SELECTING SUPERIOR PARENT TREES FOR FOREST RESTORATION PROGRAMS, MAXIMIZING PERFORMANCE WHILST MAINTAINING GENETIC DIVERSITY

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A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN BIOLOGY
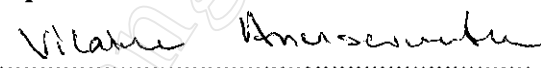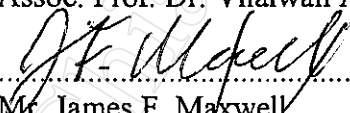
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THIS THESIS HAS BEEN APPROVED
TO BE A PARTIAL FULFILLMENT OF THE REQUIREMENTS
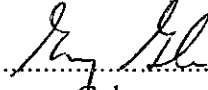FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN BIOLOGY

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7 May 2002

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Thesis Title	Selecting Superior Parent Trees for Forest Restoration Programs, Maximizing Performance Whilst Maintaining Genetic Diversity.
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ABSTRACT

The framework species method of forest restoration addresses the serious problem to tropical deforestation by planting selected tree species that accelerate the natural processes of forest regeneration and biodiversity recovery. Recent field trails have shown that the performance of framework tree species planted in deforested sites in northern Thailand is highly variable, due to variations among different seed batches originating from different parent trees. The objective of this study was to develop criteria, based on nursery and field performance of planted saplings and genetic variability, to select superior parent seed trees, to optimise production methods and performance of the 5 species studied seedlings for forest restoration projects.

The five framework tree species were *Spondias axillaris* Roxb. (Anacardiaceae), *Melia toosendan* Sieb. & Zucc. (Meliaceae), *Gmelina arborea* Roxb. (Verbenaceae), *Prunus cerasoides* D. Don (Rosaceae) and *Castanopsis acuminatissima* (Bl.) A. DC. (Fagaceae). They have all been identified as a 'framework species' for restoring evergreen forest in seasonally dry climates.

Variability in both nursery and field performance of seedlings germinated from a maximum 50 individual parent trees per species studied is reported. Relationships between seed size, germination characteristics, seedling performance in the nursery and in the field were found, but the relationships were mixed.

Seed and pyrene size of *S. axillaris*, *M. toosendan* and *C. acuminatissima* increased with increasing elevation of the parent trees, but there was no such relationship for *G. arborea* and *P. cerasoides*.

Percent seed germination of *M. toosendan* and *C. acuminatissima* increased with increasing seed size. In contrast, the percent germination of *G. arborea* increased with decreasing pyrene size and there was no relationship for *S. axillaris* and *P. cerasoides*. Mean seed size of germinating seeds of *M. toosendan* and *C. acuminatissima* was larger than those of non-germinating seeds. On the other hand mean pyrene size of germinating seeds of *G. arborea* and *P. cerasoides* was smaller than those of non-germinating seeds.

Percent germination was negatively correlated with time to germination and median length of dormancy for all species studied.

Seed and pyrene sizes was correlated with seedling size in the nursery, but only weakly correlated with relative growth rate (RGR). Seedling size (height and root collar diameter) of *M. toosendan, P. cerasoides* and *C. acuminatissima* increased with increasing seed sizes, but there was no relationship for *G. arborea* and *S. axillaris*. Seedling survival in the nursery was not correlated with seed size.

There were some correlations between seedling performance in the field and seed (pyrene) size, germination characteristics and seedling performance in the nursery. However, the correlations were equivocal and weak.

Four standards for selection of superior seed trees were recognized: (*i*) 70% or greater sapling survival in the field, (*ii*) a sapling height of 100 cm or taller after the first growing season in the field, (*iii*) 40% or greater germination in the nursery and (*iv*) 70 % or higher seedling survival in nursery. Twelve seed trees of *S. axillaris*, twenty-one for *P. cerasoides* and seventeen for *C. acuminatissima* met these standards and were therefore selected as the superior seed trees. *M. toosendan* and *G. arborea* had no seed trees that qualified in all 4 standards. Seeds for seedling production in reforestation programme should be collected from those seed trees.

The genetic diversity of *P. cerasoides* and *C. acuminatissima* was examined using microsatellite markers. This study enables a more informed selection of seed

trees in our forest restoration programmes. Firstly, the F_{ST} values indicate that there is no differentiation between the three *C. acuminatissima* populations, hence seed may be collected and moved between the three National parks. In contrast, there is significant differentiation amongst the three populations of *P. cerasoides*, indicating that for this species, seed should be collected locally, and not transferred between the National parks. Secondly, the data for both species suggests a large amount of genetic diversity, because of the high number of rare and low-frequency microsatellite alleles. Seed should therefore be collected from as many trees as possible, certainly within, or close to the FAO recommendation of 25-50 individuals per population (FAO Forest Resources Division, 1995).

Furthermore, I believe that microsatellite data can 'inform' a genetic conservation programme at this time, in the absence of more sophisticated genetic data, through the selection of individuals to capture microsatellite allelic diversity. To capture genetic diversity, two alternative algorithms were designed to: select individual seed trees based on their individual genotype (model I) and randomly select seed trees from a population of unknown genetic makeup (model II). This approach is presented and discussed fully in Chapters 6 and 7.

By combining the additional field data relating to establishment and growth rates, with the nursery performance and genetic information, I expect to have a more robust, practical procedure for identifying parent seed trees.

ชื่อเรื่องวิทยานิพนธ์	การคัดแม่ไม้ยืนต้นที่ดีเพื่อใช้ในโครงการฟื้นฟูป่าให้ได้ประสิทธิภาพสูงสุดโดยยังคงไว้ซึ่งความหลากหลายทางพันธุกรรม
ชื่อผู้เขียน	นายเกริก ผักกาด
วิทยาศาสตรดุษฎีบัณฑิต	สาขาวิชาชีววิทยา

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บทคัดย่อ

การฟื้นฟูป่าโดยวิธีพรรณไม้โครงสร้างได้นำมาแก้ปัญหาการทำลายป่าในเขตร้อน โดยการคัดเลือกปลูกชนิดของต้นไม้ที่ช่วยในการฟื้นฟูสภาพป่าตามธรรมชาติและการกลับคืนมาของความหลากหลายทางชีวภาพ จากการทดลองปลูกในแปลงทดลอง พบว่าอัตราการเจริญเติบโต อัตราการอยู่รอดของต้นกล้าพรรณไม้โครงสร้างที่ปลูกในป่าเสื่อมโทรมในเขตภาคเหนือของประเทศไทยมีความผันแปรมาก เป็นผลสืบเนื่องมาจากความแตกต่างกันของเมล็ดที่ได้มาจากแต่ละต้นแม่ วัตถุประสงค์ของงานวิจัยนี้เพื่อที่จะพัฒนาหลักเกณฑ์ในการคัดเลือกแม่ไม้ยืนต้นที่ดีที่สุดเพื่อใช้ในโครงการฟื้นฟูป่า

พรรณไม้ยืนต้นที่ทำการศึกษา 5 ชนิด ได้แก่ มะกอกห้ารู (*Spondias axillaris* Roxb. - Anacardiaceae), เลี่ยน (*Melia toosendan* Sieb. & Zucc. - Meliaceae), ซ้อ (*Gmelina arborea* Roxb. - Verbenaceae), นางพญาเสือโคร่ง (*Prunus cerasoides* D. Don - Rosaceae) และ ก่อเดือย

(*Castanopsis acuminatissima* (Bl.) A. DC. - Fagaceae) ซึ่งได้รับการคัดเลือกให้เป็นพรรณไม้โครง
สร้างที่มีศักยภาพในการฟื้นฟูป่าในเขตร้อนชื้น

ผลการศึกษาทั้งในเรือนเพาะชำและในแปลงทดลองแสดงถึงความผันแปรของอัตราการ
เจริญเติบโต อัตราการอยู่รอดของต้นกล้าซึ่งได้มาจากแม่ไม้จำนวนอย่างมากที่สุด 50 ต้น ของพรรณ
ไม้ที่ศึกษาทั้ง 5 ชนิด การศึกษาในครั้งนี้ยังพบความสัมพันธ์ระหว่าง ขนาดของเมล็ด ลักษณะการ
งอก อัตราการเจริญเติบโต อัตราการอยู่รอดทั้งในเรือนเพาะชำและในแปลงปลูก แต่ความสัมพันธ์
เหล่านี้ผสมผสานกัน

เมล็ด (pyrene) ของ มะกอกห้ารู เลี่ยน และก่อเดือย มีขนาดใหญ่ขึ้นเมื่อต้นแม่ไม้เจริญอยู่
ในระดับความสูงจากระดับน้ำทะเลที่สูงขึ้น แต่ไม่พบความสัมพันธ์นี้ในซ้อและนางพญาเสือโคร่ง

อัตราการงอกของเลี่ยนและก่อเดือยเพิ่มขึ้นเมื่อเมล็ดมีขนาดใหญ่ขึ้น แต่ในทางตรงกันข้าม
อัตราการงอกของซ้อเพิ่มขึ้นเมื่อขนาดของเมล็ดลดลง ไม่พบความสัมพันธ์นี้ในมะกอกห้ารูและนาง
พญาเสือโคร่ง ค่าเฉลี่ยขนาดเมล็ดที่งอกของเลี่ยนกับก่อเดือย ใหญ่กว่าค่าเฉลี่ยขนาดของเมล็ดที่ไม่
งอก ขณะที่ค่าเฉลี่ยขนาดเมล็ดที่งอกของซ้อและนางพญาเสือโคร่งกลับมีขนาดเล็กกว่าค่าเฉลี่ยของ
เมล็ดที่ไม่งอก

อัตราการงอกมีความแปรผกผันกับระยะเวลาที่ใช้ในการงอกและค่ากลางของระยะพักตัว
ของเมล็ด สำหรับทุกชนิดที่ทำการศึกษา

นอกจากนั้นยังพบความสัมพันธ์ระหว่างขนาดของเมล็ดกับขนาดของต้นกล้าในเรือนเพาะ
ชำ และมีความสัมพันธ์ที่ไม่เด่นชัดกับอัตราการเจริญเติบโต ขนาดรอบโคนต้นกล้าของเลี่ยน นาง
พญาเสือโคร่ง และ ก่อเดือย มีขนาดเพิ่มขึ้นเมื่อเมล็ดมีขนาดใหญ่ขึ้น แต่อย่างไรก็ตาม ไม่พบความ
สัมพันธ์นี้ในซ้อและมะกอกห้ารู อัตราการอยู่รอดของต้นกล้าในเรือนเพาะชำไม่มีความสัมพันธ์กับ
ขนาดของเมล็ด

พบความสัมพันธ์ระหว่างอัตราการเจริญเติบโต อัตราการอยู่รอดของกล้าไม้ในแปลง
ทดลอง กับขนาดของเมล็ด ลักษณะการงอกและอัตราการเจริญของต้นกล้าและอัตราการอยู่รอดใน
เรือนเพาะชำ แต่ความสัมพันธ์มีความสับสนและไม่เด่นชัด

หลักเกณฑ์ 4 ข้อในการคัดเลือกแม่ไม้ยืนต้นที่ดี ได้แก่ (1) อัตราการอยู่รอดของต้นกล้าใน
แปลงทดลอง ร้อยละ 70 หรือมากกว่า (2) หลังจากปลูกในแปลงทดลอง 1 ฤดูการเจริญเติบโต ความ
สูงของต้นกล้าสูง 100 เซนติเมตรหรือมากกว่า (3) อัตราการงอกของเมล็ดร้อยละ 40 หรือมากกว่า
(4) อัตราการอยู่รอดของต้นกล้าในเรือนเพาะชำร้อยละ 70 หรือมากกว่า ต้นแม่ที่มีคุณลักษณะเข้า
หลักเกณฑ์นี้คือ มะกอกห้ารูจำนวน 12 ต้น ก่อเดือย 17 ต้น นางพญาเสือโคร่งจำนวนชนิดละ 21
ต้น เลี่ยนและซ้อไม่มีต้นแม่ที่มีคุณลักษณะเข้าหลักเกณฑ์ทั้ง 4

การศึกษาความหลากหลายทางพันธุกรรมของนางพญาเสือโคร่งและก่อเดือย โดยใช้
เทคนิค microsatellite marker การศึกษาในครั้งนี้จะทำให้มีความรู้สำหรับการคัดเลือกแม่ไม้ใน
โครงการฟื้นฟูป่าของเราดีขึ้น ประการแรก ค่า F_{ST} แสดงให้เห็นว่าความหลากหลายทางพันธุกรรม
ของก่อเดือยไม่มีความแตกต่างกันระหว่าง 3 กลุ่มประชากร ดังนั้นจึงสามารถจะเก็บเมล็ดได้ใน
อุทยานแห่งชาติทั้ง 3 แห่ง ในทางตรงกันข้าม ความหลากหลายทางพันธุกรรมของนางพญาเสือ
โคร่งมีความแตกต่างกันระหว่างประชากร ซึ่งแสดงว่าเมล็ดควรจะเก็บในแต่ละท้องที่และไม่ควร
เคลื่อนย้ายระหว่างประชากร ประการที่สอง ข้อมูลทางด้านความหลากหลายทางพันธุกรรมของทั้ง
2 ชนิด บ่งบอกว่าต้นไม้ทั้งสองชนิดมีความหลากหลายทางพันธุกรรมสูง เนื่องจากมีอัลลีลที่หายาก
จำนวนมากและแต่ละ microsatellite อัลลีลที่มีความถี่ต่ำ ดังนั้นการเก็บเมล็ดควรจะเก็บมาจากต้น
แม่จำนวนมากเท่าที่จะทำได้ ซึ่งก็ตรงกับที่องค์การอาหารแห่งสหประชาชาติ (FAO) ได้แนะนำไว้
ว่าเมล็ดควรจะเก็บจากต้นแม่จำนวน 25-50 ต้นในแต่ละประชากร

นอกเหนือกว่านั้น ผู้วิจัยเชื่อว่าข้อมูลของ microsatellite สามารถที่จะให้ความกระจ่างชัด
เกี่ยวกับโครงการอนุรักษ์ความหลากหลายทางพันธุกรรมได้ โดยการคัดเลือกแต่ละแม่ไม้ที่มีความ
หลากหลายของ microsatellite อัลลีล เพื่อที่จะคัดเลือกความหลากหลายทางพันธุกรรมดังกล่าว จึง
กำหนดแบบแผนทางคณิตศาสตร์ขึ้น 2 โมเดล คือเลือกแม่ไม้แต่ละต้นที่ทราบ genotype แล้ว
(โมเดลที่ 1) และคัดเลือกแม่ไม้โดยสุ่มจากประชากรที่ไม่ทราบองค์ประกอบของสารพันธุกรรม
(โมเดลที่ 2) ซึ่งได้เสนอวิธีการและข้ออภิปรายปัญหาและในบทที่ 6 และ 7

เมื่อนำข้อมูลทางด้านความหลากหลายทางพันธุกรรมมารวมกับข้อมูลอัตราการเจริญเติบ
โต อัตราการอยู่รอดของกล้าไม้ทั้งในเรือนเพาะชำและแปลงทดลองก็จะทำให้หลักเกณฑ์ในการคัด
เลือกต้นแม่มีความสมบูรณ์ชัดเจนมากยิ่งขึ้น

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ABBREVIATIONS

cm	centimeter
DAK	Doi Ang Khang agriculture research station
DBH	diameter at breast height
DI	Doi Inthanon National Park
DS	Doi Suthep-Pui National Park
DS1	southern part of Doi Suthep-Pui National Park
DS2	northern part of Doi Suthep-Pui National Park
ELV	elevation of parent tree
F_{IS}	inbreeding coefficient – reduction in heterozygosity of an individual, due to non-random mating within subpopulation (Wright, 1965)
F_{IT}	overall inbreeding coefficient – reduction in heterozygosity of an individual in relation to the total population (Wright, 1965)
F_{ST}	population differentiation coefficient – reduction in heterozygosity of a subpopulation due to drift (Wright, 1965)
g	gram
GBH	girth at breast height
GP	germination period
GR	percent germination
H_E	expected heterozygosity
H_O	observed heterozygosity
JS	Jae Sawn National Park

m	meter
MLD	median length of dormancy
mm	millimeter
PGM	percent germination
PSVf	percent seedling survival in the field
PSVn	percent seedling survival in the nursery
RCD	root collar diameter
RHGR	seedling relative growth rate of height
RHGRf	sapling relative growth rate of height in the field
RHGRn	seedling relative growth rate of height in the nursery
RRGR	seedling relative growth rate of root collar diameter
RRGRf	sapling relative growth rate of root collar diameter in the field
RRGRn	seedling relative growth rate of root collar diameter in the nursery
R_{ST}	a measure of genetic differentiation analogous to F_{ST}
S:HI	seedling height
S:HIf	sapling height in the field
S:RCD	seedling root collar diameter
S:RCDf	sapling root collar diameter in the field
SD	standard deviation
TG	time to germination

CHAPTER 1

INTRODUCTION

Deforestation is one of the most serious threats to biodiversity. The causes of deforestation are varied, including population pressure, shifting cultivation, agricultural development, transmigration, forest fire and unsupervised, poor logging practices (World Resources Institute, 1991). It causes climatic change, recurrent floods, soil erosion, loss of fertility, degradation of watersheds, deterioration in the quality of life and loss of wildlife habitats. It extirpates populations within species and reduces genetic diversity within populations, (Kanowski, 1999). Between 1980 and 1990, tropical forests were destroyed at a global average rate of more than 0.8 percent per annum. This implies that tropical forests have diminished by one tenth of their area during the last twelve years (FAO, 1997).

Thailand has a total area of 513,115 km^2 and is divided into 5 regions: the Northern, North-Eastern, Eastern, Central and Southern peninsula regions. In 1950, forest cover was approximately 53%, but it has now been reduced to 22.8% or 111,010 km^2 (FAO, 1999). However, these figures do not distinguish between plantations and natural forest and unofficial estimates put Thailand's natural forest cover less than 20% (Leungaramsri and Rajesh, 1992). The rate of forest loss peaked in 1977 and reached a minimum in 1989, when commercial logging was banned. The Royal Forest Department was established in 1896, to manage the country's forests. The total area of national parks and wildlife sanctuaries is now 14.2% of the country, but large parts of many of them are deforested and fragmented. This results in loss of

species populations, reductions in remnant population sizes, changes in densities of reproductive individuals, reduced reproductive success, increased isolation of remnant populations and reduced genetic variability (*e.g.* Prober and Brown, 1994) through genetic bottlenecks and mating systems.

Founder effects, genetic drift and restriction of gene flow, enhance inbreeding occur as a result of increasing genetic isolation and divergence (Bawa, 1993; Dayanandan *et al.*, 1999; Rosane *et al.*, 1999). Such factors also influence the evolutionary potential of populations and species, such that genetic variation is reduced so much that populations can no longer respond to changing environments through natural selection (Young *et al.*, 1993), because genetic diversity provides raw material for adaptability and evolution of species and individuals (Namkoong, 1991).

Restoration often involves planting native trees species and extension of forest boundaries by artificial and natural regeneration (Bawa *et al.*, 1990). Government and non-government organizations and local communities all need to be involved in restoring forest. In the past, reforestation in Thailand meant establishing single-species plantations, mostly pines and eucalypts, which are of little value for wildlife conservation and watershed protection. Since 1993, various reforestation projects have been implemented to celebrate the Golden Jubilee of His Majesty King Bhumibol Adulyadej. The projects promoted use of a wide range of native forest tree species and restored forest is to be preserved for conservation. However, implementing such an abrupt change in policy has been considerably constrained by lack of knowledge about

which tree species are most suitable for planting in the hot, dry sunny and weedy conditions found in most deforested areas and how to grow them. (FORRU, 1998).

The Forest Restoration Research Unit (FORRU) was established in November 1994 to tackle some of the technical problems involved in re-establishing natural forest ecosystems on degraded sites within national parks and wildlife sanctuaries in Northern Thailand (Elliott *et al.*, 1995). The project is jointly managed by the Department of Biology, Faculty of Science and the Headquarters of Doi Suthep-Pui National Park (under the Royal Thai Forest Department). FORRU is situated at the Headquarters of Doi Suthep-Pui National Park, Chiang Mai Province at about 1,000 m elevation.

The aim of the unit is to determine the most effective methods to complement and accelerate natural forest regeneration on deforested sites, within conservation areas, to increase biodiversity and protect watersheds. Specific objectives include:

1. development of tools for studying the restoration of natural forest ecosystems, such as a seedling identification handbook, seedlings herbarium and database of seed, fruits and seedling morphology;

2. understanding of the ecological processes of natural forest regeneration to determine ways in which these processes might be accelerated;

3. identification of tree species suitable for planting to complement natural seedling establishment;

4. development of appropriate methods to propagate such tree species and test their performance after planting out;

5. training of interested groups in the new forest restoration techniques developed by the project.

Seedlings produced by FORRU are usually propagated from seeds of only one or two parent trees located nearest to the unit. Seedling performance is likely to be variable, depending on the parent tree from which seeds were collected. Propagation of native trees requires more reliably high performance. Also collection of seeds from so few parent trees could narrow the genetic base of seedlings planted.

Ideally the characteristics of superior parent trees include high percent germination, fast growth in the nursery and in degraded areas and high a genetic diversity.

Research Objectives

The objectives of this study were:

1. to determine the variability in performance of planted trees germinated from seeds of different individual parent trees

2. to determine if characteristics of parent trees or seed or seedling performance in the nursery can be used to predict the performance of planted trees

3. to determine the genetic relatedness of the individual parent trees and

4. to combine 1-3 to devise seed collection strategies that maximize the performance of planted trees, whilst maintaining genetic diversity.

CHAPTER 2

LITERATURE REVIEW

2.1 BACKGROUND

Reforestation is defined as the re-establishment of any kind of tree cover, including plantations and agro-forestry on land which historically has contained forest but which has been used for another purpose since last being covered by forest (Elliott, 2000). Several studies have shown that forest plantations significantly accelerate natural succession by overcoming barriers to natural regeneration (Yu *et al.*, 1994; Kuusipalo *et al.*, 1995; Parrotta, 1995).

Forest restoration is one particular form of reforestation which is defined as the re-establishment of entire forest ecosystems, as similar as possible to the original forest ecosystems that were present before deforestation occurred (Elliott, 2000). Various forest restoration methods have been developed, for example, the assisted or accelerated natural regeneration method (ANR) (Jensen and Pfeifer, 1989), the Miyawaki method (Miyawaki, 1993), the framework species method (Tucker, 2000), and the accelerated pioneer-climax series or APCS method (Sôû, 2000).

The assisted or accelerated natural regeneration method (ANR) is defined as an approach to reforestation in which human intervention accelerates natural secondary succession (Jensen and Pfeifer, 1989). This methods usually involves no, or minimal tree-planting, instead, encouraging the natural processes of forest succession

(Hardwick *et al.*, 2000). Existing naturally–established trees are protected and nurtured, by weeding, mulching or the application of fertiliser, fire protection and dispersed seed will be encouraged (Elliott, 2000). This method is very low cost (Dugan, 2000), but can only work with the trees that are already established in deforested areas (Elliott, 2000).

The Miyawaki method (Miyawaki, 1993) is a more extensive system of forest restoration, involving the planting of up to 42 climax species, designed to return the forest to its primary condition as quickly as possible. Seedlings with well-developed root systems up to 80 cm tall are planted. The soil is prepared and adequate drainage provided. Organic fertilizers and mulching with rice straw is used. Two or three years after, planting no further management is needed. The accelerated pioneer-climax series or APCS (Sôû, 2000) was developed in Vietnam. APCS is based on the ecological principle of natural succession wherein pioneer species, which have the ability to adapted to adverse planting site conditions, improved the microclimate and soil conditions, rendering them favourable for the establishment of climax species. With this method, pioneer tree species were planted into the degraded areas first and later interplanted with climax tree species. The framework species method (Goosem and Tucker, 1995; Lamb *et al.*, 1997; Tucker and Murphy, 1997; Tucker, 2000) used a mixture of 20-30 pioneer and climax species planted in a single step. This method was adapted by FORRU for use in northern Thailand, with promising results (FORRU, 1998 and 2000).

2.2 The Framework species Method of Forest Restoration

'The framework species method' of forest restoration (Goosem and Tucker, 1995; Lamb *et al.*, 1997; Tucker and Murphy, 1997), originally developed in Australia, relies on selecting tree species that have high survival rates and fast growth rates in rehabilitation plots, with dense spreading crowns that rapidly shade out competing weeds and that attract seed-dispersing wildlife, especially birds and bats. In addition, framework species must be easy to propagate in nurseries. This includes readily available seed, high, rapid and synchronous germination and production of seedlings of a suitable height (50-60 cm) before the designated planting time (FORRU, 1998). High quality seedlings of 20-30 framework species, 40-60 cm tall are planted in deforested areas at the beginning of the rainy season which is in June in northern Thailand.

From initial work on more than 350 native trees species within Doi Suthep-Pui National Park, FORRU identified species with the potential to act as framework species (FORRU, 1998). They were grown in a nursery and planted out in experimental plots to determine whether the framework species method could work in the ecological and socio-economic conditions of northern Thailand.

There are three main groups of framework species (FORRU, 1998), fig trees (*Ficus* spp., Moraceae), legumes (Leguminosae), oaks and chesnuts (Fagaceae) and other individual framework species (FORRU, 1998).

Fig trees produce edible for animals inflorescences called syconia (fig) which look like fruits, consisting of a fleshy cup, with a small orifice closed by interlocking scales at one end. Enclosed within the figs are hundreds of minute flowers which, after pollination by species specific fig wasps, develop into tiny fruits. Birds are attracted and feed on planted fig trees, bring with them the seeds of other forest trees, which are deposited when they defecate, thus adding species to the tree seedling community of a regenerating forest. According to the framework species method, about 20% of seedlings planted should be figs species (Elliott *et al.*, 1998). There are at least 47 fig tree species indigenous to northern Thailand (CMU Herbarium database, 2000). The habit of figs include treelets, trees, shrubs, woody climbers and scandent.

Some legumes (Leguminosae) are fast-growing and have with spreading crowns. Many of them have nodules on the roots within which live mycrorhiza capable of fixing nitrogen from the air to make proteins. Consequently, some legumes exhibit high growth rates on degraded sites with low nutrients. There are 61 leguminous trees species indigenous to northern Thailand (CMU Herbarium database, 2000).

Oaks and chesnuts (Fagaceae), also produce dense spreading crowns and their nuts are likely to attract seed-dispersing wildlife into planted areas (FORRU, 1998). There are 40 species in northern Thailand (CMU Herbarium database, 2000).

Many tree species from other families can be included in the framework species mixture, but they should produce fruits attractive to seed-dispersers and have a high performance in open degraded areas. Some examples include *Spondias axillaris* Roxb. (Anarcardiaceae), *Bischofia javanica* Bl. (Euphorbiaceae), *Gmelina arborea* Roxb. (Verbenaceae), *Prunus cerasoides* D. Don (Rosaceae) and *Melia toosendan* Sieb. & Zucc. (Meliaceae).

After initial planting trials in 1998 and 1999, framework species suitable for planting in the deforested areas in northern Thailand were selected using criteria which included: more than 50% survival at the end of the 2nd growing season, more than 1.5 m. in height and more than 1.8 m in canopy width. In addition, resistance to fire is desirable, as fire is a serious annual hazard to tree establishment in seasonally dry tropical climate during the late dry season months (January to May) (Elliott *et al.*, in press). Elliott *et al.* (in press) reported 9 species, which ranked as "excellent" framework species, including *Ficus hispida* L. f. var. *hispida* (Moraceae), *Gmelina arborea* Roxb. (Verbenaceae), *Hovenia dulcis* Thunb. (Rhamnaceae), *Melia toosendan* Sieb. & Zucc. (Meliaceae), *Michelia baillonii* Pierre (Magnoliaceae), *Prunus cerasoides* D. Don (Rosaceae), *Rhus rhetsoides* Craib and *Spondias axillaris* Roxb. (both Anarcardiaceae). An additional 15 species qualified as "acceptable" framework species, including *Acrocarpus fraxinifolius* Wight *ex* Arn. (Leguminosae, Caesalpinioideae), *Balakata baccata* (Roxb.) Ess. (Euphorbiaceae), *Castanopsis acuminatissima* (Bl.) A. DC. (Fagaceae), *Ficus altissima Bl.*, *Ficus benjamina* L. var. *benjamina*, *Ficus glaberrima* Bl. var. *glaberrima*, *Ficus racemosa* L. var. *racemosa*, *Ficus subulata* Bl. var. *subulata* (all Moraceae), *Glochidion kerrii* Craib

(Euphorbiaceae), *Heynea trijuga* Roxb. *ex* Sims (Meliaceae), *Macaranga denticulata* (Bl.) M.-A. (Euphorbiaceae), *Machilus* bombycin King *ex* Hk. f. (Lauraceae), *Nyssa javanica* (Bl.) Wang. (Nyssaceae), *Sapindus rarak* DC. (Sapindaceae) and *Sarcosperma arboreum* Bth. (Sapotaceae).

The selected framework trees species comprise a mixture of pioneer (fast-growing in open areas) and climax species (slower-growing, normally in mature forest). By planting both types of tree species as the same time, forest succession can be short-circuited. The lack of seed dispersal appears to limit colonization of the climax tree species in the open areas. By including some climax tree species among those planted, it is possible to overcome this problem and accelerate the return of mature forest (FORRU, 2000). Pioneer tree species which grow fast will close the canopy and shade out weeds. Climax tree species which may grow slower, form a rising understorey beneath the canopy of the pioneer trees. New forest is also promoted by seed dispersal into the planted site. The seed of the other trees species situated around the planted site should be deposited into planting sites by frugivores, and germinate to form a young naturally established trees. Short-lived pioneer trees species will die naturally 10-20 years after planting. By this time, the climax tree species and naturally established tree will be ready to replace them to form a mature forest.

Five potential framework species were selected for this study: *Spondias axillaris, Melia toosendan, Gmelina arborea, Prunus cerasoides* and *Castanopsis acuminatissima.* In a study of FORRU to restore biodiversity to degraded land in

northern Thailand. Five species studied were planted into deforested areas in 1998 and

1999. Mean percent sapling survival, mean tree height and mean crown width at the

end of the second growing season were list in table below (Table 1).

Table 1. Performance of planted trees on 1998 and 1999 plot.

Tree species	1998 planting			1999 planting		
	mean % survival	mean height (cm)	mean crown width (cm)	mean % survival	mean height (cm)	mean crown width (cm)
S. axillaris	93.5	255.7	213.5	-	-	-
M. toosendan	98.3	535.1	255.2	60.4	705.8	235.2
G. arborea	75.0	160.8	146.3	60.4	180.1	159.9
P. cerasoides	86.7	241.0	188.7	47.9	303.3	241.7
C. acuminatissima	-	-	-	62.5	135.2	112.1

Source: Elliott and Anusarnsunthorn (2001)

Attempts to restore entire forest ecosystems are appropriate where wildlife

conservation, is the primary objective. Tree planting should reverse the effects of

fragmentation by creating corridors of forest to reconnect forest patches. Forest

restoration along stream–sides is also useful to prevent stream bank erosion and

siltation. (FORRU, 1998). However, actual forest restoration strategies must depend

on the results of discussion between local communities, landowners and Royal Forest

Department.

Reforestation requires a constant supply of high quality tree seed or vegetative

propagules. However, seed is often in short supply, as a result of much seed being of

low quality with variable maturity and a limited storage life (Owen, 1994).

In agriculture, collecting seed from superior parent stock has been practiced

for thousands of years. This technique results in higher yields, higher growth rate,

higher rate of survival, better plant quality and environmentally durable plants. This concept, however, has yet to gain widespread acceptance in forestry practice, to maximize yields and quality of trees for plantations, agroforestry, and other uses (Jones, 2000). Many studies have shown that field survival and productivity are related to the quality of the seeds and seedlings used.

2.3 SEEDLING QUALITY

Seedling quality has two main aspects. The first is the genetic quality or the sources of the seed. The second component of seedling quality is physical growth, which is influenced by the environment (*i. e.* nursery conditions and practices).

For high quality tree seedlings, described by Jaenicke (1999), the following characteristics are important:

- the ability to produce new roots quickly

- the speed with which seedlings get anchored in the ground, and start

  assimilating and growing after planting out

- a well-developed root system

- sun-adapted foliage

- a large root collar diameter

- a balanced shoot : root ratio, is an important measure of seedlings survival. It

  related the transpiring area (shoot) to the water absorbing area (roots). It is

  usually measured by determining root and shoot dry weights. A good ratio—

  one which indicates a healthy plant—is 1:1 to 1:2 shoot : root mass.

- good carbohydrate reserves

- an optimum mineral nutrient content

- the establishment of adequate mycorrhizal or *Rhizobium*

Size variables such as stem diameter and height are among the most common measures of seedlings quality. Zaerr and Lavender (1976) found that seedling survival rate of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) was positively related to fresh weight, which was highly correlated with stem diameter. Shiver *et al.* (1990) found a similar relationship between diameter and seedling survival rate of loblolly pine (*Pinus taeda* L.). South and Mexal (1984) studied loblolly pine and slash pine (*Pinus elliottii* Engelm.) and found a positive relationship between root-collar diameter and survival of newly planted seedlings. Large height:diameter ratios have been also been suggested as indicators of low tree vigor and of competition from overtopping and encroaching vegetation (Cole and Newman, 1987). Schneider *et al.* (1998) found that the seedling survival of Douglas-fir and ponderosa pine (*Pinus ponderosa* Dougl. *ex* Laws.) was positively related to weather conditions, stem diameter, crown width, age and precipitation and negatively related to the height-diameter ratio and competition indices for herbs, shrubs, and hardwoods.

2.4 GENETIC DIVERSITY

There are increasing concerns about maintaining biodiversity in forest ecosystems and about the possible impact of forest practices on biodiversity (Rajora, 1999). Genetic diversity is the basis of all biodiversity and is widely recognized as the key component for long-term survival of species on an evolutionary time-scale. It

provides the template for adaptation, evolution and survival of populations and species, especially in environments undergoing climate change, introduction of new pests, pathogens or competitors. (Rajora and Mosseler, 2001). Some human activities are likely to have significant genetic effects.

Millar (1999) lists the following activities:

- addition or removal a significant numbers of individuals from natural populations (*e.g.* timber harvesting, hunting etc.);

- significant changes in population sizes (especially decrease) of native species (*e.g.* livestock grazing, land clearing and habitat conversion, biological control);

- elimination of populations, especially systematically from portions of a species range (*e.g.* by urban development, dam construction);

- increases in individuals among ecologically distinct locations (*e.g.* ecological restoration);

- significant alteration of sex ratios, number of breeding individuals, reproductive capacity, fecundity, viability of individuals, or survival and mortality of certain age classes (*e.g.* grazing, timber harvest, fire suppression);

- introduction of disease vectors or insect pests, especially exotics (made worse by roads, trails and other access points into native populations);

- significant increases in the potential for hybridization (*e.g.* tree planting or introducing non-local germplasm);

- fragmentation of populations such that gene flow is drastically reduced; conversely, alteration of the population structure such that formerly isolated population regularly interbreed (*e.g.* road and dam construction);

- disruption or significant alteration of meta-population structure and other geographic patterns and related processes (timber harvest, fish stocking, dam construction).

Therefore, maintaining genetic variation is an important objective of biodiversity conservation (Thomas *et al.*, 1999). Indeed, conservation of genetic diversity may be one of the most important issues influencing future forest management practices relying on natural and/or artificial regeneration systems. Tree improvement and natural disturbance such as forest fires, can significantly impact on genetic variability in subsequent forest populations (Rajora, 1999).

However, the impact of harvesting and regeneration on genetics are very poorly understood. Studies have yielded differing results. Knowles (1985) found that genetic diversity between fire-origin and artificially regenerated stands of jack pine (*Pinus banksiana* Lamb.) and black spruce (*Picea mariana* Mill.) B. S. P. were not different. Thomas *et al.* (1999) found no difference in genetic diversity of lodgepole pine (*Pinus contorta* Dougl. *var. latifolia*) between 3 stand types (mature lodgepole pine (>100 years), 20 to 30 year old harvested stands left for natural regeneration, and 10 to 30 years old planted stands). In contrast, Gömöry (1992) reported that stands of Norway spruce had significantly less genetic diversity than unharvested or naturally regenerated stands. Even with wild seed collection, inadvertent loss of genetic

diversity or shifts in allele frequencies may result from selection during seed collection, seed processing and seedling production (El-Kassaby and Thomson, 1996). Buchert *et al.* (1997) reported genetic diversity was reduced by 25-50% in old-growth eastern white pine in the post-harvest residual gene pool. On the other hand, selection during nursery production may lead to higher levels of genetic diversity in artificially regenerated stands. (Mouna *et al.*, 1988).

2.5 SEED QUALITY

Seedling quality depends on the seed used (Jaenicke, 1999). The quality of seed planted in the nursery is of crucial importance, since seeds are the most basic input into any planting programme. It is therefore necessary to pay proper attention to quality issues when procuring and subsequently storing tree seed until planting. Seed quality is measured in two ways: firstly by the physical quality of the seed and secondly by the desired physical traits of the resultant mature tree. Seed quality usually refers to the genetic, physical and physiological states of the seeds. Seeds with good physiological quality for forest restoration have high germination percentage and produce vigorous seedlings. Physical quality refers to seed size or infestation by pathogens. Many factors can influence the physiological and physical quality of tree seeds and all of these factors affect the production of seedlings in a nursery. The advantages of good physical and physiological seed quality are improved storage characteristics, minimal seed wastage and uniform seedlings in the nursery. Genetic quality refers to the inherent capacity of a seed to produce a tree adapted to the environmental conditions at the site where it is planted (Turnbull, 1995).

2.6 SELECTING SEED SOURCES FOR COLLECTION

"The choice of seed source is one of the most important decisions faced by the forest manager. An error in judgment can lead to trees with poor stem and branch form or prone to pests and diseases. Within the genetic constitution of the seed is the potential for either good or poor tree growth, and since even small increases in growth rate or improved timber quality can lead to a much enhanced return on investment, the advantages of using the best available seed from which to grow the planting stock are considerable" (Hibberd, 1991).

When the species and provenance have been decided, then identification of a suitable seed source is the next step. "Provenance" may be defined as an area in which trees grow under similar environmental conditions. An ideal provenance is, according to Barner (1975),

1. keep in a community of potentially interbreeding trees of similar genetic constitution (and significantly different genetic constitution from other provenances);

2. is sufficiently large for collection of reproductive material in quantities significant for forest practice and

3. defined by boundaries, which can be identified in the field.

In Thailand, the Forest Genetic Resources Conservation and Management Project (FORGENMAP) is one of the most important projects concerned with seed

quality. It is based on existing organisational structures in Thailand and was funded

for the first 3 years by the Danish Cooperation for Environment and Development

(DANCED). It was established in 1997 to secure material for the propagation of forest

trees now and for the future. The aim of the project:

1. to ensure the presence of an effective, public seed supplying organisation;

2. to support community and co-operative based activities in order to make
    sustainable use of genetic resources;

3. to support the private sector and make its material and techniques generally
    available and

4. to support and promote biodiversity and forest rehabilitation

Forest tree seed is collected from the best and most vigorous trees with a

strong growth potential and distributed to the organisations (Jones, 2000). The best

site and stands were registered and established as seed sources. In addition, the genetic

base of forest trees is maintained and improved through seed banks, protection of the

original growth sites (*in situ* conservation) and through the establishment of new

growth sites (established seed sources including *ex situ* conservation areas) (Pedersen,

2001).

Often, it is important to choose as a seed source, a provenance with an

environment closely matched to that of the planting site. For example, seed from a

mountainous region should only be planted in a mountainous region and seed

originating from the lowlands will grow best in lowland conditions. Matching seed

source to the site helps ensure the adaptability of trees planted to the planting site,

maximizing resistance to diseases and pests, as well as survival and growth rate. (Friday, 2000). One very important aspect of choosing a seed source is consideration of its genetic history, as this will affect seed quality. Genetic history includes population size and structure, contamination from foreign pollen, and the natural and artificial selection to which the natural stand and subsequent plantations have been subjected (Jones and Burley, 1984).

The main criteria in selecting tree seed of a particular species described by Wilkinson and Elevitch (1999) were:

1. characteristics of the tree products, such as form, wood quality, or biochemical traits are to be passed on to offspring of a tree;

2. adaptation to site conditions such as soil, wind, elevation and rainfall. Different populations of a species have varying tolerances to environmental conditions and stresses;

3. growth rate. Studies have shown that increases of a 20-100% in overall growth rate can be achieved for a species by selecting seed from vigorous parents/superior parents;

4. resistance to pests and diseases and

5. genetic diversity.

2.7 SEED COLLECTION

2.7.1 TIMING OF SEED COLLECTION

The timing of seed collection is very important. Correct assessment of seed maturity is critical in timing collections and has a major effect on the physiological quality of tree seeds. The development of maturity indices to ensure that seed can be harvested at the most appropriate time has been identified as a high priority research topic (Turnbull, 1995). It is best to collect seed when they are fully mature. Seed of some species may remain on the trees for considerable periods after ripening, and this may account for some of the discrepancies in the literature on dates of seed collection. However even for these species, it is better to collect seed as soon as possible after it has ripened, otherwise there may be losses due to the seed being scattered, or to insect attack. The first and last fruits are often irregular, and their seeds may not produce healthy seedlings (Turnbull, 1995). The dates on which of fruits of individual trees ripen may vary considerably between different localities, as they are affected by factors such as rainfall, altitude and aspect. Dates may also vary from year to year, according to the weather. The maturity of seed can usually be judged by changes in the colour or texture of the fruits.

Fruits of some species may be best collected at the immature stage; to minimize loss of seeds to dispersal and/or predation; to avoid development of dormancy (because some types of dormancy develop only during late maturity (Mayer

and Poljakoff-Mayber, 1982); to extend the collection season or to reduce pre-processing damage.

2.7.2 METHODS OF COLLECTION

Seed collecting methods have a major effect on seed quality. The various methods of collection may be classified into the following: (1) collection of fallen fruits or seeds from the forest floor; (2) collection from crowns of felled trees; (3) collection from the standing trees with access from the ground and (4) collection from standing trees with access by climbing (Boland, 2001). The choice of method is dependent on many factors including the characteristics of fruits, trees, stands and sites and the amount of seed to be collected. Seed is usually best collected from the trees, rather than after it has fallen to the ground, because seed collected from the ground is liable to be damaged by insects. This often means climbing the trees. Small fruit-bearing twigs may be cut and the fruits allowed to fall to the ground, but this should not be taken as permitting large branches to be looped to make seed collection easier. When collecting small fruit or seed from standing trees, it may be useful to spread polythene sheets or cloth beneath the trees to make finding the fallen fruit easier, or else the ground beneath the trees can be hoed or swept clean.

2.8 EFFECTS OF SEED TRAITS ON SEEDLING PERFORMANCE

Seed traits, including seed size, seed mass, dormancy, germination and dispersal, are central components of plant life histories, and through their pronounced

and multiple fitness effects, are a critical element in the ecology and evolution of plant life histories (Jansen, 1969; Harper, 1977). Two seed traits closely related to fitness are size and germination timing. Whereas seed size variation within species may be slight compared to the 10^{11}-fold variation found among species (Westoby et al., 1992). Seed size is one element of a coevolving complex of traits (Venable and Brown, 1988) including seed dormancy, dispersal, plant mass, longevity, niche specialization, and competition among species. (Simon and Johnston, 2000). Seed size variation may produce an optimal seed shadow (Jansen, 1977) or minimize the risk of failure in heterogeneous environments (Venable and Brown, 1988). Also, variation in seed size might be adaptive if seeds of different sizes differ in genetic quality (Temme, 1986). Alternatively, seed size variation might be the result of architectural and physiological constraints (Diggle, 1995), maternal effects (Wulff, 1986), or reduced resource availability throughout the growing season (Winn, 1991).

Since theoretical considerations predict that larger seeds within a given species will yield larger, more competitive seedlings with better performance, it follows that variation in seed size may produce variation in seedling fitness. Seed size varies within and among genotypes. These variations are caused by both environmental factors (i.e. height and aspect of the fruit in the crown) and genetic factors (El-Kassaby, 2000). For example, Silen and Osterhaus (1979) and Chaisurisri et al. (1992) demonstrated significant differences in seed weight among Pseudotsuga menziesii (Mirb.) Franco and Picea sitchensis (Bong.) Carriere clones, respectively. Both studies revealed that the smallest seeds within a clone could be as large or larger than the largest seeds in another clone.

Most studies demonstrate that large seeds within a given species have many advantages over small seeds. For example, relative to small seeds, large seeds usually have higher percent germination (Black, 1959; Harper and Obeid, 1967; Cideciyan and Malloch, 1982; Wies, 1982; Morse and Schmitt, 1985; Winn, 1985; Wulff, 1986; Tripathi and Khan, 1990; Maranon and Grubb 1993; Nizam and Hossain, 1999), greater or more rapid emergence from deeper depths of sowing and less stringent requirements for emergence with respect to herbaceous cover (Winn, 1985; Castro, 1999) and drought (Baker, 1972). They also have lower mortality (Schaal, 1980; Tripathi and Khan, 1990; Bonfil, 1998) higher seedling growth rate (Stock *et al.*, 1990; Seiwa and Kikuzawa, 1991; Seiwa, 2000) and their seedlings have survive longer than seedlings from small seeds under adverse conditions such as low light (Howe *et al.*, 1985), low soil moisture (Leishman and Westoby, 1994; Manga and Yadav, 1995) and nutrient limitation (Allsopp and Stock, 1995). Consequently, large seeds may give rise to better competitors (Anderson, 1971; Wulff, 1986). In addition, large seeds may provide seedlings with better protection against herbivores and pathogens (Foster, 1986; Bodnaryk and Lamb, 1991) and they may be better able to tolerate damage (Grime and Jeffrey, 1965).

Also, larger seeds, with greater initial energy reserves (Salisbury, 1942; Leisman and Westoby, 1994) could help confer shade tolerance by: (1) allowing radicle penetration into mineral soil through deep litter layers that are often found in forest understories (Molofsky and Augspurger, 1992); (2) allowing initial establishment of a larger germinant, which would make it less susceptible to

smothering by leaf litter and could enable successful competition with short-statured vegetation (Leisman and Westoby, 1994); (3) providing reserves for the compensation of leaf loss from damaging agents (Foster, 1986), and/or; (4) delaying the onset of carbon starvation and death in low light in understories, thus increasing the likelihood of exposure to a canopy gap before death (Leisman and Westoby, 1994).

Stanton (1984) reported that seedlings were more likely to emerge from large seeds of wild radish than from small seeds, and would grow more rapidly, but seed size had no effect on emergence time. Seedlings from large seeds also produced more flowers than those from small seeds. The timing to germination of *Lobelia inflata* L. seed has been shown to be influenced by seed size which had a persistent and significant association with both final plant size and seedlings survival (Simon and Johnston, 2000). Khurana and Singh (2000) studied the influence of seed size on seedling growth of *Albizia procera* Benth. under different soil water levels. They found that seedlings from large seeds were more tolerant of long-term extreme water stress compared to those from small seeds, which were more tolerant of moderate levels of water stress.

In contrast, several studies have detected ecological factors that can lead to an opposite trend, particularly during the phase when seeds remain in the soil. Vertebrate predation is more likely with large seeds, because large seeds represent a richer energy resource or are more apparent to vertebrates than small ones (Smith, 1975; Hulme, 1993; Van der Wall, 1994).

The advantages of small seeds over larger seed include a competitive advantage over large seeds due to earlier germination (Black and Wilkinson, 1963; Anderson, 1971), greater rates of germination under some conditions (Hendrix, 1984; Cornelissen *et al.*, 1996; Reich *et al.*, 1998) and longer dispersal distances (Jackson, 1981; Howe and Richter, 1982), increasing the probability that some seeds will reach favorable microsites. (Chambers and MacMahon, 1994). Seedlings from small seed in Mediterranean annuals have a faster relative growth rate than seedling from bigger seed (Maranon and Grubb, 1993). In addition, Susko and Lovett-Doust (2000) reported that smaller seeds of *Alliaria petiolata* (M. Bieb) Cavara and Grande germinated significantly earlier and that seedlings from smaller seed produced their first primary leaves significantly later and grew significantly taller.

Other studies, however show no relationship between seed size and seedling performance (Cipollini and Stiles, 1991; Rice *et al.*, 1993), seed germination (Vaughton and Ramsey, 1997; Eriksson, 1999), seedling survival (Hendrix and Trapp, 1992), and seedling growth (Dalan, 1984; Marshall, 1986).

Seed mass varies considerably among species in different habitats and different successional stages (Foster and Jenson 1985). However, numerous studies have demonstrated that seed mass within a species or even an individual plant can vary greatly, which commonly occurs in the range of two or sixfold (Salisbury, 1942; Black, 1959; Jansen, 1977; Schaal, 1980; Howe and Richter, 1982; Wies, 1982; Hendrix, 1984; Stanton, 1984; Winn, 1985). It plays a role in the process of dispersal and population recruitment, seedlings establishment, seedling size, relative growth

rate and competitive ability (Westoby *et al.,* 1992; Thomson *et al.,* 1993). Grime and Jeffrey (1965) and Leishman and Westoby (1994) found that seed mass is positively related to short term seedling survival in shade. Gonzaleze (1993) studied the effect of seed size on germination and seedling vigor of *Virola koschnyi* Warb. He found that seeds with greater mass produced more vigorous plants, but there were no effects on percent germination. Castro (1999), Nizam and Hossain (1999) and Sawaminathan and Sivagnanm (1999) reported that seed germination and seedling growth increase with increasing seed mass. Seedlings of heavier-seeded species tend to survive longer when grown in the absence of any mineral nutrients are able to emerge from greater depths in the soil than seedlings from lighter-seeded species. Castro (1999) found that the initial growth of the shoot was positively correlated with seed mass. However, Paz *et al.* (1999) reported that seed mass did not have a general effect on emergence success and the effects of seed mass on seedling emergence are driven by external ecological factors more than by intrinsic effects of seed mass.

Timing of emergence is influenced by seed size and light conditions (Simon and Johnston, 2000). It is well known that timing of germination can be controlled by light quantity or quality; temperature; water; available nutrients, especially nitrates; and a combination of these (Bewley and Black, 1994). Time of emergence is one factor that effects seedling performance, through effects on seedling growth rate. Seedlings that germinate and emerge early in the growing season have an advantage over those that emerge later (*e.g.* greater survival, Jones *et al.*, 1997) by (1) capturing a disproportionate share of environmental resources such as light, nutrients, and water compared with late-emerging neighbors in grasslands or old fields, (2) receiving

ephemeral light for longer before canopy closure and (3) reducing pathogen and predator loads (Seiwa, 2000).

2.9 THE BIOTECHNOLOGY OF FORESTRY

Factors that affect seedling production in nurseries include incomplete release from dormancy, germination treatments, age, condition, management of seed stands, climate, condition of mother trees during seed development, ripeness at collection and seed processing including pathogen attack during collection, cleaning and extraction, drying and storage (Poulsen, 1993). The effects of a changing environment may influence flowering, fruiting and seed set. Patterns of genetic variation should be apparent in patterns of phenotypic variation (Bawa *et al.,* 1990). Studies of flowering and fruiting phenology, morphological characteristics of adult trees, seed germination and early seedling performance in the nursery can identify superior parent trees. Seed for nursery propagation by FORRU is usually collected from a very small number of parent trees, which could cause narrowing of the genetic base or shifts in allele frequencies of the seedlings. Narrowing of the genetic base, endangers the species in future generations. Therefore genetic studies are essential.

During the past 20 years, techniques have developed rapidly for evaluation and genetic manipulation of populations and individual trees. Genetic markers can be divided into three main classes; morphological markers, biological markers and the more recent DNA-based markers (Glaubitz and Moran, 2000). Morphological markers display Mendelian inheritance (*e.g.* chlorophyll deficiency). Biochemical markers can

be either at the protein level (*e.g.* isoenzymes) or at the level of organic chemicals (*e.g.* terpenes). Isoenzymes are codominant markers, which have been used in forest genetics since the early 1960s and are still used in many laboratories, due to their low cost and usefulness in estimating mating systems and genetic diversity (Changtragoon, *et al.,* 1996). Since 1990, many new DNA based markers have been developed and applied. These include Polymerase-Chain-Reaction (PCR) based markers, namely amplified fragment length polymorphisms (AFLPs), random amplified polymorphic DNA (RAPDs), microsatellites (SSRs), restricted fragment length polymorphism (RFLPs) and also DNA sequencing (nuclear chloroplast and microchondrial DNA) (Burley, 2001). Today, DNA markers are preferred to determine the genetic diversity of forest populations, because they usually detect more polymorphic loci than isoenzymes (Szmidt, 1995).

The advantages of molecular markers include: *(i)* they are relatively inexpensive when considering the amount of information generated per unit time; (*ii*) they can be readily applied to any species, using universal protocols, reagents and analytical procedures; (*iii*) they involve non-destructive sampling of biological materials; (*iv*) they generate genetically interpretable data without environmental influence and (*v*) they allow for very rapid data gathering (Grattapaglia *et al.,* 2000).

Molecular markers have been used as an effective tool in forest trees for measuring genetic diversity and differentiation in natural, managed and breeding populations; estimating rate of gene flow or migration; characterising mating systems; analysing parentage; assessing seed orchard efficiency; DNA fingerprinting or

verification; quality control in breeding; studying phylogeny or taxonomy and genetic linkage mapping/quantitative trait loci analysis/marker-assisted selection (Glaubitz and Moran, 2000).

Microsatellites have become the preferred marker in many studies because of their high allelic diversity, reliability of scoring and codominant inheritance. Microsatellites, also known as simple sequence repeats (SSRs) (Tautz, 1989) and short tandem repeats (STRs) (Edwards *et al.*, 1991), have strong discriminatory power and are becoming a popular tool for studying of genetic diversity, gene flow and conservation of natural plant populations (Chase *et al.*, 1996; Dow *et al.*, 1995; Dayanandan *et al.*, 1997; Streiff *et al.*, 1998; Ueno *et al.*, 2000). Microsatellites are short stretches of tandemly repeated, simple DNA sequences such as $(GT)_n$ or $(CAC)_n$ (each generally less than 5 base pairs in length) (Zhao and Kochert, 1993). They were first documented almost 24 years ago by Hamada and colleagues. They are widely dispersed in eukaryotic genomes and are often highly polymorphic, due to variation in the number of repeated units (Bruford and Wayne, 1993). This occurs because there is a high mutation rate for the number of repeats at microsatellite loci, as DNA replicating enzymes make mistakes in the number of repeats at a relatively frequent rate. Estimates of microsatellite mutation rates in *Escherichia coli* in *in vivo* systems are about 10^{-2} event per locus per replication (Levinson and Gutman, 1987), in yeast rates are $10^{-4} - 10^{-5}$ units (Henderson and Petes, 1992; Strand *et al.*, 1993), and in *Drosophila* about 6×10^{-6} units (Schug *et al.*, 1997) and in humans, the rate has been estimated at 10^{-3} events per locus generation (Weber and Wong, 1993).

Microsatellites were first developed for use in the human genome (Weber and May 1989; White and Powell, 1997; Brunel, 1994), in which commonly repeated types are poly(A)/poly(T) stretches (Stallings, 1992). They were later found to be abundant in plants (Morgante and Olivieri, 1993) which commonly have GA/CT and AT repeats (Stallings, 1992).

The main disadvantage of using microsatellite markers 'in practice' is the relatively high up-front costs and the molecular biology expertise and infrastructure necessary for the development of specific primer sequences and the genetic characterization of allelic variation at amplified loci. Current methods for isolation of microsatellite markers involve: (1) The creation of a small insert genomic library; (2) library screening by hybridization; (3) DNA sequencing of positive clones; (4) primer design and locus-specific PCR analysis and (5) Identification of polymorphisms. (Powell *et al.*, 1996).

In recent years, microsatellites have become a popular tool for genetic mapping, analysis of mating systems, paternity and patterns of gene flow within and among populations, and maintenance of genetic diversity in plant groups. They may also prove to be very useful for quality control in tree breeding programs and for certification of genetically improved seed and planting stock. The first microsatellites developed in trees were from *Pinus radiata* D. Don (radiata pine) (Smith and Devey, 1994) and they have since been developed in many forest tree species, including *Quercus* spp. (oak) (Dow *et al.*, 1995; Barrett *et al.*, 1997; Isagi and Suhandono, 1997; Lexer *et al.*, 2000), *Eucalyptus* spp. (Byrne *et al.*, 1996), *Pinus* spp. (Echt *et al.*, 1996;

Thomas *et al.*, 1999), *Picea abies* (L.) H. Karst (Norway spruce) (Pfeiffer *et al.*, 1997), *Populus tremuloides* Michx. (Dayanandan *et al.*, 1998), *Magnolia obovata* Thunb. (Isagi *et al.*, 2000) and *Larix decilua* L. (Khasa *et al.*, 2000).

For tropical trees, microsatellites were first developed from *Pithecellobium elegans* Ducke (Mimosoideae) (Chase *et al.*, 1996) and they have since been developed in *Swietenia humilis* Zucc. (White and Powell, 1997; White *et al.*, 2000), *Gliricidia sepium* Humb. Bonpl. & Hunth (Dawson *et al.*, 1997), *Quercus petraea* (Matt.) Liebl. (Steinkellner *et al.*, 1997), *Shorea curtisii* Dyer *ex* King, *S. cordifolia* (Thw.) Ashton and other Dipterocarpaceae species (Ujino *et al.*, 1998; Stacy *et al.*, 2001), *Symphonia globulifera* L. (Preston *et al.*, 1998), *Carapa quianensis* Aublet. (Dayanandan *et al.*, 1999), *Caryocar brasiliense* Camb. (Rosane *et al.*, 1999), *Melaleuca alternifolia* Cheel (Rossetto *et al.*, 1999), *Castanopsis cuspidata* var. *sieboldii* Nakai (Ueno *et al.*, 2000), *Avicennia marina* (Forsk.) Viern. (Maguire *et al.*, 2000) and *Castanopsis fargesii* Franch. (Xu *et al.*, 2001).

Application of SSRs to population and conservation genetics of tropical forest is limited due to a paucity of DNA sequence information for many tropical tree species. However, SSR primers developed for one species may be used to detect polymorphisms in related species, minimizing laborious cloning and screening procedures (Dayanandan *et al.*, 1997; Downey and Iezzoni, 2000). Wu and Tanksley (1993) have assessed microsatellite diversity for accessions of rice and showed that the primers were capable of identifying SSRs in conspecific taxa and Thomas and Scott (1993) found similar sequence conservation among grapevine species. Kijas *et*

al. (1995) found that it was possible to identify the cultivars and species of *Citrus* and *Poncitrus* by using the SSRs from closely related taxa. Lexer *et al.* (1999) used the microsatellites primers developed from *Quercus petraea* (Matt.) Liebl. (Steinkellner *et al.*, 1997) to detect the seed contamination and inference of the seed parents from the offspring. Rosane *et al.* (1999) reported the microsatellites loci developed for *Caryocar brasiliense* Camb. can be used to the other species of *Caryocar*.

Konuma *et al.* (2000) used microsatellites primers developed from *Shorea curtisii* Dyer ex King (Ujino *et al.*, 1998) to study the gene flow in the tropical rainforest tree *Neobalanocarpus heimii* (King.) Ashton. Stacy *et al.* (2001) found that microsatellites developed from *Shorea cordifolia* (Thw.) Ashton could be successfully amplified in *S. megistophylla* Ridl. and expected that these primers may be transferable to other *Doona* species.

For the genus *Prunus*, microsatellite primer pairs are available from sweet cherry (*Prunus avium* L.) (Sosinki *et al.*, 2000), sour cherry (*P. cerasus* L.) (Downey and Iezzoni, 2000), and peach (*Prunus persica* (L.) Batsch) (Gannavarapu, 1998; Cipriani *et al.*, 1999). Cipriani *et al.* (1999) found that microsatellite repeats in peach could be successfully amplified in other species of the genus *Prunus*, with some amplification in *Malus* (apple). Furthermore, Sosinski (2000) reported that primers developed from apple and sour cherry could be amplified in peach and apricot. Testolin *et al.* (2000) used microsatellites in fingerprinting and testing the genetic origin and cultivars of peach. Downey and Iezzoni (2000), used primers developed from sweet cheery, sour cherry and peach to examine genetic diversity of black cherry

(*Prunus serotina* Ehrn.) and Cantini (2001) used microsatellites to identify accessions of sour cherry and ground cherry for germplasm preservation and use.

In Thailand, genetics markers including isoenzymes, RFLP's, RAPDs, and microsatellites have been used to study forest trees. Isoenzyme are the most popular marker, and have been used to study genetic diversity and variation of *Azadirachta* spp. (Changtragoon *et al.*, 1996), *Melaleuca cajuputii* Powell (Changtragoon and Szmidt, 1998), *Pinus merkusii* Jungh. & De Vriese and *P. kesiya* Roy. *ex* Gord. (Szmidt *et al.*, 1996); the mating system of *Acacia auriculiformis* A. Cunn. *ex* Benth. (Changtragoon, 1998) and species and clone identification of *Azadirachta* spp. (Changtragoon *et al.*, 1996). RFLP's have been used to study genetic diversity, variation and species identifications of *Pinus merkusii* Jungh. & De Vriese and *P. kesiya* Roy. *ex* Gord. (Szmidt *et al.*, 1996) and somaclonal variation of *Populus* spp. (Changtragoon, 1991). RAPDs have been used to study genetic diversity and variation of *Calamus palustris* (Changtragoon *et al.*, 1996), *Tectona grandis* L. f. (Changtragoon, 1998), and *Rhizophora apiculata* (Changtragoon and Szmidt, 1998). Microsatellite markers have been used in a several studies. Eiadthong *et al.* (1999) used them to identify Mango (*Mangifera indica* Linn.) cultivars and evaluate their genetic variation. Miwa *et al.* (2001) used them to analyse the clonal structure of *Melaleuca cajuputii* Powell (Myrtaceae) at a barren sandy site in Thailand.

CHAPTER 3

METHODS

3.1 STUDY SITES

Experimental work was conducted from June 1999 to May 2002. Seeds used in

this study were collected in Doi Suthep-Pui National Park, Doi Inthanon National

Park, Jae Sawn National Park and Doi Ang Khang agriculture research station.
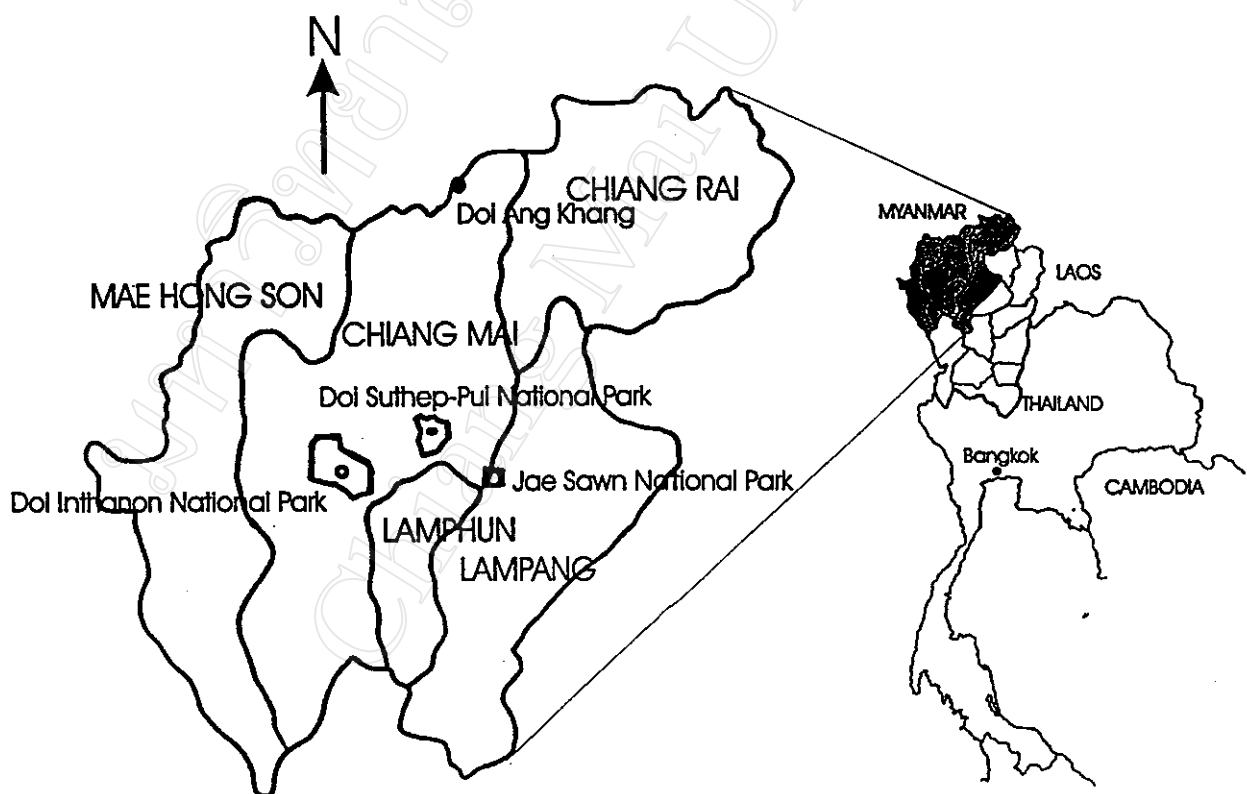
Figure 1. Locations of the study sites: Doi Suthep-Pui National Park, Doi Inthanon

National Park, Jae Sawn National Park and Doi Ang Khang agriculture

research station.

Doi Suthep-Pui National Park was established on 14 April 1981, the 24th national park to be designated in Thailand. It is situated at approximately 18° 43'-19° 08' north latitude, 98° 48' - 98° 58' east longitude. The national park extends over four districts of Chiang Mai Province: Muang District, Hang Dong District, Mae Rim District and Mae Taeng District. The area of the park is 262.5 km^2 (163,162.50 rai). The lowest elevation is 350 m and the highest is 1,685 m at the summit of Doi Pui. The bedrock of the mountain is almost entirely granite. The annual average rainfall is about 1,000 mm at the base and 2,000 mm at the summit of the mountain. The rainy season is from May to October when the highest monthly rainfall is about 2,500 mm in August. Whilst the average temperature throughout the year is 20-24 °C, the highest is 30 °C in April.

Three forest types are described by Maxwell (1988) and Maxwell and Elliott (2001). The deciduous dipterocarp-oak association, occurs between 350–750 m above sea level. Common trees include members of the Dipterocarpaceae (*e.g. Dipterocarpus obtusifolius* Teijsm. *ex* Miq. var. *obtusifolius, D. turberculatus* Roxb. var. *tuberculatus, Shorea siamensis* Miq. var. *siamensis* and *Shorea roxburghii* G. Don) and Fagaceae (*e.g. Quercus kerrii* Craib var. *kerrii* and *Q. kingiana* Craib). Many trees of this forest type support large numbers of epiphytes, including ferns (*e.g. Drynaria rigidula* (Sw.) Bedd. (Polypodiaceae)) and orchids (*e.g. Cymbidium siamense* Rol. *ex* Dow. (Orchidaceae)). The ground flora includes many perennial grasses (*e.g. Apluda mutica* L. (Gramineae)) and other herbs such as *Boesenbergia longifolia* (Wall.) O. K. (Zingiberaceae), *Geniosporum coloratum* (D. Don) O.K. (Labiatae) and *Liparis sutepensis* Rol. *ex* Dow. (Orchidaceae).
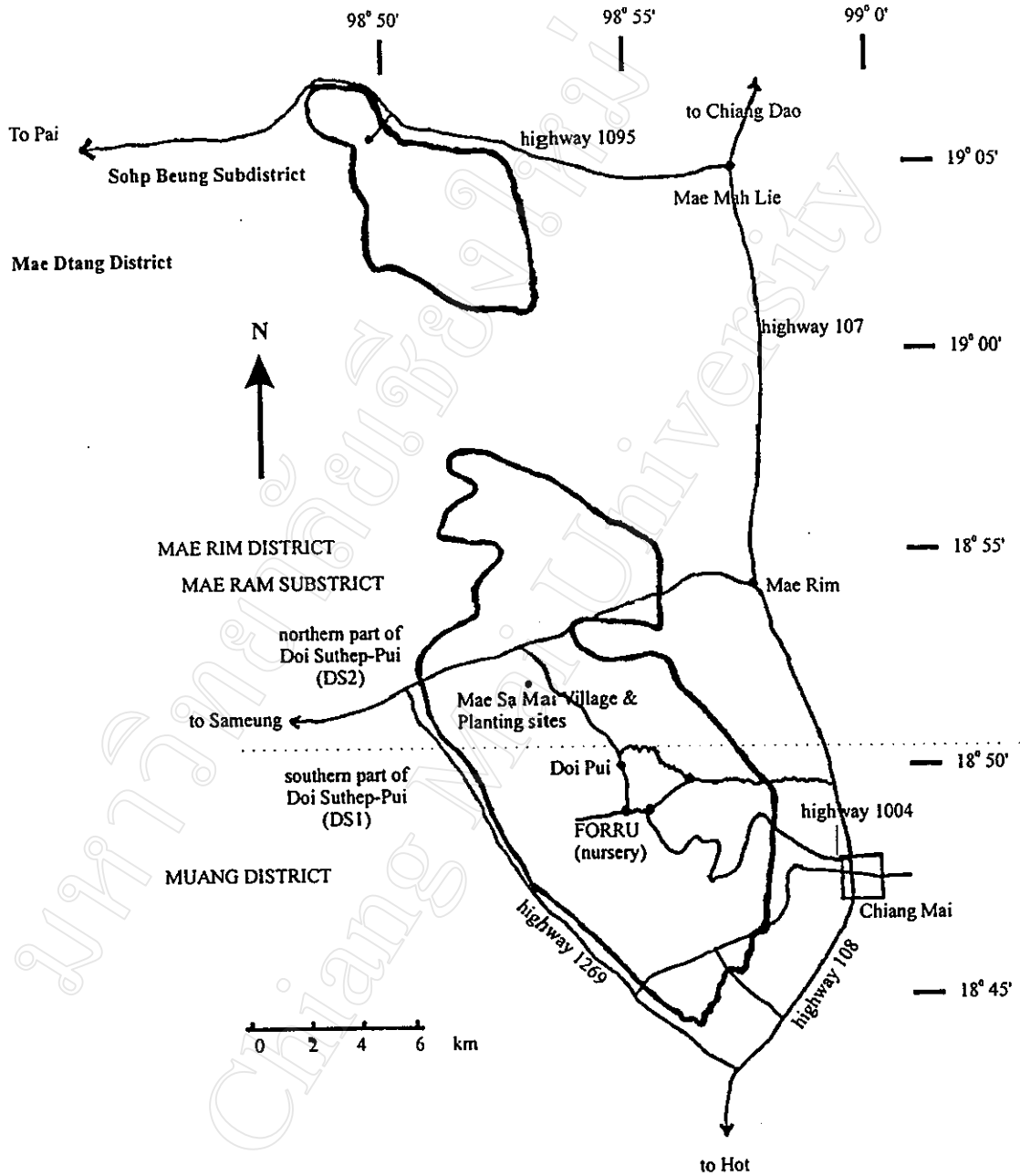
Figure 2. Doi Suthep-Pui National Park, Chiang Mai Province, Thailand.

(modified from Maxwell and Elliott, 2001)

south west of Doi Suthep-Pui National Park. The bedrock of the mountain is mostly granite and limestone with above predominantly sandy loam soils. The summit is to 2,565 m asl and totaling 272 km^2, was declared the country's sixth national park in October 1972. The climate of Doi Inthanon is monsoonal with an annual average rainfall of about 2,000 mm, most of it between May–October. The mean annual temperate in the Chiang Mai lowlands is 25.8 °C, but on the mountain it lower. With every 100 m increase in altitude, the mean temperature decreases by 0.6 °C. The coldest months are December and January when ground frost may cover exposed ridges near the summit where a low of –8 °C has been recorded.

The vegetation is similar to Doi Suthep-Pui national Park which described by Maxwell (1988) and Maxwell and Elliott (2001), except for the evergreen forest above 1800-2565 m. The dominant tree species are *Castanopsis acuminatissima* (Bl.) A. DC., *Castanopsis armata* (Roxb.) Spach, *Castanopsis diversifolia* (Kurz) King ex Hk. f., *Castanopsis tribuloides* (Sm.) A. DC., *Quercus kingiana* Craib, *Quercus brandisiana* Kurz *Lithocarpus garrettianus* (Craib) A. Camus (all Fagaceeae), *Schima wallichii* (DC.) Korth. and *Anneslea fragrans* Wall. (both Theaceae), *Rhododendron arboreum* ssp. *delavayi* (Ericaceae). The ground flora includes *Euonymus cochinchinensis* Lour. (Celastraceae), *Polygonum* spp. (Polygonaceae), *Impatiens violaeflora* Hk. f. (Balsaminaceae) and *Cyathea podophylla* (Hk.) Copel. (Cyatheaceae). Epiphytes includes mosses, liverworts, lichens, ferns and orchids.

Jae Sawn National Park was established in 1988, in Lampang Province, northern Thailand, and has an area of 768 km^2. The national park is situated in Muang, Muang Pahn, and Wahng Nua Districts and is positioned between latitudes *c.*

Muang, Muang Pahn, and Wahng Nua Districts and is positioned between latitudes *c.* 18° 30' - 19° 05' north and longitudes *c.* 99° 25' - 99° 30' east. The lowest elevation in the park is on the eastern boundary, *c.* 300-350 m, and the highest point in the park is 2,031 m above sea level in the west–central part. The bedrock in the park is granite, shale and limestone. The climate of the park is strongly seasonal. There is a hot, dry season from March to May, followed by the rainy season from June to October which has highest rainfall of *c.* 220 mm in September, and the cool, dry season from November to February. The vegetation of Jae Sawn National Park is quite similar to that found in nearby places at all elevations and on the various bedrock (Maxwell, 1997; Maxwell and Elliott, 2001).

Doi Ang Khang is located in Phang District, 19° 50' - 19° 57' north latitude and 99° 01' - 99° 06' east longitude, 25 km from the center of Phang District and 170 km from Chiang Mai Province. The total area is 91.36 km^2. The summit of the mountain is a basin. The boundary is about 1,800 m above sea level and the bottom is quite smooth, 1400 m above sea level. The basin is about 8 km in length and 1-3 km in width. The bedrocks is limestone and shale. The climate is temperate and rainy, with temperatures ranging from –3 °C to 18 °C in the cold season and 22 °C in dry season. The forest type is hill evergreen forest with pine on slopes mixed with pine forest (*Pinus merkusii* Jungh. & De Vriese and *P. kesiya* Roy. *ex* Gord.). Much forest has been cleared for agriculture land. The ground flora includes *Imperata cylindrica* (L.) P. Beauv. var. *major* (Nees) C.E. Hubb. *ex* Hubb. & Vaugh. (Gramineae), *Eupatorium adenophorum* Spreng. (Compositae), *Pteridium aquilinum* (L.) Kuhn ssp. *aquilinum* var. *wightianum* (Ag.) Try. (Dennstaedtiaceae), *Ficus semicordata* B.-H. *ex* J.E. Sm.

3.2 SPECIES STUDIED

Research carried out by FORRU has identified a number of candidate framework species, based on their growth rate in the rehabilitation plots, dense spreading crowns, potential attractiveness to seed-dispersing wildlife and ease of propagation (Blakesley *et al.*, 2000) Five species were selected for the this study; *Spondias axillaris* Roxb., *M. toosendan* Sieb. & Zucc., *G. arborea* Roxb., *P. cerasoides* D. Don and *C. acuminatissima* (Bl.) A. DC. based on their fruiting period fit on the research schedule.

Spondias axillaris Roxb. (Anacardiaceae), is a light–demanding, medium-size, deciduous tree, up to 25 m tall, with a dbh of up to 50 cm. It is fairly common in evergreen and evergreen + pine forest, often in degraded areas; at elevations of 700 to 1600 m. It is distributed in northern, north-eastern and eastern Thailand, Nepal, India, Myanmar, Hainan, Laos, Vietnam and Japan (FORRU, 2000).

The bark is thin, dark brown or dark grey, vertically cracked, often flaking. The inner bark is pale pink cream. The spirally arranged pinnate leaves are 23-28 x 31-35 cm (FORRU, 2000). The leaflets are arranged in 3-5 opposite pairs plus the terminal segments. Leaflet blades are thin, ovate to oblong-ovate with an acuminate apex an obliquely acute base. The margin is mostly entire, less often serrate. The inflorescence is axillary and paniculate with many flowers. The flowers are dull maroon. The fruit is a fleshy, ovoid drupe, usually one per infructescence. The fruit is grey–green turning yellow when ripe. The mean dimensions are 25.3 x 22.4 x 22.6 mm (FORRU, 2000).
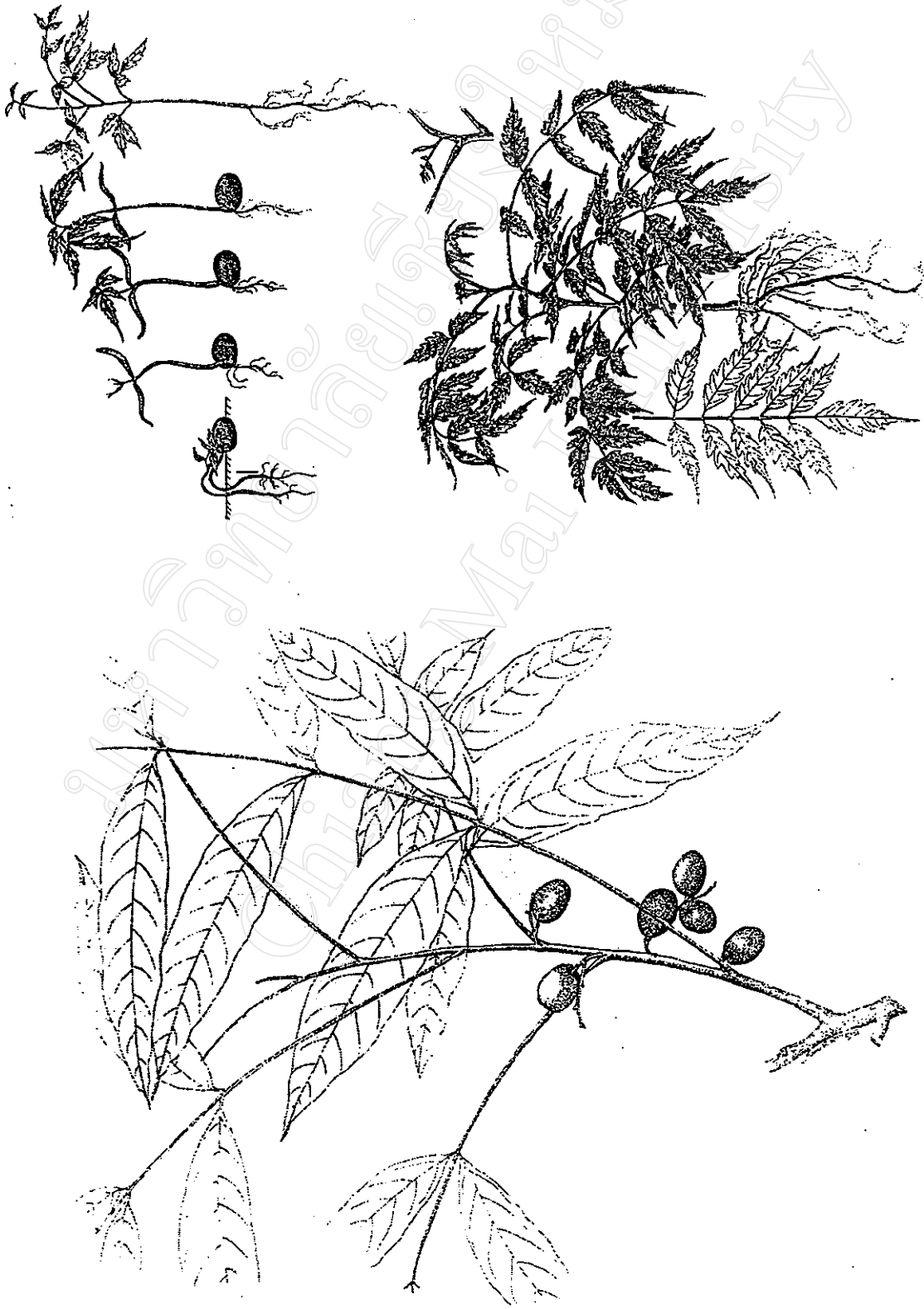
Figure 3. Foliage and various stage of seedlings of *Spondias axillaris* Roxb. (from FORRU, 2000).

The mesocarp is pulpy. Each drupe contains a solitary, obovoid pyrene with 4-5 depressions, each depression with a single, oblong, flattened, brown seed, 17.0 x 14.3 x 13.7 mm (FORRU, 2000). The wood is soft, light and used for interior finishes, drawers, crates, carvings, turnery, plywood and pulp. The leaves are used as fodder and are edible by people when boiled. The fruit is edible and is eaten both fresh and made into a variety of sweetmeats and chutney (Jackson, 1987).

Melia toosendan Sieb. & Zucc. (Meliaceae) is a medium sized, fast-growing, deciduous tree, up to 19 m tall with a dbh of up to 47 cm. It commonly establishes in degraded, fire-prone secondary growth in bamboo + deciduous, mixed evergreen + deciduous, evergreen, and evergreen with pine forest at 325-1300 m elevation. It is distributed in northern Thailand, Assam, Indo-China, Yunnan, Myanmar and Japan (FORRU, 2000). It is frequently planted as a roadside tree for ornamental purposes. The bark is grey to dark grey initially thin and developing shallow vertical cracks and brown lenticels. The spirally arranged leaves are bipinnate and vary between 20 and 45 cm in length. Each pinna has between 1 to 3 pairs of leaflets and one at the terminal segment. The leaflets are oppositely, ovate-oblong to oblong in shape with an acuminate apex, obtuse base, and an entire to slightly serrate margin. The inflorescences are 10-19 cm long with a peduncle up to 10 cm long and pedicels 4-5 mm long, which appear between January to March. The flowers are about 10 mm long and slightly fragrant The petals are white and faintly lilac coloured, with the scent of honey. The globose yellowish drupes are 21 x 21 x 20 mm (FORRU, 2000). They hang down in clusters on long stalks, making the tree very conspicuous and easy to
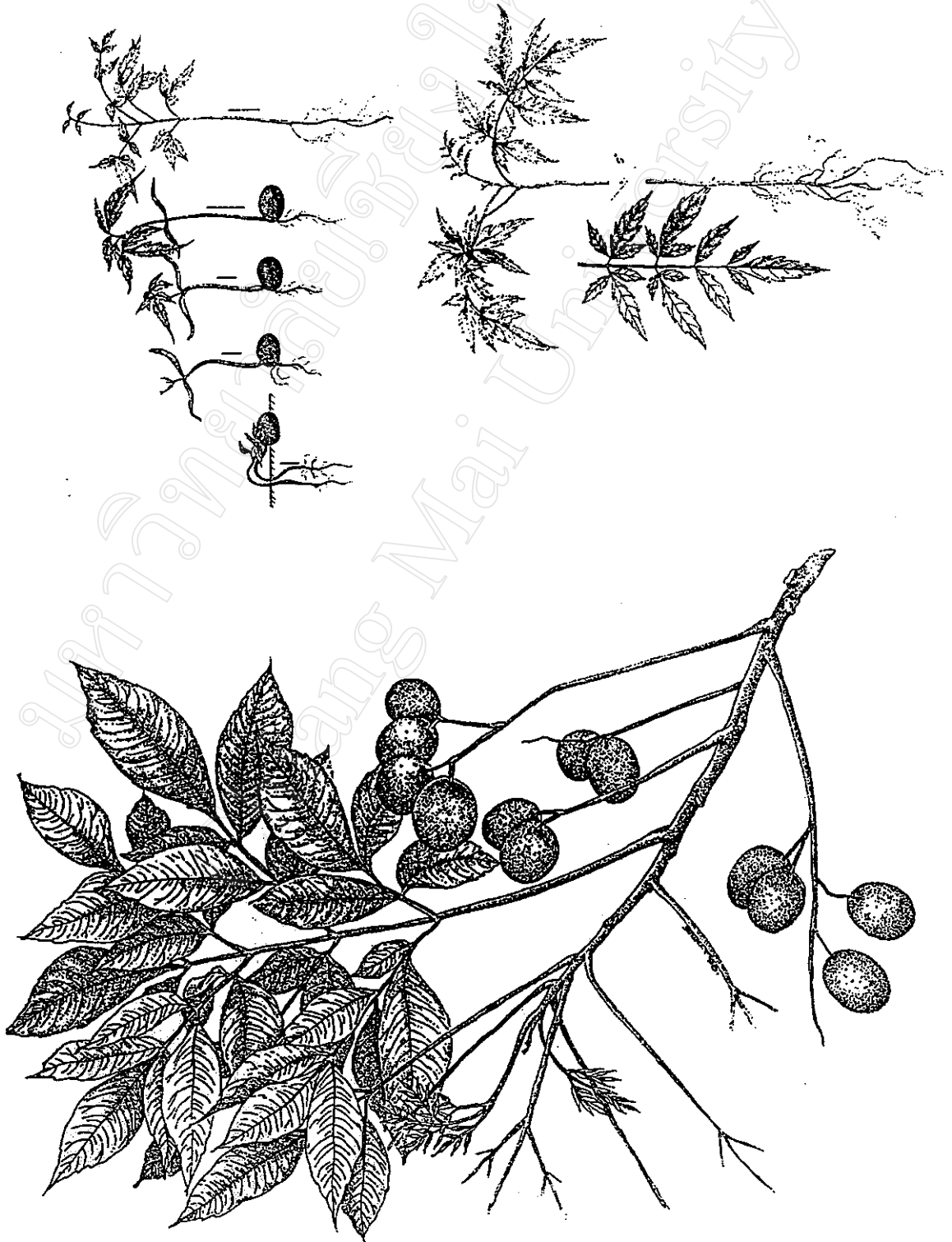
Figure 4. Foliage and various stage of seedlings of *Melia toosendan* Sieb. & Zucc. (from FORRU, 2000).

recognize when it has no leaves on the tree. The drupe are light green when unripe, turning yellow with reddish-brown spots when ripe, between September and October. Each drupe has 1 pyrene with up to 7 locules. Each locule contains one ovoid black shiny seed.

The leaves are highly nutritious, but are little used as fodder for sheep and goats. The timber is decorative and used for furniture, although rather light in weight. The bark, flowers, and fruits contain a bitter narcotic, which is used in medicine, but it may also poison animals if they eat the fruits. The nectar is attractive to wildlife, particularly sunbirds (Elliott and Anusarnsunthorn, 2001).

Gmelina arborea Roxb. (Verbenaceae) is light–demanding, fast-growing, medium-sized, deciduous tree, up to 30 m tall on favorable sites, but is relatively short-lived with a dbh of up to 64 cm (FORRU, 2000). It has a dense crown and starts to produce fruits 3-4 years after planting (Elliott and Anusarnsunthorn, 2001). It is common in secondary growth in almost all forest types. It establishes naturally in disturbed areas and is sometimes planted at elevations of 250 to 1500 m. The species is distributed throughout Thailand, India, southern Sri Lanka, Indo-China, Myanmar and Malesia. The bark is glabrous, light grey or cream coloured, corky with many black lenticels. The bark of older trees peels off in large flakes, leaving lighter patches, giving the tree a characteristic appearance. The cylindrical trunk, which has a tendency to low branching, is often horizontally banded. The branchlets are usually covered with white lenticels. The oppositely arranged, leaves are large (7-15 cm long
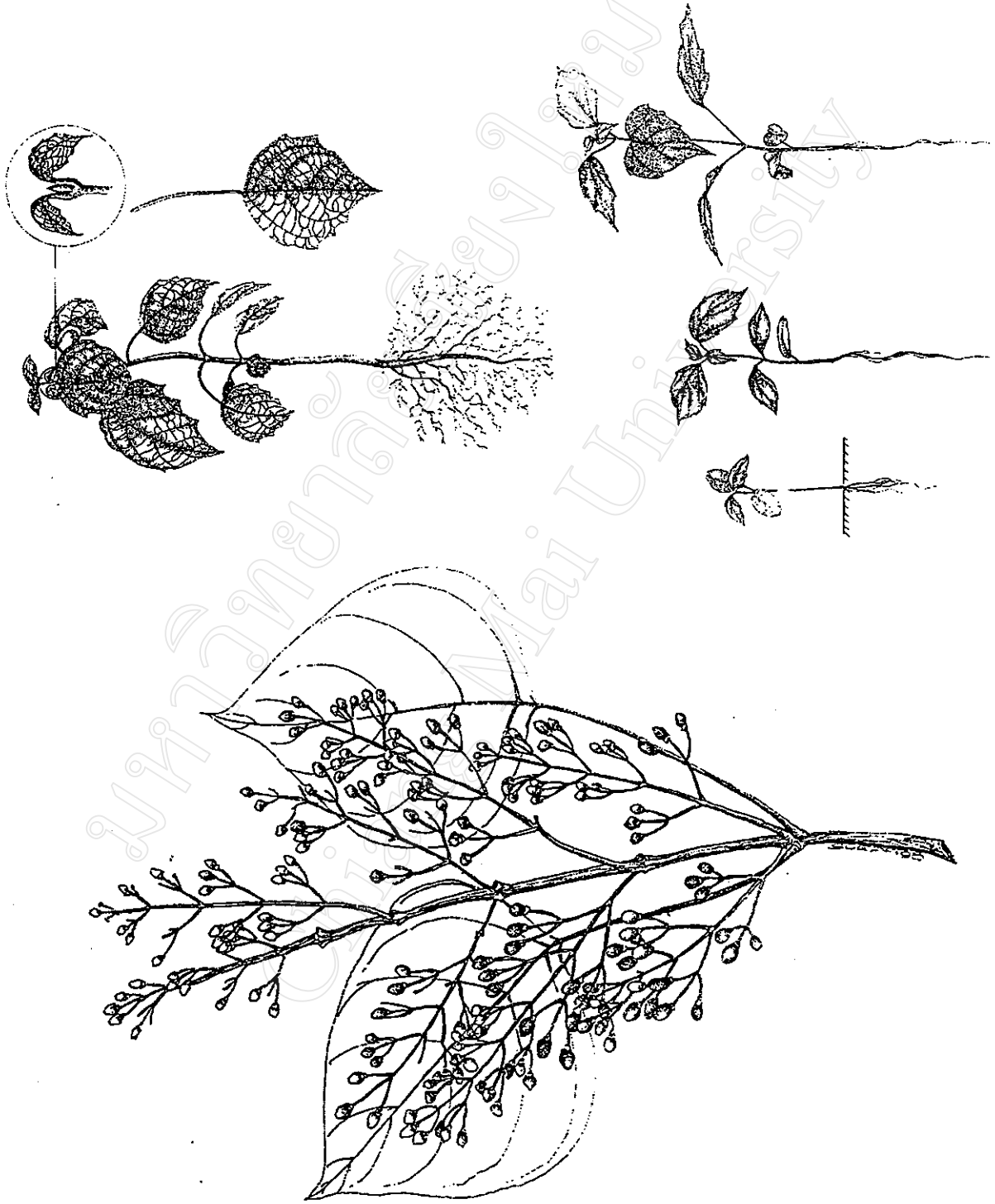
Figure 5. Foliage and various stage of seedlings of *Gmelina arborea* Roxb. (from FORRU, 2000).

and 8-10 cm wide), simple and decussate. The blades are oval or ovoid with a cuneate base and entire margins. The petiole is 4-5 cm long. The inflorescence is a 10-20 cm–long panicle, with many flowers. They mostly appear in March, when the tree is more or less leafless. Flowers are red–brown outside with yellow markings and are cream-yellow hairy on the inside and tubular shape. The obovoid, succulent drupes are about 26.3 x 18.9 x 18.1 mm (Pakkad , 1997), bright green when unripe turning yellow when ripe, between May and June. They contain 1-4 pyrenes, each with 1 seed. The tree produces a highly valued timber, easy to work and durable for its weight. It is creamy white, turning yellowish on exposure. It is used for a great variety of purposes including floors, ceilings, furniture, carvings, musical instruments, boats, tools and planks. It is also used for veneers and plywood, matches, charcoal and as a source of paper pulp. The leaves are regarded as a good fodder and deer and cattle eat the fruits. The unripe fruits are used to make an infusion to treat stomach ailments. They are also edible by humans.

Prunus cerasoides D. Don (Rosaceae), is a medium-sized, fast-growing, deciduous tree which sheds its leaves between January and March. It grows up to 18 m tall with a dbh of up to 38 cm. It is common in evergreen + deciduous, evergreen, and evergreen + pine forest in disturbed areas, elevation 1040-1700 m and is distributed in northern Thailand, Himalayas, Yunnan, Myanmar, and northern Indo-China (FORRU, 2000). The tree starts flowering and fruiting at about 3 years of age (Elliott and Anusarnsunthorn, 2001). Flowering generally occurs before the leaves develop between October to November and fruits ripen in March and April. This species is often planted along roadsides as an ornamental for its attractive flowers.
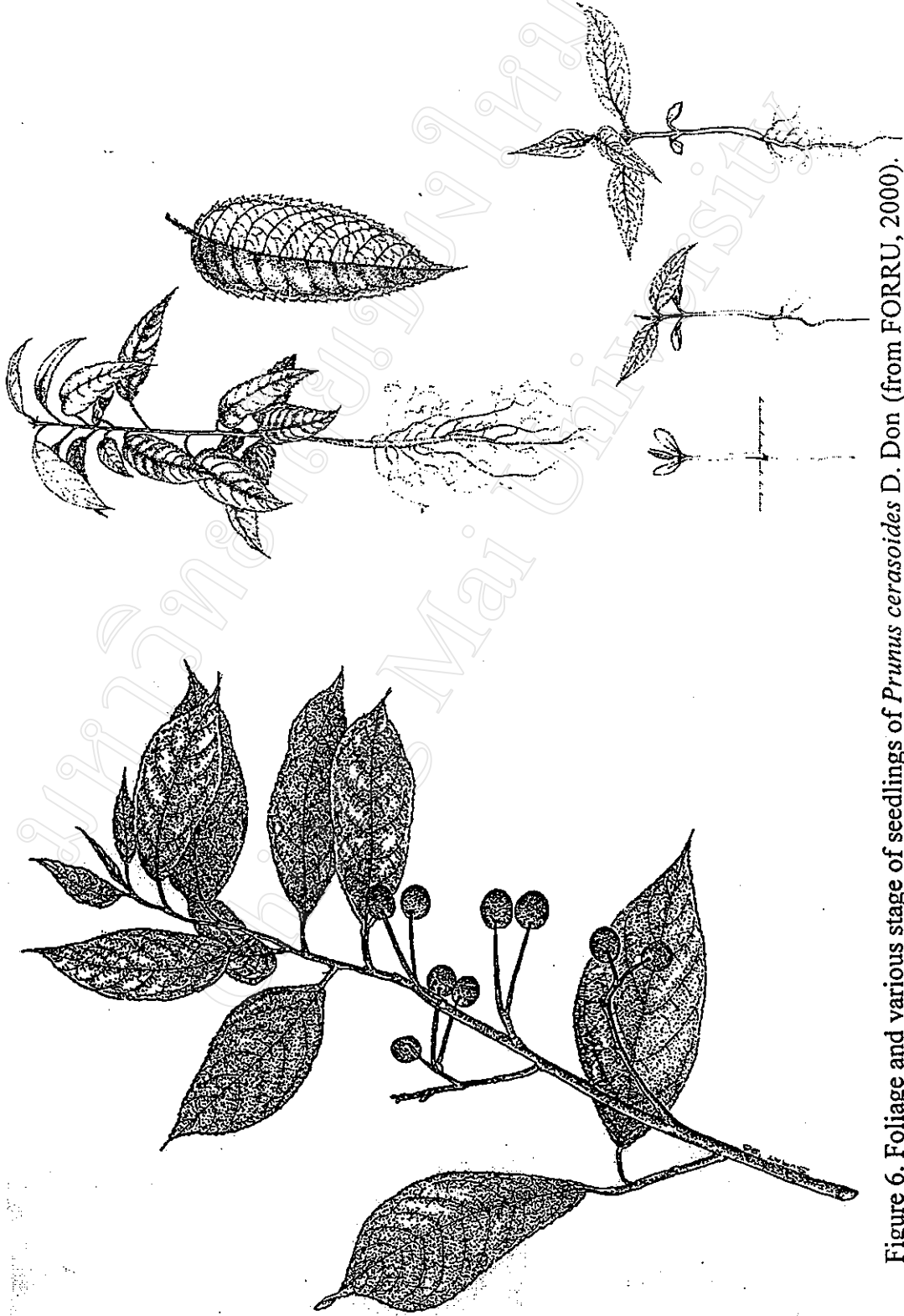
Figure 6. Foliage and various stage of seedlings of *Prunus cerasoides* D. Don (from FORRU, 2000).

The bark is red-brown and shiny, peeling in horizontal strips and with large tan lenticels. The wood is red to reddish-brown, moderately hard and mildly scented. It is occasionally used for quality products, furniture, cabinet work, interior finish, firewood, handles of knives and agricultural tools. The simple leaves are alternately arranged. The blades are glabrous, elliptic or ovate-lanceolate with an acuminate apex, broad-cuneate base, and simple or double serrate margin, 5-12 by 3-5 cm. The petiole is 8-15 mm long. The flowers are very attractive with mauve or pinkish petal, sometimes tinged with crimson and borne in cluster at the ends of branches. The fruit is a fleshy ovoid or ellipsoid drupe. The dimensions are 10.6 x 8.7 x 7.9 mm (Pakkad, 1997). It is light green, turning to bright red when ripe. Each drupe contains one rough and furrowed pyrene. The pyrene is brown. The dimensions are 9.7 x 7.5 x 6.2 mm (Pakkad, 1997).

Castanopsis acuminatissima (Bl.) A. DC. (Fagaceae) is a medium sized, evergreen tree, up to 25 m tall with a dbh of up to 102 cm. It is common in mixed evergreen + deciduous, evergreen and evergreen with pine forest, at elevation 760-2100 m. It is distributed through Thailand (except in the peninsula), north-eastern India, Indo-China, Taiwan, peninsular Malaysia, northern Sumatra, western Java, Borneo (Sabah), Sulawesi, New Guinea and New Britain (FORRU, 2000). The bark is very thick, roughly vertically cracked and ridged, dark grey-black or dark brown. The bark is used as a laxative and in Thailand is chewed with betel nut. The wood is used for medium to heavy construction, interior fitting, furniture, cabinet-making, plywood, sliced veneer and fire wood. The cut branches are used to culture mushrooms. The spirally arranged, simple, leathery leaf blades are 10-15 x 3-5 cm, oblong to lanceolate with an acuminate apex and acute base (FORRU, 2000). The

margin of the upper half is shallowly serrate. The inflorescence is axillary in erect panicles of unisexual spikes. The numerous, fragrant, male flowers are in spaced clusters, *c.* 4 mm long. The tepals are pale light yellow. The female flowers are solitary, spaced, inconspicuous, brackate and lacking tepals. The edible nut is enclosed in a spiny cupule. Rigid, branched, sharp-tiped spines partially cover the cupule epidermis. The cupules are green when unripe, turning to brown when ripe in October and November. The mean dimensions are 8-10 x 7-8 mm (FORRU, 2000). Each nut contains a single brown ovoid seed, with a mean dimension of 7.2 x 6.4 x 5.9 mm (FORRU, 2000).
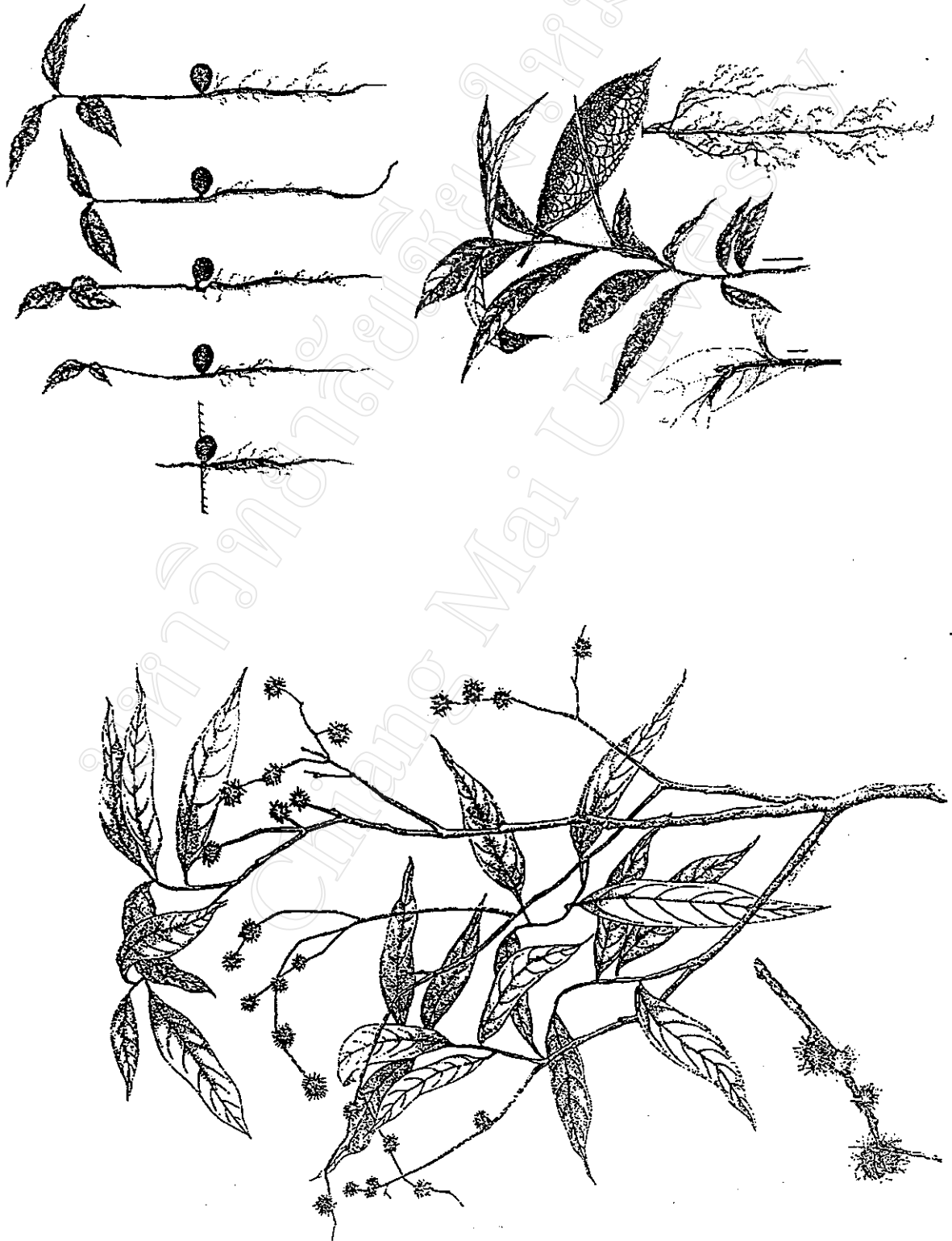
Figure 7. Foliage and various stage of seedlings of *Castanopsis acuminatissima* (Bl.) A. DC. (from FORRU, 2000).

3.3 SELECTING SEED TREES FOR FOREST RESTORATION BASED ON AN ASSESSMENT OF NURSERY AND FIELD PERFORMANCE

3.3.1 SEED AND PYRENE COLLECTION

A maximum of 50 individuals of each species, which were at least 100 m apart, were located. Characteristics of the parent trees most likely to influence seed size and quality were measured:

i) girth at breast height (gbh) as an indicator of age,

ii) habitat factors (elevation)

Mature (yellow) fruits of *S. axillaris* were collected from 27 to 29 September 1999 from 41 parent trees in Doi Suthep-Pui National Park when they fell on the ground. The pyrenes were soaked in water to remove pericarp and exposed to air until their surface was dry.

Mature (cream–light yellow) fruits of *M. toosendan* were collected from 50 parent trees in the southern part (DS1) and northern part (DS2) of Doi Suthep Pui National Park in October, November and December 1999. The fruits were collected on the ground. The fruits were exposed to air until their pericarps were dry. The woody pyrenes were cracked to remove the seeds.

Yellow fruits of *G. arborea* were collected in mid April 2000 from 49 parent trees in the southern part (DS1) and northern parts (DS2) of Doi Suthep Pui National Park on the ground. The pericarp was removed from the pyrene by rubbing the fruit

against wire mesh and putting pyrene and pulp in water, when the pyrene will sink to the bottom. The pyrenes were exposed to air until their surface was dry.

Mature (red-blackish) fruits of *P. cerasoides* were collected in March 2000 from 50 parent trees: 25 in Doi Suthep-Pui National Park, 13 at Doi Ang Khang and 12 at Doi Inthanon National Park. The pericarp was removed from the pyrene by rubbing the fruit against wire mesh and soaking pyrene and pulp in water, when the pyrene will sink to the bottom. The pyrenes were exposed to air until their surface was dry.

Brown nuts were collected from 50 parent trees included 25 from Doi Suthep-Pui National Park, 13 from Jae Sawn National Park and 12 from Doi Inthanon National Park in September 2000, when they fell on the ground.

Seventy-two seeds or pyrenes were randomly selected from each parent tree for all species studied. The dimensions of the seeds or pyrene (length, width and thickness) were individually recorded in millimeters using callipers with a vernier scale. Each seed or pyrene was individually weighed to 3 decimal places using a 3 digital balance.

3.3.2 SEED GERMINATION

Seventy two seeds or pyrenes were randomly selected from the progeny of each tree and sown within 2-3 days of collection, into modular plastic trays, on to the surface of a media of 2 parts forest soil to one part coconut husk and one part peanut

husk at FORRU's research nursery at Doi Suthep-Pui national park headquarters (at about 1050 m elevation). Seed trays were placed on the top of concrete benches, partially shaded under a transparent plastic roof, approximately 40% full sunlight (similar to the light intensity in partially regenerating gaps). Watering was carried out daily. Germination was monitored throughout the germination period and was defined as emergence of any part of the shoot. The dates on which individual seeds germinated were recorded. Non-germinated seeds or pyrenes were discarded from the experiment (*Prunus cerasoides* after 90 days, *Castanopsis acuminatissima* after 100 days, *Melia toosendan* after 120 days, *Gmelina arborea* after 60 days and *Spondias axillaris* after 200 days after sowing.

3.3.3 SEEDLING GROWTH IN THE NURSERY

Once the first pair of leaves had fully expanded for all seedlings, seedlings were pricked out and transplanted into black plastic bags, 2.5 inches in diameter by 9 inches in depth (6.5 x 23 cm), filled with a potting medium of forest soil, peanut husk and coconut husk, mixed in the ratio of 2:1:1. Seedlings were shaded the nursery under a plastic roof (approximately 40% full sunlight) for 2 weeks. Subsequently, they were placed outside, under black shade netting (slan, approximately 50% of full sunlight). Ten granules of Osmocote slow-release fertilizer (15-15-15) were applied every three months. Seedling height, root collar diameter (RCD) and health score (3=perfect health, 2=some leaves damaged or diseased, 1=nearly dead and 0=dead) were measured regularly.

3.3.4 SAPLING PERFORMANCE AFTER PLANTING OUT

3.3.4.1 Planting site

The experimental plots were located in a degraded watershed in Doi Suthep-Pui National Park (18° 52' N, 98° 51' E), 1,207-1,310 m asl. Originally, the plots would have been covered in evergreen forest, which was cleared between 1960-70. Subsequently the area was cultivated for vegetables and further degraded by frequent fires. Although a few scattered mature trees remain, the area is now dominated by weedy herbaceous vegetation such as *Pteridium aquilinum* (L.) Kuhn ssp. *aquilinum* var. *wightianum* (Ag.) Try. (Dennstaedtiaceae), *Bidens pilosa* L. var. *minor* (Bl.) Sherf, *Ageratum conyzoides* L., *Eupatorium odoratum* L. and *E. adenophorum* Spreng. (all Compositae), *Commelina diffusa* Burm. f. (Commelinaceae), and *Imperata cylindrica* (L.) P. Beauv. var. *major* (Nees) C.E. Hubb. *ex* Hubb. & Vaugh. and *Thysanolaena latifolia* (Roxb. *ex* Horn.) Honda (both Gramineae). Consequently, planted trees must be able to survive intense heat and drought conditions, particularly in the late dry season and compete with weedy vegetation. The differences in soil characteristics between planting site, and undisturbed evergreen forest (Tum Reusi, elevation 1,100 m about 9 km from the planting site) at the same elevation are shown in Table 2.

The area has two main seasons: the wet season (May – October) and the dry season (mean monthly rainfall below 100 mm, November – April). The dry season is subdivided into the cool-dry season (November to January) and the hot – dry season

(February to April). Average annual rainfall, recorded at the nearest weather station to the experiment plot at similar elevation (Kog-Ma Watershed Research Station), was 2,049.9 mm. Temperature varied from 4.5 °C in December to 35.5 °C in March (Elliott *et al.*, 2000).

Table 2. Soil characteristics of the experiment plots (n=16) compared with those in evergreen forest at the Tum Reusi.

	Experiment plots		Evergreen Forest		t-test[1]
	Mean	SD	Mean	SD	p values
pH	5.44	0.423	6.22	0.545	0.001
Organic Matter (%)	5.35	0.997	7.3	2.48	0.010
Nitrogen (%)	0.26	0.045	0.37	0.121	0.002
Potassium (ppm)	274.84	137.637	295.67	72.093	ns[2]
Moisture at field capacity (%)	34.76	2.571	35.35	4.363	ns[2]
Sand (%)	68.52	6.29	52.13	17.872	0.010
Silt (%)	18.26	3.09	22.04	5.473	0.020
Clay (%)	13.22	3.88	25.83	16.343	0.010

[1] Two-tailed Student's T-test, variances assumed equal,

[2] ns = not significant at p>0.05

Source: Elliott and Anusarnsunthorn (2001)

3.3.4.2 Site preparation and maintenance

One month before planting, weeds were cut back with hand tools and a non-residual herbicide, glyphosate, was applied. Holes about twice the size of the container were dug, with 100 g of NPK 15:15:15 mixed in with loose soil at the bottom of each planting hole.

All seedlings were hardened off in full sunlight 4 weeks before being dispatched for planting in early June of the year following sowing. Twenty saplings from every batch of seeds were randomly selected and planted out at an experiment site in the early rainy season. Seedlings of *S. axillaris* and *M. toosendan* were planted on 16 June 2000 and those of *G. arborea* and *P. cerasoides* were planted on 26 June 2001. They were planted at a density of 500 per rai, averaging a mean distance between plants of 1.8 m, in mixes of 29 or 30 species (FORRU's saplings) planted in each subplot.

All saplings were labelled in the nursery before planting. The labels used were thin bands of aluminium, manufactured for bundling electrical cables and capable of being fastened into a ring about 3 cm in diameter.

Planted trees were monitored 2 weeks after planting and again at the end of the rainy season, cool season and dry season in the first year and annually at the end of the rainy season thereafter. The root collar of each sapling was measured using callipers with a vernier scale and sapling height (soil level to the highest meristem) was also measured. Health scores were recorded (3 = perfect or nearly perfect health,

2 = slight insect damage or discoloration, 1 = severe insect damage or discoloration and 0 = believed to be dead). Weeds were cut around the saplings 3-4 times as required during the rainy season and 100 g of fertilizer was applied, once a month in July, August, September, October (in wet season) for 2 years. A fire break was established, 10-15 m wide around the planted sites in March (hot – dry season) for 2 years.

3.3.5 DATA ANALYSIS

The mean and standard deviation (SD) of seed dimensions (length, width and thickness), mass and time to germination of each parent tree were calculated. Analyses of seed dimensions (length, width and thickness), mass and time to germination among parent trees were performed with one-way analysis of variance (ANOVA). The following parameters were determined for each parent tree.

Percentage germination

$$= \frac{\text{number of germinating seeds}}{\text{number of seed sown}} \times 100 \qquad (\%)$$

Median length of dormancy (MLD)   (days)

= the number of days between sowing and 50% of total germination

Germination period (GP)   (days)

= time from germination of the first to last seed

percentage seedlings survival   (%)

$$= \frac{\text{number of surviving seedling in nursery}}{\text{number of germinating seeds}} \times 100$$

This parameter was calculated for the nursery and also in the experiment plot.

Relative growth rate of Root collar diameter (RRGR)   (% year^{-1})

$$= \frac{[\ln (RCD_2) - \ln (RCD_1)] \times 100 \times 365}{(T_2 - T_1)}$$

Whereas RCD$_2$ = RCD at time T$_2$ (at the end of measurement)

       RCD$_1$ = RCD at time T$_1$ (at the beginning of measurement)

       T$_2$-T$_1$ = Number of days between the beginning (T$_1$) and the end

            (T$_2$) time of measurement

This parameter was calculated for the nursery and also in the experiment plot.

Relative growth rate of height (RHGR)   (% year^{-1})

$$= \frac{[\ln (H_2) - \ln (H_1)] \times 100 \times 365}{(T_2 - T_1)}$$

Whereas H$_2$ = height at time T$_2$ (at the end of measurement)

       H$_1$ = height at time T$_1$ (at the beginning of measurement)

       T$_2$-T$_1$ = Number of days between the beginning (T$_1$) and the end (T$_2$)

            time of measurement

This parameter was calculated for the nursery and also in the experiment plot.

Mean seedling of root collar diameter (mm)

= sum of the root collar diameter of all surviving seedlings

number of surviving seedlings

This parameter was calculated for the nursery and also in the experiment plot.

Mean seedling of height (mm)

= sum of the height of all surviving seedlings

number of surviving seedlings

This parameter was calculated for the nursery and also in the experiment plot.

Differences in seed or pyrene length, width, thickness among germinated seeds and non-germinated seeds or pyrenes were tested using ANOVA. At the end of the nursery, experimental differences in seed or pyrene length, width, thickness, mass and time to germination of surviving seedlings and those that died were tested for significance with ANOVA, whereas only germinated seeds or pyrenes were used in the analysis of survival. Also, differences in the same parameters of surviving saplings and those that died at end of field experiment were tested for significance with ANOVA, where only planted saplings were used in the analysis of the survival. Pearson correlation was used to test correlation between seed tree characteristics, characteristics of seeds or pyrenes, germination results, nursery performance and field performance.

3.3.6 SELECTION OF PARENT TREE BASE ON SEEDLING PERFORMANCE IN THE NURSERY AND IN THE FIELD

The criteria used to identify superior parent seed trees were in order of priority; *(i)* 70% or more mean sapling survival in the field after the first growing season; *(ii)* 100 cm or taller mean sapling height in the field after first growing season; (iii) 40% or more mean germination percentage and (iv) 70% or more seedling survival in the nursery.

3.4 GENETIC ANALYSIS (USING MICROSATELLITE DNA MARKERS)

3.4.1 PLANT MATERIAL AND DNA EXTRACTION

Buds of 82 individuals of *Prunus cerasoides* D. Don (Rosaceae) and 73 individuals for *Castanopsis acuminatissima* (Bl.) A. DC. (Fagaceae) were collected. The seed trees, from which seeds were collected for studies on superior parent trees selection were included. All trees sampled were mature individuals, at a minimum distance of 100 m apart.

P. cerasoides were collected from 20 trees in Doi Inthanon National Park, 20 in Doi Ang Khang and 42 in Doi Suthep-Pui National Park. Many trees sampled were close to the roadside forest edge, suggesting that some may have been dispersed locally (collected and germinated or transplanted) by Forestry Department Officials. However, nineteen of the additional trees sampled in Doi Suthep-Pui for the

molecular studies were believed to be naturally dispersed, due to their age and location, deep in the forest.

C. acuminatissima samples were collected from 25 trees in Doi Suthep-Pui National Park, 25 trees in Doi Inthanon National Park and 23 trees in Jae Sawn National Park.

Total genomic DNA was extracted from individual trees of *P. cerasoides* and *C. acuminatissima* using the hexadecyltrimethyl ammonium bromide (CTAB) method described by Murray and Thompson (1980), with the following adaptations. Fresh leaves (0.2 g) were ground into a fine powder in liquid nitrogen using a morter and pestle and transferred to a 2.0 ml extraction tube. To this was added 800 µl CTAB buffer (2% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris (pH 8.0), 2% of PVP 40 (polyvinylpolypyrrolidone; mol wt. 40000) and 1% 2-mercaptoethanol), pre-heated to 65°C. The homogenates were mixed well by inverting, and incubated at 65°C water-bath for 3 minutes. The samples were then mixed again, extracted with an equal volume of chloroform:isoamyl alcohol (24:1) and centrifuged at maximum speed for 10 minutes. If the solution appeared cloudy the chloroform:isoamyl extraction was repeated. The supernatent was then transferred to a new tube and the DNA precipitated by adding 500 µl cold isopropanol, mixed by inversion, and centrifuged at maximum speed for 5 minutes. The DNA pellet was washed in 500 µl 70% ethanol, air dried and resuspended in 50 µl TE (10 mM Tri-HCl, 0.1 mM EDTA, pH 8.0) containing 10 µg µl^{-1} RNAse. The samples were incubated at 37°C to

remove RNA prior to storage at 4°C, or longer term storage at -20°C. The DNA was quantified against lamda standards on agarose gels stained with ethidium bromide.

3.4.2 DNA AMPLIFICATION

Microsatellite primers reported to display a high degree of polymorphism in each species were selected for the amplification reactions (Tables 7 and 14).

Eighty two individuals of *P. cerasoides* were screened with the 13 primers, comprising 5 primers from peach (*Prunus persica* (L) Batsch) (Cipriani *et al.*, 1999), 2 primers pairs from Sour cherry (*Prunus cerasus* L.) (Downey and Iezzoni, 2000) and 4 primers from sweet cherry (*Prunus avium* L.) (Sosinski *et al.*, 2000; Joobeur *et al.*, 2000).

Seventy-three individuals of *C. acuminatissima* were screened with 5 primers from *Castanopsis cuspidata* var. *sieboldii* Nakai (Ueno *et al.*, 2000)

PCR amplification was performed in a 12.5 μl reaction mix containing 10 ng of DNA, 2 μM of reverse primer, 2 μM of forward primer labelled with $\gamma^{33}P$, 2.5 mM of *Taq* DNA polymerase (GIBCO, BRL), 200 μM of each dNTP, 50 mM of $MgCl_2$ and 10X of buffer. The following conditions were used: an initial denaturation step at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, annealing temperature for 1 min, 72°C for 1 min 30 s. A final extension at 72°C for 8 min was included.

One volume of 80 % formamide containing 0.5% bromophenol blue and xylene cyanol was added to the PCR products. The microsatellites were denatured at 90°C for 3 minutes and separated on a 6 % denaturing sequencing gel in 1 x TBE buffer at a constant 65 W for 2.5 hours (Sambrook *et al.*, 1989). The gel was dried onto Whatmann 3MM paper and the microsatellites visualised following autoradiography (Kodak Biomax MR). The size of each fragment was estimated with reference to a size marker of 10 bp ladder standard (GIBCO).

3.4.3 DATA ANALYSIS

The mean observed heterozygosity (H_O, direct count estimate) and Hardy-Weinberg expected heterozygosity (H_E), and inbreeding coefficients (F_{IS}, F_{IT} & F_{ST}) (Wright, 1965) were computed using GENEPOP computer program (version 3.3, Raymond and Rousset, 1995) for each locus and averaged over all loci. A bilateral test for departure from the Hardy-Weinberg equilibrium was also performed using GENEPOP. The distribution of alleles in either two or four allele frequency classes was determined in the different populations. The four frequency classes were: high ($P \geq 0.75$); intermediate ($0.75 > P \geq 0.25$); low ($0.25 > P \geq 0.01$) and rare ($P \leq 0.01$), following Rajora *et al.* (2000). Alleles were also classified as common ($P \geq 0.05$) or rare ($P < 0.05$), as suggested by Marshall and Brown (1975).

3.4.4 ESTIMATION OF THE MINIMUM NUMBER OF TREES REPRESENTING A FULL SET OF MICROSATELLITE ALLELES

Calculations of the representation of microsatellite alleles in the sampled trees, and in an 'unknown' population were performed using two models. For each model, the trees selected had to contain most of the forest gene resources (diversity) and the sample size had to be as small as possible (parsimony). Two alternative models were investigated: selection of seed trees based on their individual genotype (model I) and random selection of seed trees from a population of unknown genetic makeup (model II). Both algorithms were implemented in GENSTAT computer program (version 5.2, Payne *et al.*, 2000).

Model I: This selection is based on knowledge of the individual genotypes. For a given sample size, it is suitable to find the most diverse set of seed trees. An algorithm was designed to find the minimum size selection, which would contain 100% of gene diversity. The "model I" algorithm considers the whole list of seed trees and includes a decision rule to keep or remove each item in turn. An item is removed from the list if its removal doesn't imply any loss of alleles. To ensure a minimum sample size at the same time, the status of genotypes concentrating the rarest alleles is checked first. The Model I algorithm is as follows:

1. A rarity ranking of each genotype is calculated (R) based on the frequency of alleles in each locus: $R_i = \Sigma_{jk} n_{ik} \cdot Ln (f_{jk}+1)$ where i, j and k denote indices for the seed genotype, the locus and the allele respectively. Σ_{jk} is a summation symbol over all loci and alleles. f_{jk} and n_{ik} denotes the frequency of allele k on locus j and

for genotype i respectively. Genotypes with some undetermined alleles are penalised, ie a missing allele is assigned a relative frequency of 1.0. R_i is the probability of appearance of a given genotype i and is interpreted as a rarity index. Genotypes with the lowest values of R_i are considered as the rarest;

2. The whole list of trees is first considered where the trees are ranked according to the rarity index (the first in the list being the rarest). The whole set of trees obviously satisfies the diversity constraint but almost surely not the parsimony one. It is assessed by an acceptation-rejection rule of the trees taken in turn from the top to the bottom of the list: if the removal of a tree implies the decrease of allelic diversity then the tree is retained, otherwise the list is shortened removing the given tree.

Model II: Selection is made without preference from a population of trees with the same relative allele frequencies as in the original data set. The population is taken as infinite so that selection of individuals doesn't affect the allelic frequency in the population. The Model II algorithm was designed to provide expected diversity as a function of the selection sample size. This is a two-level algorithm. At the first level, the selection size is varied from 1 to N (N=100). For each selection size, M samples were drawn from the reference population with replacement and their allele diversity considered (M=500). The fifth percentile of the distribution of allele diversity was computed and stored as a fair estimate of the theoretical minimum diversity for the given selection size. Such an estimation doesn't involve any assumption on the data itself. It was first introduced by Efron (1979) and is part of what is now called "bootstrap techniques".

SELECTING SEED TREES FOR FOREST RESTORATION BASED ON

ASSESSMENT OF NURSERY AND FIELD PERFORMANCE

4.1 INTRODUCTION

Forest restoration, by establishing mixed plantations of indigenous trees, usually occurs under harsh conditions, such as high temperatures and vapour pressure deficits and environmental stresses. There are several approaches to reduce the probability of plantation failure. These include intensive site preparation, fertilizer application, weed control, stress acclimation treatments and the use of containerized seedlings. However, these methods are expensive and may require imported machinery and materials (Murray *et al.*, 1995; Boyle *et al.*, 1995).

Planting high quality seedlings, which are locally adapted should increase plantation success. High quality seedlings for ecological restoration can be defined as those that survive and grow after planting out (Duryea, 1985). Planting healthy, vigorous seedlings ensures that the plants have the best chance to produce new roots quickly, in order to establish good access to soil, water and nutrient reserves, and thus cope with environmental stresses. Burdett (1983) identified several morphological and physiological characteristics that define a high quality seedling, including: the ability to rapidly produce new roots, the ability to quickly resume photosynthesis and continue growth, large fibrous root systems, sun-adapted foliage, large stem diameter,

balanced root/shoot ratios, good carbohydrate reserves, an optimum mineral nutrient content, tolerance of stress and mycorrhizal or rhizobium infection established.

Several systems have been devised to select the best seedlings. Rose *et al.* (1990) developed the concept of a 'target seedling' for *Pinus taeda* L. seedlings that combined structural and physiological traits that are quantitatively linked to successful reforestation. A target *Pinus taeda* L. seedling is identified by its height (20-25 cm), stem diameter (>4 mm), needle type, stem and bud forms, root form, volume (> 3.5 ml), and high root growth potential. However, research is required to define targets for individual species and perhaps, regions, and it is crucial that attention is paid to detail, from seed selection through to and after planting (Hawkins, 1995).

Jaenicke (1999) stated that seedling quality also depends on the seed used. Seed quality is measured in two ways; firstly by the quality of the seed and secondly by the desired physical traits of the resultant mature tree. Seed quality includes the genetic, physical and physiological states of the seeds. Seeds with good physiology have high germination percentage and vigour. Physical quality includes seed size and infestation by pathogens. Genetic quality refers to the inherent capacity of a seed to produce a tree adapted to the environmental conditions at the planting site (Turnbull, 1995).

Jones (2000) stated that seeds from healthy, well formed trees usually provide greater assurance that resulting seedlings will have good form and will survive and

better resist the stressful conditions at planting sites. Also, high quality seedlings should originate from high quality seed collected from the best parent trees.

In previous FORRU planting trails, field performance of potential framework species planted in experimental plots was highly variable. Elliott and Anusarnsunthorn (2001) found that percent sapling survival of framework species showed considerable variation both among and within species. The survival rate of individual tree species after 1 growing season ranged from 37.0 for *Diospyros glandulosa* Lace (Ebenaceae) to 98.3 % for *Melia toosendan* Sieb. & Zucc. (Meliaceae). Within species, *e. g. Prunus cerasoides* D. Don (Rosaceae) had a sapling survival in plots planted in 1998 was 87%, but only 48% in plots planted in the following year. Some of this variability (*e.g.* seedling survival and growth rate) may have been influenced by the parent trees, from which the original seed was collected.

In this study, I attempted to quantify variability of seeds, pyrenes and seedling performance in the nursery and in the field, and attribute this to the parent stock. Then the target seedling concept was modified and adapted to suit the specific requirements of ecological restoration (rather than economic forestry, which was the focus of previous seedling selection systems)

The criteria used to identify superior parent trees were: (1) mean sapling survival in the field by the end of first growing season; (2) mean sapling height in the field by the end of first growing season; (3) mean germination percentage and (4) mean seedling survival in the nursery. Sapling survival in the field was ranked as the most important criterion. A standard of 70% or higher was considered acceptable.

High percent sapling survival is essential to avoid expensive replanting. Sapling growth in the field was of secondary importance. Sapling height after a defined period of growth constitutes a morphological measurement (Hawkins, 1995). A sapling height of 100 cm or more by the end of first growing season was considered acceptable. Fast-growing saplings have a high chance of survival in degraded areas dominated by weeds. Germination percentage was the third criterion. This was included in the criteria because some forest tree species have low percent germination, which limits seedling production. Furthermore, if the trees in the restoration plots will themselves be the source of seed for neighboring deforested sites in future years, good germination would be important also. Ideally, germination characteristics of tree seed are high and synchronous germination. This reduces operational costs and improves seedling quality. In this study, a percent germination of 40 % or higher was considered acceptable. Seedling survival in the nursery was the fourth criterion. A standard of 70% or higher was considered acceptable. Seedling survival in the nursery may be influenced by seed characteristics, time to germination and MLD.

The objectives of this chapter were therefore:

1. to quantify the variability in seed characteristics, germination characteristics and seedling performance both in the nursery and in the field, amongst progeny from different parent trees

2. to develop and apply standard criteria to select superior parent tree for forest restoration programs.

4.2 RESULTS

4.2.1 *Spondias axillaris* Roxb. (ANACARDIACEAE)

4.2.1.1 Pyrene characteristics

The yellow fruits of 41 seed trees were collected from the ground in Doi Suthep-Pui National Park (for exact locations see Appendix I, Table 21) on 27th to 29th September 1999. The fruit from different seed trees did not ripen synchronously. The pericarp of some seed trees was easily removed from the pyrenes due to partial decomposition. Fruits which had recently fallen however required squeezing by hand and soaking in water to remove the pericarp.

Following extraction from the drupe, the wet mass and dimensions of all pyrenes were measured. The pyrene dimensions and wet mass were highly variable and there were significant differences amongst the pyrene batches ($P<0.0001$; ANOVA) (Appendix IV, Table 35). Across all pyrenes, a mean wet mass of 3.1 ± 0.6 g (SD) was recorded. The mean pyrene mass of individual batches ranged from 2.2 ± 0.3 g (SD) to 3.9 ± 0.5 g (SD). The mean length of pyrenes amongst the pyrene batches ranged from 16.6 ± 1.1 mm (SD) to 20.2 ± 1.2 mm (SD), the mean width ranged from 12.7 ± 0.87 mm (SD) to 16.2 ± 1.5 mm (SD), and the mean thickness ranged from 12.4 ± 0.83 mm (SD) to 15.6 ± 0.9 mm (SD) (Table 3).

Table 3. Summary of seed or pyrene characteristics of the 5 species studied.

	S. axillaris	M. toosendan	G. arborea	P. cerasoides	C. acumi-natissima
seed or pyrene length (mm)					
range (across all seeds or pyrenes)	12.0-24.0	6.7-14.8	9.4-22.0	7.0-14.2	6.8-16.4
maximum mean of individual tree	20.2±1.2	12.1±0.7	18.4±1.40	12.9±0.6	11.6±0.8
minimum mean of individual tree	16.6±1.1	9.5±0.8	13.1±0.98	9.1±0.3	8.2±0.6
mean across all trees	18.6±1.6	10.6±1.6	15.7±1.09	10.5±0.8	10.1±0.8
seed or pyrene width (mm)					
range (across all seeds or pyrene)	11.3-22.0	2.0-5.0	5.6-11.7	5.8-10.8	5.2-12.9
maximum mean of individual tree	16.2±1.1	4.1±0.3	10.3±0.63	9.1±0.5	10.4±0.9
minimum mean of individual tree	12.7±0.9	3.1±0.2	7.5±0.51	6.9±0.3	6.5±0.6
mean across all trees	14.4±1.3	3.7±0.2	8.8±0.62	7.6±0.5	9.1±0.9
seed or pyrene thickness (mm)					
range (across all seeds or pyrenes)	10.6-21.8	1.5-4.2	5.6-11.3	4.4-8.2	5.1-11.4
maximum mean of individual tree	15.6±0.9	3.0±0.4	9.4±0.63	7.4±0.2	10.0±0.7
minimum mean of individual tree	12.4±0.8	2.3±0.2	6.7±0.48	5.4±0.2	6.0±0.6
mean across all trees	14.0±0.9	2.7±0.2	8.0±0.62	6.1±0.4	8.5±0.8
seed or pyrene wet mass (g)					
range (across all seeds or pyrenes)	1.67-5.54	0.5-0.89	0.87-1.81	0.77-1.28	0.72-1.91
maximum mean of individual tree	3.93±0.49	0.8±0.02	1.46±0.14	1.17±0.05	1.49±0.15
minimum mean of individual tree	2.23±0.27	0.7±0.08	1.02±0.07	0.83±0.02	0.92±0.07
mean across all tree	3.13±0.42	0.8-0.02	1.24±0.10	0.98±0.08	1.23±0.14

4.2.1.2 Germination

The pyrenes were sown whole, without the individual seeds being extracted. Each pyrene may produce up to five seedlings, but a minimum of one germinated seedling qualified as successful germination for a given pyrene. Consequently, the time to germination was recorded for the first seedling to emerge. The germination percentage ranged considerably, from just 8.3% to 91.7% amongst pyrene batches (Table 4). The mean germination percentage, average across all batches, however, was 42.3 ± 22.3 % (SD). Fifty four percent of pyrene batches had a germination percentage of 40% or greater, which I considered to be the minimum acceptable standard for efficient nursery production, *e. g.* batches from seed tree nos. 1, 13, 15

and 17 (Appendix II, Table 26). Some pyrene batches had a very low percent germination, with only 8 germinated pyrenes, *i. e.* batches from seed tree nos. 14, 18 and 27. The mean time to germination, germination period and MLD also showed considerable variation, both within, and amongst pyrene batches (Figure 8). The mean time to germination ranged from 23.7 ± 8.4 to 161.5 ± 20.9 days (SD). Twenty pyrene batches had a mean time to germination of more than 100 days, *e. g.* batches from seed tree nos. 1, 2, 3, 4, 14, 15, 16, 17, 18 19, 20, 21 and 22 (Appendix II, Table 26). The germination period ranged from 13 days to 183 days among the pyrene batches, with an overall mean of 134.8 ± 34.9 days (SD). Most of pyrene batches had a germination period more than 100 days, with the exception of batches from seed tree nos. 3, 14, 34, 39 and 40 (Appendix II, Table 26). The MLD was also high, ranging from 23 days to 169 days, with an overall mean of 98.7 ± 47.2 days (SD) (Table 4). Twenty-three pyrene batches had MLD's of more than 100 days, *e.g.* batches from seed tree nos. 1, 2, 3, 4, 10, 14, 15, 16, 17, 18, 19, 20, 21 and 22. This was quite similar to mean time to germination (Appendix II, Table 26.).

From the germination results, pyrene batches can be divided into 4 groups including:

1. Pyrene batches with a long time to germination, MLD and germination period, *i. e.* batches from seed tree nos. 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21 ,22, 23, 24, 25, 26, 27 ,28, 29, 30, 31, 32, 33, 35, 36, 37 and 38.

2. Pyrene batches with a long time to germination and MLD but short germination period, *i. e.* batches from seed tree nos. 3 and 14.

3. Pyrene batches with a short time to germination and MLD but long germination period, *i. e.* batches from seed tree nos. 39, 40 and 41.

4. Pyrene batches with short time to germination, MLD and germination period, *i. e.* batches from seed tree nos. 34.

Table 4. Summary of germination behavior of the 5 species studied.

	S. axillaris	*M. toosendan*	*G. arborea*	*P. cerasoides*	*C. acumi-natissima*
percent germination (%)					
minimum mean of individual tree	8.30	0	0	7.50	18.1
maximum mean of individual tree	91.7	70.8	63.9	87.5	97.2
mean across all trees	42.3±22.3	49.3±14.9	20.2±18.8	55.0±20.5	79.5±14.4
time to germination (days)					
time of 1st seed germinated	10	15	9	11	3
time of last seed germinated	198	112	57	82	99
minimum mean of individual tree	23.7±8.4	28.3±13.8	10.4±2.1	20.8±10.5	25.9±8.8
maximum mean of individual tree	161.5±20.9	88.5±10.9	38.0±26.9	48.5±12.0	59.2±11.2
mean across all trees	97.6±35.1	58.5±14.6	15.5±4.6	33.3±6.2	38.6±8.3
germination period (days)					
range	13-188	6-97	2-47	26-66	26-82
mean across all trees	134±34.9	60.4±17.2	14.5±11.1	43.2±9.4	50.6±11.9
median length of dormancy (days)					
range	23-169	18-92	9-20	17-51	23-62
mean across all trees	98.7±47.2	54.3±18.3	13.3±2.4	31.2±8.0	36.9±9.3
	January	December	May	June	November
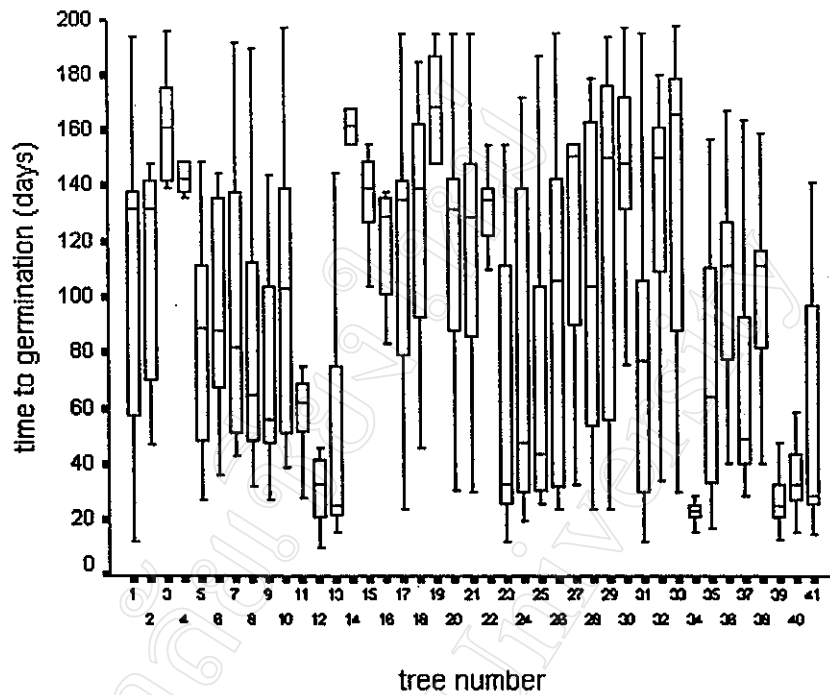
Figure 8. The germination period and median length of dormancy (MLD) of

individual seed trees of *Spondias axillaris* Roxb. Each box contains 50 %

of the seed germinated, and the line in the box shows the MLD. Vertical

lines below and above the boxes represent 0-25%, and 75-100% of seeds

which germinated respectively. Extreme values are at both bounds.

4.2.1.3 Seedling growth in the nursery

All germinated seedlings were pricked out on 20 December 1999. Most

seedlings were ready for planting at the start of the wet season, 25 weeks after

pricking out. At this time (9 June 2000), the majority of the seedlings had reached a

mean height of 40 to 60 cm, which is considered suitable for planting (FORRU 1998),

with the exception of batches from seed tree nos. 3, 14, 19, 27, 30, 33 and 36

(Appendix II, Table 26). The mean height of seedlings exhibited considerable variation among batches, ranging from 27.8 ± 11.2 cm (SD) to 73.1 ± 20.4 cm (SD) and also within individual pyrene batches (Table 5). For example, mean seedling height of batches from seed tree no. 18 varied from 12 to 91 cm. The mean root collar was similarly variable, ranging from 1.8 ± 0.4 mm (SD) to 4.4 ± 1.5 mm (SD) (Table 5). This was reflected in the wide ranging growth rates, of both height and root collar diameter (Table 5). Mean seedling RHGR across pyrene batches was 620.3 ± 99.3 % year[-1] (SD), higher than the mean seedling RRGR which was only 267.3 ± 51.6 % year[-1] (SD) (Table 5).

Table 5. Summary of seedling performance in the nursery of the 5 species studied.

	S. axillaris	M. toosendan	G. arborea	P. cerasoides	C. acumi-natissima
seedling height (cm)					
range (across all seedlings)	6.0-108.0	8.5-89.0	9.0-132	7.0-150.0	2.0-61.0
maximum mean of individual tree	73.1±20.4	71.0	125	113.1±27.2	25.8±11.1
minimum mean of individual tree	27.8±11.2	30.9±11.4	31	40.7±21.1	13.0±5.9
mean across all trees	52.6±11.4	44.9±6.8	80.4±18.2	74.6±26.1	19.8±2.8
seedling root collar diameter (cm)					
range (across all seedlings)	1.0-8.0	1.1-7.0	1.2-10.6	0.5-7.8	0.3-6.5
maximum mean of individual tree	4.4±1.5	3.7±1.0	7.5±1.8	4.8±1.6	2.2±0.8
minimum mean of individual tree	1.8±0.4	2.0±0.0	2.1±1.6	1.7±0.9	1.3±0.4
mean across all trees	2.9±0.1	3.0±0.4	5.2±1.1	3.1±1.3	1.7±0.2
seedling RHGR (% year[-1])					
range (across all seedlings)	23.9-1471.1	11.7-1081.1	-742.6-1041.6	0-998.9	-392.2-507.0
maximum mean of individual tree	836.5±23.9	1081.1	740.7±57.2	673.8±148.7	196.5±93.5
minimum mean of individual tree	408.7±125.1	509.2±194.3	78.7±691.5	374.7±164.2	115.2±56.9
mean across all trees	620.3±99.3	678.2±96.4	475.7±114.4	544.0±60.9	147.7±19.7
seedling RRGR (% year[-1])					
range (across all seedlings)	16.8-801.4	18.3-710.5	0.0-649.7	0-772.0	0-354.5
maximum mean of individual tree	342.3±166.3	393.5	474.6±141.3	397.8±200.3	109.7±70.7
minimum mean of individual tree	167.2±122.8	95.8	39.7	197.8±144.7	24.4±48.5
mean across all trees	267.3±51.6	288.5±44.4	247.8±90.5	306.6±54.9	69.5±15.3
seedling survival (%)					
maximum mean of individual tree	100.0	78.3	100	93.8	92.7
minimum mean of individual tree	61.9	0	0	26.7	41.1
mean across all trees	81.2±8.8	42.7±19.4	76.2±27.1	74.7±11.7	78.1±11.5

Seedling survival in the nursery, measured just prior to planting on 9 June 2000, was high, ranging among batches from 61.9 to 100%, with a mean of 81.2 ± 8.8 % (SD) (Table 5). The causes of mortality included: (*i*) the cotyledon of germinated seedlings failed to emerge from the pyrene (*ii*) seedlings were too underdeveloped at pricking out due to late germination and (*iii*) seedlings were infected by damping off disease and insects. Thirty seven batches (90%) had a survival rate of 70% or greater, considered to be acceptable for framework species (Appendix II, Table 26).

4.2.1.4 Sapling establishment in the field

The number of saplings of each seed tree transplanted into the plots on 16 June 2000 ranged from 5 to 37, due to variation in germination, nursery mortality and sapling height (Appendix III, Table 31). At the end of the wet season (3 November 2000), sapling survival amongst the pyrene batches ranged from 22.7 to 94.7%, with a mean of 71.2 ± 14.0 % (SD) (Table 6). Saplings from twenty-seven pyrene batches had a survival rate of 70% or greater, *e. g.* batches from seed tree nos. 5, 6, 7, 8, 9, 10 and 15 (Appendix III, Table 31). Most of the saplings that died did so within 2 weeks after planting out (monitored on 30 June 2000).

After 5 months in the plots, the mean height of all saplings was 103.4 ± 14.3 cm (SD), nearly double the size when planted. Mean sapling heights ranged from 69.0 ± 36.3 cm (SD) to 143.3 ± 11.6 cm (SD) (Table 6), exhibiting similar variation to that found in the nursery. The mean root collar diameter of all saplings was 17.3 ± 2.7 mm

(SD), more than 6 times greater than when planted, ranging from 11.4 ± 7.2 mm (SD)

to 23.0 ± 2.7 mm (SD) (Table 6). Consequently, RGRs also showed considerable

variation, from 41.4 ± 105.9 % year^{-1} to 348.2 ± 296.3 % year^{-1} (SD) for height, and

289.0 ± 217.9 % year^{-1} (SD) to 607.3 ± 131.5 % year^{-1} (SD) for root collar diameter

(Table 6).

Table 6. Summary of sapling performance in the field of the 4 species studied.

	S. axillaris	M. toosendan	G. arborea	P. cerasoides
sapling height (cm)				
range (across all seedlings)	17.0-207.0	17.030.0	12-154	19.0-195.0
maximum mean of individual tree	143.3±11.6	210.0±14.5	105.0±20.0	156.3±6.4
minimum mean of individual tree	69.0±36.3	71.0±42.6	20.0	87.9±36.8
mean across all trees	103.4±14.3	142.2±30.3	72.2±15.2	110.0±12.8
sapling root collar diamter (cm)				
range (across all seedlings)	1.1-35.0	2.1-35.0	3.5-29.0	3.0-21.0
maximum mean of individual tree	23.0±2.7	27.4±3.5	20.2	11.7±3.7
minimum mean of individual tree	11.4±7.2	11.0±4.58	6.3±2.2	6.3±2.1
mean across all trees	17.27±5.7	18.5±3.8	11.9±3.0	8.57±1.1
sapling RHGR (% year^{-1})				
range (across all seedlings)	-408.5-747.4	-312.8-872.6	-483.5-608.0	-482.0-653.25
maximum mean of individual tree	248.2±296.3	511.8±95.7	352.4±36.7	292.4±9.2
minimum mean of individual tree	41.4±105.9	65.5±274.0	-192.7	77.6±144.8
mean across all trees	188.2±63.6	326.8±84.1	58.3±150.3	179.0±44.3
sapling RRGR (% year^{-1})				
range (across all seedlings)	0-867.8	14.1-881.9	0-730.4	0-693.8
maximum mean of individual tree	607.3±131.5	737.7±110.6	364.9	343.9±163.1
minimum mean of individual tree	289.0±217.9	377.9±161.6	31.39±44.39	94.57±63.8
mean across all trees	463.9±65.1	485.7±69.6	212.87±80.4	212.6±46.9
seedling survival (%)				
maximum mean of individual tree	94.7	100	100	100
minimum mean of individual tree	22.7	0	0	37.5
mean across all trees	71.2±14.0	33.58±21.13	67.6±26.2	81.6±11.5

4.2.1.5 Selection of seed trees

Twenty-seven pyrene batches had a mean percent sapling survival in the field of 70% or higher which satisfied the first standard. Also, 7 pyrene batches grew to a height of 100 cm or taller in the first growing season (meeting the second criterion). The third criterion evaluated applied was mean percent germination. Only twelve pyrene batches achieved satisfied 40% or more germination percentage, but all of them had 70 % or higher seedling survival in the nursery. Twelve out of 41 pyrene batches (29.3%) satisfied all four standard criteria for this species. They were batches from seed tree nos. 7, 11, 17, 21, 23, 25, 26, 29, 31, 34, 35 and 39.

4.2.2 *Melia toosendan* Seib. & Zucc. (ANACARDIACEAE)

4.2.2.1 Seed characteristics

The fruit of *M. toosendan* usually ripen from November to April (FORRU, 2000). The ripe fruits remain on the trees for a long time, sometimes until the beginning of rainy season, so the seed collection period is potentially a long one. The fruit is yellow and smooth at first, but it becomes wrinkled as it gets older. The yellow fruits of 50 seed trees were collected on the ground from the southern (DS1) and northern parts (DS2) of Doi Suthep-Pui National Park (for exact locations and collection dates see Appendix I, Table 22). The fruit contains a single pyrene in which there are usually five seeds.

The seeds were extracted from the pyrenes, all seeds from all pyrenes of individual seed trees were mixed together. Seventy-two seeds were randomly selected. The wet mass and dimensions of all seeds were measured, and found to differ significantly amongst the seed batches ($P<0.0001$; ANOVA) (Appendix IV, Table 36). The mean wet mass across all seeds was 0.80 ± 0.02 g (SD). The mean seed mass of individual batches ranged from 0.74 ± 0.08 g (SD) to 0.84 ± 0.02 g (SD). The mean length of seeds amongst the seed batches ranged from 9.5 ± 0.7 mm (SD) to 12.1 ± 0.7 mm (SD), the mean width ranged from 3.13 ± 0.2 mm (SD) to 4.13 ± 0.3 mm (SD), and the mean thickness ranged from 2.31 ± 0.2 mm (SD) to 2.98 ± 0.4 mm (SD) (Table 3).

4.2.2.2 Germination

The germination percentage was highly variable, since one batch completely failed to germinate (seed tree no. 27). Germination percentage ranged from 0% to 70.8% with a mean of 49.3 ± 14.9 % (SD) (Table 4). Twenty-seven (54%) seed batches had a germination percentage of 40% or greater, which was considered the minimum acceptable standard for efficient nursery production, e. g. batches from seed tree nos. 3, 4, 7, 8, 9 and 10 (Appendix II, Table 27). The percent seed germination of seed batches from DS1 was 56.0 ± 9.9% (SD), significantly higher than those from DS2 which was 43.1 ± 16.2% (SD).

The time to germination, germination period and the MLD also showed considerable variation, both within, and amongst the seed batches (Figure 9). The mean time to germination of individual batches ranged from 28.3 ± 13.8 days (SD) to

88.5 $\pm$ 10.9 days (SD) (Table 4). The mean germination period of individual batches ranged from 6 to 97 days, with an overall mean of 60.4 $\pm$ 17.2 days (SD) (Table 4). he MLD ranged from 18 to 92 days, with an overall mean of 54.3 $\pm$ 18.3 days (SD) (Table 4).

The time to germination and MLD of seed batches from DS1 was 47.6 $\pm$ 10.9 days (SD) and 43.0 $\pm$ 9.9 days (SD) respectively, significantly less than those from DS2 which was 68.9 $\pm$ 9.1 days and 64.8 $\pm$ 18.1 days (SD) respectively. In contrast, the germination period of seed batches from DS1, which was 68.7 $\pm$ 18.3 days (SD), was significantly higher than those from DS2 which was 52.4 $\pm$ 11.6 days (SD).

Figure 9. The germination period and median length of dormancy (MLD) of individual seed trees of *Melia toosendan* Sieb. & Zucc. Each box contains 50 % of the seed germinated, and the line in the box shows the MLD. Vertical lines below and above the boxes represent 0-25%, and 75-100% of seeds which germinated respectively. Extreme values are at both bounds.

4.2.2.3 Seedling growth in the nursery

Seedling growth in the nursery was measured on 10 June 2000, 25 weeks after pricking out. Seedling height and RCD were highly variable, which was reflected in the wide ranging growth rates, of both height and root collar diameter (Appendix II, Table 27). Most seedling were 30 to 60 cm tall, considered a suitable height for

planting at the start of the wet season. Mean seedling height exhibited considerable variation amongst batches, ranging from 30.9 ± 11.4 cm (SD) to 71.0 cm (SD) (Table 5). Forty-one seed batches had a mean seedling height of 40 cm or higher, with the exception of batches from seed tree nos. 3, 6, 12, 29, 40, 41, 44, 45 and 46 (Appendix II, Table 27). The mean root collar was also variable, ranging from 2.0 ± 0.0 mm (SD) to 3.7 ± 1.0 mm (SD) (Table 5). Mean seedling RHGR across seed batches was 678.2 ± 96.4 % year^{-1}(SD), higher than the mean seedling RRGR which was 288.5 ± 44.4 % year^{-1} (SD) (Table 5).

Seedling survival in the nursery of seedlings exhibited considerable variation among batches, ranging from 0 to 78.3%, with an overall mean of 42.7 ± 19.4 % (SD) (Table 5). Some seed batches had a low percent seedling survival (Table 5), *e.g.* batches from seed tree nos. 16, 24 and 30 had no surviving seedlings in the nursery (Appendix II, Table 27). Only three seed batches had a percent seedling survival more than 70 % , which I considered to be very good for a framework species, *i. e.* batches from seed tree nos. 10, 21 and 23 (Appendix II, Table 27). Seed batches from DS1 had a percent seedling survival 48.7 %, significantly higher than those from DS2 (37%). Most of seedling dies between those were hardened off in full sunlight 4 weeks before planting. The main cause of mortality of seedling was damping-off disease.

4.2.2.4 Sapling establishment in the field

Saplings were planted on 16 June 2000. The number of saplings of each seed tree transplanted into the plots ranged from 0 to 36, due to variation in nursery

mortality (Appendix III, Table 32). After one growing season in the plots (measured on 3 November 2000), sapling survival amongst the progenies ranged from 0 to 100%, with a mean of 33.6 ± 21.1 % (SD) (Table 6). Saplings from only one seed batches had a survival rate of 70% or greater, *viz.* the batch from seed tree no. 16 (only 1 planted seedling). Most of the saplings died within 2 weeks after planting out. The main cause of sapling mortality was damping-off disease, since saplings were infected before planting. Some saplings died because their stems were damaged during transportation to the planting site.

After 5 months in the plots, the mean height of all saplings was 142.2 ± 30.3 cm (SD), more than triple the size when planted. Sapling heights ranged from 71.0 ± 42.6 cm (SD) to 210.0 ± 14.5 cm (SD), exhibiting similar variation to that found in the nursery (Table 6). Most sapling batches had mean heights of 100 cm or higher, which met the standard criteria for superior parent trees, exception for seed batches nos. 31, 36 and 41 (Appendix III, Table 32). The mean root collar diameter of all saplings was 18.5 ± 3.8 mm (SD), more than 6 times greater than when planted, ranging from 11.0 ± 4.6 mm (SD) to 27.4 ± 3.5 mm (SD) (Table 6). Consequently, RGRs also showed considerable variation among and within seed batches, ranging from 65.5 ± 274.0 % year^{-1} to 511.8 ± 95.7 % year^{-1} (SD) for height, and 377.9 ± 161.6 % year^{-1} (SD) to 737.7 ± 110.6 % year^{-1} (SD) for root collar diameter (Table 6).

4.2.2.5 Selection of seed trees

M. toosendan had the lowest mean sapling survival rate in the field of the 5 species studied (33.6%). Only one seed batch had a percent sapling survival in the

field of 70% or greater, which met the 1st standard for superior parent trees, *i. e.* batch from seed tree no. 16. *M. toosendan* did however exhibit a very high growth rate in the field. The seed batches that met the 1st standard also met the 2nd standard. When the 3rd standard were applied, no seed batches qualified, since batch from seed tree no. 16 had a percent germination only 9%. Therefore no seed batches qualified all 4 standard criteria.

4.2.3 *Gmelina arborea* Roxb. (VERBENACEAE)

4.2.3.1 Pyrene characteristics

The fruit of this species ripens between March and June (FORRU, 2000). Yellow fruits of 49 seed trees were collected from the ground from DS1 and DS2 (for exact locations see Appendix I, Table 23) on 11th to 14th April 2000. Yellow fruits ferment rapidly, and may contain insect larvae. Each fruit contains a single pyrene, in which there are usually 1-3 seeds. The pericarp was removed from the pyrene by rubbing against a wire mesh and then soaking the pyrene and pericarp in water, until the pyrene sinks to the bottom.

Following extraction from the fruit, the wet mass and dimensions of all pyrenes were measured and found to differ significantly amongst pyrene batches (P <0.0001; ANOVA) (Appendix IV, Table 37). The wet mass and dimensions were also variable within pyrene batches. Across all pyrenes, a mean wet mass of 1.24 $\pm$ 0.1 g (SD) was recorded. The mean pyrene wet mass of individual batches ranged from 1.02 $\pm$ 0.07 g (SD) to 1.46 $\pm$ 0.14 g (SD). The mean pyrenes length amongst the

pyrene batches ranged from 13.9 ± 1.0 mm (SD) to 18.4 ± 1.4 mm (SD), the mean width ranged from 7.5 ± 0.6 mm (SD) to 10.3 ± 0.6 mm (SD) and the mean thickness ranged from 6.7 ± 0.5 mm (SD) to 9.4 ± 0.6 mm (SD) (Table 3).

4.2.3.2 Germination

The pyrenes were sown whole, without the individual seeds being extracted, on 20 April 2000. Although each pyrene may produce up to three seedlings, only the first seedling to emerge was counted as germination. The time to germination was recorded for the first emerging seedling only. The germination percentage, amongst pyrene batches exhibited considerable variation, ranging from 0% to 63.9%. The mean germination percentage across batches was only 20.2 ± 18.8 % (SD) (Table 4). Twenty-two pyrene batches had a percent germination less than 10%, *i. e.* batches from seed tree nos. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 13, 15, 16, 17, 19, 20, 21, 23, 24, 25, 26 and 34 (Appendix II, Table 28). Only ten pyrene batches (20.4%) had a germination percentage of 40% or greater, which was considered to be the minimum acceptable standard for efficient nursery production, *i. e.* batches from seed tree nos. 29, 30, 33, 35, 37, 38, 41, 42, 44 and 50 (Appendix II, Table 28). All of those pyrene batches were located at DS2. Pyrene batches from DS2 had a mean percent germination of 31.8 ± 17.6 % (SD), significantly higher than those from DS1, which was only 7.0 ± 9.0 % (SD).

This species germinated rapidly and synchronously. The mean time to germination, germination period and MLD were all less than 20 days. The parameters also showed considerable variation, both within and among pyrene batches (Figure

10). The mean time to germination of individual pyrene batches ranged from 10.4 ± 2.1 days (SD) to 38.0 ± 26.9 days (SD) (Table 4). Most pyrene batches had a mean time to germination of less than 20 days, with the exception of batches from seed tree nos. 7, 20, 15, 19 and 43 (Appendix II, Table 28). The germination period was also low, but variable, ranging from 2 to 47 days among the pyrene batches, with an overall mean of 14.5 ± 11.1 days (SD) (Table 4). Most of pyrene batches had a germination period of less than 20 days, with the exception of batches from seed tree nos. 7, 9, 12, 12, 15, 18, 19, 30, 42, 43, 46 and 48 (Appendix II, Table 28). MLD's were also low, ranging from just 9 days to 20 days, with an overall mean of 13.3 ± 2.4 days (SD). Most pyrene batches had MLD's of less than 20 days, except for batches from seed tree nos. 13 and 43. (Appendix II, Table 28).

The mean time to germination of pyrene batches from DS2 was 17.2 ± 5.9 days (SD), significantly higher that those from DS1 which was 14.0 ± 2.4 days (SD), but the germination period and MLD of pyrene batches from DS1 did not differ from those from DS2.
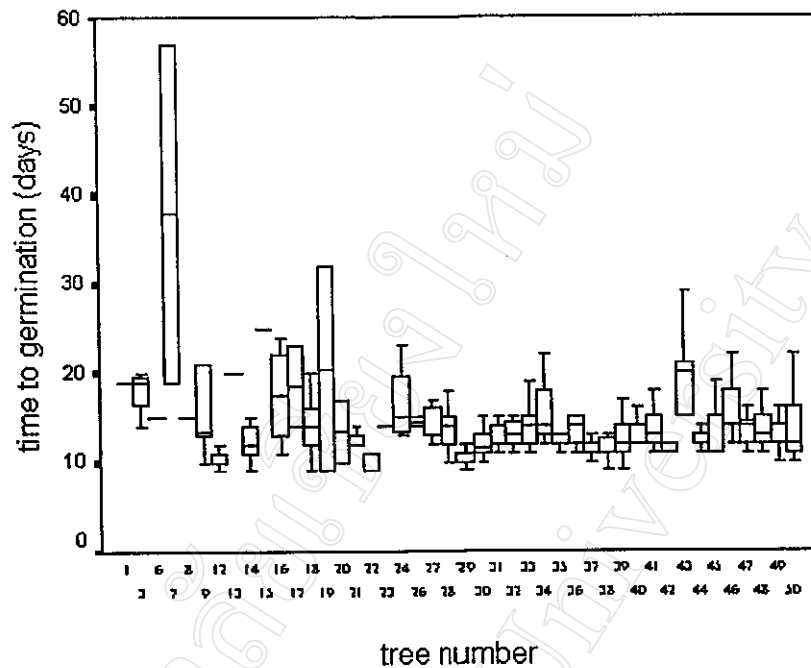
Figure 10. The germination period and median length of dormancy (MLD) of

individual seed trees of *Gmelina arborea* Roxb. Each box contains 50 %

of the seed germinated, and the line in the box shows the MLD. Vertical

lines below and above the boxes represent 0-25%, and 75-100% of seeds

which germinated respectively. Extreme values are at both bounds.

Horizontal lines indicate seed trees,which had only one germinated seed.

4.2.3.3 Seedling growth in the nursery

All germinated seedlings were pricked out on 30 July 2000. The seedling

growth rate of this species was very fast. Seedling heights were super optimal for

planting (40 to 60 cm) 4 months after pricking out, despite shoot pruning. At this time

(30 November 2000), the mean height of the seedlings exhibited considerable

variation amongst the pyrene batches, ranging from 31.0 cm to 125.0 cm. The

seedling height was also variable within pyrene batches, *e. g.* batches from seed tree no. 3 varied from 6 to 122 cm. The mean root collar was similarly variable, ranging from 2.1 ± 1.6 mm (SD) to 7.5 ± 1.8 mm (SD) (Table 5). This was reflected in wide ranging growth rates, of both height and root collar diameter (Appendix II, Table 28). Mean seedling RHGR across pyrene batches was 475.7 ± 114.4 % year^{-1} (SD), higher than mean seedling RRGR which was 277.8 ± 90.49 % year^{-1} (SD) Table 5). The RHGR was variable, since seedlings appeared to have weak stems. The shoots were vulnerable to damage by insects and wind.

The percent seedling survival was measured on 30 November 2000. Seedling survival in the nursery, amongst the pyrene batches exhibited considerable variation, ranging from 0 to 100%, with a mean of 76.2 ± 27.1 % (SD) (Table 5). One major cause of mortality of seedlings was stem boring Coleopteran insects, Yemane leaf beetle and longhorn beetles. Twenty-nine pyrene batches (59%) had a survival rate of 70% or greater, which is very good for a framework species, *e.g.* batches from seed tree nos. 7, 8, 13, 14, 15, 16, 41, 42, 43, 44, 45, 46, 47, 48, 49 and 50 (Appendix II, Table 28). Some pyrene batches had a percent seedling survival in the nursery of 100%, but those had also had a low germination percentage, *i. e.* batches from seed tree nos. 7, 8, 13, 15, 16, 17, 19, 21, 23 and 24 (Appendix II, Table 28). The percent seedling survival of pyrene batches from DS1 was 70.7 ± 35.5, lower than those from DS2, which was 81.9 ± 12.3, but not significantly so.

4.2.3.4 Sapling establishment in the field

Saplings were planted on 26 June 2001. The number of saplings of each seed tree transplanted into the plots varied from 0 to 38, due to variation in seed germination and nursery mortality (Appendix III, Table 33). Saplings were monitored on 9 November 2001. After 5 months in the plots, sapling survival amongst the batches exhibited considerable variation, ranging from 0% (*i. e.* batches from seed tree nos. 3, 8 and 13) to 100% (*i. e.* batches from seed tree nos. 5, 6, 7, 15, 19, 21, 23, 24 and 27 (Appendix III, Table 33) with a mean of 67.6 $\pm$ 26.1% (SD) (Table 6). Saplings from twenty-two pyrene batches had a survival rate of 70% or greater, *e. g.* batches from seed tree nos. 5, 6, 7, 8, 9, 10 and 15 (Appendix III, Table 33). Most saplings died within 2 weeks of planting out. The causes of mortality included: (*i*) seedling damage during transportation, (*ii*) water logging following heavy rain after planting out, (*iii*) weed competition (*iv*) insect damage.

After 5 months in the plots, the mean height of all saplings was 72.2 $\pm$ 15.2 cm (SD) (Table 6), exactly the same size as when planted. New stems had however been produced in this time on many seedlings. Mean sapling heights were highly variable, ranging from 20.0 cm to 105.0 $\pm$ 20.0 cm (SD) (Table 6), exhibiting similar variation to that found in the nursery. Only one pyrene batches had a mean sapling height in the field more than 100 cm, which met the standard for selection of superior parent tree, *i.e.* batches from seed tree no 27. The mean root collar diameter of all saplings was 11.9 $\pm$ 3.0 mm (SD), more than 2 times greater than when planted, ranging from 6.3 $\pm$ 2.2 mm (SD) to 20.2 mm (Table 6). Consequently, RGR's also showed considerable variation both amongst and within pyrene batches. The RGR was ranged from -192.7

% year^{-1} to 352.4 $\pm$ 36.7 % year^{-1} (SD) for height and 31.4 $\pm$ 44.4 % year^{-1} (SD) to

364.9 % year^{-1} (SD) for root collar diameter (Table 6).

4.2.3.5 Selection of seed trees

Thirty-two pyrene batches produced saplings which had a mean percent

survival in the field of 70% or higher, which satisfied the 1st standard. However, some

batches which had a 100% survival rate in the field, had only 1 or 2 seedlings planted,

since they had only 1 or 2 germinated seedlings (Appendix III, Table 33). The 2nd

criterion was met by only 1 pyrene batch, *i. e.* batches from seed tree no. 27.

However, pyrene batch no. 27 did not qualify for the 3rd standard, since it had a

percent germination of only 11.1%. Therefore, no pyrene batch qualified for all 4

standards.

4.2.4 *Prunus cerasoides* D. Don (ROSACEAE)

4.2.4.1 Pyrene characteristics

The fruit ripens between March to April (FORRU, 2000). Red fruits of 50

seed trees were collected, when hanging on the tree from 3 locations, comprising 25

seed trees in Doi Suthep-Pui National Park, 13 seed trees from Doi Ang Khang, and

12 seed trees from Doi Inthanon National Park on 19th to 29th March 2000 (Appendix

I, Table 24). The fruit contains a single pyrene in which there is a single seed. The

pericarp was removed from the pyrene by rubbing the fruit against wire mesh and soaking the pyrenes and the pericarp in water until the pyrenes sank.

The wet mass and dimensions of all pyrenes were measured. The Pyrene dimensions and wet mass showed considerable variation amongst pyrene batches, and there were significantly differences amongst the pyrene batches ($P<0.0001$; ANOVA) (Appendix IV, Table 38) and also within pyrene batches. The mean wet mass across all pyrenes was 0.98 ± 0.08 g (SD). The mean pyrene mass of individual batches ranged from 0.83 ± 0.02 g (SD) to 1.17 ± 0.05 g (SD). The mean length of pyrenes amongst the pyrene batches ranged from 9.1 ± 0.3 mm (SD) to 12.9 ± 0.6 mm (SD), the mean width ranged from 6.9 ± 0.3 mm (SD) to 9.1 ± 0.5 mm (SD), and the mean thickness ranged from 5.4 ± 0.2 mm (SD) to 7.4 ± 0.2 mm (SD) (Table 3).

4.2.4.2 GERMINATION

The pyrenes were sown whole on 5 April 2000, without the individual seed being extracted. Germination percentage amongst pyrene batches showed considerabe variation, ranging from just 7.5% to 87.5%, with an overall mean of 55.0 ± 20.5 % (SD) (Table 3). Thirty-nine (78%) pyrene batches had a germination percentage of 40% or greater, which was considered to be the minimum acceptable standard for efficient nursery production *e.g.* batches from seed tree nos. 1, 4, 5, 9, 10, 11 and 14 (Appendix IV, Table 35). Percent germination of pyrene batches from DI was $46.0 \pm 22.0\%$ (SD), significantly lower than those from DAK, which was $64.3 \pm 17.1\%$ (SD), but not different from DS ($54.4 \pm 19.9\%$ (SD)).

The mean time to germination, germination period and the MLD also showed considerable variation, both within, and amongst pyrene batches (Figure 11). The mean time to germination of individual pyrene batches ranged from 20.8 ± 10.5 days (SD) to 48.5 ± 12.0 days (SD) (Table 4). Most pyrene batches had a mean time to germination of less than 40 days, except for batches from seed tree nos. 2, 3, 5, 33 and 47 (Appendix II, Table 29). The germination period among the pyrene batches ranged from 26 days to 66 days, with an overall mean of 43.2 ± 9.4 days (SD) (Table 4). Most pyrene batches had asynchronous germination. The mean MLD across all pyrene batches was 31.2 ± 8.0 days (SD) (Table 4), ranging from 17 to 51 days. Twenty-three pyrene batches had MLD's of less than 40 days, except for batches from seed tree nos. 2, 3, 5, 8, 24, 35, 46 and 47 (Appendix II, Table 29). The mean time to germination, germination period and MLD of pyrene batches did not differ between the 3 locations.
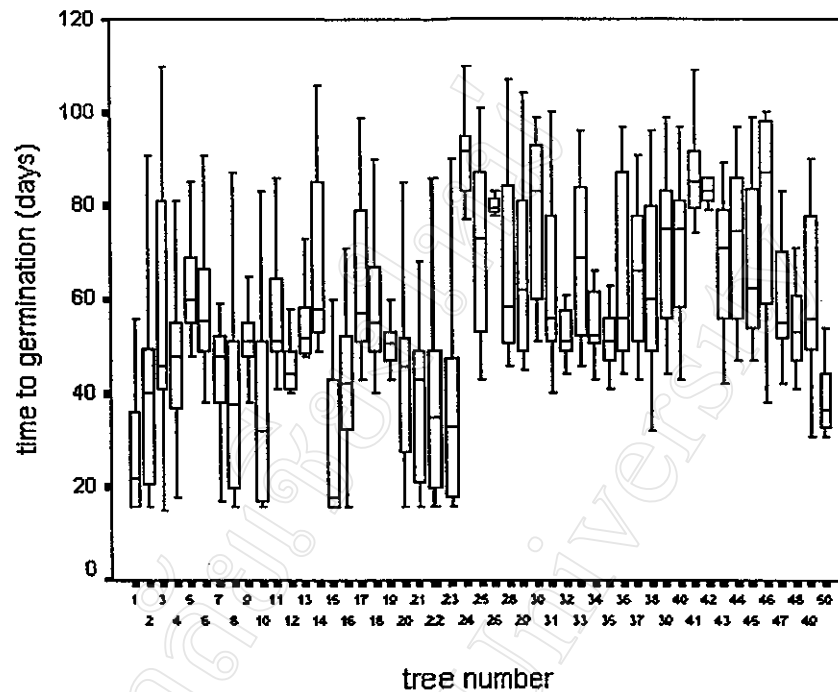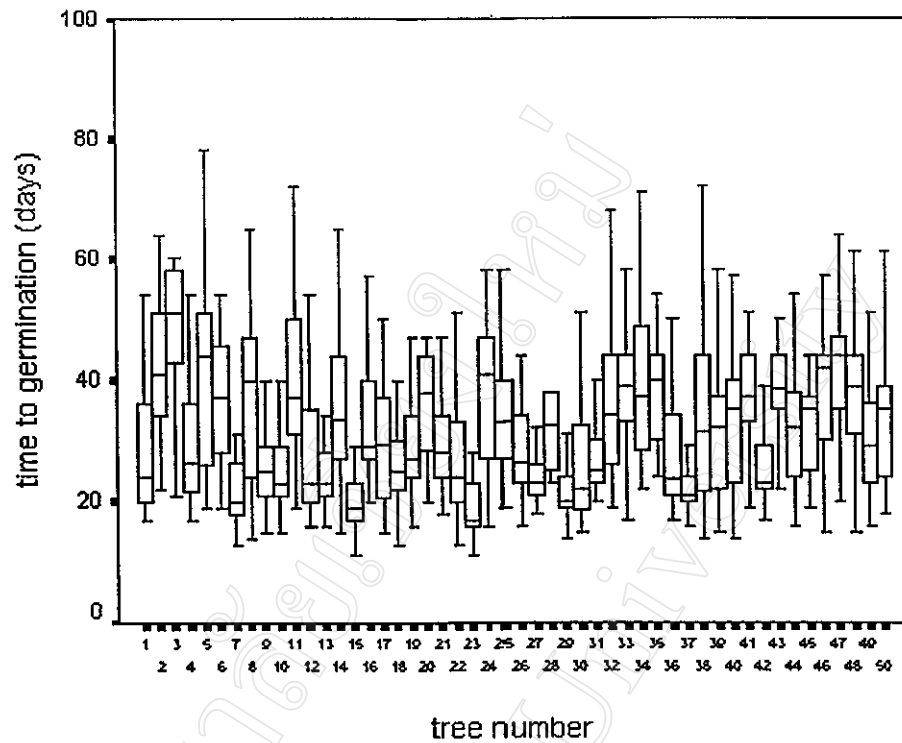
Figure 11. The germination period and median length of dormancy (MLD) of

individual seed trees of *Prunus cerasoides* D. Don. Each box contains 50

% of the seed germinated, and the line in the box shows the MLD.

Vertical lines below and above the boxes represent 0-25%, and 75-100%

of seeds which germinated respectively. Extreme values are at both

bounds.

4.2.4.3 Seedling growth in the nursery

All germinated seedlings were pricked out on 1 August 2000. Seedling growth

rate of *P. cerasoides* was high compared to the other species studied. Seedling height

was super optimal for planting (40 to 60 cm) 4 months after pricking out, despite

shoot pruning. At this time (1 December 2000), the mean seedling heights showed

considerable variation amongst the pyrene batches, ranging from 40.7 ± 21.1 cm (SD)

to 113.14 ± 27.2 cm (SD) (Table 5). Seedling height was also variable within pyrene batches, for example, seedling height of pyrene batch no. 28 varied from 30 to 120 cm. The mean root collar was similarly variable, ranging from 1.7 ± 0.9 mm (SD) to 4.8 ± 1.6 mm (SD) (Table 5). This was reflected in the wide ranging growth rates of both height and root collar diameter. Mean seedling RHGR across pyrene batches was 544.0 ± 60.9 % year^{-1} (SD), higher than the mean seedling RRGR which was only 306.6 ± 54.9 % year^{-1} (SD) (Table 5).

Seedling survival in the nursery was also measured at this time. The overall seedling survival was high (74.7%) with a high variable, ranging from 26.7 to 93.8%. Causes of mortality included (*i*) some may have been pricked out too early (*ii*) stem borer damage. Thirty-five pyrene batches (70%) had a survival rate of 70% or greater, *e. g.* batches from seed tree nos. 1, 3, 4, 5, 7, 8, 9 and 10 (Appendix II, Table 29).

4.2.4.4 Sapling establishment in the field

The number of saplings from each seed tree transplanted into the plots on 26 June 2001, ranged from 2 to 20, due to variation in seed germination and nursery mortality (Appendix III, Table 34). After 5 months in the plots (10 November 2001), sapling survival in the field was high. Sapling survival amongst the pyrene batches exhibited considerable variation, ranging from 37.5 to 100%, with a mean of 81.6 ± 11.5 % (SD) (Table 6). Saplings from forty-six pyrene batches (92%) had a survival rate of 70% or greater, which met the standard criteria for selection superior parent tree, except for batches from seed tree nos. 3, 12, 36 and 41. Most saplings died within 1 month after planting out (monitoring on 27 July 2001). The main causes of

mortality included: (*i*) seedlings being depressed by weeds, (*ii*) seedlings being destroyed by sediment and flooding, due to heavy rains after planting out and (*iii*) stem borers.

After 5 months in the plots, mean sapling height across all batches was 110.0 $\pm$ 12.8 cm (SD), ranging from 87.9 $\pm$ 36.8 cm (SD) to 156.3 $\pm$ 6.4 cm (SD) (Table 6), exhibiting similar variation to that found in the nursery. Saplings from thirty-nine pyrene batches had a mean height of 100 cm or more, which met the standard for selection of a superior parent trees. The mean root collar diameter of all saplings was 8.6 $\pm$ 1.1 mm (SD), ranging from 6.27 $\pm$ 2.1 mm (SD) to 11.7 $\pm$ 3.7 mm (SD) (Table 3). Consequently, RGRs also showed considerable variation; from 77.6 $\pm$ 144.8 % year^{-1} (SD) to 292.4 $\pm$ 19.2 % year^{-1} (SD) for height, and 94.6 $\pm$ 63.8 % year^{-1} (SD) to 343.9 $\pm$ 163.1 % year^{-1} (SD) for root collar diameter (Table 6).

4.2.4.5 Selection of seed trees

P. cerasoides had the highest mean sapling survival in the field (81.6%), compared with other species studied. Forty-six (92.0%) pyrene batches produced saplings with a mean survival rate in the field of 70% or higher, which met the 1st standard for this species. Also, *P. cerasoides* had a high growth rate. Thirty-six pyrene batches exceeded the 2nd standard. The 3rd standard was 40% or more mean percent germination. Twenty-seven pyrene batches met this standard, of which twenty-one also met the 4th standard and satisfied the other 3 standards. They were

batches from seed tree nos. 1, 4, 5, 10, 11, 15, 19, 21, 22, 23, 24, 25, 26, 27, 30, 31, 40, 42, 43, 44 and 50.

4.2.5 *Castanopsis acuminatissima* (Bl.) A. DC. (FAGACEAE)

4.2.5.1 Seed characteristics

The fruit ripens between September and November (FORRU, 2000). Nuts usually separate from the cupules when mature. Brown nuts of 50 seed batches were collected from the ground from 3 locations: comprising 25 seed batches from Doi Suthep-Pui National Park, 12 from Doi Inthanon National Park and 13 from Jae Sawn National Park. The collection dates were 19th to 27th September 2000 (for exact locations and collection date see Appendix I, Table 25). Each nut contains a single seed.

The wet mass and dimensions of all nuts were measured, and found to differ significantly amongst seed batches ($P<0.0001$; ANOVA) (Appendix IV, Table 39). A mean wet mass across all nuts was 1.23 $\pm$ 0.14 g (SD). The mean nut wet mass of individual batches ranged from 0.92 $\pm$ 0.07 g (SD) to 1.49 $\pm$ 0.15 g (SD). The mean length of nut amongst the seed batches ranged from 8.2 $\pm$ 0.6 mm (SD) to 11.6 $\pm$ 0.8 mm (SD), the mean width ranged from 6.5 $\pm$ 0.6 mm (SD) to 10.4 $\pm$ 0.9 mm (SD), and the mean thickness ranged from 6.0 $\pm$ 0.6 mm (SD) to 10.0 $\pm$ 0.7 mm (SD) (Table 3).

4.2.5.2 Germination

Nuts from 50 seed trees were sown on 3 October 2000. *C. acuminatissima* had a highest germination percentage, compared with the other species studied. The percent germination showed considerable variation among seed batches. The germination percentage ranged from 18.1% to 97.2% with a mean of 79.5 $\pm$ 14.4 % (SD) (Table 4). Forty-nine seed batches (98%) had a germination percentage of 40% or greater, which was considered the minimum acceptable standard for efficient nursery production, except batch form seed tree no. 16 (Appendix IV, Table 39). The percent germination of seed batches from DS was 72.67 $\pm$ 16.52% (SD), significantly lower than those from DI (84.38 $\pm$ 8.04% (SD)) and JS (88.14 $\pm$ 5.88% (SD)). However, the mean time to germination of seed batches from DI did not differ from JS.

The time to germination, germination period and MLD also showed considerable variation, both within, and amongst seed batches (Figure 12). The mean time to germination of individual batches ranged from 25.9 $\pm$ 8.8 days (SD) to 59.15 $\pm$ 11.2 days (SD) (Table 4). The time to germination of seed batches from DS was 43.7 $\pm$ 7.7 days (SD), significantly higher than those from DI and JS, which were 34.3 $\pm$ 4.1 and 32.8 $\pm$ 6.3 days (SD) respectively. The time to germination of seed batches from DI did not differ from JS.

The mean germination period of individual batches ranged from 26 days to 82, with an overall mean of 50.6 ± 11.9 days (SD). The mean germination period of seed batches was not different among the 3 locations.

MLD ranged from 23 days to 61 days, with an overall mean of 36.92 ± 9.3 days (SD) (Table 4). The MLD of seed batches from DS was 42.3 ± 8.7 days (SD), significantly higher than those from DI and JS, which were 32.9 ± 6.3 days (SD) and 30.3 ± 6.6 days (SD) respectively. The MLD of seed batches from DI was not different to JS.
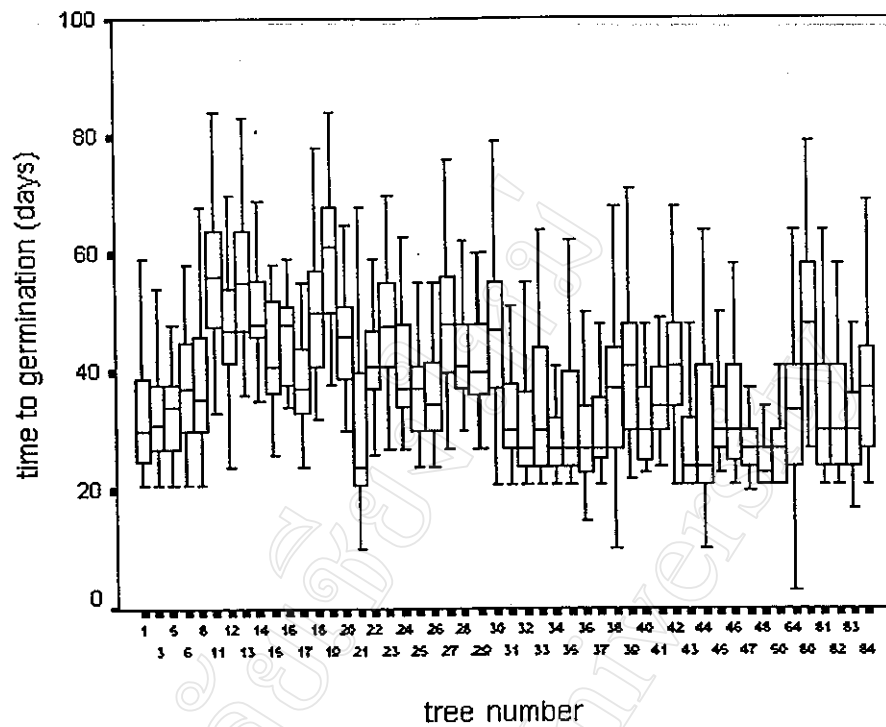
Figure 12. The germination period and median length of dormancy (MLD) of individual seed trees of *Castanopsis acuminatissima* (Bl.) A. DC. Each box contains 50 % of the seed germinated, and the line in the box shows the MLD. Vertical lines below and above the boxes represent 0-25%, and 75-100% of seeds which germinated respectively. Extreme values are at both bounds.

4.2.5.3 Seedling growth in the nursery

Seedling growth in the nursery was measured on 23 November 2001, 43 weeks after pricking out. Seedlings were not ready for planting in the 1st rainy season after seed collection, due to their very slow growth rate. Seedling growth rate was lower than the other species studied. The mean height of seedlings exhibited considerable variation amongst the seed batches, ranging from 13.0 ± 5.9 cm (SD) to 25.8 ± 11.1 cm (SD) (Table 5). Seedlings from twenty-three (46%) seed batches had a mean seedling height 20 cm or higher, which was considered the minimum acceptable

standard for selection superior parent trees. The mean root collar was also high variable, ranging from 1.3 ± 0.4 mm (SD) to 2.2 ± 0.8 mm (SD) (Table 5). The mean seedling RHGR and RRGR across seed batches were 147.7 ± 19.7 % year^{-1} (SD) and 69.5 ± 15.3 % year^{-1} (SD) respectively, the lowest compared with the other species studied.

Seedling survival in the nursery exhibited considerable variation amongst the seed batches, ranging from 41.1 to 92.7%, with a mean of 78.1 ± 11.5 % (SD) (Table 5). Forty seed batches had a percent germination 70% or greater, which met the standard for this species. The causes of seedling mortality included (*i*) some seedlings may have been too young when pricked out, since they germinated late, and (*ii*) seedlings being damaged by the larvae of moth, which bores into the stems.

4.2.5.4 Selection of seed trees

The standard criteria for this species differed from those of the other species studied, since seedlings had the lowest growth rate, compared with the other species studied. Seedlings were not ready for planting in the 1st year after seed collection. The 1st standard was a mean seedling height in the nursery of 20 cm or more. Twenty-three seed batches met this standard. The 2nd standard was 70% or more germination. Twenty-one seed batches met this standard. The 3rd standard was 70 % or more seedling survival in the nursery, which met by seventeen seed batches. They were batches from seed tree nos. 3, 15, 20, 22, 25, 26, 35, 37, 38, 39, 40, 41, 44, 47, 48, 50 and 81.

4.3 DISCUSSION

4.3.1 VARIATION IN SIZE OF SEEDS OR PYRENES FROM DIFFERENT SEED TREES

Seed size and mass varies considerably among and within species in different habitats, and different stages of succession (Salisbury, 1942). In this study, seed or pyrene size (length, width and thickness) and wet mass all differed significantly among batches for all 5 species studied (ANOVA). *S. axillaris* had the biggest pyrene length, followed by *G. arborea* (pyrene), *M. toosendan* (seed), *P. cerasoides* (pyrene) and *C. acuminatissima* (seed). However, the seed width, thickness and wet mass of *C. acuminatissima* were 2nd biggest. This species has an ovoid nut, ahead by *S. axillaris* (obovoid drupe), followed by *G. arborea* (obovoid drupe), *P. cerasoides* (ellipsoid drupe) and *M. toosendan* (flatted seed) respectively.

A number of hypotheses have been proposed to account for variation in seed size. Smith and Fretwell (1974) suggested that a trade-off between seed number and seed mass should occur, where seed number varies with changing resources status, while seed mass remained relatively constant. Seed size variation may produce an optimal seed shadow (Jansen, 1977) or minimize the risk of failure in heterogenous environments (Venable and Brown, 1988). Also, variation in seed size might be adaptive if seeds of different sizes differ in genetic quality (Temme, 1986).

Several studies have noted the pattern of seed mass variation with respect to both altitude (Baker, 1972; Lord 1994) and latitude (McWilliams *et al.*, 1968), but the relationship was equivocal. In this study, seed size and wet mass of seed trees from

higher elevations had a bigger size than those from lower elevations. This is in agreement with Vera (1997). In contrast, Baker (1972) found that mean seed size and mass decreased with increasing altitude. Mean seed or pyrene size and wet mass of *S. axillaris*, *M. toosendan* and *C. acuminatissima* were positively and significantly correlated with elevation of seed tree. On the other hand, seed size and wet mass of the other species studied were not correlated with elevation of seed tree.

4.3.2 VARIATION IN GERMINATION RESPONSE OF SEEDS FROM DIFFERENT SEED TREES

Species from a wide range of plant families, life cycle types, and plant communities exhibit differences in germination characteristics of seed collected in different locations. Depending on the species, germination responses vary with latitude, elevation, soil moisture, soil nutrients, temperature, kind and density of plant cover and degree of habitat disturbance of the sites when the seed matured (Baskin and Baskin, 1998)

Germination is determined by seed quality (germination capacity and vigour), pretreatment (release from dormancy) and germination conditions such as water, temperature, substrate, light, and freedom from pathogens (Schmidt, 2000). The three main factors which influence germination including light, temperature and moisture.

In this study, there was considerable variability in germination characteristics both within and among the 5 species tested. *C. acuminatissima* had the highest germination percentage (79.50±14.40), followed by *P. cerasoides* (54.98±20.51), *M.*

toosendan (49.28±14.94), *S. axillaris* (42.34±22.25), and *G. arborea* (20.16±18.82). The germination percentages of 3 of the 5 studied species were lower than 50%, which may to limit nursery production of seedlings.

S. axillaris had an overall mean percent germination of only 42%, remarkably similar to previously reported values in nurseries under very similar conditions (including artificial watering) namely 43% (FORRU, 2000) and approximately 50% (Hardwick, 1999). Apparently, *S. axillaris* pyrenes rapidly loose viability during storage. Hau (1999) conducted germination trials, using pyrene that had been stored for 5-7 months, and obtained a germination percent of only 16–18.8% in a nursery with supplementary watering. In the field, under natural conditions of rainfall, germination was a meager 1.7-6.7%. Hardwick (1999) also obtained low germination percentages for pyrenes sown in a nursery plot with no supplementary watering under various levels of shade, even though the pyrenes had not been stored for any length of time. Watering considerably increased germination rates. Therefore, frequent watering and maintenance of high levels of soil moisture are probably essential in achieving even moderate rates (40-50%) of germination.

For *M. toosendan*, low germination percentage also appears to limit nursery production of seedlings. Percent germination of this species was only 49.28% with a high variability (14.94), lower than previously reported values in nurseries under very similar conditions, *e. g.* 68% (FORRU, 2000). Woods (unpublished data) reported that scarifying the seeds and then soaking them in hot water over night significantly improved germination percentage. However, the germination percentage was still very low (14%).

The elevation of seed trees was positively correlated percent germination. Seed from trees at higher elevations tended to germinate better than those from lower elevation. Seed trees from DS1 were situated at a higher elevation than DS 2 (1170.45 cf. 837.40) and also seed from DS 1 had more percent germination (DS 1, 56.03; DS 2, 43.53). This result is in agreement with the findings of Lord (1994) and Vera (1997) who reported that seeds collected from the higher altitudes had the higher percent germination than those from lower altitude. Germination characteristics of seeds collected from difference sites can vary in many way, but one of the most common is the degree of dormancy, as reflected by germination percentage of fresh seeds (Baskin and Baskin, 1998). Twenty-three seed batches had a percent germination lower than 40%, which would result in them being rejected as the superior seed trees. Some seed batches had a very low percent germination, i. e. batch from seed tree no. 42 (15%) or zero germinating (tree no. 27).

The seed trees from DS2 also had a mean time to germination and median length of dormancy higher than seed trees from DS1. One reason for the low percent germination and high time to germination and median length of dormancy (Figure 9) of seed trees from DS2 might be that seeds lost viability before collection, since seeds of seed trees from DS2 were collected later than DS1 about 2 weeks (Appendix 1, Table 22). Perhaps collecting fruits at the very end of its long fruiting period increases the chances of fungal infection. The fruits ripens between November to April (FORRU, 2000), the ripe fruits remain on the trees for a long time, so the possible period for collecting seeds is a long one. The fruit is yellow at first and smooth, but it becomes wrinkled as it get older. Woods (unpublished data) found that

percent germination of *M. toosendan* seeds dropped to 7% after 6 months storage. Future research should be aimed at increasing the germination rate. Seed should be collected when they are yellow and still on the tree.

G. arborea had the lowest germination percentage (20.16±18.82), which is a serious problem for seedling production. Germination percentage in this study was lower than previous reported by Royal Forest Department (1987) and FORRU (2000). Royal Forest Department (1987) obtained a germination percentage of *G. arborea* of 78. FORRU (2000) reported germination percentage of this species of 83% for one batch. However, germination percentage of a second batch was only 39%. Only 4 pyrene batches had percent germination more than 50%, *i. e.* batches from seed tree nos. 29, 30, 37 and 42. Twenty-one pyrene batches had a percent germination lower than 10%, *i. e.* batches from seed tree nos. 1, 2, 3, 4, 5 and 6, including pyrenes derived from seed trees nos. 4 and 25 that failed to germination. Pyrene batches that came from the DS2 location tended to germinate better than those from DS1. Mean percent germination of pyrene batches from DS1 was only 6.92%, lower than DS2, which was 35.28%. The location of seed trees might have affected germination of this species, a phenomenon reported in other species, *e. g. Abies cephalonica* Loud. (Fady, 1992), *Betula papyrifera* Marshall (Bevington, 1986) and *Prunus serotina* Ehrh. (Pitcher, 1984). Woessner and McNabb (1979) reported that germination of this species may drop by 22% after a day and nearly to zero after a week. Jackson (1987) reported that yellow fruits fermented rapidly and after only one or two days, the percent germination was greatly reduced. To improve germination rate, the fruit pulp (exocarp and mesocarp) should be removed from the pyrene as soon as possible after collection. All the pericarp must be removed from the pyrenes before they are spread

out in the sun to dry. Woessner and McNabb (1979) found that thoroughly cleaned pyrene gave 10% better germination.

Prunus cerasoides had a fairly high percent germination (54.98) with a wide range (20.51). The overall percent germination in this studied was lower than previously reported by FORRU (2000) and Hardwick (1999), which was 76% and 68% respectively. The best nursery practice is to sow the pyrenes immediately after collection. Jackson (1987) suggested to improve germination percentage by soaking the pyrenes in water for 1-2 days. Kopachon (1998) found that germination percentage of *P. cerasoides* pyrenes subjected to a dry heat treatment was higher than a control and a wet heat treatment. Kessler (1981) and Cambell (1983) reported that *P. cerasoides* pyrenes rapidly loose viability after 5 months and 1 year storage respectively. In this study, pyrenes of trees from differences locations did not differ in their germination percentage. This is in disagreement with the findings of Lord (1994) and Vera (1997).

Castanopsis acuminatissima had the highest percent germination of the tree species in this study (79.50), also with a wide range. This result is in agreement with that reported by Savasti (2000). Percent germination did not limit seedling production. Overall, percent germination is this study was higher than previously reported by FORRU (2000), which was only 50%. The location of seed trees might have affected percent germination. This result is in agreement with that reported for other species *i. e. Astragalus granatensis* Lam. (Trillo and Carro, 1993), *Castilleja fissifolia* (Smith, 1975), *Eucalyptus pauciflora* (Beardsell and Mullett, 1984), *Pinus contorta* (Haais and Thrupp, 1931). Seeds of trees from higher elevations had a higher germination

percentage than those from lower elevations. This was supported by the conclusion of Lord (1994) and Vera (1997. This was probably because seeds from trees at higher elevations were larger and also had higher germination percentage.

Median length of dormancy (MLD), time to germination (TG) and germination period (GP) are also important factors in seedling production. Ideally, a nursery manager would select seed trees that produce seed with short MLD's and which germinate synchronously, for maximum efficiency and uniformity of planting stock In this study, median length of dormancy, time to germination and germination period varied considerably among seed batches from different parent trees, leading to uneven production of non-uniform seedlings. Seed batches with shorter MLD's had greater percent germination than those with long MLD's. This correlation was significant and positive for *S. axillaris*, *G. arborea* and *C. acuminatissima*, but not significant for *M. toosendan* and *P. cerasoides*.

G. arborea had the lowest MLD, time to germination and germination period. Germination period of *G. arborea* in this study was 15 days, lower than the findings of the Royal Forest Department (1987), which was 25 days. *S. axillaris* had the highest values of all 3 variables, probably because it had the largest pyrene. Jackson (1987) also reported that the germination period of *S. axillaris* was highly variable, ranging from 8 days to over 90 days. However, germination period he reported was lower than found in this study, which ranged from 13 to 188 days.

When MLD was compared with mean seedling size at time of planting, it was found that seedlings from seed batches with long MLD's were smaller than those

derived from seed batches with shorter MLD's. Seedlings that germinated early had a longer time to develop into larger seedlings by planting time. Seed or pyrene batches which had short MLD's and large seedling size (height) of *S. axillaris* were from seed tree nos. 19, 33, 14, 3 and 27; for *M. toosendan* batches from seed tree nos. 3, 6, 12, 29, 40, 41, 45 and 46; for *G. arborea* batches from seed tree nos. 7, 13, 43, 45 and 49; for *P. cerasoides* batches from seed tree nos. 8, 24 and 35 and for *C. acuminatissima* batches from seed tree nos. 18, 19, 80. This was confirmed by correlation analysis (Pearson's correlation coefficients). MLD was significantly and negatively correlated with seedling height for *S. axillaris*, *M. toosendan* and *G. arborea*, but not significant for *P. cerasoides* and *C. acuminatissima*. However, there were exceptions, *e.g.* for *S. axillaris,* pyrene batch no. 26 had a short MLD, but resulting seedlings were quite small. In contrast, pyrene batch no. 32 had a long MLD, but the resulting seedlings were larger than those from no. 26.

4.3.3 SEEDLING PERFORMANCE IN NURSERY

It is generally accepted that seedlings derived from larger seeds have an advantage over those derived from small seeds. Seed size often plays a role in seedling performance in the early stages of life. Relatively large or heavy seeds are an indication of abundant food reserves from the parent tree. Larger seeds have the ability to store greater amounts of carbohydrate in their endosperm or cotyledons than small seeds (Milberg and Lamont, 1997). Seeds with large food reserves ensure the seedlings a longer period for establishment in the new environment before becoming dependent on their own assimilation (Schmidt, 2000). In addition, large seeds may provide seedlings with better protection against herbivores and pathogens. Foster

(1986) also suggested that a larger seed could provide sufficient reserves to produce secondary compounds to defend a persistent seedling, or sufficient energy to replace lost or damaged tissue.

The advantages of larger seed size can be divided into broad categories. First, larger seeds generally develop into large seedlings more quickly and probably have a competitive advantage (Black, 1958; Gross and Werner, 1982; Gross, 1984). Secondly, seedlings from larger seeds may be able to cope better with a temporary carbon deficit during early development (Denslow, 1980; Foster, 1986).

Growth of seedlings in the nursery is important for seedling production. Ideally for seedling production, seedlings should reach a suitable height for planting (50-60 cm tall , FORRU (2000)) by the optimal planting time (*i. e.* the beginning of the rainy season, June in northern Thailand). Considerable variation was found among seed batches in growth and growth rate of seedlings.

By planting time, *S. axillaris* seedlings had a mean height suitable for planting (53 cm tall). This result is very similar to that obtained by Elliott *et al.*, (in press). They reported that *S. axillaris* seedlings, germinated from seed collected in September, reached a mean height of 56.9 cm (SD 14.4) and a mean root collar diameter of 6.6 mm (SD 1.3) by the following June, 227 days after pricking out. Therefore, plants needed to be kept in the nursery for only 9 months. This result contrasts with a previous report by FORRU (2000), which stated that pyrenes should be collected in March and seedlings kept in the nursery for 15 months. The research reported here shows that collecting pyrenes late in the rainy season is adequate to

produce a crop of saplings of a size suitable for planting at the optimal time of year, whilst shortening the nursery care period to 9 months and considerably reducing labour required and expenses. However, seedlings from some pyrene batches exhibited slow growth rates, which meant that seedlings would not be ready for planting out in the next rainy season, *i.e.* batches from seed tree no. 3, 14, 19, 27, 30, 33 and 36. Most of these batches also had a low percent germination.

M. toosendan also had a suitable size for planting 7 months after sowing date (December 1999). The mean height and root collar diameter of seedlings were 44.88 cm and 3.01 mm respectively. The height of seedlings was lower than the 50 cm, recommended by FORRU (2000), but they were still suitable for planting, because *M. toosendan* is fast-growing species. This result was similar to that reported in FORRU (2000). They found that seeds collected in November developed into seedlings ready for planting in the first planting season after germination (June 2000).

P. cerasoides is the fast-growing tree. Pyrenes were collected in March and sown in April, shortly before the planting time. This means that seedlings would will stay in the nursery for about 14 months, because seedlings were not ready for planting by mid June. *P. cerasoides* pyrenes germinate relatively quickly and its seedlings grow rapidly in containers (Blakesley *et al.,* 2000). This was confirmed in this study. Eight months after sowing, the mean seedling height and RCD were 75 cm and 3 mm respectively. Seedlings of some seed tree had a height of more than 100 cm, *i. e.* batches from seed tree no. 25, 30 and 37. Therefore, seedlings were suitable for planting 6 months ahead of the planting time. Consequently, shoot pruning was used to control seedling height. In this study, shoot pruning was carried out twice such that

the overall mean seedling height and RCD at planting time were 63 cm and 4.4 mm respectively. Blakesley *et al.* (2000) suggested that storing pyrenes and sowing them nearer to the dispatch date or by sowing pyrenes in a sterile (zero nutrient) mix and delaying potting. However, when pyrenes were stored in nursery, they will loose viability. Campbell (1983) reported that after 1-year storage the germination percentage of *P. cerasoides* pyrenes dropped to about 50. Kessler (1981) stated that the *P. cerasoides* pyrenes can be stored for up to 5 months, but with much reduced germination.

The fast growth of *G. arborea* seedlings also presented a problem with seedling production. Seven months after sowing (April), overall mean seedling height and RCD were 80 cm and 5 mm respectively. Some pyrene batches produced seedlings more than 100 cm tall, *i. e.* batches from seed tree nos. 8, 21, 40 and 41. Most seedlings grew taller than the suitable size for planting, and required shoot pruning. Shoot pruning was carried out only one time (compared with twice for *P. cerasoides*), because the growth rate of *G. arborea* was lower than that of *P. cerasoides* and seedlings of *G. arborea* take a long time to grow back after shoot pruning. The problem of over-growth of seedlings might be solved by storing pyrene and sowing them in December (six months later than in this study). Pyrenes will germinate rapidly (mean time to germination across seed batches was 16 days) and seedlings should reach a suitable height within six months after germination. Woessner and McNabb (1979) reported that *G. arborea* pyrenes are successfully stored in sealed containers at 5 °C and 6-10 % moisture content. The fresh pyrenes have a germination of 90% and after two years of storage germination is still 80%. This result is in agreement with the observation of Yap and Wong (1983) who

reported that *G. arborea* pyrenes lost 20% viability after 2 years of cold storage (4°C). Jackson (1987) also reported that dry and depulped *G. arborea* pyrenes retain viability for 6 months or longer. With cold storage (5 °C), it is possible to store pyrenes for two years or more.

C. acuminatissima was the only species in this study that was not planted in experimental plots, because of its slow growth. FORRU (2000) stated that seedlings should be ready for planting in the second planting season after germination, a fact confirmed by this study. After eleven months, the overall mean seedling height and RCD were 20 cm and 2.8 mm respectively. Twenty-three seed batches produced a seedlings with mean heights of more than 20 cm, *e.g.* batches from seed tree nos. 1, 8, 12, 14, 20, 21 and 22.

Percent seedling survival in the nursery varied considerably among seed batches. Seedling survival is one of the standard criteria for selecting superior parent trees. In this study, 4 out of the 5 species tested had seedling survival rates of more than 70%, which was acceptable for normal nursery practice (FORRU, 2000). The survival rate of *S. axillaris* seedlings in the nursery was highest, followed by *C. acuminatissima, G. arborea, P. cerasoides* and *M. toosendan* respectively.

S. axillaris had an overall percent seedling survival of 81.24 ± 8.8 % (SD). Thirty-eight batches had a seedling survival rate of more than the acceptable standard of 70%. High seedling survival could help to compensate for low germination rates in this species. Seedlings of *S. axillaris* showed high resistance to pests and diseases. Although some seedlings were attacked by catapillars, they recovered rapidly by

producing new leaves. During the two-week hardening off period, prior to planting out, seedlings of *M. toosenden* were heavily infected by damping-off disease and half of them died. However, seedlings of *S. axillaris*, that were stood down next to the *M. toosendan* seedlings were unaffected. Most of the seedlings that died were derived from large pyrene that germinated late. From the one-way ANOVA (tables 5 and 6), we should reject seedlings derived from pyrenes wet mass of 3.1 g or more and those that germinate more than 85 days after sowing.

Percent seedling survival in the nursery of *M. toosendan* appears to limit seedling production. Overall percent seedling survival in the nursery was only 42.70 ± 19.37%. Only 3 seed batches had a percent seedling survival of more than 70%, *i.e.* batches from seed tree nos. 10, 21 and 23. While, 4 had a percent seedling survival of less than 10%, *i.e.* batches from seed tree nos. 16, 24, 30 and 42. Causes of seedling death included damping-off diseases (possibly *Cercospora meliae*) and white loopers (Geometridae) (Rayden, unpublished). Damping off diseases are caused by a variety of seed and soil-borne fungi, which attack young seedlings with low vigour. Seedlings infected by damping-off diseases drop their leaves rapidly, then the shoots and stems fall over and die. More than half of seedlings died in May 2000, at the start of the rainy season. To increase seedling survival, seedlings should kept in the nursery under a roof, since spores of *Cercospora* are dispersed by rain-splash. The main preventive measures to be taken against damping-off and other fungal diseases in the nursery are adequate spacing, a substrate with good aeration, proper moisture and light management plus adequate ventilation of seedling beds (Schmidt, 2000).

Percent seedling survival of *P. cerasoides* did not limit seedling production, with an overall value of 71.69 ± 11.69% (SD). Thirty-five pyrene batches had a percent seedling survival of more than the acceptable standard of 70%, *i. e.* batches from seed tree nos. 1, 4, 5, 25, 26 and 27. Seedlings of *P. cerasoides* showed high resistance to pests and diseases. Seedlings that died tended to originate from late-germinating seeds. From the one-way ANOVA (table 6), seedlings that germinated later than 31 days after sowing should be rejected from seedling production.

Percent seedling survival of *G. arborea* was high, overall percent seedling survival was 76.17. However, there was wide variation in percent seedling survival from 100% (*i. e.* batches from seed tree no. 8, 13, 15 and 23, only 1 germinated seedling) to 0% (*i. e.* batches from seed tree nos. 1, 2, 10; only 1 germinated seedling). Causes of death included stem borers, *i.e.* Coleopteran pest, Yemane Leaf Beetle (*Calopepla leyana*) and longhorn beetle (*Glena indiana*) (Rayden, unpublished).

C. acuminatissima had the best seedling production, due to high percent germination (79.50±14.40%) and percent seedling survival in nursery (78.09±11.45). Forty seed batches had a percent seedling survival of more than 70%, which is acceptable for superior parent trees. Seedlings had a high resistance to pest and diseases. From the one-way ANOVA, there had no differences between seed size, wet mass and time to germination.

4.3.4 SAPLING PERFORMANCE IN THE FIELD

After one growing season in the field, relationships between seed characteristics, germination results and seedling performance in the nursery with sapling performance in the field were examined. Rapid early growth and establishment of saplings in the field are important factors in determining the sapling performance and sapling survival in the field.

4.3.4.1 Spondias axillaris

Growth of *S. axillaris* saplings in the field was rapid with a doubling in size, on average, 6 months after planting out (Tables 3 and 4). Hau (1999) reported exceptionally high growth rates of *S. axillaris* saplings, in comparison with other tree species planted out on hillsides in Hong Kong. He reported final stem heights (over two years after planting) of 100-156 cm. Clearly, conditions on Doi Suthep are more favourable for the growth of *S. axillaris* than those of Hong Kong, since most batches of saplings approached or exceeded the mean heights reported for Hong Kong within 6 months after planting.

Fifteen batches of saplings had a mean relative growth rate of more than 200%. Most of those sapling batches were relatively small at planting time. However, some batches were not accepted as originating from superior parent trees, because their final heights failed to exceed 100 cm. *i. e.* batches from seed tree nos. 3 and 33. Twenty-five seed batches reached produced saplings that 100 cm in height or taller, which met this criteria.

Hardwick (1999) reported that natural establishment of *S. axillaris* saplings in deforested areas is primarily limited by lack of seed dispersal. Therefore, either direct seeding or planting of saplings is necessary to establish this species in deforested areas. In a planting experiment that tested the effects of weeding, Hardwick found that highest mortality of planted *S. axillaris* saplings occurred during the hot-dry season and that removal of surrounding weeds substantially increased sapling mortality during that season. Hau (1999) reported exceptionally high survival rates (97.5-100% over two years) of *S. axillaris* saplings planted in deforested sites in Hong Kong.

A high survival rate of out planted saplings was also demonstrated in the field. A standard of 70% survival or higher, was considered acceptable and sapling batches from twenty-seven seed trees met or exceeded this standard. Compared with most other species tested as framework species, *S. axillaris* shows exceptional promise due to its high survival and growth rates in the field, although further research needs to be carried out to determine its attractiveness to seed-dispersing wildlife. In selecting parent trees for the production of *S. axillaris* seedlings for forest restoration, it is recommended that trees that produce smaller than average pyrenes are selected. Seedling batches that reach 60 cm or more in the nursery by planting time are more likely to perform well after planting out than smaller seedlings.

4.3.4.2 Melia toosendan

Sapling growth rate in the field of *M. toosendan* was also high and highly variable. The growth rate of both height and RCD of *M. toosendan* was higher than

that of all other species studied. Overall, sapling height and RCD on 3 November 2000 (140 days after planted) was 142 cm and 6.6 mm respectively, an RGR of nearly 500% for RCD and of more than 300% for height. This result is vary similar to that obtained by Elliott and Anusarnsunthorn (2001). Most seed batches produced saplings with a mean height of more than 100 cm, which met the superior parent tree standard. However, sapling batches nos. 31, 36 and 41 had mean seedlings height of less than 100 cm, and were therefore rejected as superior parent trees.

Saplings of *M. toosendan* grew very well in the field. However, they had low percent survival. The standard criterion for selection of superior parent trees in terms of sapling survival in the field was 70% or greater. The overall sapling survival was only 34%. Only one sapling batches were met this criteria, *i. e.* sapling batch nos. 16. However, only one surviving sapling from this seed batch was planted. This result differed from that reported by Elliott and Anusarnsunthorn (2001). They obtained a percent sapling survival in the field of 98.33% in plots planted in 1998 and 68.75% in plots planted in 1999, despite a short drought immediately after planting. In contrast, in this study, the cause of sapling death was mainly flooding because there was very heavy rain after planting. In addition, saplings were weak, due to the effects of damping-off disease in the nursery.

4.3.4.3 Gmelina arborea

Sapling growth rate and survival was poor in this species. The overall sapling height and RCD on 10 November 2001 (137 days after planted) were 72 cm and 12 mm respectively. This result is very similar to that reported by Elliott and

Anusarnsunthorn (2001). The growth of *G. arborea* saplings might depend on the planting site condition and soil fertility. Jackson (1987) reported that *G. arborea* is very sensitive to soil conditions. It is capable of survival on poor dry sites, but its growth under these conditions is poor and trees on such sites are very branchy and stunted. The Royal Forest Department (1987) reported that saplings of *G. arborea* were planted at Nakornratchasima. Their mean height and RCD after 14 months in the plots was only 51 and 1.8 cm respectively. Sapling relative RCD growth rate (RRGR) was more than 200%, but relative height growth rate (RHGR) was only 58%. Low growth rate was probably due to the shoots being killed by pests and diseases. The diseases that infected and destroyed saplings in the field were root rot, sooty mold, brown leaf spot and damping off. The minimum standard sapling height in the field was 100 cm. Only one pyrene batch produced saplings that met this standard, *i. e.* batch from seed tree nos. 27.

Overall, sapling survival in the field was high (68%), and also highly variable (26%). Survival rate was similar to that reported by Elliott and Anusarnsunthorn (2001). Dupuy (1985) and Kadio (2001) reported high sapling survival (75-99%), while Khoon (1987) observed a survival rate of only 15%. This was probably due to poor study conditions. In this study, some pyrene batches produced saplings with 100% survival, although in same cases only 1 or 2 saplings were planted and survived, *i. e.* batches from seed tree nos. 5, 6, 7, 15, 17, 19, 21, 23, 24 and 27. Twenty-two superior parent trees with 70% or higher sapling survival were identified, *i. e.* seed tree nos. 5, 6, 7, 15, 17, 19 and 21. Gonzal *et al.*, (1995) found that inoculating *G. arborea* saplings with VA mycorrhiza before planting results in earlier sapling establishment and improved growth of saplings in poor soil.

4.3.4.4 Prunus cerasoides

The growth rate of *P. cerasoides* in the field was fairly high. After 137 days, overall mean sapling height and RCD were 110 cm and 8.6 mm respectively. This result is very similar to that reported by Elliott and Anusarnsunthorn (2001). Sapling RRGR and RHGR were more than 200% and nearly 200% respectively. Thirty-nine pyrene batches had a mean sapling height of more than 100 cm, considered acceptable for superior parent trees. Eleven pyrene batches were not accepted as superior parent trees, but mean sapling size was fairly high (nearly 100 cm), *i. e.* batches from seed tree nos. 29, 33 and 36. Also, the growth rate of this species was variable between sites. Schaltenbran (1982) reported on sapling growth of *P. cerasoides* on a stony soil at 700 m asl. and 1200 m asl in Nepal. They found that saplings at 700 m asl. reached a mean height of 1.5 m when 26 months old, but at 1200 m asl., the mean height was only 0.5 m, at the same age. The growth rate of this species in Nepal was slower than in northern Thailand. Elliott and Anusarnsunthorn (2001) reported a mean sapling height of 240 cm and 460 cm after 1 growing season (18 months) and 2 growing seasons (30 months) respectively. Growth rates probably vary with latitude, elevation, soil moisture, soil nutrients, temperature and kind and density of plant cover.

Percent sapling survival, during the study presented here, was also high (82%) and similar to that obtained by Elliott and Anusarnsunthorn (2001). Most of pyrene batches were accepted as superior parent trees, having a sapling survival of more than 70%, except for batches from seed trees nos. 3, 12, 36 and 41. The survival rate of this

study and of FORRU (2000) were slightly higher than in Nepal (71%, Campbell and Bharai, 1983).

4.3.5 SELECTION OF SEED TREES BASED ON NURSERY PERFORMANCE AND FIELD PERFORMANCE

The primary aim of this study was to develop criteria to select *S. axillaris, M. toosendan, G. arborea, P. cerasoides* and *C. acuminatissima* seed trees for forest restoration programmes. Ideally, selection would be made on the basis of nursery and field performance, with due consideration to genetic analysis, carried out to reduce the risk of narrowing the genetic base.

In this study, the criteria used to identify superior seed trees of 5 study were: sapling survival and growth of seedlings in the field and germination percentage and seedling survival in the nursery. Twelve seed trees attained or exceeded the standard set for these criteria for *S. axillaris*, zero seed tree for *M. toosendan* and *G. arborea*, 21 seed trees for *P. cerasoides* and 21 seed trees for *C. acuminatissima*. Although, the number of satisfied seed tree was less than 50 suggested by Brown and Marshall (1995), and in this study, I suggest twenty trees should be sufficient for seed collection, based on the results from model II in Chapter 6. Twenty seed trees of *P. cerasoides* and *C. acuminatissima* were selected.

For *S. axillaris*, yielded only twelve satisfactory seed trees (section 4.1.5). However, seed tree nos. 6, 7, 8, 9, 10, 15, 19, 20 and 33 should also be classified as

superior parent trees. Since those seed batches had a percent sapling survival of more than 80%, mean sapling heights of nearly 100 cm and fairly good germination percentages.

No seed tree of *M. toosendan* met the standards, due to low seedling survival, both in the nursery and in the field. The overall percent seedling survival in the nursery and in the field was the lowest, compared with the other 4 species studied, since saplings died due to damping-off diseases.

G. arborea also had zero satisfactory seed trees. The problem of this species was low germination percentage and growth rate in the field. Germination percentage and sapling height in the field appears to limit selection of superior parent trees for this species.

Two suggestions to select superior parent trees of *M. toosendan* and *G. arborea* include: (*i*) the standard criteria for those species should be set lower than for the other species studied or (*ii*) expand the study more seed trees.

CHAPTER 5

PLANT TRAIT CORRELATIONS

5.1 INTRODUCTION

In the previous chapter, the performance of seed batches from many different parents was assessed. Although this approach to parent tree selection is very thorough, it takes a very long time to assess both nursery and field performance. The purpose of this chapter is to assess the extent to which parent tree or seed characters, germination behavior or early seedling growth might be used as indicators of field performance. The approach was to look for relationships between the various characteristics measured and to determine their predictive value for the 4 key criteria of parent tree selection that were identified in the previous chapter. If clear relationships could be found, it would considerably reduce the time required to select optimal seed trees.

Relationships between characteristics of the parent tree, and seed size, germination behavior and or early seedling growth have been reported before, e. g. seed size vs. percent germination (Black, 1959; Harper and Obeid, 1967; Cideciyan and Malloch, 1982; Wise, 1982; Morse and Schmitt, 1985; Winn, 1985; Wulf, 1986; Tripathi and Khan, 1990; Maranon and Grubb, 1993 and Nizam and Hossain, 1999), seed size vs. seedling grow rate (Stock *et al.*, 1990; Seiwa and Kikuzawa, 1991 and Seiwa, 2000) and seed size vs. time to germination (Castro, 1999).

In this chapter, a one way ANOVA, Pearson correlation coefficient and multiple regression were used to identify relationships among various plant traits for the 5 species studied.

The objectives of this chapter are:

1. to determine the relationships among characteristics of parent trees, seeds, and seedling performance both in the nursery and in the field.

2. to determine if characteristics of parent trees or seedling performance in the nursery can be used to predict the performance of planted trees.

5.2 RESULTS

5.2.1 DIFFERENCES BETWEEN GERMINATING AND NON GERMINATING SEEDS

All seeds (pyrenes) from all seed trees of each species were divided into 2 groups, *i. e.* germinating and non-germinating seeds. There were some significant differences in seed or pyrene dimensions (length, width and thickness) between germinating and non-germinating seeds for all studied species, but the results were mixed.

Germinating seeds or pyrenes of *S. axillaris*, *G. arborea* and *P. cerasoides* were significantly smaller than those that failed to germinate (Appendix IV, Tables 40, 42, and 43). In contrast, germinating seeds of *M. toosendan* and *C.*

acuminatissima were longer than those that failed to germinate (Appendix IV, Tables 41, 44).

5.2.2 SEED OR PYRENE SIZE – DID SEEDLINGS THAT DIED IN THE NURSERY ORIGINATE FROM SMALLER SEEDS?

Seed or pyrene size variables (length width, thickness and wet mass) of all surviving seedlings from all seed or pyrene batches were compared with the same parameters of all seedlings that died from all seed batches. There were some significant differences for all studies species, but the results were mixed.

Surviving seedlings of *S. axillaris* and *P. cerasoides* in the nursery tended to originate from smaller pyrenes (Appendix IV, Tables 45 and 48), and seedlings of *M. toosendan* that survived in nursery tended to originate from larger seeds (Appendix IV, Table 46). However, seed or pyrene size had no clear effect on seedling survival in the nursery for *G. arborea* and *C. acuminatissima* (Appendix IV, Tables 47 and 49).

5.2.3 TIME TO GERMINATION – DID SEEDLINGS THAT DIED IN THE NURSERY MOSTLY ORIGINATE FROM SEEDS WHICH GERMINATED LATE ?.

Time to germination of all surviving seedlings from all seed batches were combined with time to germination of all seedlings that died from all seed batches. Time to germination appeared to be related with seedling mortality on nursery.

Seedlings that survived tended to originate form seeds that germinated rapidly for all species studied.

Time to germination of surviving seedlings in the nursery was significantly shorter than that of seedlings which died, for *S. axillaris*, *M. toosendan* and *P. cerasoides* (Appendix IV, Tables 45, 46 and 48), but the result was not significant for *G. arborea* and *C. acuminatissima* (Appendix IV, Tables 47 and 49).

5.2.4 SEED OR PYRENE SIZE – DID SAPLINGS THAT DIED IN THE FIELD, ORIGINATE FROM SMALLER SEEDS?

Seed or pyrene size (length, width, thickness and wet mass) of all surviving saplings (after one growing season in the plots) from all seed or pyrene batches were compared with the same variables of all saplings that died from all seed or pyrene batches. Seed or pyrene size had no clear an effect on sapling survival in field for all species studied. Surviving saplings of *M. toosendan* in the field had a mean seed size (seed width and thickness) greater than of those that died (Appendix IV, Table 51). The seed or pyrene size of surviving saplings of *S. axillaris*, *G. arborea* and *P. cerasoides* did not differ from of those that died (Appendix IV, Tables 50, 52 and 53).

5.2.5 TIME TO GERMINATION – DID SAPLINGS THAT DIED IN THE FIELD ORIGINATE FROM LATE GERMINATING SEEDS?.

The time to germination of all surviving saplings (after one growing season in the plot) from all seed batches was compared with that of all seedlings that died from

all seed batches. Surviving saplings tended to originate from rapidly germinating seeds. Mean time to germination of surviving saplings was shorter than of those that died, but the result was not significant for all species studied (Appendix IV, Tables 50, 51, 52 and 53).

5.2.6 SAPLING SIZE AT PLANTING – DID SAPLINGS THAT DIED IN THE FIELD, ORIGINATE MOSTLY FROM SMALL SEEDLINGS

Sapling size variables (root collar diameter and height) at planting of all surviving saplings from all seed batches were compared with the same parameters of those that died, from all seed batches, in the field for all species studied. Surviving saplings tended be larger at planting time than those that died. Sapling size (both RCD and height) at planting of surviving saplings was significantly larger than those that died for all species studied (Appendix IV, Tables 54, 55, 56 and 57).

5.2.7 CORRELATIONS

Correlations between parent tree characteristics (elevation and GBH), seed or pyrene size (length, width, thickness and wet mass), germination results, nursery performance and field performance were investigated by using Pearson correlation coefficient (Table 7).

There were some correlations between seed trees characteristics and seed or pyrene size, but the results were mixed. Seed or pyrene size of *S. axillaris*, *M. toosendan* and *C. acuminatissima* was positively and significantly correlated with

elevation of seed tree, but the correlation was not significant for *P. cerasoides* and *G. arborea*. The girth at breast height (GBH) of seed tree was not correlated with seed or pyrene size for all species studied.

The correlation between seed or pyrene size and percent germination was mixed. Seed size of *C. acuminatissima* (all parameters) and *M. toosendan* (seed length) was positively and significantly correlated with percent germination, but negatively and significantly correlated for *G. arborea* (width and thickness). However, pyrene size of *S. axillaris* and *P. cerasoides* was not correlated with percent germination.

In general, increased of time to germination was associated with decreased percent germination. The time to germination was negatively and significantly correlated with percent germination for all studied species, so the correlation between seed size and time to germination contrasted with percent germination. Seed size of *C. acuminatissima* (all parameters) and *M. toosendan* (length) was negatively and significantly correlated with time to germination, but positively and significantly correlated with time to germination for *G. arborea*. Pyrene size of *S. axillaris* and *P. cerasoides* was not correlated with time to germination.

Many studies have demonstrated that large seeds have many advantages over small seeds, including seedling size. In this studied, there were some correlations between seedling size in the nursery and seed or pyrene size. Large seedlings tended to originate from larger seeds. Seed or pyrene size of *M. toosendan*, *P. cerasoides* and *C. acuminatissima* was positively and significantly correlated with both seedling

height and RCD in the nursery, but not correlated for *S. axillaris* and *G. arborea*. In contrast, time to germination had a negative correlation with seedling size in the nursery. Times to germination of *S. axillaris, M. toosendan* and *G. arborea* were negatively and significantly correlated with both seedling height and RCD in the nursery, but not correlated for *P. cerasoides* and *C. acuminatissima*.

The correlation of relative growth rate with seed or pyrene size and time to germination was not clear. Pyrene size of *S. axillaris* (length and wet mass) was positively and significantly correlated with RHGR, but negatively and significantly correlated with RRGR. In contrast, pyrene size of *P. cerasoides* (length) was positively and significantly correlated with RRGR. Time to germination was also positively and significantly correlated with RHGR for *S. axillaris* and with both RRGR and RHGR for *C. acuminatissima*.

Seedling survival should also be correlated with seed size and time to germination. Seedling survival in the nursery of *M. toosendan* was positively and significantly correlated with seed size, and negatively and significantly correlated with time to germination. The other species studied did not show correlations between seedling survival in the nursery with seed size and time to germination.

There were some correlations between sapling performance in the field, seed or pyrene size and time to germination, but not strongly. Seed or pyrene sizes of *S. axillaris, M. toosendan* and *G. arborea* and *P. cerasoides* were not correlated with both sapling RCD and height in the field, except for wet mass of *P. cerasoides*, which was positively correlated with sapling height in the field. For *G. arborea*, pyrene size

(thickness) was negatively and significant correlated with RHGR in the field, but not correlated for the other species studied.

Time to germination was also correlated with sapling size and relative growth rate in the field, but the results were mixed. Time to germination was negatively and significant correlated with both sapling RCD and height of *M. toosendan* and sapling height of *G. arborea*, but not for the other species studied. Time to germination was positively and significant correlated with both sapling RCD and height of *S. axillaris*, but not for the other species studied.

Sapling survival in the field was negatively and significantly correlated with time to germination of *M. toosendan*, but did not correlate with the other species studied.

5.2.8 MULTIPLE REGRESSION ANALYSIS

Multiple regression analysis was used to identify the independent variables that had greatest influence on the dependent variables used to define parent superiority including:

dependent variable	independent variable
percent germination	elevation of seed tree, GBH of seed tree and seed or pyrene width
seedling survival in the nursery	elevation of seed tree, GBH of seed tree, seed or pyrene width and time to germination
sapling height in the field	elevation of seed tree, GBH of seed tree, seed or pyrene width and time to germination
sapling survival in the field	elevation of seed tree, GBH of seed tree, seed or pyrene width and time to germination

PERCENT GERMINATION

The multiple regression model of percent germination was emerged and significant only for *M. toosendan* (Adjusted R square = 0.166; $F_{3,45}$=4.188, p<0.05) and *C. acuminatissima* (Adjusted R square = 0.145; $F_{3,46}$=3.781, p<0.05). The best predictor for *M. toosendan* was elevation of seed trees (*Beta*=0.424; p<0.01), which was negatively correlated with percent germination. The best predictor for *C. acuminatissima* was seed width (*Beta*=0.395; p<0.05), which was positively correlated with percent germination.

SEEDLING SURVIVAL IN THE NURSERY

The multiple regression model of seedling survival in the nursery was emerged and significant for *M. toosendan* (Adjusted R square = 0.160; $F_{4,43}=3.245$, $p<0.05$)and *C. acuminatissima* (Adjusted R square = 0.162; $F_{4,45}=3.361$, $p<0.05$). The best predictor for *M. toosendan* was seed width (*Beta*=0.260; $p=0.083$), which was positively correlation. The best predictor for *C. acuminatissima* was elevation (*Beta*=0.293; $p<0.05$) and GBH of seed tree (*Beta*=0.292; $p<0.05$), which was positively correlated with nursery survival.

SAPLING HEIGHT IN THE FIELD

The multiple regression model of seedling height in the field was not emerged for all species studied.

SAPLING SURVIVAL IN THE FIELD

The multiple regression model of seedling survival in the field was emerged only for *M. toosendan* (Adjusted R square = 0.152; $F_{4,42}=3.057$, $p<0.05$), The best predictor was time to germination (*Beta*=-0.415; $p<0.05$), which was positively correlated with seedling survival in the field.

Table 7. Plants traits correlation of 5 species studied.

		S. axillaris		M. toosendan		G. arborea		P. cerasoides		C. acuminatissima	
		Trait values		Trait values		Trait values		Trait values		Trait values	
		r	P	r	P	r	P	r	P	r	P
ELV	length	0.37	*	0.52	**			0.16	n.s.	0.34	*
	width	0.38	*	0.35	*			0.12	n.s.	0.34	*
	thick	0.36	*	0.11	n.s.			-0.09	n.s.	0.35	*
	mass	0.38	*	0.13	n.s.			-0.14	n.s.	0.30	*
	TGM	0.23	n.s.	-0.64	**			0.14	n.s.	-0.31	*
	PGM	-0.26	n.s.	0.46	**			-0.04	n.s.	0.22	n.s.
GBH	length	-0.20	n.s.	0.16	n.s.	-0.07	n.s.	0.10	n.s.	0.09	n.s.
	width	0.05	n.s.	0.11	n.s.	-0.26	n.s.	0.10	n.s.	-0.04	n.s.
	thick	0.04	n.s.	0.06	n.s.	-0.24	n.s.	0.21	n.s.	0.00	n.s.
	mass	-0.03	n.s.	0.10	n.s.	-0.16	n.s.	0.09	n.s.	-0.11	n.s.
	TGM	0.02	n.s.	-0.12	n.s.	-0.16	n.s.	0.23	n.s.	0.14	n.s.
	PGM	0.04	n.s.	0.09	n.s.	0.09	n.s.	-0.03	n.s.	-0.09	n.s.
PGM	length	-0.07	n.s.	0.31	*	0.07	n.s.	-0.12	n.s.	0.33	*
	width	-0.09	n.s.	0.15	n.s.	-0.42	**	-0.21	n.s.	0.43	**
	thick	-0.10	n.s.	-0.17	n.s.	-0.30	*	-0.18	n.s.	0.42	**
	mass	-0.21	n.s.	0.18	n.s.	-0.17	n.s.	-0.09	n.s.	0.35	*
	TGM	-0.50	**	-0.35	*	-0.48	**	-0.36	*	-0.40	**
TG	ELV	0.23	n.s.	-0.63	**			0.14	n.s.	-0.31	*
	GBH	0.02	n.s.	-0.12	n.s.	-0.16	n.s.	0.23	n.s.	0.14	n.s.
	length	0.27	n.s.	-0.41	**	0.02	n.s.	-0.27	n.s.	-0.29	*
	width	0.03	n.s.	-0.27	n.s.	0.39	**	0.02	n.s.	-0.41	**
	thick	0.05	n.s.	-0.20	n.s.	0.31	*	-0.02	n.s.	-0.38	**
	mass	0.24	n.s.	-0.30	*	0.16	n.s.	-0.10	n.s.	-0.43	**
S:RCD	length	-0.17	n.s.	0.37	n.s.	0.06	n.s.	0.48	**	0.62	**
	width	0.12	n.s.	0.55	**	-0.21	n.s.	0.37	**	0.65	**
	thick	0.11	n.s.	0.55	**	-0.21	n.s.	0.36	**	0.62	**
	mass	0.00	n.s.	0.28	n.s.	-0.06	n.s.	0.30	*	0.62	**
	TGM	-0.77	**	-0.61	**	-0.41	**	-0.22	n.s.	-0.13	n.s.
S:HI	length	-0.14	n.s.	0.03	n.s.	0.14	n.s.	0.41	**	0.63	**
	width	0.08	n.s.	0.35	*	-0.23	n.s.	0.38	**	0.57	**
	thick	0.07	n.s.	0.39	**	-0.21	n.s.	0.37	**	0.52	**
	mass	-0.04	n.s.	0.10	n.s.	0.00	n.s.	0.29	*	0.57	**
	TGM	-0.81	**	-0.33	*	-0.35	*	-0.23	n.s.	-0.21	n.s.
RRGRn	length	-0.33	*	-0.02	n.s.	0.06	n.s.	0.33	*	0.28	n.s.
	width	-0.31	*	0.23	n.s.	-0.12	n.s.	0.20	n.s.	0.28	*
	thick	-0.33	*	0.39	**	-0.16	n.s.	0.22	n.s.	0.20	n.s.
	mass	-0.31	*	0.03	n.s.	-0.06	n.s.	0.26	n.s.	0.31	*
	TGM	-0.12	n.s.	-0.13	n.s.	-0.24	n.s.	-0.14	n.s.	-0.09	n.s.
RHGRn	length	0.32	*	-0.18	n.s.	0.14	n.s.	0.09	n.s.	0.09	n.s.
	width	0.06	n.s.	-0.01	n.s.	-0.11	n.s.	0.11	n.s.	0.03	n.s.
	thick	0.09	n.s.	0.06	n.s.	-0.13	n.s.	-0.02	n.s.	0.05	n.s.
	mass	0.34	*	-0.10	n.s.	0.04	n.s.	0.13	n.s.	0.13	n.s.
	TGM	0.54	**	0.14	n.s.	-0.23	n.s.	0.02	n.s.	-0.28	n.s.

Table 7. Plants traits correlation of 5 species studied (continued).

		S. axillaris Trait values		*M. toosendan* Trait values		*G. arborea* Trait values		*P. cerasoides* Trait values		*C. acuminatissima* Trait values	
		r	*P*	*r*	*P*	*r*	*P*	*r*	*P*	*r*	*P*
PSVn	length	-0.15	n.s.	0.34	*	0.06	n.s.	0.15	n.s.	0.09	n.s.
	width	-0.24	n.s.	0.30	*	0.06	n.s.	0.11	n.s.	-0.01	n.s.
	thick	-0.26	n.s.	0.21	n.s.	0.10	n.s.	-0.02	n.s.	0.00	n.s.
	mass	-0.30	n.s.	0.31	*	0.05	n.s.	-0.06	n.s.	-0.02	n.s.
	TGM	0.13	n.s.	-0.41	**	0.07	n.s.	-0.17	n.s.	0.20	n.s.
S:RCDf	length	-0.11	n.s.	0.07	n.s.	0.30	n.s.	0.16	n.s.		
	width	-0.27	n.s.	0.04	n.s.	-0.02	n.s.	0.05	n.s.		
	thick	-0.28	n.s.	0.17	n.s.	0.04	n.s.	0.11	n.s.		
	mass	-0.26	n.s.	0.28	n.s.	0.00	n.s.	0.17	n.s.		
	TGM	-0.32	*	-0.22	n.s.	0.05	n.s.	-0.17	n.s.		
S:HIf	length	-0.20	n.s.	0.28	n.s.	-0.07	n.s.	0.19	n.s.		
	width	-0.21	n.s.	0.09	n.s.	-0.10	n.s.	0.23	n.s.		
	thick	-0.24	n.s.	0.23	n.s.	0.01	n.s.	0.19	n.s.		
	mass	-0.25	n.s.	0.20	n.s.	-0.01	n.s.	0.28	*		
	TGM	-0.33	n.s.	-0.22	n.s.	-0.39	*	-0.04	n.s.		
RRGRf	length	0.27	n.s.	-0.25	n.s.	0.38	*	-0.07	n.s.		
	width	0.06	n.s.	-0.24	n.s.	0.06	n.s.	-0.02	n.s.		
	thick	0.05	n.s.	-0.07	n.s.	0.11	n.s.	-0.07	n.s.		
	mass	0.22	n.s.	0.19	n.s.	0.05	n.s.	0.01	n.s.		
	TGM	0.32	*	0.09	n.s.	0.20	n.s.	-0.01	n.s.		
RHGRf	length	0.29	n.s.	0.23	n.s.	-0.05	n.s.	0.07	n.s.		
	width	0.12	n.s.	0.00	n.s.	0.30	n.s.	0.20	n.s.		
	thick	0.12	n.s.	0.03	n.s.	0.37	*	0.08	n.s.		
	mass	0.25	n.s.	0.19	n.s.	0.19	n.s.	0.14	n.s.		
	TGM	0.46	**	0.07	n.s.	0.19	n.s.	0.12	n.s.		
PSVf	length	0.06	n.s.	0.19	n.s.	0.12	n.s.	-0.14	n.s.		
	width	0.21	n.s.	0.14	n.s.	0.01	n.s.	-0.17	n.s.		
	thick	0.25	n.s.	0.00	n.s.	-0.02	n.s.	-0.10	n.s.		
	mass	0.14	n.s.	0.08	n.s.	-0.08	n.s.	-0.03	n.s.		
	TGM	-0.15	n.s.	-0.47	**	0.16	n.s.	-0.13	n.s.		

* = $p < 0.05$, ** = $p < 0.01$, n.s. = not significant

5.3 DISCUSSION

5.3.1 SEED OR PYRENE SIZE VS PERCENT GERMINATION

Seed size is an important characteristic in the life cycle and evolution of plants, because it affects seed dispersal, persistence in the soil, seedling establishment and fitness (*e. g.* Salisbury, 1942; Harper *et al.,* 1970; Venable and Brown, 1988; Westoby *et al.,* 1992; Thompson *et al.,* 1993; Seiwa and Kikuzawa, 1991). Most studies of intra-specific variation in seed size and seed mass demonstrated the advantages of large seeds over small ones in the early stages of seedling establishment, including germination characteristics (Black, 1956 and 1959; Cideciyan and Malloch, 1982; Harper and Obeid, 1967; Haskins and Gorz, 1975; Nizam and Hossain, 1999; Weis, 1982; Wulff, 1986).

Also, in this study, seed size and wet mass affected percent germination and time to germination. From an ANOVA on seed characteristics between germinated seeds and those that failed to germinate. I found both positive and negative correlated between seed size and percent germination. Germinated seeds of *M. toosendan* and *C. acuminatissima* tended to originated from big seeds, whilst those of *S. axillaris, G. arborea* and *P. cerasoides* tended to originate from small pyrenes. For *S. axillaris*, the result was in disagreement with the findings of Jackson (1987), who reported that big pyrenes germinate better than small ones.

The fruit type of *S. axillaris, G. arborea* and *P. cerasoides* was a drupe with a hard endocarp containing the seeds (pyrene). For this group, smaller pyrenes tended to

germinated better than larger pyrenes. Water may diffuse into the lighter, smaller pyrenes and stimulate seed germination more easily than into the heavier, larger ones. Harper and Benton (1966) noted that as seed size increases, the ratio of seed surface to volume decreases. As a result, large seeds may be unable to obtain adequate water for germination in drier soils which are suitable for the germination of small seeds. Therefore, one guideline for the selection of superior parent trees of species with hard endocarps (pyrene) for efficient nursery production of seedlings would be to choose trees that produced slightly smaller pyrenes. Another approach might be to develop pre-sowing treatments to accelerate transport of water into the pyrene for imbibition by the seeds contained therein. For example Xueying *et al.* (2001) reported high (but unspecified) rates of germination of *S. axillaris* pyrenes after soaking them in hot water. Scarification and acid treatments are also worth investigating.

On the other hand, I found that the germinated seeds of *M. toosendan* and *C. acuminatissima* tended to be the larger and heavier ones. This result is in agreement with those reported by Black (1956 and 1959); Harper and Obeid (1967); Twamley (1967); Austenson and Walton (1970); Anderson (1971); Haskins and Gorz (1975); Schaal (1980); Cideciyan and Malloch (1982); Wies (1982) and Nizam and Hossain (1999). The fruit type of *M. toosendan* was a drupe, containing one pyrene. However, in this study I extracted the seeds from the pyrene and sowed them directly. The seed testa of *M. toosendan* is thin and water can diffuse into the embryo easily. The fruit type of *C. acuminatissima* was a nut. The pericarp of the nut was thin and easily broken so water could diffuse through pericarp and the seed coat to embryo easily. Also, the seed coat of *M. toosendan* and the pericarp of *C. acuminatissima* had effect on water diffusion on to embryo.

In this study, the moisture did not limit germination since I watered the germination trays everyday. The large and heavier seeds had a high water content. Schmidt (2000) stated that very dry seeds sometimes have slower imbibition rates than more moist ones, because water movement in dry tissue tends to be physically restricted. Therefore, larger and heavier seeds of *M. toosendan* and *C. acuminatissima* germinate rapidly than smaller and lighter ones. The guideline for the selection of superior parent trees of *M. toosendan* and *C. acuminatissima* for efficient nursery production of seedlings would be to choose trees that produced larger seeds.

An acceptable germination standard needs to be established and seeds should be collected only from those trees that meet the standard. In this study, I suggest a standard germination percentage of 40 or higher for *S. axillaris* and *G.* arborea; 50 or higher for *M. toosendan* and *P. cerasoides*; and 70 or higher for *C. acuminatissima*.

5.3.2 SEED OR PYRENE SIZE VS TIME TO GERMINATION

Seed or pyrene size and wet mass affected time to germination. The overall, mean time to germination of *S. axillaris* was the longest (97.55 ± 35.06) days, followed by *M. toosendan* (58.47 ± 14.62), *C. acuminatissima* (38.62 ± 8.33), *P. cerasoides* (32.38 ± 6.18) and *G. arborea* (15.51 ± 1.63). Jurado and Westoby (1992) and Westoby *et al.* (1992) found that larger, heavier seeds tended to germinate more rapidly than smaller and lighter seeds. The results of studies on the effects of seed or pyrene size and wet mass on time to germination are mixed. Time to germination increased with decreasing percent germination with the exception of *P. cerasoides*.

Also, there were both negative and positive relationship between seed or pyrene size and time to germination.

Times to germination of *S. axillaris* and *G. arborea* were slightly positively correlated with pyrene size. The relationship was statistically significant for *G. arborea* (pyrene width and thickness), but not statistically significant for *S. axillaris*. In contrast, there was a negative correlation with *M. toosendan* (statistically significant for seed length and wet mass), slightly for *P. cerasoides* (not statistically significant) and *C. acuminatissima* (statistically significant for all).

5.3.3 Seed or pyrene size vs seedling performance in nursery

Black (1956); Harper and Obeid (1967); Twamley (1967); Wies (1982); Howe and Richter (1982); Stanton (1984) and Wulff (1986) also stated that larger seeds produce larger seedlings. In this study, I found that seedling root collar diameter and height increased with increasing seed or pyrene size. The positive relationship between seed size and seedling size (RCD and height) was statistically significant for *M. toosendan, P. cerasoides* and *C. acuminatissima*, but not for *S. axillaris* and *G. arborea* (Table 7). This was probably because larger seeds can store more energy reserves that enhances emergence and initial vertical growth (Seiwa, 2000; Baskin and Baskin, 1998). Seed or pyrene batches, which had a large seed and resulting large seedlings of *P. cerasoides* were *e. g.* seed tree nos. 7, 25, 26 and 37; *e.g.* seed tree nos. 2 and 10 for *M. toosendan*; and *e.g.* seed trees nos. 25, 37 and 38 for *C. acuminatissima*

5.3.4 SEED OR PYRENE SIZE VS SEEDLING RELATIVE GROWTH RATE (RGR)

Seed size and seedling relative growth rate (RGR) together determine the size of a seedling at any given time after germination (Swanborough and Westoby, 1996). Reports on the relationship between seed size and RGR are equivocal. While Atkinson (1973); Grime and Hunt (1975); Fenner (1978); Grime *et al.* (1988); Fenner and Lee (1989); Shipley and Peter (1990); Jurado and Westoby (1992); Maranon and Grubb (1993); Cornelissen *et al.* (1996) and Reich *et al.* (1998) observed a negative relationship between seed size and mean RGR, Stock *et al.* (1990) and Seiwa and Kikuzuwa (1991) found no relationship between the two attributes. In this study, the result was also equivocal. Both negative and positive relationships and non relationship between seed or pyrene size and RGR were found among different species tested.

For *S. axillaris*, there was a negative correlation between pyrene size (length, width, thickness and wet mass) and seedling RRGR, but a positive correlation with seedling RGR of height (only statistically significant for pyrene length and wet mass). Only one species had a negative correlation between seedling RGR and pyrene size. The other species had positive correlations. For *M. toosendan*, only seed thickness was positively correlated with seedling RRGR. *G. arborea* had no relationship between pyrene size and seedling RCD. For *P. cerasoides*, only pyrene thickness was positively correlated with seedling RRGR. For *G. arborea*, there was no relationship between pyrene size and seedling RCD. For *C. acuminatissima*, only seed width and seed wet mass were negatively correlated with seedling RRGR, but there was no relationship with seedling RHGR.

5.3.5 SEED OR PYRENE SIZE VS SEEDLING SURVIVAL IN THE NURSERY

It has been observed that survivorship of seedlings from large seeds may be higher than that of seedlings from small seeds (Howe *et al.*, 1985; Jurado and Westoby, 1992; Allsopp and Stock, 1995; Manga and Yadav, 1995; Baskin and Baskin, 1998). In this study, I also found that seed or pyrene size had an effect on seedling survival in nursery. The results of the one-way ANOVA demonstrated that seed size or pyrene of the surviving seedlings significant differed from those that died in the nursery for *S. axillaris* and *M. toosendan*, but the results was not statistically significant for *G. arborea*, *P. cerasoides* and *C. acuminatissima*.

However, only the results from *M. toosendan* supported the hypothesis that seedlings from larger seeds tended to survive better than those from smaller seeds, *i. e.* seed tree nos. 4, 10 and 17. This result was similar to result of germinated vs. non-germinated seeds. Salisbury (1942) and Baker (1972) argued that larger food reserves might allow seedlings to establish a larger root system, with better chances of surviving. Larger seeds tended to germinate better than smaller seeds. This makes sense that I would chose seed tree that produced the larger seed for seed collection. This was confirmed by the result of the Pearson correlation.

In contrast, surviving seedlings of *S. axillaris* tended to originate from smaller pyrenes. This was confirmed that smaller pyrenes gave a better yield than larger ones, *i.e.* seed tree nos. 3, 16, 23, 27 and 34.

5.3.6 TIME TO GERMINATION VS SEEDLING SURVIVAL IN THE NURSERY

Time to germination is known to have a substantial impact on size hierarchies in plant populations (Ross and Harper, 1972; Howell, 1981). Time to germination is influenced by seed size and light conditions (Simon and Johnston, 2000). It is well known that timing of germination can be controlled by: (1) light quantity or quality; (2) temperature; (3) water; (4) available nutrients, and (5) a combination of these (Bewley and Black, 1994). It affects seedling performance, and also seedling survival. Recent studies have shown that seedlings that germinate and emerge early in the growing season have greater survival over those that emerge later (*e.g.* , Langdon, 1958; Morse and Schmitt, 1985; Shipley and Peters, 1990; Jones *et al.*, 1997).

In this study, it was quite clear that surviving seedlings in the nursery of all the species studied tended to germinate more rapidly than those that died. The time to germination of surviving seedlings was shorter than those that died, a statistically significant result for *S. axillaris, M. toosendan* and *P. cerasoides*. Differences in the time to germination, between surviving seedlings and those that died was not statistically significant for *C. acuminatissima* and *G. arborea*. Although, the time to germination of surviving seedlings was shorter than for those that died.

Several hypotheses have been proposed to explain why time to germination has an effect on seedling survival. The hypotheses include (1) maternal and genetic effects; and (2) environmental effects. Maternal and genetic explanations propose that seeds that are larger inherently more vigorous, or better adapted genotypes, tend to germinate earlier (McDaniel, 1969; Dunlap and Barnett, 1982). The environmental

explanations propose that early germination takes advantage of ephemerally available resources, such as light, water, or nutrients (Jones and Sharitz, 1989).

5.3.7 SAPLING SURVIVAL IN THE FIELD VS SEEDLING SIZE AT PLANTING

Planting suitable sapling sizes may enhance the sapling survival rate in the field. FORRU (2000) suggested that the suitable seedling height for planting in northern Thailand of 50-60 cm tall or 30 cm for fast-growing species. Rose *et al.* (1990) also identified the *Pinus taeda* L. seedlings that 20-25 cm tall and > 4 mm RCD as suitable size for planting. In this study, surviving saplings tended be larger at planting time than those that died. The sapling size (both RCD and height) at planting of surviving saplings was significantly larger than those of that died for all species studied. This was probably because initial large saplings size enhance ability to capture the planting site, against neighboring weeds and secure nutrients and water. Several studies have found a relationship between sapling size and sapling survival, especially RCD. Zaerr and Lavender (1976) found that *Pseudotsuga menziesii* (Mirb.) Franco survival was positively related to fresh weight, which was highly correlated with stem diameter. Shiver *et al.* (1990) found a similar threshold relationship between diameter and survival of loblolly pine. South and Mexal (1984) also found a positive relationship between RCD and survival of newly planted seedling loblolly pine and slash pine.

5.3.8 SAPLING PERFORMANCE IN THE FIELD

Seed characteristics had no significant effect on sapling growth and sapling survival in the field for all species. This lack of relationship was not surprising since by the time saplings are planted in the field, they are entirely independent on food reserves stored in the seed. This result is in agreement with the conclusion of Wulff (1986) and Bonfil (1998).

Seedling growth in the nursery was related with sapling growth in the field. For all study species, I found that seedling size (height and RCD) was positively correlated with those in the field. However, this result was statistically significant only for *S. axillaris* and slightly for *M. toosendan*, but was not for the other species. On the other hand, the seedling height and RCD in the nursery was negatively correlated with RRGR and RHGR in the field, also was statistically significant for *S. axillaris* and *M. toosendan*. This is probably because small saplings had more proportion size between initial size and last size.

5.3.9 MULTIPLE REGRESSION ANALYSIS

The predictors (independent variable) in the multiple regression model of time to germination, germination percentage, seedling survival in the nursery and in the field were equivocal and differed amongst species by species. The multiple regression model was not a good model, because the adjusted R^2 values were quite low. In this study, the results suggested that the multiple regression did not adequately predict percent germination, time to germination, seedling survival in the nursery and in the

field. However, there was a correlation for some of the individual variables which could be used to predict seedling performance in the nursery and also in the field. For example, seed length of *M. toosendan* was negatively correlated with time to germination and time to germination was negatively correlated with percent sapling survival in the field. Therefore the seed length can be used to predict the sapling survival in the field. Surviving saplings of *M. toosendan* in the field tended to originate from bigger seeds.

CHAPTER 6

GENETIC ANALYSIS (USING MICROSATELLITE DNA MARKERS)

6.1 INTRODUCTION

The most important issue which should be addressed by any forest restoration programme is the selection of parental plant material. Genetic variation, particularly adaptive variation in a founding population is critical, especially if restored areas are not within the range of wild pollen sources. The Convention on Biological Biodiversity (Rio de Janeiro, Brazil, 1992) emphasised the importance of maintaining intraspecific genetic diversity and evolutionary potential. The collection of plant material from a few individuals can result in low effective population size, severe inbreeding depression and a decease in the adaptive evolutionary potential of the population (Barrett and Kohn, 1991). Molecular techniques provide a valuable tool for measuring the genetic diversity of trees, thus contributing to decisions to enable better genetic management for forest restoration.

The results presented in chapter 4 were designed to enable the selection of superior parent trees for forest restoration programmes using four standard criteria. Those criteria were based on nursery and field performance. In this chapter, microsatellite DNA markers were used to examine the genetic diversity of seed trees for *C. acuminatissima* and *P. cerasoides*.

Microsatellites, which consist of tandemly reiterated, short DNA sequence motifs are highly informative, PCR-based molecular markers. (Aldrich *et al.*, 1998). Microsatellites are considered by many authors to be neutral DNA markers (*e.g.* Nauta and Weissing, 1996) which should therefore be used with caution in conservation genetics (Hedrick, 2001). Whilst microsatellites have been used to assess genetic variation in tropical tree species in threatened forests and forest fragments, there have been no reports of their use to contribute to the selection of parent seed trees with a broad genetic base from which to collect seed for replanting to restore natural forest.

The generation of a library and sequencing necessary to generate polymorphic microsatellite primers in a 'new' species is likely to be both time consuming and costly. Consequently, for forest restoration programmes where the emphasis lies on practical tree planting, rather than studies of genetic diversity *per se*, amplification using published primers from other species would be most appropriate.

In this study, two simple algorithms were also designed and employed. The first indicates the minimum number of trees in each population which would represent 100% of the available gene pool (based on the microsatellite analysis) when individual genotypes are identified (model I); and the second indicates the likely conservation of allelic diversity when trees are selected 'blind' from a population, assuming that the population has the same relative allele frequencies as in the original data set (model II). This information will contribute to the selection of parent trees for supplying seed to forest restoration projects.

The objectives of this chapter are:

1. to assess genetic diversity of *Prunus cerasoides* and *Castanopsis acuminatissima* within, and between three national parks in the north of Thailand

2. to use the microsatellite data generated to contribute to the design of a seed collection strategy for these species, which conserves genetic variation

6.2 RESULTS

6.2.1 *Prunus cerasoides* D. Don

6.2.1.1 Genetic diversity

Three primer pairs from *P. avium* (PS12A02, PS05CO3, PS09FO8) 2 from *P. cerasus* (PceGA34, PceGA77) but only 1 of the 5 from *P. persica* (UDP98-409) amplified polymorphic loci (Table 8). Five of these primers were used for this study, as PceGA77 was impossible to score due to a complex banding pattern. The remaining 5 loci were believed to be monomorphic, as the banding pattern indicated that a microsatellite sequence was amplified, but this was not confirmed by sequencing. No more than 2 fragments were amplified for each tree/primer pair combination.

Four to 12 alleles were detected at the 5 polymorphic loci, with a total of 41 alleles identified among the 82 trees collected from the 3 sites. The allele frequencies

are presented in Table 9. The average number of alleles per locus per location was 2.667 for PceGA34, 8.0 for PS12A02, 6.667 for PS05CO3, 3.333 for PS09F08 and 5.667 for UDP98-409 (Table 10). The observed heterozygosity (H_O) for each locus ranged from 0.250 to 0.580, and deviated significantly from Hardy-Weinberg expectations for locus PS12A02, UDP98-409, PS09F08 (heterozygote deficiency, P <0.001) and PS05CO3 (heterozygote deficiency, P<0.05). Based on Wright's F_{IS} values (Table 14) the deviations are due to an excess of homozygotes (positive F_{IS} values).

The allelic richness and the mean number of alleles per locus were very similar between the three sites (Table 11). For each locus, the number of unique alleles was 2 for PceGA34, 4 for PS12A02, 4 for PS05CO3, 6 for UDP98-409 and none for PS09F08. In total, 7 of the alleles present in the Doi Inthanon sample were unique, in comparison to 6 in Doi Ang Khang and 3 in Doi Suthep-Pui. The distribution of alleles into allele frequency classes was expressed in two ways. When alleles were classified into 4 classes (Table 13), the distribution of the majority of the alleles in the rare frequency varied little between the 3 sites (79.4% to 86.2%). However, when classified into 2 classes, the majority of the alleles were distributed in the common class (66.7% to 89.7% across the 3 sites). All of the trees had a unique set of alleles, and consequently could be distinguished from each other.

For each site, over all the loci, the expected heterozygosity (H_E) was significantly higher than the observed heterozygosity (H_O) (P<0.05), leading to positive inbreeding coefficients (F_{IS}). For each location, the observed heterozygosity ranged from 0.480 to 0.574, with an average heterozygosity of 0.539 (Table 11),

indicating considerable genetic diversity within the locations. The levels of inbreeding for each population ranged from 0.133 to 0.192, with an average inbreeding coefficient of 0.162 (Table 11), indicating a low level of inbreeding. To assess the degree of population differentiation between the three sites, the infinite alleles theoretical model (F_{ST}) was used. Over the three sites, F-statistic analysis showed positive levels of F_{IS}, F_{IT}, and F_{ST} (0.162, 0.259 and 0.115 respectively) (Table 14). The overall F_{ST} for the three populations was 0.115 (significantly higher than zero, P <0.001), indicating that 11.5% of the variation was attributable to differentiation among the populations.

6.2.1.2 Genetic diversity within Doi Suthep-Pui

Within the 42 trees selected in Doi Suthep-Pui, 19 were believed to be naturally dispersed, due to their size and location, deep in the forest. Consequently, splitting the Doi Suthep-Pui site into two sub-locations allowed a further comparison to be carried out between trees believed to be naturally dispersed with those of unknown dispersal (Table 12). Of the 27 alleles detected in Doi Suthep-Pui, 26 were found in unknown dispersed trees, and only 18 in the naturally dispersed trees, which represents a 31% reduction in allelic richness . The one unique allele found in the naturally dispersed trees (loci UDP98-409) was also unique within the three sites (Tables 9 and 12). The mean number of alleles ranged from 5.2 (unknown dispersal) to 3.6 (naturally dispersed). The observed heterozygosity of the naturally dispersed trees (0.491), was lower than that of the trees of unknown dispersal (0.636). In the trees of unknown dispersal the expected heterozygosity (H_E) was significantly higher

(P<0.001) than the observed heterozygosity (H_O), leading to a positive inbreeding coefficient (F_{IS}).

6.2.1.3 Estimation of the minimal number of trees representing a full set of microsatellite alleles

Model I showed that a minimum of 10 trees would represent all of the alleles in the Doi Inthanon sample, 10 trees would represent all of the alleles in the Doi Suthep-Pui sample and just 7 trees would represent all of the alleles in the Doi Ang Khang sample.

Table 8. Summary of primer pair characteristics

Species	Locus	Repeat motif	Annealing temp (°C)	Size range of products (bp)	No. of alleles
Sour cherry	PceGA34*	$(GA)_{25}$	57.0	148-170	4
	PceGA77*	$(AG)_{13}$	51.0	160-163	3
Sweet cherry	PS12A02**	$(GA)_{22}$	55.0	151-177	11
	PS08E08**	CAA GTT 7	58.0	172	1
	PS05CO3**	$(GA)_{30}$	50.0	108-136	10
	PS09F08***	$(GA)_{17}$	50.0	138-148	4
Peach	UDP98-406[†]	$(AG)_{15}$	50.0	105	1
	UDP98-405[†]	$(AG)_{9}$	55.0	105	1
	UDP96-003[†]	$(CT)_{11}(CA)_{28}$	55.0	87	1
	UDP98-409[†]	$(AG)_{19}$	55.0	150-184	12
	UDP96-018[†]	$(AC)_{21}$	56.4	250	1

* Downey and Iezzoni 2000; ** Sosinski *et al.*, 2000; *** Joobeur *et al.*, 2000; [†] Cipriani *et al.*, 1999

Table 9. Allele frequencies of five microsatellite loci in; Doi Inthanon; Doi Ang

Khang; and in Doi Suthep-Pui as a single location, and when split into two

sub-locations based on dispersal.

		Frequency				
		Location				
					Doi Suthep-Pui	
Locus	Allele (bp)	Doi Inthanon	Doi Ang Khang	Doi Suthep-Pui	unknown dispersal	natural dispersal
PceGA34	148	0.875	0.9	0.622	0.7	0.529
	150	0.075	0	0	0	0
	154	0.025	0.1	0.378	0.3	0.471
	170	0.025	0	0	0	0
UDP98-409	150	0.05	0.125	0.15	0.167	0.132
	154	0.075	0.125	0.013	0.024	0
	156	0.2	0.175	0.575	0.476	0.684
	162	0.05	0.4	0.138	0.167	0.105
	166	0.2	0	0	0	0
	170	0.175	0.075	0.062	0.119	0
	172	0.15	0.025	0.013	0.024	0
	174	0.05	0	0	0	0
	180	0.05	0	0	0	0
	184	0	0.075	0	0	0
	152	0	0	0.013	0.024	0
	160	0	0	0.038	0	0.079
PS05C03	108	0	0	0.013	0.022	0
	110	0	0.075	0	0	0
	116	0.05	0	0.013	0.022	0
	118	0.175	0.3	0.312	0.391	0.206
	120	0.375	0.2	0.2	0.217	0.176
	124	0	0.025	0	0	0
	126	0.225	0.175	0.062	0.109	0
	128	0.125	0.15	0.4	0.239	0.618
	130	0.025	0.075	0	0	0
	136	0.025	0	0	0	0
PS09F08	138	0.775	0.6	0.308	0.348	0.25
	140	0.1	0.25	0.397	0.304	0.531
	142	0.125	0	0.051	0.022	0.094
	148	0	0.15	0.244	0.326	0.125
PS12A02	151	0.5	0	0	0	0
	153	0.05	0.15	0.103	0.13	0.063
	155	0.2	0.05	0.256	0.13	0.438
	157	0	0.125	0.013	0.022	0
	159	0.175	0.125	0.013	0.022	0
	161	0	0.05	0	0	0
	163	0	0.125	0.192	0.304	0.031
	167	0.05	0.075	0.308	0.348	0.25
	171	0	0.075	0	0	0
	177	0.025	0.2	0.115	0.043	0.219
	152	0	0.025	0	0	0

Table 10. Descriptive statistics for the five microsatellite loci studied over all

locations

Locus	Total number alleles	Mean A [†]	H_E	H_O
PceGA34	4	2.667	0.292	0.250
PS12A02	11	8.000	0.689	0.420***
PS05CO3	10	6.667	0.623	0.580*
PS09F08	4	3.333	0.509	0.390***
UDP98-409	12	5.667	0.636	0.490***

[†] Mean A-mean no alleles per locus per population

significant departure from Hardy-Weinberg equilibrium (*P<0.05; ***P<0.001)

Table 11. Measure of microsatellite DNA genetic diversity in the three locations

	Doi Suthep-Pui	Doi Ang Khang	Doi Inthanon	Total
No. of trees	42	20	20	82
Allele number over 5 loci	27	29	29	41
Unique alleles	3	6	7	16
Alleles common to all populations	19	19	19	19
Mean no. of alleles per loci	5.4	5.8	5.8	8.2
H_E	0.662	0.655	0.595	0.697
H_O	0.574*	0.530***	0.480*	0.539***
F_{IS}	0.133	0.192	0.193	0.162

significant departure from Hardy-Weinberg equilibrium (*P<0.05; ***P<0.001)

Table 12. Measure of microsatellite DNA genetic diversity within Doi Suthep-Pui

National Park, when split into two subpopulations; comparing trees

believed to be naturally dispersed with those of unknown dispersal

	Dispersal	
	unknown	natural
No. of trees	23	19
Allele number over 5 loci	26	18
Unique alleles	9	1
Alleles common to both populations	17	17
Mean no. of alleles per loci	5.2	3.6
H_E	0.678	0.589
H_O	0.636	0.491***
F_{IS}	0.061	0.162

significant departure from Hardy-Weinberg equilibrium (***P<0.001)

Table 13. Distribution of 41 alleles in allele frequency classes in three locations

Allele frequency class	Number of alleles (% of total number of alleles)		
	Doi Suthep-Pui	Doi AngKhang	Doi Inthanon
Total no. alleles	27	29	29
Four classes			
High ($P \geq 0.75$)	0	1(0.034)	2(0.069)
Intermediate ($0.75 > P \geq 0.25$)	8 (0.296)	4 (0.138)	2 (0.069)
Low ($0.25 > P \geq 0.01$)	19 (0.704)	24 (0.828)	25 (0.862)
Rare ($P \geq 0.01$)	0	0	0
Two classes			
Common ($P \geq 0.05$)	18 (0.667)	26 (0.897)	24 (0.828)
Rare ($P < 0.05$)	9 (0.333)	3 (0.103)	5 (0.172)

Table 14. F-Statistic analysis estimates of the parameters F_{IS}, F_{IT}, and F_{ST}.

Locus	F_{IS}	F_{IT}	F_{ST}
PceGA34	0.042	0.192	0.156
PS12A02	0.332	0.421	0.133
PS05CO3	0.040	0.086	0.049
PS09F08	0.158	0.274	0.137
UDP98-409	0.163	0.265	0.122
All	0.162	0.259	0.115

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 35 37 38 39 40 41 42 43 44 45 47 48 49 50 52 53 54 55 56 57 58 59 60 61 62 63
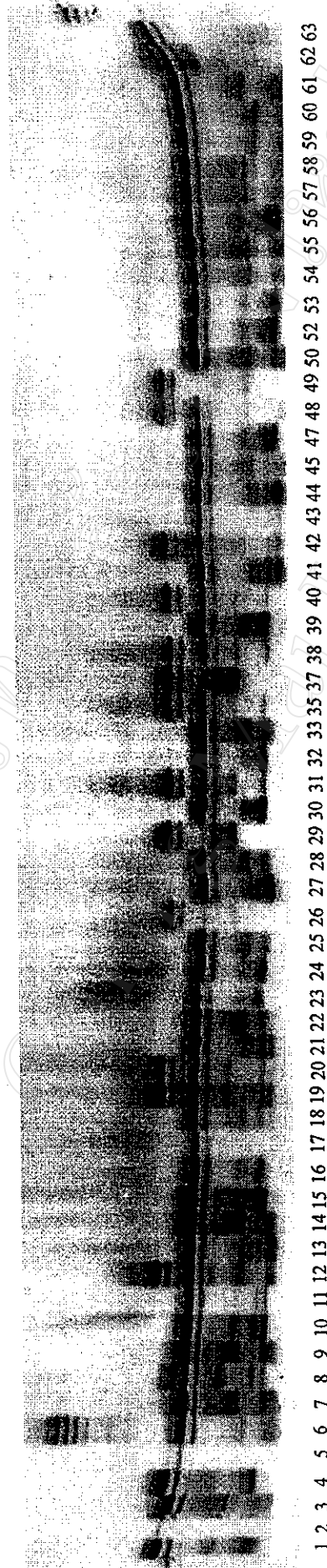
Figure 13. Autoradiogram of a polyacrylamide-gel separation of PCR-amplification products of ^{33}P-labeled PceGA34 primer pairs

from individual trees of *Prunus cerasoides* D. Don .

3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 41 42 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63
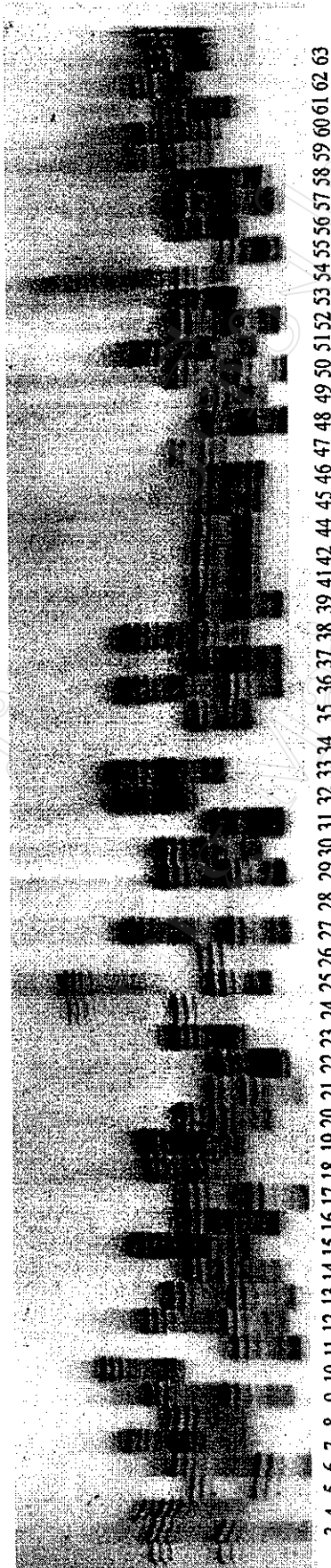
Figure 14. Autoradiogram of a polyacrylamide-gel separation of PCR-amplification products of ^{33}P-labeled UDP98-409 primer pairs

from individual trees of *Prunus cerasoides* D. Don.

6.2.2 Castanopsis acuminatissima (Bl.) A. DC.

6.2.2.1 Genetic diversity

All seven primer pairs from *Castanopsis cuspidata* amplified polymorphic loci, although two, Ccu22F30 and Ccu9T20 were not included in the study due to complex banding patterns. No more than two fragments were amplified for each tree/primer pair combination. The five loci were found to be highly polymorphic, with 6 to 18 alleles detected. A total of 54 alleles were identified among the 72 trees, representing the 3 populations. The allele frequencies are given in Table 16. The average number of alleles per locus per population was 8.0 for Ccu16H15, 4.0 for Ccu17F15, 6.33 for Ccu28H18, 12.67 for Ccu33H25 and 8.33 for Ccu5F45 (Table 17). The observed heterozygosity (H_O) for each locus ranged from 0.286 to 0.750 (Table 17). The observed heterozygosities significantly deviate from Hardy-Weinberg expectations for locus Ccu17F15, Ccu28H18 and Ccu5F45 (heterozygote deficiency, $P<0.001$). Based on Wright's F_{IS} values (Table 18) the deviations are due to an excess of homozygotes (positive F_{IS} values).

The three populations exhibited similar degrees of allelic richness, ranging from 37 alleles in Jae Sawn to 41 alleles in Doi Inthanon and Doi Suthep-Pui (Table 18). The mean number of alleles per locus was also similar between the three populations (Table 18). For each locus, the number of unique alleles was 2 for Ccu16H15, 2 for Ccu17F15, 2 for Ccu28H18, 6 for Ccu33H25 and 2 for Ccu5F45. In total, 6 of the alleles present in the Doi Inthanon population were unique to that population, in comparison to 5 in Doi Suthep-Pui and 3 in Jae Sawn.

The distribution of alleles into frequency classes in the populations were expressed in two ways. When alleles were classified into four classes (Table 19), alleles occurred in just the intermediate or low classes, with 75.7% to 85.4% of alleles in the former. When classified into two classes, the alleles showed similar distributions between the 3 populations, and were almost equally distributed between the common class and the rare class. (Table 19). All of the trees had a unique combination of alleles, and consequently could be distinguished from each other.

For each population, over all the loci, the expected heterozygosity (H_E) was significantly higher ($P<0.01$) than the observed heterozygosity (H_O) leading to positive inbreeding coefficients (F_{IS}). For each population, the observed heterozygosity ranged from 0.483 to 0.537, with an average heterozygosity of 0.519 (Table 18), indicating considerable genetic diversity within the populations. The levels of inbreeding for each population ranged from 0.205 to 0.340, with an average inbreeding coefficient of 0.276 (low level of inbreeding) (Table 18). Over the three populations, the F-statistic analysis showed positive levels of F_{IS}, F_{IT}, and F_{ST} (0.270, 0.280 and 0.006 respectively) (Table 20). The overall F_{ST} for the 3 populations was 0.006 (not significantly greater than zero), indicating that only 0.6% of the variation was attributable to differentiation among the populations. Consequently the data shows that there is no significant genetic differentiation among the populations. This is confirmed by the R_{ST} values, which ranged from -0.033 to 0.026, with the overall value for the 3 populations of -0.018 (Table 20), confirming that there is no significant genetic differentiation among the populations.

6.2.2.2 Estimation of the minimal number of trees representing a full set of

microsatellite alleles

Model I showed that a minimum of 16 trees would represent all of the alleles in the Doi Inthanon sample, 15 trees would represent all of the alleles in the Doi Suthep-Pui sample and 13 trees would represent all of the alleles in the Jae Sawn sample. Model II was employed to predict the number of trees which would capture a given percentage of genetic diversity of a random sample of trees, assuming the whole set of seed trees would describe a true infinite population. Predictive curves plotted for each of the three populations were very similar (Figure 15). Fifteen trees for example, sampled blind would capture at least 60 % of microsatellite genetic diversity, compared with 100% under model I assumptions (Figure 15).

Table 15. Summary of primer pair characteristics

Locus	Repeat motif	Annealing temp (°C)	Size range of products (bp)	No. of alleles
Ccu16H15	(TC)16	60	115-139	10
Ccu17F15	(TC)34	52	89-103	6
Ccu28H18	(CT)26	65	100-136	9
Ccu33H25	(TG)11(TC)15	67	186-236	18
Ccu5F45	(CT)40	45	292-320	11
Ccu22F30	(TC)31	65	*	*
Ccu9T20	(TC)27	45	*	*

*highly polymorphic but impossible to score in routine assays

Table 16. Allele frequencies of five microsatellite loci in three populations

of *Castanopsis acuminatissima*.

| Locus | Allele (bp) | Frequency | | |
| | | Population | | |
		Doi Suthep-Pui	Doi Inthanon	Jae Sawn
Ccu16H15	115	0.10	0.06	0.07
	117	0.20	0.24	0.25
	119	0.02	0.00	0.00
	123	0.46	0.48	0.34
	125	0.08	0.06	0.09
	127	0.00	0.06	0.02
	129	0.10	0.06	0.16
	131	0.02	0.02	0.05
	133	0.00	0.00	0.02
	139	0.02	0.02	0.00
Ccu17F15	89	0.50	0.50	0.45
	91	0.46	0.40	0.50
	93	0.00	0.02	0.00
	99	0.00	0.06	0.02
	101	0.04	0.00	0.02
	103	0.00	0.02	0.00
Ccu5F45	292	0.02	0.02	0.00
	296	0.31	0.22	0.23
	298	0.15	0.35	0.32
	300	0.08	0.04	0.09
	302	0.10	0.13	0.16
	304	0.15	0.13	0.21
	306	0.02	0.00	0.00
	308	0.10	0.02	0.00
	314	0.02	0.00	0.00
	318	0.02	0.04	0.00
	320	0.02	0.04	0.00
Ccu33H25	186	0.08	0.19	0.10
	188	0.62	0.35	0.33
	190	0.04	0.04	0.05
	192	0.06	0.10	0.26
	194	0.02	0.02	0.05
	196	0.00	0.04	0.00
	198	0.02	0.04	0.00
	200	0.02	0.02	0.02
	202	0.06	0.00	0.02
	204	0.02	0.02	0.05
	206	0.02	0.00	0.02
	208	0.00	0.06	0.00
	210	0.00	0.02	0.02
	212	0.00	0.02	0.00
	214	0.00	0.00	0.05
	220	0.02	0.04	0.02
	224	0.02	0.00	0.00
	236	0.00	0.02	0.00

Table 16. Allele frequencies of five microsatellite loci in three

populations of *Castanopsis acuminatissima* (continued).

Locus	Allele (bp)	Frequency		
		Population		
		Doi Suthep-Pui	Doi Inthanon	Jae Sawn
Ccu28H18	100	0.58	0.40	0.48
	102	0.00	0.00	0.02
	108	0.17	0.40	0.25
	110	0.02	0.00	0.02
	112	0.00	0.04	0.09
	120	0.13	0.13	0.02
	122	0.02	0.00	0.02
	124	0.06	0.04	0.09
	136	0.02	0.00	0.00

Table 17. Descriptive statistics for the five microsatellite loci studied over all

populations

Locus	Total number alleles	Mean A†	H_E	H_O
Ccu16H15	10	8.00	0.743	0.750
Ccu17F15	6	4.00	0.566	0.329***
Ccu28H18	9	6.33	0.683	0.286***
Ccu33H25	18	12.67	0.769	0.729
Ccu5F45	11	8.33	0.821	0.493***

†Mean A-mean no alleles per locus per population

significant departure from Hardy-Weinberg equilibrium (*** $P<0.001$)

Table 18. Measure of microsatellite DNA genetic diversity in the three populations

	Doi Suthep-Pui	Jae Sawn	Doi Inthanon	Total
No. of trees	25	22	25	72
Allele number over 5 loci	41	37	41	54
Unique alleles	5	3	6	14
Alleles common to all populations	25	25	25	25
Mean no. of alleles per loci	8.2	7.4	8.2	10.8
H_E	0.675	0.740	0.731	0.716
H_O	0.537**	0.537***	0.483***	0.519***
F_{IS}	0.205	0.274	0.340	0.276

significant departure from Hardy-Weinberg equilibrium (**$P<0.01$; ***$P<0.001$)

Table 19. Distribution of 54 alleles in allele frequency classes in three populations

Allele frequency class	Number of alleles (% of total number of alleles)		
	Doi Suthep-Pui	Jae Sawn	Doi Inthanon
Total no. alleles	41	37	41
Four classes			
High ($P \geq 0.75$)	0	0	0
Intermediate ($0.75 > P \geq 0.25$)	6 (0.146)	9 (0.243)	7 (0.171)
Low ($0.25 > P \geq 0.01$)	35 (0.854)	28 (0.757)	34 (0.829)
Rare ($P \leq 0.01$)	0	0	0
Two classes			
Common ($P \geq 0.05$)	21 (0.512)	19 (0.514)	20 (0.488)
Rare ($P < 0.05$)	20 (0.488)	18 (0.486)	21 (0.512)

Table 20. Estimates of the parameters F_{IS}, F_{IT}, F_{ST} and R_{ST}.

Locus	F_{IS}	F_{IT}	F_{ST}	R_{ST}
Ccu16H15	-0.005	-0.012	-0.006	-0.017
Ccu17F15	0.4278	0.415	-0.022	-0.026
Ccu28H18	0.579	0.583	0.010	-0.033
Ccu33H25	0.028	0.064	0.038	0.002
Ccu5F45	0.401	0.399	-0.003	-0.012
All	0.276	0.280	0.006	-0.018
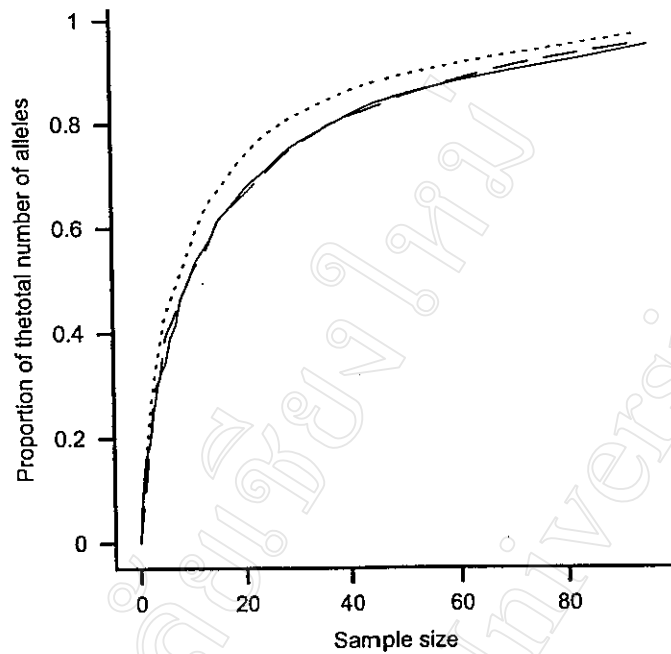
Figure 15. Selection of parent trees from a population with the same relative allele

frequencies as Doi Suthep-Pui (solid line), Jae Sawn (dotted line) and Doi

Inthanon (dashed line), using model II. Expected proportion of the total

number of alleles for sample sizes from 1 to 100 are shown. Each sample

element was drawn with replacement assuming an infinite size population.

Empirical distributions were obtained from 500 samples for each size and

each site. Expected proportions displayed on the figure are 5th percentiles

of these distributions.

Figure 16. Autoradiogram of a polyacrylamide-gel separation of PCR-amplification products of ³³P-labeled Ccu16H15 primer pairs from individual trees of *Castanopsis acuminatissima* (Bl.) A. DC.
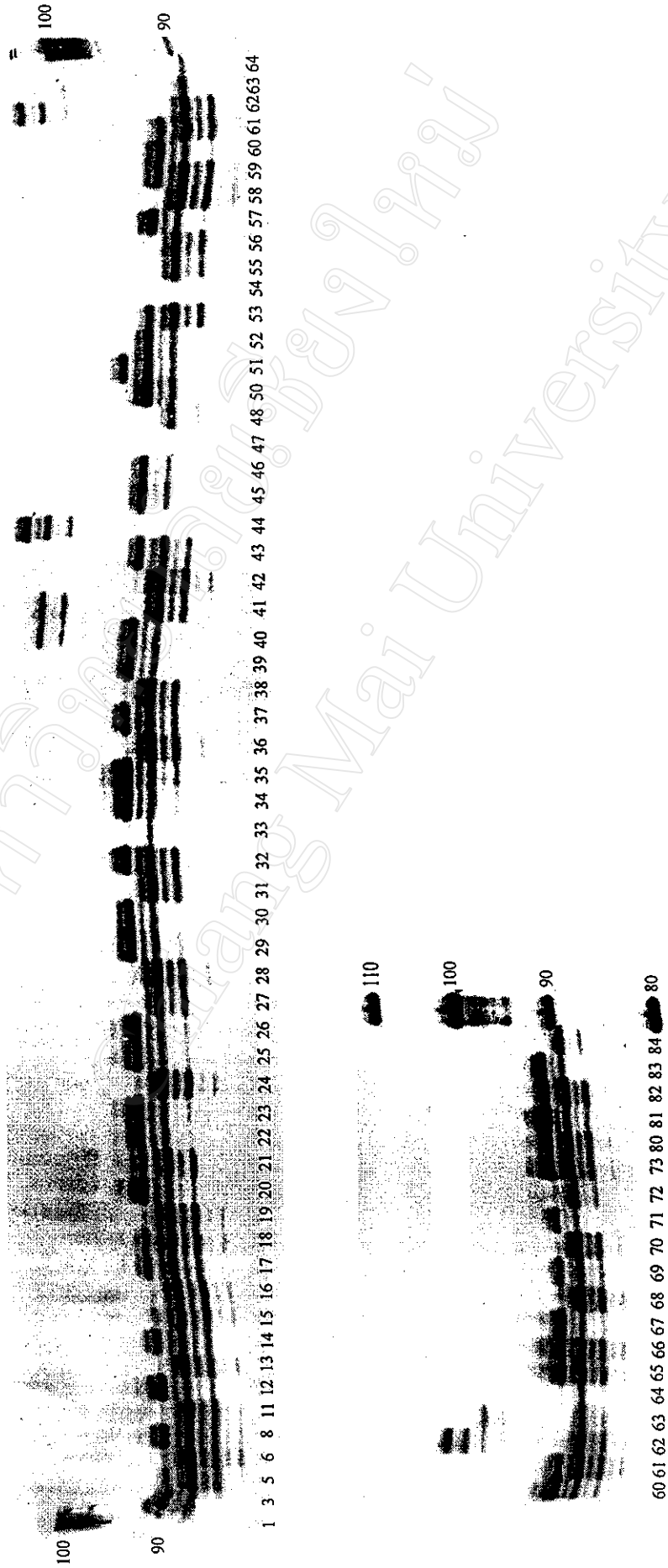
Figure 17. Autoradiogram of a polyacrylamide-gel separation of PCR-amplification products of ^{33}P-labeled Ccu17F15 primer pairs from individual trees of *Castanopsis acuminatissima* (Bl.) A. DC.

Figure 18. Autoradiogram of a polyacrylamide-gel separation of PCR-amplification products of ³³P-labeled Ccu28H18 primer pairs from individual trees of *Castanopsis acuminatissima* (Bl.) A. DC.
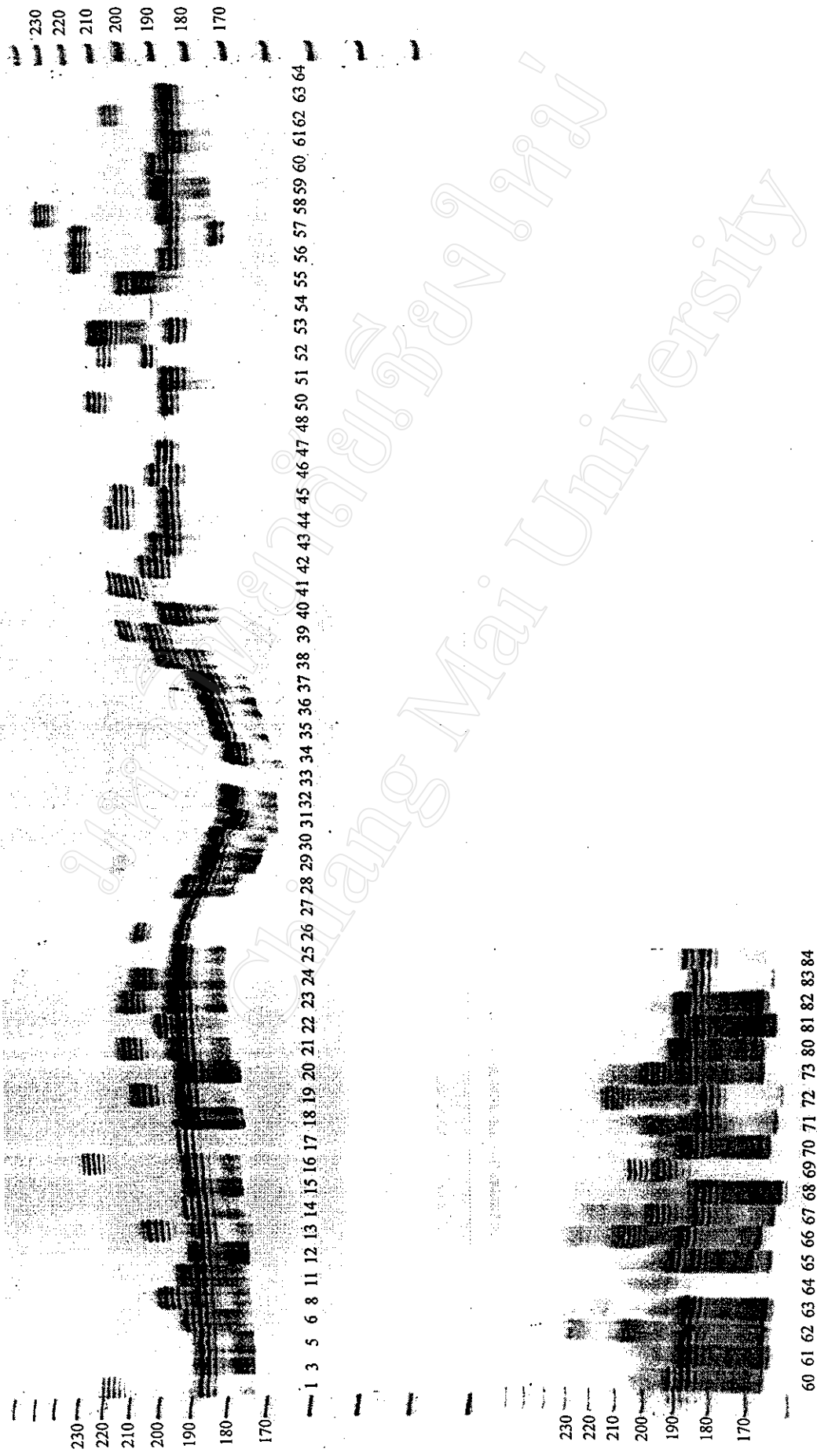
Figure 19. Autoradiogram of a polyacrylamide-gel separation of PCR-amplification products of ^{33}P-labeled Ccu33H25 primer pairs from individual trees of *Castanopsis acuminatissima* (Bl.) A. DC..
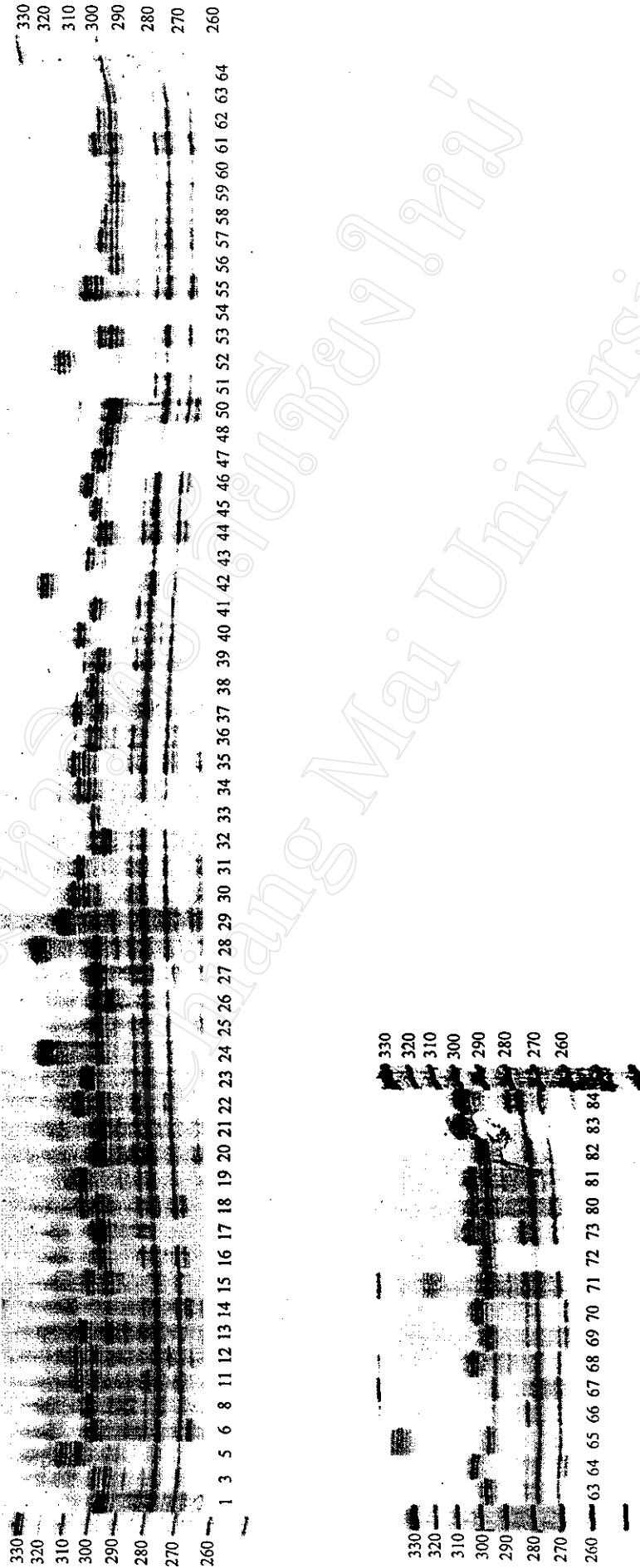
Figure 20. Autoradiogram of a polyacrylamide-gel separation of PCR-amplification products of ^{33}P-labeled Ccu5F45 primer pairs from individual trees of *Castanopsis acuminatissima* (Bl.) A. DC.

6.3 DISCUSSION

6.3.1 GENETIC DIVERSITY

This chapter represents the first report of the use of molecular markers to examine levels of genetic diversity in *Castanopsis acuminatissima* and *Prunus cerasoides*. All seven *C. cuspidata* microsatellite primers (Ueno *et al.*, 2000) showed cross species amplification with C. *acuminatissima* and amplified polymorphic loci. Three primer pairs from *P. avium*, two from *P. cerasus* and one from *P. persica* amplified polymorphic loci with *P. cerasoides*. For practical conservation programmes such as forest restoration, the generation of new microsatellite primers is likely to be prohibitively expensive. This study has demonstrated how useful heterologous amplification using published primers can be in allowing some genetic analysis to be undertaken to support the practical approach. The five microsatellite loci employed in the study of *C. acuminatissima* detected a total of 54 alleles ($n = 72$), and five microsatellite employed for *P. cerasoides* detected 41 alleles ($n=82$). The informativeness of the microsatellite loci in our study varied from 6 to 18 alleles, with an average of 10.8 alleles found over all loci for *C. acuminatissima* and varied from 3 to 12 alleles, with average of 8.2 alleles found over all loci for *P. cerasoides*. Ueno *et al.* (2000) detected a total of 70 alleles in *C. cuspidata* with these five primers, with an average of 14.0 alleles found over the five loci, and 13.9 over seven loci ($n = 32$). For both *Castanopsis* species, this is relatively high, and accords with the mean number of alleles per locus in other tropical tree species, for example 8.75 alleles found over four loci in *Neobalanocarpus heimii* (King) Ashton (Dipterocarpaceae) ($n = 30$) (Konuma *et al.*, 2000), 9.7 alleles found over four loci in *Swietenia humilis*

Zucc. (Meliaceae) ($n = 88$) (White and Powell, 1997). For *C. acuminatissima,* the longest repeat, Ccu5F45, a $(CT)_{40}$ repeat detected 11 alleles, although the more informative Ccu33H25, a $(TG)_{11}(TC)_{15}$ repeat is much smaller. This was similar to that found in *P. cerasoides,* where the longest repeat, PS05CO3, a $(GA)_{30}$ repeat detected 10 alleles, although the more informative UDP98-409, a $(AG)_{19}$ repeat detected 12 alleles. There did not appear to be a direct relationship between allele number and the average number of repeats, which contrasts with Weber (1990).

The range of heterozygosity is quite broad for both species, which suggests that overall diversity values, as a measure of observed heterozygosities, are dependant on which loci are selected. There were significant deviations from Hardy-Weinberg expectations at 3 out of 5 loci for *C. acuminatissima* and 4 out of 5 loci for *P. cerasoides.* With *Swietenia humilis,* White and Powell (1997) also reported that some individual loci exhibited significant deviations from Hardy-Weinberg expectations, which were due to an excess of homozygotes. No significant deviations from Hardy-Weinberg expectations were found for the mean observed heterozygosities in the three populations however. The range of heterozygosity was broad for both *C. acuminatissima* and *P. cerasoides.* The levels of heterozygosity (H_O) detected in the present study for each population, over all loci indicate that each had a high level of genetic variation. Based on Wright's *F*-values, the populations appeared to be outbreeding, with some inbreeding for both *C. acuminatissima* and *P. cerasoides.* However, if there was a considerable amount of inbreeding, it might be expected to be evident across all of the loci, which was not the case here. It is possible that the deviations from Hardy-Weinberg and the homozygote excesses which we observed with three loci for *C. acuminatissima* and four loci for *P. cerasoides* is due to the

presence of null alleles. These are microsatellite alleles which occasionally yield no amplification product due to a point mutation arising in the primer sites (Weber and May 1989; Pemberton *et al.*, 1995).

The value of F_{ST} over the three populations of both *C. acuminatissima* and *P. cerasoides* was 0.006 and 0.115 respectively, indicating that the vast majority of genetic diversity was contained within the populations of each species, and that there was relatively little differentiation between the three populations. However, The F_{ST} value of *P. cerasoides* is significantly higher than zero, so it would be safer to consider the three national parks as genetically distinct, local populations. This accords with Hamrick and Godt (1990) who generalised that in outcrossing species, the majority of genetic diversity resides within populations, rather than between populations (F_{ST}), as would be the case with a selfing species. In other members of the Fagaceae, Dane *et al.* (1999), using allozymes reported high levels of heterozygosities within populations of *Castanea pumila* (L.) Mill. var. *ozarkensis* (Ashe), but no significant differences in genetic diversity among populations. These authors also found that approximately 10% of genetic diversity in *Castanea dentata* (Marsh.) resided among populations, based on 18 isoenzyme loci (Huang *et al.*, 1998).

6.3.2 SEED COLLECTION STRATEGIES

Seed collection is a crucial element of any forest restoration programme. Aside from the considerations of nursery and field performance (adaptive traits), there is always a risk of narrowing the genetic base of a given species. This is particularly pertinent in sites which are isolated from good forest. General guidelines for seed

collection which consider the capture of biodiversity have been published in a number of texts (*e.g.* Brown and Marshall, 1995; Guarino, 1995; Schmidt, 2000). For provenance or progeny trials, it has been recommended that 10- 20 individuals are sampled, which may be increased to 25-50 individuals per population for *ex situ* conservation purposes (FAO Forest Resources Division, 1995). Brown and Marshall (1995) suggest a baseline figure of 50 individuals, although this may be modified depending on the aims of the sampling strategy. However, there is no clear advice to help seed collectors select seed trees from tracts of intact forest for use in small scale forest restoration programmes for biodiversity conservation. I have established that a relatively simple study of genetic diversity, performed with microsatellites does enable a more informed selection of seed trees in our practical forest restoration programmes. In this study, I have shown that the overall F_{ST} for *C. acuminatissima* is very low, which indicates that the samples from all three parks are essentially part of the same population, and hence there appears to have been no restrictions on gene flow over the distances covered in this study. This is encouraging, since it means that our reforestation programme on Doi Suthep should be extending the range of an existing population rather than establishing a new one. However, because there has been substantial forest fragmentation in recent years, it is possible that gene flow between the three parks has been cut off, although there has not been enough time for this to show up. Nevertheless, gene flow over small distances within Doi Suthep-Pui National Park is likely to be unrestricted.

I have shown that whilst a sample of 13-16 *C. acuminatissima* trees can represent the full set of microsatellite alleles detected in a given population (calculated using Model I), in a normal 'blind' collection it is more likely to represent

approximately 60% of microsatellite allele diversity (Model II). Furthermore, there is evidence for a large amount of overall/general genetic diversity in *C. acuminatissima* because of the high number of rare and low-frequency microsatellite alleles, which gives some reflection of the whole genome. It would be rather unexpected if this was accompanied by a low diversity in growth parameters. Therefore the higher recommendations made in the past with respect to seed collection, such as up to 50 parent seed trees (Brown and Marshall 1995) would seem to be a suitable number, especially if the seedlings are to be planted in isolation from a natural population. This number of trees would also represent in excess of 80% of the microsatellite allele diversity calculated using Model II. One cautionary note here is that relatively low sample sizes of 22-25 *C. acuminatissima* trees were collected from relatively large areas of forest, and many of the alleles found were present in very low frequencies. Ideally, a larger sample of trees would give a more accurate measure of allele frequencies. Furthermore, Model I could be used in the future to select actual individual parent trees, given a larger sample number and hence more accurate estimates of rare allele frequencies.

For the *P. cerasoides*, there is significant differentiation amongst the three populations, indicating that for this species, seed should be collected locally, and not transferred between the National parks. Hence the recommendation for *P. cerasoides* based on my genetic data alone is that seed is also collected from up to 50 seed trees, and bulked for the purposes of forest restoration. However, for small scale forest restoration activities, this scale of seed collection may not always be feasible; model II gives some indication of the implications on genetic diversity of collecting from fewer parent trees. It is also important to consider the molecular data alongside the

study of seed progeny performance in the field (adaptive traits). This will be discussed in more detail in Chapter 7.

Genetic diversity is the basis for the ability of any organism to adapt to environmental changes through natural selection. Consequently it is important that, in any conservation programme, some consideration is given to the conservation of 'adaptive genetic diversity'. Microsatellites are considered by many authors to be neutral DNA markers (*e.g.* Nauta and Weissing, 1996). Neutral variation has been defined as a balance predicted by a reduction in variation from genetic drift and an increase in variation from mutation. Although most recent conservation genetics research has focussed on neutral molecular markers, for example the identification of evolutionary significant units (ESUs) or management units (MUs), their application must be viewed with some caution (Hedrick, 2001). The pattern of neutral genetic variation has been used to guide the amount of adaptive variation (Hedrick, 2001), as in the case of the present study. However, some authors have stated that neutral genetic markers such as microsatellites are not useful for measuring adaptive genetic diversity. In contrast, other authors have suggested that microsatellites can provide a considerable amount of quantitative genetic variation that might be important for Darwinian selection, based on mutations that are frequent, site specific and reversible (Kashi *et al.,* 1997; Kashi and Soller, 1999; King and Soller, 1999). Recent evidence presented in a study of *Triticum dicoccoides* (Fahima *et al.,* 2002) supports the hypothesis that microsatellite polymorphisms are at least partly adaptive and are determined by natural diversifying selection.

Other ways of interpreting my data would be to consider allele frequency, rather than attempting to maximise genetic variation as discussed above. It has been suggested that a sample of individuals should include at least one copy of 95% of the alleles which occur at frequencies greater than 0.05 (Marshall and Brown, 1975). However, this does not take into account rare alleles. Krusche and Geburek (1991) commented that to maintain the genetic multiplicity of forest trees, and hence their adaptability, allele losses must be minimised. In order to capture rare alleles satisfactorily, it has been suggested that a sampling target in the order of 20 copies should be set to avoid potential problems of inbreeding (Namkoong *et al.*, 1988; Yanchuk, 2001). Such a strategy would guard against the future loss of rare alleles, and present them in a variety of genetic backgrounds. However, it would also require in the order of several thousand individuals, which is clearly beyond the scope of a forest restoration programme such as our own. A further strategy might be to obtain a sample which accurately reflects the frequencies of alleles in the original source population. We have not calculated this for the data reported here, but Brown and Marshall (1995) report that a sample of size in the order of 200 individuals would be required. Again, this is beyond the scope of a forest restoration programme such as our own Brown (1978) stated that the aim of sampling for genetic conservation should be to obtain as much genetic variation as possible, and it is this ethos that we have followed.

The genetic information reported in this study will clearly influence the selection of *C. acuminatissima* and *P. cerasoides* seed trees for practical forest restoration in these three locations. My work has only sampled the mother trees, and future work will consider the contribution of the male parent to allelic richness

through analysis of seed. Furthermore, molecular studies should ideally be carried out in conjunction with morphological and phenotypic studies (Brown, 1978). Seed from many of the parent trees sampled in this study has been trialled in the nursery and in field plots (Chapter 4), and is discussed in the context of the genetic data in Chapter 7.

CHAPTER 7

CONCLUSIONS

7.1 INTRODUCTION

This study aimed to develop criteria to select superior seed trees of 5 framework species for forest restoration programmes, based on nursery performance, field performance and genetic diversity estimated using microsatellite markers. Seed traits, including seed size, seed mass, dormancy, germination and dispersal, are central components of plant life histories and have pronounced and multiple effects on fitness (Jansen, 1969; Harper, 1977). The choice of seed trees may have profound effects on the efficiency of nursery operations and the ability to achieve production targets (Blakesley *et al.*, 2000). Ideal characteristics of seed trees include high percent seed germination, high seedling survival and rapid growth both in the nursery and in the field and high genetic diversity.

7.2 CONCLUSIONS

This study arose from the observations that the performance of planted trees germinated from seeds of different individual parent trees is variable and from the supposition that characteristics of parent trees or seeds or seedling performance in the nursery might be used to predict the performance of planted trees in the field.

The results of this study demonstrated that seed or pyrene sizes, germination characteristics, seedling performance in the nursery and in the field varied both among and within seed trees for all species studied. Significant correlations were found between seedling performance in the field and seed or pyrene sizes, germination characteristics and seedling performance in the nursery. However, these correlations were equivocal. The lack of strong relationships was probably due to complex interactions among many factors including (1) characteristics of seed trees such as elevation, age (indicated by GBH), habitat, soil moisture, soil nutrients, temperature and genotype; (2) variability in nursery practices, which could not be strictly controlled, *i. e.* potting, standing down, boundary effects, pests and diseases and (3) variability in planting practices and the characteristics of planting sites, *e.g.* soil moisture, soil nutrients, temperature, slope, weed competition, flooding and pests and diseases. All these factors are sources of uncontrolled variability that produce "noise" in the data that can mask subtle relationships.

Most *G. arborea* and *P. cerasoides* seedlings died because there was a lot of rain and flooding after planting, especially on steep slopes. Some seedlings were planted under the canopy of trees, which grew naturally on the plots. Shade increased seedling mortality, because it seemed to encourage damping-off diseases and pests.

Planting practices influenced sapling performance in the field, including seedling transportation and planting methods. The shoots and stems were sometimes damaged during transportation. Planting damaged saplings would have enhanced sapling mortality and reduced growth rate.

After planting, considerable management effort is essential to maximize performance of planted trees. Planting site management included weed control, fertilizer application and fire prevention. Weeding is essential, because newly planted saplings cannot compete with vigorous weed growth and are therefore unlikely to survive longer than 2 years (FORRU, 2000). Fire is the most common cause of failure of forest restoration projects. Fire prevention is necessary during the dry season. Fertilizer application is also necessary on sites that have been extensively cultivated or subjected to soil erosion.

In this study, fire did not occur in the 2000 and 2001 plots and fertilizer application was controlled. Therefore, weeding was the main factor that influenced sapling performance in the field. Some saplings died, after their shoots and stems were slashed and their roots were damaged during weeding. Weeding also affected mean relative growth rate (RHGR) of individual seed trees. Some saplings that had their stems or shoots cut had a low or negative RHGR. Therefore, mean RHGR's of saplings from those seed trees were low. In this case, sapling performance of those seed trees was more influenced by man (weeding), than by the parent tree. Such factors are more likely to have had an effect on sapling performance in the field than seed or pyrene size, germination characteristics and seedling performance in the nursery. Therefore, the lack of a relationship between seed or pyrene size, germination characteristics and seedling performance in the nursery with sapling performance in the field was not surprising. The hypothesis that the characteristics of parent trees or seed or seedling performance in the nursery can be used to predict the performance of planted trees was therefore rejected. This was confirmed by the results

of multivariate analyses. Although multiple regression showed that some independent variables accounted for some of the variability in the dependent variables (those used to select superior parent trees), overall the models failed to substantially explain trends in seed germination and seedling performance. Therefore these models were not good indicators of superior parent trees (Chapter 5, section 5.2.8).

The lack of relationships between parent tree, seed characteristics, germination characteristics and seedling performance in the nursery and in the field shown by this study was not surprising. It has been suggested that such relationships are rare, since many factors control and affect seedling performance in the nursery and in the field, and this was supported by this study.

Despite these sources of variability, some strong correlations which could be used to select superior parent trees, or be used by a nursery manager to enhance seedling performance in the nursery. However, the correlation varied amongst the species tested.

Spondias axillaris

Low percent germination percentage is likely to limit nursery production of *S. axillaris* seedlings. The overall mean percent germination was only 42%. To maximize percent germination, smaller pyrenes could be selected, since pyrenes that germinated were significantly smaller than those that failed to germinate. Also, surviving seedlings in the nursery tended to originate from smaller pyrenes and those that germinated rapidly. Therefore, one guideline for selection of superior parent trees

for efficient nursery production of seedlings would be to choose trees that produced slightly smaller pyrenes. Seedlings, which germinate rapidly (less than 84 day after sowing) should be pricked out, and non-germinated pyrenes could be discarded at this time. The mean height of saplings that died in the field was 54 cm with a mean RCD of 2.9 mm; to maximize percent survival in the field saplings which are larger than 54 cm should be planted since larger saplings had a higher survival rate.

Melia toosendan

Production of seedlings of this species in the nursery was more problematic. The overall mean germination percentage was only 50% so clearly treatments need to be devised to increase germination. Furthermore, seedling survival in the nursery was only 43%. The main cause of mortality was susceptibility to damping-off diseases. To maximize percent germination larger seeds might be sown, since the results showed that mean seed size and wet mass of germinating seeds was greater than those that failed to germinate. The germination percent of seed batches from trees at higher elevations was higher than for seed batches from lower elevations. Therefore, seeds should be collected from trees from higher elevations to enhance germination. However, it may also be important to ensure that seeds are collected from an altitude similar to that of the planting sites, to ensure that progeny are genetically suited to the environmental conditions of planting site. Most seedling mortality occured during the two week hardening–off period. To protect seedlings from damping-off diseases, seedlings could be kept in the nursery under a roof, because spores of damping-off diseases can be dispersed by rain-splash. Larger seeds also produced healthier seedlings. There was strong positive correlation between seed size and seedling size

in the nursery. It can be concluded that big seeds of *M. toosendan* yielded better results in the nursery than smaller ones, so selecting parent trees that produce large seeds is advisable.

Sapling survival in the field of this species was very low, possibly because the saplings were weak, due to the effects of damping-off disease. However, the results from the one-way ANOVA showed that saplings that survived in the field over the first growing season tended to be larger at the time of planting than those that died. I therefore recommend that saplings less than 43 cm tall with RCD less than 2.9 mm at planting should not be planted. Two guidelines for selecting superior parent trees of *M. toosendan* would be to choose trees that produced slightly larger seed and situated at higher elevations.

Gmelina arborea

The overall germination percentage average over all pyrene batches was only 20%. To improve germination percentage, pyrene should be collected as soon as possible after they fall on the ground. Smaller pyrenes should be selected, since germinating pyrenes were a significantly smaller than those that failed to germinate. Pyrenes collected from the northern part of Doi Suthep-Pui (DS2) had a higher germination percentage than those from the southern part of Doi Suthep-Pui (DS1). Relationships between pyrene characteristics and seedling performance in the nursery with seedling performance in the field of *G. arborea* were not clear. This was probably because field performance was affected by many factors, *e.g.* planting practices and planting site condition. However, saplings surviving after 1 growing

season in the field tended to be larger at the time of planting than those that died, especially for sapling RCD. The results suggest that saplings less than 5.9 mm in RCD and less than 70 cm in height should not be planted. Therefore, a guideline for selection of superior parent trees for efficient nursery production of seedlings would be to choose trees that produce small pyrenes and seed trees from DS2. The over-growth of *G. arborea* seedlings also presented a problem with seedling production, despite top pruning. Storing pyrenes and sowing them in December (six months later than in this study) might solve this problem.

Prunus cerasoides

The overall germination percentage averaged over all batches was fairly high (55%). To maximize germination percentage, it is suggested to sow smaller pyrenes, because germinating pyrenes were significantly smaller than those that failed to germinate. In addition, seedling survival rates in the nursery and in the field were very good. Seedlings that survived in the nursery and in the field tended to originate form pyrenes that germinated more rapidly, compared with seedlings that died in the nursery. Therefore, smaller pyrenes yielded better results than larger ones. *P. cerasoides* also had a problem with respect to production scheduling. Pyrenes were collected in March and sown in April, shortly before the planting time. Seedlings were not ready for planting by mid June. This means that seedlings would stay in nursery for about 14 months, requiring pruning and risking the seedlings becoming root bound in their containers. This problem might be solved by storing pyrenes and sowing them nearer to the dispatch date or by sowing pyrenes in a sterile (zero nutrient) mix and delaying potting.

Sapling performance in the field did not show any correlations with pyrene size, germination characteristics or seedling performance in the nursery. However, saplings that survived in the field over the first growing season tended to be larger at planting time than those that died. Therefore, a guideline for the selection of superior parent trees of *P. cerasoides* for efficient nursery seedlings production would be to choose trees that produce slightly smaller pyrenes.

Castanopsis acuminatissima

C. acuminatissima had a very high germination percentage and seedling survival in the nursery. However, seedlings grew slowly, and were therefore not ready for planting by the start of the first rainy season after seed collection. Larger and heavier seeds yielded better results than smaller, lighter ones. Seeds that successfully germinated were significantly larger than those that failed to germinate. In addition, bigger seeds produced larger seedlings. Therefore, a guideline for the selection of superior parent trees of *C. acuminatissima* for efficient nursery seedlings production would be to choose trees that produced larger seeds.

7.3 GENETIC DIVERSITY

The third objective of this study was to investigate genetic diversity of seed tree populations both within and between populations of *Prunus cerasoides* and *Castanopsis acuminatissima*

The genetic diversity of *P. cerasoides* and *C. acuminatissima* seed trees were examined using microsatellite markers. All seven *C. cuspidata* microsatellite primers (Ueno *et al.*, 2000) showed cross species amplification with *C. acuminatissima* and amplified polymorphic loci. Three primer pairs from *P. avium,* two from *P. cerasus* and one from *P. persica* amplified polymorphic loci with *P. cerasoides*. The results suggested that the vast majority of genetic diversity was contained within the populations, with relatively little differentiation among populations for both *C. acuminatissima* and *P. cerasoides*.

The overall F_{ST} for *C. acuminatissima* was very low, which indicated that samples from Doi Suthep-Pui national park, Doi Inthanon national park and Jae sawn national park were essentially part of the same population, and hence there appears to have been no restrictions in gene flow over the distances covered in this study.

The overall F_{ST} for *P. cerasoides* was slightly higher than zero, indicated that the vast majority of genetic diversity was contained within the populations, and significant differentiation amongst the three populations, so it would be safer to consider the three national parks as genetically distinct, local populations.

7.4 SELECTION OF SUPERIOR PARENT TREES BASED ON SEEDLING PERFORMANCE IN THE NURSERY AND IN THE FIELD, AND ON GENETIC VARIABILITY

The fourth objective of this study was to combine results from other parts of the study to develop recommendations for seed collection strategies that maximize the performance of planted trees, whilst maintaining genetic diversity.

Genetic diversity is the basis for the ability of a forest tree, or indeed any organism to adapt to environmental changes through natural selection. Consequently it is important that, in a forest restoration programme such as ours, some consideration is given to the conservation of 'adaptive genetic diversity'. Furthermore, it is also important that when seedlings are planted in degraded areas, they not only survive, but establish well and capture the site as quickly and efficiently as possible. This will not only indicate that the seedlings are 'well adapted' to the site, but also reduce future management input, which would result from trees performing poorly, or trees which died, both of which might require replancing.

The performance of the seed progeny in the field and nursery can be used with molecular data to provide some guidance towards establishing a seed collection strategy for a given species. The consequence of this will be that the practical procedure for identifying superior parent trees for forest restoration programs of framework species should be more robust.

In the case of *P. cerasoides*, the first conclusion is that seedling performance studies clearly identified a set of 21 trees, which were best adapted to the planting site

in Doi Suthep, and therefore by definition, were best suited to capturing that site. These comprised seed tree numbers: 1, 4, 5, 10, 11, 15, 19, 21, 22, 23, 24, 25, 26, 27, 30, 31, 40, 42, 43, 44 and 50.

The second conclusion, which comes from the molecular studies, is that the F_{ST} value indicates that the majority of genetic diversity was contained within the populations, and that there was relatively little differentiation between the three populations. However, the F_{ST} value is significantly higher than zero, so it would be safer to consider the three national parks as genetically distinct, local populations. Furthermore, the data indicates a large amount of overall/general genetic diversity in *P. cerasoides* because of the high number of rare and low-frequency microsatellite alleles, which gives some reflection of the whole genome. Consequently, the molecular data instructs us that seed should be collected from as many trees as possible, certainly more than the 'handful' which are currently used. All the 21 trees listed above could be incorporated into future seed collection exercises, but the seed should be used locally, and not transferred between locations. Consequently, for Doi Suthep, more parent seed trees will need to be screened to assess the performance of their progeny in the field plots, as only 7 of my 21 superior trees were located in this National Park.

The third conclusion which could be drawn from the data is contencious, and relates to the use of neutral genetic markers such as microsatellites for 'predicting' adaptive genetic diversity. Several authors have suggested that microsatellites can provide a considerable amount of quantitative genetic variation that might be important for Darwinian selection, based on mutations that are frequent, site specific

and reversible (Kashi *et al.*, 1997; Kashi and Soller, 1999; King and Soller, 1999). Based on this premise, I have provisionally applied model I to the *P. cerasoides* populations, and have identified a set of trees which would capture all of the allelic diversity identified in each population. This model could be adapted in the future to capture commoner alleles, if it were decided that it was not necessary to attempt to capture the rarest alleles (see chapter 6). Because of the relatively low sample number of trees, and the rarity of the alleles, it might be necessary to evaluate a larger sample of trees from a given site before finally applying model I to capture the allelic diversity identified. However, taking the trees of 'unknown' dispersal in Doi Suthep as an example, it is possible to show how model I can be used, in combination with the field data to select trees.

Twenty three trees comprised this collection, all were studied in the nursery, and were investigated using microsatellites. Of these, 7 were identified as 'superior' trees, based on their field and nursery performance; tree nos. 30, 31, 40, 42, 43, 44 and 50. These trees captured 19 alleles, which represents 70% of the allelic diversity of this population. A further 5 trees collected in Doi Suthep met the first 2 criteria (tree nos. 35, 37, 39, 46 and 48), and narrowly missed selection on either the 3rd or 4th criteria. If these were included in the 'superior' category, then the representation of alleles would be increased to 22, or 81% of allelic diversity in a collection of 12 'superior' seed trees in Doi Suthep.

In the case of *C. acuminatissima*, it is not possible to conclude which trees should provide seed material, because no data is yet available from field trials.

However, a preliminary selection based on nursery data only would comprise tree nos.: 3, 15, 20, 22, 25, 26, 35, 37, 38, 39, 40, 41, 44, 47, 48, 50 and 81.

The molecular studies revealed a very low F_{ST} value, which shows that the vast majority of genetic diversity was contained within the populations, and that there was little differentiation between the three populations. Furthermore, the data indicates a large amount of overall/general genetic diversity in *C. acuminatissima* because of the high number of rare and low-frequency microsatellite alleles, which gives some reflection of the whole genome. Consequently, this molecular data also instructs us that seed should be collected from as many trees as possible. All the 17 trees listed above might be incorporated into future seed collection exercises, depending on the outcome of field perfomance trials. When field data is available, then this will be analysed alongside the molecular data, to make final recommendations on the seed collection stragey for this species.

7.5 FURTHER RESEARCH

1. The method and timing of seed collection have a significant effect on seed germination. Experiments should be carried to determine the percent seed germination of seed batches collected from various fruit stages (tree, ground, unripe, fully ripe, fermented fruits). Germination experiments should be repeated for seed trees, which had low germination percentage.

2. The flowering and fruiting phenology of all seed trees should be studied over a long period (3 years) to develop a better understanding of seasonal and yearly

fluctuations in fruit production and to develop a seed collection protocol for the 5 species studied.

3. The lack of relationships between seedling performance in the nursery and in the field may have been due to the other factors (*e. g.* nursery and planting practices and planting site conditions), than those of the parent trees. Further experimentation to control those other factors is recommended. The factors causing seedling mortality in the nursery and in the field should also be determined.

4. Variability within seed batches is due to variable paternity. In this study, genetic diversity of mother trees was examined, but the genetic diversity of each seed from the same seed trees was not examined. Each seed might have a different father tree. It would be beneficial to continue the study of the gene flow mechanisms, characterising the mating systems for *P. cerasoides* and *C. acuminatissima*. Trees established in the restoration plots should also be examined, to determine whether gene flow is taking place between these sites, and adjacent forested areas, either through pollen, or seed dispersal.

5. More research is needed to examine the genetic diversity of *S. axillaris, M. toosendan, G. arborea* and the other framework species.

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APPENDIX I: SUMMARY OF CHARACTERISTICS OF SEED TREES OF THE 5 SPECIES STUDIED.

Table 21. Elevation, GBH and pyrene characteristics of 41 seed trees of *Spondias axillaris* Roxb.

tree no	collection date	sowing date	elevation (m)	GBH (cm)	pyrene length (mm)	pyrene width (mm)	pyrene thickness (mm)	pyrene wet mess (g)
1	27-Sep-99	6-Oct-99	1100		19.1±1.3	13.8±1.1	13.1±1.1	2.92±0.46
2	27-Sep-99	6-Oct-99	1100	144	18.9±1.1	14.4±0.8	14.0±0.8	3.02±0.41
3	27-Sep-99	6-Oct-99	1100	250	17.5±1.1	14.9±0.9	14.4±0.9	3.23±0.41
4	27-Sep-99	6-Oct-99	1175	111	19.7±1.7	13.6±0.6	13.3±0.7	3.11±0.38
5	27-Sep-99	6-Oct-99	1150	141	18.4±1.1	14.5±0.7	14.1±0.6	3.30±0.32
6	27-Sep-99	6-Oct-99	1150	98	19.4±1.2	15.0±0.9	14.6±1.0	3.40±0.49
7	27-Sep-99	6-Oct-99	1150	161	18.0±1.3	14.9±1.1	14.4±1.0	3.28±0.55
8	27-Sep-99	6-Oct-99	1175	250	18.6±1.1	15.2±0.8	14.9±0.8	3.40±0.34
9	27-Sep-99	6-Oct-99	1200	138	19.3±1.0	16.2±0.9	15.6±0.8	3.93±0.49
10	27-Sep-99	6-Oct-99	1275	115	18.2±1.3	15.3±0.9	14.7±1.0	3.27±0.53
11	27-Sep-99	6-Oct-99	1300	260	18.2±0.9	13.3±0.9	12.9±0.8	2.67±0.27
12	27-Sep-99	6-Oct-99	1100	124	17.1±1.1	14.0±0.9	13.5±0.9	2.95±0.42
13	28-Sep-99	6-Oct-99	925		17.6±1.1	13.4±0.9	13.1±0.9	2.83±0.41
14	28-Sep-99	6-Oct-99	1025	111	17.1±1.3	13.3±0.6	13.0±0.6	2.64±0.28
15	28-Sep-99	6-Oct-99	1050	220	17.7±1.0	12.9±0.8	12.6±0.7	2.68±0.28
16	28-Sep-99	6-Oct-99	1525	99	17.0±1.0	13.9±0.7	13.7±0.6	2.79±0.30
17	28-Sep-99	6-Oct-99	1525	107	19.9±1.3	15.4±1.3	15.0±1.3	3.69±0.56
18	28-Sep-99	6-Oct-99	1525	104	19.8±1.9	13.9±0.8	13.4±0.8	3.01±0.47
19	28-Sep-99	6-Oct-99	1500	236	20.0±1.5	15.6±0.8	15.1±0.7	3.80±0.42
20	28-Sep-99	6-Oct-99	1500	190	18.9±1.3	14.5±1.0	14.1±1.0	3.16±0.50
21	28-Sep-99	6-Oct-99	1450	240	18.1±1.0	16.1±0.9	15.5±1.0	3.79±0.47
22	28-Sep-99	6-Oct-99	1375	120	20.2±1.2	15.5±1.2	15.2±1.2	3.90±0.55
23	28-Sep-99	6-Oct-99	1075		17.0±1.1	12.7±0.9	12.4±0.8	2.23±0.27
24	29-Sep-99	6-Oct-99	1060	119	18.2±1.3	14.1±1.0	13.9±1.1	3.04±0.49
25	29-Sep-99	6-Oct-99	975	215	19.4±1.3	14.6±0.9	14.2±0.8	3.33±0.46
26	29-Sep-99	6-Oct-99	925	160	18.0±1.1	14.2±0.7	13.9±0.6	2.96±0.34
27	29-Sep-99	6-Oct-99			16.7±1.2	13.4±0.7	13.1±0.7	2.71±0.33
28	29-Sep-99	6-Oct-99			19.2±1.1	14.3±0.8	14.1±0.8	2.97±0.39
29	29-Sep-99	6-Oct-99			20.0±1.1	13.2±0.5	12.9±0.5	2.93±0.27
30	29-Sep-99	6-Oct-99	1025	284	18.6±1.1	14.0±0.6	13.8±0.6	3.00±0.31
31	29-Sep-99	6-Oct-99	1025	165	16.8±1.0	14.7±0.7	14.3±0.8	3.13±0.39
32	1-Oct-99	6-Oct-99	1400	90	19.2±1.2	16.1±1.0	15.6±1.0	3.82±0.53
33	2-Oct-99	6-Oct-99	1350	98	20.2±1.2	13.2±0.6	13.0±0.8	2.84±0.30
34	22-Oct-99	25-Oct-99	1350	162	17.5±1.2	14.9±1.0	14.3±1.0	2.59±0.40
35	22-Oct-99	25-Oct-99	1100	105	19.4±1.6	13.1±0.7	12.9±0.7	2.57±0.32
36	2-Nov-99	3-Nov-99	1300	98	19.5±0.9	14.8±0.7	14.4±0.7	3.53±0.38
37	2-Nov-99	3-Nov-99	1300	170	17.7±1.0	12.8±0.7	12.4±0.7	2.68±0.30
38	2-Nov-99	3-Nov-99	1300	107	19.1±1.4	15.5±1.1	15.1±1.1	3.83±0.65
39	2-Nov-99	10-Nov-99	1275	212	19.3±1.1	15.3±0.9	14.8±0.8	3.31±0.41
40	9-Nov-99	10-Nov-99	1200	147	16.6±1.1	14.7±0.9	14.4±0.9	2.83±0.41
41	9-Nov-99	10-Nov-99	1200	108	19.8±1.5	14.0±0.9	13.6±0.9	3.15±0.46
MEAN					18.6	14.4	14.0	3.1
SD					1.1	1.0	0.9	0.4

Table 22. Location, collection dates, elevation, GBH and seed characteristics of 50 seed trees of *Melia toosendan* Sieb. & Zucc.

tree no	location	collection date	sowing date	elevation (m)	GBH (cm)	seed length (mm)	seed width (mm)	seed thickness (mm)	seed wet mass (g)
1	DS1	22-Oct-99	29-Nov-99	1025	206	11.3±0.9	3.7±0.3	2.6±0.2	0.81±0.01
2	DS1	22-Oct-99	29-Nov-99	1025	189	11.3±0.9	4.0±0.2	3.0±0.4	0.74±0.08
3	DS1	22-Oct-99	29-Nov-99	1100	170	10.2±0.9	3.4±0.3	2.5±0.2	0.79±0.02
4	DS1	22-Oct-99	29-Nov-99	1100	159	12.0±0.8	3.6±0.3	2.5±0.2	0.82±0.01
5	DS1	22-Oct-99	29-Nov-99	1125	110	12.1±0.7	3.7±0.3	2.7±0.3	0.82±0.04
6	DS1	22-Oct-99	29-Nov-99	1125	177	11.3±0.7	4.1±0.3	2.7±0.2	0.81±0.01
7	DS1	22-Oct-99	29-Nov-99	1175	150	11.0±0.8	3.9±0.3	2.8±0.2	0.83±0.02
8	DS1	22-Oct-99	29-Nov-99	1275	116	10.8±1.1	3.7±0.3	2.7±0.3	0.81±0.02
9	DS1	22-Oct-99	29-Nov-99	1100	97	10.6±0.6	3.8±0.3	2.7±0.3	0.81±0.01
10	DS1	22-Oct-99	29-Nov-99	1025	212	11.6±0.5	4.1±0.2	2.9±0.3	0.84±0.02
11	DS1	1-Nov-99	29-Nov-99	1250	130	10.1±0.7	3.8±0.3	2.9±0.3	0.81±0.02
12	DS1	22-Oct-99	29-Nov-99			10.4±0.8	3.1±0.2	2.3±0.2	0.79±0.01
13	DS1	22-Oct-99	29-Nov-99	1025	236	10.5±0.7	3.7±0.3	2.7±0.3	0.81±0.02
14	DS1	1-Nov-99	29-Nov-99	1125	205	11.6±0.7	3.8±0.3	2.7±0.2	0.75±0.08
15	DS1	1-Nov-99	29-Nov-99	1275	140	11.1±0.8	3.7±0.3	2.7±0.2	0.81±0.01
16	DS1	1-Nov-99	29-Nov-99	1300	124	10.3±0.7	3.7±0.2	2.7±0.3	0.80±0.01
17	DS1	2-Nov-99	29-Nov-99	1450	200	11.6±0.9	3.9±0.4	2.5±0.3	0.79±0.02
18	DS1	2-Nov-99	29-Nov-99	1300	260	11.0±0.8	3.7±0.3	2.9±0.3	0.81±0.02
19	DS1	2-Nov-99	29-Nov-99	1250	125	9.9±0.6	3.4±0.2	2.7±0.2	0.78±0.02
20	DS1	2-Nov-99	29-Nov-99	1200	228	11.0±0.9	4.0±0.3	2.9±0.3	0.83±0.02
21	DS1	2-Nov-99	29-Nov-99	1150	230	11.2±0.7	4.0±0.3	2.9±0.3	0.82±0.02
22	DS1	2-Nov-99	29-Nov-99	1150	150	10.4±0.7	3.9±0.3	2.9±0.2	0.83±0.02
23	DS1	9-Nov-99	29-Nov-99	1200	186	10.6±0.7	3.6±0.3	2.7±0.3	0.81±0.02
24	DS2	11-Nov-99	1-Dec-99	600	173	10.2±0.8	3.4±0.2	2.4±0.2	0.80±0.02
25	DS2	11-Nov-99	1-Dec-99	600	192	10.4±0.6	3.7±0.2	2.7±0.3	0.80±0.02
26	DS2	11-Nov-99	1-Dec-99	600	116	9.9±0.6	3.8±0.3	2.9±0.3	0.80±0.01
27	DS2	11-Nov-99	1-Dec-99	600	230	9.9±0.7	3.8±0.2	2.9±0.4	0.80±0.02
28	DS2	11-Nov-99	1-Dec-99	650	102	10.0±0.7	3.5±0.3	2.6±0.2	0.79±0.02
29	DS2	11-Nov-99	1-Dec-99	650	290	9.5±0.8	3.3±0.2	2.5±0.2	0.81±0.01
30	DS2	11-Nov-99	1-Dec-99	575	123	9.9±0.9	3.6±0.3	2.6±0.2	0.79±0.03
31	DS2	11-Nov-99	1-Dec-99	775	134	9.6±0.7	3.4±0.2	2.5±0.2	0.79±0.02
32	DS2	11-Nov-99	1-Dec-99	775	140	10.5±0.6	3.6±0.2	2.9±0.3	0.80±0.02
33	DS2	11-Nov-99	1-Dec-99	800	115	9.8±0.7	3.8±0.4	2.8±0.2	0.79±0.02
34	DS2	11-Nov-99	1-Dec-99	800	142	9.9±0.6	3.8±0.3	2.6±0.3	0.81±0.02
35	DS2	11-Nov-99	1-Dec-99	975	110	11.3±0.6	3.7±0.2	2.8±0.2	0.82±0.01
36	DS2	11-Nov-99	1-Dec-99	1025	165	10.4±1.0	3.5±0.3	2.5±0.3	0.80±0.02
37	DS1	16-Nov-99	1-Dec-99	1000	121	9.9±0.7	3.6±0.3	2.7±0.3	0.79±0.02
38	DS2	16-Nov-99	1-Dec-99	900	160	10.3±0.8	3.8±0.3	2.6±0.3	0.81±0.02
39	DS2	16-Nov-99	1-Dec-99	925	260	10.8±0.8	3.9±0.3	2.7±0.2	0.82±0.02
40	DS2	16-Nov-99	1-Dec-99	850	113	10.3±0.7	3.5±0.3	2.6±0.2	0.80±0.02
41	DS2	16-Nov-99	1-Dec-99	775	200	11.0±0.6	3.9±0.3	2.7±0.2	0.82±0.02
42	DS2	16-Nov-99	1-Dec-99	825	98	9.9±0.6	3.4±0.2	2.7±0.3	0.80±0.02
43	DS2	16-Nov-99	1-Dec-99	925	108	10.3±0.5	3.7±0.2	2.8±0.2	0.80±0.01
44	DS2	16-Nov-99	1-Dec-99	925	135	10.4±0.7	3.4±0.2	2.5±0.2	0.81±0.01
45	DS2	16-Nov-99	1-Dec-99	1000	110	9.6±0.5	3.5±0.3	2.5±0.2	0.80±0.01
46	DS2	16-Nov-99	1-Dec-99	980	143	10.4±1.1	3.7±0.3	2.7±0.3	0.75±0.05
47	DS2	16-Nov-99	1-Dec-99	1000	230	10.4±0.6	3.6±0.3	2.7±0.2	0.81±0.02
48	DS2	16-Nov-99	1-Dec-99	980	295	10.2±0.6	3.4±0.3	2.5±0.2	0.80±0.02
49	DS2	16-Nov-99	1-Dec-99	1000	180	10.0±0.5	3.6±0.2	2.8±0.3	0.79±0.03
50	DS1	15-Dec-99	17-Dec-99	1100	104	11.4±0.7	3.8±0.3	2.5±0.2	0.83±0.02
MEAN						10.6	3.7	2.7	0.80
SD						0.7	0.2	0.2	0.02

Table 23. Location, collection dates, elevation, GBH and pyrene characteristics of 49 seed trees of *Gmelina arborea* Roxb.

tree no	location	collection date	sowing date	GBH (cm)	pyrene length (cm)	pyrene width (cm)	pyrene thickness (cm)	pyrene wet mass (g)
1	DS1	11-Apr-00	20-Apr-00		15.0±1.0	8.9±0.5	8.2±0.7	1.23±0.10
2	DS1	11-Apr-00	20-Apr-00		16.2±1.0	8.8±0.6	7.9±0.5	1.30±0.10
3	DS1	11-Apr-00	20-Apr-00	235	14.7±1.0	9.1±0.7	8.3±0.7	1.12±0.11
4	DS1	11-Apr-00	20-Apr-00	220	14.7±1.1	7.5±0.5	6.9±0.6	1.09±0.06
5	DS1	11-Apr-00	20-Apr-00	260	16.4±1.0	9.1±0.6	7.6±0.6	1.15±0.09
6	DS1	11-Apr-00	20-Apr-00	235	14.7±1.3	8.7±0.7	7.8±0.7	1.21±0.10
7	DS1	11-Apr-00	20-Apr-00	170	15.2±1.0	10.0±0.6	8.8±0.8	1.28±0.12
8	DS1	11-Apr-00	20-Apr-00	85	15.8±1.2	9.1±0.6	8.1±0.8	1.29±0.11
9	DS1	11-Apr-00	20-Apr-00	106	16.0±1.0	8.5±0.5	7.4±0.5	1.27±0.09
10	DS1	11-Apr-00	20-Apr-00	260	16.1±1.3	8.7±0.5	7.9±0.6	1.29±0.09
12	DS1	11-Apr-00	20-Apr-00		16.5±1.3	8.7±0.6	7.6±0.6	1.26±0.11
13	DS1	11-Apr-00	20-Apr-00		15.9±1.2	9.7±0.7	9.1±0.7	1.38±0.15
14	DS1	11-Apr-00	20-Apr-00		17.4±2.0	9.2±0.7	8.2±0.7	1.36±0.13
15	DS1	11-Apr-00	20-Apr-00	110	15.9±1.3	8.5±0.7	7.5±0.6	1.25±0.11
16	DS1	11-Apr-00	20-Apr-00	130	15.1±1.0	9.8±0.7	9.1±0.8	1.42±0.14
17	DS1	11-Apr-00	20-Apr-00	120	18.1±1.5	9.4±0.8	8.7±0.8	1.24±0.14
18	DS1	11-Apr-00	20-Apr-00	120	14.8±0.9	9.5±0.7	8.5±0.7	1.38±0.11
19	DS1	11-Apr-00	20-Apr-00	108	14.2±1.0	8.2±0.6	7.3±0.6	1.05±0.09
20	DS1	12-Apr-00	20-Apr-00	112	14.9±0.9	8.6±0.6	7.4±0.6	1.22±0.08
21	DS1	12-Apr-00	20-Apr-00	115	15.7±0.8	9.4±0.5	8.7±0.6	1.35±0.09
22	DS1	12-Apr-00	20-Apr-00	106	14.5±1.2	8.1±0.5	7.6±0.5	1.08±0.07
23	DS1	12-Apr-00	20-Apr-00	103	14.2±0.9	9.1±0.6	8.2±0.6	1.31±0.10
24	DS1	12-Apr-00	20-Apr-00	185	15.2±1.1	8.9±0.6	7.8±0.5	1.22±0.09
25	DS1	12-Apr-00	20-Apr-00	210	16.1±1.2	8.6±0.7	7.5±0.6	1.21±0.10
26	DS1	12-Apr-00	20-Apr-00	160	15.2±1.2	10.3±0.6	9.4±0.6	1.27±0.14
27	DS1	12-Apr-00	20-Apr-00	132	16.1±1.4	9.3±0.7	8.3±0.8	1.14±0.12
28	DS2	14-Apr-00	20-Apr-00	165	16.9±1.0	7.7±0.5	7.1±0.5	1.19±0.08
29	DS2	14-Apr-00	20-Apr-00	400	14.6±0.9	7.5±0.5	6.7±0.5	1.10±0.06
30	DS2	14-Apr-00	20-Apr-00	130	16.4±1.2	8.7±0.7	8.2±0.8	1.33±0.12
31	DS2	14-Apr-00	20-Apr-00	130	16.5±1.2	9.7±0.8	8.4±0.6	1.26±0.12
32	DS2	14-Apr-00	20-Apr-00	260	15.4±1.1	9.2±0.6	8.2±0.6	1.33±0.10
33	DS2	14-Apr-00	20-Apr-00	220	16.4±1.0	8.2±0.4	7.5±0.5	1.23±0.08
34	DS2	14-Apr-00	20-Apr-00	180	16.0±1.0	8.7±0.7	7.5±0.6	1.23±0.10
35	DS2	14-Apr-00	20-Apr-00	175	17.9±1.2	8.2±0.6	7.7±0.6	1.26±0.09
36	DS2	14-Apr-00	20-Apr-00	300	16.6±1.2	8.7±0.6	7.8±0.6	1.27±0.10
37	DS2	14-Apr-00	20-Apr-00	155	15.8±1.2	8.1±0.6	7.1±0.6	1.09±0.08
38	DS2	14-Apr-00	20-Apr-00	150	15.8±0.9	8.8±0.6	7.9±0.5	1.27±0.09
39	DS2	14-Apr-00	20-Apr-00	335	14.5±0.8	8.8±0.5	8.3±0.8	1.23±0.07
40	DS2	14-Apr-00	20-Apr-00	205	14.7±1.1	8.1±0.6	7.3±0.5	1.15±0.07
41	DS2	14-Apr-00	20-Apr-00	100	16.5±0.9	8.8±0.5	7.6±0.4	1.29±0.07
42	DS2	14-Apr-00	20-Apr-00	120	16.3±1.3	8.4±0.6	7.9±0.6	1.23±0.09
43	DS2	14-Apr-00	20-Apr-00	95	18.4±1.4	9.6±0.7	8.8±0.7	1.46±0.14
44	DS2	14-Apr-00	20-Apr-00	105	14.3±1.1	8.3±0.6	7.6±0.6	1.19±0.09
45	DS2	14-Apr-00	20-Apr-00	80	14.6±1.2	8.8±0.9	7.6±0.7	1.12±0.12
46	DS2	14-Apr-00	20-Apr-00	220	17.6±1.3	9.0±0.7	8.0±0.5	1.35±0.11
47	DS2	14-Apr-00	20-Apr-00	200	15.9±1.3	9.2±0.6	8.2±0.7	1.32±0.12
48	DS2	14-Apr-00	20-Apr-00	125	13.9±1.0	9.0±0.7	8.1±0.7	1.12±0.11
49	DS2	14-Apr-00	20-Apr-00	145	14.4±1.0	8.1±0.5	7.1±0.5	1.02±0.07
50	DS2	14-Apr-00	20-Apr-00	117	17.3±1.3	9.9±0.8	9.3±0.8	1.38±0.13
MEAN					15.7	8.8	8.0	1.24
SD					1.1	0.6	0.6	0.10

Table 24. Location, collection date, elevation, GBH and pyrene characteristics of 50 seed trees of *Prunus cerasoides* D. Don.

tree no	location	collection date	sowing date	elevation (m)	GBH (cm)	pyrene length (mm)	pyrene width (mm)	pyrene thickness (mm)	pyrene wet mass (g)
1	DI	19-Mar-00	5-Apr-00	1300	72	10.2±0.4	7.3±0.3	6.1±0.3	0.93±0.08
2	DI	19-Mar-00	5-Apr-00	1300	80	9.2±0.5	7.1±0.4	5.6±0.4	0.93±0.04
3	DI	19-Mar-00	5-Apr-00	1300	62	10.8±0.4	8.2±0.4	6.6±0.2	1.07±0.03
4	DI	19-Mar-00	5-Apr-00	1300	88	10.6±0.4	9.1±0.4	7.4±0.2	1.14±0.03
5	DI	19-Mar-00	5-Apr-00	1300	130	10.8±0.4	8.3±0.3	6.5±0.3	1.06±0.04
6	DI	19-Mar-00	5-Apr-00	1540	35	11.4±0.7	8.0±0.7	6.1±0.4	1.02±0.06
7	DI	19-Mar-00	5-Apr-00	1500	70	11.8±1.0	8.6±0.7	6.8±0.5	1.09±0.07
8	DI	19-Mar-00	5-Apr-00	1420	50	10.3±0.6	7.0±0.3	5.5±0.4	0.93±0.03
9	DI	19-Mar-00	5-Apr-00	1480	41	10.4±0.4	8.3±0.4	6.5±0.4	1.05±0.04
10	DI	19-Mar-00	5-Apr-00	1300	70	11.0±0.5	7.5±0.4	6.0±0.3	1.00±0.04
11	DI	19-Mar-00	5-Apr-00	1300	90	9.8±0.4	7.9±0.3	6.3±0.3	0.99±0.03
12	DI	19-Mar-00	5-Apr-00	1300	45	10.6±0.5	7.9±0.4	6.1±0.3	1.02±0.03
13	DI	19-Mar-00	5-Apr-00	800	25	9.8±0.3	7.4±0.3	6.3±0.3	0.96±0.03
14	DAK	26-Mar-00	5-Apr-00	800	56	10.3±0.3	8.0±0.3	6.7±0.3	1.13±0.03
15	DAK	26-Mar-00	5-Apr-00	800	37	10.2±0.6	7.2±0.3	6.1±0.3	0.99±0.03
16	DAK	26-Mar-00	5-Apr-00	900	41	9.9±0.5	7.1±0.3	5.7±0.3	0.97±0.03
17	DAK	26-Mar-00	5-Apr-00	1680	28	10.5±0.4	7.6±0.4	6.2±0.3	0.99±0.03
18	DAK	26-Mar-00	5-Apr-00	1600	46	10.9±0.5	7.3±0.2	5.6±0.4	0.92±0.06
19	DAK	26-Mar-00	5-Apr-00	1500	61	9.6±0.5	6.9±0.4	5.4±0.2	0.93±0.02
20	DAK	26-Mar-00	5-Apr-00	1480	46	10.6±0.5	7.7±0.4	6.0±0.3	1.05±0.05
21	DAK	26-Mar-00	5-Apr-00	1450	32	10.5±0.4	7.0±0.2	5.6±0.3	0.96±0.02
22	DAK	26-Mar-00	5-Apr-00	1380	131	12.0±0.4	8.1±0.4	6.8±0.3	1.13±0.04
23	DAK	26-Mar-00	5-Apr-00	1540	50	10.7±0.4	7.2±0.2	5.7±0.2	1.01±0.03
24	DAK	26-Mar-00	5-Apr-00	1540	43	9.7±0.3	7.2±0.3	5.9±0.3	0.95±0.03
25	DAK	26-Mar-00	5-Apr-00	1500	86	11.4±0.4	7.7±0.3	6.2±0.3	1.05±0.03
26	DAK	26-Mar-00	5-Apr-00	1480	77	12.9±0.6	8.5±0.5	6.8±0.3	1.17±0.05
27	DAK	26-Mar-00	5-Apr-00	1000	51	10.8±0.5	7.4±0.3	6.1±0.3	0.99±0.03
28	DS	28-Mar-00	5-Apr-00	1100	78	9.9±0.5	7.1±0.3	6.1±0.2	0.94±0.04
29	DS	28-Mar-00	5-Apr-00	1200	64	11.4±0.5	7.3±0.3	6.2±0.2	0.95±0.04
30	DS	28-Mar-00	5-Apr-00	1200	42	10.6±0.5	7.2±0.3	5.9±0.3	0.95±0.04
31	DS	28-Mar-00	5-Apr-00	1200	60	9.8±0.4	7.0±0.3	5.4±0.2	0.96±0.03
32	DS	28-Mar-00	5-Apr-00	1400	118	9.9±0.4	7.0±0.5	5.8±0.4	0.94±0.04
33	DS	28-Mar-00	5-Apr-00	1400	165	11.2±0.6	7.3±0.3	5.8±0.3	0.96±0.06
34	DS	28-Mar-00	5-Apr-00	1440	130	10.8±0.5	7.4±0.4	6.2±0.2	0.98±0.03
35	DS	28-Mar-00	5-Apr-00	1420	140	9.7±0.5	7.3±0.3	6.1±0.3	0.99±0.02
36	DS	28-Mar-00	5-Apr-00	1420	130	10.3±0.3	7.1±0.2	5.9±0.3	0.97±0.03
37	DS	29-Mar-00	5-Apr-00	1420	185	11.9±0.6	8.4±0.4	6.7±0.3	1.08±0.04
38	DS	29-Mar-00	5-Apr-00	1400	160	9.9±0.4	7.0±0.3	5.8±0.3	0.95±0.04
39	DS	29-Mar-00	5-Apr-00	1420	127	9.1±0.3	7.0±0.2	6.1±0.2	0.83±0.02
40	DS	29-Mar-00	5-Apr-00	1480	90	9.7±0.4	7.2±0.4	5.9±0.4	0.87±0.03
41	DS	29-Mar-00	5-Apr-00	1420	72	11.3±0.4	8.1±0.3	6.2±0.2	0.89±0.03
42	DS	29-Mar-00	5-Apr-00	1420	69	10.4±0.3	7.4±0.3	5.8±0.3	0.84±0.03
43	DS	29-Mar-00	5-Apr-00	1400	64	10.5±0.5	7.6±0.2	6.0±0.3	0.85±0.02
44	DS	29-Mar-00	5-Apr-00	1400	60	10.4±0.4	7.7±0.3	6.2±0.2	0.91±0.02
45	DS	29-Mar-00	5-Apr-00	1400	51	9.7±0.3	6.9±0.3	5.6±0.2	0.85±0.02
46	DS	30-Mar-00	5-Apr-00	1600	90	9.9±0.3	7.7±0.3	6.3±0.2	0.91±0.03
47	DS	30-Mar-00	5-Apr-00	1460	67	10.4±0.4	7.5±0.4	6.3±0.2	0.90±0.03
48	DS	30-Mar-00	5-Apr-00	1460	105	9.6±0.4	7.5±0.3	6.0±0.3	1.04±0.03
49	DS	30-Mar-00	5-Apr-00	1500	82	10.4±0.4	7.6±0.3	6.1±0.3	0.90±0.04
50	DS	30-Mar-00	5-Apr-00	1500	105	10.4±0.5	8.3±0.5	6.7±0.3	0.92±0.04
MEAN						10.5	7.6	6.1	0.98
SD						0.8	0.5	0.4	0.08

Table 25. Location, collection date, elevation, GBH and seed characteristics of 50
seed trees of *Castanopsis acuminatissima* (Bl.) A. DC.

tree no	location	collection date	sowing date	elevation (m)	GBH (cm)	seed length (mm)	seed width (mm)	seed thickness (mm)	seed wet mass (g)
1	DS1	19-Sep-00	3-Oct-00	1000	245	9.5±0.6	8.4±0.6	7.9±0.5	1.17±0.08
3	DS1	19-Sep-00	3-Oct-00	980	150	9.7±0.6	9.2±0.6	8.4±0.6	1.26±0.08
5	DS1	19-Sep-00	3-Oct-00	1000	165	8.9±0.8	7.3±0.7	7.0±0.6	0.92±0.07
6	DS1	19-Sep-00	3-Oct-00	1020	170	10.0±0.5	9.1±0.6	8.4±0.5	1.24±0.09
8	DS1	19-Sep-00	3-Oct-00	1050	137	9.9±0.7	9.3±0.7	8.5±0.6	1.25±0.10
11	DS1	19-Sep-00	3-Oct-00	1100	200	9.9±0.6	8.3±0.7	7.6±0.6	1.12±0.09
12	DS1	19-Sep-00	3-Oct-00	1080	300	10.5±0.6	9.8±0.6	9.2±0.6	1.28±0.10
13	DS1	19-Sep-00	3-Oct-00	1080	140	9.5±0.8	8.8±0.6	8.3±0.7	1.17±0.13
14	DS1	19-Sep-00	3-Oct-00	1050	320	8.9±0.6	8.5±0.6	7.9±0.5	0.99±0.10
15	DS1	19-Sep-00	3-Oct-00	1100	100	10.1±0.5	9.6±0.6	8.8±0.6	1.31±0.09
16	DS1	19-Sep-00	3-Oct-00	1100	78	8.2±0.6	6.5±0.6	6.0±0.5	0.94±0.07
17	DS1	19-Sep-00	3-Oct-00	1150	165	8.7±0.4	7.6±0.5	6.7±0.4	1.01±0.07
18	DS1	19-Sep-00	3-Oct-00	1100	220	9.9±0.6	7.7±0.5	7.1±0.4	1.06±0.16
19	DS1	19-Sep-00	3-Oct-00	1100	180	10.0±0.5	8.6±0.6	8.1±0.4	1.17±0.08
20	DS1	19-Sep-00	3-Oct-00	1080	120	9.0±0.5	8.5±0.5	7.9±0.4	1.15±0.07
21	DS1	19-Sep-00	3-Oct-00	1400	170	10.0±0.7	9.5±0.8	8.7±0.7	1.29±0.13
22	DS1	19-Sep-00	3-Oct-00	1400	250	11.1±0.5	8.4±0.5	7.9±0.5	1.20±0.09
23	DS1	19-Sep-00	3-Oct-00	1420	215	10.5±0.9	8.6±0.8	8.1±0.7	1.14±0.18
24	DS1	19-Sep-00	3-Oct-00	1480	142	9.9±0.8	9.1±0.8	8.2±0.7	1.21±0.16
25	DS1	19-Sep-00	3-Oct-00	1480	220	11.6±0.7	10.4±0.9	9.3±0.6	1.49±0.15
26	DS1	19-Sep-00	3-Oct-00	1450	385	9.9±0.4	9.3±0.6	8.7±0.6	1.13±0.10
27	DS1	19-Sep-00	3-Oct-00	1300	220	10.6±0.7	9.8±0.7	9.0±0.6	1.25±0.10
28	DS1	19-Sep-00	3-Oct-00	1300	210	8.6±0.7	7.5±0.7	7.1±0.6	0.94±0.07
29	DS1	19-Sep-00	3-Oct-00	1100	120	10.7±0.7	9.9±0.7	9.2±0.7	1.37±0.14
30	DS1	19-Sep-00	3-Oct-00	1100	150	9.5±0.5	8.4±0.5	8.1±0.5	1.20±0.08
31	DI	22-Sep-00	3-Oct-00	1100	122	10.8±0.8	10.2±0.9	9.4±0.8	1.44±0.16
32	DI	22-Sep-00	3-Oct-00	1200	200	8.9±0.7	8.4±0.7	8.2±0.7	1.11±0.08
33	DI	22-Sep-00	3-Oct-00	1200	150	10.4±0.6	9.7±0.6	8.7±0.6	1.32±0.09
34	DI	22-Sep-00	3-Oct-00	1200	200	11.0±0.7	9.4±0.7	9.0±0.7	1.32±0.11
35	DI	22-Sep-00	3-Oct-00	1220	180	11.0±0.9	9.2±0.8	8.6±0.7	1.25±0.16
36	DI	22-Sep-00	3-Oct-00	1200	200	10.0±0.6	9.1±0.8	8.2±0.7	1.23±0.12
37	DI	22-Sep-00	3-Oct-00	1260	170	10.9±0.8	10.4±0.7	10.0±0.7	1.49±0.15
38	DI	22-Sep-00	3-Oct-00	1420	135	10.7±0.9	10.0±0.4	9.2±0.5	1.37±0.09
39	DI	22-Sep-00	3-Oct-00	1420	275	10.4±0.9	9.5±0.7	9.0±0.6	1.32±0.13
40	DI	22-Sep-00	3-Oct-00	1420	350	10.6±0.5	10.0±0.5	9.2±0.9	1.33±0.07
41	DI	22-Sep-00	3-Oct-00	1420	270	10.6±0.5	9.3±0.6	8.3±0.4	1.28±0.09
42	DI	22-Sep-00	3-Oct-00	1420	245	9.1±0.6	8.6±0.5	8.2±0.5	1.04±0.09
43	JS	27-Sep-00	3-Oct-00	1420	120	10.4±0.8	9.6±0.9	9.3±0.8	1.37±0.14
44	JS	27-Sep-00	3-Oct-00	1400	100	10.5±0.9	10.0±0.8	9.6±0.8	1.33±0.13
45	JS	27-Sep-00	3-Oct-00	1400	100	10.7±0.7	10.2±0.7	9.2±0.7	1.33±0.16
46	JS	27-Sep-00	3-Oct-00	1400	170	10.2±0.8	9.0±0.5	8.7±0.6	1.30±0.09
47	JS	27-Sep-00	3-Oct-00	1300	200	9.8±0.6	9.4±0.5	8.8±0.6	1.24±0.09
48	JS	27-Sep-00	3-Oct-00	1240	150	11.0±0.5	10.4±0.6	9.5±0.6	1.47±0.12
50	JS	27-Sep-00	3-Oct-00	1240	80	10.4±0.6	9.6±0.7	8.4±0.5	1.19±0.12
64	JS	27-Sep-00	3-Oct-00	1480	60	9.3±0.4	8.9±0.4	8.3±0.4	1.24±0.10
80	JS	27-Sep-00	3-Oct-00	1250	50	9.4±0.6	9.0±0.5	8.5±0.6	1.10±0.08
81	JS	27-Sep-00	3-Oct-00	1300	80	10.4±0.6	9.5±0.7	8.4±0.5	1.19±0.12
82	JS	27-Sep-00	3-Oct-00	1300	150	8.5±0.6	7.9±0.7	7.3±0.6	1.05±0.08
83	JS	27-Sep-00	3-Oct-00	1400	100	10.7±0.7	10.1±0.6	9.5±0.6	1.35±0.12
84	JS	27-Sep-00	3-Oct-00	1220	180	10.4±0.8	9.7±1.0	8.9±0.8	1.35±0.14
MEAN						10	9.1	8.4	1.23
SD						0.8	0.9	0.8	0.14

APPENDIX II: SUMMARY OF GERMINATION RESULTS AND SEEDLING PERFORMANCE IN THE NURSERY OF THE 5 SPECIES STUDIED.

Table 26. Germination results and seedling performance in nursery of seedling, derived form 41 seed trees of *Spondias axillaris* Roxb.

tree no	TG (days)	MLD (days)	GR (%)	GP (days)	% survival	seedling RCD (mm)	seedling height (cm)	RRGR (% year^{-1})	RHGR (% year^{-1})
1	109.0±49.1	132	76.4	188	83.6	2.6±0.9	50.9±20.9	297.8±139.0	625.4±163.3
2	104.4±40.3	132	25	101	83.3	2.6±0.9	48.5±25.1	188.1±112.1	552.6±127.3
3	161.5±20.9	145	15.3	57	90.9	2.2±0.8	27.8±11.2	272.4±202.6	569.1±273.7
4	138.5±35.7	144	22.2	149	75	2.6±0.7	54.5±15.7	248.9±105.5	756.3±135.7
5	85.5±40.2	89	22.2	122	87.5	3.3±1.6	54.9±28.9	312.6±124.6	658.5±142.0
6	96.4±37.9	88	33.3	109	79.2	2.9±0.9	58.2±18.6	226.5±142.8	677.5±165.7
7	97.3±45.3	82	48.6	149	85.7	3.0±0.8	59.1±22.2	267.8±103.9	617.5±190.7
8	81.2±45.7	65	26.4	158	73.7	3.4±1.4	61.5±25.9	257.7±100.3	525.3±159.1
9	74.3±39.5	56	44.4	117	75	3.4±1.2	60.8±21.7	265.9±119.2	520.5±192.0
10	99.6±51.7	103	45.8	158	78.8	3.3±1.4	58.2±28.8	282.8±144.8	575.8±161.6
11	70.2±4.1	62	41.7	117	93.3	3.4±1.0	66.2±21.5	297.1±92.3	564.0±172.8
12	44.1±38.2	33	22.2	132	68.8	4.3±1.3	72.9±19.3	321.1±86.9	582.8±151.8
13	49.6±43.4	25	72.2	129	84.6	4.4±1.4	73.1±20.4	324.2±92.0	541.2±172.8
14	161.5±9.2	162	8.3	13	83.3	1.8±0.4	31.3±3.9	319.9±46.8	697.7±114.2
15	126.8±39.4	139	61.1	163	81.8	2.5±1.0	43.9±21.4	326.1±175.2	661.0±245.3
16	113.6±34.9	129	16.7	113	91.7	2.4±0.5	53.8±15.5	299.0±147.1	759.0±163.5
17	117.9±43.0	135	68.1	171	89.8	3.4±1.0	57.4±21.9	338.4±175.1	674.3±199.1
18	127.5±58.4	140	8.3	139	100	2.7±1.4	42.4±31.5	267.1±123.9	452.1±304.6
19	152.7±54.3	169	11.1	146	87.5	2.5±0.6	32.2±14.7	197.9±102.7	836.5±23.9
20	116.6±48.6	132	29.2	164	61.9	2.7±0.8	53.9±22.8	273.9±120.4	741.1±182.3
21	117.6±49.8	134	40.3	165	72.4	2.6±0.9	52.8±26.1	178.0±107.1	603.6±210.8
22	125.1±33.6	135	23.6	132	76.5	2.3±0.6	50.5±20.8	167.7±122.8	725.4±169.7
23	60.2±49.6	33	83.3	143	90	3.3±1.2	61.2±23.1	317.9±107.0	597.3±244.7
24	81.0±55.5	48	47.2	152	73.5	3.1±1.2	56.5±26.9	258.2±101.0	518.6±132.4
25	64.6±43.9	44	40.3	161	82.8	3.5±1.0	65.4±22.9	292.2±95.4	603.4±107.1
26	96.9±58.1	106	54.2	171	79.5	3.1±1.3	55.6±27.8	304.3±126.9	568.0±177.9
27	122.5±59.8	151	8.3	122	100	2.2±1.2	32.9±25.3	338.0±177.1	535.2±196.7
28	110.1±56.5	104	29.2	155	76.2	2.3±1.1	43.7±29.4	234.3±108.4	568.8±250.5
29	120.9±62.7	148	45.8	170	81.8	2.7±1.1	49.6±26.0	302.1±178.3	664.7±240.1
30	138.4±41.6	148	54.2	152	89.7	2.1±0.9	36.1±18.8	207.6±100.4	695.1±247.6
31	75.8±51.9	77	72.2	183	78.8	3.5±1.2	65.6±24.5	320.9±149.4	692.0±196.5
32	128.4±47.9	150	51.4	153	75.7	2.7±0.7	49.0±21.8	256.3±127.9	742.5±185.3
33	133.1±61.0	166	34.7	168	68	1.8±0.6	31.2±19.4	198.2±99.2	627.0±192.1
34	23.7±8.4	23	91.7	69	90.9	3.8±1.3	63.7±23.8	283.7±92.6	408.7±125.1
35	71.8±38.3	64	86.1	140	88.7	3.3±1.2	58.7±22.6	307.5±97.7	637.5±212.9
36	107.4±36.8	111	37.5	127	74.1	2.2±0.7	38.7±19.3	179.3±127.6	637.6±205.1
37	64.9±35.8	44	48.6	135	68.6	2.6±1.2	53.8±25.4	206.2±104.3	598.3±183.4
38	102.1±31.1	112	55.6	119	75	3.3±1.3	51.6±18.0	342.3±166.2	808.3±224.2
39	30.4±16.7	25	65.3	91	70.2	3.4±1.0	62.6±20.3	240.0±102.6	466.0±154.9
40	39.6±24.4	33	33.3	97	75	2.8±1.1	54.7±21.7	168.0±96.1	430.4±92.4
41	56.7±43.4	29	34.7	126	88	3.1±1.1	62.1±29.4	272.3±118.4	713.7±255.4
mean	97.5	99	42.3	135	81.2	2.9	52.6	267.3	620.3
SD	35.1	47	22.2	35	8.8	0.6	11.4	51.6	99.3

Table 27. Germination results and seedling performance in nursery of seedling, derived form 50 seed trees of *Melia toosendan* Sieb. & Zucc..

tree no	TG (days)	MLD (days)	GR (%)	GP (days)	% survival	seedling RCD (mm)	seedling high (cm)	RRGR (% year⁻¹)	RHGR (% year⁻¹)
1	28.3±13.8	22	50	41	55.6	3.6±0.9	49.5±14.1	288.7±130.0	647.1±134.7
2	41.1±24.9	40	51.39	79	45.9	3.7±1.0	52.6±16.7	314.8±119.0	727.3±124.5
3	53.3±26.4	46	70.83	96	33.3	2.5±0.8	36.2±16.1	273.4±128.5	614.0±245.6
4	48.7±22.8	48	65.28	91	59.6	3.1±1.2	40.6±15.0	266.0±178.4	652.0±120.2
5	63.7±12.9	42	48.61	48	40	3.0±0.7	41.5±15.0	231.7±132.0	643.0±126.3
6	59.1±14.2	56	38.89	54	14.3	3.1±0.3	38.3±14.5	302.3±42.4	587.1±155.3
7	44.1±14.3	46	59.68	66	45.9	3.1±1.0	45.6±15.7	260.4±158.3	631.9±166.3
8	41.3±24.8	38	60	72	36.7	3.2±0.9	41.9±12.4	296.9±121.8	655.6±134.9
9	51.7±8.1	49	63.89	54	54.3	3.6±0.9	50.5±14.4	320.9±145.8	685.3±186.8
10	36.4±22.1	32	63.89	97	78.3	3.7±0.7	51.3±11.4	288.8±107.1	639.2±118.0
11	58.4±16.1	51	40.28	59	44.8	3.5±0.8	54.4±15.2	322.2±93.7	697.8±143.7
12	43.5±8.4	44	68.06	42	59.2	2.8±0.6	38.3±12.0	277.9±97.8	639.9±148.8
13	53.0±23.2	52	61.11	88	59.1	3.1±0.7	44.1±13.2	291.0±129.6	653.7±151.6
14	68.8±17.1	56	54.17	58	33.3	2.7±0.7	40.2±17.2	265.9±108.4	669.1±185.3
15	31.2±24.7	18	51.39	75	56.8	3.1±1.1	46.2±18.1	267.1±158.2	726.6±154.9
16	43.8±20.9	42	31.43	70	9.1	3.0±	71	243.9	886.9
17	63.6±15.5	55	68.06	57	61.2	3.0±0.8	42.9±14.4	274.9±101.3	658.2±166.3
18	60.5±17.0	53	59.72	73	60.5	3.2±1.0	43.2±17.0	300.2±129.1	641.0±206.8
19	52.6±10.1	49	55.56	51	30	2.8±0.7	49.8±12.3	289.5±106.0	778.3±114.7
20	44.4±22.0	46	47.22	81	47.1	3.3±1.0	45.2±15.5	323.8±117.7	639.6±180.2
21	38.1±18.6	43	54.17	83	74.4	3.5±0.7	47.9±13.3	307.4±132.8	612.0±174.0
22	37.5±20.9	35	59.72	83	53.5	3.3±0.8	43.5±15.5	289.8±92.8	614.0±149.8
23	38.2±26.0	33	65.28	93	72.3	3.3±1.0	49.6±12.4	333.4±126.7	721.7±118.0
24	88.5±10.9	92	30.56	56	9.1	2.0±0.0	43.0±24.0	95.8	910.5
25	70.2±19.8	73	38.89	59	32.1	3.1±0.6	50.4±16.3	350.8±121.2	768.5±136.6
26	80.0±7.8	80	61.9	6	69.2	2.8±0.7	59.7±14.6	393.5	1081.1
27									
28	67.1±20.1	59	18.06	62	23.1	2.5±0.5	44.3±9.3	275.4±7.7	668.0±90.6
29	66.5±17.8	62	55.56	60	22.5	2.7±0.5	30.9±11.4	282.5±60.5	509.2±194.3
30	78.0±17.0	83	31.94	49	8.7	3.5	40		
31	63.7±15.4	56	37.5	61	44.4	2.8±0.9	42.0±15.3	308.9±125.7	695.3±226.7
32	56.8±14.6	51	25	54	38.9	3.3±0.5	46.9±18.8	327.6±86.9	704.2±130.8
33	68.7±16.4	69	63.89	51	63	2.9±0.8	46.7±14.5	333.3±110.3	716.1±163.0
34	59.8±17.3	53	57.5	59	17.4	3.3±0.6	51.5±20.9	261.9±62.2	674.1±207.6
35	55.5±13.4	51	48.61	49	57.1	3.1±0.9	48.6±11.7	275.7±138.8	688.4±112.2
36	65.6±19.7	56	43.06	54	41.9	3.1±0.6	40.2±7.7	368.1±77.2	665.5±129.6
37	64.9±14.4	66	56.94	49	63.4	2.7±0.5	43.9±13.1	241.8±118.4	662.3±182.9
38	63.6±17.4	60	52.78	65	57.9	2.8±0.8	40.6±12.2	254.2±132.0	652.6±156.7
39	71.2±14.2	75	62.5	56	66.7	3.1±1.1	45.5±12.9	270.8±138.9	666.0±150.9
40	71.3±14.5	75	56.94	55	46.3	2.5±0.5	37.1±12.4	273.7±84.9	586.0±197.3
41	86.7±9.7	85	52.78	38	36.8	2.7±0.7	38.0±9.4	306.4±123.1	622.1±109.2
42	81.3±12.3	83	15.71	49	0				
43	66.8±14.2	71	40.28	48	27.6	2.9±0.6	41.3±15.8	321.7±140.1	594.0±251.6
44	72.4±16.7	71	52.78	51	55.3	2.6±0.8	39.1±12.4	313.8±93.9	615.7±157.3
45	68.3±16.6	63	59.72	53	41.9	2.5±0.8	38.7±17.6	273.2±153.1	664.0±156.6
46	78.2±21.1	87	37.5	63	14.8	2.5±0.5	35.7±3.1	288.1±55.4	551.7±11.7
47	60.5±14.3	55	31.94	59	13	3.3±0.6	52.0±13.0	281.8±53.6	841.0±276.0
48	55.5±11.4	52	48.61	43	34.3	2.7±1.0	42.0±14.0	244.0±132.8	681.8±167.9
49	61.6±15.6	56	38.89	60	39.3	3.1±0.7	43.5±13.1	245.7±98.7	575.9±176.7
50	40.7±10.4	37	55.56	38	42.5	3.1±0.6	48.3±14.6	337.9±115.0	658.0±142.0
mean	58.5	54.3	49.3	30.4	42.7	3.0	44.9	288.5	678.2
SD	14.6	18.3	14.9	17.2	19.4	0.4	6.8	44.4	96.4

Table 28. Germination results and seedling performance in nursery of seedling,
derived form 49 seed trees of *Gmelina arborea* Roxb.

tree no	TG (days)	MLD (days)	GR (%)	GP (days)	% survival	seedling RCD (mm)	seedling height (cm)	RRGR (% year^{-1})	RHGR (% year^{-1})
1	19	19	1.4	1	0				
2									
3	17.7±3.2	19	5.6	7	25	4.5±2.8	71.0±48.6	225.7±172.0	78.7±691.5
4									
5			2.8		50	5.8±0.6	90.0±15.6	176.1	432.0
6	15.0±0.0	15	5.6	1	50	4.8±2.2	89.3±37.1	230.7±132.8	436.7±165.6
7	38.0±26.9	19	4	39	100	2.1±1.1	34.5±34.6	130.0±22.5	245.7±288.3
8	15	15	1.4	1	100	4.6	125.0	197.0	615.4
9	17.8±9.6	14	8.3	37	50	5.1±2.8	94.7±44.8	228.5±183.6	496.5±93.6
10				1.4					
12	12.1±5.7	11	27.8	28	90	4.4±1.8	61.8±24.2	192.1±156.2	378.7±125.5
13	20	20	1.4	1	100	4.2	67.0	39.7	391.1
14	12.3±1.9	12	13.9	7	90	6.2±2.0	93.8±23.9	252.1±142.9	509.4±89.7
15	25	15	1.7	1	100	5.3	98.0	200.2	542.0
16	17.5±5.7	15	5.6	14	100	3.9±0.7	54.5±16.5	90.8±52.7	293.5±54.6
17	18.5±6.4	14	3.3	10	100	4.1±1.8	92.0±22.6	240.4±10.2	664.5±65.5
18	15.6±6.8	14	29.2	26	42.9	4.9±1.8	81.4±37.4	207.7±112.0	447.4±167.9
19	20.5±16.3	9	2.8	24	100	6.0±4.8	92.0±31.1	168.6	507.1
20	13.5±4.9	10	4.2	8	66.7	6.6±3.0	94.3±31.6	421.8±162.7	623.6±53.0
21	12.7±1.2	12	4.2	3	100	4.7±1.0	114.3±2.1	151.5±87.9	601.2±36.4
22	10.4±2.1	9	29.2	7	90.5	5.8±1.9	83.2±25.5	284.4±119.2	476.9±135.9
23	14	14	1.4	1	100	7.2	92.0	435.9	550.0
24	16.5±4.5	14	5.6	11	100	3.9±2.2	63.0±29.6	136.1±122.5	398.6±110.7
25									
26	14.5±0.6	14	6.9	2	80	7.0±3.2	73.5±18.9	351.8±226.6	438.1±99.4
27	14.8±2.2	16	11.1	6	62.5	4.4±2.2	74.8±39.5	168.9±185.3	443.7±160.5
28	13.9±2.7	14	31.9	12	65.2	6.0±2.1	94.9±23.1	281.2±143.2	475.9±95.0
29	10.8±1.2	11	55.6	7	90	5.3±2.1	73.8±27.3	249.0±144.3	409.0±210.4
30	12.7±4.2	12	56.9	23	68.3	5.2±2.2	80.8±32.9	186.5±140.6	373.2±172.5
31	13.4±1.4	14	18.1	5	61.5	6.0±0.8	79.7±18.8	326.9±57.3	507.4±96.5
32	13.1±1.5	13	22.2	5	81.3	5.4±2.4	91.1±34.7	191.5±127.9	444.2±133.6
33	14.4±2.5	14	50	12	88.9	7.2±2.0	97.5±24.7	282.4±122.4	454.8±105.0
34	16.0±5.3	14	8.3	11	66.7	4.8±3.2	62.2±36.6	207.9±331.7	337.3±200.9
35	13.1±2.3	12	40.3	9	93.1	5.4±1.8	88.8±21.1	305.5±117.8	489.2±77.0
36	14.0±3.0	14	31.9	13	78.3	5.3±2.1	76.8±24.3	233.4±146.8	436.7±158.5
37	11.9±1.2	12	63.9	6	82.6	4.5±1.6	83.9±26.8	188.0±128.6	473.2±149.0
38	12.0±2.0	11	48.6	9	80	5.3±2.1	84.1±31.9	194.8±135.6	474.1±115.4
39	12.3±2.5	12	31.9	9	95.7	5.1±1.8	90.5±25.7	197.7±110.1	451.1±128.1
40	12.9±1.4	12	19.4	5	50	6.9±2.5	102.5±27.0	279.6±143.3	515.9±98.7
41	13.7±2.0	13	48.6	8	82.9	6.3±2.0	102.7±22.5	294.6±116.8	509.4±67.8
42	12.5±4.3	11	51.4	24	94.6	4.9±1.7	78.9±29.2	199.1±128.3	426.9±133.6
43	22.1±11.7	20	16.7	37	91.7	5.7±1.1	75.1±19.2	353.0±83.9	572.5±97.6
44	13.0±3.2	12	47.2	19	94.1	3.9±2.1	58.0±26.3	258.2±169.9	499.1±171.8
45	13.7±2.6	15	30.6	9	86.4	4.8±2.0	59.5±24.0	370.0±211.8	645.9±199.5
46	17.4±6.4	14	19.4	21	92.9	7.5±1.8	89.5±19.3	474.5±141.3	583.0±95.5
47	13.4±1.8	14	12.5	6	77.8	5.0±1.7	73.6±26.1	368.2±71.0	740.7±57.2
48	17.9±13.9	13	31.7	47	78.9	5.3±2.1	67.6±23.8	343.8±147.9	601.1±122.5
49	13.8±2.7	14	29.2	12	95.2	5.4±1.9	68.6±24.6	323.3±157.7	516.2±158.9
50	13.9±3.4	12	41.7	13	86.7	4.8±2.1	66.5±25.5	264.6±149.0	421.9±281.7
mean	15.5	13.3	20.2	15.5	76.2	5.2	80.4	247.8	475.7
SD	4.6	2.4	18.8	11.1	27.1	1.1	18.2	90.5	114.4

Table 29. Germination results and seedling performance in nursery of seedling, derived form 50 seed trees of *Prunus cerasoides* D. Don.

tree no	TG (days)	MLD (days)	GR (%)	GP (days)	% survival	seedling RCD (mm)	seedling height (cm)	RRGR (%.year^{-1})	RHGR (%.year^{-1})
1	28.4±10.4	24	68.1	38	89.8	2.7±1.2	67.7±27.4	258.6±157.6	499.5±162.3
2	41.8±13.0	41	26.4	43	63.2	3.7±1.2	99.0±27.7	360.3±110.3	506.4±158.5
3	48.5±12.0	51	16.7	40	75	3.1±1.1	75.1±22.0	314.0±103.1	534.6±76.1
4	30.0±11.0	27	69.4	47	80	3.7±1.4	99.0±30.3	344.6±159.7	456.3±133.5
5	40.6±15.5	44	58.3	60	83.3	3.0±1.3	72.6±29.0	288.5±172.9	537.4±172.8
6	36.7±17.5	37	7.5	36	66.7	2.9±2.4	63.5±37.5	283.8±352.6	569.1±98.2
7	24.8±13.1	20	34.7	46	76	3.5±1.1	86.5±23.2	317.2±151.4	582.5±126.3
8	38.5±14.1	40	27.8	52	80	1.7±0.9	40.6±21.1	213.4±148.4	514.0±174.6
9	26.2±8.6	25	70.8	44	90.2	2.2±1.0	57.2±22.7	197.8±144.7	540.9±174.3
10	26.5±9.7	23	62.5	37	77.8	3.4±1.3	80.9±23.3	305.0±143.9	537.2±128.2
11	39.6±11.3	37	61.1	54	81.8	2.6±1.3	67.0±30.0	278.7±187.2	527.6±186.0
12	28.6±11.8	23	59.7	50	76.7	3.2±1.3	81.7±26.7	356.7±170.3	633.0±146.3
13	25.3±7.1	23	34.7	32	84	2.9±1.1	76.1±20.1	368.0±146.9	652.5±109.8
14	36.4±13.6	34	62.5	58	26.7	2.9±1.4	58.5±26.7	311.7±238.2	583.3±229.7
15	22.3±10.4	19	47.2	54	79.4	3.3±1.7	74.0±34.0	309.5±194.3	514.6±168.7
16	33.8±10.6	29	77.8	46	48.2	2.8±1.2	61.9±22.9	397.8±200.3	668.7±157.6
17	29.3±9.3	30	61.1	36	61.4	3.3±1.0	78.8±21.1	386.7±144.7	673.8±148.7
18	26.2±7.5	25	63.9	32	65.2	2.5±1.0	57.2±22.0	363.0±168.6	609.9±196.8
19	29.2±8.0	27	80.6	36	81	2.4±1.1	64.1±27.2	202.4±149.9	477.6±149.0
20	36.1±10.1	38	18.1	28	76.9	3.4±1.3	81.8±27.6	352.9±154.1	658.7±130.4
21	29.9±8.4	28	80.6	34	87.9	2.5±1.3	63.1±31.9	250.2±183.5	481.4±182.0
22	26.9±8.9	24	77.8	39	78.6	3.4±1.3	76.1±25.3	384.9±148.3	596.3±148.3
23	20.8±10.5	17	76.4	62	76.4	3.2±1.4	81.4±28.5	320.1±152.6	551.9±169.9
24	37.5±11.6	41	66.7	43	72.9	2.6±1.2	58.0±25.6	306.6±186.0	506.8±168.1
25	33.8±9.5	33	56.9	40	82.9	4.8±1.6	113.1±27.2	357.6±143.3	485.5±101.0
26	28.6±8.3	27	54.2	42	82.1	4.3±1.4	99.7±29.9	351.7±128.8	547.9±136.8
27	24.8±6.2	23	76.4	33	78.2	3.3±1.2	78.3±24.2	300.6±152.2	526.3±112.0
28	37.2±17.9	33	12.5	50	66.7	3.4±2.1	78.6±41.8	336.9±181.9	464.8±129.7
29	22.8±6.9	20	69.4	38	68	3.3±1.6	74.6±31.1	319.3±171.9	462.4±146.1
30	26.6±12.2	22	47.2	50	76.5	4.3±1.4	103.2±23.6	372.5±144.0	486.1±83.3
31	27.6±7.5	25	83.3	39	93.3	3.1±1.1	74.8±22.0	319.8±145.9	588.1±120.0
32	36.3±14.0	34	51.4	50	62.2	2.9±1.2	74.2±24.1	339.7±159.8	603.7±135.3
33	40.4±13.2	39	63.9	66	82.6	2.7±1.0	69.9±27.7	320.8±163.9	587.5±172.5
34	38.4±12.3	37	44.4	50	65.6	3.2±1.5	71.5±29.9	325.3±174.2	434.5±137.0
35	38.4±8.7	40	63.9	31	69.6	2.5±1.1	59.8±25.2	237.0±163.3	522.9±151.3
36	28.6±12.2	23	65.3	55	72.3	2.5±1.1	71.5±23.0	215.5±147.7	596.6±173.1
37	24.8±10.4	21	25	39	77.8	4.1±0.9	100.2±14.4	388.1±83.4	503.7±67.0
38	35.3±15.8	32	55.6	59	72.5	2.4±1.1	61.4±26.2	243.3±146.1	521.2±161.8
39	29.9±9.2	32	72.2	44	67.3	2.7±1.4	65.3±32.4	276.7±181.6	374.7±164.2
40	33.7±10.9	35	81.9	44	88.1	2.9±1.0	70.8±21.1	264.4±138.8	549.9±128.0
41	36.6±8.8	37	56.9	33	85.4	3.6±1.3	77.4±22.2	310.7±137.5	549.7±118.1
42	26.1±7.0	23	47.2	28	82.4	4.4±1.2	98.1±23.0	395.2±122.0	523.6±102.7
43	37.7±7.7	39	47.2	32	73.5	3.4±1.2	76.7±26.5	347.6±137.9	552.1±132.5
44	32.1±9.3	33	87.5	49	76.2	2.4±1.1	59.9±25.6	211.8±168.2	524.4±169.4
45	32.6±7.4	35	77.8	26	66.1	2.4±0.9	60.2±19.9	234.9±150.3	543.9±161.2
46	38.1±11.7	44	22.2	43	93.8	3.2±1.2	72.4±23.8	329.7±175.3	586.3±135.9
47	42.5±12.8	44	48.6	45	77.1	2.5±1.0	71.6±22.1	264.5±122.5	621.3±138.9
48	38.3±10.5	39	51.4	47	64.9	2.9±1.3	67.1±26.2	283.8±184.4	526.1±226.6
49	30.2±8.8	29	29.2	36	57.1	2.4±1.2	71.2±27.6	227.0±191.0	539.3±182.2
50	33.4±10.0	35	47.2	44	73.5	3.6±1.2	85.6±29.6	281.5±122.9	562.3±117.2
mean	32.4	31.2	55.0	43.2	74.7	3.1	74.6	306.6	544.0
SD	6.2	8.0	20.5	9.4	11.7	0.6	14.2	54.9	60.9

Table 30. Germination results and seedling performance in nursery of seedling, derived form 50 seed trees of *Castanopsis acuminatissima* (Bl.) A. DC.

tree no.	TG (days)	MLD (days)	GR (%)	GP (days)	% survival	seedling RCD (mm)	seedling height (cm)	RRGR (% year^{-1})	RHGR (% year^{-1})
1	33.2±9.7	30	84.7	40	70.5	1.6±0.5	18.2±8.0	67.4±68.0	175.7±102.9
3	34.1±10.5	31	75	48	81.5	2.0±0.6	22.7±9.8	90.3±80.7	176.2±79.3
5	33.9±8.4	34	95.8	38	72.5	1.4±0.4	16.5±6.9	44.9±60.6	154.2±64.9
6	39.7±14.4	37	79.2	79	89.5	1.7±0.5	17.9±6.9	62.5±63.3	128.0±111.4
8	38.0±11.3	36	87.5	48	84.1	1.7±0.6	17.5±7.9	56.7±76.8	152.4±71.6
11	56.6±11.0	56	63.9	52	76.1	1.8±0.7	21.1±10.5	86.6±83.7	139.3±114.5
12	47.1±10.2	47	70.8	51	86.3	1.7±0.4	19.9±7.1	71.3±57.0	166.1±68.3
13	56.4±10.8	55	62.5	48	77.8	1.9±0.7	20.9±9.0	69.8±63.3	132.8±63.2
14	50.4±10.1	48	55.6	47	67.5	1.9±0.8	19.2±8.8	63.3±72.1	125.2±91.5
15	43.8±10.6	41	75	58	75.9	1.8±0.5	23.5±9.4	71.1±62.9	123.8±81.0
16	45.5±8.5	48	18.1	26	61.5	1.4±0.3	17.6±5.9	84.6±60.9	182.6±59.2
17	38.4±7.4	37	93.1	32	80.6	1.6±0.5	17.5±6.9	68.6±71.7	140.3±77.6
18	51.7±12.8	50	80.6	56	91.4	1.5±0.4	15.5±6.1	53.5±62.0	115.2±56.9
19	59.1±11.2	61	69.4	47	92	1.4±0.4	16.2±4.3	60.1±68.2	140.3±62.2
20	45.4±8.7	46	77.8	42	89.3	1.8±0.6	20.8±8.4	90.4±74.9	166.4±70.6
21	29.7±12.4	24	44.4	59	90.6	1.9±0.6	23.2±8.5	81.4±67.7	168.9±91.3
22	43.0±7.8	41	70.8	38	80.4	1.7±0.5	23.7±8.9	64.7±56.7	161.9±73.8
23	48.4±13.4	47	62.5	60	86.7	1.7±0.5	18.9±7.5	53.2±52.3	131.6±64.2
24	40.7±9.6	39	86.1	70	88.7	1.6±0.4	18.9±7.7	76.2±72.6	142.1±85.3
25	37.6±9.0	37	80.6	39	74.1	2.2±0.8	25.8±11.1	76.3±62.4	133.7±73.3
26	36.6±9.0	35	77.8	37	91.1	1.8±0.6	22.7±8.7	63.4±63.9	149.2±76.7
27	49.1±12.4	48	88.9	58	89.1	1.8±0.5	19.9±7.2	89.3±74.1	136.7±74.1
28	44.4±13.5	41	61.1	59	77.3	1.6±0.4	17.9±5.2	73.3±67.6	146.3±78.1
29	42.5±9.6	40	79.2	43	68.4	1.9±0.4	19.7±5.9	34.3±39.1	124.3±51.6
30	47.4±13.6	48	76.4	65	80	1.6±0.5	17.0±6.3	78.3±73.7	130.3±51.4
31	32.2±8.0	30	77.8	31	41.1	1.8±0.7	23.3±10.0	72.7±81.3	164.3±83.9
32	32.1±10.8	27	73.6	55	52.8	1.6±0.5	14.3±5.9	48.6±61.9	116.8±65.0
33	34.7±12.5	30	97.2	54	60	1.7±0.5	20.0±8.0	68.5±72.5	140.8±84.6
34	28.9±6.9	27	87.5	39	85.7	1.9±0.6	19.5±7.5	66.3±67.7	137.3±62.8
35	32.9±12.7	27	75	49	70.4	1.8±0.6	22.9±7.3	73.9±68.3	188.0±41.7
36	30.1±11.6	44	79.2	65	63.2	1.9±0.6	21.7±7.2	90.2±67.0	165.5±50.0
37	33.0±12.0	27	84.7	52	78.7	2.0±0.6	24.5±10.1	81.5±64.8	196.5±93.5
38	36.9±11.8	37	90.3	67	87.7	2.1±0.6	23.0±7.8	86.2±78.7	142.6±68.6
39	42.0±13.4	41	93.1	62	89.6	1.8±0.6	23.8±9.1	72.2±74.6	150.1±89.7
40	32.5±8.2	30	94.4	40	85.3	1.7±0.5	21.7±7.2	76.8±66.4	155.9±73.0
41	34.8±8.0	34	77.8	45	91.1	1.6±0.5	22.9±8.3	75.9±64.3	188.3±62.6
42	42.1±12.8	41	81.9	58	91.5	1.5±0.4	19.3±6.6	54.5±59.6	164.9±58.8
43	28.9±9.7	24	86.1	42	82.3	1.5±0.4	18.1±8.5	49.1±50.0	156.1±92.6
44	33.1±17.2	24	94.4	82	76.5	1.9±0.6	22.3±8.5	72.7±71.0	141.3±67.4
45	34.4±11.8	30	81.9	58	83.1	1.8±0.6	18.2±8.4	70.6±69.1	144.7±65.1
46	32.7±8.8	30	94.4	47	92.6	1.7±0.5	19.0±6.7	85.1±69.3	158.2±67.8
47	28.5±8.0	27	84.7	45	85.2	1.6±0.5	20.2±9.8	56.6±57.4	163.6±72.0
48	25.9±8.8	23	93.1	48	76.1	1.9±0.6	21.3±7.1	109.7±70.7	146.8±78.0
50	28.4±8.7	27	93.1	42	76.1	1.6±0.5	21.4±9.7	77.8±59.3	135.5±78.3
64	34.5±12.4	34	88.9	66	68.8	1.4±0.4	16.1±6.1	56.3±54.5	118.0±122.5
80	50.7±13.5	48	95.8	60	63.8	1.6±0.9	15.4±7.8	61.3±71.0	129.6±129.5
81	28.2±9.4	30	88.9	52	73.4	1.5±0.4	21.0±9.6	72.3±57.5	133.4±79.1
82	34.7±11.5	30	83.3	48	76.7	1.2±0.4	13.0±5.8	24.4±48.5	133.6±115.1
83	29.5±7.7	30	84.7	32	55.7	1.8±0.5	18.3±5.7	75.1±72.1	128.4±84.3
84	36.9±10.8	37	76.4	49	74.5	1.7±0.5	19.7±5.4	64.3±70.4	142.8±60.6
mean	38.6±0.7	36.92	79.5	50.56	78.1	1.7	19.9	69.5	147.7
SD	8.32	9.34	14.4	11.88	11.5	0.2	2.8	15.3	19.7

APPENDIX III: SUMMARY OF SEEDLING PERFORMANCE IN THE FIELD OF THE 5 SPECIES STUDIED.

Table 31. Sapling performance of seedling derived from 41 seed trees after 1 growing season in the field of *Spondias axillaris* Roxb.

tree no.	no. of planted	no. of survival	%survival	seedling RCD (mm)	seedling height (cm)	RRGR (% year^{-1})	RHGR (% year^{-1})
1	22	5	22.7	19.0±6.3	107.5±29.7	531.4±107.8	196.3±125.4
2	15	8	53.3	16.7±8.3	92.6±37.8	456.3±218.9	227.1±188.2
3	10	4	40	12.9±5.1	81.5±13.5	500.1±168.2	262.5±107.9
4	12	7	58.3	17.7±4.3	99.0±28.4	525.3±113.7	150.6±148.9
5	14	10	71.4	13.6±6.1	95.8±30.5	359.4±157.7	186.8±124.6
6	19	16	84.2	16.3±7.3	92.9±25.0	439.6±90.2	157.1±121.6
7	17	16	94.1	21.1±6.5	125.9±29.0	506.0±163.0	212.3±154.5
8	14	12	85.7	15.7±6.1	91.8±31.5	393.1±99.7	115.2±130.4
9	19	16	84.2	14.3±5.1	95.8±25.9	381.5±119.2	133.1±124.1
10	19	18	94.7	14.5±6.5	97.7±26.2	414.9±161.2	158.1±142.2
11	22	17	77.3	19.2±7.5	107.4±39.2	445.9±149.9	172.4±193.1
12	11	8	72.7	20.5±4.7	119.2±34.2	431.5±48.9	134.3±103.5
13	32	19	59.4	21.9±6.0	119.8±30.0	430.3±116.2	103.5±115.7
14	5	3	60	23.0±2.6	143.3±11.5	386.3±59.5	118.5±21.3
15	19	15	78.9	15.0±6.8	85.4±37.3	500.2±140.4	180.4±139.9
16	11	8	72.7	15.7±4.6	93.6±21.9	504.2±119.6	180.5±135.0
17	22	17	77.3	16.9±7.0	105.9±24.6	431.4±169.3	174.5±136.1
18	6	4	66.7	18.2±1.8	121.0±31.6	524.5±198.5	258.2±101.9
19	6	5	83.3	16.2±4.0	100.4±14.9	607.3±131.5	318.3±101.7
20	13	11	84.6	15.4±4.5	100.8±30.0	451.6±93.7	205.4±147.6
21	21	15	71.4	15.2±3.7	101.6±27.6	502.2±161.8	245.2±188.9
22	13	8	61.5	17.0±4.4	88.5±35.6	552.0±112.2	262.6±129.3
23	32	24	75	21.9±7.7	118.1±29.3	439.1±109.6	138.2±96.7
24	20	14	70	15.0±5.3	96.2±28.3	414.7±142.9	164.8±129.0
25	20	14	70	16.1±5.8	102.9±35.0	408.1±133.2	125.9±163.3
26	20	15	75	19.5±5.4	116.3±35.8	520.1±93.1	225.2±147.6
27	6	4	66.7	13.9±2.6	103.3±18.8	425.8±185.7	319.6±166.2
28	16	9	56.3	11.4±7.2	69.0±36.3	289.0±217.9	125.9±265.9
29	19	14	73.7	17.2±5.6	104.4±38.3	443.3±157.9	168.1±217.7
30	19	16	84.2	14.1±6.2	82.7±26.6	480.2±116.9	180.7±85.2
31	21	15	71.4	16.2±6.1	102.5±32.1	368.0±115.7	41.4±105.9
32	21	14	66.7	17.7±6.7	90.3±31.4	494.6±122.4	177.5±167.1
33	16	13	81.3	16.0±6.4	87.5±30.0	527.2±187.8	295.4±141.4
34	37	27	73	20.0±5.9	117.2±22.8	394.6±79.0	116.8±68.4
35	25	22	88	19.7±7.0	116.4±32.1	445.0±136.3	170.4±146.6
36	18	14	77.8	20.5±6.2	101.8±34.4	586.4±143.5	348.2±296.2
37	21	10	47.6	17.3±4.9	102.1±24.0	490.5±120.1	168.7±98.3
38	13	9	69.2	20.3±7.6	119.2±25.4	527.4±145.9	219.4±101.6
39	28	21	75	18.2±6.2	114.2±35.2	472.5±91.7	199.2±136.3
40	18	14	77.8	17.8±5.5	104.1±37.3	527.1±195.9	206.1±139.0
41	20	13	65	19.4±6.3	122.8±38.6	492.5±131.0	176.5±154.5
MEAN			71.2	17.3	103.4	463.9	188.3
SD			14.0	2.7	14.3	65.1	63.6

Table 32. Sapling performance of seedling derived from 50 seed trees after 1 growing season in the field *Melia toosendan* Sieb. & Zucc.

tree no	no. of planted	no. survival	% survival	seedling RCD (mm)	seedling high (cm)	RRGR (% year^{-1})	RHGR (% year^{-1})
1	20	12	60	17.0±7.5	141.3±58.7	389.3±119.8	270.6±100.3
2	17	10	58.8	18.1±5.7	141.8±68.9	456.1±129.5	279.9±226.7
3	17	7	41.2	15.9±8.8	122.7±62.0	471.9±126.6	309.3±231.8
4	28	9	32.1	23.8±8.8	182.7±78.7	519.6±159.4	371.5±159.7
5	14	6	42.9	18.2±8.0	164.2±76.6	505.5±136.6	384.1±79.3
6	4	-	-	-	-	-	-
7	17	7	41.2	19.2±5.9	141.3±36.3	525.8±108.7	434.3±220.4
8	11	3	27.3	15.0±9.2	122.3±50.2	476.6±101.3	341.3±47.2
9	25	17	68	18.4±7.2	139.9±54.6	462.8±115.5	316.4±146.7
10	36	21	58.3	20.3±7.3	146.5±55.8	453.9±113.6	348.9±140.6
11	13	6	46.2	20.1±4.8	113.0±50.8	498.8±128.1	136.5±230.2
12	29	11	37.9	15.5±7.4	111.5±39.9	424.7±120.6	299.5±132.0
13	26	10	38.5	21.6±6.3	151.5±52.5	573.7±80.3	354.6±125.3
14	13	5	38.5	13.9±7.5	130.2±57.4	405.6±205.1	369.0±232.7
15	21	11	52.4	21.3±7.0	175.1±53.5	523.6±162.7	331.3±123.1
16	1	1	100	22	154	493.8	268.3
17	30	13	43.3	15.8±8.8	132.2±72.5	422.7±188.8	340.5±188.1
18	26	9	34.6	16.5±8.2	123.9±71.0	420.2±161.7	293.6±159.7
19	12	3	25	16.6±2.7	119.7±14.5	520.6±102.3	256.2±66.9
20	16	5	31.3	18.2±7.1	162.6±31.1	468.9±112.8	361.5±114.2
21	29	12	41.4	19.2±7.1	160.2±62.5	447.7±155.2	301.9±125.0
22	23	10	43.5	19.6±10.5	125.6±84.1	463.2±216.6	272.4±250.0
23	34	10	29.4	22.1±4.9	188.0±53.6	540.7±133.3	381.2±106.4
24	2	-	-	-	-	-	-
25	9	5	55.6	15.2±4.2	134.2±40.6	467.9±147.3	292.3±131.6
26	9	-	-	-	-	-	-
27	0	-	-	-	-	-	-
28	3	2	66.7	21.5±7.8	147.5±31.8	460.3±83.1	335.2±173.4
29	9	4	44.4	24.2±2.3	158.8±64.2	737.7±110.6	511.8±95.7
30	2	-	-	-	-	-	-
31	12	4	33.3	14.5±7.7	71.0±42.6	431.4±243.4	65.5±274
32	7	-	-	-	-	-	-
33	29	10	34.5	20.5±7.7	148.1±47.1	501.0±100.0	382.4±95.5
34	4	-	-	-	-	-	-
35	20	3	15	26.7±4.2	210.7±14.5	526.1±110.9	411.8±65.5
36	13	4	30.8	13.5±5.4	98.5±36.4	396.6±164.9	239.5±114.6
37	26	9	34.6	14.7±7.2	103.9±44.7	453.7±191.1	300.6±195.5
38	20	6	30	20.6±6.0	164.7±42.4	549.0±89.2	392.7±87.1
39	30	15	50	17.9±7.0	130.1±42.0	530.3±148.3	364.8±125.0
40	19	3	15.8	14.9±6.4	191.7±112.7	497.1±168.4	455.3±216.8
41	14	3	21.4	11.0±4.6	81.3±27.6	377.9±161.6	260.3±43.2
42	-	-	-	-	-	-	-
43	8	3	37.5	27.4±3.5	194.3±8.1	621.5±38.0	463.92±52.7
44	21	7	33.3	24.5±7.8	150.9±24.2	599.1±140.8	403.8±85.8
45	18	4	22.2	12.4±5.1	101.5±59.6	475.6±293.1	186.6±313.6
46	4	-	-	-	-	-	-
47	3	-	-	-	-	-	-
48	12	5	41.7	19.0±3.5	158.8±21.4	506.7±86.1	361.7±70.5
49	11	2	18.2	15.1±8.6	161.0±108.9	386.9±136.1	310.0±217.6
50	17	6	35.3	17.6±7.0	130.0±63.7	442.1±178.7	311.4±168.4
MEAN	16	6	33.6	18.5	142.2	485.7	326.8
SD	10	5	21.1	3.8	30.3	69.6	84.1

Table 33. Sapling performance of seedling derived from 49 seed trees after 1 growing season in the field of *Gmelina arborea* Roxb.

tree no	no. of planted	no. of survival	% survival	seedling RCD (mm)	seedling height (cm)	RRGR (% year^{-1})	RHGR (% year^{-1})
1							
2							
3	1	0	0				
4							
5	1	1	100	10.0	53.0	363.5	23.1
6	2	2	100	7.0±1.4	70.5±23.3	31.444.4	57.8
7	2	2	100	8.4±6.5	56.5±33.2	297.0±153.9	352.4±36.7
8	1	0	0				
9	3	1	33.3	12.0	73.0	185.6	-168.3
10							
12	16	8	50.0	11.2±5.0	58.6±25.2	224.1±179.6	51.0±261.6
13	1	0	0				
14	9	5	55.6	13.5±3.8	84.0±23.9	220.3±117.5	82.4±192.9
15	1	1	100	20.2	20.0	364.9	-92.7
16	4	2	50.0	10.3±3.2	59.5±10.6	245.7±207.4	183.5±117.0
17	2	2	100	8.8±4.6	54.0±50.9	174.248.9	20.3±28.9
18	9	5	55.6	8.6±2.0	75.8±28.8	107.979.5	70.0±248.9
19	2	2	100	9.1±4.9	73.5±70.0	57.9±26.7	25.1±6.9
20	2	1	50	17.2	74.0	186.7	34.2
21	2	2	100	18.5±3.5	97.5±3.5	346.1±11.9	175.4±74.4
22	19	15	78.9	13.5±6.4	79.5±25.5	246.0±182.3	139.8±149.8
23	1	1	100	10.0	80.0	76.8	17.7
24	4	4	100	6.3±2.2	41.5±12.8	101.1±38.0	145.6±149.3
25							
26	4	3	75.0	9.8±4.3	69.0±22.9	83.9±6.8	117.8
27	3	3	100	15.1±7.6	105.0±20.2	254.0±70.2	110.2±119.4
28	15	10	66.7	11.0±5.8	76.6±23.2	214.2±204.1	87.5±130.9
29	36	22	61.1	11.1±5.1	71.9±28.6	207.7±138.6	21.5±177.8
30	28	20	71.4	13.2±5.3	82.6±24.3	281.8±155.5	97.4±192.5
31	8	6	75.0	9.0±1.7	64.222.3	116.6±94.3	45.1±316.2
32	13	8	61.5	11.6±6.4	79.3±27.6	217.7±182.6	31.0±65.2
33	32	16	50.0	13.9±6.5	89.9±31.7	158.8±146.7	67.5±164.7
34	3	2	66.7	14.0±4.2	83.0±7.1	245.3±249.1	42.7±205.2
35	27	22	81.5	11.8±5.4	75.3±25.6	229.7±179.8	39.7±218.7
36	18	12	66.7	13.0±5.9	92.0±22.4	247.7±160.2	139.1±122.0
37	38	23	60.5	9.6±4.4	58.9±22.4	193.4±134.0	80.3
38	28	15	53.6	12.7±6.0	73.724.4	282.0±174.9	99.2±113.4
39	22	12	54.6	12.7±5.3	86.8±16.9	250.4±141.0	136.2±161.6
40	7	6	85.7	17.4±7.9	92.229.1	276.5±114.6	3.1±106.4
41	26	19	73.1	11.6±4.7	67.226.4	167.6±127.8	1.8±199.2
42	35	28	80.0	10.9±3.6	68.921.3	238.2±136.4	121.5±250.7
43	11	8	72.7	13.7±5.9	79.0±24.2	300.1±173.7	117.5±168.6
44	32	16	50.0	11.3±5.1	70.4±34.0	259.7±140.3	94.0±111.5
45	19	11	57.9	13.6±6.1	75.8±20.4	272.5±170.6	136.6±102.4
46	13	11	84.6	13.8±3.6	74.6±19.4	217.6±118.8	1.11±170.40
47	7	5	71.4	7.6±1.5	58.0±23.9	81.5±100.0	154.9±223.0
48	15	9	60.0	13.5±5.9	79.2±33.0	232.0±154.6	60.9±112.3
49	20	10	50	11.9±3.7	72.7±27.4	215.3±102.9	128.0
50	26	19	73.1	10.7±4.2	64.3±18.2	254.1±161.8	15.7±137.3
MEAN			67.6	11.9	72.2	212.9	58.3
SD			26.2	3.00	15.2	80.4	95.1

Table 34. Sapling performance of seedling derived from 50 seed trees after 1 growing season in the field of *Prunus cerasoides* D. Don.

tree no.	no. of planted	no. of survival	% survival	seedling RCD (mm)	seedling height (cm)	RRGR (% year^{-1})	RHGR (% year^{-1})
1	20	17	85	9.3±3.3	111.6±27.5	231.4±122.0	162.1±110.0
2	11	9	81.8	7.8±2.3	111.8±25.9	170.3±111.5	180.4±85.4
3	8	3	37.5	10.7±2.2	156.3±6.4	242.2±147.4	292.3±19.2
4	20	16	80	8.1±2.7	110.9±24.4	180.1±107.8	172.4±102.0
5	20	14	70	8.8±3.5	109.6±31.1	207.4±132.3	179.1±113.4
6	2	2	100	8.0±4.2	112.5±60.1	293.5±31.1	270.3±71.8
7	19	15	78.9	8.3±2.7	119.1±28.8	214.3±138.4	206.3±95.3
8	16	13	81.3	9.1±3.1	117.0±27.1	343.9±163.1	256.2±108.9
9	20	15	75	6.3±2.1	90.9±45.7	256.5±129.6	182.3±172.2
10	20	17	85	8.7±4.1	110.5±32.7	211.2±161.7	141.4±110.7
11	20	18	90	9.1±3.1	118.1±33.3	260.4±136.3	196.9±94.3
12	20	13	65	8.1±3.1	111.5±33.9	246.1±132.0	228.6±79.4
13	20	18	90	10.1±3.3	121.0±33.3	286.1±124.5	195.2±105.1
14	6	6	100	9.5±3.2	111.8±33.1	171.9±95.0	152.5±149.1
15	19	16	84.2	11.7±3.7	127.9±32.4	260.4±120.6	207.2±128.2
16	11	10	90.9	6.5±2.5	91.4±26.0	110.3±100.8	79.9±135.7
17	18	14	77.8	8.1±2.0	114.1±31.1	165.9±85.5	138.8±127.9
18	13	11	84.6	8.1±2.2	108.4±33.0	186.5±136.6	126.6±150.3
19	20	17	85	8.8±2.2	115.8±33.6	244.5±130.0	200.0±190.2
20	9	7	77.8	7.8±1.9	107.1±8.0	237.3±123.8	248.8±63.3
21	19	17	89.5	10.0±4.3	122.2±32.7	219.4±155.6	195.6±80.4
22	20	15	75	9.3±2.3	125.1±37.2	190.5±76.7	193.2±128.1
23	20	14	70	9.2±3.3	106.8±29.2	204.7±112.8	122.4±107.9
24	20	17	85	8.3±3.5	110.1±31.8	186.9±136.0	155.5±99.9
25	20	17	85	9.7±2.4	129.1±33.9	183.2±111.9	174.7±115.6
26	19	18	94.7	9.9±3.1	125.7±32.5	215.4±128.6	199.9±109.0
27	20	15	75	9.0±3.3	111.5±35.7	217.9±147.0	180.4±137.0
28	5	4	80	6.5±2.9	89.5±53.1	94.6±63.8	77.6±144.8
29	20	16	80	7.5±2.9	98.8±38.6	120.1±104.1	137.0±139.0
30	20	18	90	10.0±2.3	120.9±16.2	248.3±101.7	218.3±90.4
31	20	19	95	7.8±3.0	110.6±31.7	151.3±131.3	162.8±102.5
32	20	17	85	8.6±3.9	92.5±26.3	208.3±151.4	117.4±72.5
33	20	16	80	8.8±4.0	98.4±33.0	256.2±179.5	160.5±127.6
34	20	16	80	7.5±3.5	90.5±44.5	188.8±167.0	145.9±158.0
35	20	19	95	7.4±2.9	100.9±31.9	187.5±137.9	195.5±170.6
36	20	12	60	8.3±4.4	97.2±30.1	254.7±181.5	219.4±104.6
37	14	12	85.7	8.6±3.2	101.9±34.0	228.9±146.7	155.9±117.5
38	20	17	85	7.9±3.1	98.0±31.9	232.7±156.9	168.5±81.9
39	20	16	80	9.2±1.8	114.8±23.0	247.1±84.9	199.9±87.0
40	20	18	90	9.7±2.9	124.4±24.0	265.0±104.8	233.8±92.0
41	19	9	47.4	8.1±2.6	102.3±27.4	116.1±79.8	144.9±112.8
42	20	16	80	9.1±3.5	118.5±23.9	175.6±117.0	211.1±74.7
43	19	16	84.2	7.5±3.2	103.1±28.9	149.6±116.1	151.5±128.0
44	20	18	90	8.8±3.6	105.3±37.3	268.8±157.5	172.7±134.0
45	20	17	85	7.3±2.9	88.9±22.8	225.7±170.4	131.8±59.4
46	14	11	78.6	8.8±3.4	117.7±31.9	247.3±160.3	245.1±97.5
47	20	17	85	7.2±3.7	87.9±36.8	207.9±142.4	140.3±140.2
48	20	17	85	8.4±3.2	108.6±32.1	202.0±137.4	161.2±117.0
49	14	10	71.4	8.0±3.2	113.0±25.3	211.2±157.8	205.5±112.0
50	20	19	95	9.1±3.2	107.7±31.7	201.6±107.1	152.8±99.3
MEAN			81.6	8.6	110.0	212.6	179.0
SD			11.5	1.1	12.8	48.7	44.3

APPENDIX IV: ANALYSIS OF ONE-WAY ANOVA

Table 35. Summary of the one-way ANOVA on pyrene characteristics of *Spondias axillaris* Roxb.

		Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	3370.230	40	84.256	56.993	.000
	Within Groups	4303.479	2911	1.478		
	Total	7673.709	2951			
pyrene width	Between Groups	2625.745	40	65.644	86.747	.000
	Within Groups	2202.823	2911	0.757		
	Total	4828.569	2951			
pyrene thickness	Between Groups	2313.095	40	57.827	78.552	.000
	Within Groups	2142.981	2911	0.736		
	Total	4456.076	2951			
pyrene wet mass	Between Groups	496.494	40	12.412	71.102	.000
	Within Groups	508.179	2911	0.175		
	Total	1004.673	2951			

Table 36. Summary of the one-way ANOVA on seed characteristics of *Melia toosendan* Sieb. & Zucc.

		Sum of Squares	df	Mean Square	F	Sig.
seed length	Between Groups	1408.215	49	28.739	54.070	0.000
	Within Groups	1769.936	3330	0.532		
	Total	3178.1518	3379			
seed width	Between Groups	160.159	49	3.269	43.079	0.000
	Within Groups	252.657	3330	0.076		
	Total	412.817	3379			
seed thickness	Between Groups	78.962	49	1.611	26.399	0.000
	Within Groups	203.272	3330	0.061		
	Total	282.234	3379			
seed wet mass	Between Groups	1.207	49	0.025	38.950	0.000
	Within Groups	2.107	3330	0.0006		
	Total	3.314	3379			

Table 37. Summary of the one-way ANOVA on pyrene characteristics of *Gmelina arborea* Roxb.

		Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	4012.310	48	83.590	63.367	.000
	Within Groups	4512.793	3421	1.319		
	Total	8525.103	3469			
pyrene width	Between Groups	1306.987	48	27.229	69.278	.000
	Within Groups	1344.574	3421	0.393		
	Total	2651.560	3469			
pyrene thickness	Between Groups	1294.961	48	26.978	66.0125	.000
	Within Groups	1398.112	3421	0.409		
	Total	2693.073	3469			
pyrene wet mass	Between Groups	33.387	48	0.696	66.177	.000
	Within Groups	35.946	3420	0.011		
	Total	69.332	3468			

Table 38. Summary of the one-way ANOVA on seed characteristics of *Prunus cerasoides* D. Don.

		Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	1935.169	49	39.493	183.711	0.000
	Within Groups	756.282	3518	0.215		
	Total	2691.451	3567			
pyrene width	Between Groups	929.895	49	18.977	148.306	0.000
	Within Groups	450.168	3518	0.128		
	Total	1380.064	3567			
pyrene thickness	Between Groups	578.057	49	11.797	140.777	0.000
	Within Groups	294.807	3518	0.084		
	Total	872.865	3567			
pyrene wet mass	Between Groups	22.291	49	0.455	322.106	0.000
	Within Groups	4.969	3518	0.001		
	Total	27.260	3567			

Table 39. Summary of the one-way ANOVA on seed characteristics of *Castanopsis acuminatissima* (Bl.) A. DC.

		Sum of Squares	df	Mean Square	F	Sig.
seed length	Between Groups	2257.787	49	46.077	102.914	0.000
	Within Groups	1589.428	3550	0.448		
	Total	3847.215	3599			
seed width	Between Groups	2836.600	49	57.890	132.967	0.000
	Within Groups	1545.568	3550	0.435		
	Total	4382.168	3599			
seed thickness	Between Groups	2335.908	49	47.672	126.939	0.000
	Within Groups	1332.816	3549	0.376		
	Total	3668.724	3598			
seed wet mass	Between Groups	74.992	49	1.530	120.826	0.000
	Within Groups	44.827	3539	0.013		
	Total	119.819	3588			

Table 40. Differences in pyrene characteristics between pyrene that germinated and those that did not of *Spondias axillaris* Roxb.

		germinate	non-germinate	Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	18.535±1.552	18.581±1.651	1.530	1	1.530	0.588	0.443
	Within Groups			7672.179	2950	2.601		
	Total			7673.709	2951			
pyrene width	Between Groups	14.358±1.360	14.367±1.223	0.0623	1	0.062	0.038	0.845
	Within Groups			4828.507	2950	1.637		
	Total			4828.569	2951			
pyrene thickness	Between Groups	13.974±1.295	13.988±1.184	0.12485	1	0.125	0.083	0.774
	Within Groups			4455.951	2950	1.510		
	Total			4456.076	2951			
pyrene wet mass	Between Groups	3.093±0.609	3.150±0.565	2.283	1	2.283	6.718	0.0095
	Within Groups			1002.390	2950	0.340		
	Total			1004.673	2951			

Table 41. Differences in seed characteristics between seeds that germinated and those that did not of *Melia toosendan* Sieb. & Zucc.

		germinate	not germinate	Sum of Squares	df	Mean Square	F	Sig.
seed length	Between Groups	10.683±0.972	10.496±0.959	29.598	1	29.598	31.755	0.000
	Within Groups			3148.553	3378	0.932		
	Total			3178.151	3379			
seed width	Between Groups	3.702±0.348	3.653±0.349	2.020	1	2.020	16.611	0.000
	Within Groups			410.797	3378	0.122		
	Total			412.817	3379			
seed thickness	Between Groups	2.692±0.279	2.680±0.298	0.131	1	0.131	1.572	0.210
	Within Groups			282.103	3378	0.084		
	Total			282.234	3379			
seed wet mass	Between Groups	0.805±0.030	0.801±0.033	0.0111	1	0.011	11.394	0.000
	Within Groups			3.303	3378	0.001		
	Total			3.314	3379			

Table 42. Differences in pyrene characteristics between seeds that germinated and those that did not of *Gmelina arborea* Roxb.

		germinate	non-germinate	Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	15.971±1.635	15.685±1.545	45.853	1	45.853	18.754	0.000
	Within Groups			8479.250	3468	2.445		
	Total			8525.103	3469			
pyrene width	Between Groups	8.739±0.883	8.855±0.871	7.634	1	7.634	10.013	0.002
	Within Groups			2643.926	3468	0.762		
	Total			2651.560	3469			
pyrene thickness	Between Groups	7.896±0.895	7.952±0.877	1.776	1	1.776	2.289	0.130
	Within Groups			2691.296	3468	0.776		
	Total			2693.073	3469			
pyrene wet mass	Between Groups	1.249±0.144	1.240±0.141	0.052	1	0.052	2.605	0.107
	Within Groups			69.280	3467	0.020		
	Total			69.332	3468			

Table 43. Differences in pyrene characteristics between pyrenes that germinated and those that did not of *Prunus cerasoides* D. Don.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	10.451±0.847	10.496±0.895	1.775	1	1.775	2.353	.125
	Within Groups			2689.676	3566	.754		
	Total			2691.451	3567			
pyrene width	Between Groups	7.523±0.604	7.587±0.642	3.334	1	3.334	8.635	.003
	Within Groups			1376.730	3566	.386		
	Total			1380.064	3567			
pyrene thickness	Between Groups	6.089±0.505	6.131±0.480	1.560	1	1.560	6.385	.012
	Within Groups			871.304	3566	.244		
	Total			872.865	3567			
pyrene	Between Groups	0.976±0.087	0.975±0.088	0.0004	1	0.0004	.056	.813
pyrene wet mass	Between Groups	0.976±0.087	0.975±0.088	0.0004	1	0.0004	.056	.813
wet mass	Within Groups			27.259	3566	0.0076		
wet mass	Within Groups			27.259	3566	0.0076		
	Total			27.260	3567			
	Total			27.260	3567			

Table 44. Differences in seed characteristics between seeds that germinated and those that did not of *Castanopsis acuminatissima* (Bl.) A. DC.

		germinate	non-germinate	Sum of Squares	df	Mean Square	F	Sig.
seed length	Between Groups	10.096±1.013	9.858±1.091	33.336	1	33.336	31.449	0.000
	Within Groups			3813.879	3598	1.060		
	Total			3847.215	3599			
seed width	Between Groups	9.190±1.058	8.867±1.232	61.144	1	61.144	50.913	0.000
	Within Groups			4321.023	3598	1.201		
	Total			4382.168	3599			
seed thickness	Between Groups	8.535±0.975	8.245±1.107	49.307	1	49.307	49.001	0.000
	Within Groups			3619.417	3597	1.006		
	Total			3668.724	3598			
seed wet mass	Between Groups	1.249±0.177	1.169±0.190	3.725	1	3.725	115.090	0.000
	Within Groups			116.094	3587	0.032		
	Total			119.819	3588			

Table 45. Differences in pyrene characteristics between seedlings that survived and those that died in nursery of *Spondias axillaris* Roxb.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	18.484±1.560	18.775±1.501	14.278	1	14.278	5.945	0.015
	Within Groups			2807.813	1169	2.402		
	Total			2822.091	1170			
pyrene width	Between Groups	14.324±1.345	14.514±1.428	6.071	1	6.071	3.284	0.070
	Within Groups			2161.168	1169	1.849		
	Total			2167.240	1170			
pyrene thickness	Between Groups	13.937±1.267	14.151±1.412	7.747	1	7.747	4.629	0.032
	Within Groups			1956.349	1169	1.674		
	Total			1964.096	1170			
pyrene wet mass	Between Groups	3.063±0.604	3.213±0.620	3.576	1	3.576	9.703	0.002
	Within Groups			430.768	1169	0.368		
	Total			434.344	1170			
time to germina-tion	Between Groups	84.795±52.910	99.463±57.468	36386.360	1	36386.360	12.602	0.000
	Within Groups			3375268.392	1169	2887.313		
	Total			3411654.751	1170			

Table 46. Differences in seed characteristics between seedlings that survived and those that died in nursery of *Melia toosendan* Sieb. & Zucc.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
seed length	Between Groups	10.796±0.953	10.586±22.056	18.541	1	18.541	19.853	0.000
	Within Groups			1572.719	1684	0.934		
	Total			1591.260	1685			
seed width	Between Groups	3.731±0.352	3.676±0.343	1.259	1	1.259	10.443	0.001
	Within Groups			202.954	1684	0.121		
	Total			204.213	1685			
seed thickness	Between Groups	2.699±0.284	2.689±0.275	0.061	1	0.061	0.780	0.377
	Within Groups			131.294	1684	0.078		
	Total			131.354	1685			
seed wet mass	Between Groups	0.809±0.026	0.803±0.029	0.0147	1	0.015	17.301	0.000
	Within Groups			1.430	1684	0.001		
	Total			1.445	1685			
time to germina--tion	Between Groups	54.209±22.654	58.830±22.056	8356.949	1	8356.949	16.760	0.000
	Within Groups			785855.877	1576	498.640		
	Total			794212.826	1577			

Table 47. Differences in pyrene characteristics between seedlings that survived and those that died in nursery of *Gmelina arborea* Roxb.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	15.990±1.681	15.906±1.468	0.871	1	0.871	0.326	0.568
	Within Groups			1883.788	704	2.676		
	Total			1884.659	705			
pyrene width	Between Groups	8.727±0.890	8.779±0.861	0.323	1	0.323	0.413	0.520
	Within Groups			549.756	704	0.781		
	Total			550.079	705			
pyrene thickness	Between Groups	7.912±0.903	7.842±0.870	0.613	1	0.613	0.765	0.382
	Within Groups			564.366	704	0.802		
	Total			564.980	705			
pyrene wet mass	Between Groups	1.251±0.146	1.244±0.136	0.007	1	0.007	0.331	0.566
	Within Groups			14.559	704	0.021		
	Total			14.566	705			
time to germina--tion	Between Groups	13.565±4.902	14.008±5.717	19.484	1	19.484	0.760	0.384
	Within Groups			16568.294	646	25.648		
	Total			16587.778	647			

Table 48. Differences in pyrene characteristics between seedlings that survived and those that died in nursery of *Prunus cerasoides* D. Don.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	10.470±0.837	10.395±0.874	2.087	1	2.087	2.913	.088
	Within Groups			1419.074	1981	.716		
	Total			1421.161	1982			
seed width	Between Groups	7.534±0.611	7.500±0.584	.424	1	.424	1.163	.281
	Within Groups			723.080	1981	.365		
	Total			723.504	1982			
pyrene thickness	Between Groups	6.089±0.507	6.088±0.502	2.022E-04	1	2.022E-04	.001	.978
	Within Groups			506.127	1981	.255		
	Total			506.127	1982			
pyrene wet mass	Between Groups	0.976±0.089	0.976±0.087	7.174E-06	1	7.174E-06	.001	.975
	Within Groups			14.944	1981	7.543E-03		
	Total			14.944	1982			
time to germina--tion	Between Groups	30.359±11.014	35.066±13.048	7463.287	1	7463.287	56.107	.000
	Within Groups			245820.847	1848	133.020		
	Total			253284.135	1849			

Table 49. Differences in seed characteristics between seedlings that survived and those that died in nursery of *Castanopsis acuminatissima* (Bl.) A. DC.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
seed length	Between Groups	10.105±0.998	10.063±1.062	0.859	1	0.859	0.838	0.360
	Within Groups			2937.044	2865	1.025		
	Total			2937.903	2866			
seed width	Between Groups	9.183±1.083	9.215±1.122	0.528	1	0.528	0.473	0.492
	Within Groups			3202.214	2865	1.118		
	Total			3202.743	2866			
seed thickness	Between Groups	8.532±0.964	8.541±1.011	0.035	1	0.035	0.037	0.847
	Within Groups			2717.288	2864	0.949		
	Total			2717.323	2865			
seed wet mass	Between Groups	1.249±0.174	1.248±0.188	0.001	1	0.001	0.041	0.840
	Within Groups			89.900	2862	0.031		
	Total			89.901	2863			
time to germina--tion	Between Groups	37.997±13.365	38.191±13.669	17.273	1	17.273	0.096	0.757
	Within Groups			502921.777	2789	180.323		
	Total			502939.050	2790			

Table 50. Differences in pyrene characteristics between saplings which survival in the field and those which died of *Spondias axillaris* Roxb.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	18.487±1.601	18.531±1.576	0.232	1	0.232	0.091	0.763
	Within Groups			1840.680	723	2.546		
	Total			1840.912	724			
pyrene width	Between Groups	14.375±1.346	14.324±1.375	0.322	1	0.322	0.176	0.675
	Within Groups			1321.823	723	1.828		
	Total			1322.145	724			
pyrene thickness	Between Groups	14.005±1.259	13.939±1.276	0.533	1	0.533	0.334	0.563
	Within Groups			1153.382	723	1.595		
	Total			1153.915	724			
pyrene wet mass	Between Groups	3.084±0.603	3.109±0.601	0.077	1	0.077	0.212	0.645
	Within Groups			262.458	723	0.363		
	Total			262.535	724			
time to germina-tion	Between Groups	72.099±48.461	80.919±53.892	9054.116	1	9054.116	3.670	0.056
	Within Groups			1704509.677	691	2466.729		
	Total			1713563.792	692			

Table 51. Differences in seed characteristics between saplings which survival in the field and those which died of *Melia toosendan* Sieb. & Zucc..

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
seed length	Between Groups	10.880±0.980	10.740±0.948	3.584	1	3.584	3.891	0.048
	Within Groups			714.773	776	0.921		
	Total			718.357	777			
seed width	Between Groups	3.763±0.348	3.710±0.355	0.514	1	0.514	4.135	0.042
	Within Groups			96.422	776	0.124		
	Total			96.936	777			
seed thickness	Between Groups	2.716±0.283	2.689±0.285	0.134	1	0.134	1.646	0.200
	Within Groups			62.956	776	0.081		
	Total			63.089	777			
seed wet mass	Between Groups	0.810±0.028	0.808±0.025	0.0003	1	0.0003	0.539	0.463
	Within Groups			0.529	776	0.0006		
	Total			0.530	777			
time to germina-tion	Between Groups	53.106±23.119	54.873±22.427	526.974	1	526.974	1.023	0.312
	Within Groups			367198.824	713	515.005		
	Total			367725.799	714			

Table 52. Differences in seed characteristics between saplings which survival in the field and those which died of *Gmelina arborea* Roxb.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	16.052±1.696	15.910±1.590	2.583	1	2.583	0.938	0.333
	Within Groups			1558.964	566	2.754		
	Total			1561.547	567			
pyrene width	Between Groups	8.753±0.911	8.660±0.829	1.106	1	1.106	1.416	0.235
	Within Groups			442.057	566	0.781		
	Total			443.163	567			
pyrene thickness	Between Groups	7.956±0.911	7.8077±0.871	2.869	1	2.869	3.564	0.060
	Within Groups			455.661	566	0.805		
	Total			458.530	567			
pyrene wet mass	Between Groups	1.254±0.144	1.237±0.140	0.039	1	0.039	1.891	0.170
	Within Groups			11.527	566	0.020		
	Total			11.566	567			
tine to germination	Between Groups	13.390±4.828	13.654±4.908	8.450	1	8.450	0.358	0.550
	Within Groups			12542.862	532	23.577		
	Total			12551.313	533			

Table 53. Differences in pyrene characteristics between saplings which survival in the field and those which died of *Prunus cerasoides* D. Don.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
pyrene length	Between Groups	10.498±0.844	10.568±0.836	0.659	1	0.659	0.928	0.336
	Within Groups			619.487	873	0.710		
	Total			620.146	874			
pyrene width	Between Groups	7.547±0.601	7.632±0.631	0.945	1	0.945	2.567	0.110
	Within Groups			321.493	873	0.368		
	Total			322.438	874			
pyrene thickness	Between Groups	6.110±0.500	6.163±0.486	0.370	1	0.370	1.479	0.224
	Within Groups			218.440	873	0.250		
	Total			218.810	874			
pyrene wet mass	Between Groups	0.975±0.087	0.980±0.087	0.002	1	0.002	0.322	0.571
	Within Groups			6.657	873	0.008		
	Total			6.659	874			
time to germination	Between Groups	29.873±11.014	32.386±12.354	788.085	1	788.085	6.203	0.013
	Within Groups			105447.356	830	127.045		
	Total			106235.441	831			

Table 54. Differences between saplings that survived and those that died in terms of root collar diameter and height of saplings at planting time of *Spondias axillaris* Roxb..

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
saplings height	Between Groups	63.13±24.46	53.97±25.46	10033.980	1	10033.98	16.483	0.00
	Within Groups			431613.595	709	608.76		
	Total			441647.575	710			
saplings RCD	Between Groups	3.32±1.28	2.84±1.24	28.088	1	28.09	17.375	0.00
	Within Groups			1146.149	709	1.62		
	Total			1174.237	710			

Table 55. Differences between saplings that survived and those that died in terms of root collar diameter and height of saplings at planting time of *Melia toosendan* Sieb. & Zucc.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
sapling RCD	Between Groups	3.32±0.87	2.89±0.82	34.3761	1	34.376	48.480	0.00
	Within Groups			535.351	755	0.709		
	Total			569.729	756			
sapling height	Between Groups	47.94±14.01	42.12±14.51	6273.351	1	6273.351	30.685	0.00
	Within Groups			154354.972	755	204.444		
	Total			160628.324	756			

Table 56. Differences between saplings that survived and those that died in terms of root collar diameter and height of saplings at planting time of *Gmelina arborea* Roxb.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
saplings RCD	Between Groups	6.60±2.07	5.89±1.93	59.421	1	59.421	14.530	0.000
	Within Groups			2114.252	517	4.089		
	Total			2173.673	518			
saplings height	Between Groups	74.78±24.23	69.92±24.61	2770.035	1	2770.035	4.668	0.031
	Within Groups			306822.755	517	593.468		
	Total			309592.790	518			

Table 57. Differences between saplings that survived and those that died in terms of root collar diameter and height of saplings at planting time of *Prunus cerasoides* D. Don.

		survival	dead	Sum of Squares	df	Mean Square	F	Sig.
saplings RCD	Between Groups	4.29±1.24	3.90±1.30	19.665	1	19.665	12.623	0.000
	Within Groups			1294.540	831	1.558		
	Total			1314.205	832			
saplings height	Between Groups	83.46±28.07	79.76±29.89	1711.817	1	1711.817	2.121	0.146
	Within Groups			670804.980	831	807.226		
	Total			672516.797	832			

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