietia rostrata* D. X. Li et R. Z. Zhou in det.	CPC 4520	200	Immature seeds with green and mature seeds with brilliant red
3	*Kmeria septentrionalis* Dandy	CPC 4514	80	Mature seeds with brilliant red.
4	*Michelia balansae* (A.DC.) Dandy	CPC 4515	150	Mature seeds with orange to brilliant red
5	*Taxus chinenis* (Pilg.) Rehd	CPC 4535	200	No embryo
6	*Xanthocyparis vietnamensis* Farjon & N. T. Hiep	CPC 4553	200	No embryo

3. Propagation Equipment and Facilities

3.1. Requirements for propagation environment

In general, seed germination requires moisture, warmth, sterile germination or growing medium, and sometime light. In addition, seed and vegetative propagation also requires nutrients. Further, control of environmental variables is also important for propagation including:

1) Aerial environment: humidity, light, temperature, and air quality.
2) Growing medium: moisture level, temperature, aeration, pH and nutrient level.

3.2. Equipment

Based on the requirement for the propagation environment, we set up two types of cold frame[1] for use during propagation trials of a number of species of Magnolia and Conifer. The theoretical principal behind the cold frame is a heat exchange mechanism whereby the transparent top admits sunlight and prevents heat escape via convection that would otherwise occur, particularly at night. Cold air usually expands and rises as it heats during the day. By opening the panes of the cold frame during warm weather, some of the warm air is allowed to escape and the temperature inside the cold frame remains reasonably cool. The main function of the cold frame is to raise seedlings early in the season. A cold frame may also be used to expose hardy seeds such as those of Magnolia and other tree species to a period of winter cold. Additional benefits include accommodating a large number of pots or trays, hence expanding the greenhouse size.

There are two types of cold frames that we used in the CPC facilities:

(1). **Lac Long Quan Facility**
The permanent cold frame was set up and provides a nursery bed for seedlings and cuttings. It was built with cement blocks with a panel of clear plastic over a metal frame. At the base of the cold frame, we lined a thick layer of coarse gravel for drainage. For the seedling medium, we added about 20 cm of sterile river sand. In addition, the permanent cold frame was located under a shady tree.

(2). **Bat Trang Facility**
A moveable cold frame was set up for this facility. The theoretical principle and construction of both structures are almost the same, with the major difference being that the transparent panel is made of plastic with a metal frame and may be over placed over prepared soil in the garden to form a nursery bed.

List of Equipment:

- Cold frame
- Plastic pots: 4-6-8 cm diameter
- pH tester
- Plastic container
- Plastic tray
- Polyester bags: 3-10 cm diameter
- Thermostat
- Refrigerator

[1] transparent-roofed enclosure, built low to the ground, used to protect plants from cold weather - miniature greenhouse

3.3. Growing/Seed bed medium

We used two types of propagation medium i.e. locally available river sand (Red River) and volcanic rock because they both hold moisture, have neutral pH, aerate easily, and are easy to sterilise. In addition, it is easy to buy and available in Vietnam.

4. Propagation Methodology

4.1. Magnolia

4.1.1. Pre-treatment of collected seed

After the fruit aggregates are collected, the ripe fruit are gathered to dry until the fleshy seeds come away freely. Aggregate fruits were placed in a shaded and cool area to dry.

4.1.2. Extracting Magnolia seed

Seeds were soaked in water for one to two days, then squeezed and agitated in a sieve to remove the sarcotestae (outer seed coat). After the sarcotestae was removed, seed is washed in warm water with some liquid detergent to remove the waterproof coating, and then rinsed several times in fresh water.

4.1.3. Testing seeds for viability

Two methods to test seed viability were employed prior to sowing and applied depending on the species. Large seeds were placed in water and if viable will sink, whilst any insect-infested and empty seeds will float. This method will not work with seeds of all Magnolias. A second alternative test involves cutting a sample of the seeds in half. Non-viable seeds are hollow or have a small amount of resin; viable seeds have a fat, usually white, embryo.

4.1.4. Seed sowing

Seeds were sown in the clean river-sand medium or volcanic rock at a depth of 2 cm. Seeds were sown under cover during winter, at an average temperature of around 20^{o} Celsius.

4.1.5. Transplanting germinated seed

Seedlings were transplanted when they reached a height of about 4 to 7 cm.

Transplanting seedling

4.2. Conifers

4.2.1. Seed Extraction

Place the ripe cones in a paper lined cardboard box, and label. Leave the box in a warm closet or at room temperature until the scales open. When the cones fully open, tip out the winged seeds. Use tweezers to remove any seeds lodged between the scales or shake the cone until all are removed. The seed of Taxus sp. and some other conifers have a fleshy coat. It is not essential to clean this off because it should break down naturally, however, removing it may hasten germination.

4.2.2. Testing seeds for viability

Normally, conifer cones are recognized by the seed colours. Dark seed is more likely to be viable than pale ones. If a colour difference is not apparent, there are two methods of testing the seeds before sowing. Place large seeds in water and viable seed will sink, while any insect-infested and empty seeds will float. This method may not work with seeds of all Conifers, such as Fir. An alternative test involves cutting a sample of the seeds in half. Non viable seeds are hollow or have little resin; viable seeds have a fat, usually white, embryo.

Taxus chinenis (Pilg.) Rehd and *Xanthocyparis vietnamensis* Farjon & N. T. Hiep

5. Results

5.1. Magnolia

5.1.1. Magnolia liliifera (L.) Baill. var. liliifera

From a total of 73 seeds, 52 floated and 21 sank, with those identified as being viable sown on 22 October 2011. Seedling results are shown in Table 2.

Magnolia liliifera (L.) Baill. var. *liliifera* (from left to right): Seeds with sarcotestae and without sarcotestae

Table 2. *Magnolia liliifera* (L.) Baill. var. *Liliifera*

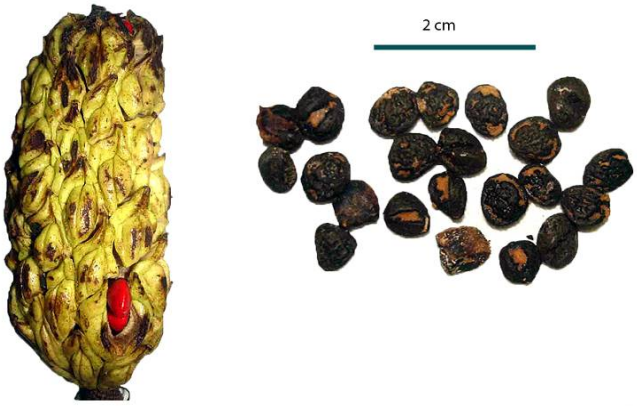
Date	January 1, 2012	February 15, 2012	March 28 to April 15, 2012	Total
Float seeds	3	1	18	22
Sink seeds	0	2	11	13
Total	**3**	**3**	**29**	**35**

5.1.2. Manglietia rostrata D. X. Li et R. Z. Zhou in det.

From a total of 138 seeds, 118 seeds were sown in sand and 20 seeds sown in the volcanic rock on October 22, 2011. Seedling results are shown in Table 3.

Table 3. *Manglietia rostrata* D. X. Li et R. Z. Zhou in det.

Date	November 10, 2011	December 12, 2011	January 5, 2012	March 28, 2012	Total
Sand batch	7	6	6	33	52
Volcanic rock batch	0	11	0	3	14
Total	**7**	**17**	**6**	**36**	**66**



Manglietia rostrata D. X. Li et R. Z. Zhou in det. (from left to right): Fruit and seeds without sarcotestae.

5.1.3. Kmeria septentrionalis Dandy

From a total of 30 viable seeds (sank) sown on 22 October 2011 only one germinated (Table 4).

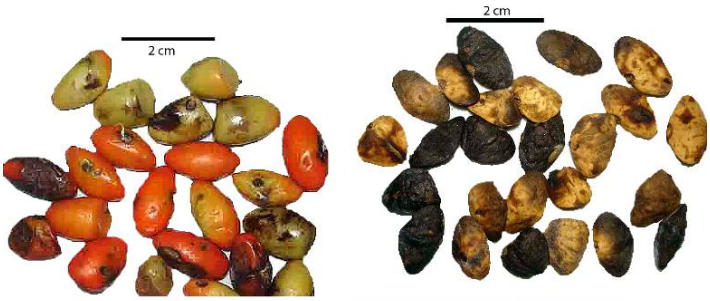
Kmeria septentrionalis Dandy (from left to right): Fruit and seed without sarcotestae

Table 3. *Kmeria septentrionalis* Dandy

Date	March 28, 2012	Total
Sink seeds	1	1
Total	**1**	**1**

5.1.4. Michelia balansae (A.DC.) Dandy

No germination occurred with seed collected from the Sinh Long Forest Area for this species, however, seed collected from Ha Giang Province germinated well.



Michelia balansae (A.DC.) Dandy (from left to right):
Seeds with sarcotestae and without sarcotestae

5.2. Conifers

Seed from both conifer species were sown on the 22 October. However, after five months (from sowing), no germination of either species i.e. *Taxus chinenis* (Pilg.) Rehd and *Xanthocyparis vietnamensis* Farjon & N. T. Hiep, were recorded.

6. Discussions

From the four magnolia species, we only chose two species, *Magnolia liliifera* and *Manglietia rostrata*, for the seed propagation experiments. These species had been collected in good quantity and their widely distribution.

6.1. *Magnolia liliifera* (L.) Baill. var. *liliifera*

In the principle of seed test, viable seeds will sink, and fertile or insect-infested and empty seeds will float. However this method is less accurate than the X-ray method, and waste resources. In this experiment, we tested the seed quality on the river sand medium. Interestingly, it took 11 weeks for floating seeds to germinate, which is four weeks shorter than those that sank. However, the germination rate of sinking seed is 20% higher than floating. Data from this experiment is recorded in Table 4 below.

Table 4. *Magnolia liliifera* (L.) Baill. var. *Liliifera*

	Quantity of sown seeds	Quantity of seed germination	Germination rate (%)
Float seeds	52	22	42
Sink seeds	21	13	61

6.2. *Manglietia rostrata* D. X. Li et R. Z. Zhou in det.

In this experiment, we tested the germination quantity of sinking seeds with two difference growing mediums i.e. river sand and volcanic rock. Results showed that with volcanic rock germination took 7 week i.e. four weeks longer than with river sand. Data from this experiment is recorded in Table 5 below.

Table 5. *Manglietia rostrata* D. X. Li et R. Z. Zhou in det.

	Quantity of sown seeds	Quantity of seed germination	Germination rate (%)
Sand batch	118	52	44
Volcano batch	20	14	70

Table 6 below provides a summary of the germination/propagation trials with suggested follow up propagation measures in the future.

Table 6. Germination/Propagation measures for each of the species

Species	Number of seedling	Germination rate (%)	Propagation measure
Magnolia liliifera	35	47.1	Seed coat is thick and may take time to germinate. After 11 weeks, seeds continue to germinate.
Manglietia rostrata	65	44.61	Seed coat is much thinner than that of other species; therefore, it took seeds less time to germinate. After 7 weeks, seeds continue to germinate.
Kmeria septentrionalis	1	0.3	Small seeds with thick coats may take longer time to germinate. Thus, germination test must continue to be observed.
Michelia balansae	0	0	No seed germinated. Might be caused by immature seeds. More seed collection required in season.
Taxus chinensis	0	0	No seed germinated. Might be caused by immature seeds. More seed collection required in season.
Xanthocyparis vietnamensis	0	0	No seed germinated. Might be caused by immature seeds. More seed collection required in season.
Total	**101**		

7. Recommendations

- Due to the low germination rate and seed quality of *Kmeria septentrionalis*, *Michelia balansae*, *Taxus chinensis* and *Xanthocyparis vietnamensis*, we highly recommend further field surveys (to identify alternative population gene pools) and seed collection are required.
- Seed collection of *Kmeria septentrionalis*, *Michelia balansae*, *Taxus chinensis* and X*anthocyparis vietnamensis* need to be observed from October to November.
- In addition to the selection of seedling medium, anything that can retain moisture, has neutral pH, is well aerated, and easy to buy may be used for propagation.
- With the low germination rate, testing to break seed dormancy is also recommended.

8. Conclusions

- Over 1,000 seeds from six target species of Magnolia and Conifer were collected in September, 2011 and trialled for propagation.
- Only 605 seeds including 73 *Magnolia liliifera*, 138 *Manglietia rostrata*, 30 *Kmeria septentrionalis*, 64 *Michelia balansae*, 100 *Taxus chinensis* and 100 *Xanthocyparis vietnamensis* were used for the CPC propagation trial. About 400 seeds have been stored in refrigerator *at* 1 - 4^0C for future propagation.
- The CPC has trained and established a network of local people to assist in the identification of plants and to identify seed collection times. This initiative is highly

important because obtaining high quality ripe seeds is a critical element for propagation activities.

- Due to the short duration of the survey, lack of information about species distribution and low success rate of germination/propagation trials, we are not in a position to recommend suitable technical guidance on how local communities can participate in the propagation of plants for conservation and plantation purposes. Further, technical germination/propagation trials are recommended before application on the field with local communities.
- In light of the previous conclusion above, CPC at the moment is unable to calculate the costs of developing community-oriented nurseries and to conduct enrichment planting with Magnolia and Conifer species. Further consultation with local communities and government is required to select appropriate land and train labour. These are also limiting factors in the calculation of nurseries and enrichment planting at the moment.