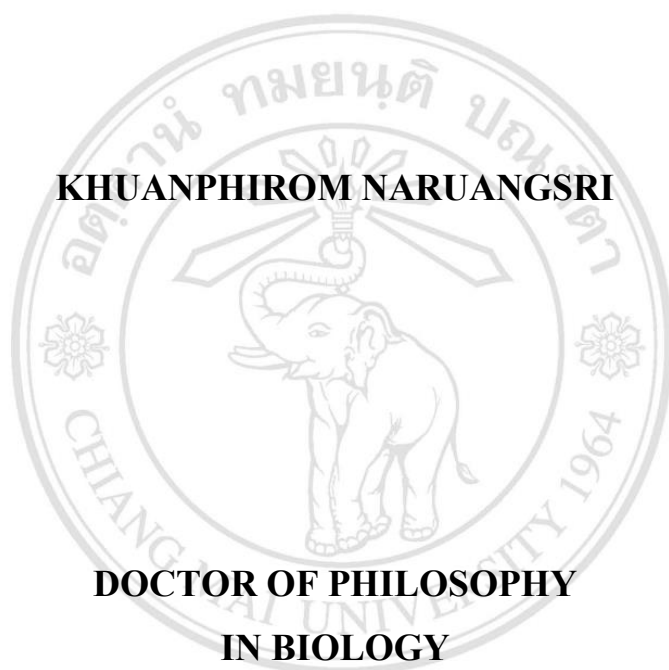


**DEVELOPING TECHNIQUES FOR DIRECT-
SEEDING FOR FOREST RESTORATION
IN NORTHERN THAILAND**

KHUANPHIROM NARUANGSRI



**DOCTOR OF PHILOSOPHY
IN BIOLOGY**

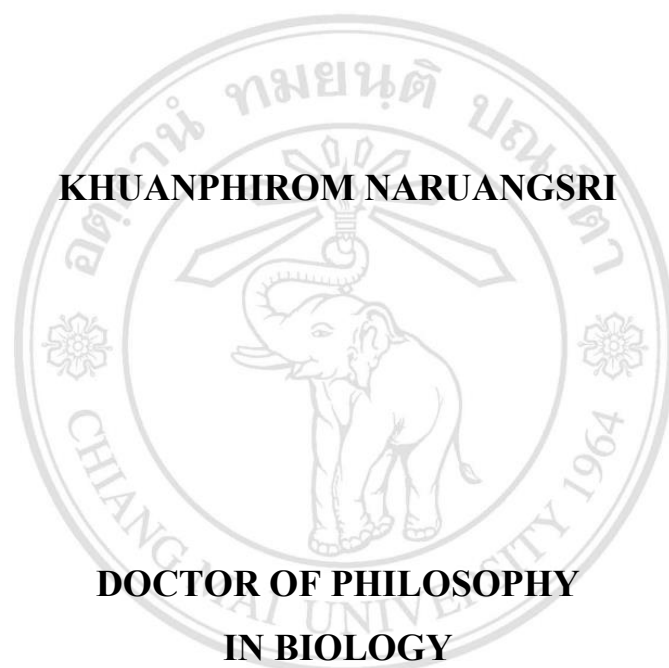
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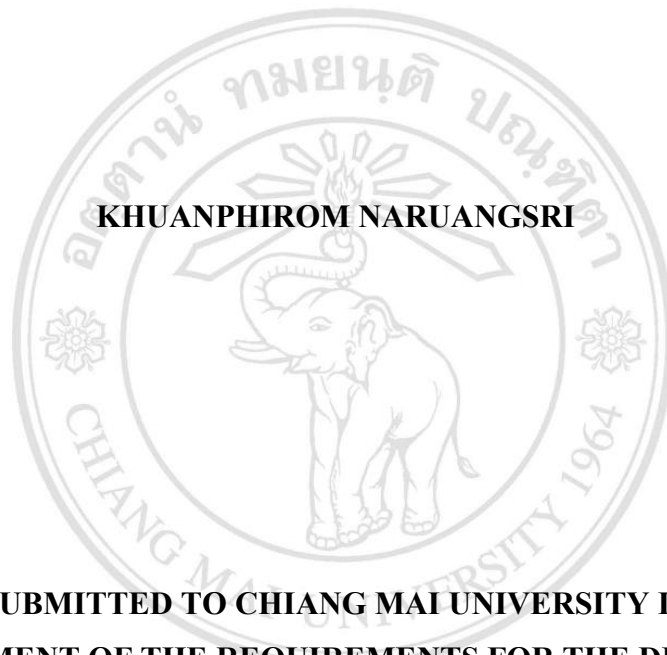
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**A THESIS SUBMITTED TO CHIANG MAI UNIVERSITY IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN BIOLOGY**

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DECEMBER 2023**

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KHUANPHIROM NARUANGSRI

THIS THESIS HAS BEEN APPROVED TO BE A PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
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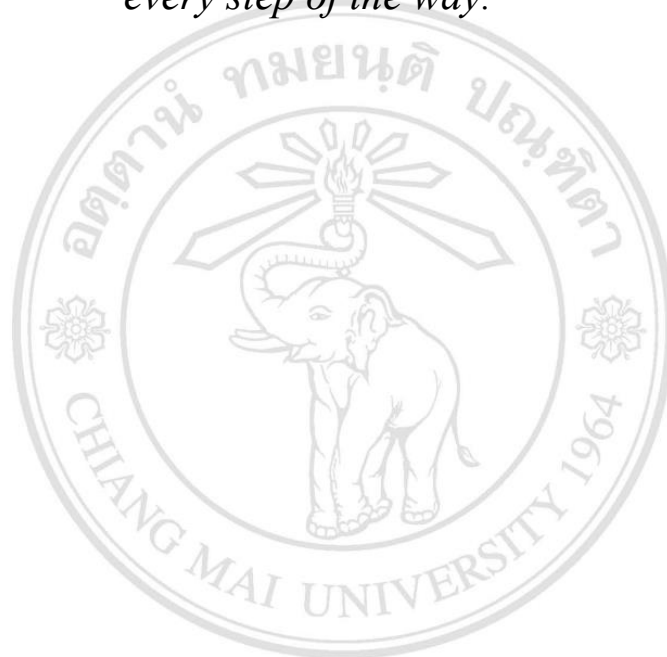
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12 December 2023

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To

*All living things involved in my research —
people, trees, and animals— for encouraging me
every step of the way.*



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Khuanphirom Naruangsri



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หัวข้อคุณูปการ	การพัฒนาเทคนิคสำหรับการหยอดเมล็ดโดยตรงเพื่อ การฟื้นฟูป่าในภาคเหนือของประเทศไทย	
ผู้เขียน	นางสาวขวัญกิริมณัฏ ณะเรืองศรี	
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บทคัดย่อ

การฟื้นฟูป่าโดยวิธีการหยอดเมล็ดเป็นวิธีการที่มีศักยภาพในด้านต้นทุนเมื่อเทียบกับการปลูกต้นไม้เพื่อการฟื้นฟูระบบนิเวศป่าเขตร้อนในสเกลที่ใหญ่ขึ้น อย่างไรก็ตามข้อจำกัดที่อาจเป็นอุปสรรคต่อความสำเร็จของวิธีการนี้ คือ เมล็ดถูกทำลายโดยสัตว์ผู้ล่าเมล็ด การงอกของเมล็ดและการตั้งตัวของต้นกล้าต่ำ อันเนื่องมาจากสภาพแวดล้อมที่ไม่เหมาะสมในพื้นที่เสื่อมโทรม อีกทั้งยังขาดข้อมูลสำหรับชนิดที่เหมาะสมสำหรับการหยอดเมล็ดโดยเฉพาะ ดังนั้น วัตถุประสงค์หลักสำหรับการศึกษานี้คือ เพื่อทดสอบความเหมาะสมของพรรณไม้ป่าพื้นเมือง 23 ชนิด สำหรับการหยอดเมล็ดเพื่อฟื้นฟูป่าดิบเขาในภาคเหนือของประเทศไทย ซึ่งเป็นป่าที่อุดมด้วยความหลากหลายทางชีวภาพ และเพื่อทดสอบวัสดุเคลือบเมล็ดที่มีประสิทธิภาพป้องกันการล่าเมล็ดและส่งเสริมการตั้งตัวของต้นกล้าในพื้นที่เสื่อมโทรม

งานวิจัยนี้ได้ดำเนินการในพื้นที่เสื่อมโทรม 2 แห่ง ได้แก่ พื้นที่ม่อนแจ่ม (MC) และบ้านแม่จิ (BMK) รวมไปถึงสภาพควบคุมภายในเรือนเพาะชำกล้าไม้ โดยแต่ละพื้นที่ศึกษาจะมีการแบ่งพื้นที่ศึกษาเป็นแปลงย่อย สำหรับ 3 ซ้ำ แล้วทำการสุมหยอดเมล็ด 20 เมล็ดต่อชนิดต่อแปลงย่อย นอกจากนี้ยังมีการเลือกเมล็ด 5 จาก 23 ชนิด เพื่อใช้ทดสอบการเคลือบเมล็ดที่แตกต่างกัน 2 กลุ่ม กลุ่มแรกเป็นการเคลือบแบบชั้นหนา หรือ seed ball ประกอบด้วยชุดการทดลองที่ต่างกัน 3 แบบ ได้แก่ biochar ดินผสม และ polysaccharide mixture และกลุ่มที่ 2 เป็นการเคลือบเมล็ดแบบชั้นบาง หรือการเคลือบเมล็ดด้วยจุลินทรีย์ (Microbial seed coating) 2 ชนิด ได้แก่ *Streptomyces antibioticus* และ *S. thermocarboxydus* isolate S3 แล้วนำเมล็ดไปสุมหยอดด้วยวิธีเดียวกันกับการหยอดเมล็ดข้างต้น

หลังจากนั้นมีการติดตามการงอกนำเมล็ดออกไป และการงอกของเมล็ดทุกสัปดาห์ จนกระทั่งการงอกหยุดลงเป็นเวลาสามสัปดาห์ จากนั้นจึงติดตามผลผลิตต้นกล้า (Seedling yield) การเจริญเติบโต และคะแนนประสิทธิภาพสัมพัทธ์ (Relative performance index: RPI) ของแต่ละชนิดในช่วงเวลาที่เหมาะสม นอกจากนี้ ในการศึกษาครั้งนี้ยังได้มีการเก็บข้อมูลลักษณะของเมล็ดแต่ละชนิด เช่น ลักษณะทางสัณฐานวิทยาของเมล็ด/ต้นกล้า พฤติกรรมการจัดเก็บของเมล็ด (Seed storage behavior) และสถานะของชนิดสำหรับการเปลี่ยนแปลงแทนที่ (Successional status)

เก้าเดือนหลังหยอดเมล็ด พบว่า ความรุนแรงของการล่าเมล็ดมีน้อยมาก พบความสัมพันธ์เชิงลบระหว่างขนาดเมล็ดและการงอกนำเมล็ด โดยเมื่อขนาดของเมล็ดเพิ่มขึ้นการงอกนำเมล็ดจะลดลง มีเมล็ด 8 ชนิด ที่ไม่สามารถงอกได้ ชนิดที่มีอัตราการงอกสูงที่สุด ได้แก่ มะกล่ำต้น (*Adenanthera microsperma*) และ ฝาละมี (*Alangium kurzii*) หลังจากผ่านฤดูแล้งแรก ต้นกล้าที่งอก 2 ชนิดล้มเหลวในการตั้งตัวในพื้นที่ ความเป็นไปได้ของการงอกและการตั้งตัวในพื้นที่เสื่อมโทรมได้รับอิทธิพลจากลักษณะที่แตกต่างกันของแต่ละชนิด โดยเฉพาะขนาดเมล็ด (Seed size) พฤติกรรมการจัดเก็บเมล็ด (Seed storage behavior) และสถานะของชนิด (Successional status) การศึกษานี้ เสนอชนิดที่มีศักยภาพสำหรับการหยอดเมล็ด โดยพิจารณาจากดัชนีประสิทธิภาพของชนิดที่คำนวณจากการรอดและการเติบโตของต้นกล้า ได้แก่ มะกล่ำต้น (*A. microsperma*) มะกอกป่า (*Spondias pinnata*) และมะกอกหัวรู (*Choerospondias axillaris*) การเลือกชนิดที่เหมาะสมสำหรับการหยอดเมล็ด พิจารณาได้จากลักษณะของเมล็ดแต่ละชนิด โดยเลือกเมล็ดที่มีขนาดกลางถึงใหญ่ และเป็นเมล็ดที่ไม่สูญเสียความมีชีวิตในสภาพแห้ง (Orthodox seed) หากจำเป็นต้องใช้เมล็ดที่ไม่ทนต่อสภาพแห้ง (Recalcitrant seed) ควรนำเมล็ดไปหยอดทันทีหลังจากการเก็บเมล็ด

สำหรับการทดลองเคลือบเมล็ด พบว่า seed ball โดยเฉพาะอย่างยิ่ง biochar มีประสิทธิภาพในการป้องกันเมล็ดจากสัตว์ผู้ล่าเมล็ด ช่วยลดการล่าเมล็ดลงเมื่อเทียบกับเมล็ดที่ไม่เคลือบ อย่างไรก็ตาม ชั้นของวัสดุเคลือบเมล็ดที่หนาอาจจำกัดการเข้าถึงของน้ำ ออกซิเจน และแสง ซึ่งจำเป็นต่อการงอกของเมล็ด ส่งผลให้การงอกของเมล็ดลดลงอย่างมีนัยสำคัญ อีกทั้งวัสดุเคลือบเมล็ดยังไม่สามารถส่งเสริมให้ต้นกล้าอยู่รอดและเจริญเติบโตได้ดีขึ้น เช่นเดียวกันกับการเคลือบเมล็ดด้วยจุลินทรีย์ แอคติโนแบคทีเรีย *Streptomyces antibioticus* และ *S. thermocarboxydus* isolate S3 ไม่ได้ส่งเสริมการงอกของเมล็ด การอยู่รอดของต้นกล้า และการเจริญเติบโตของต้นกล้า ดังนั้น จึงต้องพิจารณาความสมดุลระหว่างการลดการล่าสัตว์และความสามารถในการซึมผ่านของวัสดุหุ้มเมล็ดเมื่อมีการพัฒนาวิธีการเพื่อเพิ่มความสำเร็จในการฟื้นฟูป่าโดยวิธีการหยอดเมล็ด

Dissertation Title	Developing Techniques for Direct-seeding for Forest Restoration in Northern Thailand	
Author	Miss. Khuanphirom Naruangsri	
Degree	Doctor of Philosophy (Biology)	
Advisory Committee	Asst. Prof. Dr. Pimonrat Tiansawat	Advisor
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ABSTRACT

Forest restoration by direct seeding is potentially a more cost-effective technique than tree-planting, for upscaling restoration of tropical forest ecosystems. Unfortunately, its success is limited by seed predation, low seed germination and low seedling establishment, due to the harshness of environmental conditions on restoration sites, and particularly by lack of information about species suitability. Consequently, the main objective for this study was to test the suitability of 23 native forest tree species for direct seeding, to restore biodiversity-rich, upland, evergreen forest in northern Thailand and to find effective coating materials to prevent seed predation and promote seedling establishment.

Experiments were carried out in two-degraded areas at Mon Cham (MC) and Ban Mae Khi (BMK), and under controlled conditions in a tree nursery. Three replicate seed batches with 20 seeds for each species were sown randomly on each site. Five of the 23 seeds species were selected for testing two different seed-coating treatments: three treatments of thick-layer seed coating (or “seed balls”); biochar, soil mixture and polysaccharide mixture and two treatments of thin-layer seed coating or microbial seed coating: *Streptomyces antibioticus* and *S. thermocarboxydus* isolate S3, then sown as the same method. Seed removal and germination were monitored weekly, until germination had ceased for three weeks. Seedling yield, growth and species-performance scores were

also monitored at appropriate intervals. Moreover, various species traits were also recorded.

Nine months after sowing, the intensity of seed predation was low, seed removal decreased with increasing seed size. Among 23 tree species, eight species failed to germinate, two species including *Adenantha microsperma* and *Alangium kurzii*, were ranked as having high germination. After the first dry season, two germinating species failed to establish. Germination and establishment were influenced by seed size, seed storage behavior and successional status. Thus, the species recommended for direct seeding, based on their high species-performance index, were *A. microsperma*, *Spondias pinnata* and *Choerospondias axillaris*. The study also suggested that opting for desiccation-tolerant seeds, with medium to large seeds, could enhance the likelihood of successful seedling establishment. To maintain seed viability, especially for recalcitrant seeds, a potential solution would be to sow them immediately after collection.

Biochar seed balls were the most effective treatment at reducing seed removal compared to non-coated seeds. However, seed germination of the coated seeds was less than that of non-coated seeds, probably because the thick coating reduced permeation of water, oxygen and light to the embryo. Microbial seed coatings also did not promote seed germination, seedling yield and growth. Therefore, the balance between predation reduction and seed coat permeability must be considered when developing treatments to enhance overall direct-seeding success.

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CONTENTS

	Page
Acknowledgement	d
Abstract in Thai	f
Abstract in English	h
List of Tables	l
List of Figures	n
Statement of Originality in Thai	t
Statement of Originality in English	u
Chapter 1 Introduction	
1.1 Historical Background	1
1.2 Research Objectives	4
1.3 Usefulness of the research	4
Chapter 2 Literature review	
2.1 Forest restoration and direct seeding	5
2.2 Thick-layer seed coating: seed ball	7
2.3 Microbial seed coating	8
2.4 Seed viability and storage behaviors	9
Chapter 3 Methodology	
3.1 Study sites and measurement of site conditions	11
3.2 Species selection	15
3.3 Experimental design and data collection	18
3.4 Data analysis	30
Chapter 4 Results	
4.1 Species selection	33
4.2 Seed ball	40
4.3 Microbial seed coating	45
4.4 Seed storage behavior	50

CONTENTS (continued)

	Page
Chapter 5 Thick-layer seed pelleting: Seed ball	
5.1 Species selection for direct seeding	67
5.2 Seed ball	71
5.3 Microbial seed coating	74
5.4 Microbial seed coating for forest restoration	75
5.5 Seed storage behaviors	76
5.6 Recommended species from this research	80
Chapter 6 Conclusion and recommendation	82
6.1 Conclusion	82
6.2 Recommendation	83
References	84
Appendices	
Appendix I Publication	101
Appendix II Species traits	102
Appendix III Description of studied-tree species	106
Curriculum vitae	152

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
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LIST OF TABLES

		Page
Table 3.1	The quality of soil at two study sites; Mon Cham and Ban Mae Khi in 2019. Column do not share the same letter indicated significant different between two study sites tested by t-test ($P < 0.05$).	14
Table 3.2	Tree seed species using for direct seeding (POWO, 2023)	16
Table 3.3	The species tested with pelleting materials.	20
Table 3.4	The species tested with pelleting materials.	24
Table 3.5	The species list using for storage experiment and classification of diaspore follow Gardner et al. (2000) and FORRU (2005).	27
Table 4.1	Seedling yield (%) seedling size and corresponding relative growth rate (RGR % per year). N indicated the number of surviving trees that are ordered from highest to lowest seedling yield. Values not sharing the same superscripts within columns are significantly different among species.	39
Table 4.2	Seedling yield, growth and corresponding relative growth rate (RGR % per year). Values not sharing the same superscripts within columns are significantly different among species.	44
Table 4.3	Mean size and growth of surviving trees (N: number of surviving trees) and relative performance score (RPI) at 9 months after direct seeding - root collar diameter (RCD), height and crown width (CW)- and relative growth rates (RGR: percent per year) of corresponding growth variable. Species are ordered from highest to lowest RPI. Values not sharing the same superscripts within columns are significantly different among species.	49

LIST OF TABLES (continue)

		Page
Table 4.4	The category of seed storage behavior: orthodox, intermediate, and recalcitrant seed, followed their germinability in each moisture level (Species ordering by lowest-to-highest initial moisture content). Columns not sharing the same superscript indicate significant differences among dry treatments tested by GLM (Binomial) and Turkey Contrasts ($R = 3$).	65
Table 5.1	Comparing the results with the previous studies. 'NA' indicates no information from the respective research studies.	77
Table 5.2	Summarizing recommendation for seed practical for direct seeding.	81

LIST OF FIGURES

		Page
Figure 3.1	The location of study sites, Chiang Mai, Thailand (inset map top left). The legends (red dot) indicated different locations used for experiments at Ban Mae Khi and Mon Cham (middle map), and the boundaries of each site are shown in the right photos to indicate the conditions surrounding the plots.	13
Figure 3.2	The physical characteristics of two field studied sites; Ban Mae Khi plot (BMK) ground photo (a) and bird's eye view (b) and Mon Cham plot (MC) ground photo (c) and bird's eye view (d).	14
Figure 3.3	Annual rainfall and average temperature at the Nong Hoi Royal Project station from January 2018 to December 2020 (Meteorological Department of Hnong Hoi Royal Project, 2023). Dark blue bar represents amount of rainfall during the period of experiment.	15
Figure 3.4	The five native species applied to three different seed pelleting treatments.	19
Figure 3.5	Methodology of microbial seed coating experiments (applied from Lasudee et al., 2018).	25
Figure 3.6	Diagram of a protocol to determine seed viability (% seed germination) responding to each desiccation/storage treatments.	28
Figure 3.7	Seed germination experiment at DNSC tree nursery.	29
Figure 4.1	Seed removal ($\% \pm 1$ SE) compared among sites: tree nursery (TN), Ban Mae Khi (BMC) and Mon Cham (MC). Five species in the top row of the figure had no seed removal. Species panels are arranged in order to increase removal rates.	34

LIST OF FIGURES (continue)

		Page
Figure 4.2	Percent seed germination (± 1 SE) across study sites. Eight species which failed to germinate are not included. Columns not sharing the same superscript indicate significant differences among sites.	36
Figure 4.3	Comparison of relative rank score of seedling yield (a), relative seedling root collar diameter (RCD) (b) and relative percent performance index (RPI) (c) across studied species, ranking from the highest to lowest RPI.	38
Figure 4.4	The percentage of seed removal (± 1 SE), that average across two field sites for each tested species. The different shapes represent individual species. Additionally, ** indicates the significantly lowest seed removal of the biochar pelleting treatments compared to control (Multiple Comparisons of Means: Tukey Contrasts at $P < 0.01$).	40
Figure 4.5	Percentage of seed germination (± 1 SE), two species failed to establish in the area, so they were not included in the data analysis. The germination percentage significantly differs among pelleting treatments, seed species and study sites. Ban Mae Khi and Mon Cham had significantly lower percent seed germination compared to control condition in tree nursery at significant level 0.001 (***) and 0.05 (*), respectively. The treatments not sharing the same letter indicated significant difference ($P < 0.05$).	42

LIST OF FIGURES (continue)

		Page
Figure 4.6	The percentage of seed removal (± 1 SE) in different study sites, species, and treatments. Each bar represents various treatments; control (■), coating with <i>S. antibioticus</i> (■), coating with <i>S. thermocarboxydus</i> isolate S3 (■) and sterile seed (■). *** represents significantly different among study sites at $P < 0.001$, species that do not share the same superscripts within the x-axis are significantly different.	45
Figure 4.7	Percent seed germination (± 1 SE) of five seed species in three study sites (represents by different colors), with various microbial coating treatments; SA (coating with <i>S. antibioticus</i>), S3 (coating with <i>S. thermocarboxydus</i> isolate S3), ST (sterile without microbial coating), and CO (control). Species that do not share the same are significantly different. * indicates the significantly highest seed germination compared among treatments ($P < 0.05$).	46
Figure 4.8	The percentage of seedling yield (± 1 SE) occurred in different study sites, species, and treatments. Each bar represents various treatments; control (■), coating with <i>S. antibioticus</i> (■), coating with <i>S. thermocarboxydus</i> isolate S3 (■) and sterile seed (■). Species that do not share the same superscripts are significantly different, *** indicated significantly higher seedling yield at Mon Cham sites ($P < 0.001$).	47
Figure 4.9	Average cumulative percent seed germination ($R = 3$) of <i>A. fraxinifolius</i> at 5% MC in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.	50

LIST OF FIGURES (continue)

	Page
Figure 4.10	51
Average cumulative percent seed germination (R = 3) of <i>A. microsperma</i> at dry and moist storage treatments; compared to initial germination.	
Figure 4.11	52
Average cumulative percent seed germination (R = 3) of <i>A. kurzii</i> at various storage moisture content in ambient temperature, and one-month-freezing (at temperature -20°C) compared to initial germination.	
Figure 4.12	53
Average cumulative percent seed germination (R = 3) of <i>A. lacucha</i> at various levels of moisture content in the seed and different storage conditions compared to initial germination, treatment without seed germination was not included on the graph.	
Figure 4.13	54
Average of cumulative percent seed germination (R = 3) of <i>B. baccata</i> at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination, treatment without seed germination was not included on the graph.	
Figure 4.14	55
Average of cumulative percent seed germination (R = 3) of <i>C. axillaris</i> at various levels of moisture content in the seed and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month compared to initial germination, treatment without seed germination was not included on the graph.	

LIST OF FIGURES (continue)

	Page
Figure 4.15	56
Average of cumulative percent seed germination (R = 3) of <i>C. bakeriana</i> storing in the freezer (T -20°C) for one month compared to initial germination.	
Figure 4.16	57
Average of cumulative percent seed germination (R = 3) of <i>D. glandulosa</i> at various level of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination. Treatment without seed germination was not included on the graph.	
Figure 4.17	58
Average of cumulative percent seed germination (R = 3) of <i>G. arborea</i> at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.	
Figure 4.18	59
Average of cumulative percent seed germination (R = 3) of <i>M. baillonii</i> at various level of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination, treatment without seed germination was not included on the graph.	
Figure 4.19	60
Average of cumulative percent seed germination (R = 3) of <i>P. cerasoides</i> at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.	

LIST OF FIGURES (continue)

	Page
Figure 4.20	61
Average of cumulative percent seed germination (R = 3) of <i>P. emblica</i> at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.	
Figure 4.21	62
Average of cumulative percent seed germination (R = 3) of <i>S. fruticosum</i> at various level of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination. The treatment without seed germination was not included on the graph.	
Figure 4.22	63
Average of accumulative percent seed germination (R = 3) of <i>S. rarak</i> at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.	
Figure 4.23	66
The biplot based on LD1 and LD2, to separate the observation among storage behaviors with 100% accuracy estimation.	
Figure 5.1	72
The pelleting seed or seed ball could not germinate before the end of study.	
Figure 5.2	79
Seasonal variation in seed dispersal: a comparison of recalcitrant (■) and orthodox (■) seeds throughout the year (data generated by G. Pakkad and S. Elliott, in total of 328 species in FORRU database).	

ข้อความแห่งการริเริ่ม

1. วิทยานิพนธ์นี้นำเสนอข้อมูลเกี่ยวกับการหยอดเมล็ดโดยตรง ซึ่งเป็นเทคนิคการฟื้นฟูป่าให้มีประสิทธิภาพในบริเวณที่ใหญ่ขึ้น สำหรับการฟื้นฟูภูมิทัศน์ของป่า สามารถนำไปประยุกต์ใช้กับการโปรยเมล็ดทางอากาศด้วยโดรนหรือเครื่องบิน ลักษณะของพืชแต่ละชนิดจะนำไปใช้เป็นการคัดเลือก เพื่อให้ได้ชนิดที่มีศักยภาพดีที่สุดเหมาะสำหรับการหยอดเมล็ด
2. เมล็ดที่นำมาทดสอบเป็นพันธุ์พื้นเมืองทั้งหมด และพบกระจายได้ทั่วไปในอุทยานแห่งชาติดอยสุเทพ-ปุย เป็นชนิดที่ให้ผลและเมล็ดแก่ก่อน-ฤดูฝนซึ่งเป็นช่วงเวลาที่เหมาะสมในการปลูก
3. นอกจากนั้น การวิจัยยังได้ทดสอบประสิทธิภาพของวัสดุเคลือบเมล็ดเพื่อป้องกันการล่าเมล็ดและสนับสนุนการงอกของกล้าไม้ ซึ่งเป็นเทคนิคที่มีการปรับเปลี่ยนดัดแปลงมาจากการปฏิบัติทางการเกษตร

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STATEMENTS OF ORIGINALITY

1. This thesis presents data on direct seeding, to contribute towards development of technique to scale up forest restoration in the landscapes using seed delivery by drone or aircraft. The species traits aimed to be criteria for species selection, in order to selected best performance species suitable for direct seeding.
2. The tested species are all native species commonly found on Doi Suthep-Pui National Park. The species produced mature fruits and seeds before-during the rainy season which is the suitable time to plant.
3. Additionally, the research also tested the efficiency of seed coating materials to prevent seed predation and support seedling establishment. The techniques were modified from agriculture practices.

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CHAPTER 1

INTRODUCTION

1.1 Historical background

Active ecological restoration strategies are a priority to achieve maximum biomass, structural complexity, biodiversity and ecological functionality during forest ecosystem restoration (Elliott et al., 2017), particularly in highly threatened areas, where soil seed banks, natural regenerants and natural recovery potential are limited (Elliott et al., 2017; Besseau *et al.*, 2018; Dimson and Gillespie, 2020). Tropical forests are among the most biodiverse of terrestrial ecosystems with carbon sinks that remove 30% of anthropogenic carbon dioxide (CO₂) emissions from the atmosphere through photosynthesis (Bellassen and Luysaert, 2014). Restoring tropical forests on degraded areas could therefore contribute substantially towards preventing species extinctions and sequestering a significant amount of atmospheric CO₂. Consequently, forest restoration is being promoted globally by the United Nations (UN) and national governments (Besseau et al., 2018). However, it is often practiced on a small scale, because conventional tree-planting is costly and labor intensive, involving seedling production in nurseries and transportation of heavy containerized seedlings to planting sites. Techniques to scale up forest restoration remain under-developed or untested (Goldapple, 2017).

Direct-seeding involves sowing various forest tree seeds directly into the ground of degraded areas, to re-establish original forest ecosystems (Elliott et al., 2006; Harrington, 1972). The seeds are normally sown at the beginning of the rainy season, when conditions for seed germination are optimal, allowing maximum time for root-system development before onset of the dry season (Waiboonya and Elliott, 2020). The method costs less than conventional tree planting because it does not require funding of tree nurseries and it is less labor-intensive (Willoughby et al., 2007; Woods and Elliott, 2004; Souza, 2022). This technique is enables practice of forest restoration in remote area.

Carrying tiny seeds is more manageable, compared to carrying heavy containerized saplings. Furthermore, direct-seeded seedlings have higher growth rates, compared with planted ones (Naruangsri, 2017; Freitas et al., 2019), due to better root development onsite.

The use of native tree species is strongly advocated for forest restoration, as it is an essential key to restoring ecological functionality (Rout et al., 2009; Lu et al., 2017). In Northern Thailand, the Framework Species Method (FSM) is used to restore moderately degraded sites, where natural seed dispersal still occurs (FORRU, 2005). The method involves planting multiple indigenous forest tree species, including both climax and pioneer species, to encourage rapid growth, shade out weeds and attract animal seed dispersers (Aerts and Honnay, 2011; Elliott et al., 2002). The selection of native species according to their functional groups requires knowledge about traits, their reproductive biology, phenology, and propagation (Piekarska-Stachowiak et al., 2014; Thomas et al., 2014; Manohan et al., 2023). Moreover, genetic variation and inbreeding between species in small population size must be considered (Thomas et al., 2014).

Direct seeding of native tree species has been demonstrated for restoring various ecosystems, including broadleaved woodland (Willoughby et al., 2004), coniferous forests (Nilson and Hjältén, 2003), beech and oak forests (Birkedal, 2010), pastureland (Douglas et al., 2007), limestone mines (Barton et al., 2015), seasonal semideciduous forests (Brancalion et al., 2016) and tropical rain forest (Tunjai and Elliott, 2012). Many authors claim that direct seeding rapidly achieves restoration goals at reduced costs, while also offering the possibility of scaling up to restore large areas (Grossnickle and Ivetic 2017; Suaza, 2022). Willoughby et al. (2004) showed that the technique is cheaper than tree planting. Brancalion et al. (2016) demonstrated achievement of tree densities by direct-seeded seedling four times higher than by tree planting at within 3 years. Moreover, direct seeded sites form more complex canopy structure within 4 years (Freitas et al., 2019), with high aboveground biomass (Brancalion et al., 2016; Freitas et al., 2019).

Despite direct seeding being more cost-effective than conventional tree planting, the approach is not widely implemented on a large scale worldwide due to limitations. Failures of direct-seeding are common. Achieving high seedling density and species diversity in the short term is challenging and is often attributed to variability in species

performance (Suaza and Engel, 2018). Tree establishment can be limited by low seed germination percentage and high seedling mortality, due to drought and competition with weeds (Naruangsri, 2017; Waiboonya. and Elliott, 2019; Willoughby et al., 2019).

Seed removal/predation by animal predators can also prevent success of direct seeding in open landscapes (Naruangsri, 2017; Woods and Elliott, 2004). Seeds on the ground are subject to removal and predation which leads to a low number of seeds being available for seedling establishment. Seed-predation intensity varies, according to the predator communities present (Wells and Bagchi, 2005). Techniques to reduce seed removal/predation and increase germination must be developed, to maintain seed availability after sowing (Naruangsri et al., 2023). Two approaches, to overcome the seed removal/predation and seedling mortality, are (1) to cover seeds with enclosing materials and (2) to promote early seedling survival, by coating seeds with enhancement substances.

Furthermore, it is necessary to select suitable tree species to increase the probability of seedling establishment (Lamb, 2005; Tunjai and Elliott, 2012). Selecting species based on their functional traits is useful and successful for ecological restoration (Laughlin and Laughlin, 2013; Beckman and Tiansawat, 2020; Wang et al., 2020; Manohan et al., 2023). Seed functional traits are important, because some traits are related to seedling survival and establishment e.g., seed size, shape, moisture content and their storage behavior (Tunjai and Elliott, 2012; Waiboonya, 2017; Suaza and Engel, 2018). Species with high and rapid seed germination can contribute to high seedling density (Hossain et al., 2014; Dias Laumann et al., 2023). Seedling type may also be important. Hypogeal seedlings exhibit greater success, as they can emerge from deeper soil depths (Dias Laumann et al., 2023). However, data on the relationships between seed and seedling functional traits and species performance are still lacking. Consequently, knowledge gained from study presented here could be used to improve species selection for direct-seeding for forest restoration in Thailand.

This research study addresses four main research questions. -

- 1) What native tree species are suitable for direct seeding in degraded areas of Northern Thailand?

2) What coating materials reduce seed removal/predation and increase seed germination rate and percentage following direct-seeding of degraded sites?

3) What morphological characteristics of seeds and seedlings contribute to fast seedling growth and high survival following direct-seeding of degraded sites?

4) To what extent do site conditions affect seed germination and seedling survival and growth?

1.2 Research Objectives

1) To compare seed-removal percentages and the efficiency of three different coating materials in protecting seeds from rats and insects seed predators.

2) To compare seed germination, seedling survival and establishment of the twenty-three native tree species after direct seeding in two study sites.

3) To determine the effectiveness of microbial seed coatings on seed germination, seedling survival and growth of five seed species.

4) To determine relationships between seed/seedling traits of twenty-three native tree species and their field performance following direct seeding.

1.3 Usefulness of the Research

1) This study provides suitable techniques to protect seeds from seed predators and to increase seed germination for each tree species.

2) The techniques will be helpful for forest restoration in degraded areas. The results help in selecting suitable tree species for direct-seeding.

3) This study provides information for site selection for direct seeding.

CHAPTER 2

LITERATURE REVIEW

2.1 Forest restoration and direct seeding

Planting a wide variety of native forest tree species is recommended, to rapidly accumulate biomass and recover forest structure, biodiversity and ecological functioning in restoration forests (Lu et al., 2017). However, tree-planting is costly and labor-intensive. It entails collecting seeds from the reference forest ecosystem, establishing a nursery to produce containerized planting stock (usually saplings 30 - 50 cm tall) and transporting heavy containerized saplings to restoration sites. Tree planting and subsequent weeding and fertilizer application are all highly labour intensive. Furthermore, sites available for restoration are mostly on steep, difficult terrain, far from vehicular access, i.e., those unsuitable for agriculture.

Direct seeding circumvents some of these logistical limitations, and provides a means to upscale forest restoration projects, to meet the needs of the global initiatives mentioned above (Cole et al., 2011; Grossnickle and Ivetic, 2017). The method involves simply sowing or burying tree seeds directly into the ground. People become seed-dispersal agents, where natural seed-dispersal is limited. Direct seeding requires no nursery costs, and it is far less labor-intensive than conventional tree planting; transportation costs are also much reduced (Woods and Elliott, 2004; Willoughby et al., 2007; Cole et al., 2011). It is easier to carry bags of seeds onto steep or remote sites than to haul baskets of containerized saplings. Moreover, seedlings from direct seeding often grow better in the field than nursery-produced saplings, because they develop better root systems and transplantation shock is avoided (Naruangsri, 2017).

Direct seeding has been widely trialed in several countries with mixed results (Ruiz-Jaen and Aide, 2005). For example, Silva et al. (2015) reported average emergence of around 52% for mixed species of tree seeds sown into neotropical savannas, whilst Grossnickle and Ivetić (2017) reported 17% establishment, following direct seeding of

tropical forest tree species. In Thailand, the potential of direct seeding for forest restoration was tested in northern seasonally dry forests (e.g., Woods and Elliott, 2004; Tunjai, 2005; Hossain et al., 2014; Naruangsri, 2017; Waiboonya and Elliott, 2020) and in southern evergreen forests (e.g., Tunjai and Elliott, 2012) with the average seedling establishment ranging from 0 up to 89%. Success appears to be highly species-specific. In southern Thailand, Tunjai and Elliott (2012) concluded that large, round seeds (> 5 g) with thick seed coats (> 0.4 mm) are likely to be more successful in the seasonally dry tropics. Waiboonya and Elliott (2020) reported that the optimal time to sow seeds for restoration of upland evergreen forest in northern Thailand was at the beginning of the rainy season.

In a meta-analysis of 30 studies, including both tropical and temperate forests (but none in Thailand), Ceccon et al. (2016) reported overall seed germination was 20%, and approximately 28% of the studied species exceeded 20% seedling establishment. Outcomes were not significantly affected by climate, species successional status nor the application of pre-sowing treatments. Success increased with seed size, and with the application of physical protection from seed predators. More recently, in a global bibliometric analysis of 81 publications on direct seeding for forest restoration, Souza (2022) reported that forests, established by direct seeding, are rarely monitored for long-term outcomes. He concluded that the technique has great potential to attain restoration goals, but that it is insufficiently studied and is, therefore, a promising area for research, to determine its applicability around the world. He attributed its lack of wide adoption in the tropics thus far (Ceccon et al., 2016; Grossnickle and Ivetic, 2017) to low seedling emergence, establishment and growth; low seed availability, lack of knowledge of seed biology (desiccation tolerance -orthodoxy vs recalcitrance), storage conditions; optimal seeding densities and times—all limitations that are ultimately determined by species choice. For restoring tropical forests, species selection for direct seeding is more complex and challenging than it is for tree planting. Susceptibility to seed predation is crucial, along with germinability, tolerance of very young seedlings to the harsh conditions on deforested sites and their resilience following damage (Meli et al., 2014; Lu et al., 2017).

2.2 Thick-layer seed coating: seed ball

Seed coating has been suggested as a way to conceal seeds from potential predators. Repel them or make seeds more difficult to handle and consume. Seed coating is routinely and reliably used in modern agriculture (Zhang et al., 2022). Different coating agents have been applied for various purposes e.g., pesticides, water absorbent gels, plant hormones, fertilizers etc. have been tested to prevent diseases, promote germination and enhance seedling survival (Gorim, 2014; Williams et al., 2016; Su et al., 2017; Taylor, 2019; Zhang et al., 2022). Notably, numerous studies have successfully applied seed coating techniques to various crops (e.g., Turner et al., 2006; Liu et al., 2010; Gorim, 2014; Williams et al., 2016; Su et al., 2017; Taylor, 2019; Baroni and Vieira, 2020). In general, seed coating holds significant promise in overcoming challenges associated with seed protection and enhancement.

Covering seeds with protective materials can be a useful approach to keep them safe from animal predators while still allowing enough seeds to germinate at the target site. For example, the study in grass species for rangeland reforestation project by Taylor (2019) suggested that effect from seed predation can be reduced using a simple clay and polymer seed covering which is cheaper and safer than extra deterrent substances. Likewise, a study of Liu et al. (2010) claims that seed coating with polysaccharide agents can promote seedling emergence and growth. Despite extensive research about seed coating on grass and herbaceous plants, there is still a paucity of information about the use of seed coating for tree seed species, particularly in tropical seasonal forests.

Currently, numerous agencies and organizations are actively engaged in the advancement of seed coating methods specifically for forest restoration endeavors (Pedrini et al., 2020) for example the seed balls Kenya team, ICIMOD, We Grow Forest Foundation, Department of forestry and Forest Restoration Research Unit. Seed coatings can be classified based on their physical attributes, including weight, size, and sorting properties of the coated seeds (Pedrini et al., 2020; Javed et al., 2022). Some common types of seed coatings are film coating, encrusting, and pelleting (Afzal et al., 2020; Javed et al., 2022). The study in question primarily emphasis on the pelleting technique, that would be particularly useful for aerial seeding conducted by unmanned aerial vehicles

(UAV). Consequently, the term "pelleting" is used instead of "coating" to highlight the specific method being investigated.

2.3 Microbial seed coating

Seed coating is used by horticultural and crop industries worldwide (Pedrini et al., 2020). One type of seed coating is microbial seed coating. It involves application of a thin layer of beneficial microorganisms to the surface of seeds (Rocha et al., 2019), including beneficial bacteria, fungi and algae (Khan et al., 2016). The use of microbial seed coatings has gained popularity in recent years, as it offers many advantages rather than traditional seed treatments. The microbial seed coatings can improve seed germination, plant growth, development, and yield by providing bioactive compounds, nutrients, and protection from environmental stressors (Barka et al., 2016; Khan et al., 2016; Rocha et al., 2019). Therefore, the application of bacteria can effectively enhance crop productivity, whilst also decreasing reliance on agrochemicals, thereby demonstrating its eco-friendly potential for sustainable agriculture (Boukhatem et al., 2022).

One of the microorganisms identified in plant growth-promoting rhizobacteria is actinobacteria. The gram-positive bacteria belong to Streptomycetaceae family, *Streptomyces* genus, which is the most abundant and arguably the most important actinomycetes (Sousa and Olivares, 2016; Law et al., 2018). The *Streptomyces* is commonly found in soil, making up roughly 10 to 50% of the microbial population in the soil (Olanrewaju and Babalola, 2019). *Streptomyces spp.* have been recognized for their ability to colonize plant roots (Tufail et al., 2022), playing a crucial role as nitrogen-fixing bacteria which is essential for soil ecosystem functioning (Dahal et al., 2017; Paravar et al., 2023). The *Streptomyces* also help plant to produce valuable bioactive compounds (Tufail et al., 2022; Nazari et al., 2023) and various kinds of phytohormones crucial for plant growth (Nazari et al., 2023). These phytohormones, produced by *Streptomyces*, accelerate plants' responses to biotic and abiotic stresses, such as salinity, drought, soil contamination, and the management of weed, pathogens, and diseases (Olanrewaju and Babalola, 2019; Tufail et al., 2022; Nazari et al., 2023; Paravar et al., 2023).

2.4 Seed viability and storage behaviors

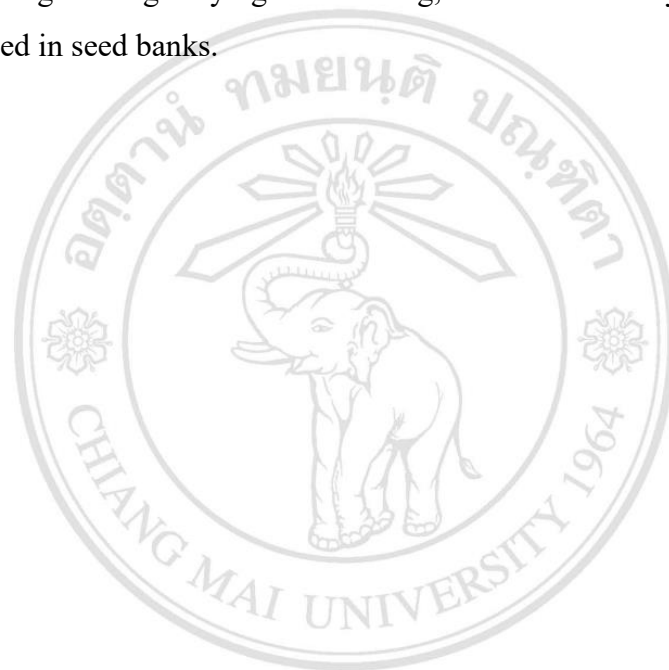
Seed storage is necessary to maintain seed viability and quality from harvest until sowing (Dadlani and Yadava, 2023). Generally, seeds are best stored in dry and cool places (at minus degree Celsius). However, the exact moisture content and temperature suitable for each species and accession within a species vary. The ideal environmental conditions to store a species of seeds are called the 'storage behavior' (Hong et al., 1996; Baskin and Baskin, 2014).

The responses of seed to desiccation and chilling determine their classification as orthodox, recalcitrant and intermediate seeds (Hong et al., 1996). 'Orthodox' seeds are desiccation tolerant—they survive when moisture content is reduced to 5% (Yulianti et al., 2020; Matilla, 2021)—and they can be frozen. "Recalcitrant" seeds are desiccation-intolerant and cannot survive are killed by freezing. "Intermediate" seeds possess functional characteristics that lie between those of orthodox and recalcitrant seeds (Baskin and Baskin, 2014; Yulianti et al., 2020). They can tolerate desiccation to a certain extent (typically maintaining a moisture content of around 7 - 10% during dry seed storage) (Gold and Hay, 2014; Yulianti et al., 2020), but are sensitive to freezing. Therefore, understanding the storage behavior of seeds becomes crucial when conducting direct seeding outside of the fruiting season of the target species.

In general, most tropical pioneer species have orthodox seeds but many climax species have recalcitrant or intermediate seeds. Moreover, the proportion of each storage behavior varies among forest types, with recalcitrant behavior being common in moist forests but rare in arid and dry forests (Tweddle et al., 2003). More than 25% of plant species worldwide produce recalcitrant seeds (Li and Pritchard, 2009), which including a high proportion of tropical trees and many species of conservation concern (Dadlani and Yadava, 2023). Across all forest types in Thailand, it is estimated that 46% of the country's native forest tree species are likely to be recalcitrant, whereas approximately 54% possess orthodox and intermediate seeds (Wyse and Dickie, 2018). However, in Northern Thailand where seasonal dry forest dominates, about 75% of species tend to have orthodox seeds, with only 21% having recalcitrant seeds (Tweddle et al., 2003). Therefore, the majority group of tree species in the north produces orthodox seeds, which are likely to remain viable

during storage, while a minority of species have recalcitrant seeds that requires careful consideration of suitable storage methods before sowing.

Under suitable conditions, it is possible to maintain viability of orthodox seeds with conventional storage techniques—normally dry and frozen at -20°C —for an extended period. On the other hand, maintaining the viability of seeds of recalcitrant or intermediate species is challenging. Short-term storage is usually the best that can be achieved (Waiboonya, 2017; Yulianti et al., 2020). Such species are not suitable for traditional seed storage through drying and freezing, which is currently the main method of preservation used in seed banks.



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CHAPTER 3

METHODOLOGY

3.1 Study sites and measurement of site conditions

Field experiments were conducted in two different sites, under the authority of Nong Hoi Royal Project. Both sites are in Mae Rim District in Chiang Mai, Northern Thailand (Figure 3.1). At each site, the experiments cover an area of 4,800 m².

The first site (hereafter Mom Cham: MC) was a degraded site near Mon Cham viewpoint, a tourist attraction at 1,300 m above sea level (18° 56' 18.0" N, 98° 49' 16.7" E). Most of the study sites faced the northeast (NE) with the mean slope of 27.1 ± 2.5 degrees. Mon Cham is a seasonal evergreen forest located on the upper watershed site bordering Doi Suthep-Pui National Park (Figure 3.2, a-b). This area was previously used as agricultural land but was subsequently earmarked for forest restoration by the Royal Project in 2012. Approximately 8,600 m² of the site used for direct seeding experiments had been planted with trees. Unfortunately, due to lack of budget for weeding and fertilizer, survival rate of the planted seedlings was low. The restored site was dominated by weeds such as *Ageratina adenophora*, *Eupatorium odoratum* and *Pteridium aquilinum*. A rapid site assessment carried out in 2019 found 15 tree species with 237 trees per rai. The recommended density of seedlings for accelerating forest recovery is 500 trees per rai (FORRU, 2005), the aim was to interplant among the surviving trees to bring the density back up to about 500 trees per rai.

The second site (hereafter Ban Mae Khi: BMK) was a bamboo plantation near Ban Mae Khi at 925 m above sea level (18° 57' 34.0" N, 98° 48' 33.4" E). The aspect was predominantly northwest (NW) to north (N) with a relatively gentle slope of 12.4 ± 7.5 degree and a flat area for the first replicate. Ban Mae Khi is a mixed deciduous forest. Some bamboos and fruit trees had been planted, but the top of the ridge remained largely bare (Figure 3.2, c-d). According to a survey, the density of natural regenerants

was 85 trees per rai (1,600 m²). The dominant weeds included *Eupatorium odoratum*, *Imperata cylindrica*, and *Chrysopogon aciculatus*.

Soil samples were randomly collected in two study sites at the beginning of the experiment and then submitted to the Faculty of Agriculture Laboratory in Chiang Mai to assess soil nutrients: nitrogen (N), phosphorus (P), potassium (K), and soil pH (Table 2.1). The soil quality at Mon Cham was found to be superior to that of the Ban Mae Khi plot, with higher levels of essential nutrients and a more favorable pH balance. The amount of P ($t(2) = -7.3$, $P = 0.02$) and K ($t(4) = -3.9$, $P = 0.02$) was significantly higher in Mon Cham), whereas N was higher at Ban Mae Khi but not significantly different ($t(4) = 1.6$, $P = 0.19$). Additionally, soils were acidic in both study sites, the Ban Mae Khi plot had a lower pH compared to Mon Cham.

In the year 2019, rainfall was lower than usual. The total rainfall was 1,638.5 mm (average 125 mm per month) with 21°C average annual temperature and 87% air humidity. During the experiment period from July 2019 to May 2020, a three-month period from January to March 2020 without rainfall. Overall, the amount of rainfall during the experiment was significantly lower than the amounts recorded in both 2018 and 2020, which both exceeded 14,700 mm (Figure 3.3).

In addition to the field experiments, seed germination tests were conducted at a research nursery, located in Doi Suthep-Pui National Park (18° 48' 3.7" N, 98° 54' 59.6" E, at about 1,000 m above sea level) and Ban Mae Sa Mai, Mae Rim (18° 52' 34.2" N, 98° 50' 52.3" E, at about 980 m above sea level). Tree seedlings were looked after and watered by FORRU's staff.



Figure 3.1 The location of study sites, Chiang Mai, Thailand (inset map top left). The legends (red dot) indicated different locations used for experiments at Ban Mae Khi and Mon Cham (middle map), and the boundaries of each site are shown in the right photos to indicate the conditions surrounding the plots.

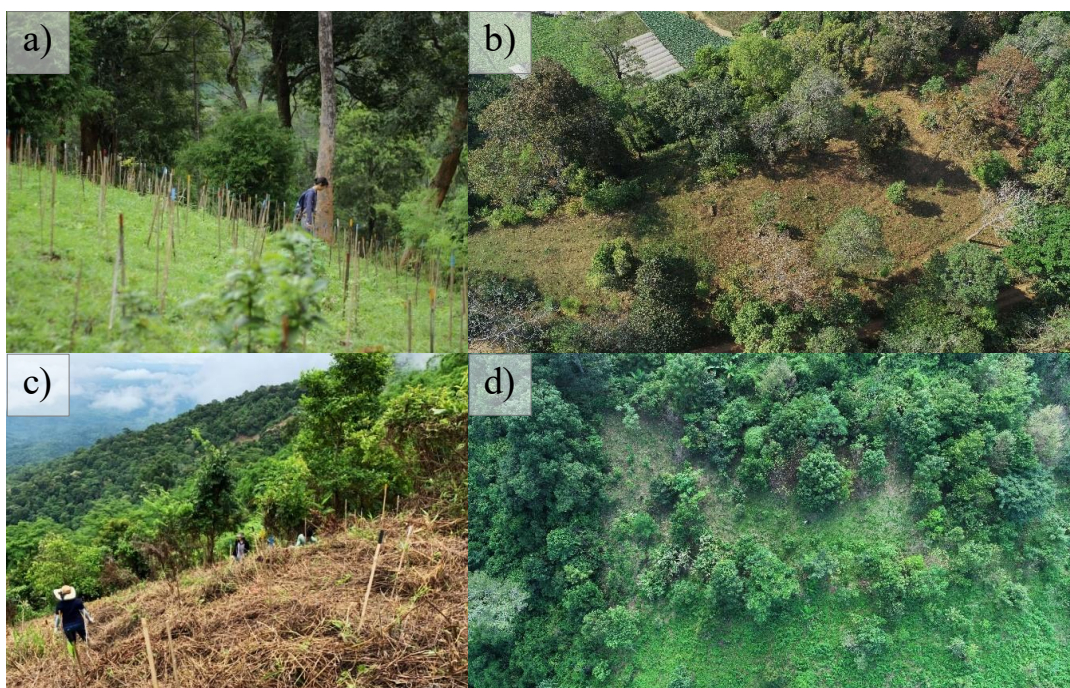


Figure 3.2 The physical characteristics of two field studied sites; Ban Mae Khi plot (BMK) ground photo (a) and bird's eye view (b) and Mon Cham plot (MC) ground photo (c) and bird's eye view (d).

Table 3.1 The quality of soil at two study sites; Mon Cham and Ban Mae Khi in 2019. Column do not share the same letter indicated significant different between two study sites tested by t-test ($P < 0.05$).

Soil properties	Mon Cham	Ban Mae Khi
	Mean \pm SD	Mean \pm SD
Phosphorus (mg/kg)	20.5 \pm 4.3 ^a	2.4 \pm 0.4 ^b
Potassium (mg/kg)	419.4 \pm 75.5 ^a	195.8 \pm 65.1 ^b
Nitrogen (%)	0.18 \pm 0.03 ^a	0.23 \pm 0.04 ^a
pH	5.4 \pm 0.16 ^a	4.92 \pm 0.16 ^b
OM (%)	4.8 \pm 1.23 ^a	6.0 \pm 1.2 ^a
*Moisture (%)	20.0 \pm 4.4	18.9 \pm 5.7

*Soil moisture was average across 3 seasons in a year.

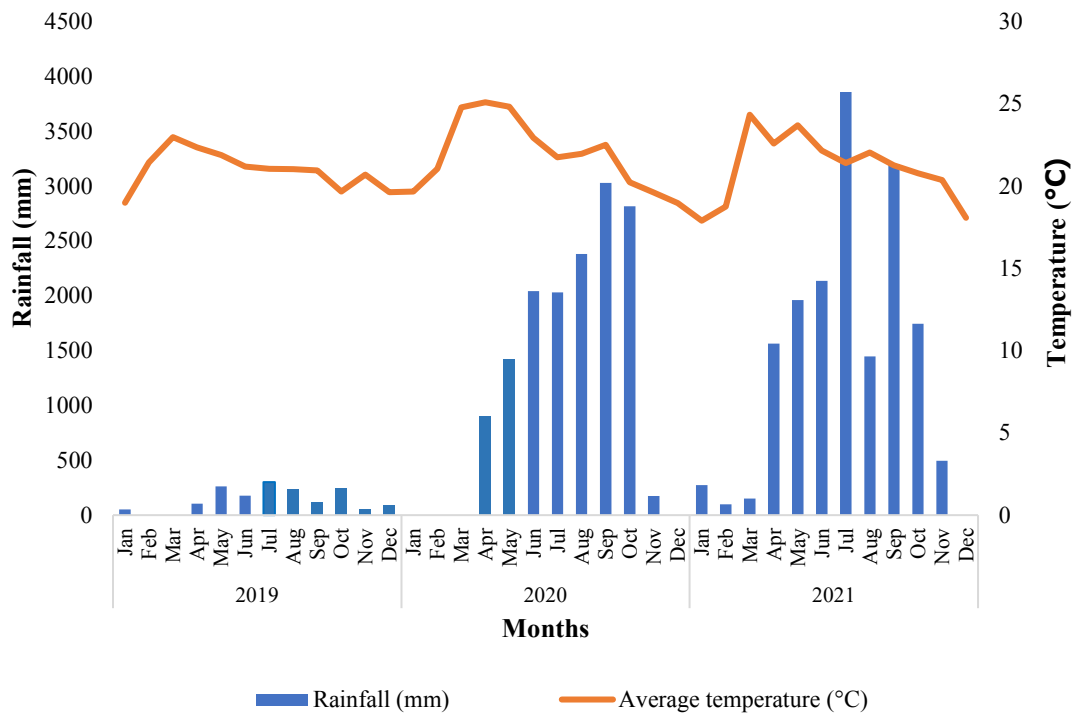


Figure 3.3 Annual rainfall and average temperature at the Nong Hoi Royal Project station from January 2018 to December 2020 (Meteorological Department of Hnong Hoi Royal Project, 2023). Dark blue bar represents amount of rainfall during the period of experiment.

3.2 Species selection

Twenty-three native tree species of Northern Thailand were used in the experiments (see in Appendix III for further details of the species). All species grow at high altitudes (about 900 - 1,500 m above sea level). The species are suitable for conventional tree planting to restore forest to degraded areas (Elliott et al., 2003). Tree species were also selected on their seed availability before and during rainy season, which are postulated to be suitable conditions for direct-seeding (Tunjai and Elliott, 2012).

Table 3.2 Tree seed species using for direct seeding (POWO, 2023)

No.	Thai name	Scientific name	Family	Elevation range		Propagule	Successional guilds
				Lower	Upper		
1	สะเตาะช้าง	<i>Acrocarpus fraxinifolius</i> Wight ex Arn.	Leguminosae	500	1250	Seed	Climax
2	มะกกลำต้น	<i>Adenanthera microsperma</i> Teijm & Binn.	Leguminosae	200	1100	Seed	Pioneer
3	ฝาละมี	<i>Alangium kurzii</i> Craib.	Alangiaceae	600	1400	Pyrene	Pioneer
4	หาค	<i>Artocarpus lacucha</i> Roxb.	Moraceae	200	1500	Seed	Pioneer
5	สลีนก	<i>Balakata baccata</i> (Roxb.) Esser	Euphorbiaceae	375	1500	Seed	Pioneer
6	กัลปพฤกษ์	<i>Cassia bakeriana</i> Craib	Leguminosae	800	1350	Seed	Pioneer
7	มะกอกหัวรู	<i>Choerospondias axillaris</i> (Roxb.) B.L. Burtt&A.W. Hill	Anacardiaceae	460	1600	Pyrene	Climax
8	กล้วยฤๅษี	<i>Diospyros glandulosa</i> Lace	Ebenaceae	650	1650	Seed	Climax
9	ชะมวง	<i>Garcinia cowa</i> Roxb.	Guttiferae	60	1500	Seed	Climax
10	ช้อ	<i>Gmelina arborea</i> Roxb.	Verbenaceae	200	1475	Pyrene	Pioneer
11	หมอนหิน	<i>Hovenia dulcis</i> Thunb.	Rhamnaceae	1025	1300	Seed	Climax
12	จำปีป่า	<i>Magnolia baillonii</i> Pierre	Magnoliaceae	650	1350	Seed	Climax
13	เตียน	<i>Melia azedarach</i> Linn.	Meliaceae	500	1450	Seed	Pioneer
14	มะตุ๊กตง	<i>Phoebe cathia</i> (D. Don) Kosterm.	Lauraceae	550	1600	Seed	Climax
15	มะขามป้อม	<i>Phyllanthus emblica</i> L.	Euphorbiaceae	60	1700	Seed	Pioneer
16	ยางโอบ	<i>Polyalthia viridis</i> Craib	Annonaceae	500	800	Seed	Climax

Tabel 3.2 (continue)

No.	Thai name	Scientific name	Family	Elevation range		Propagule	Successional guilds
				Lower	Upper		
17	นางพญาเสือโคร่ง	<i>Prunus cerasoides</i> D. Don	Rosaceae	1050	1750	Pyrene	Pioneer
18	ก้อสีเสียด	<i>Quercus brandisiana</i> Kurz.	Fagaceae	750	1300	Seed	Climax
19	มะขี้ก	<i>Sapindus rarak</i> DC.	Sapindaceae	200	1620	Pyrene	Intermediate
20	มะยง	<i>Sarcosperma arboreum</i> Bth.	Sapotaceae	550	1500	Seed	Climax
21	จันทอน	<i>Scieropyrum pentandrum</i> (Dennst.) Mabb.	Santalaceae	60	1600	Pyrene	Climax
22	มะกอกป่า	<i>Spondias pinnata</i> (L.f.) Kurz	Anacardiaceae	60	1200	Pyrene	Pioneer
23	หัวจี่กวาง	<i>Syzygium fruticosum</i> DC.	Myrtaceae	200	1525	Seed	Climax

Successional guild classification: Pioneer = fast growing and light demanding, Climax = slow growing and shade tolerant



In this study, the word “seed” is used to include all propagules, including pyrenes (one or more seeds contained within a hard endocarp) (Table 3.2). Seed dry mass varied from 0.02 g to 4.30 g. For each species, approximately 3,000 seeds were collected from at least five mother trees. After collection, seeds of all mother trees were cleaned, air-dried, and stored at 4°C until used (Waiboonya, 2017). Seeds were separated into three lots:-

- (1) seeds to be sown in field and nursery experiments,
- (2) seeds to be used for seed coating experiments and
- (3) seeds to be studied for their morphological traits and storage behavior.

3.3 Experimental design and data collection

3.3.1 Suitable species for direct seeding

Three replicates of 23 tree species, each of 20 seeds, were hand-sown into both field sites during the rainy season of 2019. Bamboo tubes (about 10 cm long and 5 - 10 cm diameter) were buried 5-cm deep into the soil near a bamboo marker stick, established a meter apart from one another. In each tube, one seed was pressed into the soil and buried about 0.5 cm deep. A paper tag, indicating the identity of the seed in each tube, was attached. A total of 1,380 seeds from 23 species were sown in each study site.

Percent seed removal and germinated seeds was recorded weekly for nine months from sowing time in July 2019. Seed was recorded as removed when the evidence of seed predation was observed such as a whole seed being removed from the bamboo tube, a seed was bitten by an animal, seeds were cracked and removed from the bamboo tube, and a seed was damaged by insects. This study used seed removal to indicate intensity of seed predation (Vander Wall et al., 2005). Seed removal comprised both destroyed and dispersed seeds, both of which reduced seeds remaining in the study plots.

Germination was defined as emergence of a primary root, cotyledon, or hypocotyl visible on the surface of the soil. Monitoring ceased when no further germination had occurred for more than a month. At the end of the experiment, non-germinated seeds were dug up, and a cutting test was used to determine if they were still alive. Additionally, any evidence of predation, such as holes in the seeds or insect burrowing, was noted. Seeds that had disappeared or were unobserved were recorded as 'no data'.

Three replicates of twenty seeds of each species were also sown in a tree nursery, in modular germination trays under 50 - 70% shade, in parallel to the field experiments. This determined germination rates under ideal conditions and without predation. Germination was recorded in the same way as in field trials.

During the first year of field experiments, weeds were removed by hand, and fertilizer was applied, in November 2019 and again in May 2020 (at the end and beginning of the rainy season, respectively). The number of surviving seedlings was recorded during such maintenance procedures. Root collar diameter (RCD)—stem diameter where shoot meets root—was measured at the widest point using Vernier-scale calipers. Seedling (or sapling) height (from root collar to apical meristem) and crown width (at broadest axis) were measured with a ruler (as outlined in Elliott et al., 2013).

3.3.2 Thick layer seed coating (seed balls)

Studied species

Five native tree species were used in the experiments. Their seed sizes ranged from 0.03 to 1.34 g dry mass (Table 3.3).

Seed pelleting treatments

Three pelleting treatments with three main different materials were tested: biochar, soil mixture, and polysaccharide mixture (Figure 3.4). The control was non-coated seeds. The biochar and soil mixture treatments were tested to see whether coating materials help to make seeds less attractive to seed predators. The biochar from longan woods were ground and mixed with clay soil with the ratio 1:1 of biochar and clay and applied to the seeds. The biochar cannot be used alone due to high basicity (pH 12 - 13) (Shafer, pers. comm.).

For soil mixture treatment, equal portions of clay soil, coconut husk and peanut shell were mixed together. All materials for soil mixture are material used as potting media in seedling production in tree nurseries (FORRU, 2005).

Table 3.3 The species tested with pelleting materials.

No.	Scientific name	Family name	Seed size (g dry mass)	Storage behavior	Successional stage*
1	<i>Hovenia dulcis</i> Thunb.	Rhamnaceae	0.03	Orthodox	Climax
2	<i>Acrocarpus fraxinifolius</i> Wight & Arn.	Leguminosae	0.03	Orthodox	Climax
3	<i>Syzygium fruticosum</i> DC.	Myrtaceae	0.38	Recalcitrant	Climax
4	<i>Gmelina arborea</i> Roxb. ex Sm.	Verbenaceae	0.52	Orthodox	Pioneer
5	<i>Sarcosperma arboreum</i> Buch.-Ham. ex C.B.Clarke	Sapotaceae	1.34	Recalcitrant	Climax

Identify of successional stage; Climax (late successional, shade-tolerant) & Pioneer (early successional, light loving) species (Waiboonya et al., 2019).

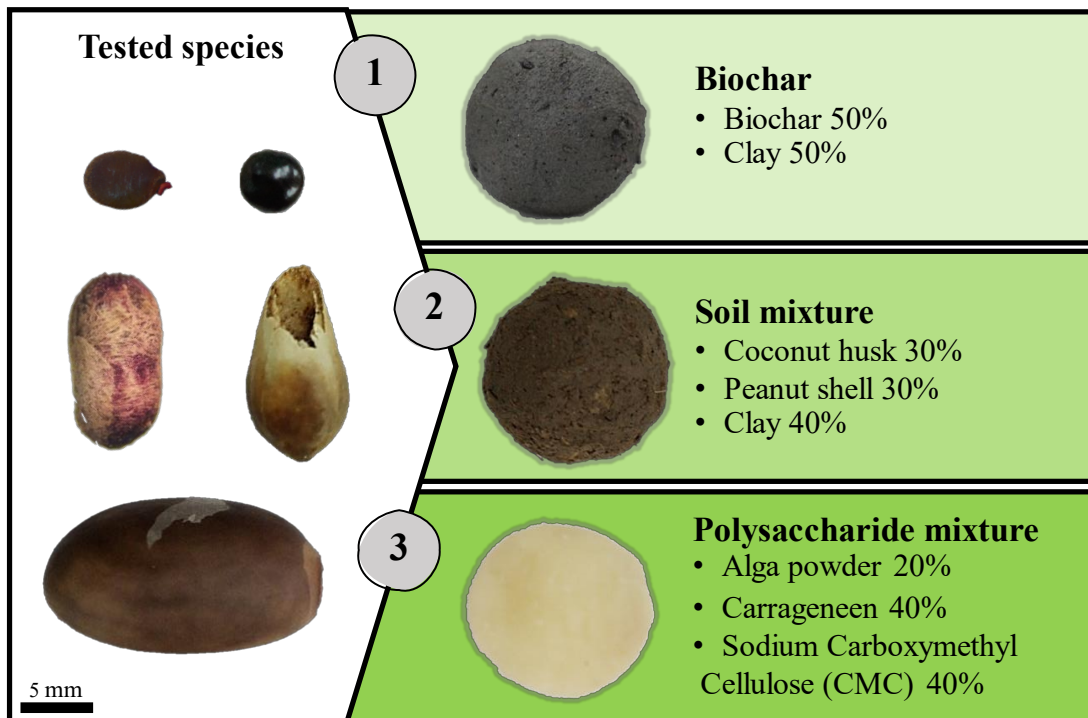


Figure 3.4 The five native species applied to three different seed pelleting treatments.

The polysaccharide mixture was chosen as a primary treatment to facilitate the germination process and enhance the overall survival rate of the seedlings. For the polysaccharide mixture treatment, the active constituents are carrageenan, sodium carboxymethyl cellulose (CMC) and alga powder. The mixture ratio of pelleting material is 4:4:2 of carrageenan, sodium carboxymethyl cellulose and alga powder that can provide the stickiness for seed pelleting process (Liu et al., 2010). Previous studies reported that pelleting seeds with polysaccharide mixture helps to keep the moisture and water supply for seed germination (Liu et al., 2010).

The cleaned seeds were enclosed with three different materials by hand. The weight of pelleting materials for individual seeds varied among species due to differences in seed size. The thickness of pelleting was 5 - 10 mm from seed surface.

Field experiment and data collection

After applying coating materials, the coated seeds were divided into two groups. The first group of seeds was sown by hand directly in two degraded areas – Mon Cham and Ban Mae Khi. There were three replicates of 20 seeds per treatment. For each replicate, various colors of 1.2 m bamboo stick, used to identify different treatments, were randomly established at a meter apart from one another. Bamboo tubes were buried five cm deep into the soil near bamboo stick. In each tube, one seed was pressed into the soil inside the bamboo tubes and buried them about one centimeter from soil surface.

The number of seeds that are removed, germination and dead were observed every week. The monitoring was finished when no further germination occurs for four consecutive weeks.

To collect data on effects of pelleting and collecting materials on seed germination and seedling growth in a control environment, the second group of coated seeds were sown in a tree nursery in parallel to the field experiments. Three replicates of 20 seeds from each treatment were sown on germination trays filled with forest soils. The tree nursery received 50% sunlight.

3.3.3 Microbial seed coating

Studied species

Five native tree species, varying in seed size from 0.03 to 1.43 g dry mass, were selected for the experiments (Table 3.4).

Seed coating treatments

The seeds were surface sterilized, using 6% sodium-hypochlorite solution, followed by 3% sodium-hypochlorite solution (NaOCl), and then with 95% and 70% ethanol (Lasudee et al., 2018). The duration of sterilization varied according to the size of the seeds—five minutes each step for *C. axillaris* and two minutes for other species. Subsequently, the seeds were washed three times with distilled water for a minute each time.

Seeds of each species were inoculated with different microbial seed coatings. The thin-layer coating is intended to support small seedlings after germination from seeds. Seeds were coated with actinobacteria that were reported to be beneficial for early seedling growth and seedling tolerance (Paravar et al., 2023).

There were four treatments in this experiment.

- 1) *Streptomyces antibioticus* (SA)
- 2) *Streptomyces thermocarboxydus* isolate S3 (S3)
- 3) Sterilized seeds (testing effect of sterile solution on seed viability) (ST)
- 4) Non-sterilized seeds (Control; CO)

The actinobacteria used were *Streptomyces thermocarboxydus* isolate S3 and *S. antibioticus* (Lasudee et al., 2018). The isolates of two species of actinobacteria were supplied by Dr. Wasu Pathom-Aree, Department of Biology, Chiang Mai University. The sterilized seeds were mixed with 10^8 CFU ml⁻¹ of *S. antibioticus* and *S. thermocarboxydus* isolate S3 solution in a shaker at 120 rpm for 2 hours. The inoculated seeds were dried under laminar air flow cabinet before testing them in the field and nursery (Figure 3.5). For sterilized seeds, we used

them for testing the effects of sterile solution on seed viability. Beside the coating treatments, cleaned seeds without any treatments or surface sterilization were sown in the same area to serve as a control.

After coating, for each treatment, the seeds were separated into two groups of 60 seeds each to be shown by hand directly into two degraded areas, same as the species selection section. To differentiate between treatments in each replicate, various colors of 1.2 m bamboo sticks were randomly placed 1 m apart from one another as the same method as 3.3.2. The number of seeds that are removed and the number of germinated seeds were recorded. The number of surviving seedlings was monitored approximately nine months after sowing, specifically after the first dry season.

To gather data on the effects of coating materials on seed germination and seedling survival in a controlled environment, the second group of coated seeds was also sown in a tree nursery, concurrently with the field experiments. For each treatment, three replicates of twenty seeds were sown in germination trays filled with sterile soils. The autoclave cycle for soil sterilization was 30 minutes at 121°C. The experimental area was exposed to 50% of normal sunlight. Seed germination was determined based on radicle emergence, following the same method used in the field.

Table 3.4 The species tested with pelleting materials.

No.	Scientific name	Family name	Seed mass (g)	Successional stage
1	<i>Hovenia dulcis</i> Thunb.	Rhamnaceae	0.03	Climax
2	<i>Acrocarpus fraxinifolius</i> Wight & Arn.	Leguminosae	0.03	Climax
3	<i>Alangium kurzii</i> Craib.	Alangiaceae	0.18	Climax
4	<i>Gmelina arborea</i> Roxb. ex Sm.	Verbenaceae	0.52	Pioneer
5	<i>Choerospondias axillaris</i> (Roxb.) B.L.Burt & A.W.Hill	Anacardiaceae	1.43	Pioneer

Identify of successional stage; Climax (late successional, shade-tolerant) & Pioneer (early successional, light loving) species (Waiboonya et al., 2019).

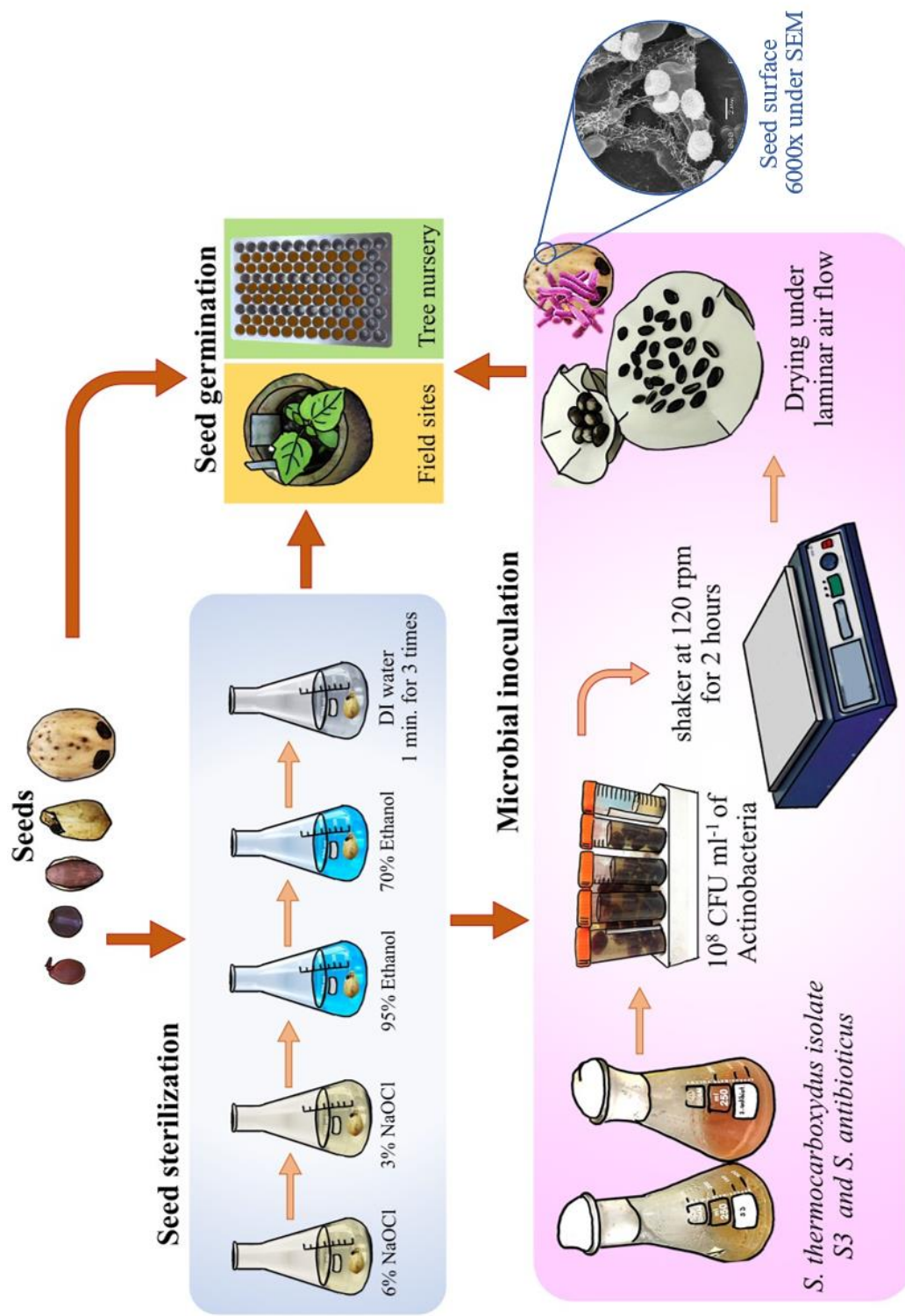


Figure 3.5 Methodology of microbial seed coating experiments (applied from Lasudee et al., 2018).

The number of surviving seedlings was recorded and represented in terms of seedling yield, which is the percentage of seedling survival per the total number of seeds sown. The size of the seedlings including root collar diameter (RCD), height, and crown width (CW) was measured at each time of fertilization to determine the performance of the direct-seeded seedlings.

3.3.4 Seed storage behaviors

Studied species

Seeds of 14 native tree species were tested (Table 3.5)—all of them native to Northern Thailand. In this study, two species had pyrenes: *C. axillaris* (around five seeds/pyrene) and *A. kurzii* (2 seed/pyrene).

At least 600 seeds from 3 - 5 maternal trees were collected, mixed, cleaned, and dried at room temperature. The fresh-dry mass and initial moisture content (MC) were measured for each species following ISTA rule (ISTA, 2006). Twenty seeds were randomly selected to record the fresh weight using the weight scale accurate to 1/10,000th of a gram. Then, seeds were then dried in hot air oven at 103° for 17 hours, after that their dry weights were measured. Seed moisture content was calculated using the equation described by Schmidt (2008).

$$\text{Moisture content (\%MC)} = \frac{\text{Wet weigh} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

The set of seeds was also used to record their morphological characteristics using digital vernier caliper accurate to 1/100th of a millimeter; seed length (the longest axis), width (the second axis, which is perpendicular to the length), and depth (the third axis, which is perpendicular to the length and width). In addition, the seed was cut to measure seed coat thickness, under a light microscope (Leica EZ4W) with LAS V4.9.

Table 3.5 The species list used for storage experiment and classification of diaspore follow Gardner et al. (2000) and FORRU (2005).

No.	Scientific name	Family name	Diaspore used	Collection date
1.	<i>Acrocarpus fraxinifolius</i> Wight ex Arn.	Leguminosae	Seed	6-Jun-20
2.	<i>Adenanthera microsperma</i> Teijm & Binn.	Leguminosae	Seed	23-Jul-20
3.	<i>Alangium kurzii</i> Craib	Alangiaceae	Pyrene	25-Jun-20
4.	<i>Artocarpus lacucha</i> Buch. -Ham.	Moraceae	Seed	8-Jun-20
5.	<i>Balakata baccata</i> (Roxb.) Esser	Euphorbiaceae	Seed	5-Jul-20
6.	<i>Cassia bakeriana</i> Craib	Leguminosae	Seed	6-Jun-20
7.	<i>Choerospondias axillaris</i> (Roxb.) B.L.Burt&A.W.Hill	Anacardiaceae	Pyrene	18-Jun-20
8.	<i>Diospyros glandulosa</i> Lace	Ebenaceae	Seed	2-Oct-20
9.	<i>Gmelina arborea</i> Roxb.	Verbenaceae	Pyrene	23-Apr-21
10.	<i>Michelia baillonii</i> (Pierre) Finet & Gagnep.	Magnoliaceae	Seed	29-Jul-20
11.	<i>Phyllanthus emblica</i> L.	Euphorbiaceae	Seed	23-Apr-21
12.	<i>Prunus cerasoides</i> D. Don	Rosaceae	Pyrene	29 Apr 21
13.	<i>Syzygium fruticosum</i> DC.	Myrtaceae	Seed	15-Jul-20
14.	<i>Sapindus rarak</i> DC.	Sapindaceae	pyrene	20-Feb-21

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Seed desiccation experiment

Seed storage experiment was set up at Department of Biology, Faculty of Sciences, Chiang Mai University. Both dry storage and moist storage treatments were applied to test desiccation tolerance (Hong and Ellis, 1996) (Figure 3.6). The number of treatments applied to each species varied according to initial seed moisture content. For dry storage, seeds were separated into batches of 45 - 100, depending on seed availability and initial moisture content. Batches were kept in a container with drying beads (Rhino Research Co.). The mass of the drying beads equaled total seed weight. Seed batches were tested for germinability at

moister levels of 40%, 20%, 10% and 5%. Species that remained viable at 5% moisture content were then stored at -20°C for a month.

Moist storage treatments were set up simultaneously, with the numbers of seeds per batch and the numbers of batches being the same as for the dry storage experiments. Seed batches were placed in plastic boxes with moist filter papers to maintain 100% humidity.

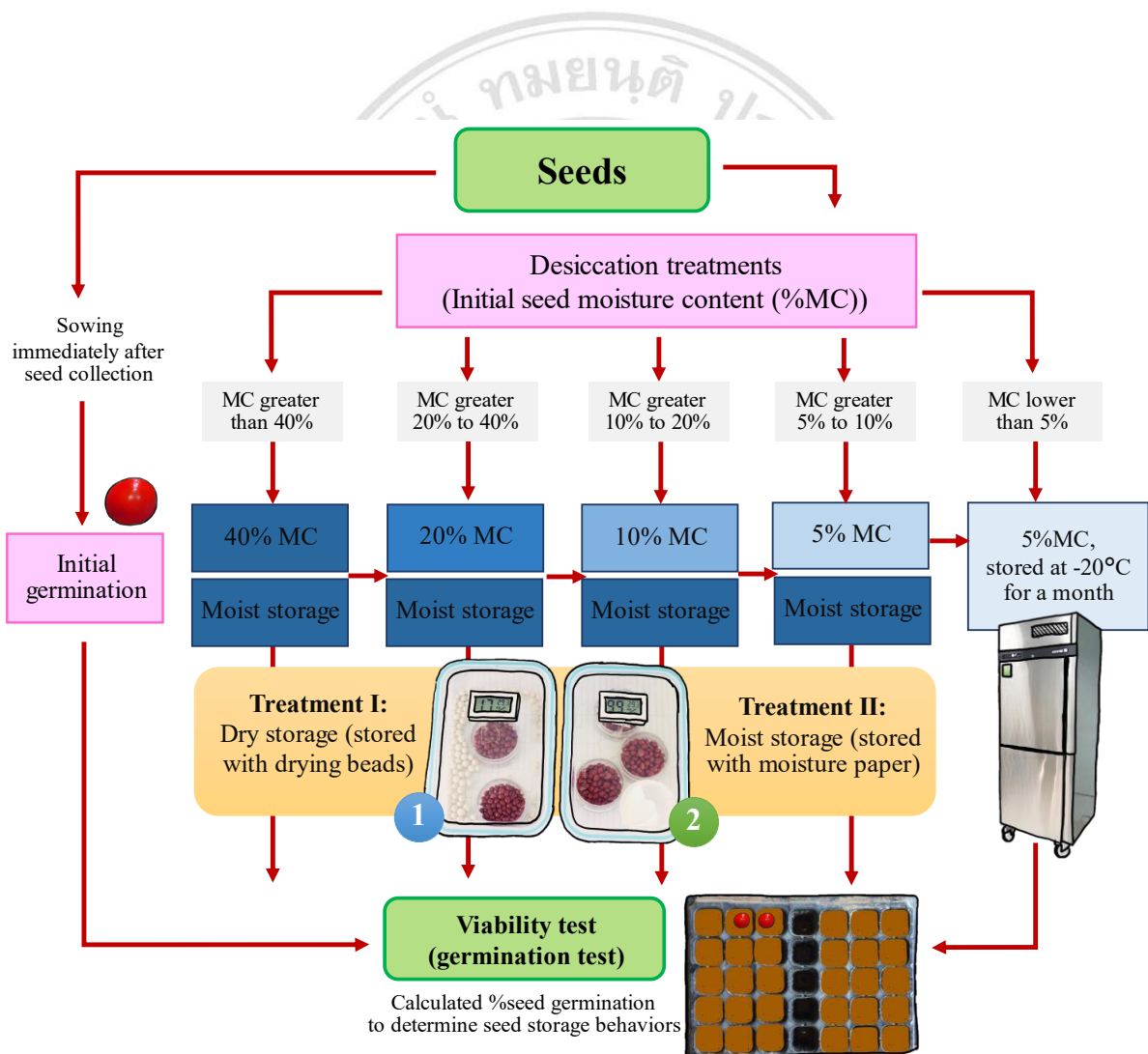


Figure 3.6 Diagram of a protocol to determine seed viability (% seed germination) responding to each desiccation/storage treatments.

Seed germination and viability test

Seeds were germinated in Doi Suthep Nature Study Center (DNSC) tree nursery (350 m altitude), to determine their viability (Figure 3.7). Thirty seeds were put in soil in a germination tray— one germination tray per replicate, three replicates per species per treatment. The number of seeds germinating was recorded every week, till no further seeds had germinated for at least three weeks. Non-germinated seeds were subjected to a cut test to determine their viability. Seeds of every species were also tested for initial germination of fresh seeds, immediately after collection. Initial germination was compared with that of seeds after storage treatments had been applied.



Figure 3.7 Seed germination experiment at DNSC tree nursery.

3.4 Data analysis

All statistical analyses were performed using R version 4.0.2 (R Core Team, 2020), applying significance level of $P < 0.05$.

3.4.1 Seed removal, germination, and survival

Seed removal, germination and seedling yield were calculated as a percentage of the total number of seeds sown. A Generalized Linear Model (GLM) with a logit link function was applied to determine the significance of treatment effects on seed removal, germination, and seedling survival (yield). The independent variables were species and sites. The dependent variable was the proportion of seed removal, germination, and survival. When significant effects were found, significant differences between means were determined by Tukey's multiple comparison test (at 95% confidence interval).

3.4.2 Seedling growth

Growth of seedling height, root-collar diameter (RCD) and crown width (CW) were monitored twice on 24th November 2019 and after dry season on 22nd May 2020 (over a total of 180 days). For each species, relative growth rates (RGRs) were determined for all seedlings, using differences in height (RGR-H), root collar diameter (RGR-RCD) and crown width (RGR-CW) between the two monitoring dates and formula below:

$$\text{RGR (\% per year)} = \frac{\ln(\text{final size}) - \ln(\text{initial size})}{\text{number of days between measurements}} \times 365 \times 100$$

Daily proportional growth relative to the average plant size over the measurement interval was multiplied by 365, to derive an annual value, and by 100 (to convert to a percent) (modified from Hoffmann and Poorter, 2002).

Analysis of variance (ANOVA) was used to test whether study site and/or species affected seedling performance variables: absolute values and RGR of height, crown width (CW), root collar diameter (RCD). Independent variables

were studied site and species. Dependent variables were height, CW and RCD and their %RGR per year. When ANOVA indicated presence of differences, Tukey's multiple was applied to determine which of the independent variable had significant effects (at 95% confidence level). When the assumptions of ANOVA were not met, Kruskal-Wallis nonparametric test was performed instead of ANOVA.

3.4.3 Relative performance index (RPI)

To determine whether species were suitable for direct seeding, a relative performance index (RPI) was devised which combined both seedling yield and growth into a single indicator. Seedling yield was the proportion of seeds that became established seedlings. Average RCD (mm) was used to represent seedling size (e.g., Naruangsri et al., 2023) as it is closely and positively correlated with seedling height, crown width and plant biomass (Tian et al., 2017).

A raw performance index was calculated by multiplying the relative seedling yield in combination with the relative size of the RCD. The score was transformed into a relative score (RPI), by expressing each raw score as a percent of the highest species score (Tunjai and Elliott, 2012). The RPI is unitless.

3.4.4 Effect of species traits on direct seeding

Pearson's correlation analysis was conducted to investigate the relationship between various species traits and field data, including seed removal, germination, survival and growth (Tunjai and Elliott, 2012). A generalized linear model (GLM) was constructed to identify the impact of these traits on each field-performance-related variable and to determine the most predictive traits. Independent variables were dry propagule mass, seed/propagule storage behavior and successional guild. The dependent variables were seed removal, germination, survival, and growth. In addition, ANOVA was utilized to detect the effects of these traits on seedling growth, including crown width (CW), height (H), root collar diameter (RCD), relative growth rate (RGR), and performance score. Post-hoc analyses, specifically Tukey's HSD test, were conducted to compare the

means of each parameter. Furthermore, the non-parametric Kruskal-Wallis test was applied to test the effects of seed size and successional guild the score of relative performance index (RPI).

3.4.5 Determining seed storage behavior.

Percent seed germination was calculated as the number of seedlings emerged, divided by total number of seeds sown x100. Differences in mean percent seed germination (averaged across three replicates) among storage treatments were evaluated using the Generalized Linear Model (Family Binomial) and separated to group using Turkey Contrasts. Seed storage behavior was classified as orthodox, recalcitrant, or intermediate following the criteria of Hong and Ellis (1996), Schmidt (2008) and FORRU database.

3.4.6 Relationship between seed storage behavior and the morphological traits

Seed-coat thickness correlated significantly with seed mass, seed depth and width ($P < 0.01$). Initial moisture content did not correlate well with other seed traits not. A Linear Discriminant Analysis (LDA) was performed to identify the relationships between species traits (see in Appendix II) and seed storage behavior. The independent variables were seed traits (seed coat thickness and moisture content); the dependent variable was seed storage behavior.

CHAPTER 4

RESULTS

4.1 Species selection

4.1.1 Seed removal

Percent seed removal varied among the 23 species and two sites. Zero removal was recorded for five species: *A. microsperma*, *Q. brandisiana*, *S. arboreum*, *S. pentandrum* and *S. pinnata*. For 18 species, mean percent removal ranged from 0.8% (± 1.2 SE) (for *A. fraxinifolius*, *C. axillaris*, *D. glandulosa* and *P. cathia*) up to a maximum of 9.2% (± 5.8 SE) for *C. bakeriana* (Figure 4.1). Percent removal, averaged across species, was $< 5\%$ at all studied sites. The highest removal was recorded at BMK (4.3%, ± 0.5 SE), followed by MC (2.5%, ± 0.4 SE) and the tree nursery (0.1%, ± 0.1 SE). The GLM indicated a significant effect of study site on percent seed removal, but no species effect (Coefficient estimate \pm SD = -7.0 ± 1.0 , $z = -7.0$, $P < 0.001$). The probability of seed removal at the three study sites was 0 to 0.04. Smaller seeds (e.g., *C. bakeriana* and *H. dulcis*) were more likely to be removed than larger ones (e.g., *S. pentandrum* and *S. pinnata*). Linear regression also indicated that percent seed removal significantly decreased with increasing seed mass (Coefficient estimate \pm SE = -0.6 ± 0.3 $t = -2.2$, $P = 0.04$) but the relationship was extremely weak (R-squared = 0.2).

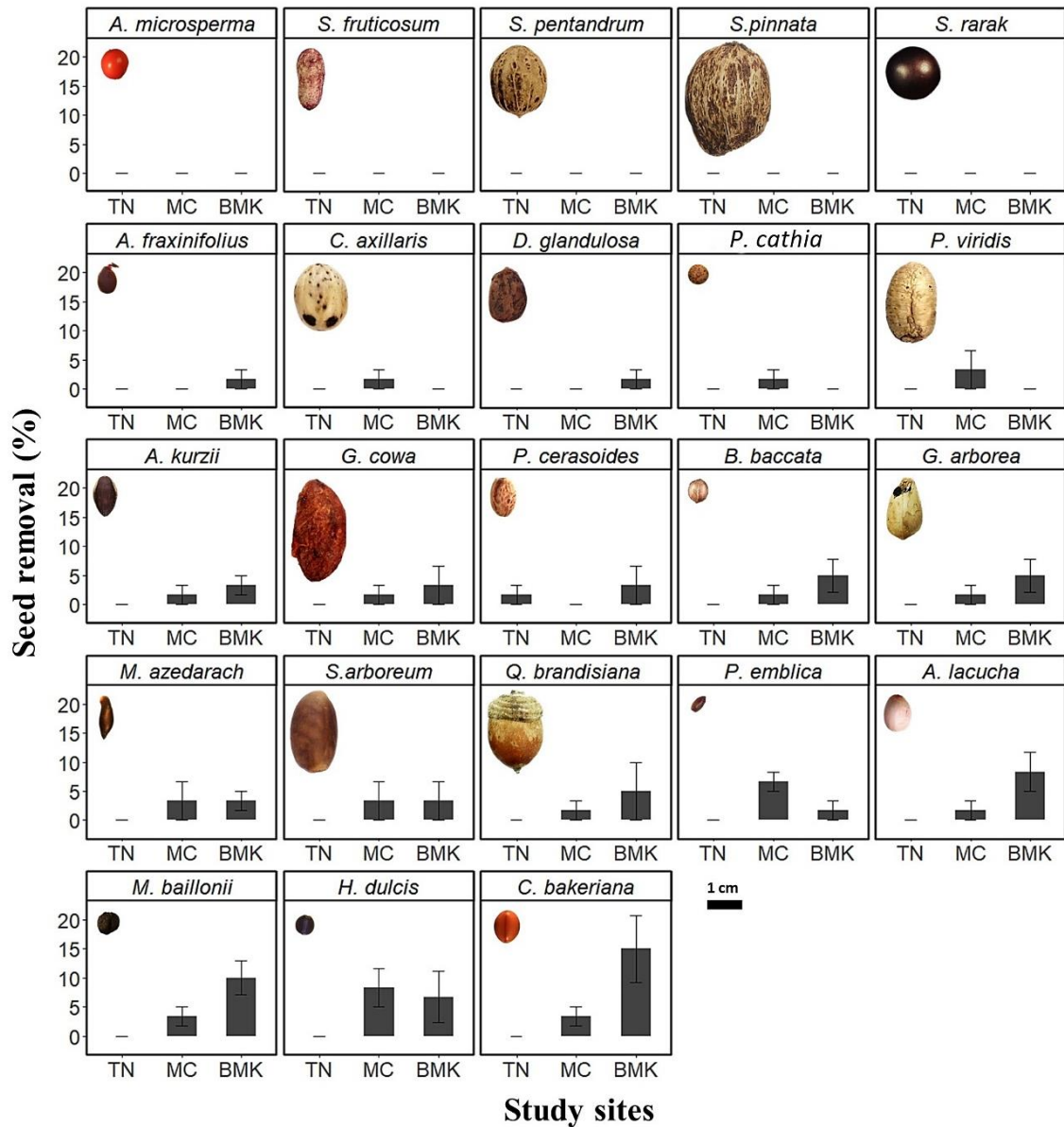


Figure 4.1 Seed removal ($\% \pm 1$ SE) compared among sites: tree nursery (TN), Ban Mae Khi (BMC) and Mon Cham (MC). Five species in the top row of the figure had no seed removal. Species panels are arranged in order to increase removal rates.

4.1.2 Seed germination

Eight species (35% of studied species) failed to germinate. Non-germinating species were excluded from further analyses. Species were categorized into three groups: high, medium, and low germination (Figure 4.2). Eight species had low percent seed germination ($< 20\%$ and a group average of 8.82% , ± 1.4 SE), ranging from 0.6% (± 0.6 SE) for *B. baccata* to 16.1% (± 8.7 SE) for *P. cathia*. Five species achieved moderate seed germination ($20 - 50\%$ germination, with a group average of 25.4% (± 1.7 SE), ranging from 21.7% (± 5.9 SE) for *P. emblica* to 30.6% (± 13.8 SE) for *C. axillaris*. Only two species attained germination percent higher than 50% : *A. kurzii* at 68.8% (± 7.5 SE) with *A. microsperma* being the highest 85% (± 3.5 SE) (group average: 76.4% , ± 7.6 SE).

The GLM showed a significant interaction effect between species and study site on seed germination (Coefficient estimate \pm SE = -3.4 ± 0.7 , $z = -4.6$, $P < 0.001$). Seed germination was different at different sites, indicating a site-specific effect. *C. axillaris*, *S. pinnata* and *P. emblica* achieved higher seed germination in the field sites, whereas seeds of *A. kurzii* and *H. dulcis* germinated better in the tree nursery (Figure 4.2).

Among all species studied, the GLM showed a significant effect of successional guild on seed germination (Coefficient estimate \pm SE = -5.0 ± 1.4 , $t = -3.6$, $P = 0.003$) and a significant interaction effect of successional guild and seed size (Coefficient estimate \pm SE = 17.0 ± 6.6 , $t = 2.6$, $P = 0.02$). Late successional species had higher germination probability. Furthermore, percent germination increased with increasing seed size. Moreover, the GLM also indicated a significant interaction effect of seed size and seed storage behavior on seed germination (Coefficient estimate \pm SE = -58.7 ± 27.1 , $t = -2.2$, $P = 0.049$). The effect of seed storage behavior on probability of seed germination was marginally significant (Coefficient estimate \pm SE = 10.2 ± 4.7 , $t = 2.2$, $P = 0.05$). Germination failure (zero probability of germination) was more likely for recalcitrant seeds than for orthodox ones. The probability of seed germination decreased with decreasing seed size.

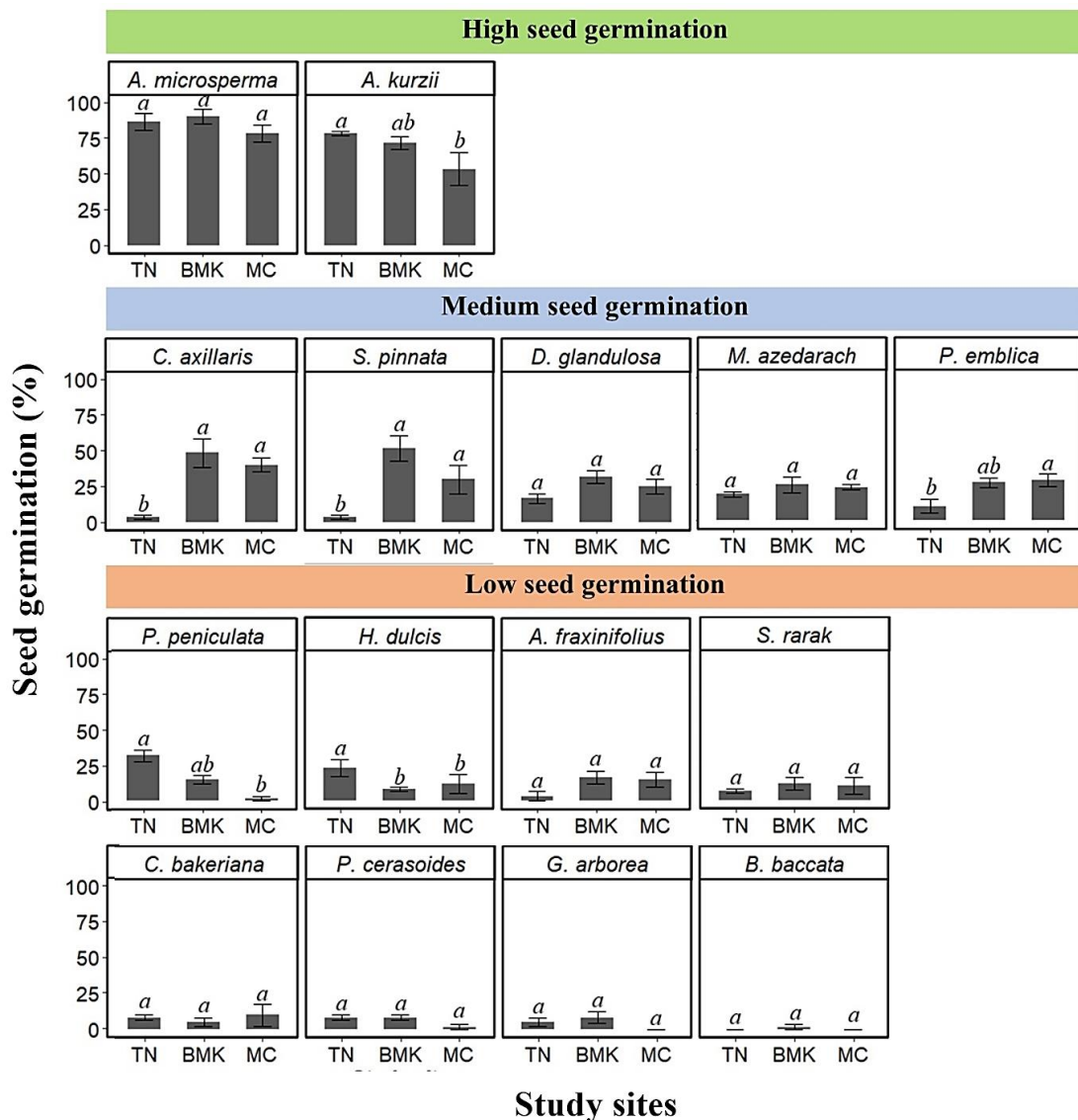


Figure 4.2 Percent seed germination (± 1 SE) across study sites. Eight species which failed to germinate are not included. Columns not sharing the same superscript indicate significant differences among sites.

4.1.3 Seedling yield

Overall seedling yields across surviving species were similar between the two field sites: averaging 21.2% (± 14.0 SE) at MC and 20.1% (± 20.3 SE) at BMK. The GLM indicated no significant effect of study site on seedling yield

(Coefficient estimate \pm SE = -0.0 ± 0.1 , $Z = -0.1$, $P = 0.95$). However, the effect of species on seedling yield was statistically significant ($P < 0.05$).

Of the 15 species that germinated, two (*B. baccata* and *M. bombycine*), failed to establish any seedlings. Differences in seedling yield among the other 13 species were statistically significant ($P < 0.05$), such that the species could be divided into three groups. A single species stood out as having by far the highest seedling yield: *A. microsperma* (66.7%, ± 8.3 SE). Four species had moderate seedling yields (with a group average of 25.2% (± 3.5 SE) ranging from 17.5% (± 0.8 SE) for *P. emblica* to 33.3% (± 8.3 SE) for *S. pinnata*. Nine had poor seedling yields below 15% (Table 4.1), ranging from 5% (± 0 SE) for *G. arborea* to 15% (± 0 SE) for *D. glandulosa* (with a group average of 10.3% (± 1.3 SE)) (Table 4.1).

Based on the GLM, three species traits - seed storage behavior, successional guild, and seed size - significantly influenced seedling yield, without any interaction effects ($P < 0.05$). The seedling yields significantly increased with increasing seed size (Coefficient estimate \pm SE = 0.4 ± 0.2 , $t = 2.4$, $P = 0.03$). The seedling yield was 35% for large seeds and 6% for small seeds. Furthermore, pioneer species had a lower seedling yield, compared with climax species (Coefficient estimate \pm SE = 1.9 ± 0.6 , $t = -3.4$, $P = 0.004$). Orthodox species had significantly higher seedling yield (17%) than recalcitrant species did (zero seedling yield) (Coefficient estimate \pm SE = 1.8 ± 0.8 , $t = 2.2$, $P = 0.04$).

4.1.4 Seedling growth

Seedling growth varied greatly among the 13 surviving tree species, nine months after seed sowing. ANOVA indicated significant differences in mean seedling height, CW and RCD among species. *H. dulcis* (N = 2) grew tallest; *M. azedarach* (N = 11) had the broadest canopy. *S. rarak* (N = 8) achieved the highest mean RCD (Table 4.1).

RGRs of height, CW and RCD exceeded 50% per year for most species. The fast-growing species were *C. axillaris* and *H. dulcis*, with RGRs of RCD,

height and *CW* exceeding 100% per year (Table 3.2). Furthermore, two other species: *A. fraxinifolius* and *M. azedarach*, also achieved fast growth with RGR-CWs and RGR-Hs exceeding 100% per year. In contrast, *S. rarak* and *S. pinnata* were slow-growing, despite having large seedlings at nine months (Table 4.1).

4.1.5 Relative performance indices

RPIs ranged from seven to 100. There were no significant effects of seed size and successional guild on relative performance index. Two of the 13 species that were established had RPIs exceeding 50, performing more than half of the best species score. *A. microsperma* achieved the highest raw performance score and was assigned as the 100 benchmarks. In comparison *S. pinata* achieved an RPI of 64 whilst *C. axillaris* scored of 46 (Figure 4.3). Relatively low performing species included *P. cerasoides*, *C. bakeriana*, *G. arborea* and *A. fraxinifolius* with RPIs lower than 20.

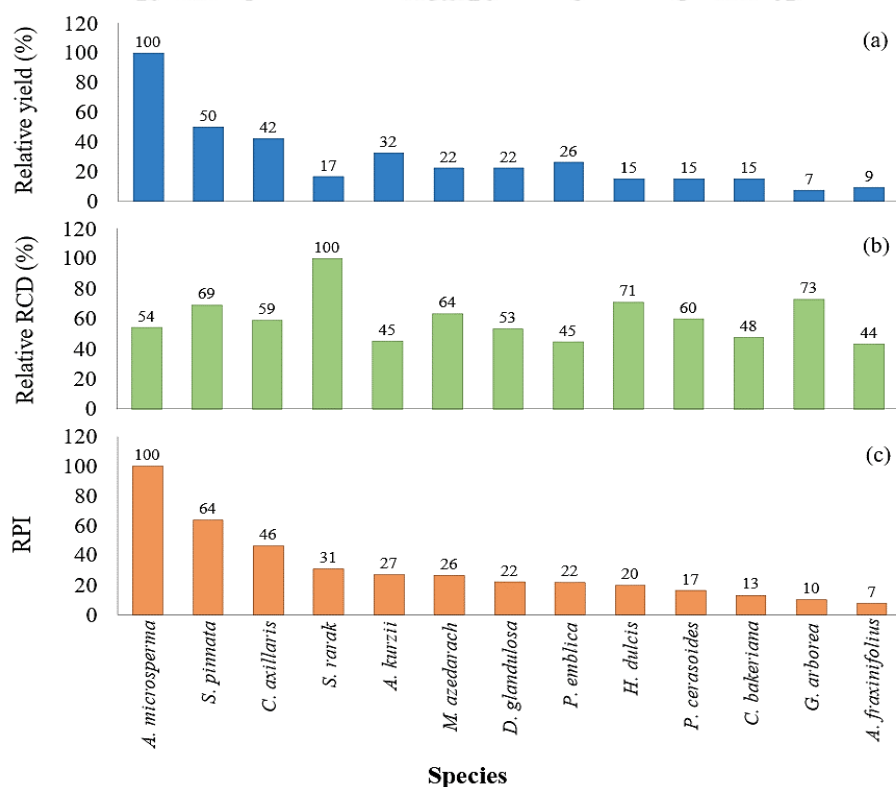


Figure 4.3 Comparison of relative rank score of seedling yield (a), relative seedling root collar diameter (RCD) (b) and relative percent performance index (RPI) (c) across studied species, ranking from the highest to lowest RPI.

Table 4.1 Seedling yield (%) seedling size and corresponding relative growth rate (RGR % per year). N indicated the number of surviving trees that are ordered from highest to lowest seedling yield. Values not sharing the same superscripts within columns are significantly different among species.

Species	N	Yield		Root collar diameter			Height			Crown width					
		Mean (%)	± SE	Mean (mm)	± SE	RGR (% per year)	Mean (cm)	± SE	RGR (% per year)	Mean (cm)	± SE	RGR (% per year)	± SE		
<i>A. microsperma</i>	72	66.7	± 8.3 ^a	2.2	± 0.0 ^b	5.07	± 2.0 ^{bc}	12.8	± 0.3 ^{cd}	83.6	± 1.8 ^{cd}	13.5	± 0.5 ^{cd}	74.0	± 3.6 ^{bc}
<i>S. pinnata</i>	32	33.3	± 8.3 ^b	2.8	± 0.0 ^b	25.2	± 6.2 ^c	11.2	± 0.4 ^d	50.9	± 9.7 ^{de}	9.6	± 0.8 ^d	44.2	± 6.3 ^c
<i>C. axillaris</i>	23	28.3	± 1.7 ^b	2.4	± 0.1 ^b	136.1	± 18.8 ^{ab}	22.4	± 1.6 ^a	134.0	± 12.3 ^b	19.0	± 0.9 ^{ab}	124.1	± 5.5 ^{ab}
<i>A. kurzii</i>	20	21.7	± 1.7 ^c	1.8	± 0.1 ^b	70.6	± 13.6 ^{bc}	9.6	± 0.7 ^d	113.5	± 4.0 ^{bc}	8.0	± 0.4 ^d	75.2	± 20.4 ^{bc}
<i>P. emblica</i>	17	17.5	± 0.8 ^{cd}	1.8	± 0.1 ^b	64.1	± 7.4 ^{bc}	18.4	± 1.6 ^{abc}	74.8	± 12.2 ^{cde}	15.4	± 1.4 ^{bcd}	75.3	± 12.1 ^{bc}
<i>M. azedarach</i>	11	15.0	± 5.0 ^{de}	2.6	± 0.3 ^b	47.4	± 7.3 ^{bc}	20.4	± 2.6 ^{ab}	101.7	± 15.9 ^{bcd}	22.6	± 3.7 ^a	146.4	± 20.4 ^a
<i>D. glandulosa</i>	8	15.0	± 0.0 ^{ef}	2.2	± 0.1 ^b	70	± 6.4 ^{bc}	11.6	± 0.4 ^d	84.6	± 11.2 ^{cd}	11.2	± 1.1 ^{cd}	33.0	± 12.1 ^c
<i>S. rarak</i>	8	11.2	± 3.8 ^{ef}	4.0	± 0.4 ^a	47.1	± 20.7 ^{bc}	17.0	± 1.3 ^{abcd}	65.8	± 23.5 ^{cde}	17.0	± 2.0 ^{abcd}	26.9	± 6.6 ^c
<i>H. dulcis</i>	2	10.0	± 0.0 ^{ef}	2.9	± 0.5 ^{ab}	160.8	± 66.9 ^a	26.2	± 9.4 ^a	236.2	± 42.4 ^a	11.6	± 4.6 ^{cd}	136.7	± 68.4 ^{ab}
<i>P. cerasoides</i>	2	10.0	± 0.0 ^{fg}	2.4	± 0.3 ^b	52.6	± 37.6 ^{bc}	25.5	± 1.5 ^a	66.0	± 7.6 ^{cde}	17.5	± 0.1 ^{abc}	25.5	± 13.9 ^c
<i>C. bakeriana</i>	8	10.0	± 2.5 ^{fg}	1.9	± 0.1 ^b	15.3	± 5.2 ^c	8.2	± 1.1 ^d	20.4	± 7.7 ^e	7.3	± 1.0 ^d	50.3	± 29.2 ^{bc}
<i>A. fraxinifolius</i>	4	6.2	± 1.2 ^g	1.8	± 0.4 ^b	157.4	± 13.1 ^a	10.1	± 2.0 ^d	107.6	± 14.7 ^{bcd}	8.9	± 2.6 ^d	79.8	± 2.0 ^{abc}
<i>G. arborea</i>	1	5.0 ^g		2.9 ^{ab}		139.2 ^{ab}		14.0 ^{bcd}		58.3 ^{cde}		12.0 ^{cd}		37.0 ^c	

4.2 Seed ball

4.2.1 Seed removal

Evidence of seed predation (such as bitten seeds, empty seed husks or shells, damaged seeds inside or around bamboo tubes, and the presence of ants or termites colonizing at sowing point) was observed, with an average of 3% of seed removal overall.

Removal varied across the five species, ranging from 6.4% (± 1.0 SE) for *H. dulcis* up to 9.2% (± 3.0 SE) for *S. arborea*. Across the study sites, seed removal was higher at Ban Mae Khi (8.3%, ± 1.2 SE), compared to Mon Cham (7.1%, ± 0.6 SE) (Figure 4.4). However, the GLM indicated no significant effects of species and study site on percentage of seed removal ($P < 0.05$).

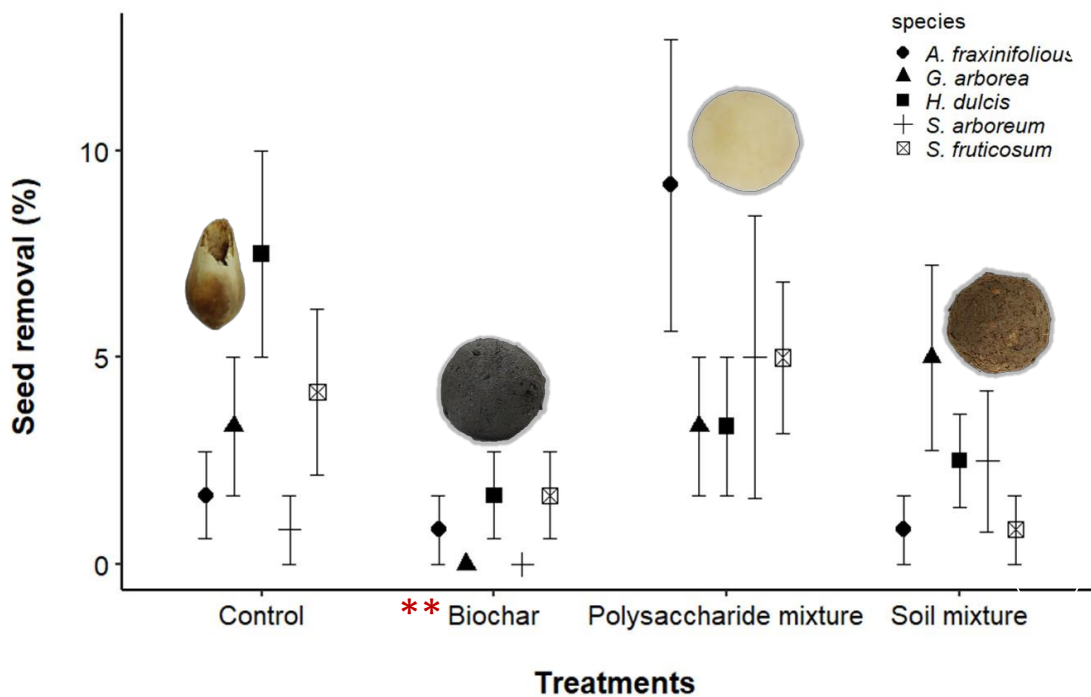


Figure 4.4 The percentage of seed removal (± 1 SE), that average across two field sites for each tested species. The different shapes represent individual species. Additionally, ** indicates the significantly lowest seed removal of the biochar pelleting treatments compared to control (Multiple Comparisons of Means: Tukey Contrasts at $P < 0.01$).

The lowest seed removal was achieved with biochar (3%, ± 1.2 SE) and soil mixture (6.5%, ± 1.5 SE) compared to the control (6.8% ± 1.5 SE), whereas the polysaccharide mixture (10%, ± 2.4 SE) increased seed removal. The GLM indicated that biochar was the best pelleting material. It significantly reduced percent seed removal, compared to other treatments (Coefficient estimate \pm SE = -1.5 ± 0.5 , $z = -2.9$, $P = 0.003$), with 0.06 % probability of seed removal. The highest probability of seed removal was 0.3 for polysaccharide mixture and 0.2 for soil mixture and control treatments.

4.2.2 Seed germination

For seed germination, *S. arborea* and *S. fruticosum* failed to germinate in the field, so those two species were excluded from the data analysis. The GLM indicated that the interaction effect of species and study sites on seed germination was significant (Coefficient estimate \pm SE = -3.3 ± 0.5 , $t = -6.1$, $P < 0.01$), suggesting that the response of seed germination of species varied among different sites. The probability of germination of *H. dulcis* was significantly lower in the two field sites (0.06) compared to the tree nursery (0.10). On the other hand, *A. fraxinifolius* showed the highest seed germination rate at the Ban Mae Khi field site (0.14 probability of germination), better germination compared to the tree nursery (0.02 probability of germination).

The average percent germination was highest in the control group (6.1%, ± 0.4 SE), followed by the soil mixture group (3.2%, ± 1.4 SE), the biochar group (3%, ± 0.7 SE), and the polysaccharide mixture group (2.6%, ± 0.3 SE), respectively (Figure 4.5). The GLM showed significant differences in the percent seed germination among treatments ($P < 0.05$).

Every pelleting material reduced germination. Lowest germination was obtained with the polysaccharide mixture (Coefficient estimate \pm SE = -1.0 ± 0.3 , $t = -3.2$, $P < 0.001$, probability of germination = 0.38), followed by biochar (Coefficient estimate \pm SE = -0.8 ± 0.3 , $t = -2.8$, $P < 0.001$, probability of germination = 0.45) and soil mixture (Coefficient estimate \pm SE = -0.7 ± 0.3 , $t = -2.6$, $P < 0.001$, probability of germination = 0.48). Covering the seed with

protective material decreased the probability of seed germination by almost 58% from the control.

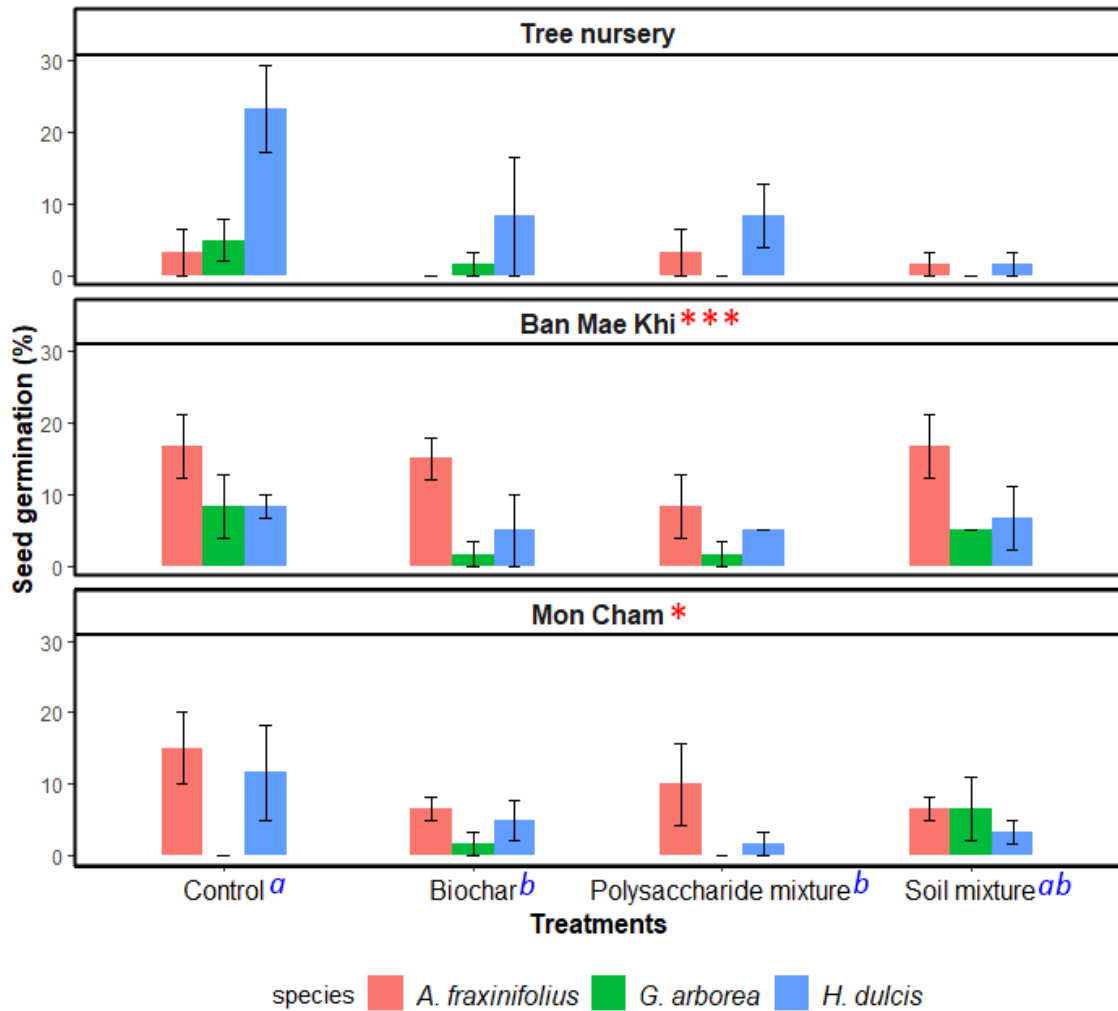


Figure 4.5 Percentage of seed germination (± 1 SE), two species failed to establish in the area, so they were not included in the data analysis. The germination percentage significantly differs among pelleting treatments, seed species and study sites. Ban Mae Khi and Mon Cham had significantly lower percent seed germination compared to control condition in tree nursery at significant level 0.001 (***) and 0.05 (*), respectively. The treatments not sharing the same letter indicated significant difference ($P < 0.05$).

4.2.3 Seedling yield

The pelleting materials used in this study did not enhance seedling establishment in the degraded areas. Seedling yield was highest in control (7%, \pm 1.2 SE) and soil mixture (7%, \pm 2.0 SE), followed by polysaccharide mixture (6.2%, \pm 1.2 SE) and biochar (6%, \pm 1.0 SE). The GLM showed no statistically significant differences among the treatments ($P > 0.05$).

The GLM indicated that differences in percent seedling yields among species (Coefficient estimate \pm SE = -3.3 ± 0.2 , $z = -12.9$, $P < 0.001$) were significant. The predicted probability of seedling yield varied among the different species tested, with *A. fraxinifolius* having the highest predicted probability of seedling yield at 3.6%, followed by *G. arborea* with 2%, and *H. dulcis* with 1.2%.

4.2.4 Seedling growth and performance score

For seedling growth, the pelleting material did not significantly increase seedling growth in terms of height ($F_{(3)} = 2.2$, $P = 0.1$), CW ($F_{(3)} = 0.6$, $P = 0.6$), and RCD (nonparametric test $\chi^2(2) = 4.6$, $P = 0.004$).

Among the tested species, height and CW did not differ. However, *G. arborea* attained the highest RCD (significantly) compared with the other species. However, RGR of *G. arborea* seedlings was the slowest compared to other species. *H. dulcis* attained the highest RGR (based on height, CW, and RCD) exceeding 100% per year (Table 4.2).

All the species tested had poor performance in terms of survival and seedling size. The species performance score varied among species, ranging from 37.5% for *H. dulcis*, 75% for *A. fraxinifolius* and 100% for *G. arborea* (Table 4.2).

Table 4.2 Seedling yield, growth and corresponding relative growth rate (RGR % per year). Values not sharing the same superscripts within columns are significantly different among species.

Species parameters	<i>A. fraxinifolius</i>	<i>G. arborea</i>	<i>H. dulcis</i>
	Mean ± SE	Mean ± SE	Mean ± SE
Number of trees	10	4	3
Seedling yield (%)	3.6 ± 0.4 ^a	2.9 ± 2.1 ^b	1.2 ± 0.7 ^b
Height (cm)	9.2 ± 1.1 ^a	11.2 ± 1.9 ^a	18.8 ± 10.6 ^a
RGR-H (% per year)	106.8 ± 14.0 ^a	17.0 ± 23.1 ^a	236.2 ± 42.4 ^a
CW (cm)	9.6 ± 1.5 ^a	9.2 ± 2.4 ^a	8.7 ± 4.1 ^a
RGR-CW (% per year)	86.0 ± 24.8 ^a	50.2 ± 33.4 ^a	136.7 ± 68.4 ^a
RCD (mm)	1.6 ± 0.2 ^b	2.9 ± 0.1 ^a	2.3 ± 0.6 ^{ab}
RGR-RCD (% per year)	117.3 ± 25.2 ^a	99.9 ± 20.4 ^a	160.8 ± 66.9 ^a
Relative Performance Index	75	100	37.5

4.3 Microbial seed coating

4.3.1 Seed removal

During a study period of over nine months, microbial seed coating did not affect the probability of seed removal. However, differences among study sites were significant (Coefficient estimate \pm SE = -1.4 ± 0.3 , $z = -4.6$, $P < 0.001$). The Mon Cham site had a significantly lower probability of seed removal (0.01) than the Ban Mae Khi site (0.04). Furthermore, the effect of species on seed removal was significant ($P < 0.05$). *H. dulcis* had the highest seed removal rate of $9.5 (\pm 1.3$ SE), while *C. axillaris* had the lowest seed removal rate of $1.7 (\pm 0.7$ SE) (Figure 4.6).

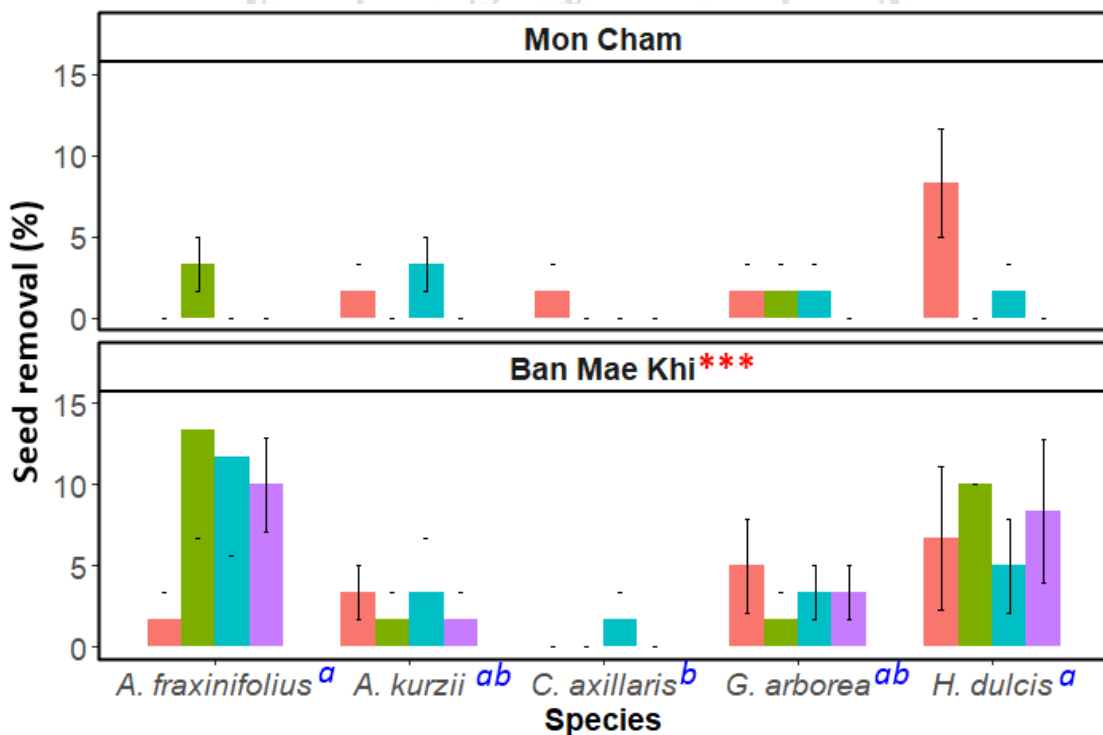


Figure 4.6 The percentage of seed removal (\pm 1 SE) in different study sites, species, and treatments. Each bar represents various treatments; control (red), coating with *S. antibioticus* (green), coating with *S. thermocarboxyodus* isolate S3 (cyan) and sterile seed (purple). *** represents significantly different among study sites at $P < 0.001$, species that do not share the same superscripts within the x-axis are significantly different.

4.3.2 Seed germination

Seed germination was influenced by specific species, treatments, and study sites. The GLM indicated significant interaction effects between various treatments, species, and study sites ($P < 0.05$). The sterilized seed treatment (ST) significantly decreased percent seed germination compared to control (CO). ST reduced seed germination particularly of *C. axillaris* (Coefficient estimate \pm SE = -3.8 ± 1.6 , $t = -2.3$, $P = 0.02$). Furthermore, *C. axillaris* completely failed to germinate after inoculated by *S. antibioticus* (SA) treatment and *S. thermocarboxydus* isolate S3 (S3) treatment. The SA and S3 treatments significantly reduced germination, compared with the control (Coefficient estimate \pm SE = -4.1 ± 1.2 , $t = -3.4$, $P < 0.001$), resulting in a zero probability of seed germination for both field sites (Figure 4.7). When no treatment applied, percent seed germination was the highest, particularly noticeable in the cases of *A. kurzii*, *H. dulcis*, and *C. axillaris*.

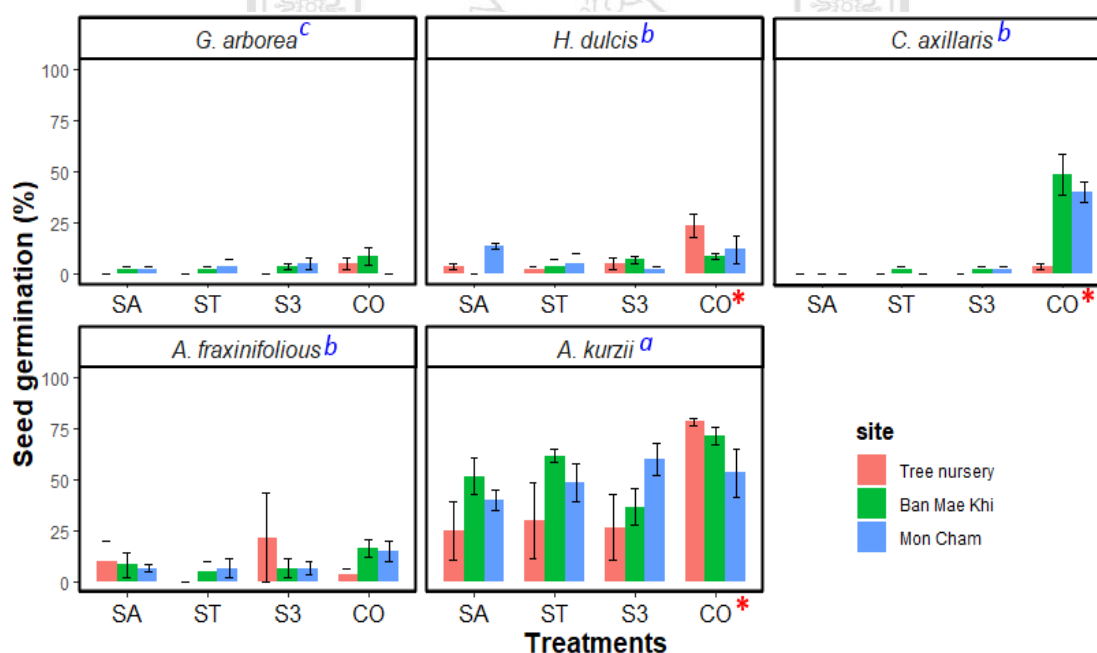


Figure 4.7 Percent seed germination (± 1 SE) of five seed species in three study sites (represents by different colors), with various microbial coating treatments; SA (coating with *S. antibioticus*), S3 (coating with *S. thermocarboxydus* isolate S3), ST (sterile without microbial coating), and CO (control). Species that do not share the same are significantly different. * indicates the significantly highest seed germination compared among treatments ($P < 0.05$).

Among the studied species, *A. kurzii* had the significantly highest germinability (Coefficient estimate \pm SE = 2.6 ± 0.5 , $t = 5.1$, $P < 0.001$, over all probability of germination = 48%). However, for *A. kurzii*, the S3 treatment significantly decreased the percentage of seed germination (Coefficient estimate \pm SE = -1.2 ± 0.6 , $t = -2.0$, $P < 0.001$), half of the control. Neither of the microbial seed coating treatments enhanced seed germination compared to control.

4.3.3 Seedling yield

At the end of the study, seedling yield was lower for microbial seed coating treatments (Figure 4.8). The highest seedling yield was 17.5% (\pm 2.7 SE) for control, followed by S3 (16.7%, \pm 5.5 SE), SA (14.4%, \pm 4.4 SE) and ST (11.9%, \pm 3.1 SE).

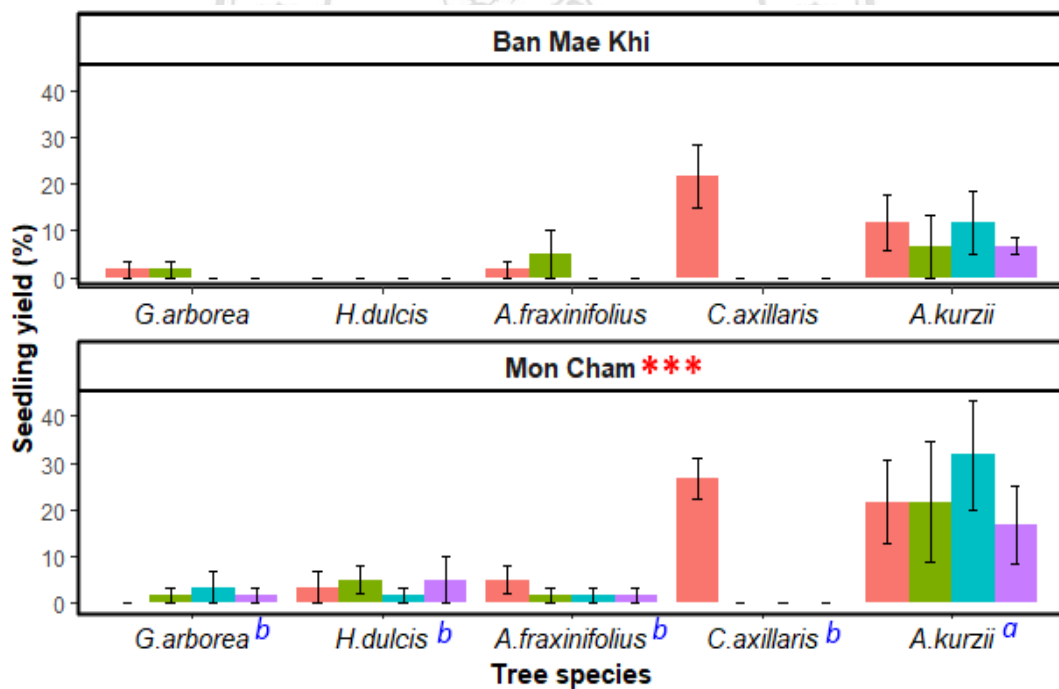


Figure 4.8 The percentage of seedling yield (\pm 1 SE) occurred in different study sites, species, and treatments. Each bar represents various treatments; control (■), coating with *S. antibioticus* (■), coating with *S. thermocarboxydus* isolate S3 (■) and sterile seed (■). Species that do not share the same superscripts are significantly different, *** indicated significantly higher seedling yield at Mon Cham sites ($P < 0.001$).

The GLM indicated that all the microbial coating treatments especially the ST treatment, significantly reduced percent seedling yield (Coefficient estimate \pm SE = -1.2 ± 0.4 , $t = -3.0$, $P = 0.004$). The probability of seedling yield in this treatment was 0.03 which was three times lower than the control.

Apart from the treatments, differences in seedling yield among the five species were significant ($P < 0.05$). The seedling yield of *A. kurzii* (16% probability) was significantly greater (Coefficient estimate \pm SE = 2.3 ± 0.6 , $t = 4.4$, $P < 0.001$) than that of other species. Moreover, the effects of the study sites on seedling yield were significant ($P < 0.05$). Mon Cham (28.1%, ± 7.8 SE) had significantly higher seedling yield than Ban Mae Khi (22.8%, ± 6.6 SE) (Coefficient estimate \pm SE = 0.9 ± 0.3 , $t = 3.0$, $P = 0.003$).

4.3.4 Seedling growth

The microbial seed coating treatments did not significantly improve the growth of seedlings in terms of height (H), crown width (CW), and root collar diameter (RCD) compared to the control. The ANOVA revealed a significant effect of species on seedling height ($F_{(4, 28)} = 49.6$, $P < 0.001$) and CW ($F_{(4, 28)} = 15.0$, $P < 0.001$). The species that performed best in terms of seedling growth was *C. axillaris*, which produced the tallest seedlings with the broadest CW and largest RCD by the end of the study period. In addition, *C. axillaris* achieved the highest relative growth rate of RCD (RGR-RCD), which was greater than 100% per year (Table 4.3). *H. dulcis* also performed well, with the highest relative growth rates of height (RGR-H) and crown cover (RGR-CW). On the contrary, *A. fraxinifolius* seedlings were the smallest size of height, CW and RCD compared to other species. Despite the seedling size being different among species, there was no significant difference in growth rate among the species and among treatments.

Table 4.3 Mean size and growth of surviving trees (N: number of surviving trees) and relative performance score (RPI) at 9 months after direct seeding - root collar diameter (RCD), height and crown width (CW)- and relative growth rates (RGR: percent per year) of corresponding growth variable. Species are ordered from highest to lowest RPI. Values not sharing the same superscripts within columns are significantly different among species.

Species	N	Height		CW		RCD		RPI
		Mean (cm)	SE (% per year)	Mean (cm)	SE (% per year)	Mean (mm)	SE (% per year)	
<i>A. kurzii</i>	74	9.4 ± 0.6 ^b	126.6 ± 9.5 ^a	8.6 ± 0.6 ^b	81.6 ± 13.1 ^a	2.1 ± 0.1 ^a	92.4 ± 12.1 ^a	100
<i>C. axillaris</i>	23	23.0 ± 2.6 ^a	133.8 ± 19.0 ^a	19.8 ± 1.9 ^a	113.9 ± 20.17 ^a	2.5 ± 0.2 ^a	139.0 ± 22.0 ^a	45.1
<i>H. dulcis</i>	9	14.8 ± 3.8 ^b	216.1 ± 20.7 ^a	7.9 ± 1.8 ^b	157.4 ± 29.9 ^a	1.8 ± 0.3 ^a	130.7 ± 28.1 ^a	20.6
<i>G. arborea</i>	5	8.5 ± 2.0 ^b	131.7 ± 73.4 ^a	8.1 ± 1.8 ^b	84.4 ± 47.5 ^a	1.9 ± 0.4 ^a	130.9 ± 8.3 ^a	11.8
<i>A. fraxinifolius</i>	9	8.3 ± 1.0 ^b	105.7 ± 11.8 ^a	7.8 ± 1.2 ^b	94.3 ± 15.6 ^a	1.4 ± 0.2 ^a	122.1 ± 41.6 ^a	9.8

4.4 Seed storage behavior

4.4.1 Desiccation treatments

Acrocarpus fraxinifolius

Highest germination of *A. fraxinifolius* seeds was achieved at 5% moisture content and storage at -20°C —significantly higher than initial germination (Coefficient estimate \pm SE = 1.6 ± 0.4 , $Z = 4.0$, $P < 0.001$; Figure 4.9). Desiccation treatment resulted in higher seed germination, compared to control (Table 4.4), meaning that desiccation and freezing had no effect on seed viability. Therefore, the species was classified as orthodox.

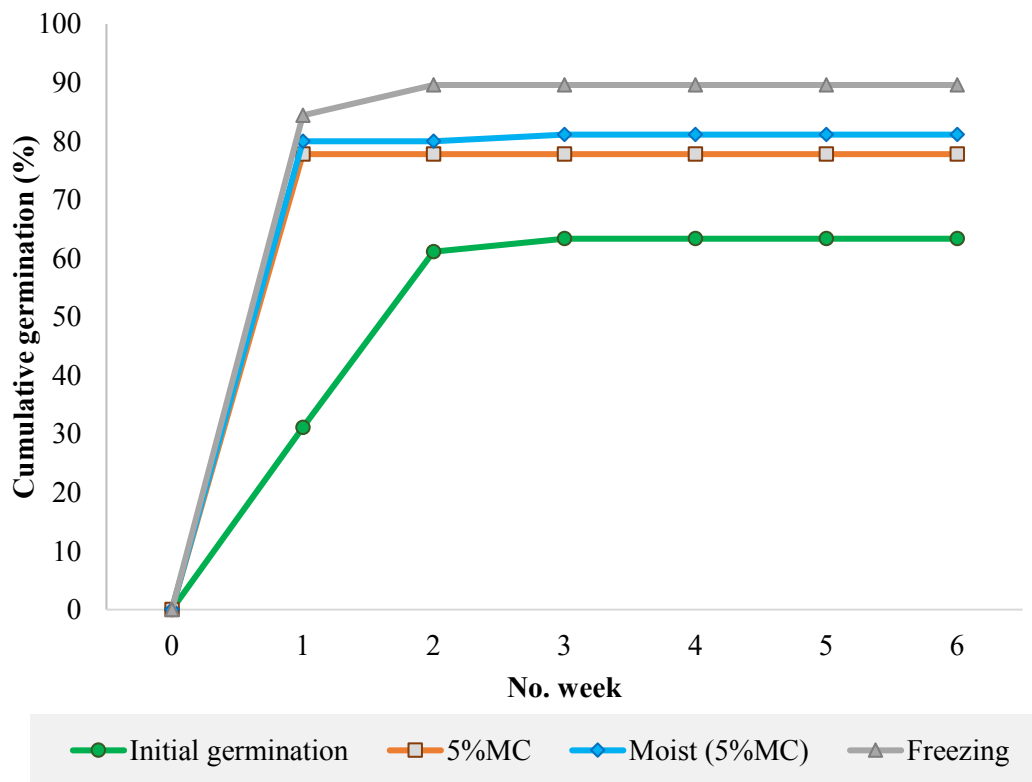


Figure 4.9 Average cumulative percent seed germination ($R = 3$) of *A. fraxinifolius* at 5% MC in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.

Adenanthera microsperma

Highest germination of *A. microsperma* seeds was achieved at 5% MC and storage at -20°C —the only treatment that significantly increased germination above initial germination (Coefficient estimate \pm SE = 1.3 ± 0.5 , $Z = 2.9$, $P = 0.004$; Figure 4.10). For other storage treatments, germinability did not differ from initial seed germination, meaning that there was no desiccation and storage condition effect on seed viability. The species was desiccation tolerant and able to maintain seed viability under dry and cool conditions. Therefore, this species was classified as orthodox.

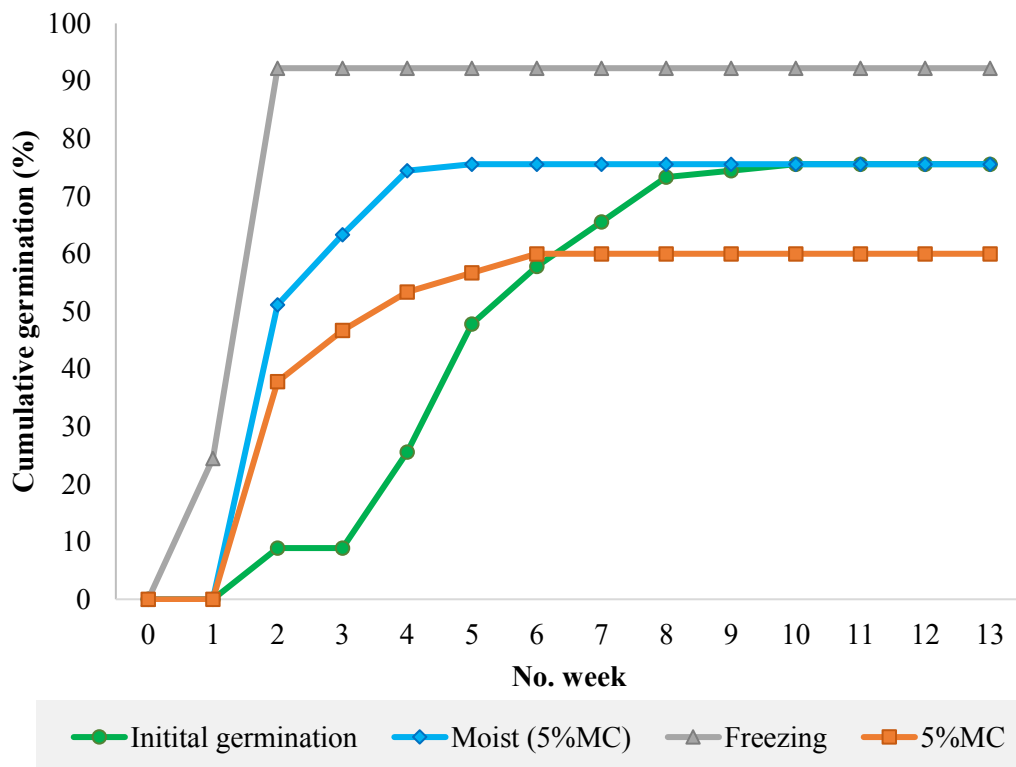


Figure 4.10 Average cumulative percent seed germination ($R = 3$) of *A. microsperma* at dry and moist storage treatments; compared to initial germination.

Alangium kurzii

Highest mean germination of *A. kurzii* seeds was achieved after wet storage but it was not significant different compared to initial germination (Coefficient estimate \pm SE = 0.5 ± 0.4 , $Z = 1.3$, $P = 0.2$). Reducing seed water content to 5% did not affect percent seed germination. Freezing substantially and significantly reduced germination (Coefficient estimate \pm SE = -3.4 ± 0.4 , $Z = -8.3$, $P < 0.001$; Figure 4.11). Therefore, the species was able to be dried but was sensitive to low-temperature storage. This shows intermediate seed storage behavior.

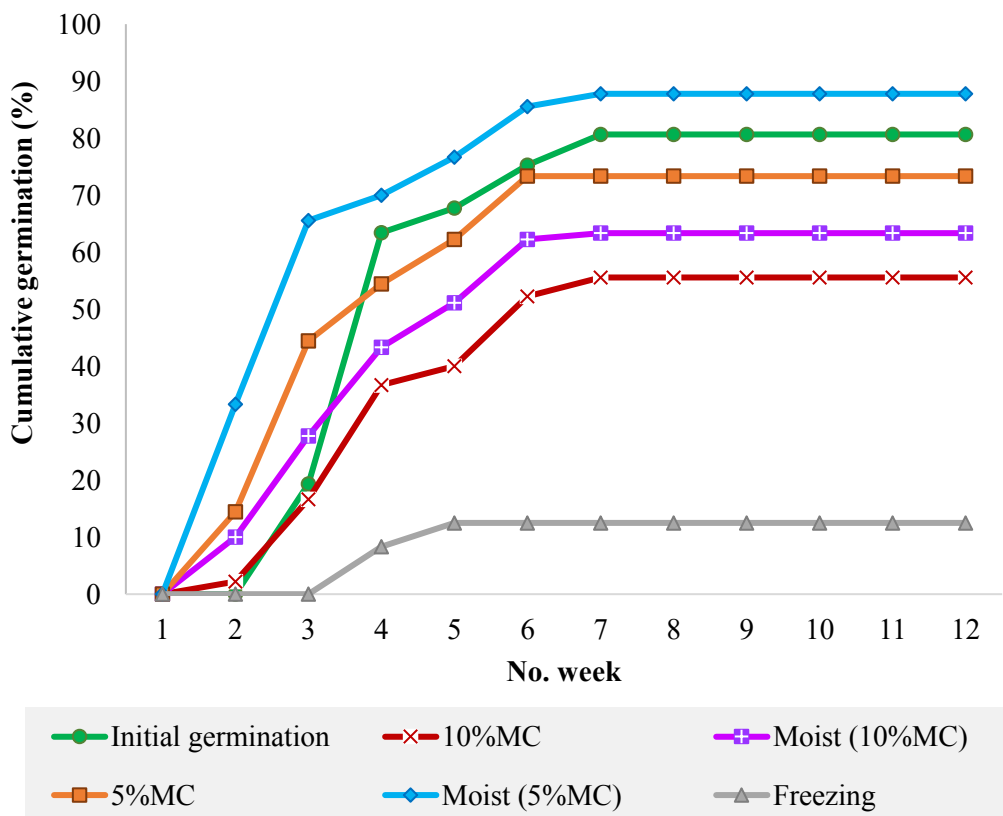


Figure 4.11 Average cumulative percent seed germination ($R = 3$) of *A. kurzii* at various storage moisture content in ambient temperature, and one-month-freezing (at temperature -20°C) compared to initial germination.

Artocarpus lacucha

Mean germination of *A. lacucha* seeds was highest after wet storage at 40% MC at six days of storing, although germination was not significantly different from initial germination (Coefficient estimate \pm SE = -0.3 ± 0.3 , $Z = -1.0$, $P = 0.3$; Figure 4.12). All desiccation treatments significantly reduced germination compared with the control (Table 4.4). The wet storage treatment preserved seed viability for at least 45 days, but seed viability decreased with increasing storage times. Therefore, this species was classified as recalcitrant.

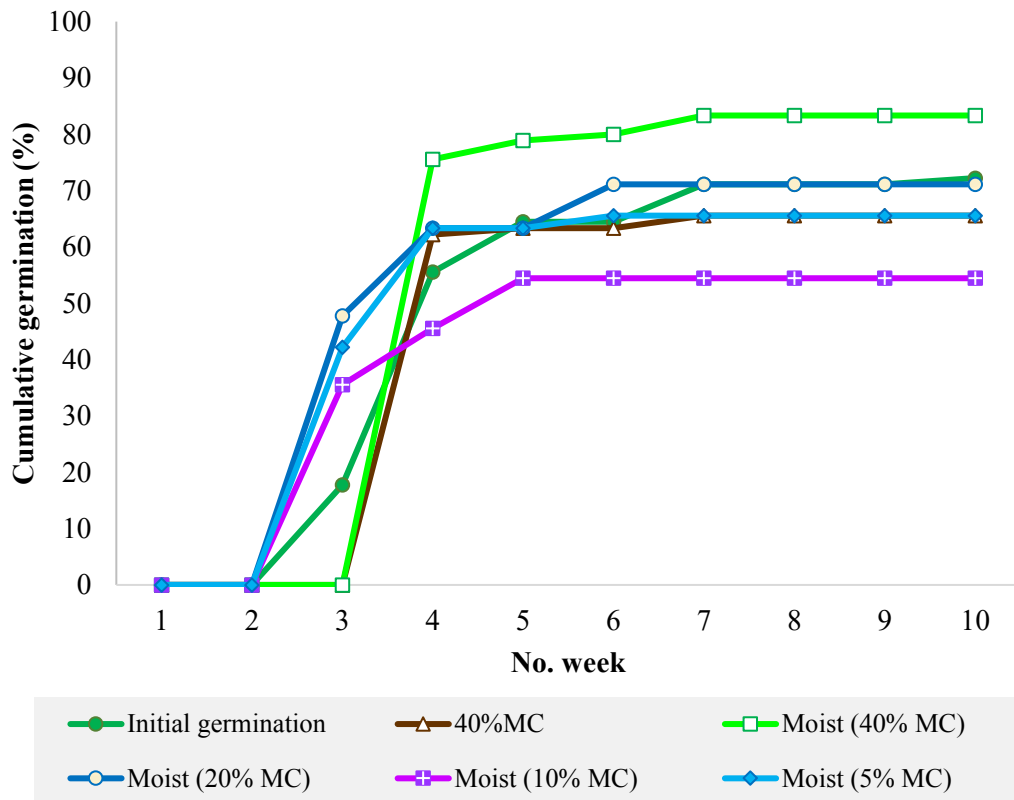


Figure 4.12 Average cumulative percent seed germination ($R = 3$) of *A. lacucha* at various levels of moisture content in the seed and different storage conditions compared to initial germination, treatment without seed germination was not included on the graph.

Balakata baccata

Initial germination of *B. baccata* seeds was low—12.2% (± 6.8 SE) (Table 4.2). Germination at 10% MC (16.7%, ± 14.5 SE) was slightly increased, but not significantly so compared with the control (Coefficient estimate \pm SE = 0.4 ± 0.4 , $Z = 0.8$, $P = 0.4$). Desiccation, freezing and wet storage substantially reduced germination (Coefficient estimate \pm SE = -1.8 ± 0.8 , $Z = -2.3$, $P = 0.02$) (Figure 4.13). This species is therefore recalcitrant, and seeds must be sown immediately after collection.

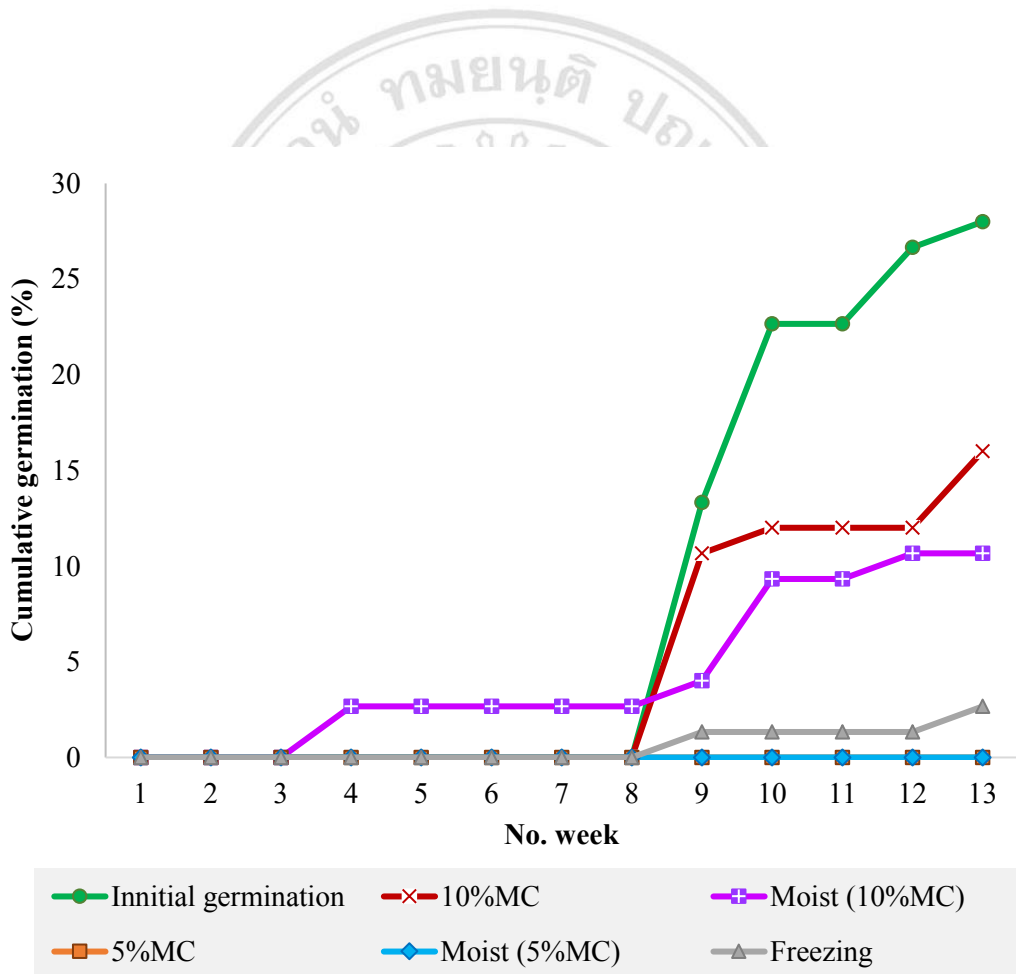


Figure 4.13 Average of cumulative percent seed germination ($R = 3$) of *B. baccata* at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination, treatment without seed germination was not included on the graph.

Choerospondias axillaris

The highest seed germination of this species was 70% (± 3.3 SE) achieved after moist-storage (pair treatment to 5% MC at 84 days) that percent seed germination was double from initial germination (37.8%, ± 25.0 SE). Reducing seed moisture content to 5% significantly increased seed germination compared to initial germination (Coefficient estimate \pm SE = 0.9 ± 0.3 , $Z=2.8$, $P = 0.005$, Figure 4.14). Furthermore, seed viability was not decreased after dry-storage and one-month-storing in the freezer (Table 4.4). Therefore, this species is classified as orthodox.

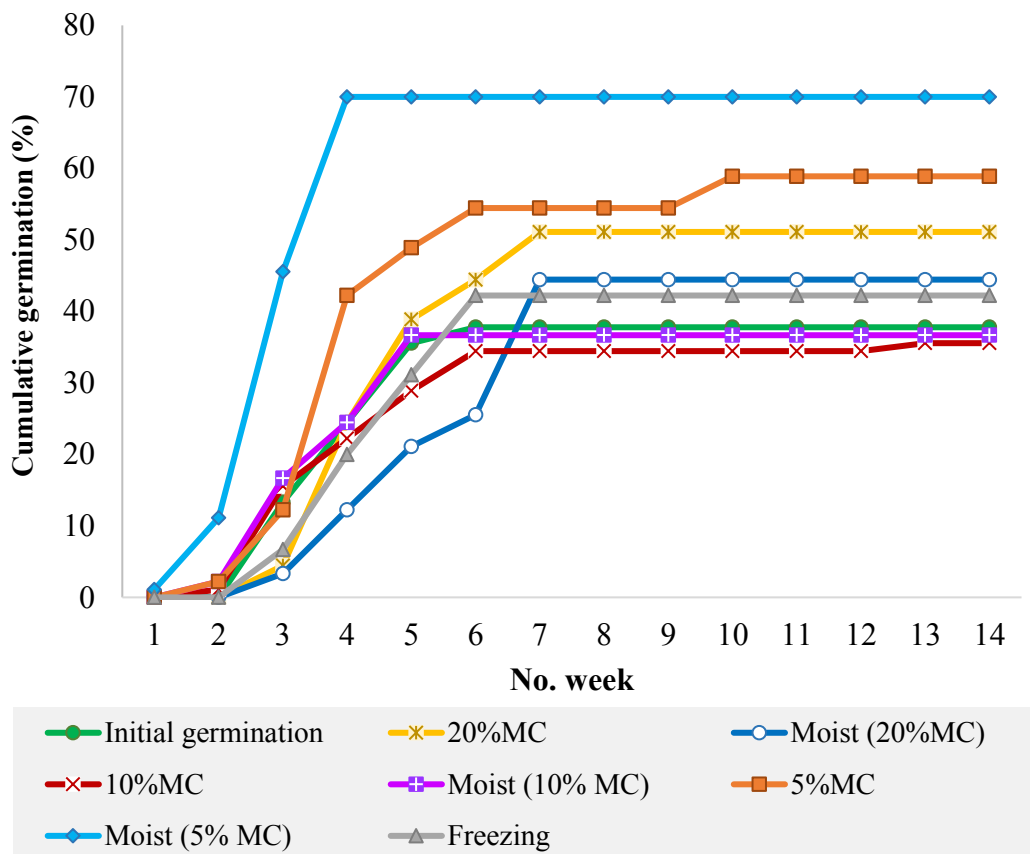


Figure 4.14 Average of cumulative percent seed germination ($R = 3$) of *C. axillaris* at various levels of moisture content in the seed and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month compared to initial germination, treatment without seed germination was not included on the graph.

Cassia bakeriana

The initial seed moisture content of *C. bakeriana* is lower than 5% MC, so there was no desiccation treatment for this species. Highest mean seed germination was after one-month storage in the freezer, but it was not significantly higher than initial seed germination (Coefficient estimate \pm SE = 0.6 ± 0.4 , $Z=1.5$, $P = 0.1$, Figure 4.15). Therefore, the species was classified as orthodox.

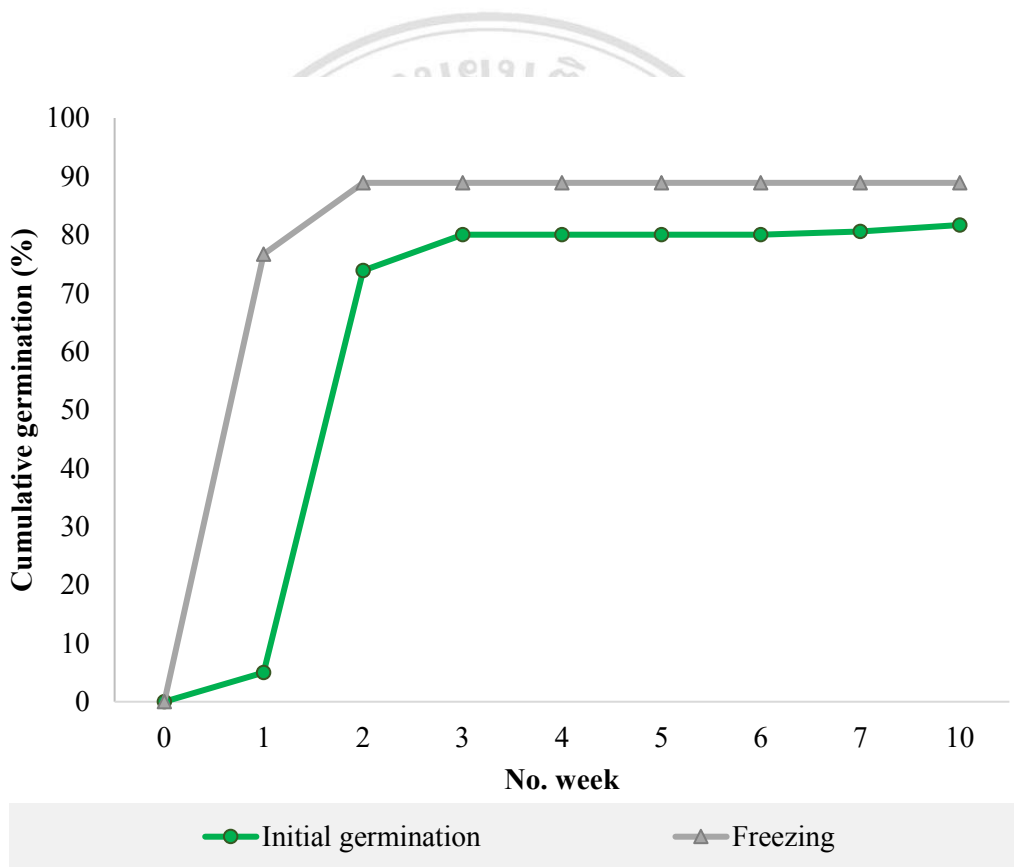


Figure 4.15 Average of cumulative percent seed germination (R = 3) of *C. bakeriana* storing in the freezer (T -20°C) for one month compared to initial germination.

Diospyros glandulosa

Highest mean seed germination of *D. glandulosa* was 66.7% (± 12.0 SE) achieved at 20% MC—significantly higher than initial germination (Coefficient estimate \pm SE = 0.9 ± 0.3 , $Z = 3.0$, $P = 0.003$; Table 4.4). Reducing MC to 5% and freezing had no effect on seed viability (Figure 4.16). Therefore, this species was classified as orthodox.

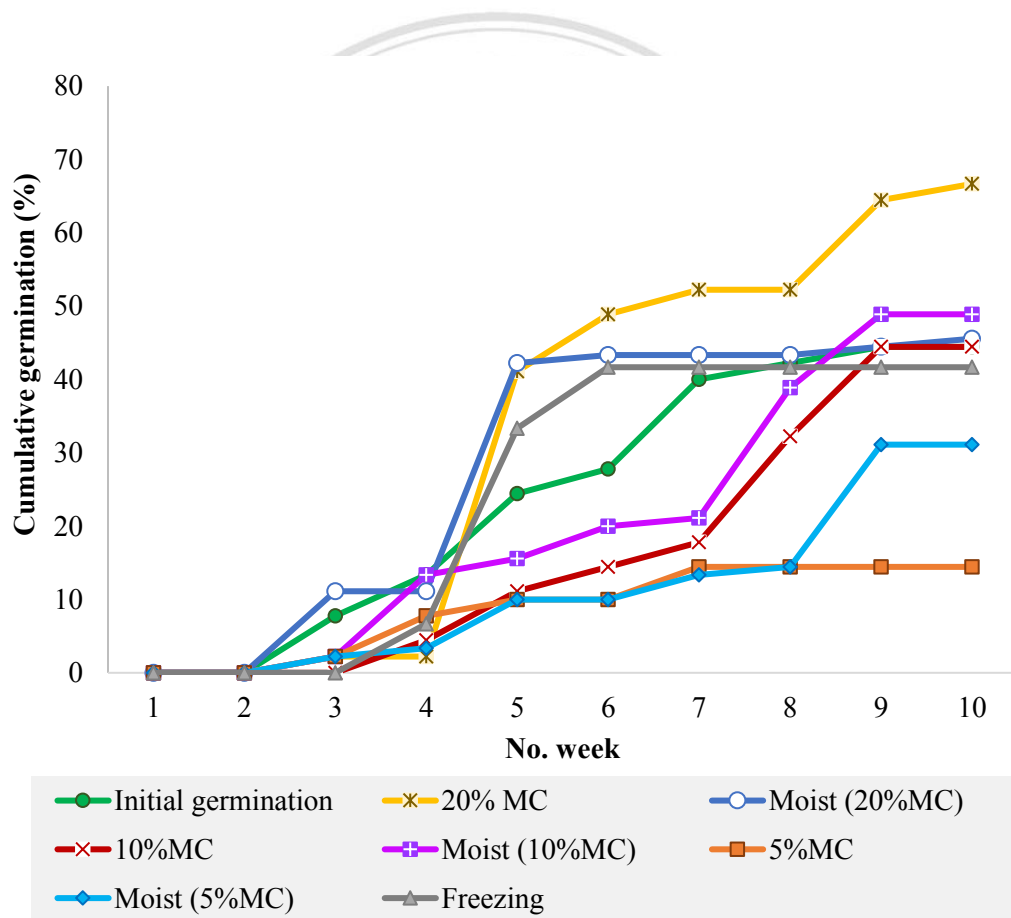


Figure 4.16 Average of cumulative percent seed germination ($R = 3$) of *D. glandulosa* at various level of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination. Treatment without seed germination was not included on the graph.

Gmelina arborea

G. arborea had low initial mean seed germination of just 10% (± 5.8 SE). Desiccation and freezing had no significant effects on percent seed germination (Coefficient estimate \pm SE = -1.1 ± 0.8 , $Z = -1.4$, $P = 0.2$; Figure 4.17). Therefore, the species was classified as orthodox.

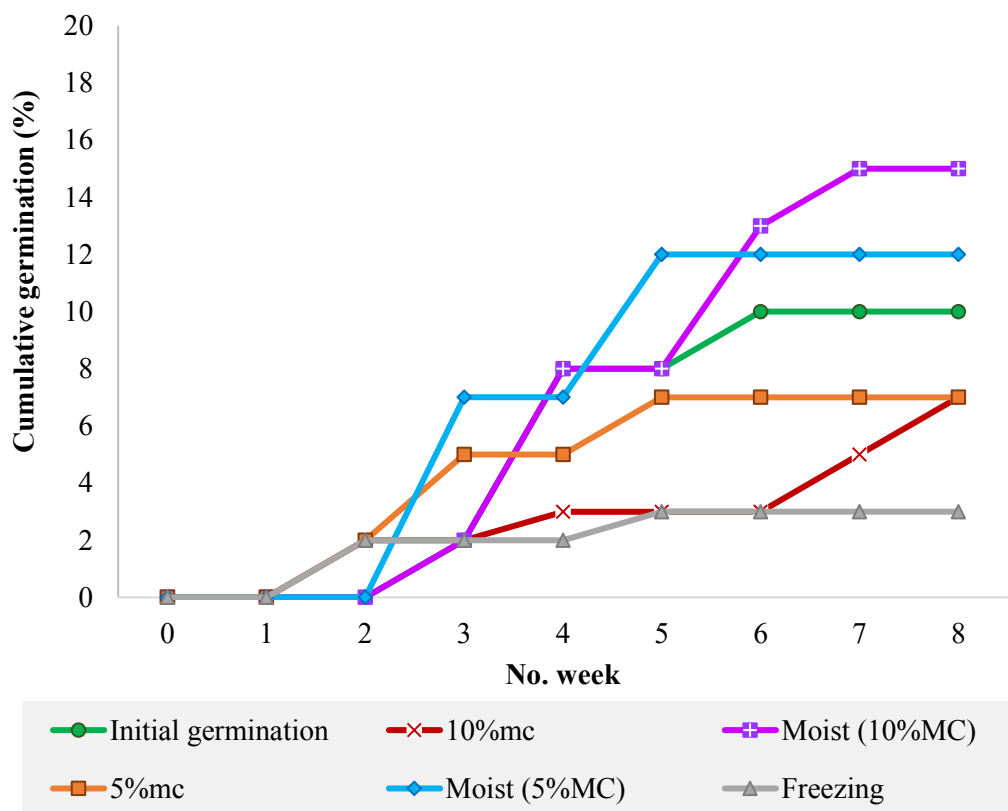


Figure 4.17 Average of cumulative percent seed germination ($R = 3$) of *G. arborea* at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.

Michelia baillonii

The highest germination was 28% (± 17.4 SE), achieved immediately after seed collection. Reduction of MC to 5% caused complete germination failure. Freezing at 5% MC substantially reduced germination (Coefficient estimate \pm SE = -2.6 ± 0.8 , $Z = -3.5$, $P < 0.001$), (Figure 4.18). The response of *M. baillonii* seed to desiccation and storing conditions is still unclear.

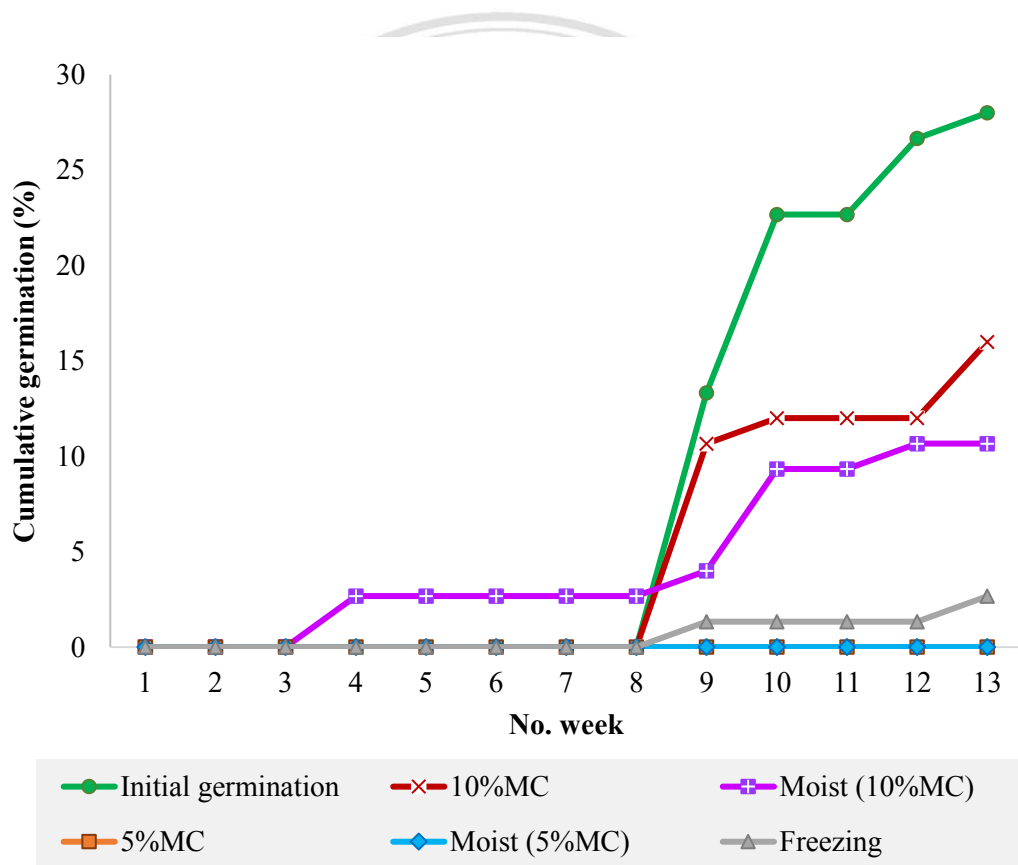


Figure 4.18 Average of cumulative percent seed germination ($R = 3$) of *M. baillonii* at various level of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination, treatment without seed germination was not included on the graph.

Prunus cerasoides

The highest mean seed germination of this species (63.3%, ± 8.8 SE) was achieved by the wet storage treatment (pair treatment to 5% MC). Reducing water content on the seed to 5% MC had no effect on seed viability (Figure 4.19). However, viability was significantly decreased after one-month-storing in the freezer (Coefficient estimate ± SE = -0.8 ± 0.3, $Z = -2.6$, $P = 0.01$; Table 4.4), (30%, ± 12.0 SE). Therefore, this species was classified as an intermediate seed.

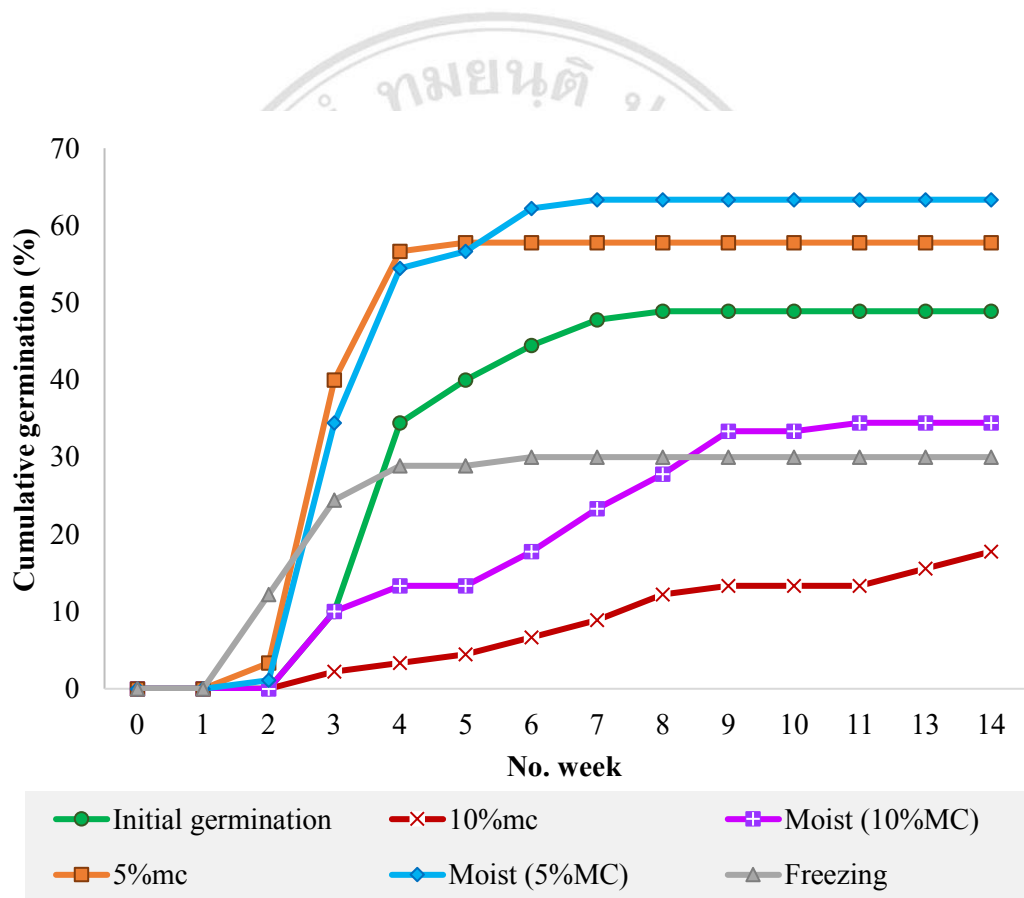


Figure 4.19 Average of cumulative percent seed germination (R = 3) of *P. cerasoides* at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.

Phyllanthus emblica

Highest mean germination capacity of *P. emblica* seeds was achieved at 5% MC and storage at -20°C—substantially and significantly higher than initial germination (Coefficient estimate \pm SE = 2.0 ± 0.4 , $Z= 5.0$, $P < 0.001$; Figure 4.20). Desiccation and freezing did not significantly reduce germinability (Table 4.4). Therefore, this species was classified as orthodox.

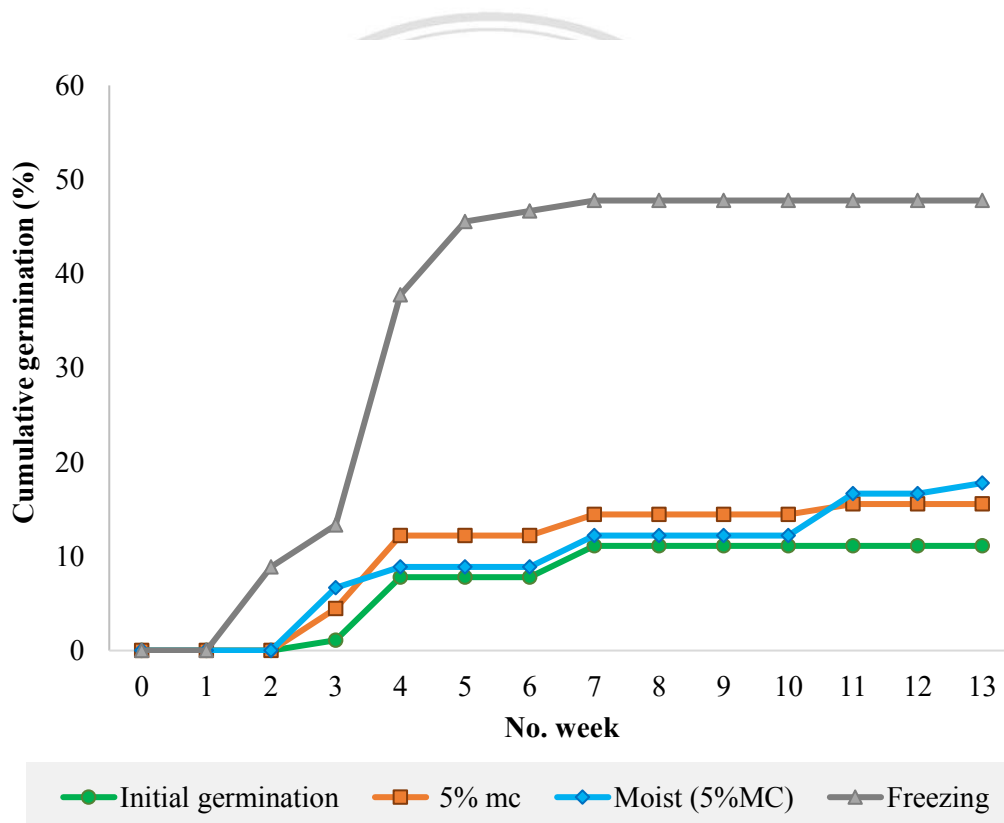


Figure 4.20 Average of cumulative percent seed germination ($R = 3$) of *P. emblica* at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.

Syzygium fruticosum

The ability to germinate of *S. fruticosum* was highest under wet storage (pair treatment with 20% MC and 40% MC). Some seed viability was retained at 10% MC, but it was significantly decreased from initial germination (Coefficient estimate \pm SE = -4.3 ± 0.8 , $Z_c = -5.8$, $P < 0.001$; Figure 4.21). Reduction of MC to 5% and freezing killed all seeds (Table 4.4). Therefore, this species was classified as recalcitrant.

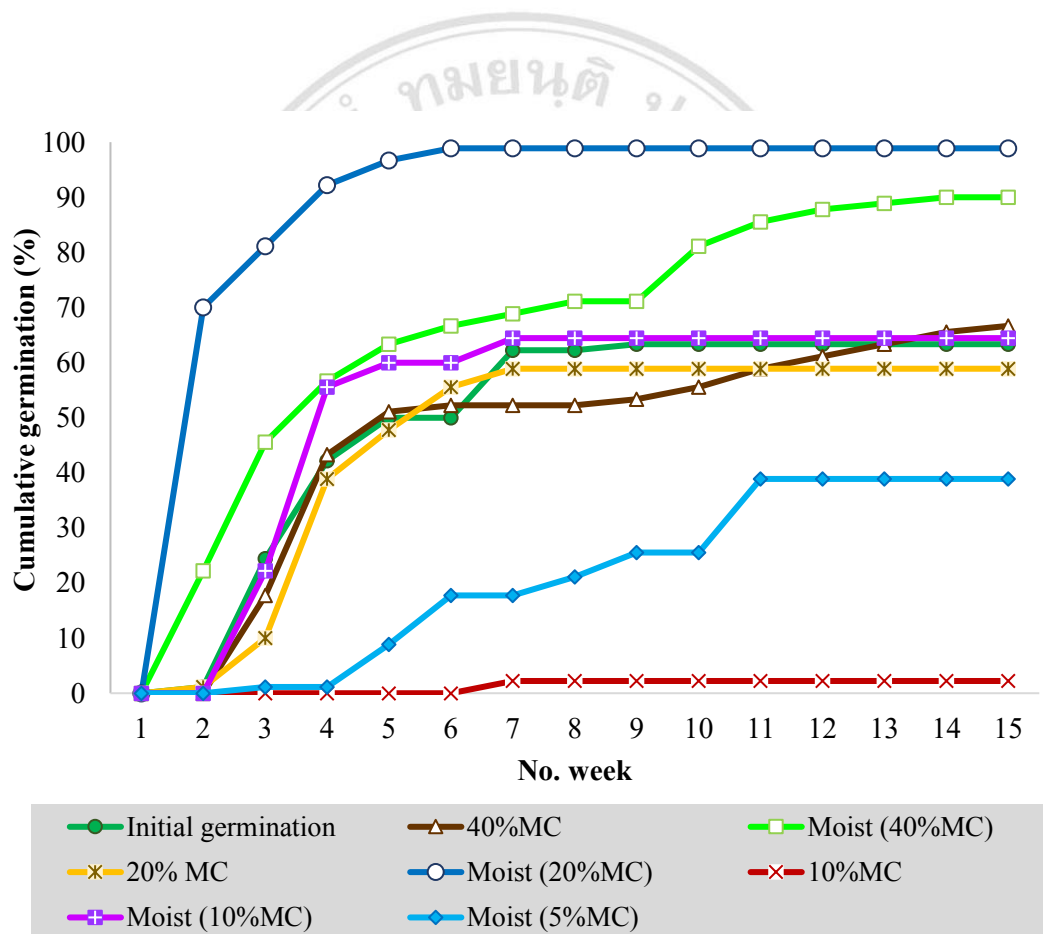


Figure 4.21 Average of cumulative percent seed germination ($R = 3$) of *S. fruticosum* at various level of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination. The treatment without seed germination was not included on the graph.

Sapindus rarak

Highest mean germination of *S. rarak* was 55.6% (± 16.8 SE) with wet storage (pair treatment to 5% MC). Reducing MC to 5% and freezing decreased percent seed germination but not significantly so compared with initial seed germination (Coefficient estimate \pm SE = -0.4 ± 0.3 , $Z = -1.4$, $P = 0.2$; Figure 4.22). Therefore, this species was classified as orthodox.

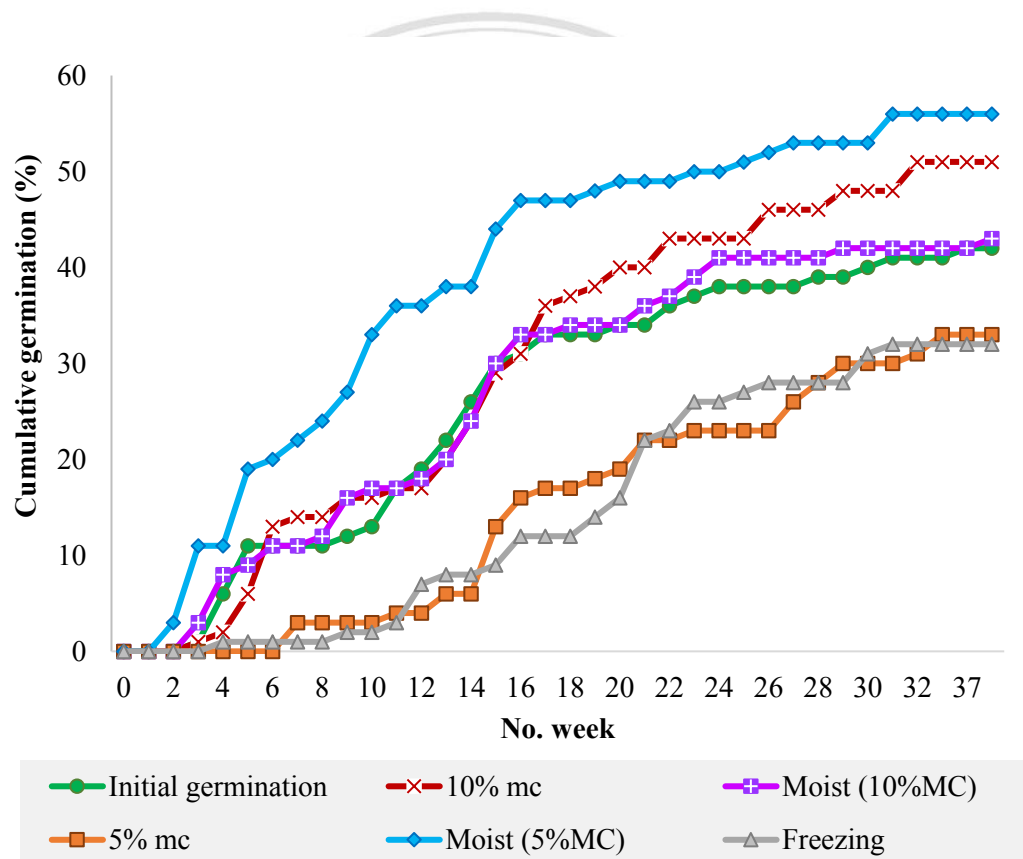


Figure 4.22 Average of accumulative percent seed germination ($R = 3$) of *S. rarak* at various levels of moisture content and different storage conditions in ambient temperature and storing in the freezer (at temperature -20°C) for a month, compared to initial germination.

4.4.2 Seed storage behaviors

The species could be categorized according to seed responses to storage conditions (Table 4.4); -

Seeds of eight species (57% of species tested) tolerated drying to 5% MC and freezing at -20°C for a month, with little or no loss of viability: *C. bakeriana*, *A. fraxinifolius*, *A. microsperma*, *P. emblica*, *S. rarak*, *G. arborea*, *C. axillaris*, and *D. glandulosa*. Consequently, they were classified as orthodox.

Seeds of four species (29% of species tested) had high-water content, when collected and lost viability when the moisture level was decreased ($P < 0.05$): *M. baillonii*, *B. baccata*, *S. fruticosum* and *A. lacucha*. Storing these seeds in moist conditions-maintained viability for at least 45 days before sowing. Therefore, the species were classified as recalcitrant seed.

Seeds of two species *P. cerasoides*, and *A. kurzii* (14% of the studied species) tolerated drying to 5% MC, but lost viability when frozen -20 °C ($P < 0.05$). Therefore, these three species were classified as intermediate, meaning that they have a limited ability to withstand sub-zero temperatures without losing viability.

Table 4.4 The category of seed storage behavior: orthodox, intermediate, and recalcitrant seed, followed their germinability in each moisture level (Species ordering by lowest-to-highest initial moisture content). Columns not sharing the same superscript indicate significant differences among dry treatments tested by GLM (Binomial) and Turkey Contrasts (R = 3).

Species	%MC of seed (%) ± SE	Seed germination					
		Initial (%) ± SE	Dried 40%MC (%) ± SE	Dried 20%MC (%) ± SE	Dried 10%MC (%) ± SE	Dried 5%MC (%) ± SE	Dried 5% MC and stored at -20°C (%) ± SE
Orthodox							
<i>C. bakeriana</i>	4.6 ± 0.4	81.7 ± 1.9 ^a	-	-	-	-	88.9 ± 1.1 ^a
<i>A. fraxinifolius</i>	5.2 ± 0.6	63.3 ± 15.75 ^b	-	-	-	-	89.6 ± 5.5 ^a
<i>A. microsperma</i>	6.0 ± 0.5	75.6 ± 8.7 ^b	-	-	-	68.9 ± 11.8 ^b	92.2 ± 2.9 ^a
<i>P. emblica</i>	6.4 ± 1.9	11.1 ± 9.6 ^b	-	-	-	15.5 ± 7.7 ^b	47.8 ± 19.2 ^a
<i>S. rarak</i>	10.3 ± 2.5	42.2 ± 5.1 ^a	-	-	51.1 ± 1.9 ^a	33.3 ± 15.3 ^a	32.2 ± 10.2 ^a
<i>G. arborea</i>	15.5 ± 2.5	6.7 ± 6.7 ^a	-	-	4.4 ± 1.9 ^a	4.4 ± 3.8 ^a	2.2 ± 1.9 ^a
<i>C. axillaris</i>	27.4 ± 0.5	37.8 ± 14.4 ^b	-	51.1 ± 19.0 ^{ab}	35.6 ± 13.1 ^b	58.9 ± 14.4 ^a	42.2 ± 6.2 ^{ab}
<i>D. glandulosa</i>	37.1 ± 0.8	44.4 ± 2.9 ^b	-	66.7 ± 6.9 ^a	44.4 ± 10.6 ^b	14.4 ± 4.4 ^c	41.67 ± 5.8 ^b
Intermediate							
<i>P. cerasoides</i>	19.6 ± 3.0	48.9 ± 10.2 ^a	-	-	17.8 ± 13.5 ^b	57.8 ± 1.9 ^a	30.0 ± 12.0 ^b
<i>A. kurzii</i>	19.8 ± 0.3	80.6 ± 14.8 ^a	-	-	55.6 ± 27.8 ^b	73.3 ± 13.3 ^{ab}	12.5 ± 8.3 ^c
Recalcitrant							
<i>B. baccata</i>	25.7 ± 3.7	12.2 ± 6.8 ^a	-	6.7 ± 3.3 ^{ab}	16.7 ± 8.4 ^a	2.2 ± 1.1 ^b	2.2 ± 2.2 ^b
<i>M. baillonii</i>	15.3 ± 0.4	28.0 ± 10.1 ^a	-	-	16.0 ± 8.3 ^{ab}	0 ^c	2.7 ± 2.7 ^b
<i>S. fruticosum</i>	43.7 ± 1.1	63.3 ± 10.0 ^a	66.7 ± 17.3 ^a	58.9 ± 10.6 ^a	2.2 ± 2.2 ^b	0	0
<i>A. lacucha</i>	51.6 ± 1.7	72.2 ± 2.9 ^a	65.6 ± 13.5 ^a	0	0	0	0

4.4.3 Factors associated with seed storage behaviors

The Linear Discriminant Analysis (LDA) was clear for the categorized between recalcitrant and orthodox seeds. There was no overlap between the orthodox seeds in green and recalcitrant seeds in blue. We can also see that seed coat thickness is in a positive direction for orthodox seed whereas the contrary was for seed moisture content (Figure 4.23). Therefore, species with thick seed coats and low moisture content tend to exhibit greater desiccation tolerance, while species with thinner seed coats and high moisture content were more likely to be desiccation-sensitive.

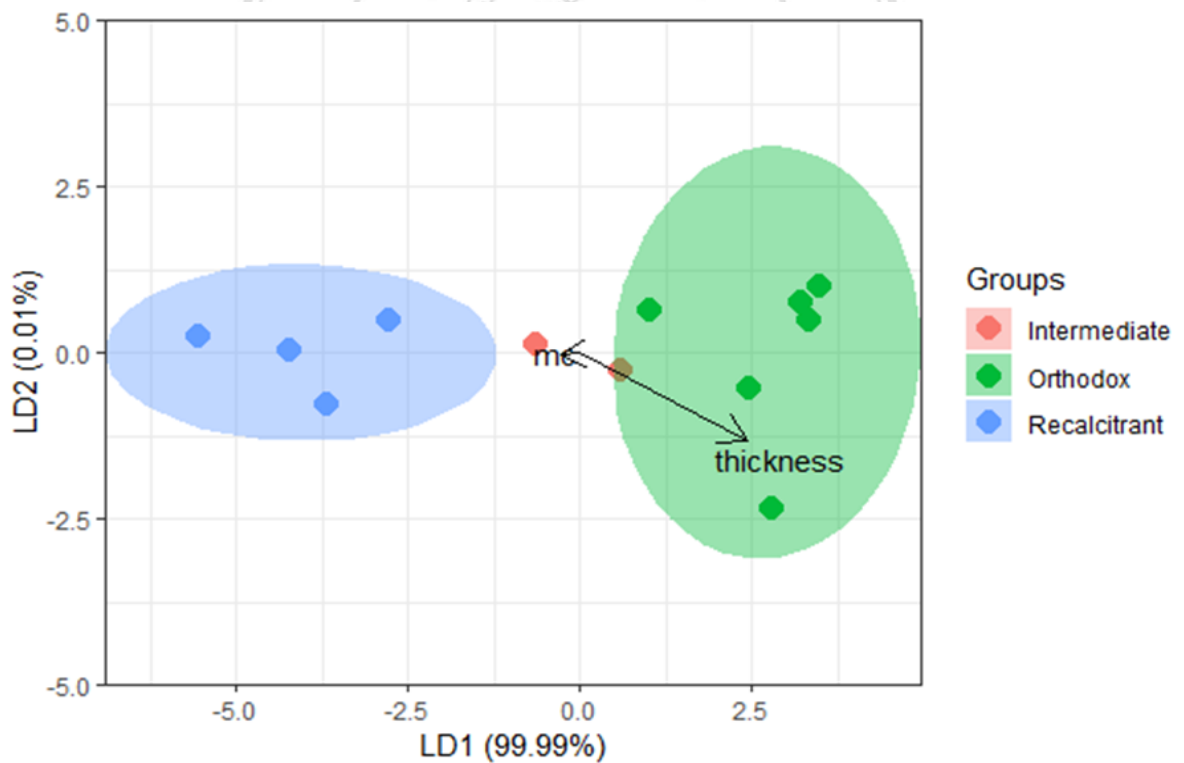


Figure 4.23 The biplot based on LD1 and LD2, to separate the observation among storage behaviors with 100% accuracy estimation.

CHAPTER 5

DISCUSSION

5.1 Species selection for direct seeding

This study evaluated the suitability of 23 tree species for direct seeding, to restore upland evergreen forest ecosystems in Northern Thailand. We investigated the intensity of seed predation and species performance, in terms of seedling yield and growth after the first dry season. Although seed predation was low, 10 out of the 23 species studied failed to establish. Seedling yields of those that were established were mostly low (averaging 20%). The study revealed an interplay of various factors that contributed to low seedling establishment including effects from study sites, species traits (seed size and storage behavior) and successional guild.

5.1.1 Seed removal

Small seeds tended to be more vulnerable to seed removal than larger ones, most probably due to seed predation. This agrees with a study by Dylewski et al. (2020), who reported that in tropical forests, seed removal rates decrease with increasing seed mass. This may be because smaller seeds are easier to move than large ones, and they tend to lack protective structures, such as thick coverings (Hau, 1997; Ruxton and Schaefer, 2012). None of the largest propagules: *S. pinata* and *S. pentandrum* were removed from the experiment. They are both pyrenes with tough coverings, derived from the fruit endocarp. This observation suggests that large propagules, particularly those with hard coverings, are resistant to predation (Hau, 1997; Naruangsri, 2017).

However, the results of this study contrasted with those of a predator-exclusion experiment, performed at the same Mon Cham plot on 29 July 2015 - 26 July 2016, during which up to 100% removal was recorded. Large seeds were lost to rodents, but small seeds remained untouched (Naruangsri et al., 2023). The fact that seeds in the present study were more widely spaced and buried

deeper than in the previous study may explain the contrasting results. Wide spacing is known to substantially reduce seed predation by rodents (Hau, 1997; Fricke et al., 2014).

5.1.2 Seed germination

Seed germination probability increased with increasing seed mass. This was consistent with previous research (Doust et al., 2008; Tunjai and Elliott, 2012; Souza et al., 2015; Silva and Vieira, 2017). The amount of food reserved within seeds is essential for achieving successful germination (Bewley et al., 2013). Larger seeds typically have a higher concentration of nitrogen and phosphorus than small seeds (Vaughton and Ramsey, 2001), which can facilitate seedling development, even in areas with limited light and nutrients (Cordazzo, 2002; Flores et al., 2016). The small seeds, such as *A. fraxinifolius* and *M. baillonii*, have limited resources, providing better germination under sunlight (Table 3.3). Light serves as one of the determining factors to ensure that they are positioned close enough to the soil surface for successful emergence (Aud and Ferraz, 2012; Flores et al., 2016). The heterogeneity of canopy cover within the studied site and the limitation of light in bamboo tubes may affect the potential for seed germination, especially for light demanding species.

Seed storage behavior influenced germination success. Seeds of desiccation-sensitive species included *S. fruticosum*, *S. arborea*, *G. cowa*, *P. cathia*, *P. viridis*, and *Q. brandisiana*, failed to germinate both in the field and the nursery. In contrast, those classification as orthodox exhibited high seed germination (*A. microsperma* and *A. kurzii*). This underscores the importance of maintaining appropriate seed storage conditions, even for brief durations (Hau, 1997; Waiboonya, 2017).

5.1.3 Survival and yield of seedling

Seedling yield varied greatly among species but appeared to be unaffected by the study site. Seed size affected early post-germination survival. Species with medium (*A. microsperma* and *C. axillaris*) to large (*S. pinnata*) seeds

achieved high seedling yields, whilst small-seeded species (e.g., *A. fraxinifolius* and *H. dulcis*) attained lower seedling yields. Many previous studies demonstrated that larger or intermediate-sized seeds achieve higher seedling-establishment rates than smaller ones (Doust et al., 2008; Palma and Laurance, 2015; Tunjai and Elliott, 2012), mainly by prolonged provision of stored reserves, which sustain early seedling development and growth (Saverimuttu and Westoby, 1996; Coomes and Grubb, 2003). This is consistent with the larger-seed-later-commitment mechanism, validated by Kidson and Westoby (2000).

Species with high seedling yields tended to have rapid and high seed germination. Rapid germination is highly advantageous, as it reduces the amount of time available for seed predation (Naruangsri, 2017). It also maximizes the time for root growth before the start of the wet season (Yi et al., 2012). This allows roots to access water, deep down in the soil profile, to survive their first dry season and thus greatly reduces first-year mortality (Elliott et al., 2013; Naruangsri, 2017). Consequently, to ensure high seedling survival rates, species characterized by fast germination with minimal dormancy periods should be prioritized for direct seeding efforts.

5.1.4 Seedling growth

C. axillaris, *H. dulcis*, and *M. azedarach* attained large seedling sizes and high growth rates. They are all pioneer species, recommended for forest restoration by the framework tree species method (Elliott et al., 2013). With the framework species method, 30-50 cm is the recommended size for nursery-grown planting stock (FORRU, 2005). In this study, some individual seedlings of *A. microsperma*, *C. axillaris*, *M. azedarach*, and *H. dulcis* had grown taller than 30 cm by the end of study (around 7 - 8 months after emerging from seeds). Similarly, Tunjai (2005) and Waiboonya (2017) reported rapid growth of *M. azedarach* (formerly *M. toosendan*) and *C. axillaris*, with direct-seeded seedlings growing taller than nursery-raised ones, due to the better developed root systems. On the other hand, slow growing species, *C. bakeriana* and *A. fraxinifolius* produced the smallest seedlings. Even though these two species were categorized as pioneer species (FORRU, 2005), they did not perform well in the study sites.

Therefore, conventional tree planting may be the most suitable way of reintroducing the slow growing species to degraded areas.

5.1.5 Relative performance index (RPI)

This study underscored the importance of appropriate tree species selection for direct seeding to restore forest ecosystems, emphasizing the need to select species with a combination of attributes, including rapid and high seed germination, which contribute to high seedling yield, and rapid seedling growth. This study was consistent with previous studies (Tunjai, 2005; Doust et al., 2008; Yi et al., 2012; Hossain et al., 2014; Naruangsri et al., 2023).

A. microsperma stood out as the top-performing species (assigned an RPI of 100). The second-best species, *S. pinnata*, achieved an RPI of 50% that of *A. microsperma*. *C. axillaris* and *S. rarak* were considered as acceptable species, with seeds resistant to predation and relatively fast-growing seedlings. On the other hand, species considered unacceptable for direct seeding, due to their low RPI were *C. bakeriana*, *G. arborea* and *A. fraxinifolius*. They had low rates of seed germination that resulted in low seedling yields. Their slow-growing, small seedlings could not compete effectively with herbaceous weeds. However, they may potentially be used for direct seeding if seeds are pre-treated to accelerate germination (Table 5.2). Otherwise, conventional tree planting would be a better option, especially for *G. arborea* and *A. fraxinifolius*, which are considered excellent framework tree species on degraded areas in Northern Thailand (FORRU, 2000; Elliott et al., 2003).

5.2 Seed ball

The study highlights the application of protective materials through seed pelleting and demonstrated efficacy in reducing the percentage of seed removal. Within the context of this study, biochar has emerged as the most highly promising material for preventing seed predation. Extensive documentation supports the positive effects of biochar on seed germination and plant performance (Williams et al., 2016; Zhang et al., 2022), but it's rare to find the result on its effects on predation. The success of biochar in effectively safeguarding seeds against predator consumption adds intrigue (Kinyanjui, 2022). The pelleting of seeds using biochar and soil mixture can create a physical barrier that makes it more challenging for seed predators to access and consume the seeds (Jacobs, 1992). The pelleting of seeds can result in camouflage, which leads to lower levels of seed removal compared to seeds with natural colors (de Almeida et al., 2010). In addition, the technique poses challenges for animal predators, particularly rodents, in locating them through olfaction (Briggs and Vander Wall, 2004), seeds with low odor are less likely to be consumed by predators (Yi et al., 2016).

On the other hand, polysaccharide mixture increased the chance of seed being removed. The seeds coated by polysaccharide mixture can attract seed predators leading to higher seed removal compared to the seed coated with other pelleting materials and uncoated seeds. The polysaccharide mixture used in this research is a carbohydrate compound (Su et al., 2017) which explains its appeal to animals. Additionally, the function of these materials is well known for seed germination and seedling performance (Zhang et al., 2022), with no specific observations made regarding seed protection. Based on the information provided, it appears that using the polysaccharide mixture for seed protection is not recommended. The mentioned research does not highlight any observations or evidence supporting the effectiveness of the polysaccharide mixture in terms of seed protection.

An intriguing discovery from our study was the relatively low level of seed removal, even in the control group. In this experiment, the seeds were randomly distributed on the ground, spaced about a meter apart, resembling a tree planting technique. By reducing seed density and burying the seeds underground, it becomes more difficult for seed predators to access them compared to a deposition station that

resembles a buffet (Carlo et al., 2013; Egerer et al., 2018). There was only evidence of insect seed predation, particularly by ants, which colonized seed deposition points randomly. Therefore, the study emphasis that seed pelleting and sowing methods could be the solution for seed predation by animals.

Despite the efficiency of preventing seed removal, the germination of the seed could be limited compared to uncoated seed. Every pelleting influenced the ability to germinate, percent seed germination reduced almost 50% from control. Many researchers reported that the polysaccharide mixture which acts as a “mini-reservoir” around the seeds, enhances germination (Su et al., 2017; Afzal et al., 2020), the polymers did not have any positive effect on seed germination. Previous research suggested that the germination rate depended strongly on the amount of pelleting material applied to the seed (Jin et al., 2023). The inhibitory effect of the pelleting material particularly from the thickness of pelleted seeds, was the determining factor in inadvertently creating a physical barrier, preventing essential elements such as water, oxygen, and light from reaching the seed (Elliott, 2010; Gorim, 2014; Jin et al., 2023). In addition, the coatings were excessively tough for the roots or shoot to penetrate (Figure 5.1), or excessively restrictive, hampering gaseous exchange, resulting in detrimental effects on seed metabolism (Stendahl, 2005; Javed et al., 2022).



Figure 5.1 The pelleting seed or seed ball could not germinate before the end of study.

The results further indicated that the tested pelleting materials did not enhance tree-seedling establishment, growth, and overall performance in degraded areas of Northern Thailand. Despite the initial expectation that pelleting seeds with the three different materials would enhance water holding capacity and promote seedling growth,

this potential improvement was not observed. Comparing our findings with previous studies, it is clear that the use of coating materials for seedling establishment remains a complex and variable process. While many studies in crops and herbaceous plants have reported positive effects on seedling performance (Turner et al., 2006; Liu et al., 2010; Williams et al., 2016; Su et al., 2017; Baroni and Vieira, 2020). The seeds coated with various materials normally grew better than controls during the seedling stage (Su et al., 2017).

The variation in seedling performance in our study was largely explained by differentiation of species. Unfortunately, the selected species had poor seed germination even in the treatment without seed pelleting in the tree nursery. This inhibition of germination directly influenced the number of available seedlings, thereby reducing overall establishment and potentially impacting yield. Viability loss was particularly observed in the tested species *S. fruticosum* and *S. arboreum*, which exhibited complete failure to germinate due to compromised viability. The absence of seed germination in these species raised concerns regarding seed viability or quality prior to the application of seed pelleting or any kind of seed coatings. Certainly, various pretreatments such as scarification, applying heat, hot-water treatment etc., are often necessary for breaking seed dormancy and accelerating rapid germination (FORRU, 2005). Therefore, the inability of these species to initiate germination highlighted the critical importance of species selection (related to topic in CHAPTER 3) in ensuring optimal seed viability and quality before applying seed pelleting. Further study for pelleting techniques, particularly for tree seeded species, may require more processes and various practice to improve field performance.

5.3 Microbial seed coating

The microbial treatments applied in this experiment were not adequately tested. We found Microbial seed coating application did not improve germinability and increase seedling yield or did not support seedling growth for all tested species. This result did not support our hypothesis. The germination of sterile seeds decreased by 10% compared to untreated, due to surface sterilization having a negative effect on seed viability and decreased percent seed germination. This is in agreement with previous studies that have shown a negative effect of NaOCl on seed sterilization, with an increase in NaOCl concentration and exposure time leading to reduction in germination for *Ficus religiosa* (Hesami et al., 2017). Seeds were possible to lose viability before we tested with the microbials. Before applying microbial seed coating, it is essential to determine the minimum concentration and exposure time that effectively sterilize seeds without injury and reducing seed viability.

The influence of study sites and seed species were observed, rather than the effect of the microbial seed coating treatments. The higher seedling yield was observed at Mon Cham which had higher soil moisture content and nutrient availability compared to the Ban Mae Khi (see in Table 3.1, CHAPTER 3). In this study, plot maintenance included weeding and fertilization (Osmocote 13-13-13) was applied to all seedlings two times during seedling monitoring in November 2019 and May 2020, which was able to relieve the nutrient limitation. The efficiency of microbial seed coating is normally outstanding in harsh environmental conditions and non-optimal soil condition (Jamil et al., 2022; Paravar et al., 2023), which may not limit factors in our plots.

The potential of beneficial bacteria to function as plant growth promoter varies depending on the specific plant group. The response of the plant to microbial inoculation and their colonization is essential for microbial functioning (Venturi and Keel, 2016). These interactions are contingent upon plant chemical that attract microbes to their roots and soil properties (Jamil et al., 2022). Although, the two actinobacterial species; *S. antibioticus* and *S. thermocarboxydus* isolate S3 (S3), are common in natural soil and have proven effective in crops and herbaceous plants (Jamil et al., 2022; Nazari et al., 2023), this research did not observe their potential benefits for our tree seeds.

Consequently, additional research is essential to identify suitable beneficial bacteria for tree-seeded species.

The symbiosis of microbes on the roots after sowing were not guaranteed in our study. A film coating applied to the seeds creates a very thin layer devoid of an outer protective material. When these coated seeds are sown in open areas and non-sterile soil, it was a significant challenge for the successful colonization (O’Callaghan et al., 2016). Under uncontrollable conditions, the inoculated microbes may vanish during seed sowing, adversely impacting both their survival and symbiosis on the seed surface. To address this issue, inoculation of microorganism spores with hygroscopic nutrient source materials, serving as slow decomposers of the coating materials, might offer a viable solution to maintain microbes on the seed surface until the seeds develop into seedlings (Liu et al., 2010; Paravar et al., 2023).

5.4 Microbial seed coating for forest restoration

Sterilization of seeds before applying beneficial microorganisms is still recommended but more research on a large scale should be done appropriately (Akbari et al., 2011; Hesami et al., 2017; Paravar et al., 2023). To apply microorganisms on seed surface, common methods such as fluidized bed treatment, rotary coating, and rotary pan coating are normally used for treating a large number of seeds simultaneously (Javed et al., 2022; Paravar et al., 2023). The seeds are loaded into the machine, where they receive a liquid seed treatment that is atomized onto them while they rotate inside a machine’s container (Paravar et al., 2023). Liquid treatment can involve either single or co-inoculation, both of which can enhance the efficiency of microbial function in plant development (Emmanuel and Babalola, 2020).

In forest restoration, beneficial microorganisms may be used with different methods. Despite seed inoculation is the most popular method (Simon et al., 2011), microbial inoculation is possible to apply to soil, roots, and leaves, depending on tool accessibility, and inoculum availability (Paravar et al., 2023). Various methods can be used as a combination to develop techniques for forest restoration.

5.5 Seed storage behaviors

Most of the studied species had orthodox seeds, constituting 57% of the tested species, while seeds 14.3% were classified as intermediate. Most species in our study are able to store without a loss of viability, whereas special condition is required for only 8.6% recalcitrant seeds. A previous study on 16 forest tree species with some species overlap found that 68.8% were orthodox species, and 25% were classified as recalcitrant species (Waiboonya et al., 2019). These results are similar to the FORRU database, which comprises 328 species across seasonal forests in northern Thailand, with 115 (35%) recalcitrant seeds and 213 (65%) orthodox seeds. (Figure 4.18). In a different study, Tweddle et al. (2003) estimated an even higher percentage of orthodox seeds, at 75%, among a sample of 68 species in the same forest type around the world. However, the proportion of each storage behavior may vary depending on sample size and species distribution in various forests habitat (Tweddle et al., 2003). The results of this research chapter have already fulfilled the primary objectives outlined within the scope, which involved determining seed responses to desiccation levels and classifying seed storage behavior for individual species.

Most species exhibited clear storage behavior, except for *D. glandulosa*, *M. baillonii*, *P. cerasoides*, *A. kurzii* and *B. baccata* (Table 5.1). The desiccation responses of the seeds based on their germination were ambiguous for the classification of these seeds, which showed the opposite results to previous study. *P. cerasoides* and *A. kurzii*, previously identified as orthodox seeds (Waiboonya, 2017), became intermediate seeds in this study due to viability loss after one month in the freezer (-20°C). However, for study of Waiboonya (2017), seeds were stored in a 4°C refrigerator, which may have a minimal impact on seed viability compared to extremely cold storage. This emphasizes the essential role of suitable storage conditions for specific species in preserving seed viability.

Storing orthodox seeds in dry and cool condition did not destroy seed viability but in some cases, storing the seeds briefly can promote seed germination for some species including *C. axillaris*, *A. fraxinifolius*, *A. microsperma*, *C. bakeriana* and *P. emblica*. According to Waiboonya et al. (2019) and FORRU database, report long dormancy periods of the same species compared to other species, maintaining 50+%

germination for at least 100 days. Dormancy helps preserve the viability of seeds by preventing them from germinating prematurely. This is especially important for long-term storage, as it ensures that the seeds remain viable for an extended period with minimal or no loss of seed viability (Bonner and Karrfalt, 2008; Yulianti et al., 2020). However, seeds can exhibit delayed germination when used immediately after collection. Therefore, dormancy-breaking treatments are required to overcome their dormancy and promote seed germination.

Table 5.1 Comparing the results with the previous studies. 'NA' indicates no information from the respective research studies.

Species	This study	Waiboonya et al., (2019)	KEW seed information	FORRU database
<i>A. fraxinifolius</i>	Orthodox	Orthodox	NA	Orthodox
<i>A. kurzii</i>	Intermediate	Orthodox	NA	NA
<i>A. lacucha</i>	Recalcitrant	Recalcitrant	tend to be Recalcitrant	NA
<i>A. microsperma</i>	Orthodox	Orthodox	NA	Orthodox
<i>B. baccata</i>	Recalcitrant	NA	NA	tend to be Orthodox
<i>C. axillaris</i>	Orthodox	Orthodox	NA	Orthodox
<i>C. bakeriana</i>	Orthodox	NA	NA	Orthodox
<i>D. glandulosa</i>	Orthodox	Intermediate	NA	NA
<i>G. arborea</i>	Orthodox	Orthodox	Orthodox	Orthodox
<i>M. baillonii</i>	Recalcitrant	NA	tend to be Recalcitrant	tend to be Orthodox
<i>P. cerasoides</i>	Intermediate	Orthodox	tend to be Orthodox	NA
<i>P. emblica</i>	Orthodox	Orthodox	Orthodox	Orthodox
<i>S. fruticosum</i>	Recalcitrant	NA	NA	NA
<i>S. rarak</i>	Orthodox	NA	NA	tend to be Orthodox

The morphological traits of seeds are able to be the predicting factors to explain the ability for desiccation tolerance and their seed storage behaviors. Our data suggested a positive association of seed coat thickness with seed storage behavior. The species with thick seed coat tend to be more desiccation tolerant. The hard and thick seed coats found in orthodox seeds serve multiple crucial purposes, including preserving seed viability, maintaining metabolic processes, and protecting the embryos from damage during collection and conditioning (Bonner and Karrfalt, 2008; Yulianti et al., 2020). Moreover, the ability to resist water lost was related to seed mass and seed coat thickness, small seed with hard and thick seed coat appeared to be orthodox whereas large seed with thin seed coat tend to be recalcitrant (Pritchard et al., 2004; Daws et al., 2005). Larger seeds with thin layers of seed coat are able to lose viability rapidly after dried while decreasing in seed size increased more desiccation tolerance (Pritchard et al., 2004; Daws et al., 2005; Ley-López et al., 2014; Yulianti et al., 2020).

Apart from seed morphology, seeds with high moisture content appeared to be recalcitrant, while orthodox seeds typically have low moisture content. Just over one third of the studied species (*C. bakeriana*, *A. fraxinifolius*, *A. microsperma* and *P. emblica*) had orthodox seeds with below 10% moisture content (when collected). Low moisture levels prevent cell damage during freezing, enabling seeds to maintain viability even when frozen to -20°C. Conversely, high levels of moisture content in recalcitrant seeds result in cell rupture, as waters expand and contract during freezing (Chin et al., 1984; Hong et al., 1996). Desiccation sensitivity also varied depending on species; *A. lacucha* completely lost germinability at 20% MC compared with *S. fruticosum*, which showed a significant decrease in viability at 10% moisture content, with complete non-viability at 5% MC.

The recalcitrant seeds: *S. fruticosum* and *A. lacucha* did not survive the dry storage treatment while storing recalcitrant seeds in moist conditions at 99% relative humidity at room temperature can temporarily preserve seed viability. It is crucial to be aware that seed viability may be lost after a duration of 66 days, rendering the seeds non-viable. However, in moist storage, seed viability can be dramatically reduced by fungi (Martin et al., 2022). Thus, it is advisable to sow recalcitrant seeds immediately after collection to achieve higher seed germination.

Most recalcitrant species are dispersed at the beginning of the rainy season, very few a redispersed in the dry season. Unpublished data from FORRU database indicated the dispersal of recalcitrant seeds remains high throughout the rainy season, with some exceptions where recalcitrant seed disperse during the dry season from December to March (Figure 4.18). Notably, 36 and 27 species are dispersed in May and June, respectively, with 17 species being dispersed in both months, resulting in 46 species available at the beginning of the rainy season. This data supports the potential to use recalcitrant species for inclusion in direct/aerial seeding projects, provided they are dispersed at the commencement of the rainy season and sown promptly after seed collection. Furthermore, it is crucial to underscore the importance of careful handling of these species between collection and direct/aerial seeding.

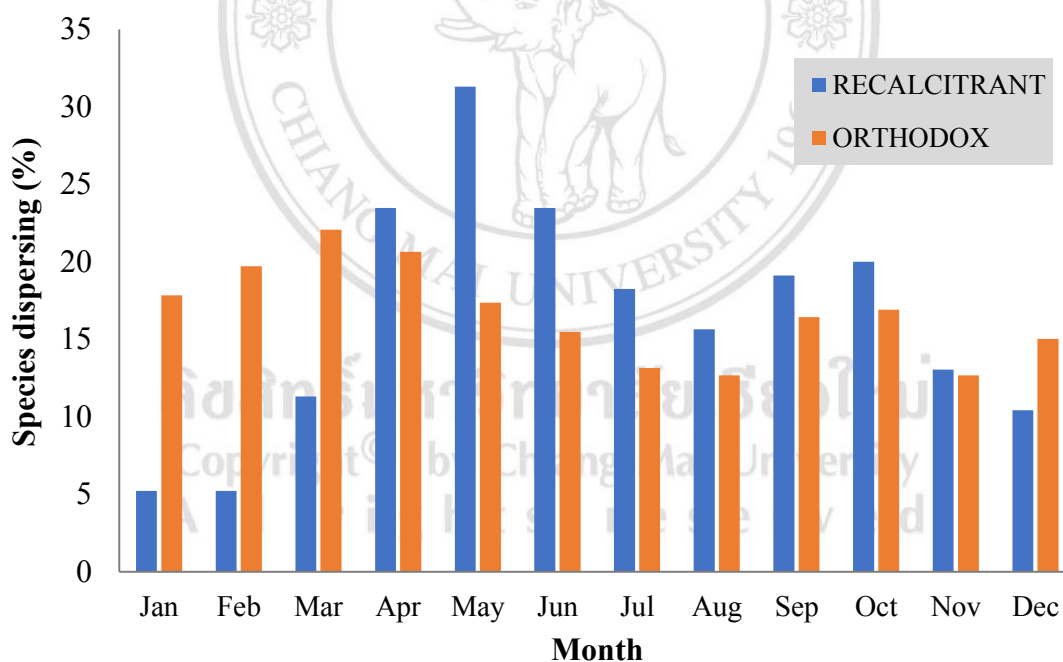


Figure 5.2 Seasonal variation in seed dispersal: a comparison of recalcitrant (■) and orthodox (■) seeds throughout the year (data generated by G. Pakkad and S. Elliott, in total of 328 species in FORRU database).

5.6 Recommended species from this research

Our study suggested that species traits can be used as criteria to make appropriate species choices for direct seeding, particularly rapid and high germination, high seedling survival and growth, with seeds tolerant of desiccation (orthodox seeds) and of medium to large size. Such criteria should also be considered in combination with site factors. For example, where seed predation is likely to be high, selecting seeds with thick, tough seed coats and sowing them far apart from each other is likely to increase overall success.

Utilizing orthodox seeds for direct seeding offers advantages in terms of both seed availability and pre-sowing storage methods. The seeds of orthodox species are more evenly dispersed throughout the year with less pronounced seasonality (Fig. S3). These seeds can be dried, stored, and sown at the beginning of the rainy season without any loss of viability. Moreover, applying seed pre-treatments that accelerate and increase seed germination may also increase success (Table 3.3).

Use of non-orthodox species for direct seeding is more problematic, as indicated by the failure of *A. lacucha*, *G. cowa*, *P. viridis*, *Q. brandisiana*, *S. arboreum* and *S. fruticosum* in this study, despite these species typically exhibiting high seed germination in the nursery (FORRU, 2005; Waiboonya, 2017). However, the use of such species for direct seeding should not be completely rejected, because including them would greatly enhance tree species richness of the restored forest ecosystems. Fortunately, most recalcitrant species disperse their seeds at or shortly before the onset of the rainy season (Fig. S3)—the optimum time for direct seeding—and germinate rapidly immediately thereafter (including those species listed above). Such species often fruit prolifically and are easily collected (FORRU, 2000). So, provided they are sown immediately after collection and they are handled with great care between collection and sowing, they may still be used to diversify restoration by direct-seeding (Waiboonya, 2017).

Table 5.2 Summarizing recommendation for seed practical for direct seeding.

Species	Collection month ³	Sowing time ^a	Storing conditions ^b	Seed pre-treatments	Light requirement for germination
<i>A. fraxinifolius</i>	Apr - Jun	RS	RT & RE ⁴	Soaking in warm water for 24 hours and scarification ^{2,5}	Full sunlight ²
<i>A. microsperma</i>	Sep - Mar	RS	RT ³	Without or with scarification ^{2,4}	Sunlight ⁴
<i>A. kurzii</i>	Jun - Sep	RS	RE ³	No	Sunlight ⁴
<i>A. lacucha</i>	Dec - Jun	IS	-	No ¹	Sunlight ^{1,4}
<i>B. baccata</i>	Apr - Dec	IS	-	Soak in warm water for 2-3 days ¹	Full sunlight ^{2,4}
<i>C. bakeriana</i>	Sep - Jun	RS	RT & RE	Scarification ¹	Sunlight ⁴
<i>C. axillaris</i>	Mar - Aug	RS	RE ³	Soaking in water for 12 hours ¹	Sunlight ⁴
<i>D. glandulosa</i>	May - Oct	RS	RT	Soaking in water for 24 hours ¹	Partial shade ¹
<i>G. cowa</i>	Sep - Jun	IS	-	No ⁶	Shade ^{4,6}
<i>G. arborea</i>	Mar - Jun	RS	RE ³	Soaking in water for 12-24 hours ^{1,3,4}	Sunlight ²
<i>H. dulcis</i>	Nov - Mar	RS	RE ³	Soak in water for 1-2 days ¹	Shade ^{1,5} or 25% sunlight ²
<i>P. cathia</i>	Jul - Sep	IS	-	No	-
<i>M. azedarach</i>	Apr - Aug	RS	RE ³ & RT ^{3,4}	Soak in water for 1-2 days ¹	Sunlight ^{1,4}
<i>M. baillonii</i>	Aug - Mar	RS	RE ³	No ¹	Sunlight ²
<i>P. emblica</i>	May - Mar	RS	RT ³	Scarification ¹	Partial sunlight ¹
<i>P. viridis</i>	Mar - May	IS	-	-	-
<i>P. cerasoides</i>	Feb - May	RS	RE ³	No	Sunlight ^{1,4}
<i>Q. brandisiana</i>	Feb - Jun	IS	-	No	Shade ⁴
<i>S. rarak</i>	Jul - Jan	RS	RT & RE	Scarification ¹	Partial sunlight ^{1,5} or full sunlight ²
<i>S. arboreum</i>	Apr-Jul	IS	-	No ⁴	Shade ⁵
<i>S. pentandrum</i>	Aug - Oct	RS	-	Scarification ⁴	-
<i>S. pinnata</i>	Sep - Mar	RS	RT ³	No	Sunlight ⁴
<i>S. fruticosum</i>	Mar - Aug	IS	-	No ²	Full sunlight ²

¹FORRU (2000); ²FORRU (2006); ³Waiboonya (2017); ⁴FORRU database; ⁵<https://plantflowerseeds.com>) and ⁶NPark flora and fauna web (<https://www.nparks.gov.sg/florafaunaweb>)

^aIS=Immediately sown at the time of collection, RS= Begin of rainy season; ^bRT= stored at room temperature, RE=stored at 4°C in a refrigerator

CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Conclusions

1) Seed removal intensity varied between degraded areas, and among seed species. The bigger seeds had a lower possibility of seed removal compared to small seeds. *A. microsperma*, *Q. brandisiana*, *S. arboreum*, *S. pentandrum* and *S. pinnata* belonged to species without evidence of seed removal. The percentage seed removal was 3.4%; low compared to previous studies.

2) *A. microsperma*, *S. pinnata* and *C. axillaris* are recommended for direct seeding due to their high relative performance. These species had low seed predation, high germinability and seedling yield, as well as the provided good performance in the fields.

3) Seed morphology (seed size), storage behavior and successional status influence tree species ability to establish from seed in degraded areas. Species with medium to large seed-size survive better than small seeds. Moreover, this research study emphasizes the crucial role of seed storage behavior influencing the viability of seeds after sowing in to degraded areas. All the recalcitrant seeds failed to establish in the areas due to their viability loss, whereas orthodox seeds had more success.

4) The pelleting materials; biochar and soil mixture in this study, were effective in decreasing seed removal to almost zero. However, thick layers of the materials affected seed germinability by providing limitation of water, oxygen, and light (for some species) that necessary for seed germination. Moreover, the materials did not promote seedling yield and growth in the degraded areas.

5) Actinobacteria coatings *Streptomyces antibioticus* and *S. thermocarboxydus* isolate S3 were not shown to affect direct seeding success.

7.2 Recommendations

1) Site conditions such as level of degradation, condition of canopy cover, ground flora, soil condition and potential seed predator should be quantified before applying direct seeding. Direct seeding strategies, such as for species selection, seed distribution/sowing and treatment to prevent seed predation, can be planned, based on site information.

2) For degraded areas with high risk of seed predation, particularly by rodents, applying pelleting materials or seed balls may protect the seeds. Biochar or soil mixture are recommended as effective materials prevent seed predation, but modification of pelleting layer is necessary.

3) Direct seeding is approved for species with rapid and high seed germination as correlated with a higher chance of successful seedling establishment. Additionally, fast-growing species that produce substantial seedlings which can outcompete weeds, are considered ideal candidates for direct seeding. Baseline data collected in the nursery can serve as valuable information for decision-making, as the germination rates of most species tend to be consistent between field conditions and tree nurseries. Additionally, further studies are needed on topics such as seed dormancy and pre-treatment methods to promote seed germination, especially for species with low seed germination rates but good seedling performance.

4) To ensure the preservation of seed viability after sowing, it is advisable to select orthodox seeds. Nevertheless, recalcitrant seeds can be employed for immediate sowing, preferably during the period just before or at the beginning of the rainy season.

The limitation of species establishment is the priority task for forest restoration by direct seeding. Additionally, it is crucial to consider potential future climate scenarios that could affect ecosystem restoration efforts. However, this approach can lay the fundamental for the widespread aerial distribution of seeds to recover degraded areas with suitable species selection and various techniques to overcome the limitations of direct seeding.

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APPENDICES

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
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APPENDIX I

Publication

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Differential seed removal, germination and seedling growth as determinants of species suitability for forest restoration by direct seeding – A case study from northern Thailand



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ABSTRACT

Background: Direct seeding is potentially a more cost-effective alternative to conventional tree planting for restoring tropical forest ecosystems. However, seed loss, due to removal and damage by animals, can substantially reduce seedling establishment. Therefore, this study examined the impact of seed predation on seedling establishment of five tree species, native to upland evergreen forests of northern Thailand: *Hovenia dulcis*, *Alangium kurzii*, *Prunus cerasoides*, *Choerospondias axillaris* and *Horsfieldia amygdalina*. We tested the hypothesis that excluding animals would significantly reduce seed removal, and increase both germination and seedling survival. The objective was to calculate a composite index of the relative suitability of the species studied for direct seeding. **Methods:** Seeds were placed on the ground in a deforested site and subjected to five predator-exclusion treatments: wire cage, insecticide, cage + insecticide, open cage and no exclusion (control). **Results:** Seed loss was highest for *H. amygdalina* (the largest seed tested). Across species, wire cages significantly reduced seed loss by 12.4% compared with controls ($P < 0.001$) suggesting that vertebrates were the major seed predators. Seed germination ranged from 0 to 77% among the species tested. Based on relative species-performance scores (combining measures of survival and seedling growth), *P. cerasoides* was the most suitable species for direct seeding, followed by *A. kurzii* and *C. axillaris*, whilst *H. dulcis* and *H. amygdalina* were unsuitable. *H. dulcis* had small seeds with low seed germination, whereas *H. amygdalina* was subjected to high seed removal. **Conclusion:** Exclusion of seed predators and the selection of suitable species may substantially increase the success of direct seeding, as a technique for restoring upland evergreen forest ecosystems. Testing more species for their suitability is needed, to provide more diverse options for forest restoration.

1. Introduction

Despite recent international commitments to “halt and reverse” deforestation by 2030 (UK Government, 2021), losses of primary tropical forest increased by 10% in 2022 to 4.12 million hectares (compared with 3.75 million the previous year), releasing 2.7 gigatonnes (Gt) of carbon dioxide into the atmosphere (Weisse et al., 2023). Such rapid deforestation also results in substantial biodiversity loss (Thomas et al., 2004; Giam, 2017; Oakley and Bicknell, 2022) and exacerbates rural poverty (Chomitz, 2007). Agriculture remains by far the most significant deforestation driver, accounting for more than 90% of forest loss globally (Seydewitz et al., 2023). To counteract such deforestation, restoration of diverse forest ecosystems on deforested/degraded areas is being

implemented on vast scales in many countries, under ambitious schemes such as the UN’s “Decade on Ecosystem Restoration” (2021–2030) (UNEP and FAO, 2020) and the Bonn Challenge, which calls for reforestation of 350 million hectares by 2030 (Wentink, 2015).

Most usually, conventional forest restoration involves growing tree saplings in nurseries, transporting them to restoration plots, planting them and maintaining them thereafter (Lamb and Gilmour, 2003; Elliott et al., 2013; Verdone, 2015), although so-called “passive” restoration (which relies on natural regeneration) is now becoming popular, despite doubts about its effectiveness (Reid et al., 2018). Even though tree-planting involves several arduous, time-consuming and expensive tasks (Elliott et al., 2013), it is still widely practiced and is often successful (Ruiz-Jaen and Aide, 2005; Elliott et al., 2013; Ceccon et al.,

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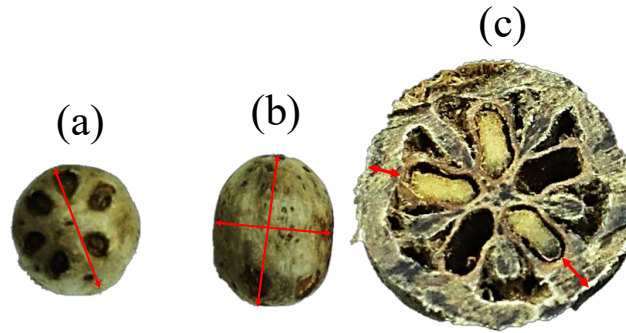
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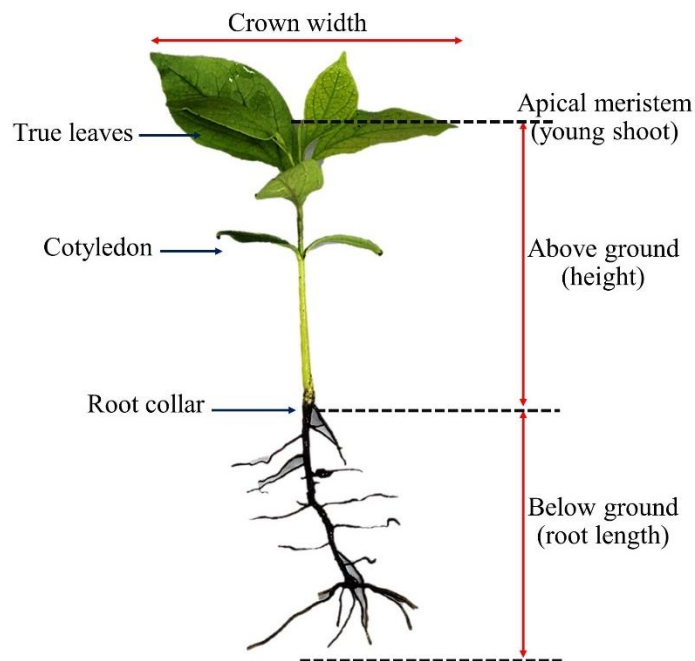
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Appendix II

Species traits



The measurements of seed traits include seed width (a), length and depth (b), and seed coat thickness (c).



The measurements of seedling traits include crown width, aboveground/stem height, belowground part (root length), and root collar diameter.

The characteristic of seeds for 23 native tree species

Scientific name	Width		Length		Depth		Coat-thickness		Wet weight		Dry weight		Moisture content	
	(mm)	±SD	(mm)	±SD	(mm)	±SD	(mm)	±SD	(g)	±SD	(g)	±SD	(%)	±SD
<i>Acrocarpus fraxinifolius</i>	4.52	±0.22	6.69	±0.51	1.71	±0.16	0.13	±0.01	0.04	±0.00	0.03	±0.00	3.69	±2.74
<i>Adenanthera microsperma</i>	6.19	±0.62	6.50	±0.66	4.09	±0.42	0.46	±0.11	0.11	±0.02	0.10	±0.02	6.97	±1.76
<i>Alangium kurzii</i>	6.64	±0.37	11.80	±0.52	4.66	±0.38	0.52	±0.07	0.22	±0.02	0.18	±0.02	21.20	±1.14
<i>Artocarpus lakoocha</i>	8.49	±0.83	10.28	±0.97	7.36	±1.29	0.14	±0.05	0.35	±0.09	0.17	±0.04	49.41	±11.19
<i>Balakata baccata</i>	6.03	±0.71	5.84	±0.44	4.64	±0.77	0.18	±0.02	0.10	±0.14	0.05	±0.01	25.72	±16.64
<i>Cassia bakeriana</i>	7.41	±0.51	9.04	±1.11	6.11	±1.24	0.38	±0.08	0.29	±0.04	0.27	±0.04	6.53	±1.51
<i>Choerospondias axillaris</i>	13.26	±0.78	17.34	±1.25	13.37	±0.71	2.35	±0.20	1.92	±0.36	1.43	±0.27	24.62	±10.69
<i>Diospyros glandulosa</i>	8.93	±1.50	14.20	±1.07	2.80	±0.27	0.25	±0.19	0.30	±0.07	0.26	±0.06	13.22	±5.68
<i>Garcinia cowa</i>	16.18	±1.69	30.25	±2.97	11.48	±1.74	0.57	±0.17	2.49	±0.58	1.76	±0.36	28.95	±4.05
<i>Gmelina arborea</i>	9.44	±0.74	15.71	±2.13	8.86	±0.65	1.11	±0.33	0.62	±0.13	0.52	±0.10	15.47	±2.52
<i>Hovenia dulcis</i>	4.24	±0.42	4.32	±0.35	2.05	±0.17	0.27	±0.02	0.03	±0.01	0.03	±0.01	5.82	±2.15
<i>Melia azedarach</i>	3.67	±0.90	10.84	±0.87	2.84	±0.17	0.24	±0.05	0.05	±0.01	0.05	±0.01	5.75	±0.56
<i>Michelia bailonii</i>	5.36	±1.17	8.06	±1.27	3.09	±0.31	0.30	±0.05	0.06	±0.02	0.05	±0.01	21.59	±3.42
<i>Phoebe cathia</i>	6.15	±0.27	5.46	±0.25	6.02	±0.35	0.02	±0.01	0.13	±0.03	0.08	±0.02	35.64	±7.15
<i>Phyllanthus emblica</i>	2.87	±0.18	4.90	±0.48	2.49	±0.19	0.21	±0.03	0.02	±0.00	0.02	±0.00	6.44	±1.94
<i>Polyalthia viridis</i>	12.71	±1.16	20.96	±2.44	12.30	±0.82	0.22	±0.04	2.72	±0.05	1.55	±0.22	42.55	±3.31
<i>Prunus cerasoides</i>	7.15	±0.80	9.38	±0.84	5.64	±0.44	0.84	±0.19	0.20	±0.04	0.18	±0.04	19.64	±2.96
<i>Quercus brandisiana</i>	14.42	±1.66	18.17	±1.76	14.23	±1.44	0.42	±0.04	2.63	±0.77	1.78	±0.54	32.69	±2.87
<i>Sapindus rarak</i>	13.99	±0.68	15.25	±0.76	13.61	±0.75	1.52	±0.17	1.99	±0.29	1.95	±0.25	10.30	±2.46
<i>Sarcosperma arboreum</i>	12.88	±0.87	20.20	±2.71	12.40	±0.83	0.22	±0.06	2.11	±0.46	1.34	±0.21	35.65	±4.70
<i>Scleropyrum pentandrum</i>	17.07	±1.64	20.65	±2.32	16.89	±1.50	0.99	±0.18	3.41	±0.67	2.53	±0.67	27.14	±7.92
<i>Spondias pinnata</i>	22.98	±1.50	31.84	±1.18	21.65	±1.20	6.18	±1.00	5.29	±1.03	4.26	±0.68	18.81	±3.98
<i>Syzygium fruticosum</i>	8.17	±0.74	15.01	±1.29	7.76	±0.65	0.15	±0.02	0.62	±0.12	0.37	±0.07	39.17	±2.93

The characteristic of above ground-part for 23 native tree species

Scientific name	Shoot (stem)					RGR in nursery							
	Height (cm)	±SD	dry weight (g)	±SD	Moisture (%)	±SD	CW (cm)	±SD	RCD (mm)	±SD	%RGR-RCD	%RGR-Height	%RGR-CW
<i>A. fraxinifolius</i>	7.2	± 1.5	0.1	± 0.0	81.6	± 2.9	7.4	± 1.8	1.7	± 0.3	292.3	303.3	335.7
<i>A. kurzii</i>	8.5	± 1.5	0.1	± 0.0	83.3	± 2.1	10.0	± 1.8	2.0	± 0.2	228.8	307.0	320.6
<i>A. lacucha</i>	9.8	± 2.0	0.1	± 0.0	85.5	± 2.8	12.2	± 3.4	1.9	± 0.4	230.6	459.8	386.5
<i>A. microsperma</i>	12.0	± 1.8	0.2	± 0.0	79.3	± 0.9	12.8	± 1.3	2.1		232.9	218.3	369.1
<i>B. baccata</i>	10.8	± 1.9	0.1	± 0.1	84.3	± 1.9	10.7	± 1.6	2.1	± 0.2	254.4	338.7	294.8
<i>C. axillaris</i>	11.8	± 2.7	0.3	± 0.1	75.5	± 7.5	11.6	± 2.4	2.3	± 0.3	114.9	307.8	253.1
<i>C. bakeriana</i>	12.7	± 2.8	0.3	± 0.3	58.4	± 55.0	17.7	± 3.9	1.8	± 0.2	184.4	343.0	272.9
<i>D. glandulosa</i>	8.2	± 0.8	0.1	± 0.0	81.8	± 1.6	8.4	± 1.5	3.3	± 0.4	40.3	336.7	373.3
<i>G. arborea</i>	7.8	± 1.2	0.1	± 0.0	83.1	± 3.6	4.9	± 1.6	1.7	± 0.2	114.8	80.0	222.4
<i>G. cova</i>	19.2	± 3.2	0.4	± 0.3	85.8	± 4.3	17.8	± 4.1	2.7	± 0.2	288.1	441.1	211.7
<i>H. dulcis</i>	10.0	± 3.0	0.1	± 0.0	82.4	± 1.5	10.9	± 2.6	1.5	± 2.3	371.3	605.6	399.2
<i>M. azedarach</i>	9.9	± 1.5	0.2	± 0.1	83.9	± 4.1	16.0	± 5.7	2.4	± 0.3	261.9	437.3	224.4
<i>P. cerasoides</i>	6.4	± 1.2	0.1	± 0.0	72.0	± 2.0	6.2	± 1.2	1.5	± 0.2	97.3	159.7	89.6
<i>P. emblica</i>	11.5	± 4.2	0.1	± 0.0	82.5	± 4.8	9.4	± 2.1	1.1	± 0.3	315.0	642.5	546.7
<i>P. cathita</i>	10.3	± 2.2	0.4	± 0.2	78.1	± 2.2	11.2	± 2.4	2.4	± 0.0	111.2	89.9	171.6
<i>P. viridis</i>	12.3	± 2.7	0.2	± 0.1	79.0	± 1.7	12.4	± 2.7	1.8	± 0.4	192.0	560.7	320.4
<i>Q. brandisiana</i>	15.8	± 1.4	0.3	± 0.1	54.3	± 3.5	9.0	± 2.5	1.8	± 0.3	69.8	4.7	-1.3
<i>S. arboreum</i>	15.0	± 2.3	0.6	± 0.2	75.7	± 2.8	27.1	± 3.6	3.2	± 0.2	130.7	255.0	204.8
<i>S. fruticosum</i>	14.2	± 2.4	0.2	± 0.0	83.0	± 2.7	17.4	± 2.8	2.5	± 0.2	179.8	177.9	132.1
<i>S. pentandrum</i>	11.2	± 1.7	0.1	± 0.0	75.0	± 2.2	5.1	± 1.0	2.9	± 0.7	45.5	108.1	161.1
<i>S. pinnata</i>	28.6	± 5.6	1.2	± 0.6	87.6	± 3.8	26.3	± 7.5	4.0	± 0.2	216.4	257.2	233.7
<i>S. rarak</i>	9.7	± 1.8	0.1	± 0.1	76.0	± 2.2	8.5	± 1.8	1.4	± 0.1	215.4	185.2	312.2

The characteristic of roots for 23 native tree species

Scientific name	Length (cm)	±SD	Dry weight (g)	±SD	%moisture	±SD	SRL*
<i>A. fraxinifolius</i>	12.5	± 4.7	0.0	± 0.0	87.7	± 1.7	549.8
<i>A. kurzii</i>	7.6	± 2.7	0.0	± 0.0	85.3	± 5.5	442.5
<i>A. lacucha</i>	13.3	± 3.9	0.0	± 0.0	88.2	± 1.2	303.2
<i>A. microsperma</i>	13.1	± 4.0	0.2	± 0.1	78.8	± 4.0	57.5
<i>B. baccata</i>	13.5	± 7.3	0.0	± 0.0	82.1	± 13.6	376.4
<i>C. axillaris</i>	17.8	± 7.8	0.1	± 0.0	83.6	± 3.2	238.5
<i>C. bakeriana</i>	12.2	± 3.8	0.1	± 0.2	85.0	± 5.1	99.5
<i>D. glandulosa</i>	6.1	± 2.5	0.0	± 0.0	85.9	± 3.1	161.2
<i>G. arborea</i>	13.7	± 4.0	0.1	± 0.0	88.4	± 3.4	192.5
<i>G. cowa</i>	21.9	± 4.9	0.2	± 0.2	85.3	± 6.0	109.2
<i>H. dulcis</i>	14.7	± 4.9	0.0	± 0.0	85.7	± 2.3	344.0
<i>M. azedarach</i>	14.7	± 3.8	0.1	± 0.0	87.5	± 9.1	268.2
<i>P. cerasoides</i>	5.4	± 0.8	0.0	± 0.0	81.4	± 2.5	170.1
<i>P. emblica</i>	11.7	± 4.9	0.0	± 0.0	87.4	± 5.5	899.9
<i>P. cathia</i>	11.2	± 4.8	0.1	± 0.0	87.2	± 1.6	186.9
<i>P. viridis</i>	12.7	± 3.3	0.0	± 0.0	86.7	± 3.1	256.1
<i>Q. brandisiana</i>	11.3	± 3.2	0.4	± 0.5	57.1	± 3.4	31.1
<i>S. arboreum</i>	16.2	± 3.5	0.1	± 0.1	86.7	± 1.8	127.5
<i>S. fruticosum</i>	14.5	± 3.4	0.1	± 0.0	87.5	± 1.5	149.9
<i>S. pentandrum</i>	7.7	± 2.6	0.1	± 0.0	77.6	± 2.7	72.3
<i>S. pinnata</i>	21.1	± 7.5	0.2	± 0.1	80.4	± 32.1	112.6
<i>S. rarak</i>	13.3	± 4.4	0.1	± 0.1	78.0	± 2.8	117.3

*SLR = specific root length in nursery (root length/dry root mass)

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APPENDIX III

Description of studied-tree species

Descriptions of each plant species in this study are based on Gardner *et al.* (2007), The Botanical Garden Organization (2011), Pakkad (1997) and FORRU (2006). Plant scientific names, family names and local names follow The Plants of the World Online (POWO 2019), The Plant List (2013) and Gardner *et al.* (2007). Other information for seed and seedling morphological characteristic were measured in this study.

Species name: *Adenantha microsperma* Teijsm.&Binn.

Common name: Maklam-Takai

Family: LEGUMINOSAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves	■	■	■	■	■	■	■	■	■	■	■	■
Flowering	■	■	■	■	■	■	■	■	■	■	■	■
Fruiting	■	■	■	■	■	■	■	■	■	■	■	■

Briefly deciduous tree to 20 m with uneven, rounded crown and large, spreading branches. Common, usually in gaps or at the forest edge at 200-110 m above sea level, often planted. Easily recognized by the bipinnate leaves with alternate leaflets and glossy red seeds.

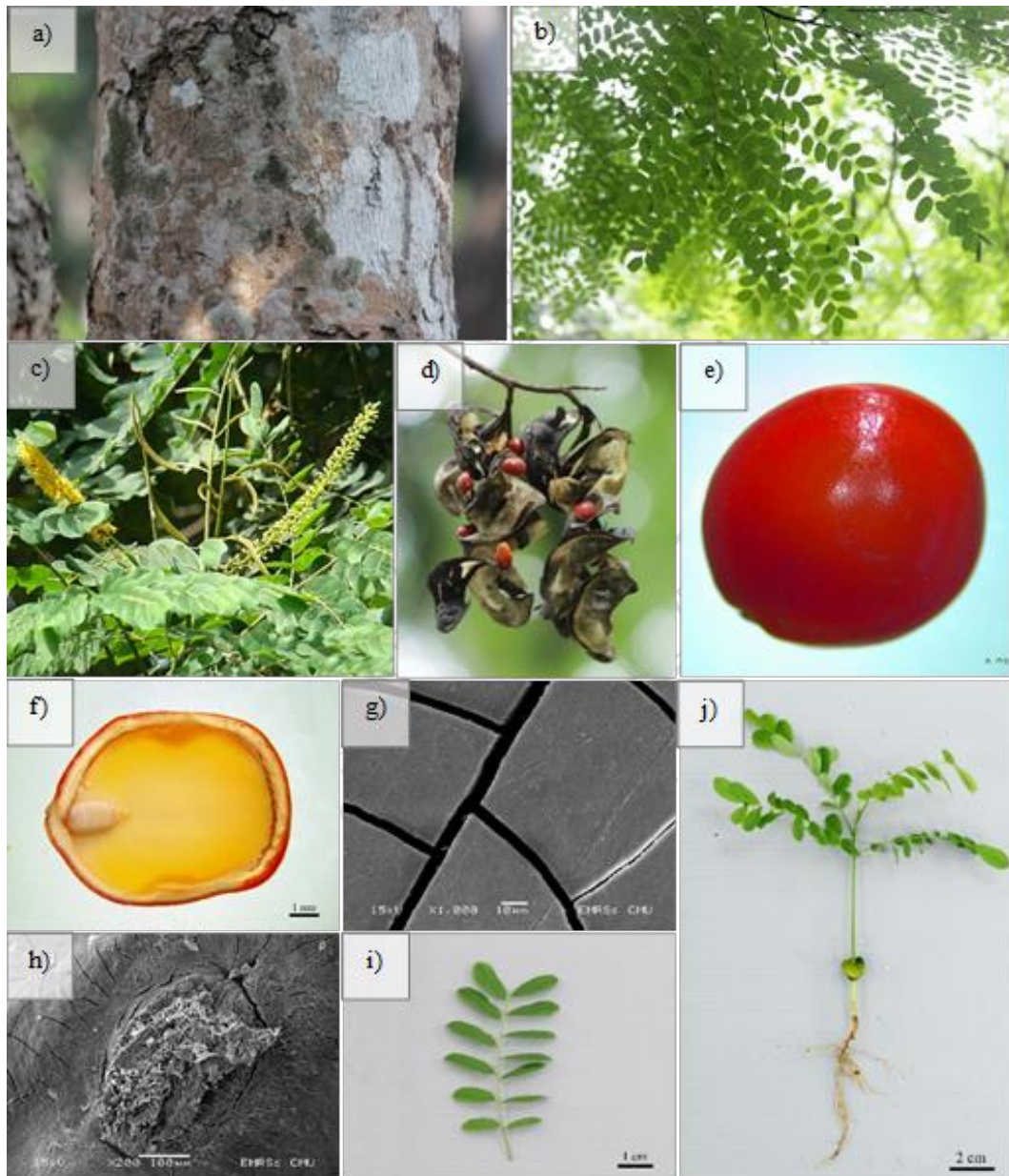
Bark: dark brown or greyish, flaking, inner bark soft, pale cream.

Leaf: bipinnate, 3-6 pairs of opposite side stalks, each with 5-8 (12) pairs of alternate leaflets, 1.5-3.5x1-2 cm, oval or oblong with blunt or rounded tip and asymmetric base. Mature leaflets smooth, dark grey-green above, paler and slightly glaucous below. Leaf stalks without glands, stipules very small, falling early.

Flower: 0.3 cm, creamy-yellow turning orange with age, in spike-like clusters at upper leaf axils or branched clusters at end of twigs, 7.5-20 cm. Flowers opening gradually from base of cluster upwards, faintly scented of orange blossoms especially in the early evening. Individual flower stalks 1.5-3 mm, silky hairy, calyx <1 mm, 5 petals

2.5-3 mm, fused at very base, narrow with pointed tips. 10 free stamens, as long as petals, anthers without hairs but with a gland at tip.

Fruit: 15-20x0.8-1.2 cm, strap-shaped, twisted in a tight coil, very thin, splitting in two strips. Seeds 5-8 mm, bright red, smooth and glossy, remaining in pods a long time.



Bark (a), leaves (b), flowers (c), dry pods (d), seed (e), crosssection of seed shows endosperm and embryo (f) surface of seed 1000x under SEM (g), hilum (h), single leaf (i) and seedling (j) of *A. microsperma* species.

Species name: *Acrocarpus fraxinifolius* Wight&Arn.

Common name: Shingle Tree

Family: LEGUMINOSAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

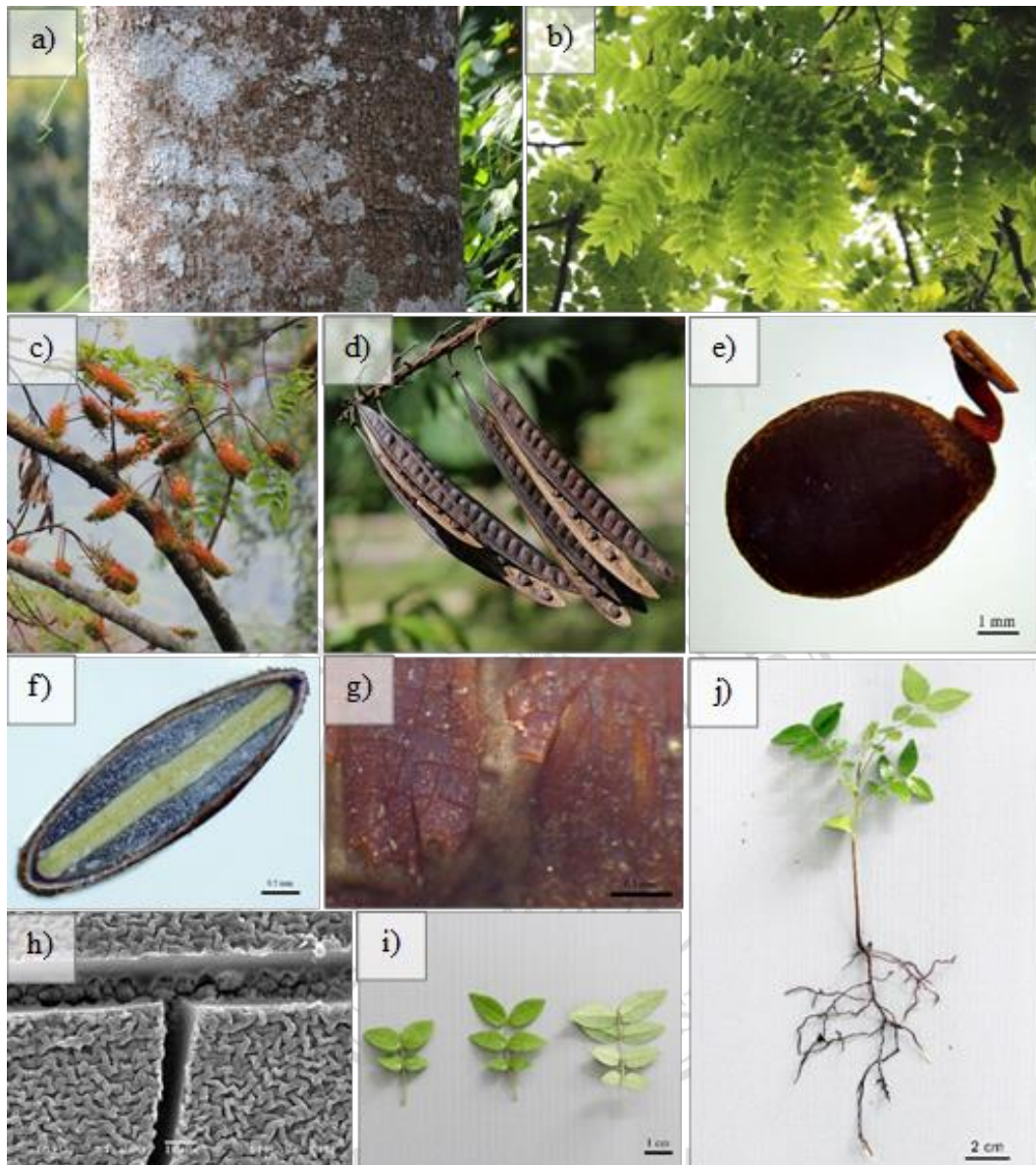
Very fast growing and large tree to 50 m and 100 cm diameter in less than 100 years, briefly deciduous at beginning of cold season. Crown irregular & rather sparse with steeply ascending main branches & a long straight trunk, often buttressed when older. One of the forest giants of Northern Thailand, a common feature of the emergent layer in moist evergreen forests, at 500-1250 meter above sea level.

Bark: pale grey, sprinkled with large brown lenticels, inner bark pinkish, heartwood dark red.

Leaf: up to 100 cm, bipinnate with 3-5 pairs of side stalks, each with 4-9 pairs of leaflets, 4-14x2-7 cm, ovate with long pointed tips & slightly asymmetric base. Young leaves pink and slightly hairy, mature leaves pale green, completely smooth. Main stalks swollen at base, with small triangular stipules which fall early.

Flower: 1-1.5 cm, in dense spike-like clusters close to tips of leafless branches, 15-25 cm, main stalks thick and fleshy, individual stalks 0.6-0.8 cm. 5 bright green sepals, 0.3-0.4 cm, rounded, slightly overlapping, fused at base, finely hairy. 5 red petals, 0.6-1 cm, narrow and pointed. 5 yellow-orange stamens, twice as long as petals. 1 short, curved pale green style with small stigma.

Fruit: 8-16 x 1-2 cm, black and shiny, flattened, pointed at both ends with a thick ridge or narrow wing along the top joint, splitting into 2 sections. 10-18 pale brown, lens-shaped seeds.



The characteristic of *A. fraxinifoliosa*; bark (a), leaves (b), flowers (c), dry pods (d), seed (e), cross-section of seed shows embryo and cotyledon (f) seed surface under light microscope (g) and 1200x under SEM (h), leaves (i) and one-month-seedling (j)

Species name: *Alangium kurzii* Craib

Common name: Sa Leek Dong

Family: CORNACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

Pioneer tree, growing up to 28 m tall. Common in evergreen forest at elevation 600-1,400 m above sea level.

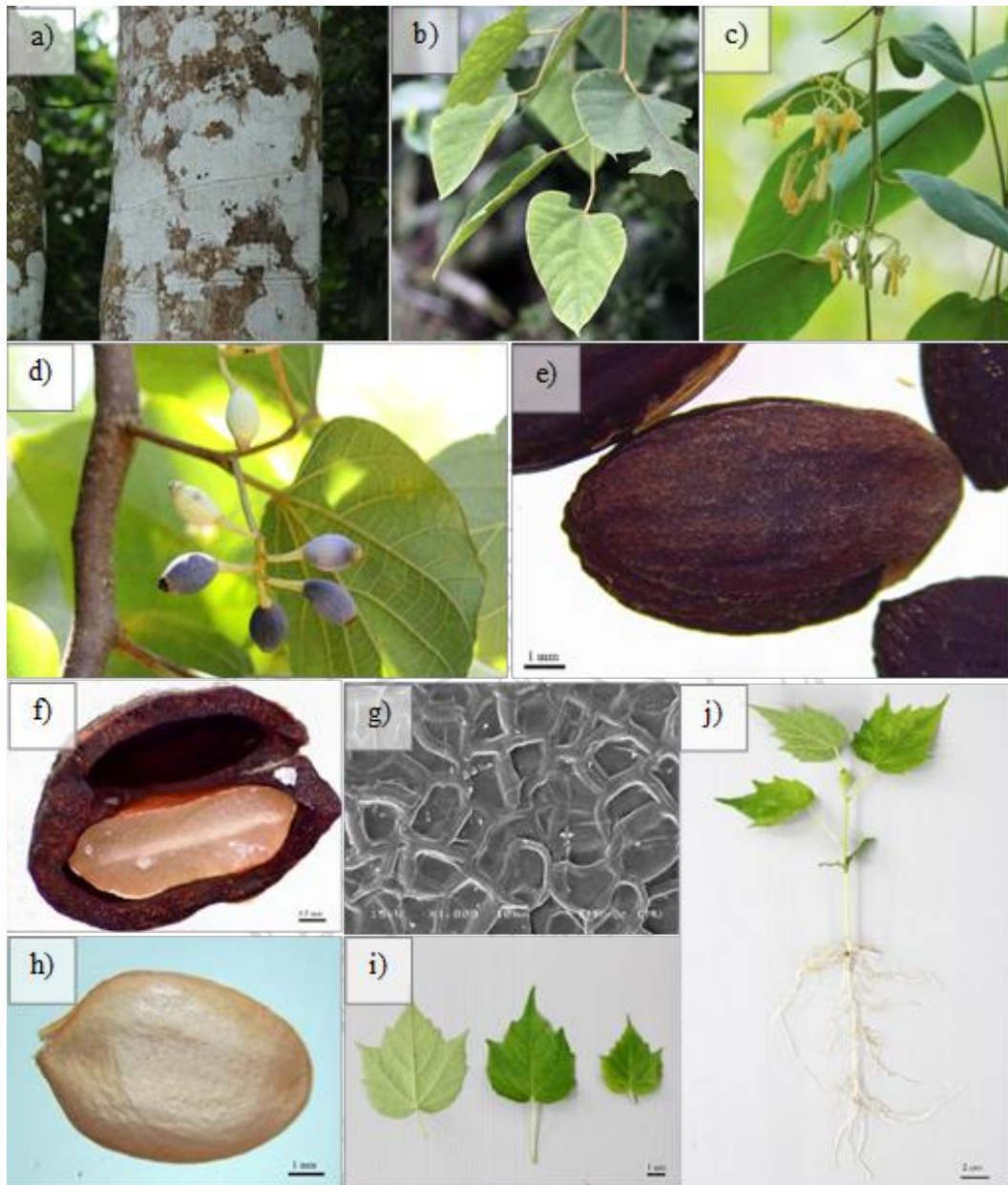
Bark: smooth, dark grey, lenticellate; inner bark orange and cream mottled.

Leaf: broadly ovate with tapering tip and heart-shaped base, obviously asymmetric; mature leaves densely covered with soft golden hair below and on veins only above.

Flower: very fragrant, main stalks to 7-9 petals, dense silvery hairs, connectives also hairy.

Fruit and seed: 1.2-1.5 cm, ellipsoid with blunt tip, smooth to thinly hairy, sometime slightly grooved, crowned by distinct disc, ripening dark purple to black (June to September), contains one black seed (7.08 x 11.83 x 4.67 m³ of seed volume), oval with pointed ends, bird-dispersed species.

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Bark (a), leaves (b), flowers (c), freshy fruits (d), pyrene (e), cross-section of pyrene shows seed's endosperm (f) surface of seed 1000x under SEM (g) shape of seed (h) single leaf (i) and seedling (j) of *A. kurzii* species.

Species name: *Artocarpus lacucha* Roxb.

Common name: Monkey Jack

Family: MORACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

Deciduous tree to 24 m. Common in semi-open areas, 200-1500 m above sea level

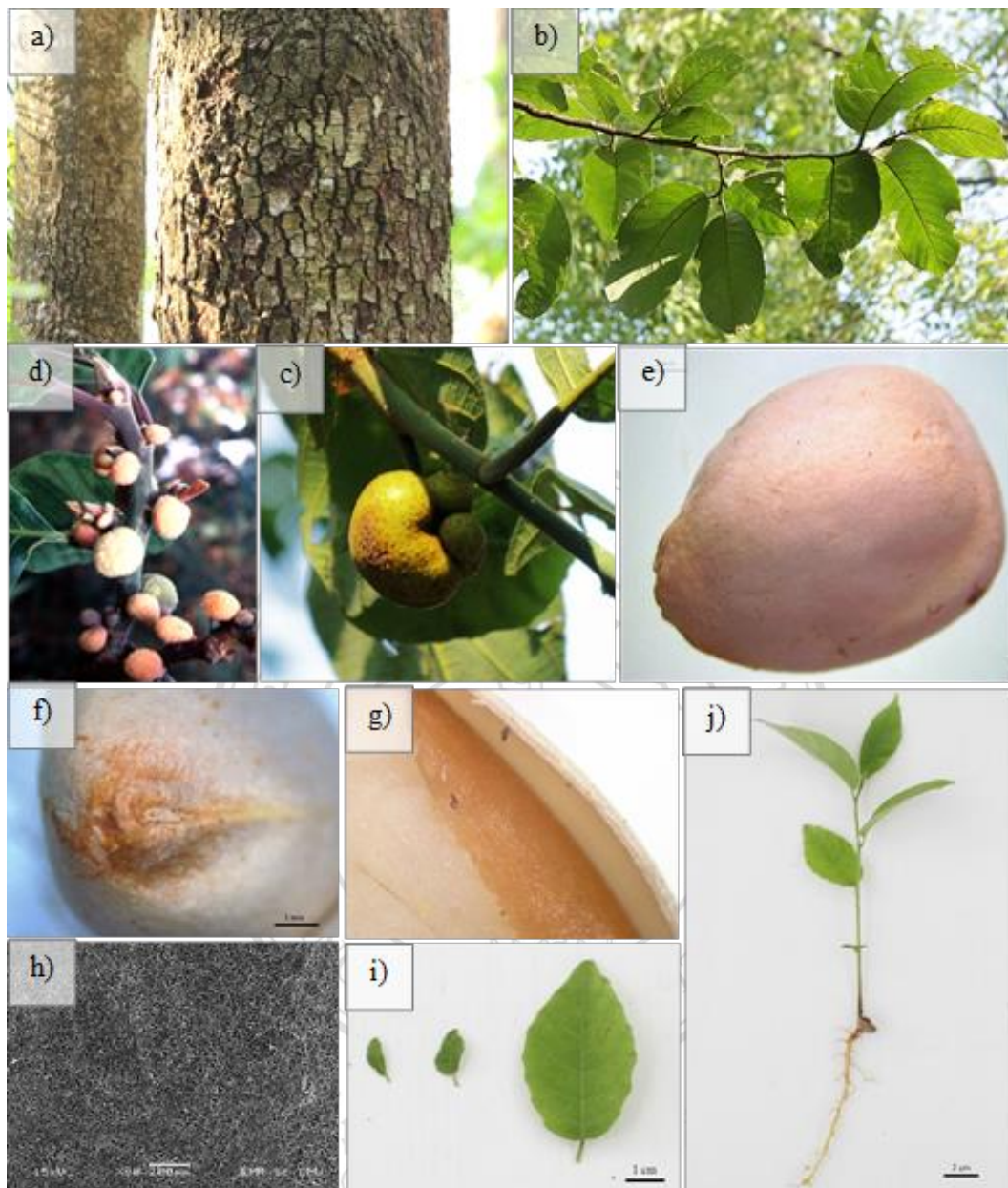
Bark: red-brown to dark brown, becoming rough and scaly with age.

Leaf: simple, alternate, \pm planar, oval to broadly ovate or obovate with blunt or shortly pointed tip and rounded or slightly heart-shaped base, often asymmetric, untoothed or with minute teeth. Young shoots densely red-brown hairy, mature leaves leathery, dark green and slightly rough above, grey-green and finely hairy below. Stalks finely brown-hairy with small lanceolate stipules which fall early. Twigs rather stout, without ring scars.

Flower: heads dirty yellow to pale pink or orange, solitary at leaf axils or just behind leaves. Male heads 0.8-2 cm, globular, stalks 0.8-2 cm. Female heads 1.2-2.3 cm, oval or oblong, usually behind leaves, stalks 2.5-3.5 cm.

Fruit: 2.5-8 cm, stalks 1.2-3.8 cm, pale yellow or orange, irregularly globose or fist-shaped, knobbly and velvety outside, pink inside with many oblong seeds, \pm 1.2 cm.

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Bark (a), leaves (b), flowers (c), fresh fruits (d), seed (e), hilum (f), cross-section of seed (g) surface of seed 80x under SEM (h) single leaf (i) and seedling (j) of *A. lacucha* species.

Species name: *Balakata baccata* (Roxb.) Esser

Common name: Mousedeers Rubber Tree

Family: EUPHORBIACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

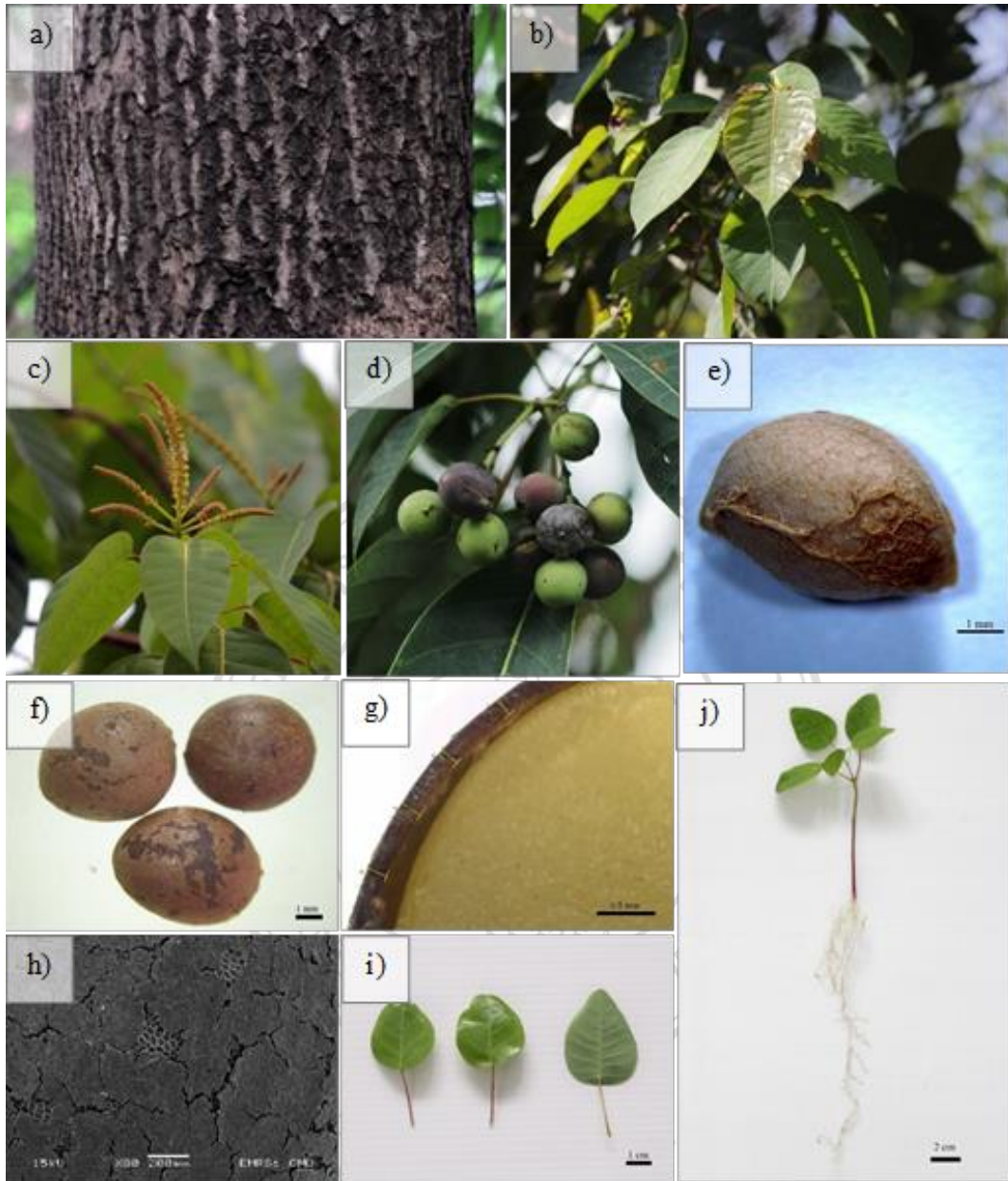
Large evergreen tree to 35 m with spreading rounded crown & thick steeply ascending branches with drooping tips. Trunk stout, up to 200 cm diameter, slightly buttressed when older. common to locally abundant in evergreen & moister deciduous forests, 375 – 1500 m above sea level. Fruits are very attractive to birds.

Bark: pale grey & quite smooth with large lenticels when young, becoming dark grey-brown & deeply fissured with age, inner bark pale yellow, no latex in trunk but often with white latex in twigs.

Leaf: 8-18x3-8 cm, alternate, spiral, elliptic or ovate with pointed or tapering tip and blunt or rounded base, slightly peltate in younger trees, untoothed, completely smooth. Young leaves red-purple, mature leaves dark green above, grey-green (glaucous) below, usually reddish along margins & on stalks, with 2 dark knob-like glands at base of leaf. 11-13 pairs of + parallel side veins, tertiary veins ladder-like. Stalks 3-7.5 cm, slender, slightly swollen at both ends, Stipules small & falling early. Old leaves clear yellow but with red stalks.

Flower: minute, in branched spike-like clusters at end of twigs & upper leaf axils, 4-22 cm, all males or with males & females in same cluster. Males in groups of 6 in axil of an obovate bract, +1 mm, flanked by 2 large oblong glands. 2-3 sepals fused into a toothed cup, +1 mm, no petals, 2 stamens, no disc. Females solitary, +5 mm, calyx as males but larger, 2 styles, slightly fused at base, 1-1.5 mm.

Fruit: 0.8-1.3 cm, dark green with greyish dusting and whitish sap when young, ripening dark purple-black. individual stalks slender, 0.6-0.9 cm, pear-shaped or subglobose, +slightly 2-lobed, with 2 small, recurved styles at top & persistent calyx at base. The outer layer is thin, not splitting, with leathery inner layer and 2 black seeds which remain attached to the central column for a long time after fruits disintegrate.



Bark (a), leaves (b), flowers (c), freshy fruits (d), pyrene (e), seed (f), cross-section of seed shows layer of seed coat and endosperm (g) surface of seed 80x under SEM (h), single leaf (i) and seedling (j) of *B. baccata* species.

Species name: *Cassia bakeriana* Craib

Thai name: Pink Shower Tree

Family: LEGUMINOCEAE

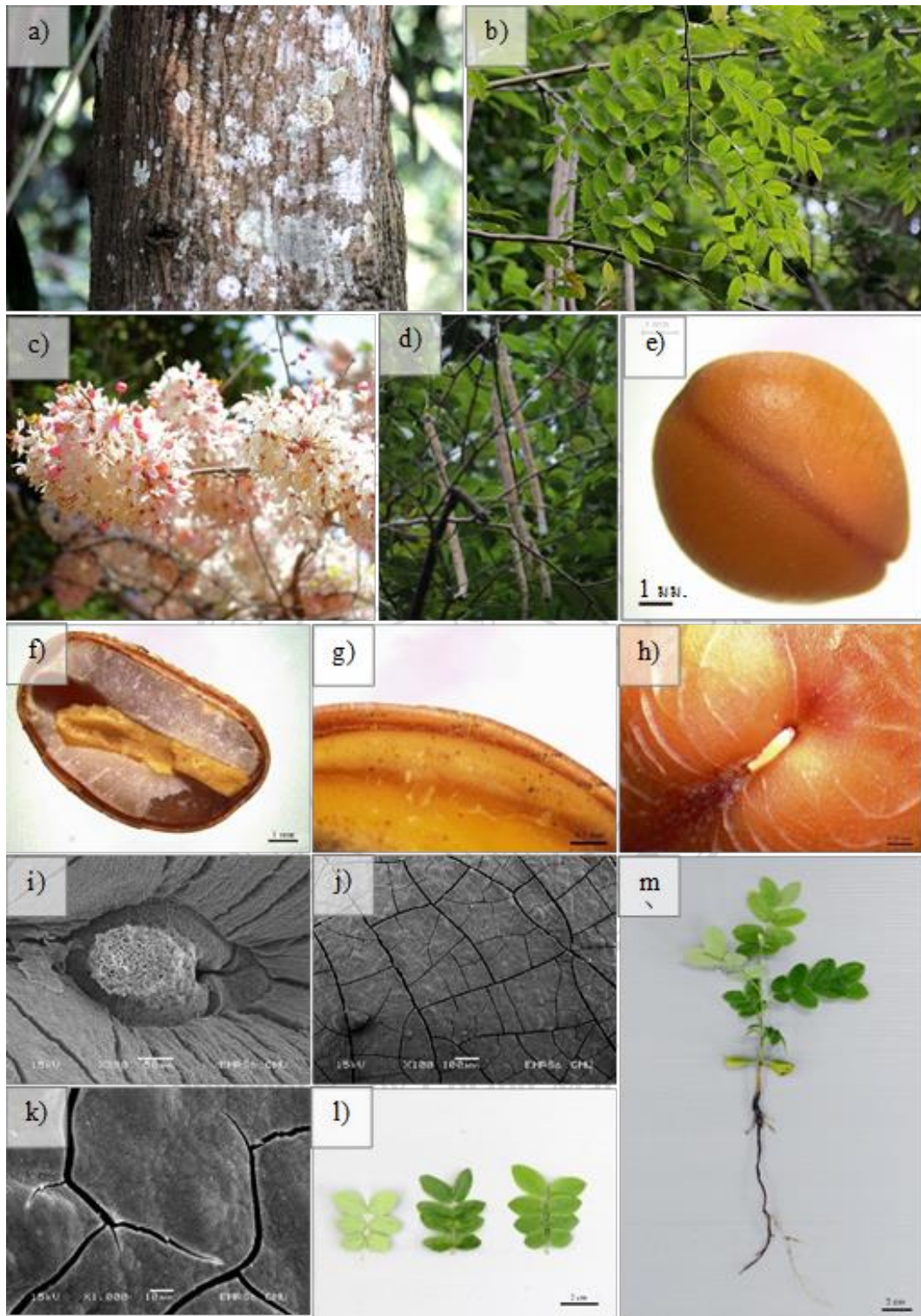
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves	■	■	■	■	■	■	■	■	■	■	■	■
Flowering	■	■	■	■	■	■	■	■	■	■	■	■
Fruiting	■	■	■	■	■	■	■	■	■	■	■	■

Small tree to 12 m with wide, spreading crown & leaves in flattened sprays. native to Northern Thailand, scattered in semi-open forests and sometimes planted, elevation 800 – 1350 m.

Leaf: 5-7 pairs of leaflets, rounded at both ends or with very short tip. Young leaves densely silky hairy, mature leaves with short velvety hairs below, no glands. Stipules narrow and pointed, attached in the middle.

Flower: in upright, unbranched clusters, usually behind the leaves, 10-20 cm. Individual stalks dark red-purple, slender, to 6 cm. Sepals 0.9-1.2 cm, hairy, dark red-purple. Petals 3-4.5 cm, pink fading to almost white. 3 stamens longer than others, filaments swollen in the middle, anthers very small.

Fruit: brown or grey, narrowly tubular, finely hairy. Stalks +6 cm.



Bark (a), compound leaves (b), flowers (c), dry pods (d), seed (e), cross-section of seed shows layer of seed coat and endosperm (f-g), hilum under light microscope (h) and SEM (i), surface of seed 100x and 1000x under SEM (j-k), single leaf (l) and seedling (m) of *C. bakeriana* species.

Species name: *Choerospondias axillaris* Roxb.

Thai name: Nepali Hog Plum

Family: ANACARDIACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

A medium-sized, pioneer, deciduous tree, growing up to 25 to 30 m tall. The common plant species, widespread in evergreen forest, evergreen forest-pine and mixed forest at elevations of 700 to 1,600 m above sea level. Planted saplings achieve very high survival and growth rates. The tree supports nesting birds from the 5th year after planting.

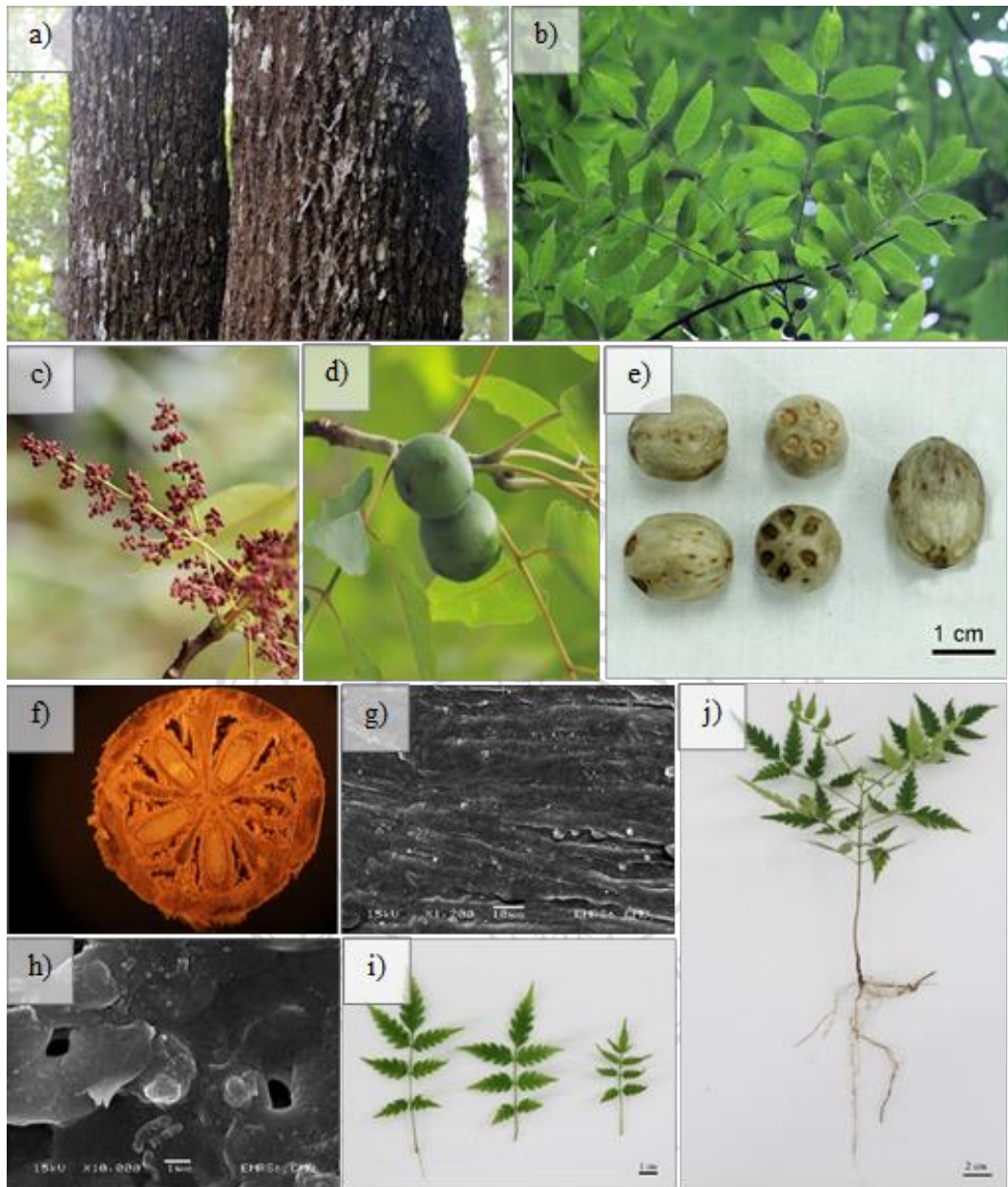
Bark: grey-brown, thin, vertically cracked.

Leaf: spirally arranged, compound, once pinnate, leaflet blades opposite or sub-opposite, ovate to ovate-lanceolate, apex acuminate.

Flower: male inflorescences 4-10 cm long; male corollas dark reddish purple, 0.4-0.5 cm; females solitary in upper leaf axils.

Fruit and seed: drupes, oval-shaped, with yellow leathery exocarp when ripe, each containing a single pyrene with 5 locules (13.81 x 18.83 x 13.67 m³ of seed volume); animal-dispersed (fruits are eaten by deer, wild pigs and bears).

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Bark (a), compound leaves (b), flowers (c), fresh fruits (d), pyrene (e), cross-section of pyrene showing seeds (f), surface of seed 1200x and 10000x under SEM (g-h), single leaf (i) and seedling (j) of *C. axillaris* species.

Species name: *Diospyros glandulosa* Lace

Common name: Streaked Ebony

Family: EBENACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

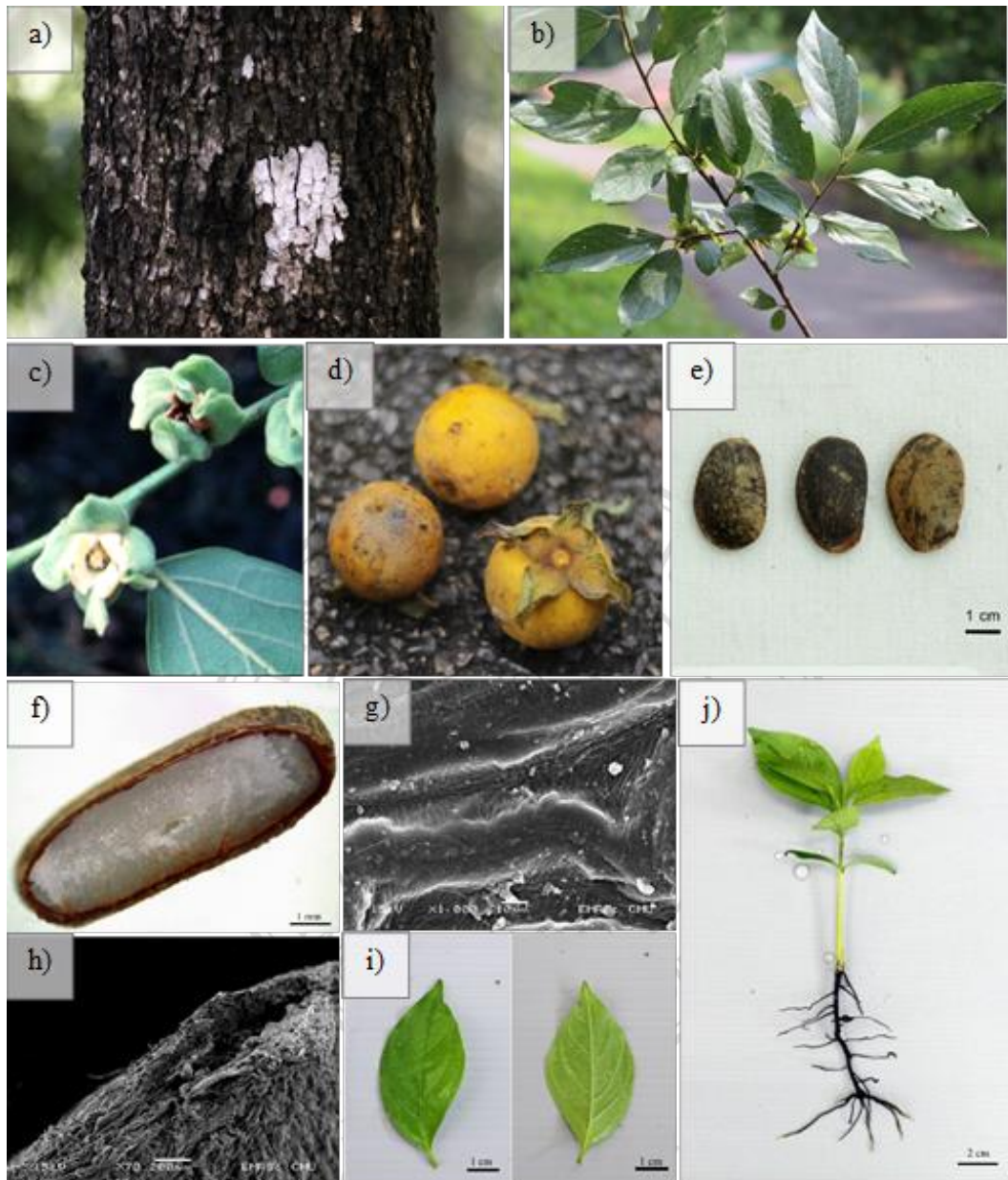
Evergreen or partly deciduous tree to 15 m. It is common in hill forests, 650 – 1650 m above sea level. The species planted as a pioneer species in reforestation projects in Thailand.

Bark: dark grey-brown or red-brown, shallowly to quite deeply cracked.

Leaf: simple, narrowly elliptic-oblong with broadly tapering tip and slightly pointed or blunt base. Young leaves densely coated with golden-brown hairs, mature leaves smooth or with scattered dark brown hairs on midvein above, densely pale brown hairy below. 4-7 pairs of curved side veins, sunken above, tertiary veins ladder-like. Stalks densely hairy.

Flower: dioecious plant. Male flower - stalks ± 2 mm, hairy. Calyx 4-6 mm, bell-shaped, divided nearly to base with 4(5) lobes, long-hairy on both sides. Corolla 6-8 mm, globose, divided $\frac{1}{4}$ - $\frac{1}{3}$, smooth on both sides except along midline. 14-30 stamens. Female flower - larger than males, 12 smooth sterile stamens, 1 hairy style with 4 stigmas, ovary hairy.

Fruit: yellow-orange, succulent, globose or oval, slightly sunken at both ends, densely coated with silky hairs which easily rub off. Stalks 0.3-0.5 cm. Calyx lobes 1.6-1.8 cm., spreading and wavy, conspicuously veined. 3-7 dark brown seeds in a star-shaped pattern.



Bark (a), simple leaves (b), flowers (c), freshy fruits (d), seed (e), cross-section of seed (f), surface of seed under SEM (g-h), single leaf (i) and seedling (j) of *D. glandulosa* species.

Species name: *Garcinia cowa* Roxb. ex Choisy

Common name: Cowa Mangosteen

Family: GUTTIFERAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

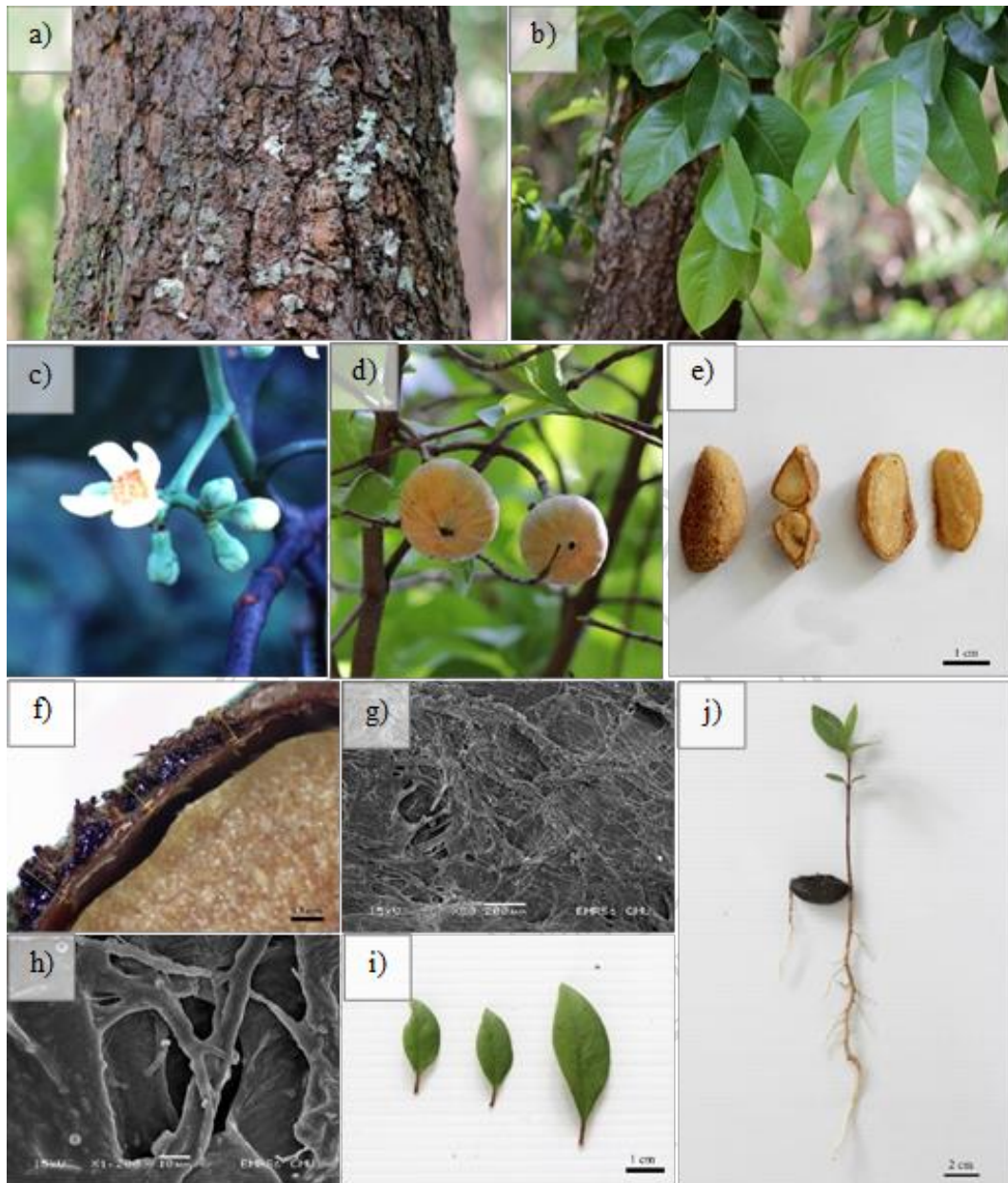
Evergreen tree, up to 20 m tall. Common in low land forest, scattered in the understory of less-disturbed forests about 60-1500 m above sea level.

Bark: bark smooth, surface greyish-brown; blaze creamy-yellow; exudation yellow, sticky, scanty; branches horizontal; branchlets quadrangular, drooping.

Leaf: 6-17 by 2.5-6 cm, oblong, usually <3x as long as wide with blunt or slightly pointed tips. Stalks to 1 cm.

Flower: males with no stigma and the stamens in single squarish mass. Females and shallowly 4-8 ridged stigma.

Fruit: 2.5-6 cm, dull orange or yellow with 5-8 shallow grooves at least near the top. Tip sunken with small black persistent calyx. 4-8 segments, each with a large 3-angled seed.



Bark (a), simple leaves (b), flowers (c), freshy fruits (d), seed (e), cross-section of seed (f), surface of seed under SEM (g-h), single leaf (i) and seedling (j) of *G. cowa* species.

Species name: *Gmelina arborea* Roxb. ex Sm.

Common name: Beechwood

Family: VERBENACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves	■	■	■	■	■	■	■	■	■	■	■	■
Flowering	■	■	■	■	■	■	■	■	■	■	■	■
Fruiting	■	■	■	■	■	■	■	■	■	■	■	■

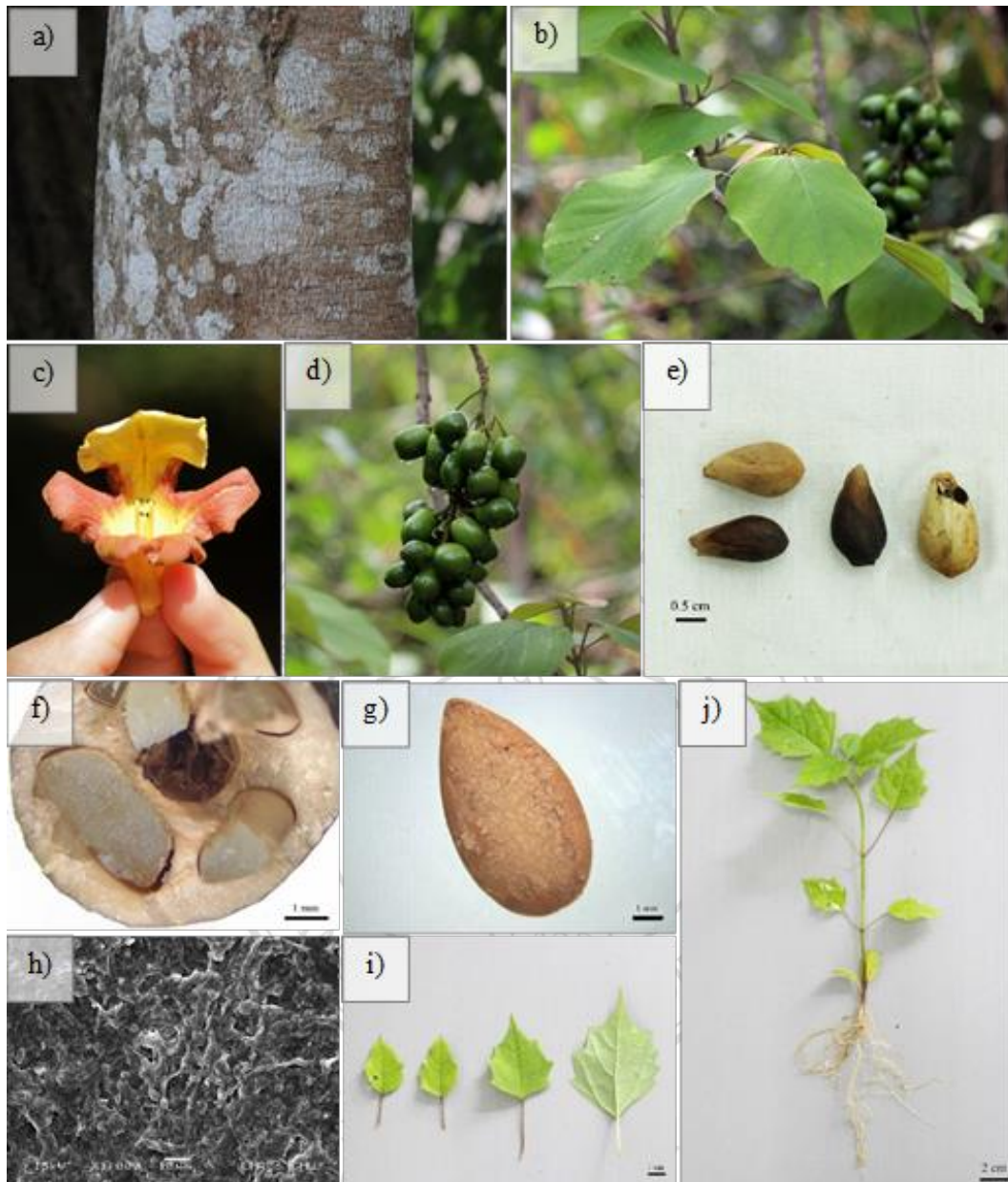
Deciduous tree to 25 m with a narrow crown and slender, drooping branches. common in semi-open deciduous forests, at elevation 200-1475 m, often with Teak.

Bark: pale creamy-brown or greyish, smooth with pale corky lenticels, becoming cracked and flaking with age, inner bark cream.

Leaf: simple, opposite, broadly ovate, cordate, glandular, glabrous above when mature and fulvous-tomentose beneath.

Flower: Yellowish-brown 5-lobed flowers, usually 1 - 3, borne on axillary and terminal panicle inflorescence, lower lobe is yellow and about 2 times as long as the rest of the lobes.

Fruit: greenish-yellow, smooth and slightly glossy, globose or obovoid with persistent calyx at base, fleshy with a hard 1-2 seeded stone.



Bark (a), simple leaves (b), flowers (c), fleshy fruits (d), pyrene (e), cross-section of pyrene (f), single seed that extracted from pyrene (g) surface of seed 1000x under SEM (h), single leaf (i) and seedling (j) of *G. arborea* species.

Species name: *Hovenia dulcis* Thunb

Common name: Japanese Raisin Tree

Family: RHAMNACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

A large, pioneer, briefly deciduous tree, growing up to 30 m tall. This species was record as rare species in evergreen forest often along stream, seasonal, hardwood forest and open disturbed roadside, at elevation of 1,025 to 1,325 m above sea level.

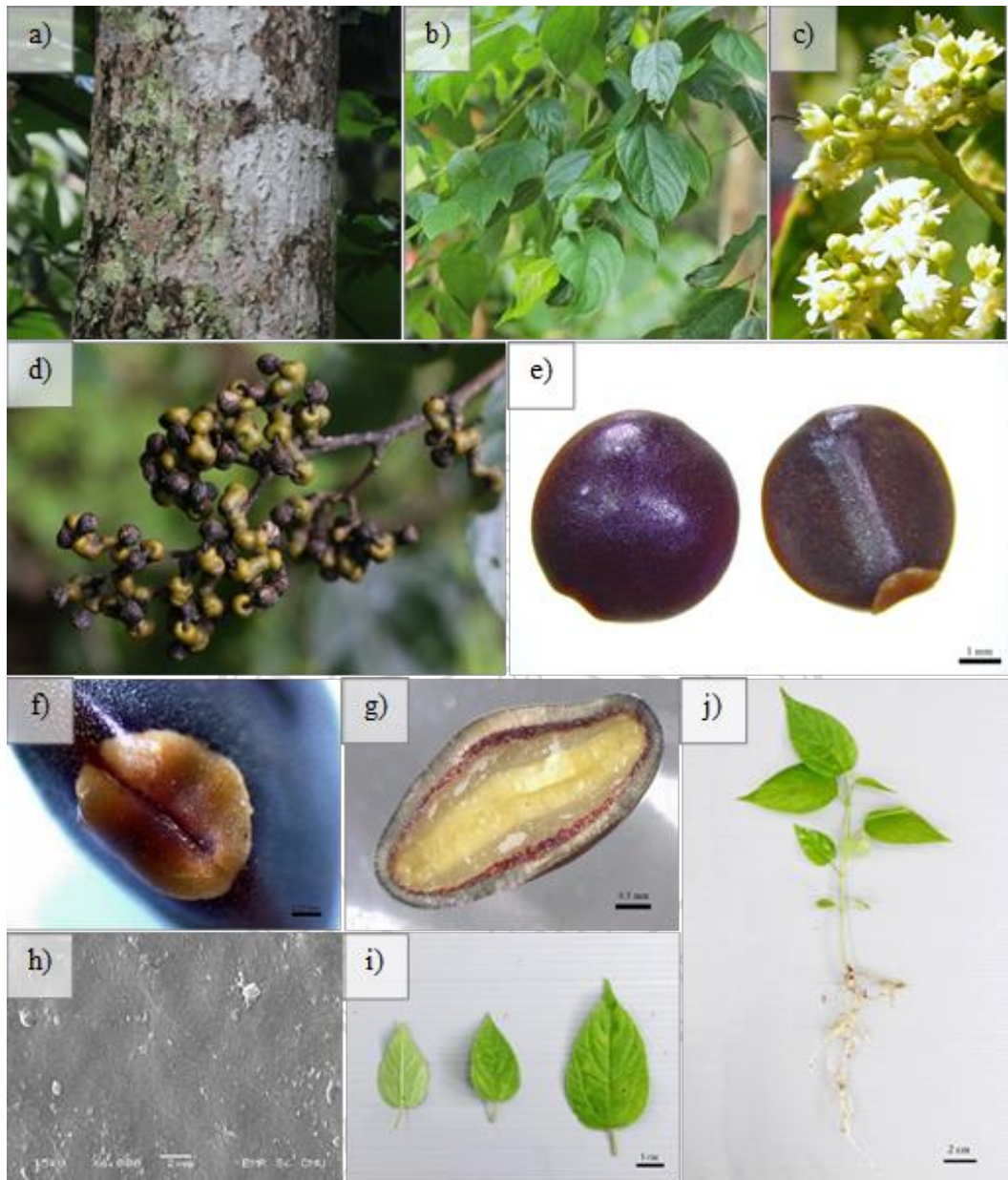
Bark: thick bark with broad, longitudinal, grey or brown ridges, separated by narrow brick-red fissures

Leaf: spirally arranged, simple blade with ovate to elliptic

Flower: in cymes, numerous, light green and cream, small.

Fruit and seed: septicidal capsule, fruit stalks very thin and curving for 2-3 mm above each fruit, swollen and fleshy, green when fruit are unripe, turning red-brown or black as fruit ripen, glossy, black seed per locule (4.60 x 4.84 x 2.13 m³ of seed volume), birds-dispersed particularly by pigeons (Kopachon *et al.*, 1996).

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Bark (a), simple leaves (b), flowers (c), fleshy fruits (d), seed (e), hilum (f), cross-section of seed (g), surface of seed 6000x under SEM (h), single leaf (i) and seedling (j) of *H. dulcis* species.

Species name: *Michelia baillonii* Pierre

Common name: Tita-Sopa

Family: MAGNOLIACEA

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves	■	■	■	■	■	■	■	■	■	■	■	■
Flowering												
Fruiting				■	■	■	■	■				

Briefly deciduous tree to 40 m. Common in hill evergreen forests throughout Northern Thailand at the area 650 – 1350 m above sea level

Bark: dark brown, corky and deeply fissured, vertically.

Leaf: simple, narrowly elliptic or oblong, pointed or tapering at both ends. Buds narrow and pointed, young leaves with dense silvery-silky hairs, mature leaves smooth or nearly so. 10-15 pairs of side veins with dense network of smaller ones. Stalks 2.5-3.5 cm, stipule scar <1/2 total length.

Flower: white, 12-18 sepals/petals, outer ones lanceolate, 2-2.5 x 0.5 cm, inner ones linear. Stamens 7-8 mm, carpels densely grey-hairy.

Fruit: 5-8 cm, yellow-green with pale spots, irregularly knobbly, breaking up when mature, leaving characteristic skeletal husks which often remain on the tree throughout the year. Seeds bright red.

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Bark (a), leaves (b), flowers (c), freshly fruits (d), ripe fruit (e) cleaned seeds (f), hilum (g), cross-section of seed (h-i), surface of seed under SEM (j-l) of *M. baillonii* species.

Species name: *Melia azedarach* L.

Common name: Chinaberry Tree

Family: MELIACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

Deciduous tree to 25 m with very open crown and widely spreading branches. The tree is common in open areas, 500 – 1450 m above sea level. Native range is Tropical and Subtropical Asia to Australia. It is used to treat unspecified medicinal disorders.

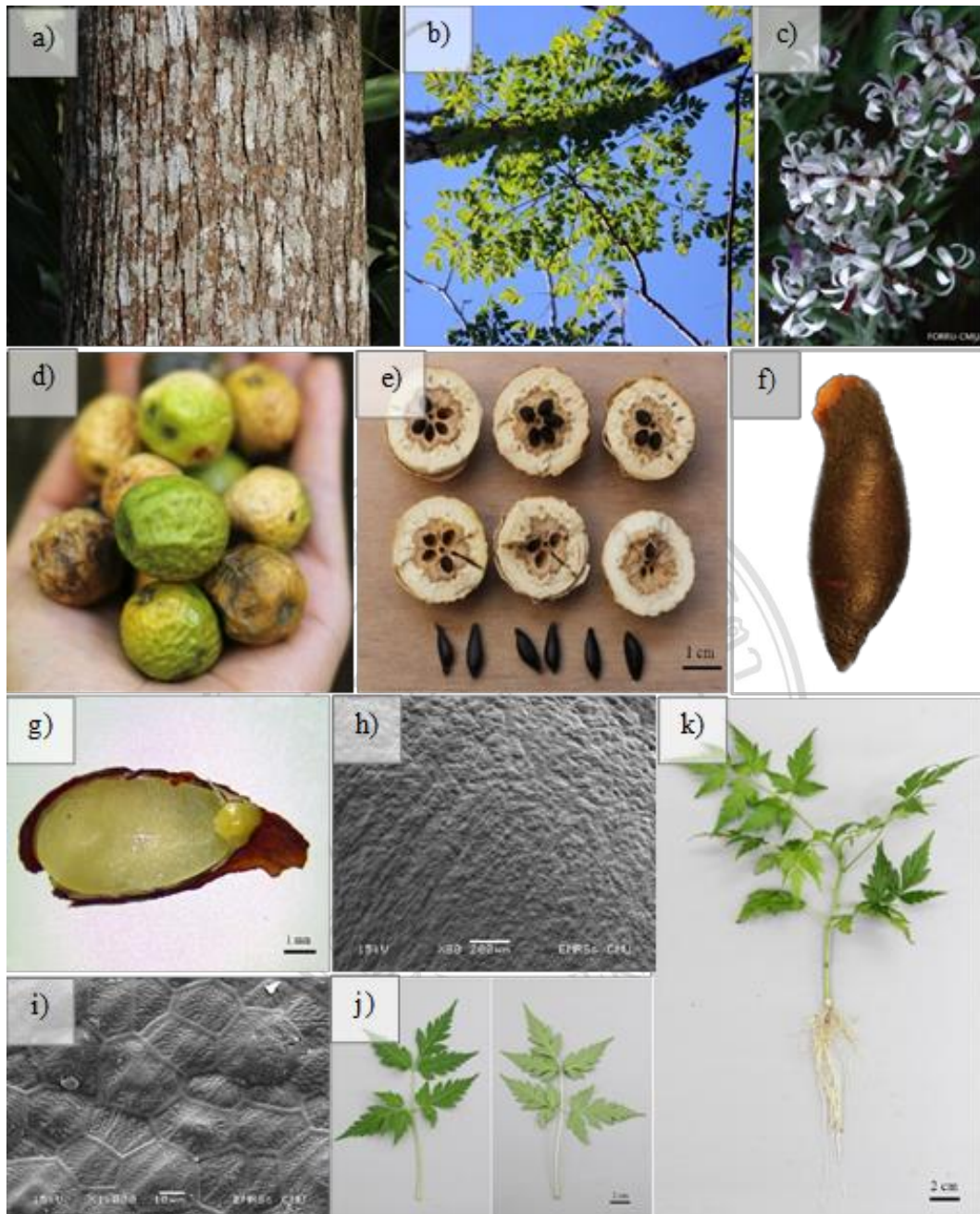
Bark: pale grey or brown with narrow fissures, inner bark cream.

Leaf: bipinnate or tripinnate, clustered near end of twigs, 4-5 pairs of side stalks each with 2-5 pairs of opposite leaflets, 3-7 x 1.2-2 cm, ovate with narrow tips, margin usually with scattered irregular teeth. Mature leaflets smooth, sometimes with whitish powder below (glaucous). Leaflet stalks 0.2-0.4 cm.

Flower: white with violet centre, in large open branched clusters grouped near end of twigs. 5-6 small triangular sepals, 5-6 white petals, curved backwards. Stamen tube violet, cylindrical, as long as petals, 8-10 anthers attached just below rim between teeth. Single slender style as long as stamen tube with unlobed stigma.

Fruit: 1.6-2 cm, green, thinly-fleshy, 6-8 lobes each with a single small stone.

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Bark (a), leaves (b), flowers (c), freshy fruits (d), ripe fruit (e) seeds (f), cross-section of seed (g), surface of seed 80x and 1000x under SEM (h-i) single leaf (j) and seedling (k) of *M. azedarach* species.

Species name: *Phyllanthus emblica* L.

Common name: Indian Gooseberry

Family: EUPHORBIACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

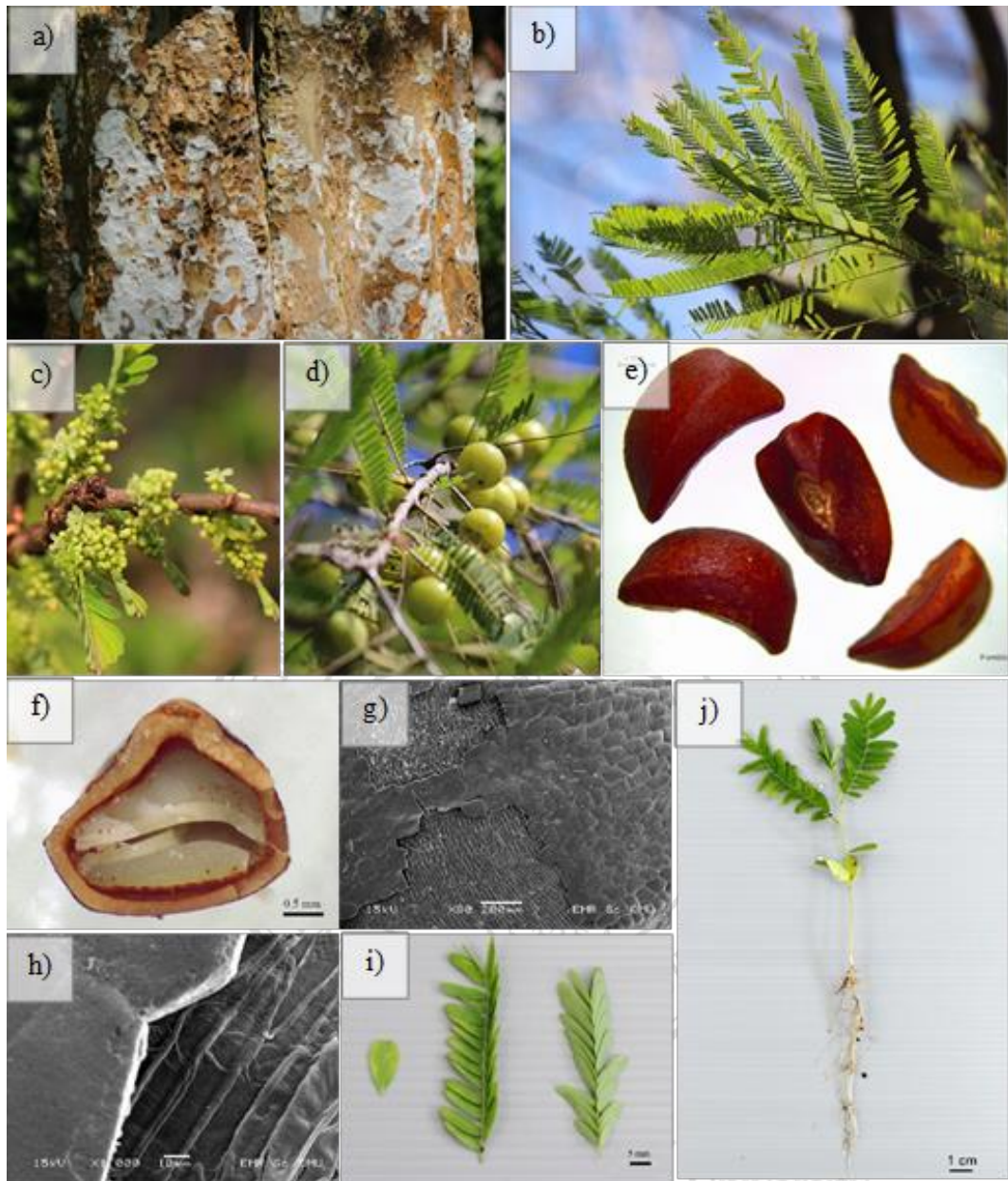
Small deciduous tree to 8 -20 m with open irregular crown and crooked trunk. Fire-resistant and common species in drier semi-open forests, at elevation 60-1700 m.

Bark: grey-brown with creamy orange patches, thin, smooth, peeling in broad flakes, inner bark pink.

Leaf: simple, alternate, simple but strongly planar and appearing pinnate, oblong or linear with blunt or slightly pointed tip and rounded base, usually asymmetric, untoothed. Young leaves finely hairy, often tinged reddish, mature leaves completely smooth. Stalks with tiny red-brown stipules.

Flower: tiny, pale green or creamy-yellow, +tinged pink, in dense simple clusters at leaf axils or behind them, usually with a few female and many males in each cluster.

Fruit: green and semi-translucent with pale veining, ripening yellowish, globose, juicy and edible but rather acidic, with a hard 3-sectioned stone, each section with (1) 2 seeds.



Bark (a), leaves (b), flowers (c), freshy fruits (d), seeds (e), cross-section of seed (f), surface of seed 80x and 10000 under SEM (g-h) single leaf (i) and seedling (j) of *P. emblica* species.

Species name: *Polyalthia viridis* Craib

Common name: Yang Own

Family: ANNONACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves	■	■	■	■	■	■	■	■	■	■	■	■
Flowering			■	■								
Fruiting			■	■								

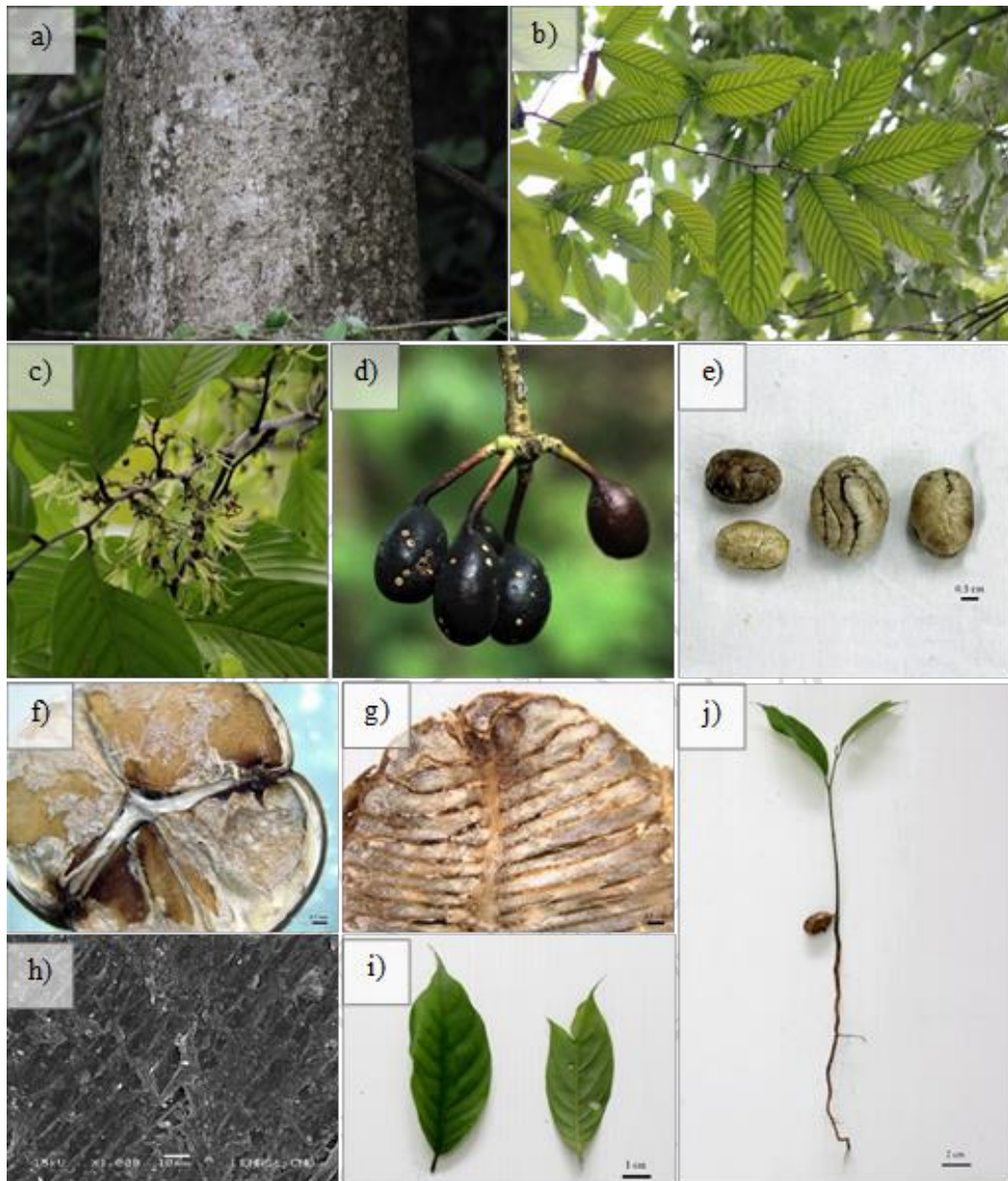
Evergreen tree to 20 m with a very narrow conical crown and a long, straight main stem, usually without branches lower down. Locally common, restricted to less disturbed forests at 500 – 800 m above sea level

Bark: greyish-brown, slightly cracked, quite thick, inner bark cream turning orange when cut.

Leaf: 20-33 x 8-12 cm, oblong, with short tips and rounded or slightly heart-shaped base, dark glossy green above, paler with scattered hairs on veins below. +15 pairs of straight, parallel side veins, faint above but obvious below.

Flower: greenish-yellow, in clusters of up to 8 star-shaped flowers on older leafless branches. Petals narrow and tapering, carpels smooth with distinct styles and a velvety stigma.

Fruit: +3 x 1.5 cm, pale orange turning dark red then black, smooth & slightly glossy, stalks as long as fruits.



Bark (a), leaves (b), flowers (c), freshy fruits (d), seeds (e), cross-section of seed (f-g), surface of seed 1000x under SEM (h) single leaf (i) and seedling (j) of *P. viridis* species.

Species name: *Prunus cerasoides* D. Don

Common name: Himalayan wild cherry

Family: ROSACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

A medium-sized, pioneer, deciduous tree, growing up to 16-18m tall. It's fairly common in evergreen forest, mixed-forest and evergreen forest-pine, often in disturbed areas, at elevations of 1,040 to 2,400 m above sea level.

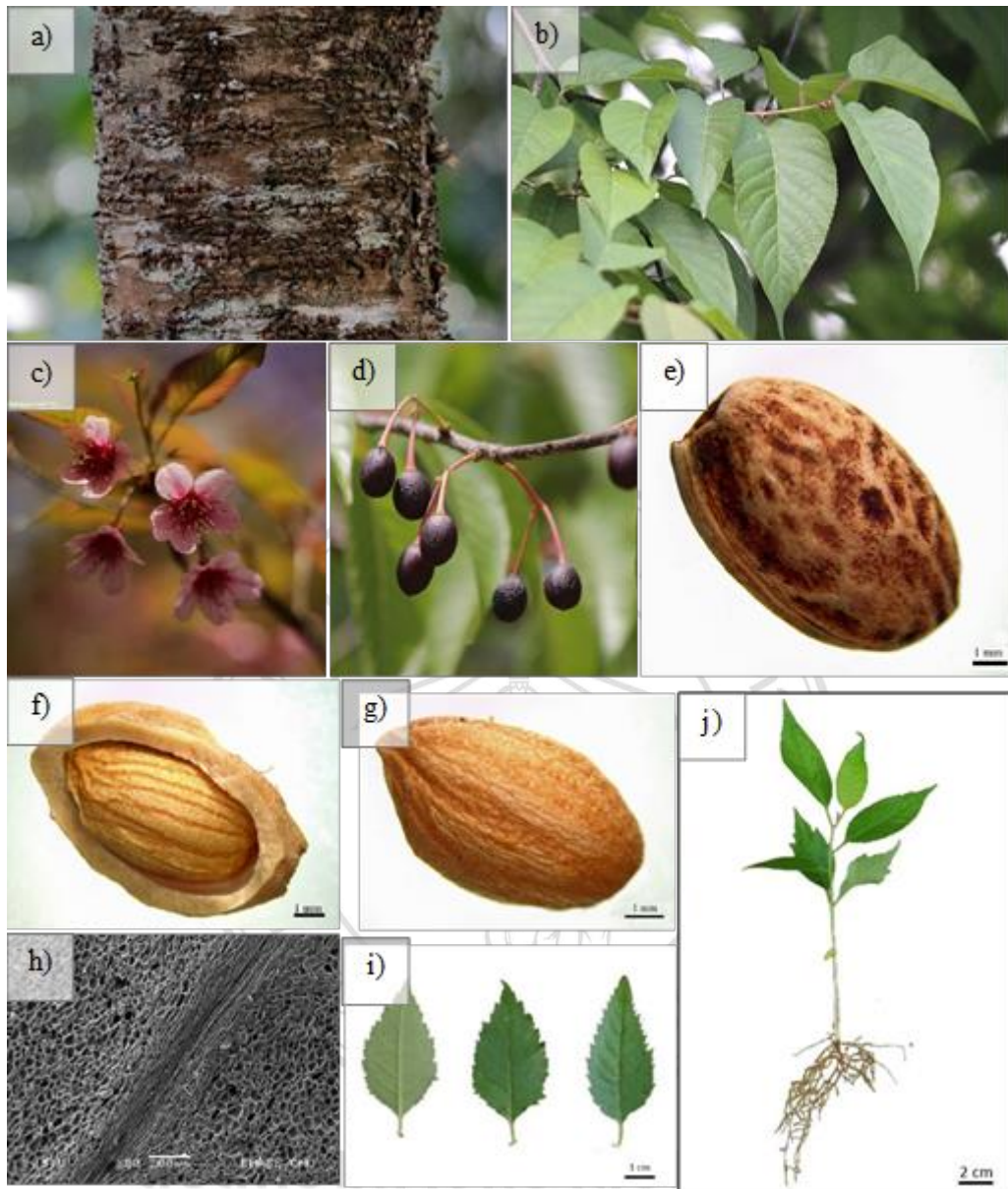
Bark: shiny, red-brown, with large, raised, brown lenticels; outer layer peeling horizontally.

Leaf: spirally arranged, simple, blades; margin finely serrate; dark red, stalked, glands where petiole meets blade.

Flower: in axillary clusters, petals, pink; on leafless trees.

Fruit and seed: drupes (small cherries), ovoid, red when ripe, 10 -15 mm, each containing a single-seeded pyrene (7.31 x 9.67 x 6.01 m³ of seed volume); dispersed by birds, squirrels and other small mammals. Birds such as Sunbirds, Spider-hunters and White-eyes feed on the nectar, whilst bulbuls eat the fruits.

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Bark (a), leaves (b), flowers (c), freshy fruits (d), seeds (e), cross-section of seed (f-g), surface of seed under SEM (h) single leaf (i) and seedling (j) of *P. cerasoides* species.

Species name: *Sapindus rarak* DC.

Common name: Soap nut, Ma Suk

Family: SAPINDACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

Fast growing and medium- tree up to 35 m. The widespread tree, 200 – 1620 m above sea level, but not usually common

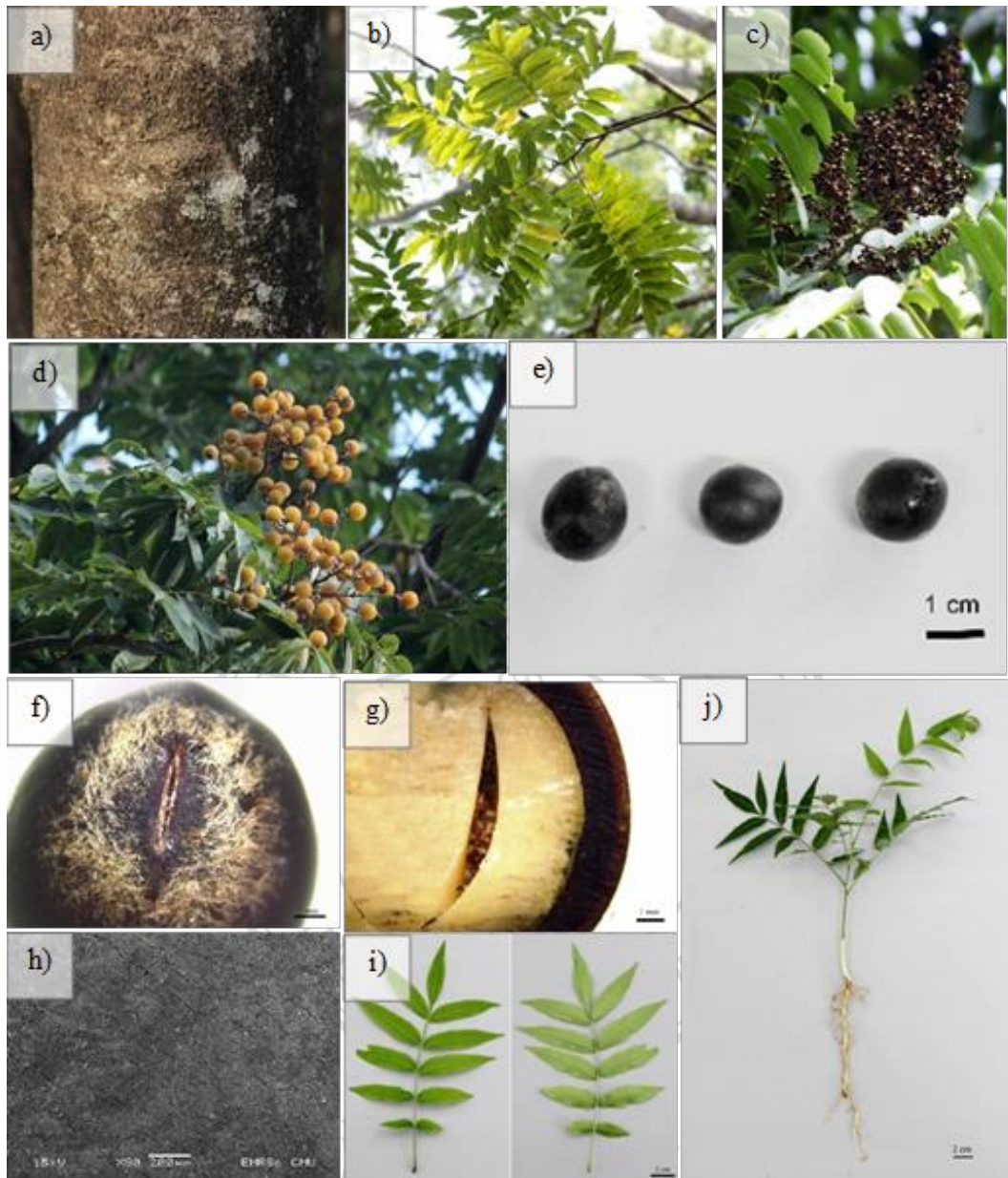
Bark: becoming thicker and roughened with fissures with age, grey to light brown.

Leaf: spirally arranged, once paripinnate, leaflets opposite to subopposite pairs, blades slightly leathery acute, margin entire, above dark green, below mid-green with sparse tiny white hairs on young blades and on margins of old blades, otherwise hairless.

Flower: branched clusters at end of twigs. 4 petals (absent fifth petal leaving an obvious gap), outside densely hairy esp. along margin, inside with hairy, 2-lobed scale. 8 stamens, slightly longer than petals, hairy at base, disc smooth, horseshoe-shaped.

Fruit: yellow-brown, smooth, leathery, 3-lobed but often only 1 developing, each lobe with a large black seed enclosed in a hard shell which is hairy near attachment of seed (placenta) inside.

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 Bark (a), leaves (b), flowers (c), freshly fruits (d), pyrenes (e), seed micropyle (f), cross-section of pyrene (g), surface of seed under SEM (h) single leaf (i) and seedling (j) of *S. rarak* species.

Species name: *Sarcosperma arboreum* Buch.-Ham. ex C.B. Clarke

Common name: Ma Yang (ມະຢາງ)

Family: SAPOTACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaves												
Flowering												
Fruiting												

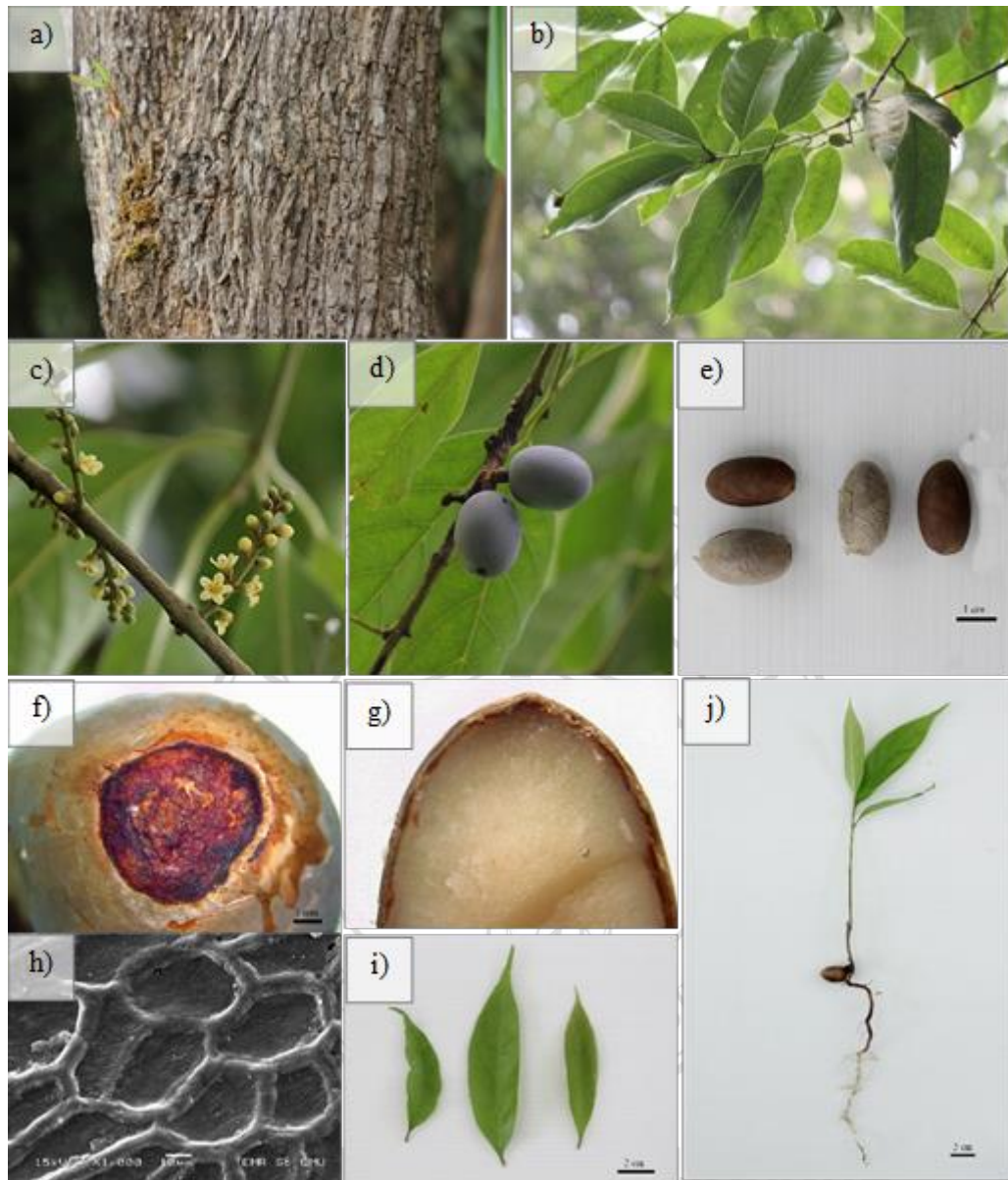
Evergreen tree, to 14 m. fairly common and widespread in less-disturbed evergreen forests, 550 – 1500 m above sea level. Previously considered a distinct family, Sarcospermataceae, with only 12 species worldwide.

Bark: red brown or creamy-brown, smooth or shallowly fissured, corky, inner bark pale cream.

Leaf: simple, opposite, oblong or lanceolate with tapering or abrupt tip and pointed base, untoothed. Mature leaves leathery, dark green above, completely smooth with colorless crater-like glands in vein axils. 6-14 pairs of prominent arching side veins, open at margin, tertiary veins ladder-like, \pm at right angles to midvein, raised both sides. Stalks flat at both ends, with inconspicuous stipules which soon fall, leaving triangular scars \pm 1 mm.

Flower: pale yellow or greenish, mildly fragrant, in slender branched or unbranched clusters at leaf axils, 5-20 cm, stalks finely hairy. Calyx 2.5-3.5 mm with 5 rounded lobes in a single row, subequal, strongly overlapping, densely hairy outside. Corolla tube \pm 2 mm with 5 rounded lobes, 2-2.5 mm, overlapping in bud. 5 fertile stamens alternating with tiny sterile ones, attached to corolla tube with short filaments and oblong anthers. Ovary smooth.

Fruit: dark purple with pale grey sheen which easily rubs off, ellipsoid with blunt tip and persistent recurved calyx at base, firmly fleshy, 1-2 dark brown seeds.



Bark (a), leaves (b), flowers (c), freshy fruits (d), seeds (e), hilum (f), cross-section of seed (g), surface of seed under SEM (h) single leaf (i) and seedling (j) of *S. arboreum* species.

Species name: *Scleropyrum pentandrum* (Dennst.) Mabb.

Common name: Kee Hnon

Family: SANTALACEAE-

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaf												
Flowering												
Fruiting												

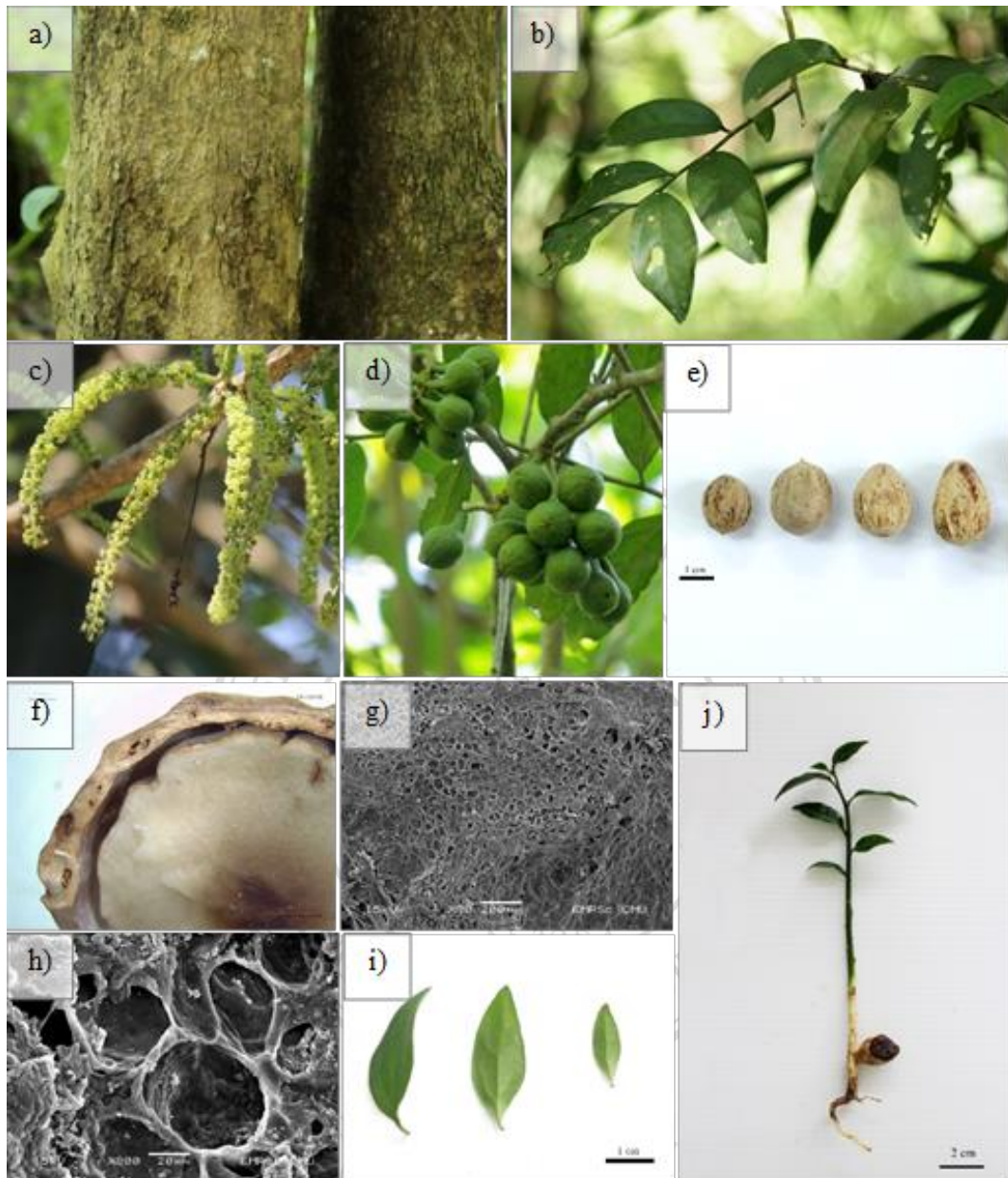
Trees 4-10 m tall. Generally found in disturbed habitats on sandy soil, as well found in semi and dry evergreen forests, in open forests near streams and in lowland dipterocarps forest; at elevations from 60 - 1,000 m

Bark: Branches grayish green, strong and thick, smooth, spines sometimes present.

Leaf: Petiole thick, 6-10 mm; leaf blade 9-17 × 5-7 cm, glabrous or sparsely pubescent, abaxially pale green, adaxially deep green, ± glossy, midvein adaxially depressed, abaxially prominent, lateral veins 3 or 4 on each side, lower 2 pairs almost reaching leaf apex, tertiary veins patent and netlike, base subrotund or cuneate, apex obtuse or acute.

Flower: Inflorescences solitary, paired, or a few in fascicles, 2-2.5 cm, yellow tomentose; bracts narrowly lanceolate, ca. 2 × 0.7 mm, villous abaxially, caducous. Perianth pale yellow to reddish yellow, ca. 3.8 × 5.5 mm, lobes 5, ovate, ca. 2 × 1.5 mm, apex subacute, abaxially villous, hair short near base or tomentose, adaxially with a tuft of hair behind each stamen. Stamen filaments ca. 1.5 mm. Disk depressed in middle, ca. 1.8 mm in diam. Style 0.8-1 mm; stigma shallowly 3- or 4-lobed, sunken in middle (April – May)

Fruit: Drupe orange or orange-red when mature, 3-3.5 × 2.3-2.5 cm, glabrous, glossy, apex nipple-like, persistent perianth not conspicuously enlarged, 2-2.5 mm in diameter (August – September).



Bark (a), leaves (b), flowers (c), freshy-young fruits (d), seeds (e), cross-section of seed under light microscope (f), surface of seed 80x and 800x under SEM (h) single leaf (i) and seedling (j) of *S. pentandrum* species.

Species name: *Spondias pinnata* (L. f.) Kurz

Common name: Hog Plum

Family: ANACARDIACEAE

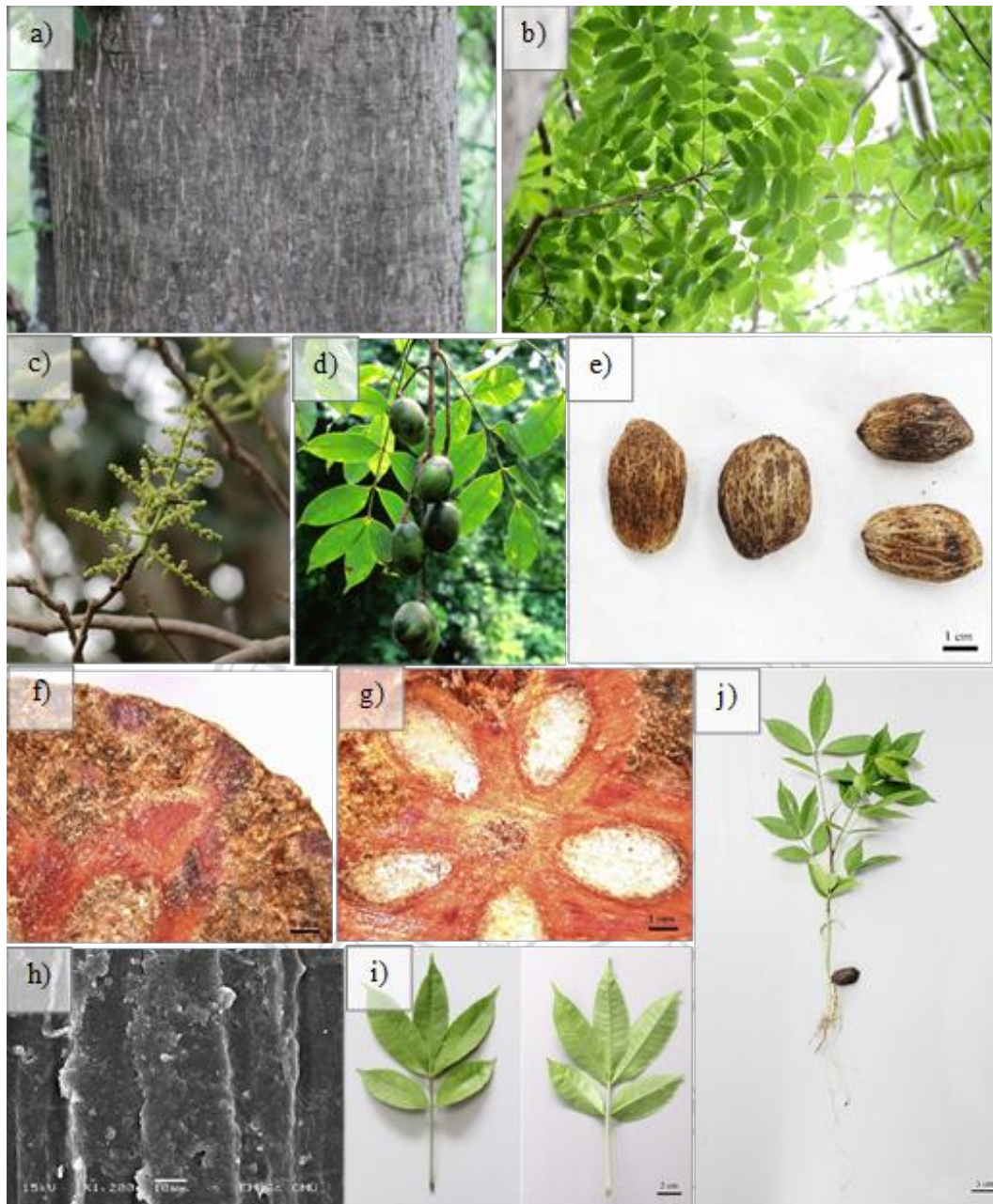
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaf												
Flowering												
Fruiting												

Deciduous tree to 20 m with open crown and slender, often drooping branches. Very common, often with bamboo at elevation 60 – 1200 m.

Leaf: 30-45 cm, odd-pinnate, alternate, 3-6 pairs of opposite or sub-opposite leaflets, 7-16 x 3-6 cm, elliptic or oblong with abruptly tapering tip & blunt or pointed base, often slightly asymmetric, no teeth, completely smooth. (10)15-20 pairs of straight, narrow side veins connecting to a distinct marginal vein, finer veins faint. Side leaflet stalks 0.3-0.8 cm, main stalk 12-16 cm, twigs stout with large leaf scars. Young leaves pink, old leaves a beautiful clear golden-yellow.

Flower: 0.5 cm, white or creamy yellow, branched clusters in upper leaf axils, 20-30 cm, individual stalks short, smooth Calyx cup-shaped with 5(4) triangular teeth, smooth, 5(4) petals, narrowly ovate with curved tips 2.5-3 mm, smooth, not overlapping in bud. 8-10 stamens, much shorter than petals, disc shallowly 10-lobed, 5(4) ovaries, pressed together but not fused, smooth, each with a short, curved style. Bisexual & unisexual flowers on same tree (January – May).

Fruit: 3-4.5 cm, green turning dirty yellow, oval, fleshy with a single large stone consisting of a very hard star-shaped core with dense fibrous material between the rays up to 5 seeds (September – March).



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Bark (a), leaves (b), flowers (c), freshly-young fruits (d), the pyrenes (e), cross-section of pyrene under light microscope (f-g), surface of pyrene 1200x under SEM (h) single leaf (i) and seedling (j) of *S. pinnata* species.

Species name: *Syzygium fruticosum* DC.

Common name: Wha Kee Kwang

Family: MYRTACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaf												
Flowering												
Fruiting												

Evergreen trees, 12 m tall. Common tree at elevation 200 – 1525 m

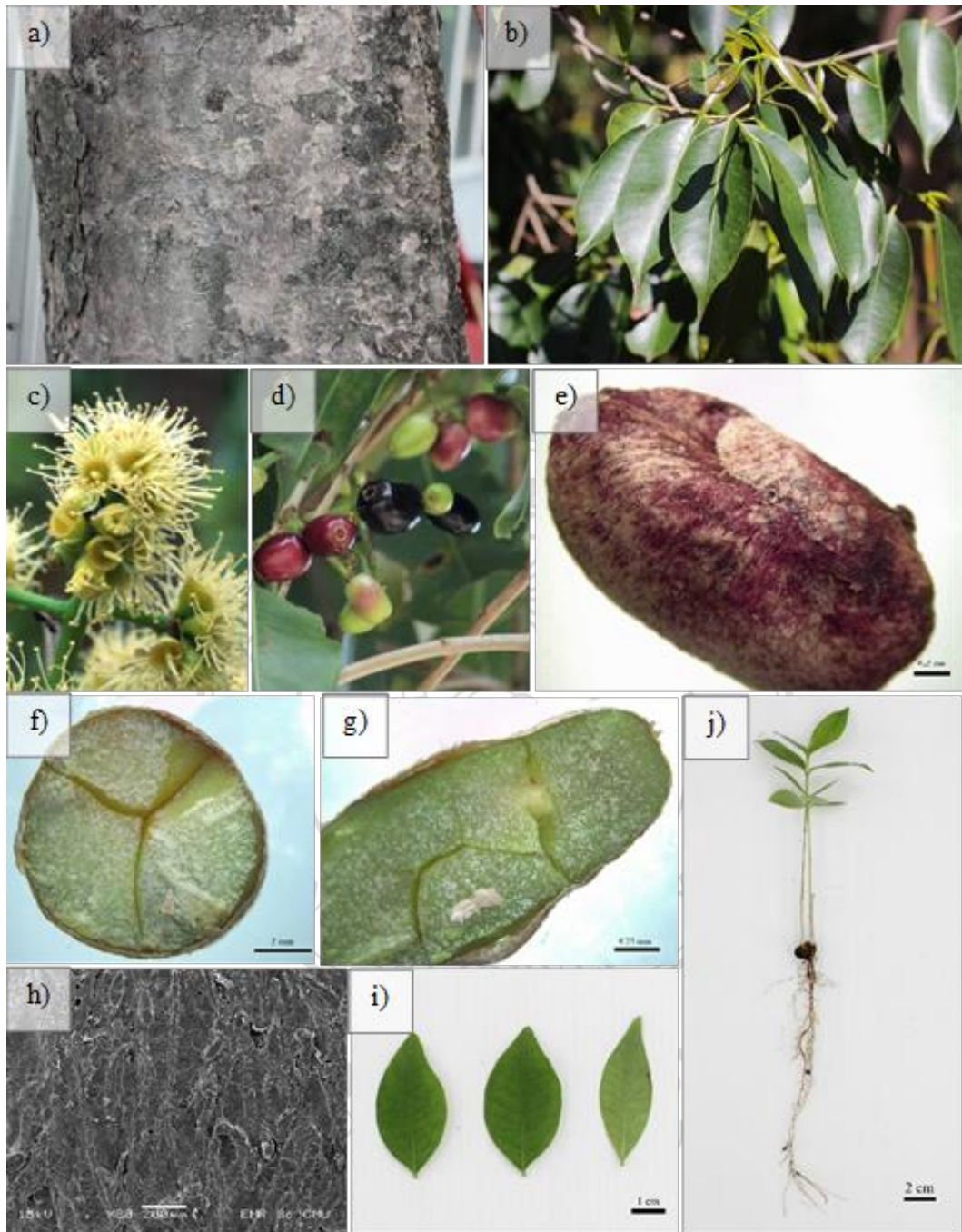
Bark: Branchlets dark brown when dry, compressed or grooved; old branches grayish white.

Leaf: Petiole 1-1.5 cm; leaf blade narrowly elliptic to elliptic, 9-13 × 3.5-5.5 cm, thinly leathery, abaxially reddish brown when dry, adaxially brown and glossy when dry, both surfaces with numerous glands, secondary veins numerous, 2-3 mm apart, and gradually extending into margin, intramarginal veins ca. 1 mm from margin, base broadly cuneate to slightly rounded, apex acuminate.

Flower: Inflorescences lateral below leaves, panicle cymes, 4-7 cm. Hypanthium obconic, 2-2.5 mm. Calyx lobes inconspicuous. Petals 4, distinct, rounded, 1-1.5 mm wide. Stamens 1.5-2.5 mm. Style as long as stamens.

Fruit: red when ripe, globose, 6-7 mm in diam., 1-seeded.

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Bark (a), leaves (b), flowers (c), freshy fruits (d), seed (e), cross-section of seed under light microscope (f-g), surface of seed 80x under SEM (h) single leaf (i) and seedling (j) of *S. fruitcosum* species.

Species name: *Phoebe cathia* (D.Don) Kosterm.

Common name: Ma Dook Dong

Family: LAURACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaf												
Flowering						No data						
Fruiting												

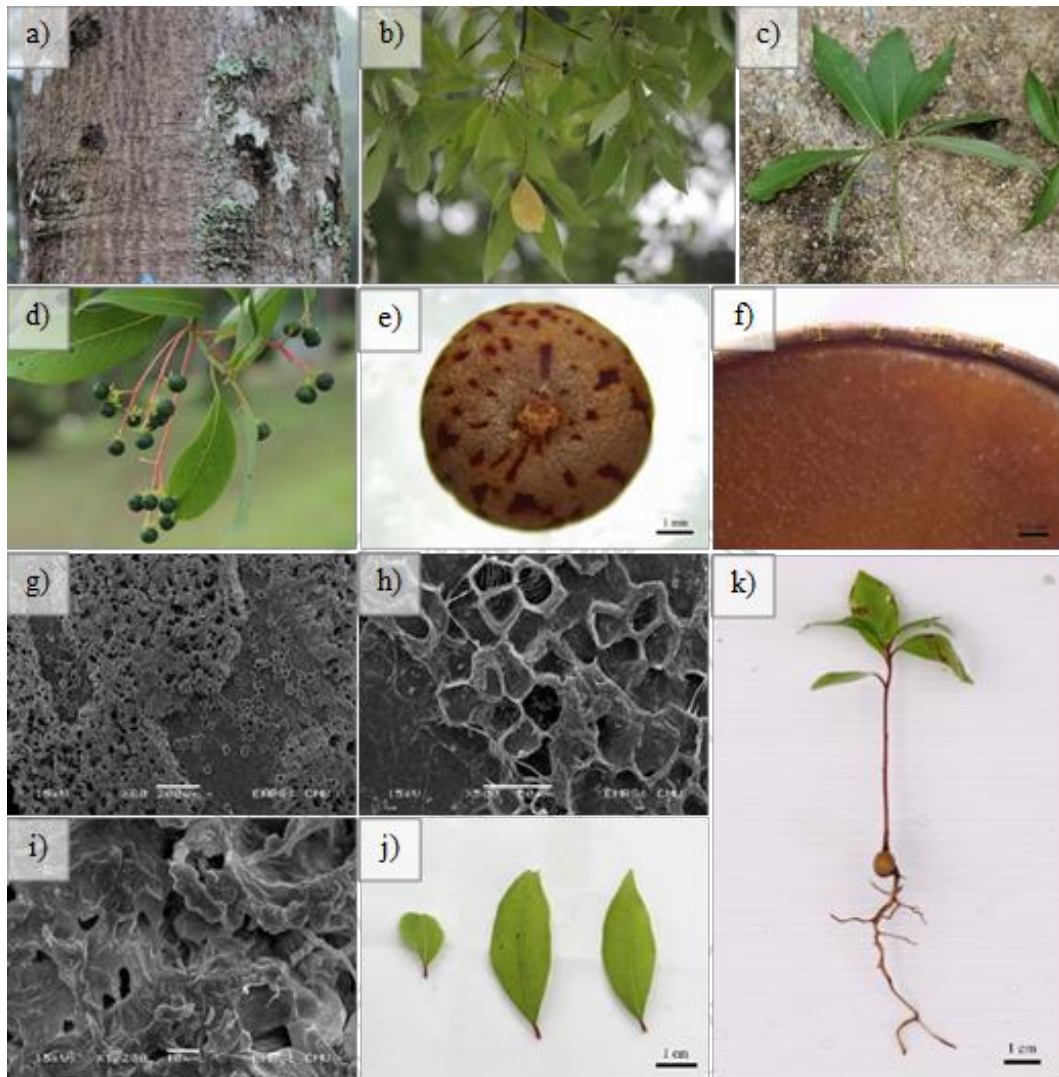
Evergreen tree to 13 m., scattered in less disturbed hill forests.

Bark: pale with short fissures & large lenticels.

Leaf: 10-30x5-9 cm, lanceolate, elliptic or narrowly obovate, tapering both ends. Young shoots brown-hairy, mature leaves sparsely hairy below & often also on midvein above. 6-12 pairs of side veins, prominent below. Stalks 0.8-2(4) cm.

Flower: small, white, in branched clusters on long slender common stalks at end of twigs & upper leaf axils, axes hairy. Individual stalks at least as long as calyx, hairy. Calyx lobes pointed, + 3mm, hairy outside, inner ones slightly shorter & rounded.

Fruit: 0.8-1.2 cm, oval, black & glossy, partly enclosed by hard persistent calyx.



Bark (a), leaves (b-c), freshy fruits (d), seed (e), cross-section of seed under light microscope (f), surface of seed 80x (g), 800x (h) and 1200x (i) under SEM, single leaf (j) and seedling (j) of *P. cathia* species.

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Species name: *Quercus brandisiana* Kurz

Common name: Kor See Sead

Family: FAGACEAE

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Leaf												
Flowering												
Fruiting												

Evergreen tree to 13m. Elevation range 750 – 1300 m

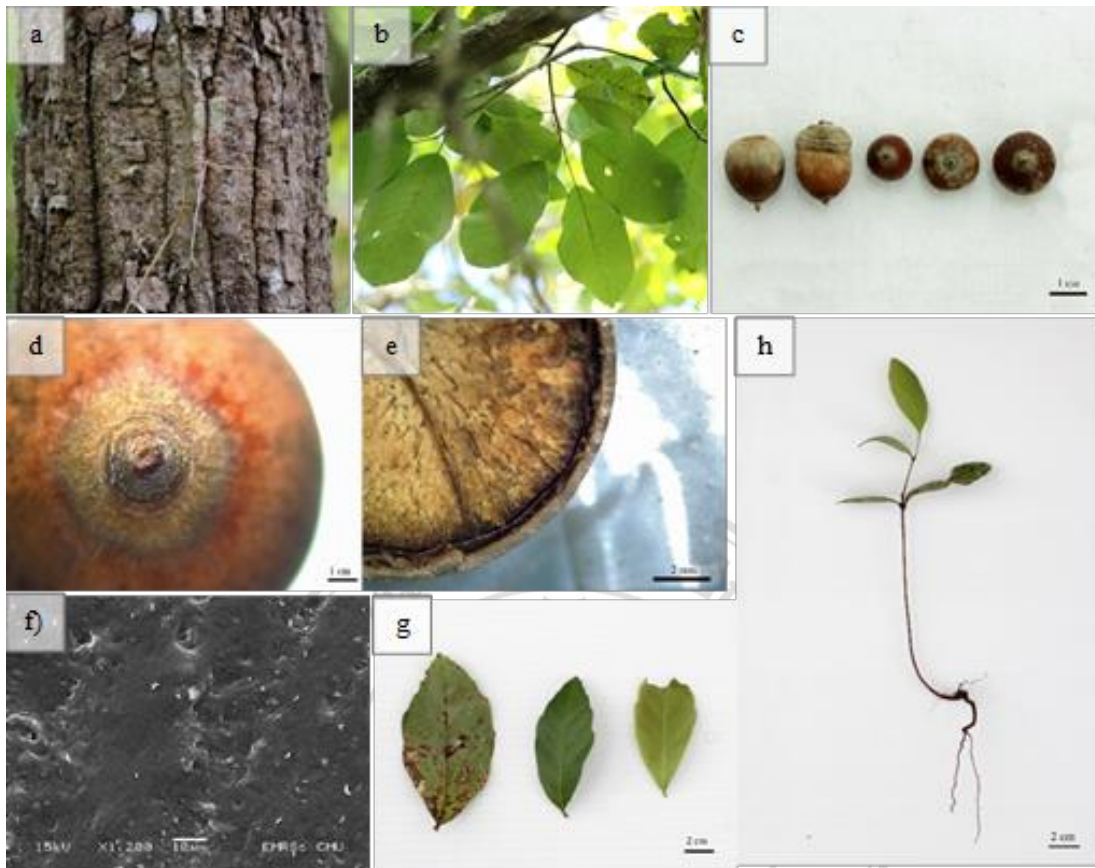
Bark: inner bark yellow-brown, fibrous fissured, corky, ± 0.8 cm thick.

Leaf: 10-20x5-8 cm (30x13 cm), narrowly ovate, obovate or elliptic-oblong with pointed or slightly tapering tip & blunt or slightly pointed base, scattered shallow teeth especially in upper half. Young shoots thinly hairy, mature leaves quite opaque both sides, smooth and wrinkled above, glaucous below with scattered fine hairs which easily rub off, becoming smooth. 10-15 pairs of straight parallel side veins, bent at margin and ending in teeth, sunken above. Stalks 1.6-3.6 cm, slender, smooth.

Flower: female flowers at the base of leaves petioles, on spikes 3 times longer than the leaf petiole; Nov - April

Fruit: spikes 2.5-5-7.5 cm, few fruited, stalks red-brown hairy, several pressed closely together in groups on short stalk. Cups 1.2-2 cm diam, plate-shaped, $\frac{1}{2}$ covering nut, greyish-velvety on both sides, with 4-6 concentric rings, irregularly deeply toothed. Young nuts depressed, becoming ovoid or conical with short point and red-brown or golden hairs near top. (February-June)

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Bark (a), leaves (b), fruits (c), remains of style (e), cross-section of fruit under light microscope (f), surface 1200x under SEM (f), single leaf (g) and seedling (h) of *Q. brandisiana* species.

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CURRICULUM VITAE

Name Miss. Khuanphirom Naruangsri

Education Background

March 2010 B.Sc (Biology), Chiang Mai University, Thailand

August 2014 M.Sc (Biology), Chiang Mai University, Thailand

Scholarship Development and Promotion of Science and Technology Talents Project (Royal government of Thailand scholarship)

Work Experiences

2014 - 2015 Teaching Assistant, Department of Biology, Faculty of Science, Chiang Mai University

2013 Intern student and Research assistance at Khao Nang Ram Wildlife Research Station, Huai Kha Khaeng Wildlife Sanctuary, Thailand.

2015 - 2018 Research Assistant, Department of Biology, Faculty of Science, Chiang Mai University

2016 Intern student at Lab of Biodiversity and Ecology, College of Life Sciences and Biotechnology, Korea University, South Korea.

2021 Research student in Doi Suthep Nature Study Center (DSNC), Chiangmai University, Thailand.

2022 Intern student at Lab of Seed Biology, University of Illinois at Urbana-Champaign, United States.

2018 – present Field Research Assistant, Forest Restoration Research Unit (FORRU), Department of Biology, Faculty of Science, Chiang Mai University

Research Participation

- 2014 Bachelor's Thesis on the topic of "Effects of forest fire on population of Pak Waan Paa (*Melientha suavis* Pierre)".
- 2017 Master's research on the topic of "Seed and Seedling Predation of Five Framework Tree Species in a Degraded Forest Area of Ban Nong Hoi, Mae Rim District, Chiang Mai Province".
- 2021 Research Assistant working on seed bank projects: Ex-situ seed banking for conserving northern Thailand tree species.

Publications

Naruangsri K., Jampeethong A. Meewasana J. and Chairuangsri S. (2014) Effects of forest fire on population of *Melientha suavis* Pierre in Huai Hong Khrai Royal Development Study Center and Mae Kuang Udomthara Dam, Amphoe Doi Saket, Chiang Mai province. *Thai Journal of Botany*, 6(Special Issue): 59-65.

Naruangsri, K. and Tiansawat, P. (2016). Potential seed predators in an abandoned agricultural area in northern Thailand. *Proceedings of the 3rd National Meeting on Biodiversity Management in Thailand*, 124–133.

Naruangsri, K., Tiansawat, P. and Elliott, S. (2023). Differential seed removal, germination and seedling growth as determinants of species suitability for forest restoration by direct seeding – A case study from northern Thailand. *Forest Ecosystems*, 10, 100133. <https://doi.org/10.1016/j.fecs.2023.100133>.