

Symposium Proceedings



IUFRO Tree Seed Symposium: Recent Advances in Seed Research and *Ex Situ* Conservation

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**IUFRO Tree Seed Symposium:
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Ex Situ Conservation**

August 16 – 18, 2010 Taipei, Taiwan

Symposium Editors

Dr. Ching-Te Chien & Dr. Fen-Hui Chen

Taiwan Forestry Research Institute

English Editor

Daniel P. Chamberlin

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TABLE OF CONTENTS

III	Opening Address	<i>Yue-Hsing Star Huang</i>
IV	Welcome Address	<i>Tannis Beardmore</i>
V	Congratulatory Address	<i>Carol C. Baskin</i>
1	Biogeography and Phylogeny of Seed Dormancy and Nondormancy in Trees	<i>Carol C. Baskin, Jerry M. Baskin</i>
11	The Role and Future Challenges of <i>Ex Situ</i> Gene Conservation Approaches for Forest Tree Genetic Resources	<i>Alvin D. Yanchuk</i>
21	<i>Ex Situ</i> Conservation Activities in Mexico: Challenges and Successes	<i>Javier Lopez-Upton</i>
27	<i>Ex Situ</i> Conservation of Tree Seeds: A Canadian Perspective	<i>Tannis Beardmore, Dale Simpson</i>
37	Germination Responses of <i>Terminalia ivorensis</i> Seeds to a Range of Alternating and Constant Temperatures Provided by the Two-Way Grant's Thermogradient Plate	<i>Joseph M. Asomaning, Moctar Sacande</i>
49	High-Temperature Effects on Seed Germination in <i>Shorea balangeran</i> , a Tropical Peat Swamp Tree in Central Kalimantan, Indonesia	<i>Tomoya Inada, Hideyuki Saito, Sampang Gaman, Takashi Inoue, Limin Suwido, Masato Shibuya, Takayoshi Koike</i>
55	Dormancy and Storage Behavior of Seeds of Thirty Tropical Fabaceae Tree Species from Sri Lanka	<i>K.M.G. Gehan Jayasuriya, Asanga S.T.B. Wijetunga, Jerry M. Baskin, Carol C. Baskin,</i>
59	<i>Ex Situ</i> Conservation Issues Relevant to the International Seed Testing Association (ISTA)	<i>Zdenka Prochazkova</i>
67	Status of <i>Ex Situ</i> Conservation of Forest Tree Germplasm in Seed Banks in Africa, Asia, and Latin America	<i>Judy Loo, Riina Jalonen, Jesus Salcedo</i>
75	Component Analysis of Acorns of <i>Quercus mongolica</i> and <i>Q. variabilis</i>	<i>Hyun-seok Lee, Chan-hoon An, Chang-soo Kim, Sang-Urk Han, Tae-heum Shim, Hyeok-Hwa Lee, Jae-Hoon Sa, Jae-seon Yi</i>
81	Desiccation Sensitivity of <i>Antiaris toxicaria</i> Axes and Reactive Oxygen Species-Scavenging Enzymes in Washed Mitochondria	<i>Hong-Yan Cheng, Song-Quan Song,</i>
83	Storage Conditions for Prolonging the Seed Viability for <i>Ex Situ</i> Conservation and Deteriorative Changes Associated with Viability Loss in <i>Dalbergia sissoo</i> Seeds	<i>Geeta Joshi, RC Thapliyal, SS Phartiyal, JS Nayal</i>
93	<i>Ex Situ</i> Conservation of Trees and Seeds in Taiwan	<i>Ching-Te Chien</i>
103	Timing of Seed Germination and Life History of Trees: Case Studies from Greece	<i>Costas A. Thanos, Christine Fournaraki, Achilleas Tsiroukis, Petros Panayiotopoulos</i>

113	Moist Chilling and Dormancy of Eastern White Pine (<i>Pinus strobus</i> L.) Seeds <i>Ben S.P. Wang, J. Dale Simpson, Bernard I. Daigle</i>
119	Seed Source Variations in Cone and Seed Traits in Three Himalayan Pines <i>Manisha Thapliyal, Ombir Singh, R.C. Thapliyal</i>
129	Desiccation Tolerance and Storage Response of <i>Bassia latifolia</i> Roxb. Seeds <i>Maitreyee Kundu, Rupnarayan Sett</i>
137	Dynamics of Imbibition, Seed Germination, and Seedling Development of Austrian Pine (<i>Pinus nigra</i> Arnold) from Populations Growing in Contrasting Habitats of Southeastern Europe <i>Milan Mataruga, Diane L. Haase, Vasilije Isajev, Yu-Jen Lin</i>
153	Seed Dormancy and Hydrotime Model for Seed Populations in Two Habitats of an Invasive Fabaceae Species, <i>Prosopis juliflora</i> <i>B.L. Ganesha Sanjeevani, K.M.G. Gehan Jayasuriya, J.H.L.D.H.C. Jayasinghe</i>
165	Seed Mycoflora, and Physicochemical and Biochemical Changes in Tree Seeds during Storage <i>V. RavishankarRai, T. Mamatha</i>
185	Effects of Light, Temperature and Applied Chemicals on Laboratory Germination of <i>Pellacalyx yunnanensis</i> Seeds <i>Hong-Yan Cheng, Hui-Ying He, Song-Quan Song,</i>
187	Efficacy of a Microbial Consortium on <i>Acacia nilotica</i> (L.) Willd. ex. Del Seeds for the Production of Quality Seedlings in the Nursery <i>Poonam Dubey, R. K. Verma</i>
201	Genetic Diversity of Mahogany for Mitigation and Adaptation to Climate Change <i>Carlos Navarro, Meryll Arias, Fernando Mora</i>
203	The Pacific Decadal Oscillation and Weevils Influence Acorn Production and Germination in the Endemic Evergreen Oak, <i>Cyclobalanopsis glaucoides</i> <i>Ke Xia, Roy Turkington, Zhe-kun Zhou</i>
205	Early Ovule Development of Taiwanese Yew (<i>Taxus sumatrana</i>) <i>Yan-Yow Lin, Ling-Long Kuo-Huang, Ching-Te Chien</i>
207	Assessment of Seed Distribution, Dissemination, and Diffusion Pathways of Priority Tree Plantation Species in the Philippines <i>Marcelino U. Siladan, Enrique L. Tolentino, Jr., James M. Roshetko, Wilfredo M. Carandang, Roberto G. Visco, Juan M. Pulhin</i>
217	Forest Seed Science Research in India <i>G.S. Rawat, Manisha Thapliyal, Geeta Joshi, A.N. Arun Kumar</i>
239	Pretreatment to Enhance Germination of Seeds of <i>Diospyros melanoxylon</i> Roxb. <i>Geeta Joshi, Arun Kumar</i>
241	Advances in Precision Seed Quality Assessment in Conifer Nurseries <i>Robert F. Keefe, Anthony S. Davis</i>
243	Seed Longevity and Deterioration in Orthodox Seeds: A Perspective Based on Structural Stability of Visco-Elastic Materials <i>Christina Walters</i>

Opening Address

Yue-Hsing Star Huang
Director General
Taiwan Forestry Research Institute
Taiwan, R.O.C.

Distinguished guests, ladies and gentlemen,

It is my honor and pleasure to welcome you to the IUFRO Tree Seed Symposium in Taiwan: Recent advances in seed research and *ex situ* conservation.

Forests cover 58% of the land area of Taiwan, and forest zones range from high elevation temperate to middle to low elevation subtropical to tropical on the southern tip of the island. These forests are the most precious and important resource of the country. Taiwan has been called “Formosa,” which means the beautiful island with dense forests. Forests influence watersheds, climate, wildlife habitats, *etc.* However, we certainly have seen some negative impacts on our forests from recent disasters such as floods and landslides; and even from climate change. Such impacts, especially those from climatic change, probably will become worse in time, and thus we must plan for solutions to them, now.

Ex situ plantations of some important species for genetic resources conservation were established in Taiwan in the 1950's. These stands promote public awareness of the importance of forests in Taiwan and serve as the basis for academic research and scientific forest management. The Tree Seed Bank at the Taiwan Forestry Research Institute has stored seeds of woody plants for long-term preservation, research and exchange with other countries. Overall, we need this effort in order to increase the quantity of healthy forests.

We believe that the relationship of forests to climatic change is significant, for example, *via* carbon sequestration. We hope this symposium will benefit future generations in terms of advancing our understanding of climatic change and of how different species of trees are adapted to it. Choosing which species to conserve, for example, through long-term seed storage, is important for maintaining genetic diversity.

Finally, I would like to express my appreciation to Dr. Tannis Beardmore of the Canadian Forest Service – Atlantic Region, Hugh John Fleming of the Forestry Centre, New Brunswick, Canada, and Dr. Ching-Te Chien and his colleagues of the Taiwan Forestry Research Institute for their hard work in organizing this symposium, which I hope all participants will enjoy. Let us work together to advance our knowledge of the biology of tree seeds and their role in *ex situ* conservation of the forests of Taiwan and the world.

Please enjoy your stay in Taiwan, and I hope you will enjoy seeing some of our beautiful forests on the symposium field trips.

Welcome Address

Tannis Beardmore

Chair of the IUFRO Unit 2.09.03 Seed Physiology and Technology

Natural Resources Canada, Canadian Forest Service- Atlantic Forestry Centre

1350 Regent St. S. / PO Box 4000

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New Brunswick Canada

Good morning and welcome to the 2010 Seed Symposium addressing advances in seed research and *ex situ* conservation.

I would first like to thank a number of organizations for making this meeting possible including, Taiwan Forestry Research Institute, Reforestation Association Republic of China, National Science Council, Forest Bureau, NTU Experimental Forest and Ministry of Foreign Affairs, Republic of China. In particular, I would like to thank Dr. Chien for all his efforts that went in to organizing this event, and I would like to thank the organizing committee.

The IUFRO “Seed Physiology and technology” working group is one of IUFRO’s older working groups. It was formed following a meeting in Finland in 1970, where interest was expressed in having a group which addressed seed physiology. The first meeting for this new working group was in 1973 in Norway and since then there have been meetings every two to three years across in locations all over the world.

The topics of the meetings have been diverse focusing on issues and areas of research that were topical at the time, many of which are still very relevant. These meetings have focused on seed processing, dormancy, issues unique to tropical seeds and nursery technology. This is the first meeting this group has had which focuses on *ex situ* conservation. *Ex situ* conservation has probably never been as important as it is today, with many of the species are in danger of extinction, threatened by habitat transformation, over-exploitation, alien invasive species, pollution and climate change. The disappearance of such vital and large amounts of biodiversity poses one of the greatest challenges for the world community.

We have a unique opportunity with so many excellent experts in seed research and in *ex situ* conservation attending this meeting for sharing and generating new ideas. I would like to thank everyone for taking the time to attend this meeting.

Congratulatory Address

Carol C. Baskin
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From the equatorial lowlands to tree line on high mountains and at high latitudes, trees account for most of the biomass in various kinds of plant communities. Trees have long been important for successful human habitation and well-being in many regions on earth. From trees, man has obtained building materials, fuel, food and medicines, resulting all too often in over-exploitation and destruction of forests. Increasingly, forests are destroyed/removed so the land can be used for agriculture, including pastures, and for growth of cities. Also, many hectares of forests are removed annually to facilitate various kinds of mining operations and to expand our global transportation network.

In many countries, there now is an increasing awareness of the need to replant/restore forests, if we are to have sustainable supplies of wood and other forest products and to conserve the rich diversity of tree and other species found in forest ecosystems. However, replanting a high-diversity forest is difficult, and there are many problems related to tree seeds that need to be solved to accomplish this task.

First, seeds of some economically-important trees in many parts of the world, especially the tropics (*e.g.*, the dipterocarps) are desiccation-sensitive and thus cannot be dried below a certain relatively high water content without loss of viability. Thus, development of methods for *ex situ* conservation of these species is urgently needed. Further, although seeds of many trees are desiccation-tolerant, they are dormant, *i.e.* there is a long delay in germination. Clearly, for these species knowledge of dormancy breaking and germination requirements would enhance efforts to propagate the species. True, seeds of some species are nondormant and germinate relatively easy; however, unless appropriate soil moisture and light conditions are available few or none of the seedlings survive.

In many countries, people working for research institutions, universities, mining companies, government agencies and conservation groups are attempting to answer questions related to seed storage, dormancy break and germination and to the successful establishment of seedlings of many tree species. Also, much work is being done to find ways to replant diversity-rich forests.

The 2010 IUFRO Tree Seed Symposium in Taipei, Taiwan, is being attended by people from 17 countries. We are united by our common desire to help solve problems and make new discoveries about tree seeds. There is always a sense of excitement when scientists come together at a meeting, for example, this symposium. Here, we can meet old friends, make new friends and surely we will learn many new things about tree seeds. The new information we obtain will no doubt stimulate much additional research on tree seeds. Also, new collaborative efforts for solving problems related to forest tree seeds

and forest conservation will be initiated from our interactions with each other at this symposium.

We congratulate each of you on your devotion to science and in particular your contributions to our knowledge of tree seeds. We also congratulate you on your desire to help answer the questions about tree seeds that must be resolved if we are to ultimately find ways to replant diversity-rich forests. Finally, please join us in extending congratulates to Tannis Beardmore and Ching-Te Chien for organizing this very important symposium, to Director General Yue-Hsing Star Huang and to Ching-Te Chien and his colleagues at the Taiwan Forestry Research Institute for all of their hard work in serving as the host for the symposium.



Biogeography and Phylogeny of Seed Dormancy and Nondormancy in Trees

Carol C. Baskin,^{1,2,3)} Jerry M. Baskin¹⁾

[Summary]

Seed dormancy in relation to the timing of germination for successful seedling establishment is an adaptation of a species to its habitat. Given that seeds are either nondormant (ND) or have one of the five classes of dormancy, we asked how the classes of dormancy (and ND) are distributed geographically and phylogenetically among trees worldwide. Seed dormancy data were compiled for 5084 species of trees growing in 13 major vegetation zones on earth (for which we have data) and used to evaluate the world biogeography and phylogenetic position of the five classes of dormancy (and ND). Seed dormancy profiles for six tropical and seven temperate vegetation zones revealed that ND accounts for 14%-53% of the species, with evergreen rainforest (48%) and sclerophyllous woodlands (53%) having the highest percentages. In the tropics, the importance of physical dormancy (PY) increases and physiological dormancy (PD) decreases with decrease in rainfall. In the temperate region, PD is consistently high (35%-71%) and PY consistently low (0%-9%). Morphological dormancy (MD) and combinational dormancy (PY+PD) are not very important (<0.5%-9%) in any vegetation zone, and morphophysiological dormancy (MPD) is most important (15%-18%) in tropical evergreen rainforests and montane forests and in temperate broad-leaved evergreen forests. ND, MD, and MPD are widely distributed on the APG III phylogenetic diagram, but PY and (PY+PD) are restricted to the fabids and malvids. Dormancy profiles based on number of individuals of each species instead of number of species only will provide additional insight into seed dormancy/ND of species in various vegetation types, as illustrated with 95 of the 468 taxa in the Xishuangbanna tropical seasonal rainforest dynamics plot in southern Yunnan Province, P R. China.

Key words: biogeography, phylogeny, seed dormancy, trees, vegetation zones.

INTRODUCTION

Ecologists have long been aware of the important role that timing of seed germination plays in determining if seedlings become established (Ratcliffe 1961). Further, long-term persistence of a strictly sexually-reproducing species at a particular site depends on successful seedling establishment. Donohue (2005) nicely summarizes the importance of timing of germination as an adaptation of a species to its habitat: "Indeed, appropriate germination responses to environmental factors are the first requirement for successful growth and adaptation in any life-history trait; no subsequent life-history trait can even be expressed if the plant does not first survive past the

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germination stage.” Thus, control of the timing of germination is one aspect of the life history adaptation of a species to its habitat. Clearly, the control of timing of germination of a species in its habitat is complicated and includes maternal effects, season of seed maturation and dispersal, environmental conditions in the habitat between dispersal and germination, ability of seeds to tolerate desiccation, presence vs. absence of dormancy, how and when dormancy is broken and what environmental conditions are required for nondormant seeds to germinate (Baskin and Baskin 1998, Donohue 2009).

As a part of the challenge of understanding the germination ecology of individual species, one would determine if freshly-matured seeds were nondormant or dormant, and if seeds were dormant what kind of dormancy was present. There are five major kinds (or classes) of dormancy, and these will be described below. Studies on the germination ecology of individual species are numerous (Baskin and Baskin 1998), and they have served as a foundation for development of other questions. One early question was about the occurrence of different classes of dormancy among the species in a plant community, and the answer was that more than one class of seed dormancy is found in a plant community (Angewine and Chabot 1979). A second question is: in what proportions do the classes of dormancy (and nondormancy) occur in trees of the major vegetation zones on earth? It seems reasonable that a good understanding of the world biogeography of seed dormancy/nondormancy would enhance our understanding of how species are adapted to the various vegetation zones on earth.

Since the early 1990's, we have been compiling information, largely from the literature, on the absence/presence of dormancy and the classes of seed dormancy of trees, shrubs, vines, and herbaceous species growing in all the major vegetation zones on earth. This work has been done in an attempt to gain a better understanding of the biogeography, and also phylogeny, of seed dormancy. We have used Walter's (1979) map of the major vegetation zones of the world as the broad outline for organizing the biogeographical information. In the tropical and subtropical regions (hereafter called tropical), Walter recognized evergreen rainforests, tropical montane forests, semievergreen rainforests, deciduous forests, savannas, and hot deserts. In the temperate and arctic regions (hereafter called temperate), there are sclerophyllous woodlands, broad leaved evergreen forests, deciduous forests, steppes, boreal/subalpine forests, tundra, mountains (montane and woodland), and cold deserts. Since the focus of this conference is on trees, we will consider only tree seed dormancy/nondormancy here. As background, we have information for trees in all of Walter's vegetation zones, except cold deserts and tundra, and we now have information on seed dormancy/nondormancy for 5084 species of trees, which is ca. 8.5% of the total number of the estimated 60,000 species of trees on earth (Tudge 2005): 4047 from the tropics and 1037 from the temperate region. In this paper, we will examine the world biogeography of tree seed dormancy/nondormancy, using the information compiled for the 5084 species of trees. Also, in any investigation of the biological traits of a large number of species, it may be informative to evaluate phylogeny in relation to variations of the trait being considered. In our case, we have qualitatively evaluated the five classes of dormancy and nondormancy in relation to the phylogenetic position of the orders to which the 5084 species of trees belong.



NONDORMANCY AND CLASSES OF DORMANCY

Before discussing the world biogeography and phylogeny of tree seed dormancy/nondormancy, we need to define the dormancy-related terms that will be used. It is well recognized that seeds of some species are **nondormant** (ND) at the time of maturity and dispersal. These seeds have well developed embryos [i.e., they are fully-elongated and no additional growth inside the seed is required before germination (radicle emergence)], have water-permeable seed/fruit coats and will germinate in a relatively short period of time, often in only a few days after sowing/dispersal, but not more than about 30 days. If such seeds require more than about 30 days to germinate, they are considered to be dormant. Since seeds of some species with shallow dormancy can undergo considerable dormancy-break and begin to germinate after about 30 days (e.g., Baskin and Baskin 1977), 30 days has been selected as an arbitrary dividing line between ND and dormant seeds (Baskin and Baskin 1998). Thus, if at maturity, seeds have underdeveloped embryos (that must grow before the seed germinates), or if they have fully-developed embryos and require more than about 30 days to germinate they are said to be **dormant**. As mentioned above, there are five classes of dormancy. In the hierarchical seed dormancy classification system, class is the highest level (Baskin and Baskin 2004, 2008). In this system, classes are divided into levels and levels into types, but these subdivisions of dormancy will not be included in the present analysis of seed dormancy in trees. The five classes of dormancy will be described briefly.

In **physiological dormancy** (PD), the seed/fruit coat is water-permeable, and the embryo is fully developed. Seeds do not germinate because there is a “physiological inhibiting mechanism” (sensu Nikolaeva 1977) in the embryo that prevents it from generating enough growth potential to overcome the mechanical restriction of the seed coat and/or other covering layers. Dormancy break occurs at cool (about 0-10°C) wet, warm (about 15°C) wet, or warm dry conditions, depending on the species. After the embryo becomes ND, it has enough growth potential to push through all the layers surrounding it. In **morphological dormancy** (MD), the seed coat is water-permeable, but the embryo is underdeveloped (small and has a low embryo length :seed length ratio), meaning that it must grow inside the seed prior to radicle emergence. In these seeds, the delay in time of germination (dormancy) is the time required for embryo growth and germination. Depending on the species (and if seeds are exposed to suitable moisture, light/dark, temperature, and oxygen conditions), embryo growth and germination occur in about 7 to 30 days. In **morphophysiological dormancy** (MPD), seeds are water-permeable and have an underdeveloped embryo that also has PD. Thus, PD must be broken, either prior to, during, or after the period of embryo elongation, and the embryo must grow before the radicle emerges. In seeds/fruits with **physical dormancy** (PY), the seed/fruit coat is water-impermeable, and the embryo is fully developed. Impermeability is due to the presence of one or more palisade layers of lignified cells in the seed coat or in the endocarp of the fruit coat (pericarp). A specialized structure (“water plug” or “water gap”) in the impermeable seed or fruit coat becomes dislodged or disrupted (i.e., an opening forms) in response to environmental cues such as heat from fire, high temperatures, or alternating temperatures, thereby creating an entry point for water into the seed (Baskin et al. 2000). In **combinational dormancy** (PY+PD), the seed/fruit coat is water-impermeable, and the embryo is fully developed but has PD.

Depending on the species, PD is broken via afterripening (relatively slow breaking of PD under dry conditions) before PY is broken (e.g., winter annuals in Fabaceae and Geraniaceae), or PD is broken via cold stratification after PY is broken [e.g., *Cercis* spp. (Fabaceae) and *Ceanothus* spp. (Rhamnaceae)] (Baskin and Baskin 1998).

BIOGEOGRAPHY OF TREE SEED DORMANCY/NONDORMANCY

Dormancy profiles

For each of the 13 vegetation zones with trees, the percentage of species with ND seeds and with MD, MPD, PD, PY, and (PY+PD) was calculated (Table 1), resulting in a profile of tree seed dormancy/nondormancy in each zone. In the tropics, ND ranged from 48% in evergreen rainforests to 14% in hot deserts. PD was the most important class of dormancy in evergreen rainforest, montane forests, semievergreen forests, and deciduous forests, and PY was the most important class of dormancy in savannas and hot deserts. MD and (PY+PD) were not very important in any tropical vegetation zone. MPD occurred in all tropical vegetation zones, including hot deserts, but it was most important in evergreen rainforests (15%) and montane forests (18%).

Table 1. World biogeography of morphological (MD) morphophysiological MPD, physiological (PD), physical (PY), and combinational (PY+PD) dormancy and of nondormancy (ND) in seeds of 5084 species of trees

Vegetation zone	Number of species	Dormancy profile (%)					
		ND	MD	MPD	PD	PY	(PY+PD)
TROPICAL							
Evergreen rainforests	2056	48	3	15	25	9	<0.5
Montane	207	21	2	18	48	10	1
Semievergreen	1092	45	1	7	35	12	<0.5
Deciduous	224	32	0	3	37	28	0
Savannas	412	34	2	4	24	35	1
Hot deserts	56	14	2	2	9	71	2
TEMPERATE							
Sclerophyllous	185	53	1	1	35	9	1
Broad-leaved	175	34	2	16	40	8	0
Deciduous	475	15	0	7	70	3	5
Steppes	7	43	0	0	57	0	0
Boreal/subalpine	77	27	0	3	70	0	0
Montane	85	26	0	3	71	0	0
Woodland	33	36	0	0	64	0	0

In the temperate region, ND ranged from 53% in sclerophyllous woodlands to 15% in deciduous forests. PD was important in all temperate vegetation zones, ranging from 35% in sclerophyllous woodlands to 71% in montane forests. PY occurred in 3-9% of the species in sclerophyllous woodlands, broad-leaved evergreen, and deciduous forests but did not occur in the other vegetation zones. MD and (PY+PD) were of minor importance (1-5%), with MD only in sclerophyllous woodlands and broad-leaved evergreen forests and (PY+PD) in sclerophyllous woodlands and deciduous forests. MPD occurred in five vegetation zones, and it was most important in the broad-leaved evergreen (16%) and deciduous (7%) forests.



From a comparison of the dormancy profiles (Table 1), we can conclude that trees with ND seeds can be found in all parts of the world where trees grow. The relatively wet tropical zones (evergreen rainforest, montane forests, and semievergreen rainforest) have more species with PD than PY. With a general decrease in rainfall from the dry deciduous tropical forest zone to the hot deserts, there is a decrease in PD and an increase in PY, with 71% of the species in the hot deserts having PY. In the temperate region, PD is relatively high in all vegetation zones and PY relatively low, or absent.

Families

The 5084 species of trees occurred in 151 plant families: 65 only in the tropics, 20 only in the temperate region, and 66 in both the tropical and temperate regions. Thus, at the family level, Sorensen's similarity index is 60.83%. The number of families in a vegetation zone ranged from 100 in evergreen rainforest to eight in the woodland forest zone on (temperate) mountains (Table 2). Some families were restricted to a particular vegetation zone, with the highest number of restricted families (36) occurring in the broad-leaved evergreen forest. The tropical evergreen rainforest had 21 families restricted to it. However, many vegetation zones had no families restricted to them.

No family occurred in all 13 vegetation zones where trees grow. The families that occurred in nine or more vegetation zones were: Anacardiaceae (9), Aquifoliaceae (9), Arecaceae (9), Fabaceae (9), Oleaceae (10), Pinaceae (10), Rhamnaceae (10), Rosaceae (11), Sapindaceae (11), and Ulmaceae (10). In some cases where trees in a family are absent from a vegetation zone, the family is represented by the shrub and/or herb life form. For example, although trees in the Fabaceae were not recorded from steppes, boreal/subalpine, montane, or woodland vegetation zones, leguminous shrubs and herbs were recorded in the steppes and leguminous herbs in all 4 of these zones.

Table 2. Number of plant families with trees occurring in the 13 vegetation zones, for which seed dormancy data have been included in this study, and the number of families occurring only in a particular vegetation zone.

Vegetation zone	Number of families	Number of restricted families
TROPICAL		
Evergreenrainforest	100	21
Montane	56	6
Semievergreen	92	6
Deciduous	38	0
Savannas	59	0
Hotdeserts	12	1
TEMPERATE		
Sclerophyllouswoodland	27	0
Broad-leavedevergreen	77	36
Deciduous	29	0
Steppes	4	9
Boreal/subalpine	9	0
Montane	19	0
Woodland	8	0

PHYLOGENY

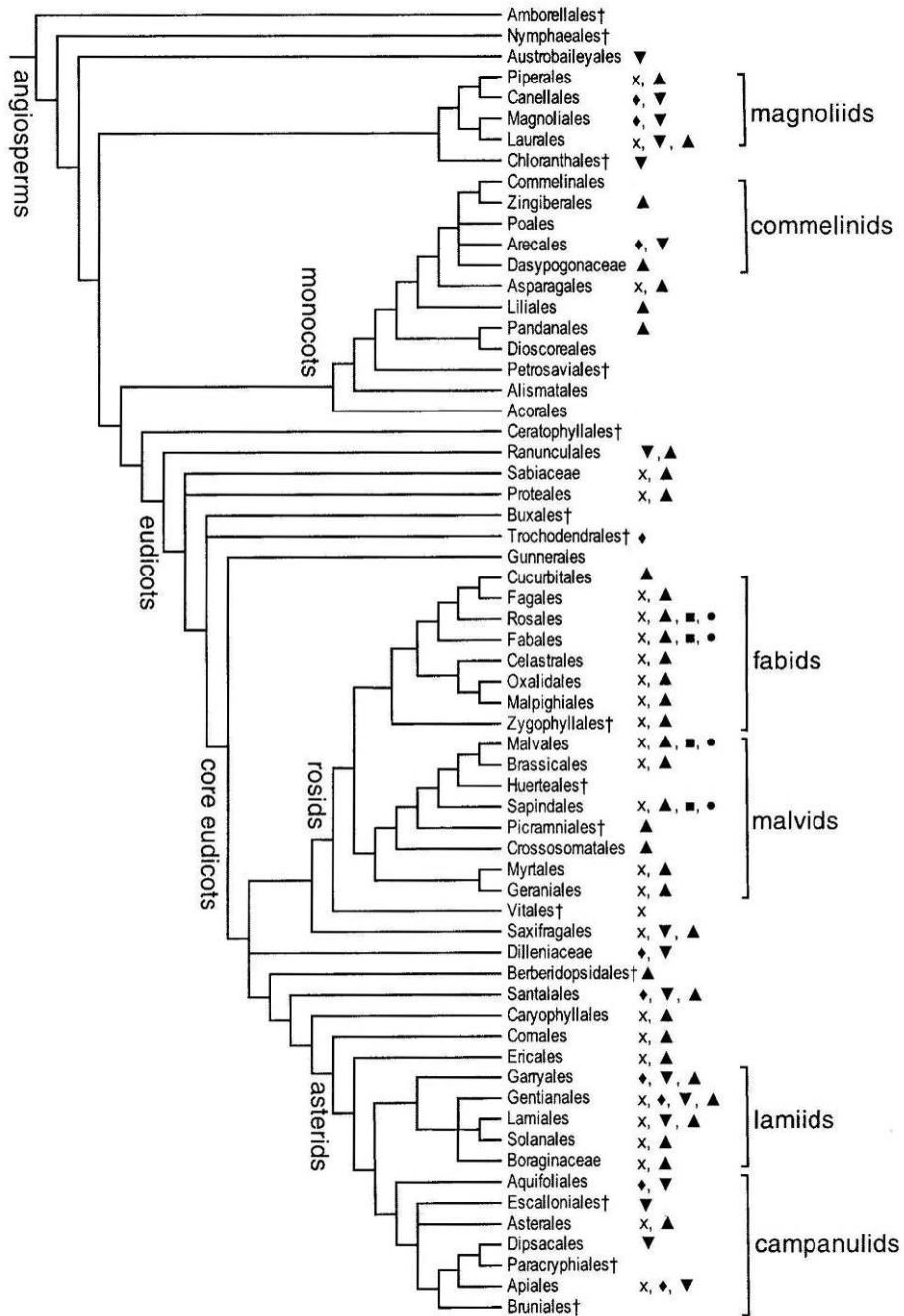
Information regarding seed dormancy/nondormancy was plotted on the angiosperm phylogeny diagram published by APG III (2009), and we had tree seed information for 49 of the 63 orders/unplaced families on the diagram (Fig. 1). No information on tree seed dormancy is included for Amborellales, Nymphaeales, Commelinales, Poales, Dioscoreales, Petrosaviales, Alismatales, Acorales, Ceratophyllales, Buxales, Gunnerales, Huerteales, Paracryphiales, and Bruniales, mainly because no trees occur in them. Members of some of these orders such as Paracryphiales and Bruniales can be small trees, but we do not have information on seed dormancy for them.

MD and MPD, i.e., trees whose seeds have underdeveloped embryos, occurred in 18 of the 63 orders/unplaced families, and they are widely distributed on the phylogenetic diagram (Fig. 1). However, trees whose seeds have MD and/or MPD did not occur in the fabids or malvids. Some orders, including Garryales, Gentianales, Lamiales, Laurales, Santalales, and Saxifragales, have trees in families with underdeveloped embryos and in other families with fully developed embryos. Trees whose seeds have fully developed embryos are widely distributed on the angiosperm phylogeny diagram, with a high concentration in the fabids, malvids, and lamiids. ND and PD occur widely across the diagram, and frequently ND and PD are in the same order, family and genus. Some examples of genera with both ND and PD include *Artocarpus*, *Baccaurea*, *Beilschmiedia*, *Canarium*, *Diospyros*, *Entandrophragma*, *Ficus*, *Garcinia*, *Helicia*, *Kagenickia*, *Lithocarpus*, *Mallotus*, *Nectandra*, *Ocotea*, *Pouteria*, *Quercus*, *Syzygium*, *Tabebuia*, *Ulmus*, and *Vatica*. Trees with PY and (PY+PD) are restricted to the Rosales and Fabales in the fabids and to the Malvales and Sapindales in the malvids. Further, the orders with PY and (PY+PD) also have families in which ND and PD occur. In fact, the Fabaceae, Malvaceae, Rhamnaceae, and Sapindaceae are the only families with trees whose seeds can have ND, PD, PY, or (PY+PD), depending on the genus. Some genera in these four families have ND and PD and others ND and PY, and only a few genera (e.g., *Berchemia*, *Cercis*, *Hovenia*, *Rhus*, and *Tilia*) have species with (PY+PD).

Are there any phylogenetic restrictions with regard to the class of dormancy or ND for the families that occur only in one vegetation zone? Plotting the restricted families from tropical evergreen rainforests and temperate broad-leaved evergreen forests on the phylogenetic diagram, revealed that seeds of these trees mostly were ND or had PD, with an occasional occurrence of MD and MPD (data not shown). Further, with regard to trees from both the evergreen rain forest and the broad-leaved evergreen forest, both ND and PD are widely distributed phylogenetically. Plotting the 65 families that occurred only in the tropics and the 20 that occurred only in the temperate region (data not shown) revealed a wide distribution of ND and PD and scattered MD and MPD. None of the trees in these 85 families have PY or (PY+PD). Thus, in trees ND, PD, MD, and MPD are not restricted phylogenetically, whereas PY and (PY+PD) are restricted to the fabids and malvids.



Fig. 1. Phylogenetic position of morphological (◆), morphophysiological (▼), physiological (▲), physical (■), and combinational (●) dormancy and of nondormancy (x) in seeds of 5084 species of trees. Diagram is from APG III (2009).



FUTURE CONSIDERATIONS

The database we have compiled for the world biogeography of seed dormancy in trees gives us much information, but there may be more that we can learn from it. First, studies reported in the literature may be biased toward economically-important trees, perhaps giving us little insight into the seed dormancy/nondormancy of trees with little economic value. (2) Within the forest vegetation at a given site, there are unequal numbers of individuals of each tree species. Thus, if we wish to understand germination ecology as part of the adaptation of trees to a particular vegetation zone, we need to know how many individuals of each species are present and what class of dormancy (or ND) they have.

An excellent source of information on density of tropical tree species is the long-term monitoring plots established in many tropical countries. As an example, we will consider the Xishuangbanna Tropical Seasonal Rainforest Dynamics Plot in southern Yunnan Province, China (Cao et al. 2008). This is a semievergreen rainforest in the sense of Walter (1979). We have found seed dormancy data in the literature for 95 of the 468 species in the plot. If we compare the dormancy profile for the 95 species based on number of species only with a profile based on number of individuals per species, the results are quite different (Table 3). The profile based on number of individuals shows a decrease in ND, MD, and PY and an increase in MPD and PD, when it is compared to the one based on number of species. Thus, as our tree seed dormancy database expands, we envision being able to create dormancy profiles for tropical forests that use number of individuals of a species rather than simply number of species, which should greatly improve our understanding of the ecology of seed dormancy from a community/vegetation zone perspective.

Table 3. Dormancy profile for 95 species of trees in the Xishuangbanna Tropical Seasonal Rainforest Dynamics Plot in southern Yunnan Province, China, based on (A) number of species and (B) number of individuals of each of the 95 species in the plot.

Number of	Dormancy Profile (%)					
	ND	MD	MPD	PD	PY	(PY+PD)
SPECIES						
95	45.2	3.2	5.3	34.7	11.6	0
INDIVIDUALS						
20956	33.1	0.6	20.0	45.5	0.8	0



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The Role and Future Challenges of *Ex Situ* Gene Conservation Approaches for Forest Tree Genetic Resources

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[Summary]

Interest in genetic conservation for crop, animal, and tree genetic resources has substantially increased over the last decade, and particularly of late, due to the predicted impacts of climate change. While most major commercial crop and forest tree species have representative *ex situ* conservation collections in place, relative to crop species, there are few examples of the *ex situ* conservation of forest tree genetic resources that show how they can be actively used, routinely evaluated (e.g., through testing and seed rejuvenation), and incorporated into comprehensive genetic resource management programs.

In situ conservation, through special wild land reserves or protected areas (e.g., parks), is considered the stalwart conservation vehicle for most forest tree species; however, climate change is also changing our views on their long-term role and effectiveness. For species with good *inter situ* or *ex situ* conservation plantations (i.e., those ‘intermediate’ between *in situ* reserves and seed banks), the role and value of *ex situ* seed collections will need to be carefully reexamined, considering how they are collected, stored, evaluated, and deployed to mitigate potential climate-change impacts (i.e., ‘facilitated migration’ becomes ‘facilitated conservation’). This will greatly vary by species, but some general approaches apply.

A case study for the ecologically and commercially important species of the western larch (*Larix occidentalis* Nutt.) in British Columbia is presented which identifies some options for use of *ex situ* genetic resources. While strong theoretical and technical arguments can be made for *ex situ* seed collection, storage, and use, the main impediment will ultimately be garnering long-term institutional financial and technical capacity to utilize and regenerate *ex situ* materials under a climate-change lens, for all species of interest.

INTRODUCTION

The profile of genetic conservation for plant and animal genetic resources has increased over the last decade, primarily due to the awareness of potential negative impacts of climate change on the environment and agriculture production (Fowler 2008). To date, forest genetic resource conservation has had a somewhat lower priority relative to genetic conservation of agricultural species, but recently the Commission on Genetic Resources for Food and Agriculture acknowledged the importance of work on forest tree

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genetic resources (FAO 2009). The long delay in giving forest tree genetic resources an improved status relative to agricultural species probably originated from the large biological, historical, and economic differences between crop plants and trees, i.e., relatively little domestication in terms of time scale and differences in wild and domesticated gene pools, perceived lower economic and social importance levels, a well-established reliance on *in situ* conservation, and an overall lower perceived risk level of loss of genetic resources of forest tree populations. However, *in situ* conservation in forestry, typically considered the stalwart conservation vehicle for most forest tree species, will encounter serious challenges as most populations within the reserves they currently occupy will not have the ability to adapt and evolve *in situ* to rapid climate change that is projected for many species (St. Clair and Howe 2007, Kuparinen et al. 2010, Ledig et al. 2010).

The main genetic conservation vehicle for crops by far is *ex situ* conservation. While also a routine practice in most countries with developed forestry programs, it has generally suffered from only passive implementation, and there are few examples that show how it can effectively complement regional forest tree genetic conservation and improvement programs. *Ex situ* conservation of most crop species, particularly those that require periodic germ plasm rejuvenation, allows the crop breeder and seed bank technicians the important opportunity to examine long-term viability of accessions, and to assess whether genetic changes have occurred in neutral markers or quantitative traits.

It is also clear that genetic diversity decreases linearly over time (e.g., Fu and Somers 2009), but tends to be less dramatic for more-complex quantitative traits (e.g., Gomez et al. 2005). We should expect the situation to largely be the same for forest tree seeds, but what remains unclear in *ex situ* storage systems is what are the tradeoffs in terms of the length of time in storage, the cost of rejuvenation programs (i.e., turning over a generation in the same or some new environment), loss of low-frequency alleles to genetic drift, and changes in additive genetic variances of important adaptive traits for the future. This approach, however, also assumes we know and have set priorities among species and populations (e.g., see Yanchuk and Lester 1996, Koshy et al. 2002, FAO FLD IPGR 2004, Krakowski et al. 2009), and which ones are worthy of rejuvenation or regeneration. The latter term may be more appropriate for forest tree seeds as it may reflect multiple events required to generate a new crop of trees and then obtain seeds, rather than strictly focusing on a new crop of seeds to maintain seed viability.

Also with crops, the long period of domestication and the global reliance on a few main crop species (i.e., wheat, maize, and rice) have allowed a rather sophisticated network and seed-storage system to develop. The need for a global ‘backup system’ to support regionally managed and funded seed centers around the world in crop plants led to the building of the much-needed and very high-profile Svalbard Global Seed Vault, which has also helped accentuate the visibility and importance of *ex situ* conservation in crops (Fowler 2008). With the possible exception of radiata pine (Rogers et al. 2002), there are very few examples in forestry where intergovernmental or inter-agency efforts have resulted in targeted collections to conserve forest tree seeds. Conservation of animal genetic resources, even with their enormous contributions to the human food supply, has some interesting and similar challenges as does forestry, as both attempt to balance *ex situ* in vivo and *ex situ* in vitro efforts (FAO 2007).



Use of *ex situ* collections

The contemporary ‘perfect storm’ of limited resources and rapid climate change is now forcing those of us in forestry to clearly outline the challenges and options available to more fully integrate *ex situ* conservation into genetic management plans. Projected effects of climate change on forests will severely test our ability to utilize *in situ*, *inter situ*, and *ex situ* conservation vehicles in a complementary and technically justifiable approach for a target species.

***Ex situ* conservation in cold storage (in vitro)**

Many conifer species have seeds with long viability in appropriate cold storage (e.g., with desiccation), which makes it relatively easy to store large quantities of seeds for long periods of time. While this is a huge advantage for forest geneticists working with conifers, it also has fostered some complacency in building strategies for use and evaluation. Agencies will need periodic justifications of their investments in *ex situ* conservation facilities or field trials. Where seed storage facilities are already in place for commercial nursery and forestry programs, cold storage can be added at limited additional costs, but new facilities will be difficult to justify in many jurisdictions if both the short- and long-term values cannot be quantified. For this reason, there is almost no redundancy collection system for insurance against catastrophic losses at national and international levels for forest tree species. As such, redundancy in the field may be 1 of our safest options.

For many deciduous species and species with recalcitrant seeds which typically have shorter viability in storage, the problems are substantial, and it is likely these will require an approach similar to that for crop species, i.e., almost immediate collection, germination, and outplanting in some type of conservation stand. In essence, they only increase the immediacy of the problem, but the principles can be the same for all species.

***Ex situ* conservation plantations (in vivo)**

On the surface, it appears that a relatively easy solution to utilizing traditional *ex situ* collections is to simply grow a representative sample of seeds from the accession, and plant the trees in a conservation stand. However, this approach can be fraught with problems. For example, attempts to establish *ex situ* plantations for several hardwood species and a few tropical pines in Indonesia ended up with very high rates of failure, and most of the conservation objectives were not met (FAO FLD IPGRI 2004). Most of the problems were and will continue to be predictable, e.g.: fire, illegal harvesting and use, natural succession, small sample and outplanting sizes, poor site selection, and limited flowering.

With the decrease in investments in tree improvement programs around the world, it is even less likely that these types of plantings can be accommodated unless they are built into operational reforestation activities as part of genetic management programs for species, i.e., have the necessary features and security, and be considered part of a ‘dynamic’ aspect of genetic conservation that explicitly tests and/or mitigates climate-change impacts. In the end, it is likely that long-term institutional commitments may be the largest risk such plantings face; therefore, any type of *ex situ* plantings must be essentially ‘free-to-grow’ shortly after establishment and be subject to normal risks which any plantation must endure.

***Inter situ* conservation: What's the difference?**

While not a commonly used term in genetic conservation (*ex situ* planting is considered an equivalent term in most cases), I proposed the use of the term, *inter situ*, for forestry (Yanchuk 2001), originating largely from Blixt (1994), to make an important distinction from *ex situ* seed collections, clonal archives, and *ex situ* outplantings (in forested or semi-forestry settings). *Inter situ* sites incorporate most elements of *ex situ* field plantings, but are also purposefully undergoing natural, land race, or additional trait selection imposed by the changing climate, and typically contain more genetically informative material (see below). Such plantations also embody concepts proposed by Ericksson et al. (1993) of the need for dynamic conservation. For example, recently on Kaua'i in the Hawaiian Islands, *inter situ* conservation of native species was proposed as a means to reintroduce endemics which are near extinction, and where *ex situ* and *in situ* conservation efforts are no longer effective (Burney and Burney 2007). Their *inter situ* objectives are to create new populations that can be intensively managed, and paleoecological records support the concept as these species typically had large ranges on the island. These plantings would also be equivalent to what Jana (1991) referred to as 'mass reserves', with possibly more-heterogeneous populations, undergoing development of 'modern landraces'. In essence, *inter situ* populations would allow us to somewhat control or direct genetic changes and genetic drift, rather than the random drift effects more prevalent in crop rejuvenation programs.

Interestingly, open-pollinated progeny trials have many positive attributes as vehicles for *inter situ* conservation (Yanchuk 2001, Lipow et al. 2003). Even though they may originate from a moderate number of selections or parents, they capture a large proportion of the genetic variation in a defined geographic area, or breeding or seed planning zone. Depending upon how many parents were sampled per stand, and the distribution of the collections, open-pollinated progeny trials may sample a broader spectrum of the native gene pool than would a large number of ad hoc *ex situ* collections. For example in British Columbia, at least 10 (50 preferred) parents are required from a wild stand for a seed lot to be used in reforestation; however, documentation quality and verification vary. While any collections of this size capture over 95% of the adaptive genetic variation in the population sampled (Yanchuk 2001), typical *inter situ* populations would have several hundred genotypes.

Genetic sampling objectives

Most genetic conservation concerns for adaptive traits could be addressed by using a few hundred open-pollinated seed collections; but to ensure rare alleles are captured, the sampling number becomes more problematic. Genetic sampling requirements or numbers needed to ensure some level of genetic variation is captured are now presented or summarized in many papers, and will not be reviewed here. However, in Yanchuk (2001), some figures and tables may help illustrate some of our sampling objectives with respect to *ex situ* conservation. For example, an *ex situ* or *inter situ* conservation plantation established with seeds from 250 individual open-pollinated accessions would have an effective population size (N_e) of ~1000, assuming an approximate multiplier of 4 (for the number of open-pollinated accessions) providing a reasonable approximation of the N_e for outcrossed species (Krakowski et al. 2009). Also,



assuming that a large number of seeds per family are available and used, this would capture 20 copies of a dominant allele at a ~ 0.02 frequency, and 20 copies of a recessive allele at a 20% frequency (Yanchuk 2001).

More importantly, and probably more realistically, is what kinds of collections are possible; i.e., there might not be many trees left in a population, or no extra seeds can be collected or stored, and what may be affordable. In other words, ‘adequate’ conservation may be a misleading term, as we must aim to conserve what is available and affordable, given that the statistical distribution of allelic variations provides diminishing returns with increasing sampling effort. In some situations, existing breeding programs meet the criteria for necessary and affordable genetic management and conservation objectives; if done correctly, they can even increase genetic variations (e.g., Namkoong 1997, Cooper et al. 2001).

Case study: *ex situ* collections of *Larix occidentalis* Nutt. (western larch) in British Columbia (BC)

Western larch (*Larix occidentalis* Nutt.) is harvested and reforested for timber in BC. There is an active breeding program, and open-pollinated progeny tests have been nominated as our *inter situ* populations in 2 breeding zones. Western larch currently has good genetic conservation with adequate *ex situ* collections, *inter situ* plantations, and *in situ* reserves in place (Fig. 1) (Krakowski et al. 2009), and a facilitated migration policy is being implemented for commercial reforestation of larch to mitigate anticipated impacts of climate change (Rehfeldt and Jaquish 2010).

Based on information from population and quantitative genetics, incorporating ecological and climatic variations as surrogates for past evolutionary pressures, these 2 populations are recognized as being distinct (for genetic management purposes), with a small overlapping transition zone (which we can largely ignore for conservation purposes) (Fig. 1). Over 100 commercial stand-level seed collections were sampled for dedicated *ex situ* conservation collections (these are stored at the BC Ministry of Forests and Range Tree Seed Centre). The criteria for *ex situ* genetic conservation collections in BC are a minimum sample of 1000 viable seeds, with 3 separate samples per ecological zone to accommodate some redundancy in the event that 1 or 2 are eliminated by accident. If, for example, an *ex situ* plantation was established from these 3000 seeds, it would be rather small (approximately 2–3 ha), and it would be at risk for the reasons discussed earlier. From Fig. 1, well over 3000 seeds are available since there are 72 separate 1000-seed collections for the Nelson Low zone alone, which would be candidates to augment the diversity of any *ex situ* or *inter situ* samples.

For radiata pine in New Zealand, it was proposed that 20-ha blocks of such *ex situ* plantings be established. The equivalent for western larch in BC would require $\sim 24,000$ trees to be planted (at 1200 seedlings ha⁻¹). Of course, the block size would need to be evaluated in terms of the local area available, costs, replication, and outplanting design and location in order to appropriately challenge trees for some targeted climate conditions. In the western larch Nelson low zone, established *inter situ* populations likely contain a good level of genetic diversity (i.e., N_e 1300 (Krakowski et al. 2009), which would be similar to the values mentioned in the example above), so any new investment in *ex situ* plantings (e.g., four 5-ha blocks, or one 20-ha block) would have to be

carefully evaluated and justified for incremental benefits. More importantly now, is what could or should be done if climate projections for current *in situ* reserves (Fig. 1) show that a limited appropriate climate and habitat will exist there in the future. Modeling work is currently underway (see below) to determine this, but this brings up a few important questions.

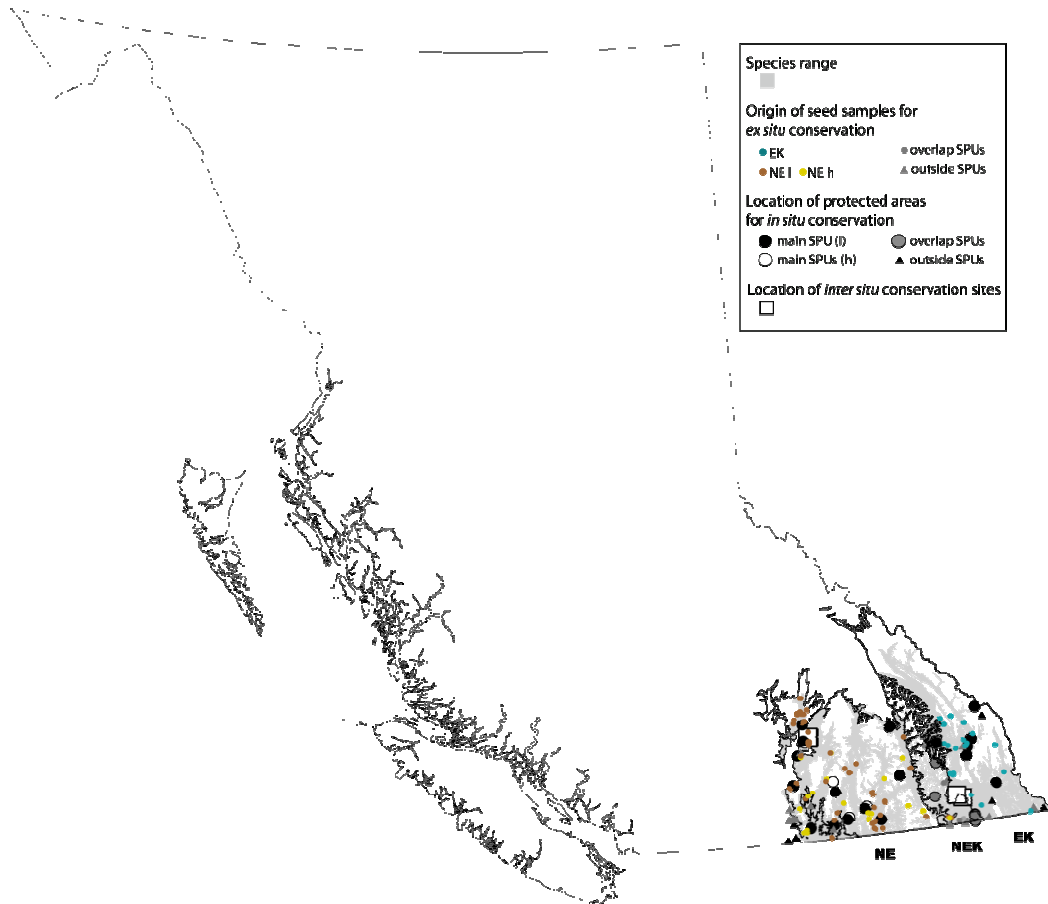


Fig. 1. Delineations of the 2 seed planning unit (SPUs) (NE, Nelson; NEK, Nelson and East Kootenay overlap; and EK, East Kootenay), and the location of select *in situ*, *inter situ*, and *ex situ* gene conservation populations of western larch in British Columbia (from Krakowski et al. 2009).

- 1) Do current *ex situ* seed collections adequately sample or provide any additional needed coverage to potentially replace *in situ* reserves that are projected to ‘fail’. If so, how many, where, and when should these be planted (i.e., in a similar climate space in a targeted time in the future)?
- 2) Are current *inter situ* plantings the most cost-effective resource to maintain (i.e., collect seeds and replant if necessary)? Would additional *ex situ* materials (either



already in storage, or new collections required) be needed to supplement new *inter situ* plantings?

Rehfeldt and Jaquish (2010) recently published projections of suitable climate space stratifying 5 genetically distinct populations of western larch, including the 2 shown in Fig. 1. These projections provide important starting points for facilitated migration for commercial forestry, as well as for conservation. For example, in Fig. 2, the Nelson low and East Kootenay low populations show larger differences in the predicted future climate space only 2 decades in the future than they currently occupy. By chance, *in situ* and *inter situ* reserves appear to be in locations which may be suitable for the projected climate in 2030; however, where and when new *inter situ* plantings should be established in the future (e.g., projected climate space in 2060) will need to be determined by further modeling. In order of importance, some options are listed here.

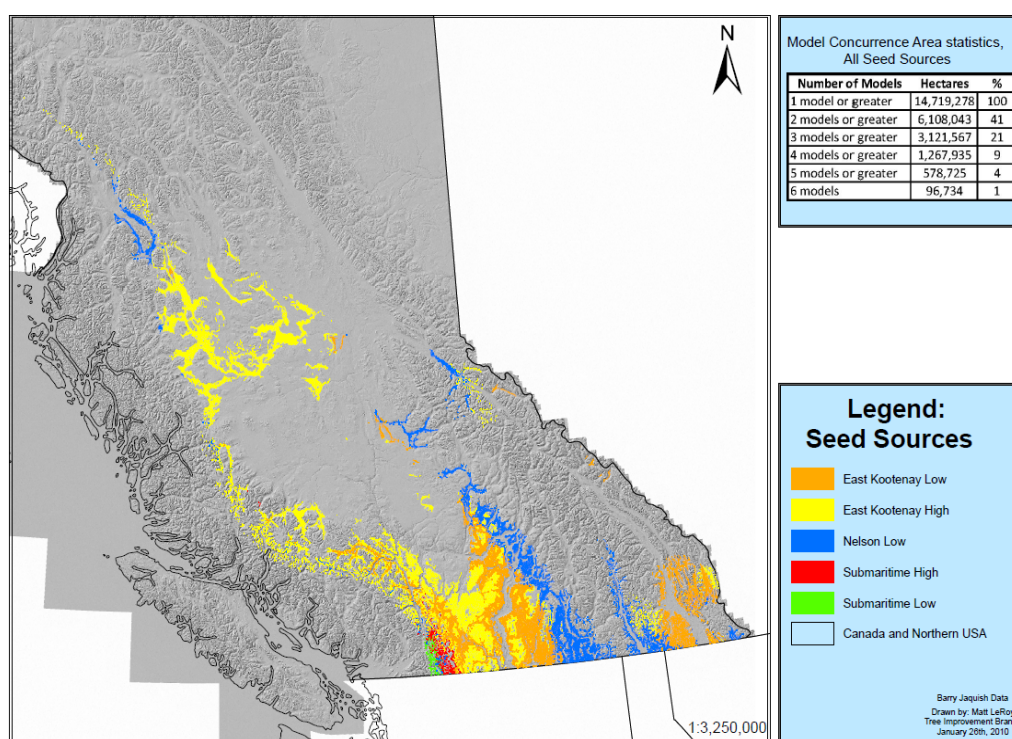


Fig. 2. Projected areas supporting appropriate climates for the 5 populations of western larch in 2030 with 2 models in congruence, based on simulations by Rehfeldt and Jaquish (2010) (figures unpublished, provided by B. Jaquish and M. LeRoy, British Columbia Ministry of Forests and Range).

- Collect as many seeds as possible from current *inter situ* trials and reestablish plantations in new areas where the projected climate is expected to be suitable (if sufficient seeds are available).
- Pool several of the *ex situ* collection seed lots from a given zone, and if they likely contain adequate genetic representation, and a better sample than *inter situ* sites,

establish new *inter situ* populations in appropriate habitats within the projected suitable climates. Family structure may be lost in such a scheme, but this might not be critical, and pedigree reconstruction (e.g., El-Kassaby et al. 2010) could be attempted if required.

- Collect new seed lots from *in situ* populations, either pooled or separated, and reestablish populations in appropriate habitats within areas likely to support the most-suitable future climates.

Of course, many questions remain regarding what we can now describe as ‘facilitated conservation’: 1) the size of each conservation plantation, 2) the number of these plantations, 3) the risks associated with establishing these plantations in areas where climate models suggest suitable climates will appear at some future date (e.g., Ledig et al. 2010), 4) the age to target for establishment and/or possible future seed collections (e.g., age 20 yr), 5) maintenance and security of records on family or provenance pedigrees, and 6) planting as pseudo-commercial stands or as more-dedicated conservation plantations.

CONCLUSIONS

Genetic conservation theory and applications have matured over the last few decades; however, the projections and implications of climate change pose enormous challenges to our traditional ideas and approaches. New and more-aggressive strategies will have to be developed and implemented, probably with fewer resources. More than ever, *in situ*, *inter situ*, and *ex situ* genetic conservation mechanisms must be considered complimentary and over the next decade, may require some redefinition as the distinctions among them blur.

For the most part, forestry is fortunate as most agencies have better access to the 3 conservation vehicles than do most crop or animal genetic managers. Conservation strategies must also build in sufficient redundancy that the impacts of chance events, errors of climate predictions, and inconsistent institutional support can be minimized. For most species, there are simply not going to be enough capacity available for the activities described above for western larch, and we will simply have to rely on *in situ* reserves, the natural processes influencing protected areas, and simply accepting higher levels of risk and loss of forest genetic resources.

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Ex Situ Conservation Activities in Mexico: Challenges and Successes

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[Summary]

Mexico ranks fourth in biology diversity. Approximately 30,000 species of plants grow in Mexico, more than 3000 trees species are native, and 1000 tree species are endemic to the country. Problems and achievements in the conservation of genetic resources in the field are discussed, with an emphasis on *ex situ* conservation. The general strategies for *in situ* and *ex situ* gene conservation are described. The Mexican government has created some rules for the use of species that are considered in danger of extinction, and supervise forest harvesting. Seed collections and some field tests (as active conservation) were made by universities and research institutions. In Mexico, there are 26 facilities with cold rooms for storage of seeds and 30 arboretums for educational purposes. Seed laboratories have adequate equipment, but not enough trained personnel to operate them. Seed collection is usually done for economically important species or for endemic tree species which are in danger across their natural range in Mexico. Coniferous species are the most common trees encountered in seed collections, while tropical and xeric species are less common. The creation of a system to secure aquatic, agricultural, forestry, livestock, and microbial germplasm and a national bank of genetic resources are explained. Some examples of advances in the management of rare tree seeds are given.

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Mexico is covered by a large variety of vegetation types, ranging from humid tropical rainforest to dry scrub and natural grasslands. The Mexican territory covers 1,972,545 km², and forests (trees > 3 m high) once covered 52.0% of the country's land area, but that has declined to 33.3% (Palacio-Prieto et al. 2000). Although Mexico is the fourteenth largest country in the world, it ranks fourth in biological diversity (CONABIO 1998). The wealth of species, both floristic and faunistic, is the result of a varied biogeographical history over a variety of climates, encompassing a Nearctic environment in the north and a Neotropical one in the south (Ramamoorthy et al. 1993). Approximately 30,000 species of plants grow in Mexico, of which over 300 genera and around 50% of the species are endemic to the country (Ramamoorthy and Lorence, 1987; Robles and Dirzo 1996). There are 2263 tree species (> 3 m high) (Palacio-Prieto et al. 2000), but Villasenor and Ibarra (1998), in an herbarium survey, counted 3639 species of 128 families that are native Mexican flowering plants with an arboreal growth form. However, a review of many taxonomic groups is still pending. There may be around 1000 tree species endemic to Mexico; however, more-precise information is needed from the tropics, where species are still being discovered and described.

If the taxonomy of Mexico's tree species is incompletely known, their distributions are even less so. Given this situation, an attempt was made to develop a database of species distributions: The Mexican 'National Commission for Knowledge and Use of Biodiversity' (CONABIO) was created in March 1992. This governmental institution has provided funds for scientific projects that compile georeferenced data on biodiversity in the form of databases for all taxonomic groups. But it still does not have the approval of taxonomists, because it lacks taxonomic verification of specimens in the traditional way, where one specialist compares (and reclassifies) collected specimens within a taxonomically related group.

For example, coniferous trees dominate a huge portion of the territory with 15 genera and over 150 species. More than 50% of the world total species of *Pinus*, 49 species, grow in México (Styles 1993), among which 21 species are endemic. Along with pines and firs, many angiosperm species grow in Mexico, including 161 species of *Quercus*, among which 109 oaks are endemic (Valencia 2004).

Mexico's biodiversity is one of the most important worldwide. Many of the crop plants used by humans have their origins in Mexico. A large portion of a critically important "gene belt" that circles the world between the Tropics of Cancer and Capricorn lies in Mexico. The scattered distribution of centers of endemism of various taxonomic groups implies that the complex geological history of Mexico has been a major evolutionary force. Epochs of orogeny contributed to fragmentation of vegetation and ancestral populations (Ramamoorthy et al. 1993). Mexico has served as both a corridor and barrier to biota with different origins (Rzedowski 1991).

The high proportion of lands under cultivation continues to be a problem in reducing forests, although the government financially supports reforestation programs. Unresolved problems of having large areas devoted to agriculture remain an issue in Mexico. Agriculture and livestock activities have had major impacts on tropical rainforests, mountain cloud forests, and lower-elevation areas of temperate forests, composed mainly of *Quercus* and *Pinus*. Large-scale habitat fragmentation has affected biodiversity, creating semi-natural grasslands and small forests surrounded by agricultural crops or pasture for livestock.



The Mexican Ministry of the Environment (SEMARNAT) has established Protected Natural Areas (ANPs), which are portions of the territory (land or marine) representative of different ecosystems, where the original environment has not been seriously altered, and there are sustainable management programs. There are 154 ANPs (federal) comprising over 18.7×10^6 ha (including 4×10^6 ha for marine areas). Overall, ANPs cover about 9.5% of the land surface. Most of country's major ecosystems are represented within the limits of the ANPs, but xeric scrublands (35.1%), temperate forests (12.4%), and tropical humid forests (9.4%) occupy a greater proportion of the protected areas. ANPs are subject to special protection, conservation, restoration, and development, according to 7 categories of management: biosphere reserves, national parks, natural monuments, protected areas of natural resources, floral protection areas, wildlife sanctuaries, and other categories.

However, the greater part of the country's forests (and therefore biodiversity), is found in areas of common property, *ejidos*, and the agrarian community land tenure system, where decisions are made by all members of the community. This socioeconomic system is based on the principle of joint ownership of property, and equality and cooperation of production, consumption, and education. A general assembly of all members formulates policy. Because of this, decisions are difficult to make in the short term. Members of *ejidos* or communities are citizens with little or no experience in forest management, and education is complex since the members of these communities (indigenous and non-indigenous) are the poorest people in Mexico.

Only 15% of forestry land that is being harvesting has a "management plan" that is regulated by the government. No legal harvesting is allowed without the permission of SEMARNAT. Each property has areas dedicated to "protection", usually where biodiversity is greatest, and areas for protection of ravines where they protect streams or where the slope is steep. In these areas, it is difficult to remove trees, so they remain untouched. SEMARNAT has also established a list of species, animals and plants, which are protected by law (NOM-059). There are 3 categories of endangered, threatened, and protected, which are similar to those of the IUCN. These species are not harvested unless they die by natural phenomena. Moreover, wherever a protected species grows, no harvest activities are permitted (except sanitation cuttings).

One of the ways to preserve these habitats is to plan collaborative research and study, involving educational and research institutions and government agencies. Some plans are underway, and local citizens are involved in conservation activities, but the majority of land owners are not aware of the wildlife richness of their surrounding environment, and other necessities are much more important to them.

Several institutions such as universities and research centers have established seed collections which are used for research and education. Seeds are usually stored in conventional refrigerators. These collections have no legal protection. This is connected to a lack of funding needed to enhance the quality of laboratory facilities and maintain the collections. Seeds of economically important species are usually collected or of endemic tree species which are in danger across their natural range in Mexico. Coniferous species are the most common trees encountered in seed collections, and tropical and xeric species are less commonly found (trees that are included on the NOM-059 list). These seed sources are used for conservation purposes and for conducting experiments such as provenance or progeny tests. In some cases, seeds are

used for commercial plantations (by private companies) and for ecological restoration efforts in anticipation of climate change (for projects by SEMARNAT).

The Mexican government recognizes that there are insufficient comprehensive *in situ* conservation plans to promote the preservation of genetic resources as an integral part of community development. But there are also a) a lack of space for adequate *ex situ* preservation of germplasm, b) a lack of adequate equipment and facilities, c) a shortage of seeds, strains, gametes, embryos, seedlings, and shoots of many species, d) a lack of knowledge of preservation methods and protocols for many species, and e) a lack of facilities and equipment for the renewal of seedlings and other germplasm that remain viable for short times. So the Mexican government decided to create the National System of Phytogenetic Resources (SINAREFI) which is divided into 5 subsystems: aquatic, agricultural, forestry, livestock, and microbial. For each species, they have identified 4 strategic lines of attention: i) conservation *in situ*; ii) *ex situ* conservation iii) the use and enhancement of genetic resources, and iv) creating and strengthening national capacities; including the full participation of producers, organizations, and institutions in coordinated mechanisms of networks, to systematize the work of collection, characterization, and improvement of genetic resources.

As part of SINAREFI, the National Center for Genetic Resources (CNRG) was created, as a depository of collections of species of strategic importance for the nation. The NCGR was built in an area of approximately 6 ha, in a town in western Mexico, and will have 9800 m² of construction. The seed bank was created following the Seed Storage Laboratory (NSSL) located in Ft. Collins, CO, USA. The basement of the main building will contain long-term facilities containing conventional facilities such as cold rooms and tissue culture and cryogenic preserve gene bank with tanks of liquid nitrogen. On the first floor will be laboratories for each subsystem and a general laboratory, and on the upper floor will be central support areas and the administration; there will also be an academic building for teaching and training. There will also be supporting external areas, consisting of 3 greenhouses and an arboretum which will house various plant species. The CNRG will have a capacity of 3×10^6 accessions in their different forms of reproduction: seed samples, semen, plants, tissues, cells, and DNA, as a valuable reserve for research, breeding, and conservation in cases of disaster and to prevent the loss of genes.

Across Mexico, CONAFOR (the National Forestry Commission) has 18 cold rooms for storage, all of which are well-equipped, but need people trained in seed management, especially in defining seed collection strategies (e.g., what and how many trees to secure) and maintenance. In general, such facilities are for short-term or operational purposes. Only 5 states (out of 32 states that form the country) have cold rooms, and 5 universities or research institutions have this kind of laboratory and long-term facilities. In general, those have enough equipment to provide storage for seeds and to conduct seed testing.

The National Institution of Research in Forestry, Livestock and Agriculture (INIFAP) has 3 cold rooms with full equipment (north for arid and temperate species, central for temperate and semiarid species, and south for tropical species). INIFAP used to have the largest seed collection in México, but the seed bank was almost forgotten as a result of several financial crises in Mexico. Further, many researchers that work in seed



management have reached the age of retirement and no replacements are in sight, since there are other fields of research with higher priority than seed management.

Much of the work to date has mainly been done on temperate species, such as pines and firs, and research on tropical and subtropical species is lagging behind; thus, our knowledge of tropical tree seed physiology is still inadequate. Information on storage conditions for medium-term storage of tropical species which are recalcitrant is needed, but also for the 106 species of *Quercus*. Usually for restoration purposes, seedling production is accomplished using fresh seeds. No commercial plantations exist for species like *Quercus*, or many tropical species where seed management information is lacking. Some research has been done in order to know whether a species shows orthodox, intermediate, or recalcitrant seed storage behavior, which is important in order to determine the most suitable storage environment and the likely duration of successful storage. *Cedrela odorata* and *Swietenia macrophylla* may be the species for which storage conditions are best documented; these species are very valuable tropical Mexican trees because the wood is highly prized. Fortunately, seeds of most tropical tree species do not require special conditions for germination. When seed production is low or seed germination is complicated, reproduction is by asexual means, usually by root cuttings.

Provenance and progeny tests have been established throughout the country as active collections. Most tests are for pines such as *P. patula*, *P. greggii*, *P. pseudostrubus*, *P. engelmannii*, and *P. montezumae* and some tropical species, *Cedrela odorata* and *S. macrophylla*. Thirty botanical gardens are reported to be functioning in the country, containing 20,000 plants. INIFAP has 3 experimental fields, with plants from more than 300 tree species, plus several Cactaceae plants, in particular from northern Mexico.

CONAFOR will be in charge of establishing protocols for each accession, for example, the amount of seeds in each sample needed to maintain genetic integrity, viability standards, parameters of storage, timing of germination re-testing, and database maintenance. Passport data will be available for all accessions in the collections. With so many tree species, the first decision was to define a list of priority species to be stored. It has established criteria for selecting the economic and ecological importance of a species, and the status of some species on the list of endangered species. Currently each state of the republic has a list of priority species. CONAFOR will begin to collect seeds from the final list. Also CNRG will request samples of seed lots that are in research institutions and universities, and check the physical and physiological conditions of each lot.

In the NOM-059 are included 36 species of angiosperms and 41 species of conifers. Sixteen species of conifers are endemic to Mexico: *Abies* (3), *Picea* (2), *Pinus* (10 species), and *Taxus globosa*. Examples are given of progress in the collections, and problems of production and germination of seeds of various species including *Taxus globosa*, *Pseudotsuga menziesii*, *Picea engelmannii* var. *mexicana*.

The challenges facing academics and policy-makers in Mexico and other countries are doubly difficult because the components of its biodiversity, one of the largest in the world, are mostly restricted and highly scattered throughout the country. Problems are compounded by an expanding population, a moderate rate of unemployment, and growing poverty, but the Mexican public recognizes the necessity of conservation.

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***Ex Situ* Conservation of Tree Seeds: A Canadian Perspective**

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[Summary]

There are a 126 tree species native to Canada, and 57% produce orthodox seeds, 23% produce orthodox? (questionable orthodox seeds) seeds, 13% produce recalcitrant seeds, and 1% produce recalcitrant? and seeds of an unknown or uncertain storage type (referred to as unknown). Over 95% of native conifer species produce orthodox seeds, while hardwood species are variable, with the majority of species producing orthodox or orthodox seeds and 25% of hardwoods producing seed which are either recalcitrant, intermediate, or unknown. The Canadian Forest Service's National Tree Seed Centre (NTSC) has national-level *ex situ* conservation collections, and 78% of seeds stored are from species which produce orthodox seeds. For species which produce orthodox, intermediate, and unknown-type seeds, 51, 50, and 33% of the species, respectively, are represented by 1 or more seed lots in the NTSC (results not shown). The NTSC has no stored recalcitrant seeds, and seeds from only 6% of recalcitrant species (*Acer saccharinum*) are stored. These results and the ability to conserve the seeds of tree species at risk are discussed.

Key words: *ex situ*, conservation, seed.

INTRODUCTION

Ex situ conservation of forest trees has probably never been as important as it is today. As climate changes at unprecedented rates and our forests are faced with increasing impacts from insects and diseases, the need to conserve these forest genetic resources is increasingly becoming more important. These threats are occurring globally. Tropical and subtropical regions, with complex and species-rich ecosystems, are rapidly being destroyed or altered by threats such as changes in land use, harvesting, and climate change, while temperate forest regions are increasingly threatened by forest pests and changing climate.

The International Union for Conservation of Nature (IUCN) estimates that approximately 12.5% of the world's vascular plants (34,000 species) are under varying degrees of threat, while approximately 25% of the world's flowering plants either are or will be threatened with extinction over the next 50 yr (UNEP 2010). Considering tree species, global surveys suggest that approximately 8000 species are threatened with extinction worldwide (Oldfield et al. 1998). This potential loss of nearly 10% of all tree species is a significant conservation issue requiring widespread collaboration and action.

A comprehensive conservation program often requires a combination of conserving populations of stands of trees in place (*in situ* conservation), with the conservation of genetic diversity in planted gene or seed banks (*ex situ* conservation). *Ex situ* conservation can take various forms including clonal archives, seed banks, in vitro

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conservation (cell and tissue cultures), and also conservation plantings such as those found in botanical gardens.

In situ conservation is regarded as the preferred way to conserve tree species, but unprecedented rates of changes in climate, and associated insect and disease impacts raise questions about the value of protected areas for maintaining valuable genetic resources. In Canada, "The Report of the Panel on the Ecological Integrity of Canada's Parks" concluded that the ecological integrity of virtually all of Canada's national parks is threatened by a variety of internal and external stressors (Parks Canada 2000). Human-mediated movement of species (referred to as assisted migration) to new managed or protected areas may be required for dozens of tree species in order to facilitate adaptation to rapid changes. The success of this approach is dependent in part on seed availability and collection, source documentation, and the seed storage strategies used. This may constitute the only insurance that valuable sources of adaptation will survive. In particular, potentially valuable genetic resources must be documented, collected, tested, and stored for important representative populations of species that may be at great risk. Storage of tree seeds is an efficient means of preserving germplasm for *ex situ* conservation, before we can develop and initiate critical 'rejuvenation' programs in the field.

There can also be significant economic benefits associated with the *ex situ* conservation of forest genetic resources, where specific traits may lead to innovation and currently unrecognized economic potential. An example of this is seen with *Taxus* spp., the natural taxanes of which are a bio-resource that has provided the basis for innovation, yielding significant economic and health benefits. *Ex situ* conservation may also become more important for the forest industry as companies integrate assisted-migration strategies into their long-term planning. These types of strategies will enhance forest productivity and commercial value under a changing environment.

In Canada, the Canadian Forest Services' National Tree Seed Centre (NTSC), established in 1967, is well placed to function as the national *ex situ* conservation tree seed bank for Canada (Canadian Forest Service 2010). The center has facilities, expertise, and people necessary for coordinating and conducting the work, and the collaborations to participate with national and international conservation programs. Currently, the NTSC has over 12,000 seed lots in storage, 4000 of which are allocated to *ex situ* conservation. The NTSC has taken the lead in establishing *ex situ* conservation collections for many tree and shrub species and has an expanding database that provides unique data on the long-term storage capabilities of seeds from many different tree species (Simpson et al. 2004).

In addition, three of Canada's jurisdictions, British Columbia (BC), Alberta, and Manitoba have productive *ex situ* conservation collections and programs for tree species. These collections consist predominantly of seeds from commercial conifer species present in their respective jurisdictions.

The purpose of this review is to assess where Canada is with regard to national-level *ex situ* conservation and to make recommendations as to how these activities can be expanded to ensure that the *ex situ* conservation of tree species is an important resource for conservation and sustainable forest management.



Canadian tree species: seed characteristics and *ex situ* collections

In Canada, there are 126 native tree species (height ≥ 10 m) (Farrar 1995). These species, which are found in forests that cover approximately half of Canada, face a variety of threats, including land-use changes, environmental changes, invasive alien species, and harvesting practices that ignore silvicultural requirements of non-commercial species. Given the size of these forests and their diversity, obtaining a Canadian-wide perspective on how individual tree species are tolerating these threats is challenging.

To identify species at risk, Canada has both federal and jurisdictional (provincial and territorial) policies and procedures to list a species as endangered, threatened, or of special concern (Environment Canada 1996, COSEWIC 2002). Among the jurisdictions, there is substantial variation in the types of legislation and in its implementation. At the jurisdictional level, 3 provinces (BC, Ontario, and Nova Scotia) have identified and listed tree species at risk (Nova Scotia Dept. of Natural Resources 2007, Ontario Ministry of Natural Resources' Species at Risk Section 2009, British Columbia Ministry of the Environment 2010). At the federal level, Canada has listed 338 forest-associated species at risk, which represent 58% of the species at risk, and 10 of these species are trees (Canadian Forest Service 2009). At a global level, the IUCN lists no Canadian tree species.

Although most tree species in Canada are in no danger of extinction or local extirpation, loss of populations with unique alleles or combination of alleles is occurring (Namkoong 1989). This can be referred to as hidden extinction, and can have adverse consequences on long-term species viability. There is a need to conserve species before they are at risk of becoming endangered. A national-level survey was conducted in 2003 with the purpose of identifying species which may be in need of conservation, before they require official risk designation (Beardmore et al. 2006). The survey identified that 52% of Canadian tree species required *in situ* and/or *ex situ* conservation measures in some portion of their range, with 37% of native tree species assessed requiring *ex situ* conservation (Beardmore et al. 2006). While Canada does have an NTSC, there has been no concerted effort to produce pan-Canadian *ex situ* collections or strategies for these collections. With such a high number of species potentially requiring *ex situ* conservation, the first step is to develop a national perspective as to where we are with regard to the ability to store seeds from these species, to identify what collections we have, and then to develop national-level *ex situ* strategies to ensure that we are capturing the genetic diversity, where appropriate.

The seed storage classification system described by Hong et al. (1998) (Table 1) and their compendium on species seed storage behavior was used to classify the tree seed storage characteristics of species native to Canada (Table 2). Of the 126 native tree species, 57% produce seeds classified as orthodox and 23% produce orthodox? (questionable orthodox seeds) seeds, and 13% and 1% produce recalcitrant and recalcitrant? (questionable recalcitrant) seeds, respectively (Fig. 1a). Only 1% of the species produce seeds that are considered intermediate.

Temperate conifers typically produce orthodox seeds, and of the 31 conifer species native to Canada, 30 species produce orthodox seeds, while 1 species, *T. brevifolia*, produces orthodox? seeds (Table 2). Conifer species are well represented in the NTSC (in terms of having 1 or more seed lots) with only *T. brevifolia* and *Larix lyalli*

not having any accessions. *Larix lyalli* has a very limited range in Canada, with small populations present in BC and Alberta. The range of *T. brevifolia* consists of isolated disjunct populations in BC. Neither BC nor Alberta has seed collections for these species in their seed banks.

Table 1. Seed storage classification¹

Category	Comments	Characteristics		
		A. desiccation tolerance longevity (moisture content)	B. Low temperature tolerance	C. Seed
1. Orthodox	Generally seeds tolerate storage.	5~12%	tolerant of sub-zero temperatures	survival for > 10 yr at below-freezing temperatures
2. Orthodox?	Seeds show orthodox seed characteristics.	10~12%	tolerant of 5~15°C	survival for ≥ 1 and < 3 yr at ambient temperatures
3. Intermediate	Seeds exhibit storage behavior intermediate between orthodox and recalcitrant characteristics.	variable 10~12%	ambient temperatures	variable
4. Recalcitrant	Seeds are generally intolerant of storage.	> 20%, killed by desiccation to 15~20% moisture content	ambient temperatures	difficult to maintain viability
5. Recalcitrant?	Seeds show recalcitrant characteristics.	variable but recalcitrant-like	variable but recalcitrant-like	difficult to maintain viability
6. Unknown	Information is lacking for making a designation, or available data for the species precludes classification into 1 of the above categories.	uncertain or unknown	uncertain or unknown	uncertain or unknown

¹ Seed storage classifications based on those described by Hong et al. (1998).

Native hardwood species have the greatest variation with regard to seed storage classification. Of the 95 species, approximately 44% produce orthodox seeds, 30% produce orthodox? seeds, 17% produce recalcitrant seeds, while 9% of the species produce seeds which are either intermediate, recalcitrant, or unknown (Fig. 1b). In total, 43% of the hardwoods are not represented by collections in the NTSC (Table 3). Those species which are represented in the NTSC have a variable number of collections on a per species basis, ranging from 1 seed lot to a maximum of 280 for *Fraxinus nigra*. A few genera, such as *Fraxinus*, are well represented with 645 seed lots (Table 2).

The NTSC stores seeds under 2 primary categories: seed banks (seeds available for research) and gene conservation. Seeds for both categories are stored under the same conditions. For the purpose of this paper, seed lots of both categories were considered. Overall 61% of tree species are represented in the NTSC with 1 or more seed lots (results not shown). Species which produce orthodox seeds are well represented in the NTSC with 78% of species having 1 or more seed lots (results not shown). The number of seed lot/species varies greatly, from 2771 for *Picea glauca* to 1 for *Betula lenta*. The location



from which these collections were made vary, with *Picea glauca* seeds in the NTSC well represented from the southern part of this species' range in Canada (results not shown), while *F. nigra* collections are predominantly from the eastern part of this species' range (results not shown). For trees which produce orthodox, intermediate, and unknown-type seeds, 51, 50, and 33%, of the species, respectively, are represented by 1 or more seed lots in the NTSC (results not shown). The NTSC has no recalcitrant seeds stored, and seeds of only 6% of recalcitrant species (*Acer saccharinum*) are stored, albeit for only a short duration.

Table 2. National Tree Seed Centre *ex situ* seed collections for native trees of Canada¹

Genus	Common name	Seed storage type (no. of species per genus for each seed type)	Species names Seed storage type ² in [] ³ No. of seed lots ⁴ in { } brackets
A) Conifers			
<i>Abies</i>	Fir	orthodox: 4	<i>amabilis</i> {5}, <i>balsamea</i> {139}, <i>grandis</i> {12}, <i>lasiocarpa</i> {6}
<i>Chamaecyparis</i>	Cypress	orthodox: 1	<i>nootkatensis</i> {1}
<i>Juniperus</i>	Juniper	orthodox: 2	<i>virginiana</i> {6}, <i>scopulorum</i> {2}
<i>Larix</i>	Larch	orthodox: 3	<i>laricina</i> {401}, <i>lyallii</i> {0}, <i>occidentalis</i> {3}
<i>Picea</i>	Spruce	orthodox: 5	<i>engelmannii</i> {14}, <i>glauca</i> {2771}, <i>mariana</i> {772}, <i>rubens</i> {182}, <i>sitchensis</i> {24}
<i>Pinus</i>	Pine	orthodox: 9	<i>albicaulis</i> {1}, <i>banksiana</i> {1122}, <i>contorta</i> {103}, <i>flexilis</i> {101}, <i>monticola</i> {7}, <i>ponderosa</i> {9}, <i>resinosa</i> {129}, <i>rigida</i> {30}, <i>strobus</i> {201}
<i>Pseudotsuga</i>	Douglas-fir	orthodox: 1	<i>menziesii</i> {28}
<i>Taxus</i>	Yew	orthodox: 1	<i>brevifolia</i> [O?] {0}
<i>Thuja</i>	Cedar	orthodox: 2	<i>occidentalis</i> {97}, <i>plicata</i> {8}
<i>Tsuga</i>	Hemlock	orthodox: 3	<i>canadensis</i> {221}, <i>heterophylla</i> {7}, <i>mertensiana</i> {6}
B) Hardwoods			
<i>Acer</i>	Maple	orthodox: 3 orthodox?: 4 intermediate: 1 recalcitrant: 1 unknown: 1	<i>circinatum</i> [O?]{0}, <i>glabrum</i> [O?] {1}, <i>macrophyllum</i> [I]{0}, <i>negundo</i> [O?]{23}, <i>nigrum</i> [Un]{0}, <i>rubrum</i> [O]{245}, <i>pensylvanicum</i> [O?]{33}, <i>saccharinum</i> [R]{7}, <i>saccharum</i> [O]{69}, <i>spicatum</i> [O]{64}
<i>Aesculus</i>	Buckeye	recalcitrant: 1	<i>glabra</i> [R]{0}
<i>Alnus</i>	Alder	orthodox: 1 orthodox?: 3	<i>rubra</i> [O]{30}, <i>rugosa</i> [O?] {0}, <i>sinuata</i> [O?]{20}, <i>incana</i> [O?] {52}
<i>Arbutus</i>	Arbutus	orthodox?: 1	<i>menziesii</i> [O?]{0}
<i>Asimina</i>	Pawpaw	recalcitrant?: 1	<i>triloba</i> [R?]{0}
<i>Betula</i>	Birch	orthodox: 8	<i>alleghaniensis</i> {158}, <i>cordifolia</i> {16}, <i>lenta</i> {1}, <i>lutea</i> {0}, <i>neoalaskana</i> {0}, <i>occidentalis</i> {2}, <i>papyrifera</i> {154}, <i>populifolia</i> {44}
<i>Carpinus</i>	Blue beech	unknown: 1	<i>caroliniana</i> [Un]{2}
<i>Carya</i>	Hickory	orthodox: 4	<i>cordiformis</i> {0}, <i>glabra</i> {0}, <i>laciniosa</i> {0}, <i>ovata</i> {0}
<i>Castanea</i>	Chestnut	recalcitrant: 1	<i>dentata</i> [R] {0}
<i>Celtis</i>	Hackberry	orthodox: 1	<i>occidentalis</i> {0}
<i>Cercis</i>	Redbud	orthodox: 1	<i>canadensis</i> ⁵ {0}
<i>Cornus</i>	Dogwood	orthodox?: 3	<i>alternifolia</i> {1}, <i>florida</i> {4}, <i>nuttallii</i> {1}
<i>Crataegus</i>	Hawthorns	orthodox?: 4	<i>crus-galli</i> {0}, <i>coccinea</i> {2}, <i>douglasii</i> {1}, <i>mollis</i> {0}
<i>Fagus</i>	Beech	orthodox?: 1	<i>grandifolia</i> {11}
<i>Fraxinus</i>	Ash	orthodox: 5	<i>americana</i> {280}, <i>nigra</i> {193}, <i>pennsylvanica</i> {170}, <i>profunda</i> {1}, <i>quadrangulata</i> {1}
<i>Gleditsia</i>	Honey locust	orthodox: 1	<i>triacanthos</i> {2}
<i>Gymnocladus</i>	Kentucky coffee-tree	orthodox: 1	<i>dioicus</i> {0}

Genus	Common name	Seed storage type (no. of species per genus for each seed type)	Species names Seed storage type ² in [] ³ No. of seed lots ⁴ in { } brackets
<i>Hamamelis</i>	Witch hazel	orthodox: 1	<i>virginiana</i> {5}
<i>Juglans</i>	Walnut	recalcitrant: 2	<i>cinerea</i> {0}, <i>nigra</i> {0}
<i>Liriodendron</i>	Tulip-tree	orthodox?: 1	<i>tulipifera</i> {2}
<i>Magnolia</i>	Cucumber tree	orthodox?: 1	<i>acuminata</i> {1}
<i>Malus</i>	Wild Apple	orthodox: 2	<i>coronaria</i> {0}, <i>fusca</i> {0}
<i>Morus</i>	Mulberry	orthodox: 1	<i>rubra</i> {0}
<i>Nyssa</i>	Black gum	unknown: 1	<i>sylvatica</i> {0}
<i>Ostrya</i>	Ironwood	unknown: 1	<i>virginiana</i> {20}
<i>Plantanus</i>	Sycamore	orthodox: 1	<i>occidentalis</i> {2}
<i>Populus</i>	Poplar	orthodox: 6	<i>augustifolia</i> {0}, <i>balsamifera</i> {52}, <i>deltoids</i> {20}, <i>grandidentata</i> {49}, <i>tremuloides</i> {66}, <i>trichocarpa</i> {1}
<i>Prunus</i>	Cherry	orthodox: 2 orthodox?: 1 intermediate: 1 unknown: 2	<i>americana</i> [O?]{0}, <i>emarginata</i> [Un]{0}, <i>nigra</i> [Un]{1}, <i>pensylvanica</i> [O]{91}, <i>seotina</i> [O]{40}, <i>virginiana</i> [I]{367}
<i>Ptelea</i>	Hop-tree	orthodox?: 1	<i>trifoliata</i> [O?]{1}
<i>Quercus</i>	Oak	recalcitrant: 11	<i>alba</i> {0}, <i>bicolor</i> {0}, <i>ellipsoidalis</i> {0}, <i>garryana</i> {0}, <i>macrocarpa</i> {0}, <i>muehlenbergii</i> {0}, <i>palustris</i> {0}, <i>prinoides</i> {0}, <i>rubra</i> {0}, <i>shumardii</i> {0}, <i>velutina</i> {0}, <i>purshiana</i> {0}
<i>Rhamnus</i>	Buckthorn	orthodox?: 1	<i>amygdaloides</i> [O?]{0}, <i>nigra</i> [O?]{11}
<i>Salix</i>	Willow (trees only)	orthodox?: 2	
<i>Sambucus</i>	Elder	orthodox?: 2	<i>cerulea</i> {0}, <i>glaucia</i> {0}
<i>Sassafras</i>	Sassafras	orthodox?: 1	<i>albidum</i> {0}
<i>Sorbus</i>	Mountain ash	orthodox?: 1	<i>americana</i> {9}, <i>decora</i> {7}
<i>Tilia</i>	Basswood	orthodox: 1	<i>americana</i> {15}
<i>Ulmus</i>	Elm	orthodox: 3	<i>americana</i> {43}, <i>rubra</i> {0}, <i>thomasii</i> {0}

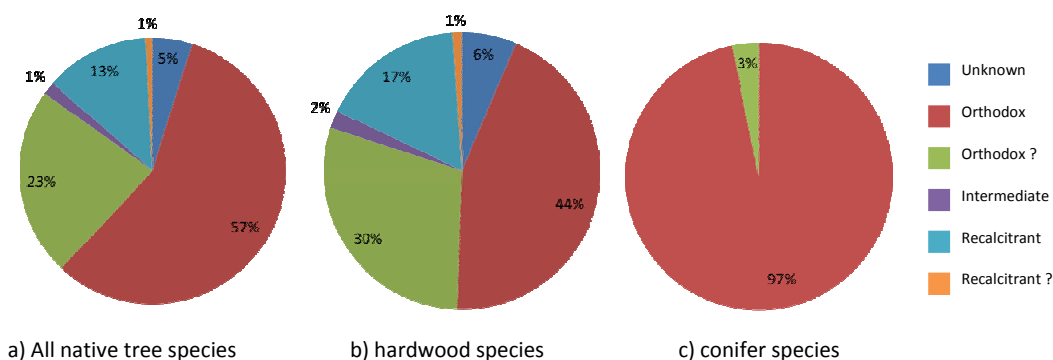
¹ These data were derived from the NTSC database and represent the total number of seed lots stored as of June 2010.

² All seed storage classifications are based on the species-specific storage designations by Hong et al. (1998).

³ If all species in a genus are of the same seed storage type, this value was not noted in this column.

⁴ A seed lot is a collection made at 1 point in time, from a specific location from either a single tree or a bulk of a number of trees.

⁵ This species is most likely extirpated.



¹ These summaries are based on the results in Table 2.

Fig. 1. Summary of the seed storage classification of Canadian tree species¹.



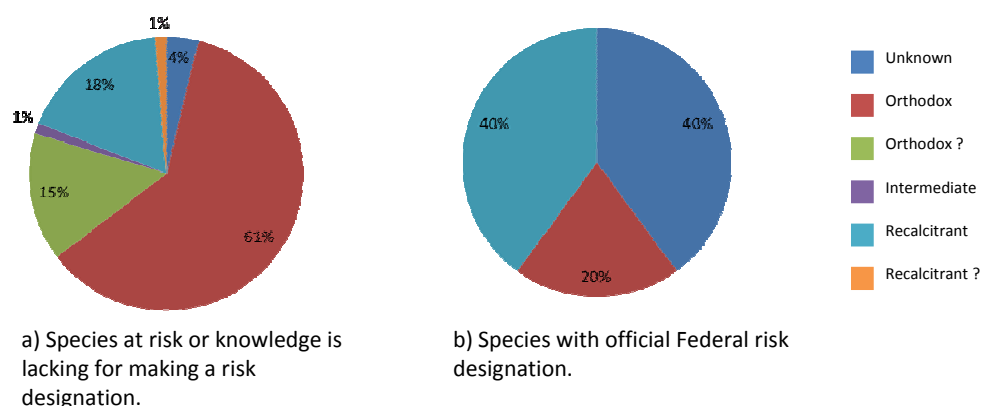
Table 3. A) Seed storage classification¹ of Canadian tree species and B) number of seed lots stored in the National Tree Seed Centre² based on seed storage classification

	Seed storage classification					
	Orthodox	Orthodox?	Intermediate	Recalcitrant	Recalcitrant?	Unknown
A)						
Conifers: no. of species	30	1	0	0	0	0
Hardwoods: no. species	42	28	2	16	1	6
Total no. of species	72	29	2	16	1	6
B)						
Conifers: no. of seed lots	6390	0	0	0	0	0
Hardwoods: no. of seed lots	1816	169	367	7	0	23
Total no. of seed lots	8206	169	367	7	0	23

¹ All seed storage classifications are based on the species-specific storage designations by Hong et al. (1998).

² These data were derived from the NTSC database and represent the total number of seed lots stored as of June 2010.

Focusing on the number of species identified by the 2003 national survey as being at risk or knowledge is lacking for making a conservation designation, 61% produce orthodox seeds, while 39% produce seeds which can either not be stored or there may be storage issues (considering orthodox, intermediate, recalcitrant, and unknown classified seeds) (Fig. 2a). Eighty percent of species with an official federal risk designation are classified as either recalcitrant or unknown (Fig. 2b). This provides a challenge for storage and an opportunity for research to develop effective storage protocols.



¹ results based on a survey conducted in 2003.

² Based on the number of tree species listed by the Canadian Species At Risk Act (SARA) in 2009.

Fig. 2. Summary of the seed classification type of Canadian tree species at risk based on a) a national survey¹ and b) species with an official federal risk designation².

Three other political jurisdictions in Canada have designated *ex situ* collections for native tree species within their respective jurisdictions: BC, Alberta, and Manitoba. The goals of these collections vary slightly, but overall they are to establish seed banks or clone banks to maintain a source of genetic diversity, and to provide insurance against catastrophic losses to *in situ* populations, in addition to providing reserve materials for expanding *in situ* reserves (Kolotelo et al. 2007).

DISCUSSION

The majority of native conifer species produce orthodox seeds, which can readily tolerate storage, and these species are well represented in the NTSC. However, the collections are variable in terms of how representative they are of the species' range and/or the species' genetic variation (if known). Seeds of many of these species are dormant (results not shown). Seeds may enter a deeper form of dormancy or break dormancy when stored at below-freezing temperatures (Beardmore et al. 2008). So while seeds can tolerate storage, there may be issues associated with alterations in dormancy which could significantly impact the quantity and quality of germinants.

For Canadian native hardwoods, seed storage characteristics range from orthodox to recalcitrant, with a few species having unknown storage characteristics. Forty-two percent of these species are represented in the NTSC, and the majority of these collections are from species with orthodox seed characteristics. An obvious gap in the NTSC collections is with non-orthodox seeds, in particular recalcitrant and questionable recalcitrant seeds. The majority of species listed at the federal level as being at risk produce this type of seed. Clearly, there is a need to focus attention on developing storage protocols, for these species that produce seeds which cannot be stored for long durations, or for which knowledge concerning how to store their seeds is lacking. This is particularly important for species which are not well represented *in situ* conservation areas.

In order to move forward, it is very important to develop national-level strategies for targeting species and collections. Specifically we need to:

1. Adopt and implement a comprehensive strategy in collaboration with the jurisdictions to collect and conserve seeds for supporting conservation and recovery of key representative natural populations, habitats, and ecosystems;
2. Develop effective deployment and rejuvenation strategies for *ex situ* collections with participating agencies; and
3. Conduct research concomitant with collections and banking activities to ensure that effective *ex situ* conservation measures are taken.

All native Canadian trees have ranges that span into the US. The degree to which this occurs varies; for example, approximately 90% of the range of *Fraxinus quadrangulata* is in the US, while only small disjunct populations of *Picea rubens* are found in the US with the majority of the range found in Canada. For a few species such as *Pseudotsuga menziesii*, the range spans 3 countries, Canada, the US, and Mexico. Given this situation, it is important to consider what the conservation activities and goals are for these species in the US and Mexico, and where it is possible to coordinate *ex situ*



strategies across these borders. This may be particularly relevant when considering optimal seeds sources for an assisted migration strategy.

Internationally, there is a strong commitment by governments and non-governmental agencies to *ex situ* conservation. Infrastructure commitments can be best depicted by, for example, the Svalbard Global Seed Vault which was built in 2007 to provide insurance against the loss of germplasm from seed banks, as well as a refuge for seeds in the case of large-scale regional or global crises. The Millennium Seed Bank Project (MSBP) of the Royal Botanic Gardens, Kew, UK, in partnership with countries such as Australia, Chile, Kenya, China, and the US, and agencies such as the Food and Agricultural Organization, and Bioversity International, was developed to enable countries to meet international conservation objectives set by the Global Strategy for Plant Conservation of the Convention on Biological Diversity and the Millennium Development Goals of the United Nations (UN) Environment Programme (UN, 2007). The current goal of the MSBP is to collect and bank seeds of 25% of the world's plant species. The NTSC has initiated collaboration with the MSBP. The EU through the European Native Seed Conservation (Bonomi et al. 2008), the United States Seeds of Success Program and the Australian Network for Plant Conservation (ANPC 1997) are examples of networked national-level *ex situ* conservation programs. Clearly it is best to conserve species across geopolitical borders. This is particularly relevant when species' ranges span borders, and when threats to the species span borders.

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Germination Responses of *Terminalia ivorensis* Seeds to a Range of Alternating and Constant Temperatures Provided by the Two-Way Grant's Thermogradient Plate

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[Summary]

A 2-way Grant thermogradient plate was used to investigate the effect of various temperature combinations on germination of *Terminalia ivorensis* with no chemical pretreatment. Seeds were harvested at full maturity in November 2005 in Ghana. Seeds were tested for germination on top of 3 sheets of filter paper moistened with deionized water in 9-cm-diameter glass Petri dishes. The thermogradient plate allows for germination testing of seeds over a wide range of single- and alternating-temperature regimes over a time continuum given 64 temperature combinations. Conditions were 40/40°C (day/night temperatures) on the high end of the plate and 5/5°C on the cool end. Two temperature gradients ranging 5~40°C were used. The first gradient, progressing from left to right on the thermogradient plate in darkness, was alternated every 12 h with the second progressing from front to back of the thermogradient plate with light. The study was repeated 2 times. Fifteen seeds were used for each replication. The various temperature combinations had significant effects on the final germination percentage, mean germination time, and time to first germination. Germination occurred at 35 of the 64 temperature combinations. The best final germination percentage at a constant temperature was 87%, and this was recorded at 30/30°C. Alternating temperature combinations of 15/35, 20/35, 30/35, 35/40, and 40/30°C gave final germination percentages of 73~86%.

Key words: *Terminalia ivorensis*, optimum germination requirement, thermogradient plate, temperature regimes, germination percentage, seed conservation.

INTRODUCTION

Temperature affects the germination capacity (germinability), germination rate, and distribution of the relative frequency of germination along the incubation time (Labouriau 1978). There is usually an optimal temperature below and above which germination is delayed or prevented (Rawat 2005). Each species requires a range of temperatures for seed germination and seedling establishment (Bradbeer 1988). For most tropical tree seeds, room temperature of 25~30°C in the tropics will be quite suitable for maximum germination (Smith 2002).

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Alternating temperatures are preferred over constant temperatures because they can overcome shallow seed dormancy and enhance uniform germination (ISTA 1996). Although natural fluctuations between day and night temperatures are less in lowland moist tropical forests than in other forest types, alternating temperatures can still affect the germination of tropical species (FAO 1985).

Studies of temperature effects on seed germination are usually limited to relatively few different temperatures because of restrictions imposed by the number of available germinators for simultaneous experiments (Larsen 1965).

The Grant thermogradient plate is a bi-directional incubator (Manger 1999). The instrument allows the germination testing of seeds over a wide range of single- and alternating-temperature regimes over a time continuum (Tarasoff et al. 2005). Within a predetermined range, this device simultaneously provides all possible alternating and constant temperature combinations (Larsen et al. 1973).

Terminalia ivorensis is among the most important of the some 200 species belonging to the genus *Terminalia* (Combretaceae). The species is native to West and Central Africa, and as a result of the quality of its timber, a number of important plantation programs were begun within its natural range in West Africa. Seeds of *T. ivorensis*, however, germinate with great difficulty. This is most probably caused by the thick, lignified seed coat (Corbineau and Côme 1993).

A more-comprehensive look at its germination responses to a broad range of alternating and constant temperatures is important for its *ex situ* conservation and its use in afforestation and reforestation programs. The present research was conducted to ascertain optimal germination requirements and all possible alternating- and constant-temperature combinations for the germination of the species.

MATERIALS AND METHODS

Seed materials

Seeds of *T. ivorensis* were harvested at full maturity from forests at Gambia no. 1 (in the Brong Ahafo Region) of Ghana in October 2005. Seeds were spread on jute sacks and cleaned of twigs, bark, foliage, and other impurities (Turnbull 1975). They were then shade-dried for 3 d and packed in cotton bags. Samples were immediately sent by air to the Seed Conservation Department of the Royal Botanic Gardens, Kew, UK, where the experiment was conducted.

Seed moisture content and equilibrium relative humidity (eRH)

determination

To establish the moisture status of seed samples on receipt at the laboratory in order to ensure their proper handling, the eRH or water activity (aw) of the seeds and their moisture contents were determined (MSBP 2002, 2005). The eRH of the seed samples was measured using a Rotronic AW-14P water activity measuring station (Rotronic Instruments, Horley, UK) set up with a DMS 100H humidity sensor.

Seed moisture contents (MCs) of the species were determined on whole seeds; 5 replicates of 10 seeds each were weighed before and after drying at 103°C for 17 h



(ISTA 1999). The moisture content was then calculated using the formula: $(IW - FW)/IW \times 100$, where IW is the initial weight and FW is the final weight.

Terminalia ivorensis seeds were then dried in silica gel for 24 h by mixing them in a 1: 1 ratio. This reduced the moisture content from the initial 18.5% to 7.4%, after which seed samples were sealed in aluminum foil laminate bags and held at 15°C until use 2 mo later (Table 1).

Table 1. Equilibrium relative humidity (eRH; %) and seed moisture content (%) of *Terminalia ivorensis* seed sample measured on receipt at the laboratory and after the seeds had been desiccated in silica for 24 h

eRH (%) of seed samples upon receipt in the UK	70.5%	eRH (%) of seed sample after desiccation in silica gel for 24 h	40.7%
Moisture content (%) of seed samples upon receipt in the UK	18.5%	Moisture content (%) of seed sample after desiccation in silica gel for 24 h	7.4%

Germination of seeds on the 2-way Grant's thermogradient plate

Seeds of *T. ivorensis* were germinated on filter paper. Each 90-mm plastic Petri dish was lined with 3 pieces of moistened filter paper. Petri dishes were arranged in an 8 x 8 array on the thermogradient plate giving 64 temperature combinations (regimes) (at 5~40°C). Conditions were 40/40°C (daytime/nighttime temperatures) on the high end of the plate and 5/5°C on the cool end. Filter papers in the Petri dishes located at the hot end of the plate were moistened daily due to excessive drying. Two temperature gradients ranging 5~40°C were used. The first gradient, progressing from left to right on the thermogradient plate in the dark, was alternated every 12 h with the second which progressed from front to back of the thermogradient plate with light. Seeds were subjected to 8 constant- and 56 alternating-temperature regimes as shown in Fig. 1. The study was repeated 2 times. Fifteen seeds were used for each replicate.

Normal seedlings were scored and removed as soon as the radical was 1 cm long and the plumule was visible. The location and time of removal were recorded as described by Larsen et al. (1973).

Seeds remained on the thermogradient plate for 30 d, and ungerminated samples were then placed at 30°C for 10 d after which the cutting test was conducted to ascertain the viability of seeds which had still not germinated ((IPGRI-DFSC 2000).

Germination indices were measured, namely, the germination percentage, the weighted mean germination time (MGT), the time to first germination, and the rate of germination (Anjum and Bajwa 2005). These are important aspects of the germination process which inform of the dynamics of the process (Bewley and Black 1994, Silveira et al. 2005).

The germination percentages at 30 d after imbibition were calculated from the total number of seeds germinated at each temperature divided by the total number of seeds used for each regime.

The MGT or average time to germination (ATG in days) was calculated as done by Yang et al. (2003):

$$\text{MGT (ATG)} = \Sigma (t \times n) / \Sigma n$$

where t is time in days starting from the day of sowing, and n is the number of seeds completing germination on day t . Results were subjected to an analysis of variance (ANOVA) to determine the main effects and interaction effects using GensStat Release 4.21 (Rothamsted Experimental Station, UK)

H1 (40/5)	H2 (40/10)	H3 (40/15)	H4 (40/20)	H5 (40/25)	H6 (40/30)	H7 (40/35)	H8 (40/40)
G1 (35/5)	G2 (35/10)	G3 (35/15)	G4 (35/20)	G5 (35/25)	G6 (35/30)	G7 (35/35)	G8 (35/40)
F1 (30/5)	F2 (30/10)	F3 (30/15)	F4 (30/20)	F5 (30/25)	F6 (30/30)	F7 (30/35)	F8 (30/40)
E1 (25/5)	E2 (25/10)	E3 (25/15)	E4 (25/20)	E5 (25/25)	E6 (25/30)	E7 (25/35)	E8 (25/40)
D1 (20/5)	D2 (20/10)	D3 (20/15)	D4 (20/20)	D5 (20/25)	D6 (20/30)	D7 (20/35)	D8 (20/40)
C1 (15/5)	C2 (15/10)	C3 (15/15)	C4 (15/20)	C5 (15/25)	C6 (15/30)	C7 (15/35)	C8 (15/40)
B1 (10/5)	B2 (10/10)	B3 (10/15)	B4 (10/20)	B5 (10/25)	B6 (10/30)	B7 (10/35)	B8 (10/40)
A1 (5/5)	A2 (5/10)	A3 (5/15)	A4 (5/20)	A5 (5/25)	A6 (5/30)	A7 (5/35)	A8 (5/40)

Fig. 1. Layout of the arrangement of treatments on the thermogradient plate. The letters A1 to H8 represent Petri dishes (treatments), and the numbers in parentheses are the 64 temperature combinations (in °C) of each treatment during the experimental period. The zero amplitude diagonal gives a gradient of constant temperatures.

RESULTS

Seed eRH (%) and MC (%) on receipt at the laboratory and after desiccation in an equal weight of silica gel for 24 h are presented in Table 1. An eRH value of 70.5% and MC of 18.5% were obtained for *T. ivorensis* on receipt at the laboratory in the UK. After desiccation in silica gel for a period of 24 h, the seed eRH and MC were 40.7% and 7.4%, respectively.

Germination temperatures significantly affected the final germination percentage (FGP) of *T. ivorensis*. Germination occurred at 35 of 64 temperature combinations provided by the thermogradient plate. No germination of *T. ivorensis* occurred in areas of low alternating temperatures and areas of low constant temperatures on the



thermogradient plate. Among these alternating temperature regimes were 5/10, 5/15, 5/20, 10/5, 10/15, 10/20, 15/5, 15/10, 20/5, and 20/10°C. Constant temperature regimes where no germination was recorded included 5/5, 10/10, and 15/15°C. Constant temperatures below 25/25°C and above 30/30°C produced very poor germination results. For instance, germination percentages at 20/20 and 35/35°C were 0% compared to 87% at 30/30°C. Alternating temperatures of 15/35, 20/35, 30/35, 35/40, and 40/30°C gave germination percentages of > 73% (Table 2, Fig. 2).

Table 2. Germination (Germ.) percentages of *Terminalia superba* seeds incubated at different temperature combinations on the 2-way thermogradient plate. Only temperature regimes at which germination occurred are shown.

Temp. (°C)	Germ. percentage (%)	Temp (°C)	Germ. percentage (%)	Temp. (°C)	Germ. percentage (%)	Temp. (°C)	Germ. percentage (%)
5/30	27.0	20/30	60.0	30/20	53.0	35/40	73.0
5/35	20.0	20/35	76.5	30/25	63.5	40/5	20.0
5/40	23.5	25/25	13.0	30/30	88.0	40/15	27.0
10/35	33.0	25/30	40.0	30/35	73.0	40/20	43.5
10/40	13.0	25/35	43.5	30/40	63.5	40/25	23.5
15/30	47.0	25/40	60.0	35/15	47.0	40/30	86.0
15/35	73.0	30/10	60.0	35/20	16.5	40/35	33.0
15/40	53.0	30/15	23.5	35/25	60.0	40/40	13.0
20/25	40.0		33.0	35/30	60.0	-	-
S.E.D = 2.51				replicates = 2			
d.f. = 35				Coefficient of variation = 5.6%			

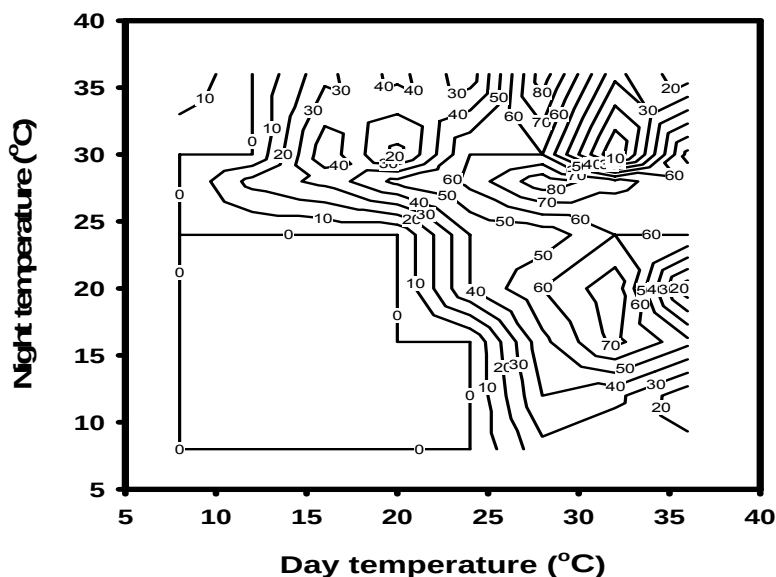


Fig. 2. A map showing final germination percentages of *Terminalia ivorensis* seeds on the thermogradient plate. Percentage germination values are shown on the isopleths.

Germination of the species was significantly influenced by "reversed" alternating temperature regimes. Reversed temperature regime pairs such as (15/35 and 35/15°C), (20/35 and 35/20°C), (30/35 and 35/30°C), (35/40 and 40/35°C), (40/30 and 30/40°C), and (25/30 and 30/25°C) among others recorded significantly different FGPs, MGTs, and times to first germination. In all cases, with the exception of the reversed-temperature regime pairs (30/40 and 40/30°C) and (25/30 and 30/25°C), low daytime temperatures followed by high nighttime temperatures resulted in significantly higher FGPs than high daytime temperatures followed by low nighttime temperatures. At incubation temperature combinations where low daytime temperatures were followed by warm nighttime temperatures, amplitudes of change between daytime and nighttime ranging 5~20°C resulted in germination percentages of 60~86%, while the amplitude of change between daytime and nighttime at > 20°C resulted in germination percentages of 13~53% (Table 2).

There were significant differences ($p < 0.001$) between MGTs at the various temperature combinations at which seeds were set for germination on the thermogradient plate. The shortest MGT recorded included 22.5 d at 30/30°C (88.0%), 23.5 d at 40/30°C (86.0%), 23.2 d at 35/40°C (73.0%), 25.5 d at 15/35°C (73.0%), and 26.5 d at 20/35°C (76.5%) (Table 3).

Table 3. Mean germination time (MGT; d) of *Terminalia superba* seeds incubated at different temperature combinations on the 2-way thermogradient plate. Only temperature regimes at which germination occurred are shown.

Temp. (°C)	MGT (d)	Temp. (°C)	MGT (d)	Temp. (°C)	MGT (d)	Temp. (°C)	MG (d)
5/30	33.3	20/30	29.1	30/20	31.0	35/40	23.2
5/35	33.0	20/35	26.5	30/25	31.4	40/5	31.4
5/40	32.5	20/40	26.4	30/30	22.5	40/15	34.5
10/35	30.9	25/25	29.0	30/35	29.5	40/20	26.5
10/40	30.0	25/30	32.0	30/40	24.4	40/25	31.0
15/30	22.8	25/35	27.2	35/15	31.9	40/30	23.5
15/35	25.5	25/40	29.9	35/20	30.2	40/35	22.6
15/40	30.5	30/10	33.5	35/25	27.1	40/40	29.0
20/25	26.3	30/15	30.3	35/30	26.6	-	-
S.E.D = 0.4573				Replicates = 2			
d.f = 35				Coefficient of variation = 1.6%			

The time to first germination of the species was significantly ($p < 0.001$) influenced by the temperature regimes at which seeds were placed on the thermogradient plate. The fastest times to first germination were 13 and 14 d, recorded with the temperatures regimes of 30/30, 30/35, and 40/30°C. These were significantly faster than times to first germination registered at other temperature combinations. The longest times to first germination were 31, 31, 31, 31, 32, and 33 d recorded at 5/30, 5/35, 5/40, 25/30, 40/15 and 30/10°C, respectively (Table 4).



Table 4. Time to first germination of *Terminalia superba* seeds incubated at different temperature combinations on the 2-way thermogradient plate. Only temperature regimes at which germination occurred are shown.

Temp. (°C)	Time to 1st germination (d)	Temp (°C)	Time to 1st germination (d)	Temp. (°C)	Time to 1st germination (d)	Temp. (°C)	Time to 1st germination (d)
5/30	31.0	20/30	23.0	30/20	27.0	35/40	15.0
5/35	31.0	20/35	16.5	30/25	27.0	40/5	29.0
5/40	31.0	20/40	17.0	30/30	13.0	40/15	32.0
10/35	28.5	25/25	21.5	30/35	14.0	40/20	23.0
10/40	28.5	25/30	31.0	30/40	19.0	40/25	28.5
15/30	19.0	25/35	17.0	35/15	24.0	40/30	13.0
15/35	19.0	25/40	19.0	35/20	17.0	40/35	15.0
15/40	24.5	30/10	33.0	35/25	18.0	40/40	27.0
20/25	21.0	30/15	25.0	35/30	17.0	-	-
S.E.D = 0.5606 d.f. = 35				Replicates = 2 Coefficient of variation = 2.5%			

Cumulative seed germination curves of *T. ivorensis* at certain selected temperatures combinations are presented in Fig. 3. Seeds placed at the various temperature combinations showed differences in increased germination percentages over time, periods of largest seed germinability, continuity in seed germination, and periods at which germination remained constant as well as periods of maximum germination (gMax).

At 15/30°C, germination percentages increased until the 19th day (73.3%), after which it remained constant. The largest germinability was recorded between the 13th and 15th days. At 30/15°C, seed germination increased until the 13th day; it then remained constant, and began slightly increasing on the 17th day, attaining gMax of 33.3% on the 21st day. Seeds placed at 20/35°C had increased germination until the 7th day, germination, then it remained constant [for x d?], and increased again to the 23rd day. The largest germinability was registered between the 9th and 11th days and 15th and 17th days. Seeds incubated at a constant temperature of 30/30°C recorded increased germination until the 7th day; it then remained constant and picked up from the 11th until the 19th day when a gMax of 87.0% was reached. The largest germinability was recorded between the 9th and 11th days. At 40/30°C, germination increased until the 13th day; it then remained constant and picked up again from the 17th day, attaining a gMax of 86.7% on the 21st day.

DISCUSSION

The eRH value of 70.5% and MC of 18.5% for *T. ivorensis* on receipt at the laboratory in the UK placed the seed lot into a "damp" seed status, and they required immediate drying to reduce the risk of fungal attack (MSBP 2005). Drying the seeds in silica gel lowered the eRH to 40.7% with a seed MC of 7.4%, placing the seed in a "dry" seed status (MSBP 2005), thereby keeping them safe from fungal attack before the germination experiment began 2 mo later.

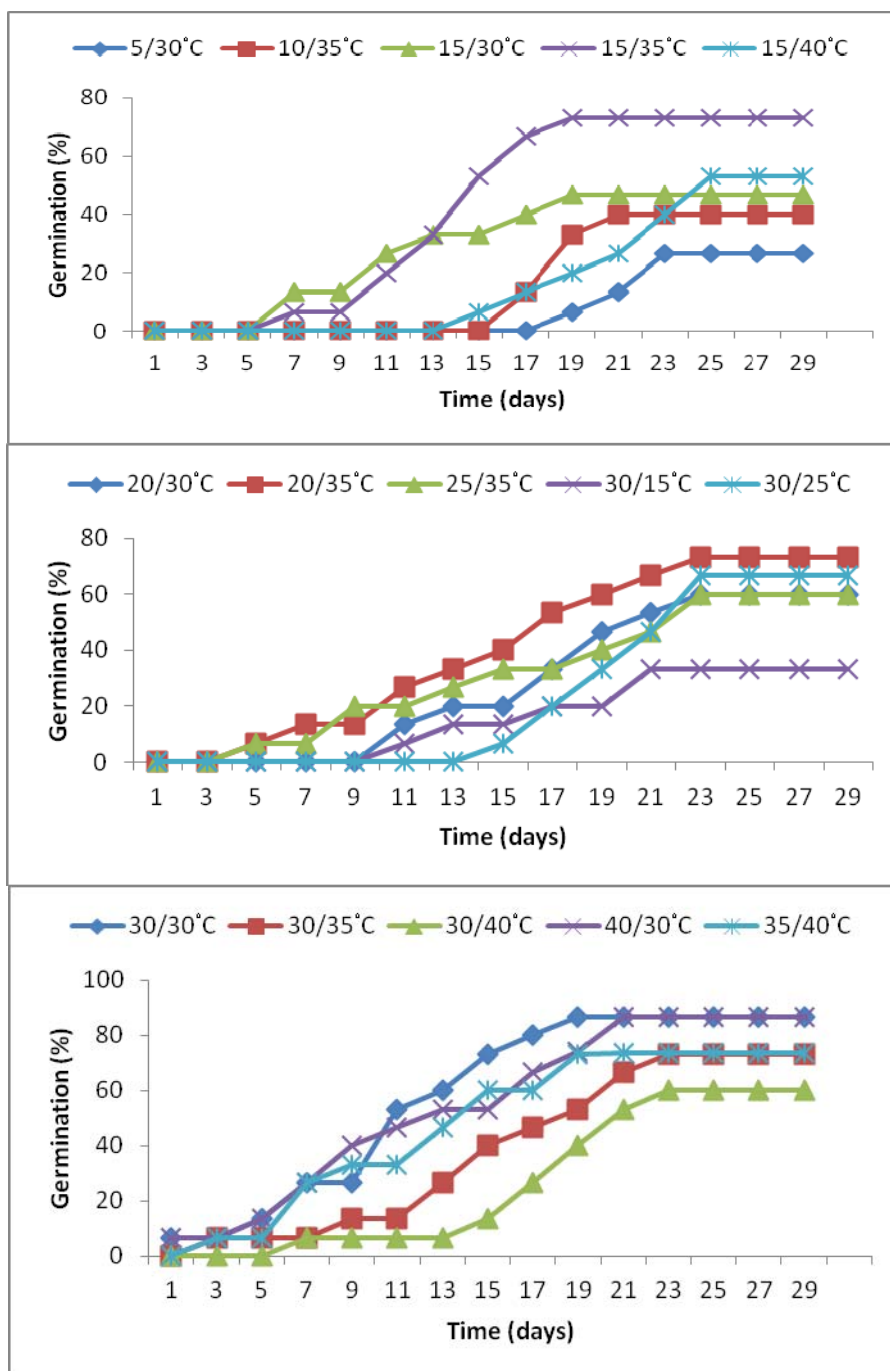


Fig. 3. Cumulative seed germination curves of *Terminalia ivorensis* at some selected temperature combinations on the thermogradient plate.



Germination of *T. ivorensis* seeds occurred at 35 of a total of 64 temperature combinations provided by the thermogradient plate. This is an indication that the species will germinate over a wide array of temperature regimes (Table 2, Fig. 2). In similar work on *T. superba*, Asomaning Sacande, and Olympio (unpubl. data) reported that the species germinated at 40 of 64 temperature combinations provided by the thermogradient plate, meaning that *T. superba* germinates over a wider array of temperature combinations than *T. ivorensis*.

Against the background that seeds of *T. ivorensis* germinate with great difficulty as a result of the presence of a thick, lignified seed coat (Corbineau and Côme 1993), *T. ivorensis* seeds in the present study showed germination percentages of 60.0~86.0% at alternating temperature combinations with relatively high components (Table 2, Fig. 2). Under a constant temperature of 30/30°C, the germination percentage was the highest; this is also a relatively warmer temperature regime (Table 2, Fig. 2). The species exhibits a typical tropical characteristic in terms of the optimum temperature for seed germination. Asomaning et al. (unpubl. data) also reported that seeds of *T. superba*, germinated better at warmer alternating and constant temperature regimes on the thermogradient plate. According to Daws et al. (2002), germination at higher temperatures is typical of tropical species because in the natural environment, dispersed seeds are likely to experience soil temperatures of 36°C or higher.

The observation that in the majority cases reversed temperature regimes with low daytime temperatures followed by high nighttime temperatures recorded higher FGPs than in situations with high daytime temperatures followed by low nighttime temperatures is an indication that seed germination in *T. ivorensis* is stimulated by the lower daytime temperatures followed by warmer nighttime temperatures.

Characteristics of the amplitude (i.e., the difference between the daily maximum and minimum temperatures) of alternating temperatures that appear to control germination were at play in the present study. It was established from this study that seeds of *T. ivorensis* incubated at alternating temperatures (low daytime temperatures followed by a warm nighttime temperatures) exhibited good germination percentages when the amplitude of change in the temperature was 5~20°C. In a similar experiment on *Khaya anthotheca*, Asomaning (2009) observed that the amplitude of change between day and night temperatures of 5~25°C resulted in very high germination percentages. Bonner (1983) observed that germination of many temperate species occurs at a wide range of temperature regimes, and that an amplitude of change between day and night of 12°C may be more important than the cardinal points.

The MGT is used to measure the spread of germination of a seed lot. A low MGT is an indication of rapid seed germination and uniform seedlings (Silviera et al. 2005). Results from the current study showed that temperature regimes which support high FGPs achieved this with a minimum MGT, while temperature regimes which recorded low germination percentages achieved this with much longer periods (Tables 2, 3). This observation corresponds to findings of Silviera et al. (2005) on *Calliandra fasciculata* (Benth.). Asomaning et al. (unpubl. data) also observed similar development of *T. superba* in a thermogradient plate experiment.

The time to first germination expresses the time of germination of the fastest seeds (Silviera et al. 2005). In general, time to first germination was invariably shortest at temperature regimes that recorded higher FGPs as well as fastest mean times

indicating rapid germination (Tables 2-4). This means that when seeds are placed at their optimal temperature regimes, they will go through the germination process faster compared to when they have been placed in unfavorable temperature regimes. This result is similar to what Asomaning et al. (unpubl. data) observed in a germination experiment with *T. superba* on the thermogradient plate.

Germination curves can be used to describe germination performances of seeds placed at various temperature regimes provided by the thermogradient plate. Germination curves at optimal germination temperatures such as 20/30, 40/30, 35/40, 15/35, 30/30, 30/25, and 30/40°C exhibited steeper slopes compared to curves generated at unfavorable temperatures such as 5/30 and 30/15°C among others (Fig. 3). Steep slope germination curves signify faster increases in germination percentages over time, consistency in continuity of seed germination, and other germination parameters which inform of the dynamics of the germination process. Labouriau (1978) and Kocabas et al. (1999) reported that temperature affects the germination capacity (germinability), germination rate, and distribution of the relative frequency of germination along the incubation time. In general, germination curves of *T. ivorensis* at all temperatures over time appeared as S-shaped curves resembling typical cumulative germination of a population of seeds over time as reported by Palazzo and Brar (1997).

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High-Temperature Effects on Seed Germination in *Shorea balangeran*, a Tropical Peat Swamp Tree in Central Kalimantan, Indonesia

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Limin Suwido,³⁾ Masato Shibuya,²⁾ Takayoshi Koike²⁾

[Summary]

To determine suitable sowing conditions for nursery operations, we investigated the effects of 5 h of heat-shock treatment at 4 temperatures of 30 (control), 40, 50, and 60°C, on germination and initial juvenile root survival in *Shorea balangeran* (Dipterocarpaceae), a tree species planted on disturbed peat land in Indonesia. Germination rates were sufficiently high at 80% at 30, 40, and 50°C. However, at 60°C, heat shock killed all of the seeds. After germination, 30°C incubation and 40°C heat shock resulted in 80% and 85% radicle survival, respectively; however, 50°C heat shock killed all radicles within 1 wk. Consequently, the threshold of survival through seed germination and seedling establishment may be between 40 and 50°C. In a previous study, we found that survival of *S. balangeran* seeds and seedlings decreased by 20% in an open area without watering, accompanied by high soil temperatures of around 50°C. Our results suggest that a high soil temperature is a serious factor reducing the efficiency of seedling emergence; thus, the sowing of *S. balangeran* seeds should be accompanied by shading and/or watering to avoid high soil temperatures.

Key words: germination, high temperature, heat shock, dipterocarp, tolerance.

INTRODUCTION

Seed germination and initial growth are important stages for the establishment of seedlings. *Shorea balangeran* Kahui is an ecologically important and useful tree species in the family Dipterocarpaceae that is often planted in tropical peat swamps in central Kalimantan, Indonesia. Seed germination and seedling establishment of this species are problematic under nursery conditions. The efficiency of seedling emergence is extremely low under sunlit conditions (Inada 2010). Generally, sunlit conditions affect a complex of environmental factors: not only light intensity, but also soil temperature and moisture. To improve the techniques of nursery operations, an understanding of the environmental factors that inhibit seed germination and initial seedling growth is needed.

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High temperatures are an important factor that inhibits seed germination and the survival and growth of seedlings (Berry and Björkman 1980, Kozłowski and Pallardy 1997, Koniger et al. 1998). Relationships of stable or alternating temperatures with germination are well studied (Baskin and Baskin 2001). Although sensitivity to high temperatures likely varies among species (Choinski and Tuohy 1991, Baskin and Baskin 2001, Fenner and Thompson, 2005), the optimum germination temperature for dipterocarp species is around 30°C (Tompsett 1998). In the tropics, temperatures fluctuate daily and can exceed 50°C, especially at midday on a sunlit surface (Saito et al. 2005), unlike the stable or moderately alternating temperature conditions of a germination experiment. A study to understand the effects of high temperature on seed germination and seedling establishment in an open nursery must assume field conditions (Fenner and Thompson 2005). However, the threshold of tolerance to heat shock is poorly understood in dipterocarp species.

Here, 2 heat-shock experiments were conducted on *S. balangeran* seeds and juvenile seedlings. We determined the high-temperature threshold for germination and radicle (juvenile root and hypocotyl) survival. Suitable conditions and nursery operations for *S. balangeran* seedling establishment are discussed.

MATERIALS AND METHODS

Shorea balangeran is widely distributed in peat swamp forests in Kalimantan and neighboring small islands in Indonesia (Soerianegara and Lemmens 1994). Its seeds are non-dormant, based on Baskin and Baskin's (2004) classification system of seed dormancy. This trait, combined with its quick germination, suggests that it is a pioneer species. Seedlings are of the phanerocotylar-epigeal-reserve morphological type (Garwood 1996). The tree reaches heights of up to 30 m with a straight bole (Soerianegara and Lemmens 1994).

The *S. balangeran* seeds used in this study were collected in March 2009 from several mother trees along the Sebangau River in Palangka Raya, central Kalimantan, and stored in a plastic bag at room temperature until used, to prevent desiccation. The 100-seed weights with and without wings were 10.3 and 6.6 g, respectively. The heat-shock experiment was begun within 1 wk of seed collection.

The germination experiment was conducted in a growth chamber under a constant temperature of 30°C and dim-light conditions (12/12 h of day/night). Seeds were subjected once to 5 h of heat-shock treatment at 4 temperatures, 30 (constant, as the control), 40, 50, and 60°C. We chose 5-h heat-shock durations assuming that the ground surface temperature is highest between 10:00 and 15:00 (data not shown). Seeds of each cultivar were sown on sterilized silicic sand in glass Petri dishes of 12 cm in diameter at a rate of 50 seeds per dish. The silicic sand was adequately watered, and the air humidity was kept near saturation. Six replicates were used for each of the 4 heat-shock treatments. Germinated seeds were counted daily for 24 d. Germination was determined to have occurred upon formation of a bend in the root.

The radicle (juvenile root and hypocotyl) survival experiment was conducted using seedlings that had germinated in the germination heat-shock treatment. The sample number per dish was 20 or 30 seedlings ($n = 5$). The temperatures and durations of heat-shock were the same as in the germination experiment. Heat-shock treatment was



applied once daily for 7 d. To evaluate the survival, root growth was also observed in a dish under a constant temperature (30°C) and dim light conditions (12/12 h of day/night). To avoid root desiccation, dishes were watered daily.

Differences in the final germination and final survival rates among the different heat-shock levels were tested using the Mann-Whitney *U*-test. The analysis was performed using SPSS vers. 12 software (SPSS, Chicago, IL, USA). The significance level was set to 5%.

RESULTS AND DISCUSSION

Incubation at 30°C resulted in $78.3 \pm 7.4\%$ germination, indicating the potential germination rate of this species (Fig. 1). It took 18 d to complete germination at 30°C. Although 40°C heat shock was likely to accelerate the timing of germination, no significant difference in the final germination rate was found compared to incubation at 30°C. The 50°C heat-shock treatment delayed germination but resulted in no significant reduction in the final germination rate ($82.1 \pm 3.7\%$). In contrast to heat shock at 30, 40, and 50°C, that at 60°C killed all of the seeds. After germination, $79.7 \pm 13.3\%$ of the radicles of sprouting seeds incubated at 30°C survived, and with 40°C heat shock, $80.8 \pm 14.9\%$ survived (Fig. 2). However, 50°C heat-shock treatment killed all of the seedlings. Comparing the results of the 2 experiments, tolerance to heat shock was lower in the developmental stage before germination than after germination, namely during the growth of the radicle and hypocotyl (Fig. 1 vs. Fig. 2). Consequently, our findings indicate that the threshold of heat-shock survival throughout seed germination and seedling establishment of *S. balangeran* is between 40 and 50°C.

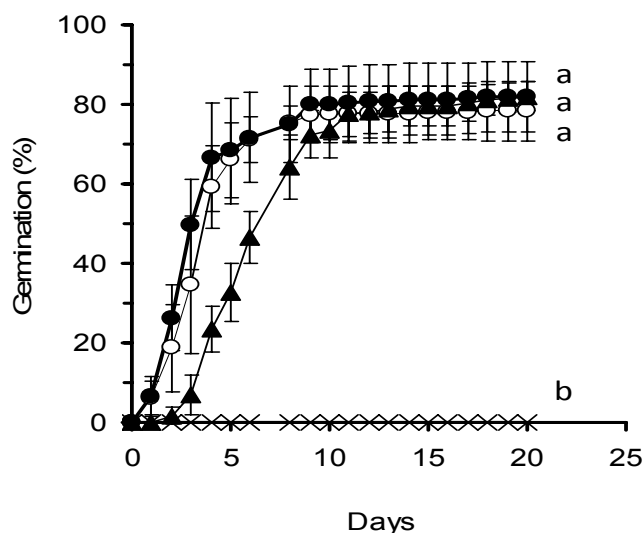


Fig. 1. Germination rates of *Shorea balangeran* seeds after a single heat-shock treatment. The symbols denote different levels of heat shock: 30 (control; ○), 40 (●), 50 (▲), and 60°C (×) for 5 h. Seeds were incubated at 30°C. Bars denotes the standard deviation ($n = 6$). Different letters denote a statistical difference (*U*-test, $p < 0.05$).

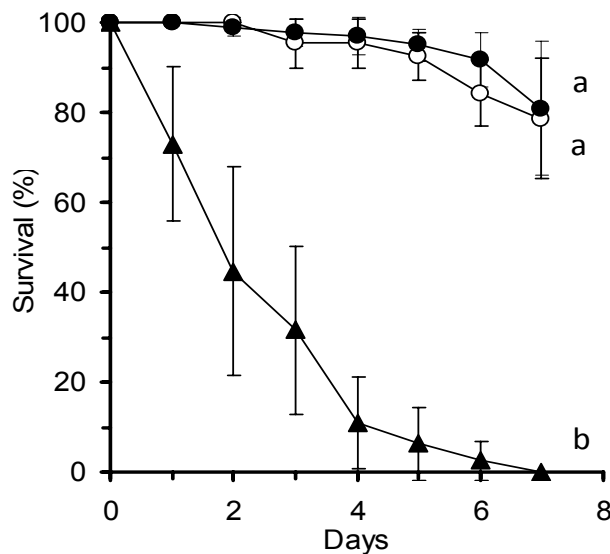


Fig. 2. Survival of the radicles of sprouting *Shorea balangeran* seeds treated with heat shock. The symbols denote different levels of heat shock: 30 (control; ○), 40 (●), and 50°C (▲) for 5 h. The temperature and duration of heat shock were the same as in the germination experiment. Heat shock was applied once daily for 7 d. Bars denote the standard deviation ($n = 5$). Different letters denote a statistical difference (U-test, $p < 0.05$).

I previously showed that survival of juvenile *S. balangeran* seedlings decreased to 20% in an open area without watering accompanied by high soil temperatures of around 50°C (Inada 2010). Because the results of the present study indicate that the threshold of survival to heat shock is between 40 and 50°C in *S. balangeran*, increased soil temperature during midday is a major factor reducing the efficiency of seedling emergence in open nurseries. The ability of a plant to cope with heat stress varies with the developmental stage (Wahid et al. 2007), and *S. balangeran* is vulnerable to high temperatures in the sprouting stage; thus, care should be taken to control temperatures during the developmental stage between the start of seed sprouting and the establishment of seedlings. In nursery operations, shading and/or watering is needed to avoid high temperatures until seedling establishment.

The results of this study explain why in open conditions, the recruitment of *S. balangeran* seedlings in nursery operations is lower. According to Tompsett (1998), the optimal germination temperatures for 31 dipterocarp species range 26~31°C. Corbineau and Come (1986) reported a broad range of optimal germination temperatures, with maxima of 30~35°C for both *Hopea odorata* and *S. roxburghii*. We found no evidence of a relationship between a stable temperature and the germination rate in *S. balangeran*; however, the heat-shock threshold, 50~60°C for germination and 40~50°C for survival of the radicle, might exceed the stable high temperature. Although numerous studies



mentioned stable high temperatures with respect to the optimal germination temperature, little information is available about the effects of heat shock (from midday higher soil temperatures under sunny conditions) on the germination of dipterocarp tree species. Further study on the dynamics of temperature effects on germination and recruitment of seedlings is needed to better understand the ecology of germination and recruitment under nursery conditions as well as regeneration dynamics in the field.

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Dormancy and Storage Behavior of Seeds of Thirty Tropical Fabaceae Tree Species from Sri Lanka

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[Summary]

The Fabaceae consists of a large number of tropical and temperate tree species, including wild relatives of important crops, shade trees, and important members of many plant communities. However, information on seed germination of tropical legumes is limited. In this study, basic information was collected on seed dormancy and storage behavior of 30 endemic, native, and introduced Fabaceae tree species in Sri Lanka. Seeds were collected throughout Sri Lanka. The seed moisture content (MC) and mass were determined. Germination and imbibition of intact and scarified seeds were compared. To test for recalcitrancy, seeds of species with > 15% MCs were dried to a low MC, stored at -1°C and then tested for germination.

Seeds of 2 species had > 15% MCs, and drying and storage experiments confirmed that they were recalcitrant. Imbibition experiments revealed that seeds of 24 species exhibited physical dormancy (PY); however, 2 of these also produced a high proportion of nondormant seeds. Seeds of another 6 species (including the 2 with recalcitrant seeds) did not exhibit PY. Germination studies showed that seeds of 3 of the 6 species were nondormant. Seeds of the other 3 species were physiologically dormant, and 1 of these (*Humboldtia laurifolia*) exhibited a new kind of (physiological) epicotyl dormancy. Thus, seeds of the 30 tropical Fabaceae tree species had either no dormancy, physical dormancy, physiological dormancy, or a new type of (physiological) epicotyl dormancy. Further, seeds of 28 species were orthodox, while those of only 2 species, both with water-permeable seeds, were recalcitrant.

Key words: Fabaceae, orthodox seeds, recalcitrant seeds, seed dormancy.

INTRODUCTION

The Fabaceae consists of a large number of tropical and temperate tree species (Mabberley 2008), including wild relatives of important crops, shade trees, important members of many plant communities, and invasive species. Information on seed dormancy and germination is important for propagating important species and controlling invasive ones. However, our knowledge about seed germination of tropical legumes is limited (Baskin and Baskin 1998). Available information suggests that seeds of most Fabaceae species are physically dormant (PY, i.e., caused by a

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water-impermeable seed or fruit coat) (Baskin and Baskin 1998). However, some tropical Fabaceae species do not produce seeds with PY; instead, the seeds are nondormant or have physiological dormancy (PD) (Baskin and Baskin 1998, Sautu et al. 2007). There are no reports of combined dormancy (PY + PD) in tropical legumes, and certainly neither morphological or morphophysiological dormancy is present in this family, since the embryo is fully developed at seed maturity (Martin 1946). However, most studies conducted on seed dormancy and germination of tropical legumes are incomplete. Thus, our main objective in this study was to collect basic information on seed dormancy and storage behavior of 30 endemic, native, and introduced Fabaceae tree species in Sri Lanka.

MATERIALS AND METHODS

Seeds were collected throughout Sri Lanka, stored in polythene bags and brought to the Univ. of Peradeniya, Peradeniya, Sri Lanka. All experiments were conducted at the Department of Botany, Univ. of Peradeniya. Experiments were initiated within 2 wk of seed collection. The seed mass was determined to the nearest 0.0001 g using a digital chemical balance. The seed moisture content (MC) was determined using an oven-drying method. To determine whether seeds had PY or not, water uptake (imbibition) of at least 10 intact and 10 manually scarified (individually with a scalpel) seeds were monitored for each species at ambient laboratory conditions. Germination of intact and manually scarified seeds was studied under ambient laboratory conditions in light/dark and in the dark. To test for recalcitrancy, seeds of species with > 15% MCs at maturity were dried to a low MC (30%, 20%, and 10%) or stored at -1°C and then tested for germination.

RESULTS AND DISCUSSION

Seeds of *Humboldtia laurifolia* and *Cynometra caulifolia* had > 15% MCs, and none of the seeds dried to 10% MC or stored at -1°C germinated. Thus, it was concluded that these seeds were recalcitrant. The seed mass was highest in the 2 recalcitrant species, as expected (Forget, 1992). The lowest seed mass observed was 0.0074 ± 0.0006 mg in *Sesbania sesban*. Imbibition experiments revealed that seeds of 24 species exhibited PY; however, 2 of these (*Senna suratensis* and *Prosopis juliflora*) also produced a high proportion of nondormant (water-permeable) seeds. Seeds of another 6 species (*H. laurifolia*, *Cynometra caulifolia*, *Copaifera officinalis*, *Erythrina variegata*, *Pterocarpus marsupium*, and *Pte. Indica*) did not exhibit PY. More than 90% of untreated and manually scarified seeds of *Cyn. caulifolia*, *Cop. officinalis*, and *Ery. variegata* germinated, and thus seeds of these 3 species were nondormant. Neither untreated nor manually scarified seeds of *Pte. marsupium* or *Pte. indica* germinated at ambient laboratory temperatures within 30 d. Thus, it was concluded that the seeds of these 2 species were physiologically dormant. There was a several-week delay of shoot emergence following radicle emergence in seeds of *H. laurifolia*. Therefore, seeds of *H. laurifolia* have a new kind of (physiological) epicotyl dormancy (Jayasuriya et al. 2010).



CONCLUSIONS

Seeds of the 30 tropical Fabaceae tree species we studied either had no dormancy, physical dormancy, physiological dormancy, or a new type of (physiological) epicotyl dormancy. Further, seeds of 28 species were orthodox, while those of only 2 species, both with water-permeable seeds, were recalcitrant. Although our sample was small compared to the species richness of the Fabaceae, it reveals that recalcitrancy is associated with a high seed mass.

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Ex Situ Conservation Issues Relevant to the International Seed Testing Association (ISTA)

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[Summary]

The aim of the International Seed Testing Association (ISTA) is to develop and publish standard procedures in the field of seed testing. ISTA pursues its vision of “uniformity in seed testing worldwide” by producing internationally agreed upon rules for seed sampling and testing, accrediting laboratories, promoting research, and providing international seed analysis certificates, training, and dissemination of knowledge in seed science and technology to facilitate seed trading domestically and internationally.

This paper gives a brief overview of ISTA's history and main activities as they relate to specific goals dealing with *ex situ* conservation in forestry.

Key words: cryogenic storage, international seed testing association, international rules for seed testing, seed storage methods, seed vigor tests.

“Seed conservation is the use of seed storage as a means of ensuring the future availability of plant germplasm. *Ex situ* conservation is a method ensuring that the seeds are stored away from where the original plant populations grow in nature or cultivation” (Black et al. 2006).

The International Seed Testing Association (ISTA) was established in 1924 to work towards a vision of uniformity in seed testing internationally. The principle objective of ISTA is to develop and standardize methods for sampling and testing of seed-quality parameters such as purity, moisture, germination, viability, and seed health, using the best scientific knowledge available. Another important aim is to promote research in all areas of seed science and technology through publications, training programs, and meetings.

ISTA's current mission is to develop, adapt and publish standard procedures for sampling and testing seeds, and to promote uniform application of these procedures to evaluate seeds moving in international trade.

ISTA's motto “uniformity in seed testing” was coined in Hamburg in 1876 and later became part of the ISTA logo (Steiner and Kruse 2006).

International rules for seed testing

The basic need of ISTA is for seed testing methods that are the reliable and reproducible among its accredited member laboratories. This goal is achieved through publication of the *International Rules for Seed Testing (ISTA Rules)* established in 1931. However, the first attempt to provide internationally acceptable methods appeared in

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1906, at the 1st International Conference for Seed Testing in Hamburg, Germany, where Prof. Voight published a manuscript entitled "Technical Rules for Quality Determination of Seeds in Trade" for participants. The manuscript also contained tables with average seed testing results for all kinds of species including forest tree seeds (Steiner and Kruse 2006).

The primary aim of the *ISTA Rules* is to provide testing methods for seeds designated for growing crops or producing plants. However, the validated methods are suitable and useable for *ex situ* conservation as well.

The first *ISTA Rules* described sampling, purity testing, germination, weight and moisture content (MC) determinations, and sanitary conditions.

In 1966, the first specific seed health testing methods were included in the *ISTA Rules* (Mathur and Jorgensen, 2002 ex Muschick 2010). Today, the *ISTA Rules* contain 21 standardized seed health testing methods, which can also be downloaded free of charge from the *ISTA* website. Among these methods, there are 2 that deal with seed-borne pathogens on forest tree seeds: 7-008: Detection of *Caloscypha fulgens* on *Picea engelmannii* and *glauca* (spruce); and 7-009: Detection of *Fusarium moniliforme* var. *subglutinans** on *Pinus taeda* and *Pin. elliottii* (pine) (*ISTA Rules* 2010).

In the same year as the Plant Diseases Committee (PDC) (1966), the topographical tetrazolium test was introduced as a standardized test in the *ISTA Rules* (Steiner 1997). This biochemical test is used to rapidly assess seed viability when seeds must be sown shortly after harvest, but especially in forestry for seeds with deep dormancy to evaluate their quality and suitability for storage.

Seed vigor tests provide information about the planting value of seed lots in a wide range of environments and also their storage potential. In 2001, the first 2 vigor methods (the conductivity test for *Pisum sativum* and the accelerated ageing test for *Glycine max*) were added to the *ISTA Rules*.

The most recent version of the *ISTA Rules* contains 15 chapters providing internationally accepted test methods for various attributes of seed quality. Among them, MC, germination, viability (tetrazolium test), and seed health determinations are basic for quality assessment of seeds stored *ex situ* in seed banks.

ISTA technical committees

In 1924, 9 *ISTA* technical committees including the Moisture Content and Drying Committee and Investigations of Genuineness of Variety and of Plant Diseases were established (Muschick 2010). In 1928, a separate PDC was founded.

Recently, the tasks are subject-focused in 18 technical committees and a task force, in which scientists from different research fields and specialists from all over the world work closely together to improve seed testing. The technical committees perform comparative studies and surveys, and exchange information about specific issues. A committee may have several working groups composed of seed specialists on particular subjects. These committees are responsible for developing and improving the *ISTA Rules* and *ISTA* handbooks on seed methods including sampling, testing, processing, and distributing seeds.

Among the *ISTA* technical committees, the Storage Committee, the Moisture Committee, and the Forest Tree and Shrub Seed Committee (FTS) can be considered the most closely related to *ex situ* conservation.



Topics related to seed storage are specifically investigated by members of the Seed Moisture and Storage Committee that was established in 1950. Terms of reference deal mainly with general aspects of storage irrespective of species although major agricultural crops are used (e.g., viability losses during storage, packing seeds for storage and shipment, short- and long-term storage of seeds, methods of seed treatment before and during storage, studies of physiological, physical, genetic, and other changes during storage, and accelerated aging techniques).

In 1980, the Seed Moisture and Storage Committee was divided into the Seed Moisture Committee and the Seed Storage Committee (STO). “*Although research into seed storage has progressed far beyond the requirements of ISTA as referred to in rule 2.8 and is, strictly speaking, no longer a primary purpose of the Association, it is unanimously felt that ISTA is the most appropriate body to coordinate research in this filed internationally*”, according to J. F. van Wyk, chairperson (Witte 1981). This separation was supported by some ECOM members, e.g., member-at-large Prof. A. Lovato of Italy who said, “*The aim of the Association is not only to reach a better uniformity in seed testing, but also to favor a better quality of seeds. We are often asked for information about the maintenance of viability of seeds and we must be prepared to give detailed instructions*”.

After its establishment, the STO's goals were related to activities studied in previous years: the physiology of aging, effects of long-term storage on genetic integrity of seed lots, storage of recalcitrant seeds, effects of provenances on seed longevity, and effects of storage-related fungi on seed longevity. With an increasing world interest in the conservation of genetic reserves in seed banks, information is needed to assess the storability of seeds (Bass 1981).

For forest tree seeds, e.g., development of storage methods for *Fagus sylvatica* seeds, monitoring of temperature during shipment of *Picea glauca* and *Pinus banksiana* seeds, and planning a comparative study of 2 storage methods (conventional and cryoconservation) of white and black spruce were included in the terms of reference (Bass and Kähre 1987). The influence of seed-coat anatomy on water and gas exchange, the validity of storage constants of 4 tree species, the incidence of chromosome breakage, and the effects of seed collection and processing on the storage potential were also studied (Boyce 1989).

In the 1990s, the committee focused on cryogenic storage of orthodox seeds and recalcitrant seed embryonic axes, storage-related fungi, and pre-storage invigoration of orthodox seeds (Chin 1992, 1995). In addition to agricultural species, forest tree seeds were included as subjects of interest. For example, committee members focused their studies on fungi on stored recalcitrant seeds of *Avicennia marina* (mangrove), screening of the storage behaviors of several wild forest plants including trees, the cryostorage procedures for somatic embryos of several palms, and the cryopreservation of *Azadirachta indica* (neem) (Berjak 1998).

In 1998~1999, the STO formally established working groups dealing with various aspects in 3 basic areas: i) orthodox seeds, ii) non-orthodox seeds, and iii) development of methodologies. For forest tree seeds, scientists mainly focused on non-orthodox (recalcitrant) seed problems: mycoflora on seeds of *Avicennia marina*, biological control of *Ciboria batschiana* during storage of *Quercus robur* acorns, responses to seed manipulation and cryoprotocols for *Q. robur*, interactions between

drying and environmental requirements for germination of papaya seeds, and short-term storage of intact seeds of 4 African tree species (in cooperation with IPGRI/DFSC) (Berjak and McLean 2001).

In the last 10 yr, the activities of the STO have concentrated on forest tree seeds much more than in previous years. For example, cryopreservation of seeds of tropical tree species of immediate or potential socioeconomic importance in the seed trade and the storage behaviors of seeds of various palm species were studied. Other investigations evaluated the effects of seed MC and storage temperature on the longevity of hybrid *Salix* seed or application of systemic fungicides to recalcitrant seeds of *Trichilia dregeana*, *Shorea hemseleyana*, and *Parkia speciosa* seeds to extend the hydrated storage lifespan. Experiments with biological control of fungi on stored *Q. robur* acorns by antagonists (e.g., *Trichoderma virens*) provided additional protection even when hot-water treatment was possible.

Recently, 15 scientists from 10 different countries (with only 6 of the 15 from the EU and North America) have been involved in the Seed Storage Committee. The common goal of the committee is to develop and/or improve effective medium- and long-term storage methods. To achieve these goals, it is important to have an appreciation of the biological processes that underlie seed storage behaviors and how these processes are affected by actual storage. This is particularly true of recalcitrant (non-orthodox) seed germplasm.

The second technical committee closely involved in *ex situ* conservation topics related to forest tree seeds is the Seed Moisture Committee (MOI). As mentioned above, activities dealing with storage and moisture content determination (methodology) were included in the agenda of the Seed Moisture and Storage Committee until 1980. This indicates the importance of MC determination for *ex situ* conservation. A good example is the evolution of oven moisture testing methods in the *ISTA Rules*. In 1924, at the founding meeting of the ISTA, a paper on the oven-drying of seeds in Scandinavia to determine the MC was presented. The first *ISTA Rules* approved in 1931 contained an oven method to determine MCs. Tree seeds first appeared in the 1959 rules when a toluene distillation method was introduced for 2 genera (*Abies* and *Picea*) and an air-oven method at 130°C for 1 h was specified for 4 species. During the 1965~1971 period, there were several activities studying the MCs of forest tree seeds (a survey of labs around the world and comparative tests between the oven and toluene methods). In 1985, tolerances for tree and shrub seed MC testing were introduced.

In 1985, the toluene method was dropped, and all tree species were thereafter tested at 103°C for 17 h with coarse grinding when necessary (Grabe 1987). At present, the air-oven method is routinely used in seed testing laboratories around the world.

The third technical committee which has a very close liaison with the Seed Moisture and Storage Committee is the Forest Tree and Shrub Seeds Committee (FTS). The FTS, established in 1950, is unique among the technical committees of ISTA as its sphere of activities covers all methods (and chapters) of the *ISTA Rules* (similar to the later Flower Seed Committee).

The first attempt to internationally standardize testing methods for forest tree seeds was made in 1937 by the IUFRO. After the first world war, the IUFRO decided to ask the ISTA to take over responsibilities for controlling the testing of the forest tree seeds, and in 1953 at the 10th ISTA Congress in Dublin, prescriptions for 33 forest tree



species were approved. In 1974, the number of forest tree species increased to 203 with 37 species being tested with tetrazolium (TT), and a special chapter for the excised embryo method was included for 15 species. A questionnaire distributed to 130 ISTA and 40 non-ISTA laboratories in 1975 revealed that at that time, only 15 ISTA-accredited laboratories were entirely dealing with forest tree seeds. About 30% of species listed in the *ISTA Rules* were undergoing fewer than 10 germination tests per year. The question arose if those species should be in the rules. The committee decided that it was useful to have some guidance when such rare samples arrive, and it was very good that the *ISTA Rules* provides such assistance; it was suggested that such unimportant species be moved to the (proposed) *Handbook on Testing Forest Seeds* (Buszewicz 1978).

Dr. A.G. Gordon, chair of the FTS, proposed reducing the number of seeds to be tested for viability (by TT or EE tests) as in many laboratories the cost of a seed test was greater than the value of the seed lot being sold. If a more-practical test had not been found, ISTA certificates would never have been more widely used in the forest tree seed trade (Gordon 1981). Recently, ISTA and non-ISTA laboratories that test only forest tree seeds, especially tropical and subtropical species, and seed banks have likely had to face the same problem.

Workshops

One of the ISTA's aims of promoting research in all areas of seed science and technology through publications, training programs, and meetings has been fulfilled by the FTS by organizing workshops and publishing handbooks dealing with the testing of forest tree seeds.

By now, 5 ISTA 'Forest Tree Seed Testing Workshops' have been held, at Guildford, UK (1973), Macon, GA, USA (1989), Prague, Czech Republic (2003), and then twice in Italy at Verona (2006) and Peri (2008). The workshops in Italy were also attended by participants from seed banks and universities involved in *ex situ* conservation programs.

The meeting agenda was a mixture of practical exercises, demonstrations, presentations and discussion sessions. The last 3 workshops were respectively under the auspices of the FTS, Tetrazolium, and Moisture and Bulking and Sampling Committees.

A special Tetrazolium workshop that only dealt with forest tree seeds was organized by the Tetrazolium and FTS Committees in Karlsruhe, Germany in 2005.

Publications

The journal *Seed Science and Technology* (SST) is published by ISTA. In 1973, a special issue of SST (vol. 1, no. 3) was devoted to seed storage and drying. In it, some fundamental papers relating to seed storage, the basic method of *ex situ* conservation, were published, e.g., papers by E.H. Roberts (Predicting storage life; Loss of seed viability: chromosomal and genetical aspects; and Loss of seed viability: ultrastructural and physiological aspects).

Since 2000, papers published in SST have been listed according to their subjects, and papers on germination, biochemical methods, dormancy, morphology, and pathology of storage have also been published.

The first special handbook dealing with tree and shrub seeds was published by the FTS in 1991 (Gordon et al. 1991). This publication covers details about seed testing

methods extracted from the *ISTA Rules*, and has much additional information about species not listed in the rules including a chapter of “Seed storage”. The FTS' plan is to update this handbook.

In 1998, the second handbook relevant to tropical and subtropical tree and shrub seeds was published by the FTS (Paulsen et al. 1998). This excellent publication provides a unique compendium of information for all handlers of tropical and subtropical tree seeds including seed banks.

Dr. Isolde D.K. Ferraz, an FTS member, co-authored a book (Camargo et al. 2008) that was awarded the 3rd place in the best Brazilian book category Health Science and Natural Sciences (JABUTI Award). This first volume deals with fruits, seeds, and seedlings of 50 trees of the Amazon and can be utilized by conservation programs. One of the proposed future goals of the FTS is to cooperate on a second volume of “Propágulos e Plântulas da Amazonia”.

In 2005~2007, another FTS member, Dr. B. Pioto, participated in a bibliography project processing information about germination of *Juniperus* species. This special activity resulted in a selection of 1010 abstracts covering the period 1893~2006 that are presented on several websites (ISTA, APAT, USDA FS NTSL, etc.).

Future goals

Future activities of the ISTA with respect to *ex situ* conservation will cover 3 main areas: i) development and standardization of sampling and testing methods including the introduction of new species, ii) publications, and iii) workshops (training programs and meetings).

For agriculture, ISTA's current mission focuses on procedures for evaluating seeds that are moving in international trade, and the situation in forestry differs. At least in the northern hemisphere, e.g., within the EU, international trade in forest tree seeds has been limited, as countries protect their forest tree seed resources against introduction of unwelcome genes. This situation is reflected in a decreasing number of ISTA certificates requested from seed testing laboratories. However, internationally recognized sampling and testing methods for new species might be desirable in *ex situ* conservation programs represented, for example, by seed banks. On the other hand, there are many tree seed centers in Africa and Latin America (some also in Southeast Asia) that store tons of tree seeds over the short term. Their objective is rarely conservation per se, but rather to support afforestation programs. Quality assurance is not what it should be in those laboratories (H. Pritchard, pers. comm.).

Basic data are available on seed germination of many tropical and subtropical tree species, but before being included in the *ISTA Rules*, they must be validated. Many of those species have large (recalcitrant) seeds or tiny seed lots, and it is practically impossible to follow the 4 x 100 seed rule to determine germination or viability. So the FTS in cooperation with the STO and Germination Committee should concentrate on procedures (substrates, number of seeds in replicates, etc.) that can fulfill the principle of species/method validation. The challenge for the future is the use of preferred methods with tolerance tables.

Revision of the *Tree and Shrub Seed Handbook* or writing of working sheets on particular forest tree species and workshops on testing of tropical and subtropical tree seeds remain among the top tasks of the FTS.



In 2010~2013, the work plan of the STO will focus on characterizing seed storage of new species, developing new technologies (cryopreservation, syn 'seeds', thermal fingerprinting, etc.), and maximizing the dry-storage potential (including seed health).

Water activity determination is a promising procedure that can particularly be utilized in *ex situ* conservation programs. This topic is ranked among the highest priorities of the FTS, MOI, and STO committees in the new triennium. On 13~15 October 2010, a Water Activity Workshop will be co-organized by the MOI, STO, and FTS Committees together with Patrick Baldet and Fabienne Colas, as local organizers in Montargis, France. This workshop symbolizes the importance of relationships among ISTA technical committees essential to achieve ISTA's aims.

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Status of *Ex Situ* Conservation of Forest Tree Germplasm in Seed Banks in Africa, Asia, and Latin America

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[Summary]

Ex situ conservation of forest genetic resources is increasingly important as forest and woodland habitats are lost to other land uses, climate change threatens the viability of natural ecosystems, and more trees are added to lists of threatened species. Pressures on forest trees are greatest in Africa, Asia, and Latin America, and seed banks provide one option for *ex situ* conservation. Other options that are more applicable for many tropical species include conservation plantations, clone banks, and conservation through use. Most commercially important tree species in the humid tropics have recalcitrant seeds, and this limits the usefulness of seed banks for long-term conservation, however seed centers also fulfill an important function in collecting and processing seeds for short-term storage before establishing *ex situ* plantations and facilitating conservation of genetic resources through use. It has proven difficult to sustain *ex situ* conservation programs for tree species in many countries after initial start-up funding ends. Many seed centers have struggled for self-sufficiency as external and often national support ended or was severely reduced, leaving the centers dependent upon seed sales for survival; conservation and research functions are commonly sacrificed. The Millennium Seed Bank has given new life to conservation efforts in many seed banks during the past decade. Nevertheless, new models are needed for *ex situ* conservation of forest tree genetic resources. Effective *ex situ* conservation models must be flexible, combining different approaches for species with different characteristics and requirements, and whenever possible, should include increased use of target species.

Key words: *ex situ* conservation, gene bank, forest genetic resources, seed bank, seed storage behavior, tropical tree species.

INTRODUCTION

The remarkable diversity of tree species in tropical and subtropical environments represents both a richness of opportunities and an immense challenge for conservation as human populations expand, resource exploitation increases, and increasing numbers of species and populations of trees are threatened. *Ex situ* conservation methods are mainly used to preserve valuable genetic samples of commercially important species, or less commonly, as a last resort when *in situ* conservation methods are failing. The vast numbers of tree species between the 2 extremes are generally expected to adequately be protected by *in situ* conservation areas and sustainable forest management. Most tropical

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and subtropical tree species are increasingly susceptible to loss of populations and associated sources of unique genetic variability, however, due to a combination of ongoing habitat loss and changing climates. Opinions vary regarding the potential for *ex situ* conservation of germplasm as a means of stemming the loss of tropical and subtropical forest tree genetic resources.

Between 1965 and 2000, about 50 tree seed centers were established in Africa, Asia, and Latin America with assistance from Denmark, Canada, the Netherlands, the UK, Switzerland, France, Belgium, Norway, the USA, Japan, and others (Graudal and Lillesø 2007). The centers were established in 2 waves, at first with the intention of supplying forest managers with seeds of commercially important species, mostly pine and eucalyptus for plantation establishment; and a second wave, beginning around 1990, was intended to meet the needs for a broader array of species for small-holder use. In the 1990s, objectives of national tree seed centers generally included conservation of genetic resources of native tree species in addition to meeting demands for seeds. The functions of the centers were both productive (seed production for planting, breeding for tree improvement, and conservation) and normative (development of policies, capacity-building, and awareness-raising) (Graudal and Lillesø 2007).

International donor support for tree seed centers declined during the past 10 yr, and in particular, interest in supporting *ex situ* conservation has fallen because of shifting priorities and the perception that *in situ* conservation is more urgent and important because it protects multiple species. It is difficult for donors focused on poverty alleviation to link *ex situ* conservation to that objective in the short term (FAO, FLD, Biodiversity International 2007). The duration of donor funding varies from just a few years to 2 decades (Graudal and Lillesø 2007). When external funding ended, most centers continued to function but had to become self-supporting, and the breeding and conservation functions were, of necessity, a lower priority than the sale of seeds from species in demand for planting (Graudal and Lillesø 2007). In some countries, seed centers continued to fulfill their broader original mandates and have maintained conservation collections. Others became entirely production-focused, and some centers no longer exist.

In the absence of external support, effective *ex situ* conservation is not likely for most tree species in many countries. Interest and awareness of the importance and urgency of conserving tree species are increasing globally, but in general, financial and human resources are inadequate for the immensity of the task. The role of donors in supporting activities and programs that do not generate income for the seed centers has been partially replaced in the last decade by Kew Garden's Millennium Seed Bank Project (Phartyal et al. 2002). During the past decade, the Millennium Seed Bank has become a key global actor in facilitating national gene-banking efforts. Agreements were negotiated with many countries around the world to define mutually agreeable conditions for collecting seed samples and adding them to the bank. In many cases, this has meant making parallel collections, one for the Millennium Seed Bank and the other for the target country. The Millennium Seed Bank Project has also assisted national seed banks acquire the equipment that they need to process and maintain seeds. In this way, the project has improved the conservation capacity and contributed to building collections in numerous countries. Some of the countries that are involved include Mexico, China, South Africa, Namibia, Mozambique, Venezuela, Morocco, Egypt, Syria, and Lebanon.



Hundreds of tree species are represented in national tree seed banks in Africa, Asia, and Latin America. In most cases, however, the species for which large quantities of seeds are stored are exotic species, and the storage is intended for production purposes, not conservation. Most of the seeds available for planting in the tropics are from a small number of Australian and temperate American species. Asian species such as teak (*Tectona grandis*) and *Gmelina arborea* are widely planted outside of their native habitats as well. Thus many of the seeds stored and flowing through national seed centers are not from native species. These exotic species are often high priorities for research, breeding, and conservation in countries where they are planted because of their commercial value, but this paper focuses on the status of *ex situ* collections of species within their native ranges.

Challenges for *ex situ* conservation via seed banks

Ex situ conservation via seed banks is not an ideal solution to the threats faced by thousands of tree species. Some of the main challenges can be summarized as: a lack of knowledge, a short duration of seed viability for many species, a lack of money to support research to determine seed behavior and understand the reasons for short periods of seed viability, and time and space required to grow-out seed samples to produce more seeds.

The seed storage and germination behaviors of many species in the tropics and subtropics have not been studied (Phartyal et al. 2002, Sacandé et al. 2004). According to Phartyal et al. (2002), the seed storage behaviors of only about 3% of tropical and subtropical tree species have adequately been studied as of 2002. Assuming that the small proportion of species that have been studied are representative, however, it seems likely that seeds of many humid tropical tree species have poor longevity and are difficult to store in seed banks. Although data for tropical species are incomplete, and it is often not known why seeds are short-lived, more than 70% of commercially valuable tropical tree species are estimated to have recalcitrant or intermediate seeds (Ouédraogo et al. 1999, Sacandé et al. 2004). Careful experimentation is important, however, as in several cases, species thought to have recalcitrant seeds were found to be orthodox or intermediate seeds after further study (Phartyal et al. 2002, Mng'omba et al. 2007). Different provenances within a species sometimes have very different storage behaviors, ranging from recalcitrant to orthodox (Schmidt 2000). The longevity of seeds is often related to the moisture conditions in the species' natural habitat, and many species with recalcitrant seed are native to the humid or semi-humid tropics, but some dryland tropical and subtropical species also have recalcitrant seeds (Schmidt 2000).

Africa has extensive woodlands where soil moisture is very low for extended periods. Although the diversity of tree species is lower in these areas than in the humid tropics, millions of people depend on diminishing tree-based resources. Little is known about seed characteristics of most of those species (Mng'omba et al. 2007), but many of them are probably orthodox and suited to *ex situ* conservation in seed banks. Seed banks are the logical location to carry out required seed-storage and germination studies, but to do so requires access to scarce research funds.

In Southeast Asia, the general feeling is that seed banks for forest trees are usually not appropriate for long-term conservation as most of the studied species produce recalcitrant seeds. A number of studies compiled by Sacandé et al. (2004) reported that

among 19 useful Asian tree species, 74% have recalcitrant seeds. Studies were carried out to evaluate the potential for long-term conservation using cryopreservation or tissue culture. Cryopreservation trials were successful for *Dipterocarpus alatus*, *Dip. intricatus*, *Swietenia macrophylla*, *Pterocarpus indicus*, *Thyrosostachys siamenis*, *Bambusa arundinacea*, *Dendrocalamus membranaceus*, and *Den. brandissi*. In addition, in vitro techniques were studied for *Swi. macrophylla*, *Swi. leprosula*, *Swi. ovalis*, *Swi. parvifolia*, *Hopea odorata*, and *Calamus manan*, and they can be maintained in vitro (Luomo-aho et al. 2004).

Alternatives to seed banks include living genebanks through arboreta, botanical gardens, conservation plantations, clone banks, and active, well-managed utilization (Kjaer et al. 2001). Conservation through use is considered to be the option most likely to succeed for many tropical tree species (Simons et al. 2000). Seed centers have an important role to play in such initiatives to ensure that seeds made available to farmers and forest managers have a wide genetic base from well-documented sources, and that seed quality is ensured. Thus, tree seed centers may serve dual roles of conserving forest tree genetic resources by establishing and maintaining conservation collections and by facilitating the use of native species for plantations, rehabilitation, and agroforestry.

Conservation of genetic resources may be static or dynamic: conserving genotypes or conserving genes. Traditionally, *ex situ* conservation was considered to be static, conserving genotypes, while *in situ* conservation was dynamic, conserving genetic diversity and the evolutionary processes that maintain diversity. Complications posed by recalcitrant seeds and the need to grow and recollect seeds even from species with orthodox seed means that *ex situ* conservation in seed banks can also be dynamic. Because most tree species have long generation lengths, they spend as much or more time out-planted as in seed banks. Although the growing-out phase is required for effective conservation, the space and care required for such long-term plantings pose additional challenges for seed banks.

***Ex situ* programs by region**

Africa

A study was conducted to evaluate the roles of 7 Sub-Saharan tree seed centers in conserving native food tree species (Ramamonjisoa 2009). Each seed center handles seeds of 30~400 species, both native and exotic (Table 1). Among identified priority species, about 1/2 are native to Africa. Most of the seed centers include conservation among their objectives, but their capacities vary. Both Senegalese and Togolese seed centers have explicit conservation objectives, for example, but neither have operational facilities for long-term storage. Some seed centers collaborate with gene banks for agricultural crops that also include seeds of some tree species. Most seed centers are associated with tree improvement programs, and living gene banks have been established for species in breeding programs. Living gene banks may be seed orchards, clone banks, or conservation plantations. Tree improvements are mainly focused on exotic species, so most living gene banks do not include more than 1 or 2 native species.



Table 1. *Ex situ* conservation of forest tree resources in national tree seed centers in selected countries in Africa

Country	Conservation focus	No. of species in seed banks	Proportion of species that are native (%)
Burkina Faso	Explicit objective; seed, clone bank, and conservation plantations	60 in seed bank 18 in conservation	78% (in conservation collection)
Ethiopia	Storage not explicitly for conservation; field gene banks for species in breeding programs	60 in seed bank	Most in seed bank; 40% of priority species
Kenya	Long-term conservation facilities in seed bank; field gene banks for breeding programs	110 in seed bank	Most in seed bank; 48% of priority species
Madagascar	Explicit objective; seed banking and field gene banks	400 in seed bank (trees and shrubs)	Most in seed bank; 25% of priority species
Senegal	Explicit objective; no cold-storage facilities in operation	59 (varies year to year)	78% of priority species
Tanzania	Storage for use; collects for Millennium Seed Bank Project	200	All identified priority species
Togo	Explicit objective	30~50 in seed bank; 3 for conservation	74% of priority species

In Africa, institutions that are participating in the Millennium Seed Bank Project include Benin's Institute National de Recherche Agronomiques (INRA); Botswana's National Tree Seed Centre (NTSC); Burkina Faso's Centre National de Semences Forestières (CNSF); Cape Verde's Institut Nacional de Investigacas Agraria (INIDA); Cote d'Ivoire's Centre National de Recherche Agronomique (CNRA); Ethiopia's Forestry Research Center (FRC); Ghana's Forestry Research Institute of Ghana (FORIG); Kenya's Kenya Forestry Research Institute (KEFRI); Madagascar's Silo National des Graines Forestières (SNGF); Malawi's National Tree Seed Centre, Forestry Research Institute of Malawi (FRIM); Mali's Centre Régional de Recherche Agronomique (CRRRA); Niger's Institute National de Recherches Agronomiques du Niger (INRAN); Nigeria's Owolowo University; Tanzania's Tanzania Tree Seed Agency (TTSA); Togo's National Tree Seed Centre (NTSC); and Uganda's National Tree Seed Centre (NTSC). Collections for the Millennium Seed Bank Project concentrate on relatively dry regions, because species there are more likely to have orthodox seeds.

Asia

Most *ex situ* conservation of tree species in tropical and subtropical Asia is via living gene banks that often serve dual purposes, both conservation and productive, instead of seed banks, because most tree species are considered to be recalcitrant. Seed centers are generally intended for production and distribution, so storage is short-term. According to a set of reports from 7 countries in South and Southeast Asia, seed storage is not considered to be an option for *ex situ* conservation (Jalonen et al. 2009). Instead, reports from Asia Pacific Forest Genetic Resources Programme (APFORGEN) member countries include seed orchards and provenance trials in descriptions of *ex situ* conservation (Table 2) (Luoma-aho et al. 2004, Jalonen, et al. 2009).

Many species are represented in botanical gardens and arboreta, and in many cases, that is the only form of *ex situ* conservation in existence. Sample sizes are very limited in botanical gardens and arboreta, so it is a species-level conservation method, at best. Such collections do not conserve intraspecific genetic resources. Seed orchards, progeny trials, and provenance tests maintain broader genetic diversity, but only for a handful of mostly exotic commercially important species.

Table 2. *Ex situ* conservation of native tree species in selected countries in Asia

Country	Approach purpose	No. of species: seed bank total/conservation
Bangladesh	Clone banks and botanical gardens; seed center has no facility for long-term storage, only for production	Clone bank: 7; hundreds in botanical gardens
Cambodia	Seed orchard and demonstration plantation	6
China	Seed orchards, clone banks, and plantations; dual conservation and production purposes	10
India	Clone banks, botanical gardens, provenance trials, and seed orchards	At least 20
Indonesia	Seed orchards, botanical gardens, arboreta, and seed banks; conservation and production purposes	Many; at least 12 in seed orchards
Lao PDR	Demonstration plots	32
Malaysia	Arboreta, cryogenic storage	About 800 in arboreta; 7 cryopreserved
Myanmar	Arboreta, botanical gardens, and seed orchards	Not known
Nepal	Seed orchards	At least 7
Philippines	Conservation plantations, botanical gardens, arboreta, and seed bank	Not known (more than 1/2 of native dipterocarps)
Sri Lanka	Conservation plantations and seed orchards	Not known
Thailand	Botanical gardens and arboreta; seed banks for production, not conservation	30
Vietnam	Arboreta and conservation plantations	More than 250 in arboreta; 9 in conservation plantings

Latin America

Seed banks are important for the *ex situ* conservation of forest tree species in Latin America. At least 67 native tree species of socioeconomic significance are stored in multiple seed banks in at least 14 countries. Among the 100 priority Latin American tree species included in the MAPFORGEN project undertaken by Latin American Forest Genetic Resources Programme (LAFORGEN), seed banks in Brazil and Honduras each have collections of 23; Costa Rica (mostly CATIE) has 19 species; Ecuador has 10; Argentina has 9; Bolivia and Colombia each have 6, and Nicaragua, Cuba, Peru, Mexico, Guatemala, Panama, El Salvador, Venezuela, Puerto Rico, and Dominican Republic each include 5 or fewer species in seed banks. Mexico is developing an ambitious new gene bank project which will significantly increase the country's capacity for storing seeds.

Reports of storage trials carried out in Latin America for 10 tropical species, as part of a joint IPGRI and DANIDA project initiated in 1995 (Sacande et al. 2004), indicated that 4 of the species had orthodox seeds, 4 had recalcitrant seeds, and 2 were intermediate. Although the sample size was too small to draw conclusions, the reason for greater use of seed banks for *ex situ* conservation in Latin America than in Asia or Africa may be a reflection of a lower proportion of recalcitrant seeds. Lists of species stored in



seed banks in Latin America include temperate (high-elevation) and dry subtropical species.

DISCUSSION AND CONCLUSIONS

This overview considers *ex situ* conservation in isolation from either *in situ* measures or the conservation value of sustainable use. Objectives and methods for *ex situ* conservation vary in different regions. Although a species sample growing in an arboretum is, by definition, “*ex situ*”, it does not constitute effective conservation of genetic resources. Likewise, a single accession in a jar in a seed bank cold room, does little to preserve the evolutionary potential of the species in question, especially if the quality of the seed is poor or not known. Conservation plantations constitute effective *ex situ* conservation only for as long as they are maintained, and as long as records are kept. All of the approaches for gene-banking have significant costs and challenges, and no one method alone is likely to be sufficient. Effective conservation requires a holistic approach, evaluating the potential of each method and using the best combination.

A lesson learned by DANIDA, after many years of assisting the establishment and functioning of seed centers, was that utilization is an essential component of conservation (Kjaer 2001). The role of seed banks in conservation is often viewed in a one-dimensional way, and seed banks are often viewed as static facilities for storing seeds. The utility of a well-functioning seed bank extends beyond its function as a storage facility, however. An equally important role in long-term effective conservation is in providing good quality seeds of many species, with a broad genetic base, made available for planting. Applied research is a vital function of any seed center that can fulfill this role. Ideally seed centers should be sources of important information about sampling strategies, storage behaviors, and germination procedures for a wide range of native species, as well as sources of viable, genetically diverse seeds.

Many species are represented in seed banks throughout the tropics and subtropics, but it seems likely that seed banks could have much-larger and more-important roles in conserving important genetic resources. The limiting factor is knowledge of the behavior of many useful but not necessarily commercially important tree species. Several studies (some cited above) revealed that some species that were labeled recalcitrant after initial testing are actually orthodox or intermediate. The high degree of genetic variability in storage behavior is both an opportunity and a disadvantage for conserving genetic resources, because it means that species that were previously considered recalcitrant might not have the same behavior across the entire species range, but storing only those seeds that have more-orthodox behavior is a selection screen and narrows the available genetic diversity of the collections.

Even if the storage behaviors of all species were known, significant challenges would still remain. Sampling strategies should aim to capture important genetic variability, but most species have not been studied with an objective to understanding genetic variability of known significance. Even the best seed centers do not have systematic approaches to define where and how much genetic variability to sample for long-term conservation. The logical approach to *ex situ* conservation is to start small and add accessions as opportunities arise. Simultaneously promoting the use of gene-banked species will enhance the value of programs for conservation.

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Component Analysis of Acorns of *Quercus mongolica* and *Q. variabilis*

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[Summary]

To compare seed components of plus trees, seed powder ground up after seed-coat removal was analyzed for 2 oak species, i.e., *Quercus mongolica* (white oak) and *Q. variabilis* (red oak), which are typical oak trees in Korea but have different physiological characteristics. Thus we attempted to analyze and compare many ingredients including minerals, sugars, fatty acids, etc. The 2 species were similar to each other in water contents, crude ash, crude protein, and free sugars, but the crude fat content in *Q. variabilis* was 2.5-times higher than that in *Q. mongolica*. Crude proteins of clone 124 were 1.5-times higher than those of clone 75 of *Q. mongolica*. The highest value of the crude fat content was found in clone 0511 of *Q. variabilis*, and more phosphate and iron were found in *Q. mongolica* than in *Q. variabilis*. Glucose comprised > 90% of the total sugars in the 2 species, and galactose and arabinose were also found. Unsaturated fatty acids comprised more than 80% of the total fatty acid contents. Oleic acid was higher in *Q. variabilis*, while linoleic acid was higher in *Q. mongolica*. Differences in the contents of phosphate, iron, crude fat, and fatty acids were found between the 2 species and among clones of each species.

Key words: fatty acids, mineral, polysaccharide, *Quercus mongolica* seed, *Q. variabilis* seed, seed components.

INTRODUCTION

Oak trees (*Quercus* spp.), distributed widely in Korea, occupy 75% of natural broadleaf forests (Suh and Lee, 1998). However, oak trees have not received much attention because they are considered useless. But recently, their forest stands are becoming more valuable and important as new demands for oak timber increase based on advancements in utilization technology. *Quercus mongolica*, *Q. variabilis*, *Q. serrata*, and *Q. acutissima* together account for about 27% of the total growing stock in Korea (Korea Forest Service 1998, Korea Tree Breeding Institute 1995).

Thus, oak trees are dominant species in Korean forest ecosystems, and they play important roles in sustaining ecosystems mainly because of their high environmental adaptability. From economic aspects, they are highly valuable because they can be used

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as materials for construction, furniture, pulp, charcoal, cork, and food (acorns only) (Lee 1997). The Korea Forest Service worked out "a broad-leaved forest plan" in the 1990s and made seed orchards of offspring from superior, plus trees based on these advantages and increasing demand for timber.

Even though much work that focused on volume growth for wood production was carried out, only small differences were recognized in growth patterns, age, and sites. Recently, the public's interest in eco-friendly, traditional foods such as tofu made from organically produced soybean, is rapidly growing. A kind of jelly made from acorn powder, which is called "muk" in Korean and is similar in general features to tofu but is dark brown in color, is very popular in Korea. Thus oak acorns are considered very good for health and are becoming more expensive because of a limited supply of acorn production. Commonly, acorns of all oak trees were treated equally for cooking and thought to have the same flavor, tastes, and nutrition, despite many differences in size, color, and maturity processes. Therefore, it has become necessary to identify the ingredients of acorns of each species.

In Korea, *Q. mongolica* and *Q. variabilis* are widely distributed and found in continuous patches in forests, but they have different physiological characteristics. *Quercus mongolica* is a white oak with light-colored bark together with *Q. serrata*, *Q. aliena*, and *Q. dentata*, and its flowering and fruition are achieved in 1 yr (Miller and Lamb 1985). However, *Q. variabilis* is a red oak with dark-colored bark together with *Q. acutissima*, and fruition is completed in 2 yr (Song 2002). Taste is an important factor for evaluating acorns as a food resource as its powder is the source of muk production in Korea. As far as taste, acorns of red oaks are astringent and bitter; but those of white oaks are smooth and not as bitter (Bonner and Vozzo 1987). It is said traditionally in Korea that acorn jelly of *Q. serrata* has a better taste than any other acorn jelly. It can be argued that components of acorns may greatly influence the taste. Thus some research was carried out on the components of oak acorns. Shim et al. (2004) studied the components of minerals and polysaccharides of oak acorn powder; Jeong et al. (2007) analyzed the components of oak acorn powder. The effects of saccharides on the texture and retrogradation of acorn starch gels were also studied (Lee et al. 1998). But in those studies, mixed acorn powder was used from different families or species, or the acorn species was not known.

The objectives of this study were to compare the components of acorns of 2 oak species, *Q. mongolica* (white oak) and *Q. variabilis* (red oak), and to determine differences in several ingredients among the 2 species.

MATERIALS AND METHODS

Plant materials

Acorns were collected from trees in seed orchards located in Hwaseong, Korea. These seed orchards, established in 1994, consist of grafted seedlings of plus trees selected for timber production. Each orchard includes 24 superfamilies of *Q. mongolica* and 66 superfamilies of *Q. variabilis*. We used acorns which were not injured by insects, removed the internal and external coats, and then mixed them. After being dried in the sun, the acorns were cleaned and ground up in a pulverizer before analysis.



Analysis of general components

The moisture content (MC) was measured by pressure-drying at 105°C; crude fat was determined by the soxhlet extraction method; crude protein was measured on a protein automatic analyzer (2300 Kjeltac Analyzer Unit, Foss Tecator, Sweden; with a protein coefficient of 6.25), and crude ash was analyzed in Muffle's furnace at 550°C. Nitrogen-free sugar was calculated by subtracting the sum (%) of the contents of moisture, crude fat, crude protein, and crude ash from 100%.

Mineral analysis

Samples were resolved by sulfuric acid (the nitric acid wet digestion method), and measured for iron, zinc, magnesium, manganese, potassium, copper, and sodium using atomic extinction spectrophotometry (Analytik jena AG Nov AA-300, Germany). For the analysis of calcium and lead, potassium chloride (KCl) was added to the resolved solution to avoid phosphate interference and measured by an atomic absorption spectrophotometric (AAS) method using nitrous oxide and acetylene gas. Phosphate was analyzed by molybdenum blue colorimetry using ultraviolet (UV)/visible (VIS) spectrophotometry (DH800, Beckman Coulter, USA).

Fatty acid analysis

Crude fats were extracted by the method mentioned above and were purified by the Bligh-Dyer method (1959) using a chloroform: methanol (2: 1, w/v) solution, and they were retreated by the Metcalfe method using 14% boron trifluoride methylation. After that, samples were analyzed using gas liquid chromatography (GLC; Hewlett-Packard GC Model 5890 series II, USA).

Polysaccharide content analysis

Polysaccharides were quantified by the Blakeney method (1983). Ten milligrams of sample was taken, mixed with 125 µl of 72% sulfuric acid (H₂SO₄), and left at room temperature for 45 min. They were processed by hydrolysis through neutralization to separation and then analyzed using GLC (J&W Scientific, Folsom, CA, USA).

RESULTS

Analysis of general components

Moisture and crude fat contents were higher in *Q. variabilis* than in *Q. mongolica*; in particular, *Q. variabilis* had an average 2.50% crude fat which was 2.5-times higher than that of *Q. mongolica*. On the other hand, *Q. mongolica* had less ash, crude protein, and nitrogen-free sugar contents than *Q. variabilis*.

In the clones, the MC of *Q. variabilis* clone 0616 (47.00%) was 10% higher than that of *Q. mongolica* clone 0511 (36.56%), which was the lowest for *Q. mongolica*. *Quercus variabilis* clone 0511 (3.81%) had a 5-fold higher crude fat content than *Q. mongolica* clone 129 (0.70%). In *Q. mongolica*, moisture and crude ash contents were the highest in clone 129 (43.04% and 1.65%, respectively), and the lowest in clone 828 (36.56% and 1.31%, respectively). The crude fat content was the highest in the clone 77

(1.28%) and the lowest in clone 129 (0.70%) in *Q. variabilis*. Crude contents were the highest in clone 124 (6.14%) and the lowest in clone 75 (3.68%).

In *Q. variabilis*, the MC was the highest in clone 0616 (47.00%) and the lowest in clone 0511 (39.00%). But the crude fat content showed the opposite trend. The highest was found in clone 0511 (3.81%) and the lowest in clone 0616 (1.40%). The clone with the highest crude ash content was San-cheong (1.39%) and the lowest was clone 0220 (0.94%). The clone with the highest crude protein content was 0511 (4.96%), and the lowest was clone 0110 (3.52%).

Mineral content analysis

On average, compared to those of *Q. variabilis*, contents of *Q. mongolica* showed higher rates of zinc (Zn, 0.52 mg/100 g), copper (Cu, 0.30 mg/100 g), sodium (Na, 62.94 mg/100 g), and calcium (Ca, 49.68 mg/100 g). For calcium, the latter was 2-fold the value of the former. *Quercus variabilis* showed higher contents of iron (Fe, 2.18 mg/100 g), magnesium (Mg, 44.75 mg/100 g), manganese (Mn, 3.89 mg/100 g), potassium (K, 208.10 mg/100 g), and phosphorus (P, 686.20 mg/100 g). Among these, especially the phosphorus content *Q. variabilis* revealed 2-time higher rate than *Q. mongolica*.

In *Q. mongolica*, the Ca content was 10 times higher in clone 513 (110.84 mg/100 g) compared to that of clone 76 (13.13 mg/100 g). Clone 76 showed the lowest rates of Ca, Fe, Zn, Mg, Mn, P, K, and Cu. In *Q. variabilis*, it was shown that 1 or 2 mineral contents were higher in some clones, but such clones did not show the highest values in all contents. Clone 0110 showed lower contents in the elements, Fe, Zn, P, Cu, and Na.

In both species, mineral contents showed high values of P, K, Na, Mg, Ca, Mn, Fe, Zn, and Cu in that order.

Fatty acid analysis

We analyzed 23 fatty acids normally found in food. Palmitic acid (16:0) content, one of the saturated fatty acids, did not significantly differ between the 2 species, although it was a little bit higher in *Q. variabilis* (14.9%) than in *Q. mongolica* (12.7%). Stearic acid (18:0), another saturated fatty acid, was a little bit higher in *Q. mongolica* (1.8%) than in *Q. variabilis* (1.6%).

In unsaturated fatty acids, oleic acid (18:1 (cis)) appeared 2-fold higher (47.9%) in *Q. variabilis* and linoleic acid (18:2) was 1.8-fold higher (51.7%) in *Q. mongolica* than in the other species. Linolenic acid (18:3) was 2.5-times higher in *Q. mongolica* (8.5%) than in *Q. variabilis* (2.8%).

Fatty acids showed small differences in *Q. mongolica*, but little differences in *Q. variabilis* among all analyzed clones.

Polysaccharide analysis

Contents of ribose, arabinose, mannose, and galactose were similar in the 2 species. Rhamnose was 3-times higher in *Q. mongolica* than in *Q. mongolica*. Allose was only detected in *Q. mongolica* clone 124 (1.52 g/100 g). More glucose was found in *Q. variabilis* (18.59 g/100 g) than in *Q. mongolica* (16.04 g/100 g).



DISCUSSION

As found in the results, acorn components showed differences in many ingredients between species and among clones of each species. Established seed orchards must be good sources for selecting high-quality seed production families although they were intended to develop families and/or individuals for high wood productivity.

Compared to the analysis of components using mixed acorns without identifying species (Shim et al. 2004), our analysis showed higher rates of moisture, crude protein, and crude fat contents. Those researchers used dry powder in storage, but we used raw powder just after pulverizing. This difference may have led to different analytical results. In our results of mineral content analysis, phosphorus showed the highest content, but the potassium content was 5-times higher than phosphorus and calcium in another report (Shim et al. 2004). Also among polysaccharides, glucose occupied more than 85% in Shim et al.'s research, but more than 97% in our analysis. In raw chestnut research (Nha and Yong 1996), it was mentioned that fructose and glucose decreased with the storage period, and acorn powder in storage showed very low water content.

In research on acorns of *Castanopsis cuspidata* which grows naturally in southern coastal area and islands of the Korean peninsula and provides "muk" material for residents, the compositions of mineral and fatty acids (Lee et al. 2009) were found to be similar to those of our results, even though the species used for both composition analyses belong to different genera but show similar features. It may be inferred that powder storage conditions can be an important factor for maintaining ingredients in a stable condition.

In the seed production process of selected, superior trees, levels of each clone contributing to pollination are very important factors for fertilization and fruiting. Sometimes it is very difficult to obtain high-quality seeds even from a seed orchard because of this kind of uneven pollination (Han et al. 2001). Therefore, seed orchards should carefully be established, especially for the production of seeds of high yield and proper quality for food sources like oak acorns.

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Desiccation Sensitivity of *Antiaris toxicaria* Axes and Reactive Oxygen Species-Scavenging Enzymes in Washed Mitochondria

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[Summary]

The axes of *Antiaris toxicaria* seeds and washed mitochondria from the axes were used as experimental materials. Changes in desiccation sensitivity, the subcellular structure, activities of reactive oxygen species (ROS)-scavenging enzymes, and contents of thiobarbituric acid (TBA)-reactive products in whole axes and washed mitochondria during dehydration were studied. Axes gradually lost desiccation tolerance with dehydration, and desiccation tolerance of the epicotyls was higher than that of the radicles. Water contents at which 50% of epicotyls and radicles were killed by dehydration were approximately 0.59 and 0.66 g g⁻¹, respectively. The subcellular structure of newly collected axes was integrative and gradually damaged by dehydration. Cytochrome *c* oxidase (CCO) activities of washed mitochondria increased during the early stages of dehydration, and then markedly decreased. Latencies of CCO activities of washed mitochondria slightly decreased during dehydration. Activities of superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, and dehydroascorbate reductase of whole axes and washed mitochondria increased in the early stages of dehydration, and then rapidly decreased. The activities of these 5 enzyme were more sensitive to dehydration in washed mitochondria than in whole axes. Contents of TBA-reactive products in whole axes and washed mitochondria dramatically increased with dehydration. These results show that *A. toxicaria* seeds are recalcitrant in nature, and that the ROS-scavenging enzyme activities in mitochondria can be a sensitive parameter for studying seed recalcitrance.

Key words: *Antiaris toxicaria*, cytochrome *c* oxidase, mitochondria, reactive oxygen species scavenging enzymes, recalcitrant seeds, subcellular structure, TBA-reactive products.

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Storage Conditions for Prolonging the Seed Viability for *Ex Situ* Conservation and Deteriorative Changes Associated with Viability Loss in *Dalbergia sissoo* Seeds

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[Summary]

Seed viability during storage can be prolonged by manipulating the storage temperature and seed moisture content (MC). To determine optimal conditions for long-term seed storage of *Dalbergia sissoo*, seeds were stored at 6 temperatures (40, 25, 15, 5, -5°C, and room temperature) and 3 different seed MCs (12.50, 8.0, and 5.60%). Viability was determined by germination at bimonthly intervals, survival curves were drawn, and the rate of loss of viability was calculated. Viability was completely lost by 60 d for seeds stored at 40°C and a 12.5% seed MC, and the rate of loss of viability was maximum. The germination percent and survival curve revealed that maximum viability was recorded for seeds with a 5.6% MC stored at 5 and -5°C after 665 d of storage. The results revealed that *D. sissoo* seeds are orthodox in nature, as the rate of loss of viability was minimum at a reduced MC and low temperature. Thus these conditions are ideal for the long-term storage of seeds for *ex situ* conservation. The ultrastructure of embryos studied through transmission electron microscopy revealed that viable seeds had rigid cell walls with granular deposition and all cell organelles. In non-viable seeds, the cell wall had disintegrated, the granular material had disappeared, the lipid bodies had coalesced, the number and size of vacuoles had increased, and all cell organelles were completely disorganized. Analysis of proteins by SDS-PAGE showed that the number of polypeptides decreased from 61 in viable seeds to 13 in nonviable seeds.

Key words: seed viability, temperature, moisture content, survival curve, ultrastructure.

INTRODUCTION

Dalbergia sissoo Roxb. of the family Leguminosae is an important broadleaf leguminous tree of the Indo-Pakistan subcontinent, growing naturally from the Himalayan hills to the plains of Afghanistan, Pakistan, India, and Malaysia and has been planted in several African and South Asian countries (Bangarwa et al. 1996). About 80% of tropical and subtropical plantations of *D. sissoo* are in India in an area of 4.94 x 10⁶ ha (Varmola and Carle 2002). Owing to its multipurpose uses for timber, fodder, fuel, and as a nitrogen fixer, the species has been introduced in many parts of India. It is

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among the principal tree species commonly recommended for plantation programs, as it is environmentally and socioeconomically acceptable (Lodhiyal et al. 2002). *Dalbergia sissoo* is 1 of 3 major species selected for plantations programs in India, and 10% of its seeds are from genetically improved sources. More than 41,000 kg of seeds are collected for *D. sissoo* annually (Katwal et al. 2003), but the demand is increasing.

A limited viability period of seeds poses problems in maintaining germplasm during the period when the accessions are being built up for testing, evaluation, and long-term conservation. Efforts are therefore immediately needed to extend the storage for the short (5 yr) to medium term (10~15 yr). Viability can be prolonged for longer periods if we understand the factors responsible for and changes taking place during deterioration. To maintain seeds at the highest possible viability and vigor, determining the best conditions for storage is critical. The ideal storage conditions are those which reduce the processes of respiration and transpiration to the lowest possible degree, without impairing the inherent vitality or strength of the seed embryo in any way. Dry, cold conditions provide superior longevity compared to warm, humid conditions, but there is a debate about the optimal environment for seed storage. Although the seed moisture content (MC) and temperature are interrelated, high temperatures hasten the deterioration of seeds with high MCs by increasing the metabolic activity of hydrolyzed substrates and enzymes. High temperatures exert only minimum deteriorative effects on low-moisture seeds. Temperature and MC are negatively correlated with seed longevity. The rate of respiration is lower at lower temperatures, and thus the life span of the seed in storage is longer (Willan 1985).

Storage of seeds under adverse conditions results in the production of ‘aged’ seeds, and a number of structural, biochemical, physiological, cellular, and metabolic changes in cell tissues may be associated with the aging of stored seeds. The most visible symptoms of seed deterioration are observed first at the whole-seed morphological level and then during germination and seedling growth. However, these are preceded by numerous ultrastructural and physiological changes, the symptoms of which are not as readily apparent but can be detected by sophisticated techniques that attempt to identify changes in the deteriorating seed at the physiological level. In this study, we determined the optimum storage conditions for *D. sissoo* seeds and documented changes that occur during loss of viability.

MATERIALS AND METHODS

Seed storage studies

For storage studies, seeds were collected from the Shakumbra forest range in the Dehra Dun India. Samples were taken to determine the initial MC according to the method described by ISTA (1993). The MC was reduced by drying seeds in the laboratory under a fan. Seeds at 3 MCs of 12.50, 8.00, and 5.60% were stored at 6 different temperatures of 40, 25, 15, 5, -5°C and room temperature (RT) ($25 \pm 5^\circ\text{C}$). The initial germination of each lot was recorded before storage. During storage, samples were taken once every 2 mo to assess viability by a germination test. Germination studies were carried out at 30°C, and 4 replicates of 50 seeds each were taken. Seeds that had germinated by the end of the germination period (21 d) were counted, and the cumulative germination was expressed as the percentage of the total number of seeds sown. By a



probit transformation of the mean germination percentage, the expected germination was calculated. Survival curves were drawn between the expected germination and storage time. The rate of loss ($-V/D$) of viability was determined from the slope of the linear least squares fit of the probit germination vs. storage time. A 3-way analysis of variance (ANOVA) was carried out to determine the effects of temperature, MC, time of storage, and their interactions on viability.

Qualitative estimation of protein

Stored seeds were tested for viability after 18 mo, and 8 samples with different viability percentages were selected. In these samples, viability varied from 96 to 0% (i.e., 96, 88, 76, 68, 54, 22, 16, and 0%). Qualitative estimation of protein was carried out by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Laemmli 1970).

Ultrastructural studies

Ultrastructural changes were documented in the embryonic axis of fresh (fully viable) and aged seeds (nonviable) through transmission electron microscopy (TEM). Samples were fixed at 0~4°C in freshly prepared 0.1 M sodium cacodylate buffer (pH 7.3 ± 0.1) containing 3.00% glutaraldehyde and 2.00% paraformaldehyde. Samples were then put in 0.1 M cacodylate buffer, post-fixed in 1.00% osmium tetroxide in 0.1 M cacodylate buffer for 2~3 h, washed 3 or 4 times in distilled water to remove excess osmium tetroxide, and dehydrated in ascending gradations of ethanol (30, 50, 70, 90, and 100%), an ethanol-isoamylacetone mixture (2:1, 1:1, and 1:2), and 100% isoamylacetone. Ultrathin section cut with an LKB ultratone 8800 were individually collected on clean 400-mesh grids, stained for 30 min with 1.00% uranyl acetate, followed for 5~10 min with lead citrate in a CO₂ free atmosphere (using KOH pellets), and photographed on a Philips EM 410LS electron microscope.

RESULTS

Seed storage studies

With an increase in temperature, the viability decreased. The minimum mean germination of 11.66% was recorded at 40°C and the maximum of 84.09% at -5°C. Mean germination increased as the temperature decreased from 40 to -5°C (Table 1). The rate of the loss of viability was highest at 40°C at all MCs (Table 2). The effects of temperature on germination were statistically significant ($p = 0.05$). As the MC was reduced from 12.50 to 5.60%, the mean germination increased from 61.55 to 71.85% (Table 1), while the rate of the loss of viability decreased. Interactions of different combinations of storage temperature and seed MC had significantly different effects on viability ($p = 0.05$). The minimum mean germination of 7.50% was recorded for seeds with a 12.50% MC stored at 40°C, while the maximum mean germination of 85.15% was recorded for seeds stored at -5°C with a 5.60% MC (Table 1), and this was on a par with a germination of 84.17% for seeds stored at 5°C with the same MC.

The interaction of storage temperature, seed MC, and time revealed that complete loss of viability was recorded only at a 40°C storage temperature. Survival curves at this temperature had a deep slope due to rapid loss of viability at all seed MCs (Fig. 1). After

665 d, seeds with a 12.5% MC stored at RT had minimum germination (24.0%). Maximum germination (85.5%) was at a 5.6% seed MC and -5°C storage temperature and was similar to the remaining 3 storage temperatures. The survival curve with an almost straight line revealed that viability loss was minimum at 5 and -5°C storage temperatures at a 5.6% MC (Fig. 1). The rate of loss of viability (Table 2) decreased with reductions in storage temperature and seed MC.

Table 1. Effects of seed moisture content (MC; 12.5, 8.0, and 5.6%), storage temperature (40°C, room temperature (RT), 25, 15, +5 and -5°C) and their interactions on mean germination

Temp.(°C) MC (%)	40°C	RT	25°C	15°C	5°C	-5°C	Mean MC
12.50	0.750 (6.14)	56.83 (48.90)	65.47 (54.58)	73.71 (59.88)	82.60 (65.72)	83.19 (66.31)	61.55 (50.32)
8.00	08.82 (7.47)	73.98 (60.01)	79.46 (63.55)	80.76 (64.88)	83.10 (66.12)	83.93 (66.73)	68.25 (54.70)
5.60	19.21 (17.51)	77.06 (62.01)	82.39 (65.54)	83.14 (66.08)	84.17 (66.99)	85.15 (67.73)	71.85 (57.64)
Mean temp.	11.66 (10.57)	69.28 (56.97)	75.77 (61.22)	79.20 (63.44)	83.29 (66.28)	84.09 (67.73)	

Values in parentheses were arcsine-transformed.

Treatment and interactions	Coefficient of determination value
MC	0.34
Temperature	0.49
MC x temperature	0.84
MC x temperature x time (d)	5.09

Table 2. Rate of loss of viability (-V/D) for seeds under different combinations of storage temperature and seed moisture content

Temperature (°C)	Moisture content (%)		
	12.5	8.0	5.6
40	0.143	0.143	0.133
Room temperature	0.049	0.022	0.007
25	0.031	0.009	0.002
15	0.020	0.008	0.001
+5	0.0005	0.0008	0.0008
-5	0.0007	0.0005	0.0008

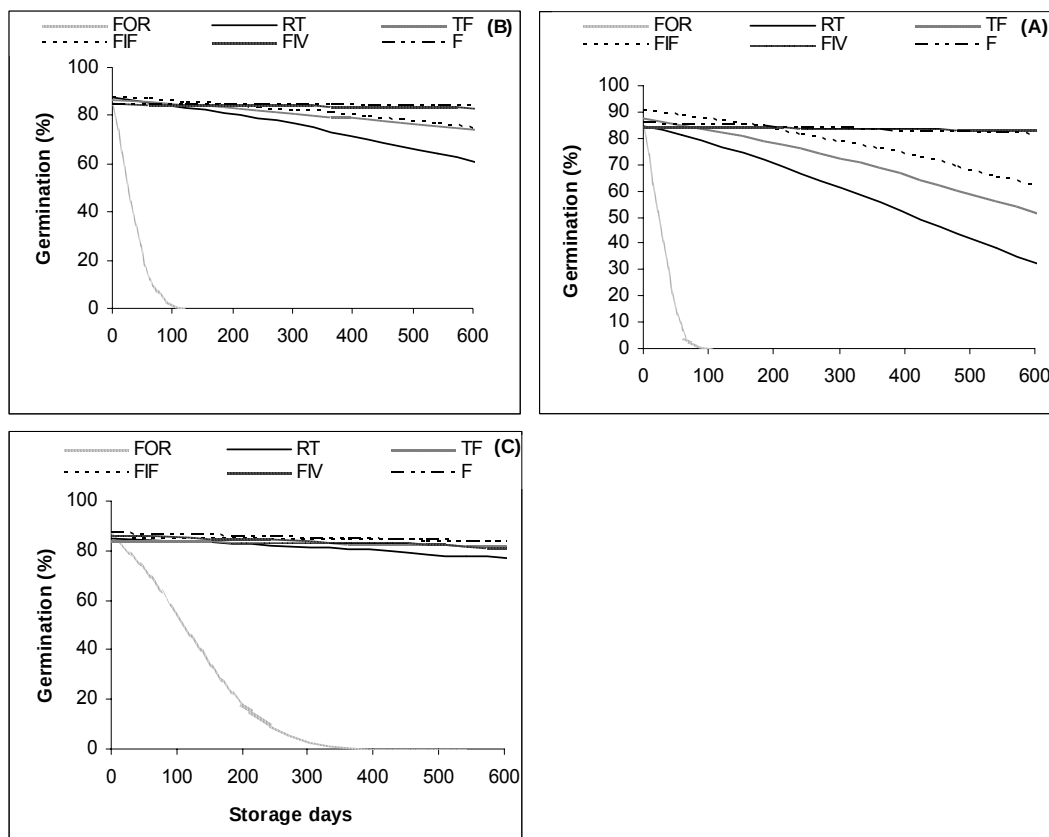


Fig. 1. Survival curves for seeds stored at 3 moisture contents (A) 12.5, (B) 8.0, and (C) 5.6% and 5 temperatures. (FOR, 40°C; RT, room temperature; TF, 25°C; FIF, 15°C; FIV, 5°C; F, -5°C.)

Qualitative estimation of protein

There was a decrease in the number of polypeptides with deterioration (Table 3). Seeds with 96% viability had a total of 61 polypeptides, while nonviable seeds had 13 polypeptides. The polypeptides were categorized into 3 groups based on their molecular weights as low ≤ 49 kDa, medium 50~99 kDa, and high ≥ 100 kDa. High-molecular-weight polypeptides decreased from 11 in seeds with 96% viability to 1 in non-viable seeds, similarly to what occurred with medium- (16 to 4) and low-molecular-weight polypeptides (34 to 8) (Table 3).

Ultrastructural studies

Ultrastructural studies of seeds revealed degenerative changes with seed deterioration. The ultrastructure of fully viable seeds (Fig. 2) had rigid cell walls with uniform deposition of some granular materials and starch granules distributed throughout the cytoplasmic matrix. Double-membraned plastids with clear matrix and Golgi bodies (Fig. 2C) were present along with mitochondria, endoplasmic reticula, and ribosomes

attached to them (Fig. 2B). Nuclei were double-membraned with a nucleolus and a clear nuclear matrix (Fig. 2D).

The ultrastructure of cells of nonviable seeds revealed disintegration of cell walls and the complete absence of granular material along the cell walls (Fig. 3A). The cytoplasmic matrix was dense, with a disorganized nucleus and swollen mitochondria with disintegrated cristae (Fig. 3B, C). The prominent structures visible at this stage were lipid bodies and autophagic vacuoles scattered throughout the dense cytoplasmic matrix (Fig. 3D).

Table 3. Total number of polypeptides and their numbers in low-, medium-, and high-molecular-weight (kDa) categories for seeds with different germination percentages

Germination (%)	Total number of polypeptides	Number of polypeptides in different molecular weight categories		
		Low (< 49 kDa)	Medium (50~99 kDa)	High (> 100 kDa)
96.0	61	34	16	11
88.0	46	31	8	7
76.0	33	22	7	4
68.0	40	24	9	7
54.0	43	29	9	5
22.0	39	25	9	5
16.0	21	15	4	2
0.0	13	8	4	1

DISCUSSION

Seed MC and storage temperature are the 2 crucial factors affecting the viability of seeds in storage. The viability was maintained for longer periods when the MC was reduced from a maximum of 12.60% to a minimum of 5.20%, suggesting the desiccation-tolerant nature of *D. sissoo* seeds. In *Fagus sylvatica* (Poulsen 1993), *Holoptelea integrifolia* (Rajput 2001), *Grewia optiva* (Nayal et al. 2002), and *Pinus roxburghii* (Gautam et al. 2005), storability increased by drying to a 5.00% MC. The essential feature of orthodox seed is a negative logarithmic relation between longevity and MC over a wide range of conditions (Ellis and Roberts, 1980). Temperature also plays an important role in maintaining the longevity of seeds: the lower the temperature is, the greater the longevity is. This trend was also seen in our results. At 5 and -5°C, maximum viability was maintained at all MCs, compared to other temperatures. Prolongation of viability at low temperatures of -5~0°C was reported for *Terminalia bellerica* (Warrier et al. 2005) and *Feronia elephantum* (Sivakumar et al. 2006). Poulsen (1993) suggested that if drying equipment is not available, reducing the storage temperature can compensate for storage at higher MCs. Overall results show that a 5.60% MC and 5 and -5°C are the best conditions for storage of *D. sissoo* seeds. The shape of the survival curve and rate of loss of viability also indicated that viability decreased with decreases in seed MC and storage temperature, which is in accordance with Roberts (1973) on the longevity of orthodox seeds.

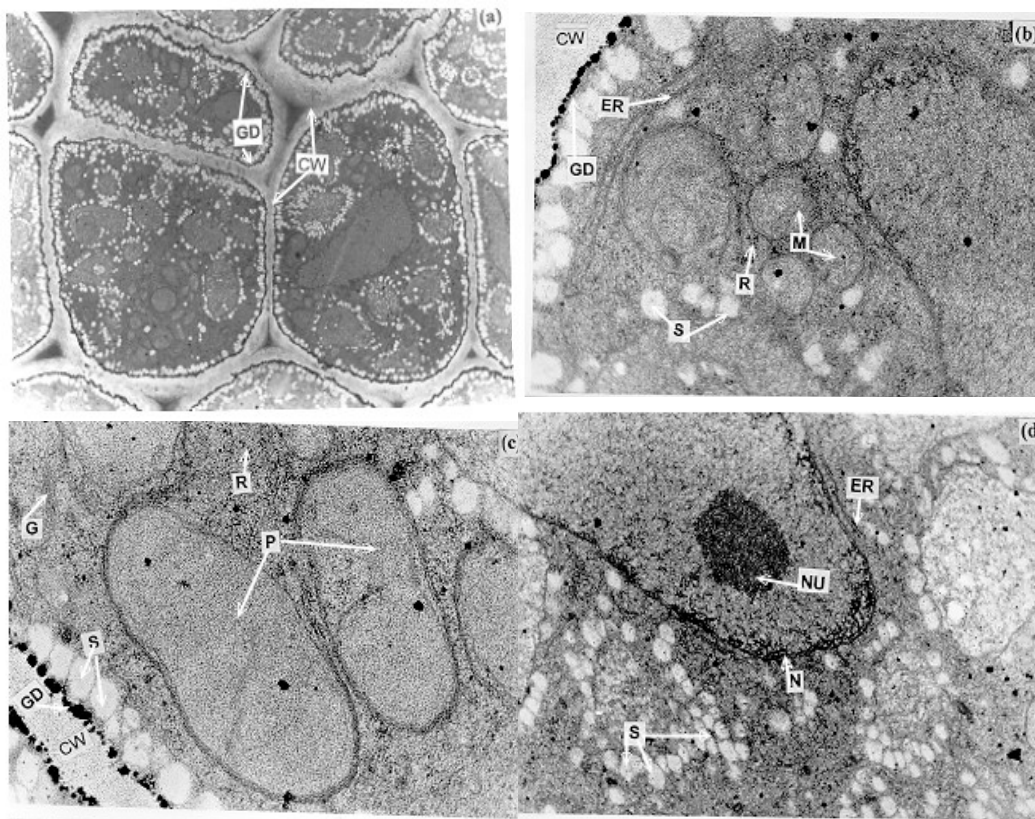


Fig. 2. Section of the embryonic axis from fresh seeds showing (A) a general view of tissue (x3000); (B) cell wall (CW), granular deposition (GR), mitochondria (M), ribosomes (R), endoplasmic reticulum (ER), and starch granules (S) (x21,000); (C) plastid (P) and Golgi bodies (G) in a clear matrix (x21,000); and (D) nucleus (N) and nucleolus (Nu) (x14,000) in a clear matrix.

Seed deterioration is an irreversible physiological phenomenon and can be defined as loss of quality, viability, and vigor (Kapoor et al. 2010). Changes in seeds as they deteriorate occur in almost every system, and affect many kinds of enzymes and almost all organelles. As a result, the seed loses its vigor (Enju et al. 1993). A qualitative estimation of proteins through electrophoresis showed that total number of polypeptides decreased from 61 to 13 as seeds lost viability from 96 to 0% (Table 3). Nautiyal and Thapliyal (1984) reported that denaturation of proteins and isozymes and the ability to synthesize them upon imbibition are lost during the loss of seed viability. In *Avicennia marina*, the protein-synthesizing capacity of the root primordia declined as seeds deteriorated in storage (Motete et al. 1997).

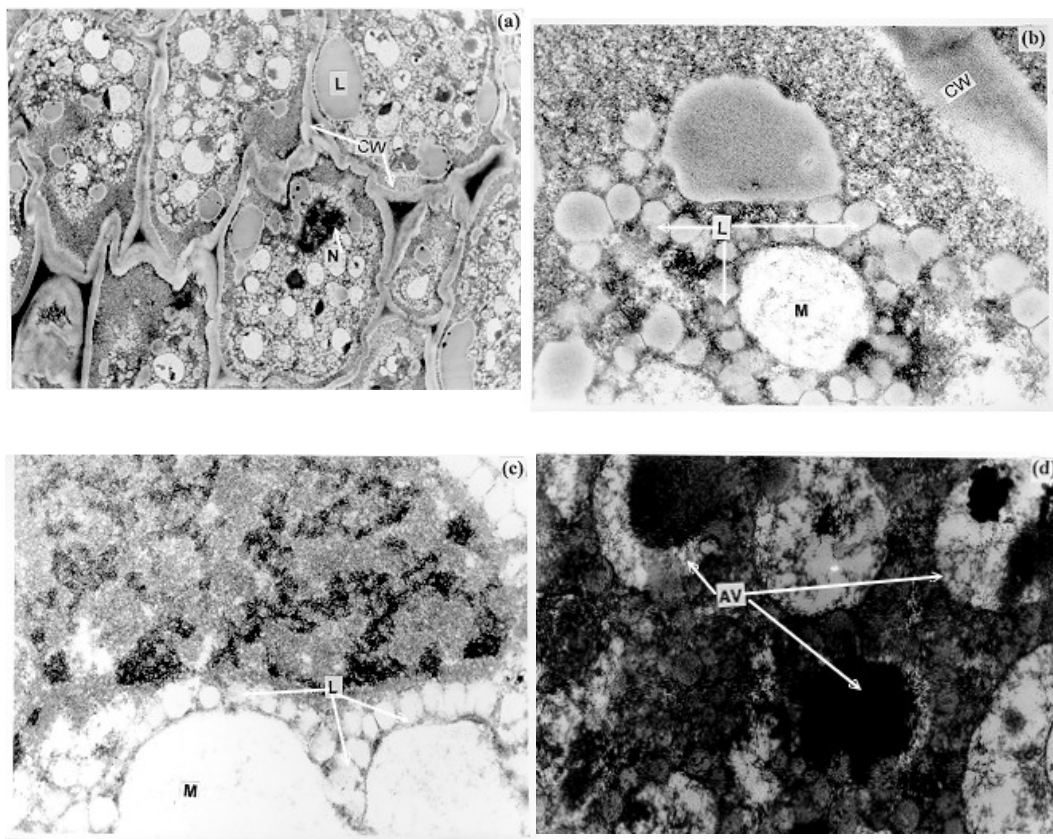


Fig. 3. Section of the embryonic axis from a nonviable seed showing (A) a general view of tissue (x3000); (B) cell wall (CW) without granular deposition, swollen mitochondria (M), and dense lipid bodies (L) (x21,000); (C) disintegrated nucleus with dense matrix (x21,000); and (D) an autophagic vacuole (AV) (x7100) in a dense cytoplasmic matrix.

Ultrastructural changes associated with aging (Fig. 3) suggest that with aging, there is degradation of all of the organelles. In *D. sissoo*, cell walls are rigid and smooth with uniform deposition of granular material in seeds from seed lots with 96% viability, but the walls became wavy, and finally lost rigidity and deposition of granular material as cells became nonviable. Ultrastructural examinations of the root tips of gymnosperms (Simola 1974, 1976), monocotyledons (Berjak et al. 1986), and dicotyledons (Dawidowicz and Podskelski 1992) confirmed that membranes undergo deteriorative changes with increasing seed age. Together with mitochondria, plastids are among the first organelles to suffer aging-related changes. Plastids, which are present as large double-membraned structures with clear matrix in cells of fully viable seeds, disappeared from cells of nonviable seeds. The endoplasmic reticula, Golgi bodies, and ribosomes were also not found in aged cells. Nuclei became irregular, and nucleoli and nuclear membranes had degenerated in dead cells. Similar results were reported in *Azadirachta indica* (Berjak et al. 1995) and *Avi. marina* (Motete et al. 1997). Abu-Shakar and Ching



(1967) observed that new seeds contained intact mitochondria as well as plastids and vesicles; however, in old material, the mitochondria appeared to have become dilated, cristae were inflated, the matrix was coagulated, and the outer membrane was distorted. Coalescence of lipid bodies was seen in cells of nonviable seeds (Fig. 3). Fernandez Gracia de Castro and Martinez-Hunduwilla (1984) reported the coalescence of lipid bodies in pine. Extensive vacuolation and confluency were reported in *Aza. indica* (Berjak et al. 1995) and *Avi. marina* (Motete et al. 1997). Thus progressive membrane deterioration, initially involving mitochondria and plastids and culminating in the dissolution of tonoplasts can explain the decline in vigor and ultimate loss of viability.

Our study confirms the orthodox nature of *Dalbergia sissoo* seeds. For *ex situ* conservation, seeds can be stored at a temperature of +5 or -5°C with a reduced moisture content of 5.60%.

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Ex Situ Conservation of Trees and Seeds in Taiwan

Ching-Te Chien¹⁾

[Summary]

The Tree Seed Bank at the Taiwan Forestry Research was established in 1956. The purpose of this gene bank is to store seeds of woody plants for long-term preservation, research, and seed exchanges with other countries. There are about 51 families, 183 species, and more than 600 seed lots stored at 5 and -20°C. However, those recalcitrant seeds that cannot be dry-stored are mixed with moist sphagnum moss and stored at 4~5°C until the next harvest time. Seeds from clonal seed orchards are also available. The Taiwan Forestry Research Institute has established > 60 long-term experimental stands, and these stands provide such services as public awareness and academic research, and are the scientific basis of forest management.

Key words: *ex situ* conservation, intermediate seed-storage behavior, orthodox seed-storage behavior, recalcitrant seed-storage behavior, tree seed bank.

INTRODUCTION

The Taiwanese vascular flora is rich and diversified. There are 4339 known species of 235 families and 1419 genera in Taiwan, including 592 trees, 434 shrubs, 252 lianas, 184 vines, and 2877 herbs and ferns. There are about 262 exotic species, most of which are pasture species. Endemic species include 178 trees, 176 shrubs, 93 lianas and vines, and 620 herbs. The proportion of endemic species increases from 17.6% at low elevations to 59.2% at high elevations, and a high positive correlation ($r^2 = 0.99$) exists between endemism and elevation (Flora of Taiwan, 2nd edition, Vol. 6, 2003). The 20 most speciose families (including numbers of species in parentheses) are as follows: Orchidaceae (336), Gramineae (248), Compositae (195), Leguminosae (180), Cyperaceae (174), Rosaceae (111), Rubiaceae (98), Euphorbiaceae (80), Dryopteridaceae (79), Scrophulariaceae (66), Labiatae (71), Lauraceae (64), Urticaceae (64), Polypodiaceae (62), Athyriaceae (57), Fagaceae (52), Thelypteridaceae (49), Liliaceae (48), Moraceae (48), and Ranunculaceae (46). The 20 most speciose genera (including numbers of species in parentheses) are as follows: *Carex* (61), *Asplenium* (42), *Rubus* (39), *Ficus* (37), *Polygonum* (36), *Dryopteris* (30), *Pteris* (30), *Fimbristylis* (28), *Polystichum* (28), *Symplocos* (27), *Cyperus* (24), *Ilex* (24), *Athyrium* (23), *Diplazium* (21), *Liparis* (21), *Lycopodium* (21), *Bulbophyllum* (21), *Clematis* (20), *Goodyera* (20), and *Calanthe* (19).

Ex situ conservation of trees and seeds is important work at the Taiwan Forestry Research Institute (TFRI). Tree conservation with resources of various species has been carried out in the field since the 1960s. The Tree Seed Bank of the TFRI is the only bank storing tree seeds in Taiwan (Fig. 1).

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Taiwan Satellite Map

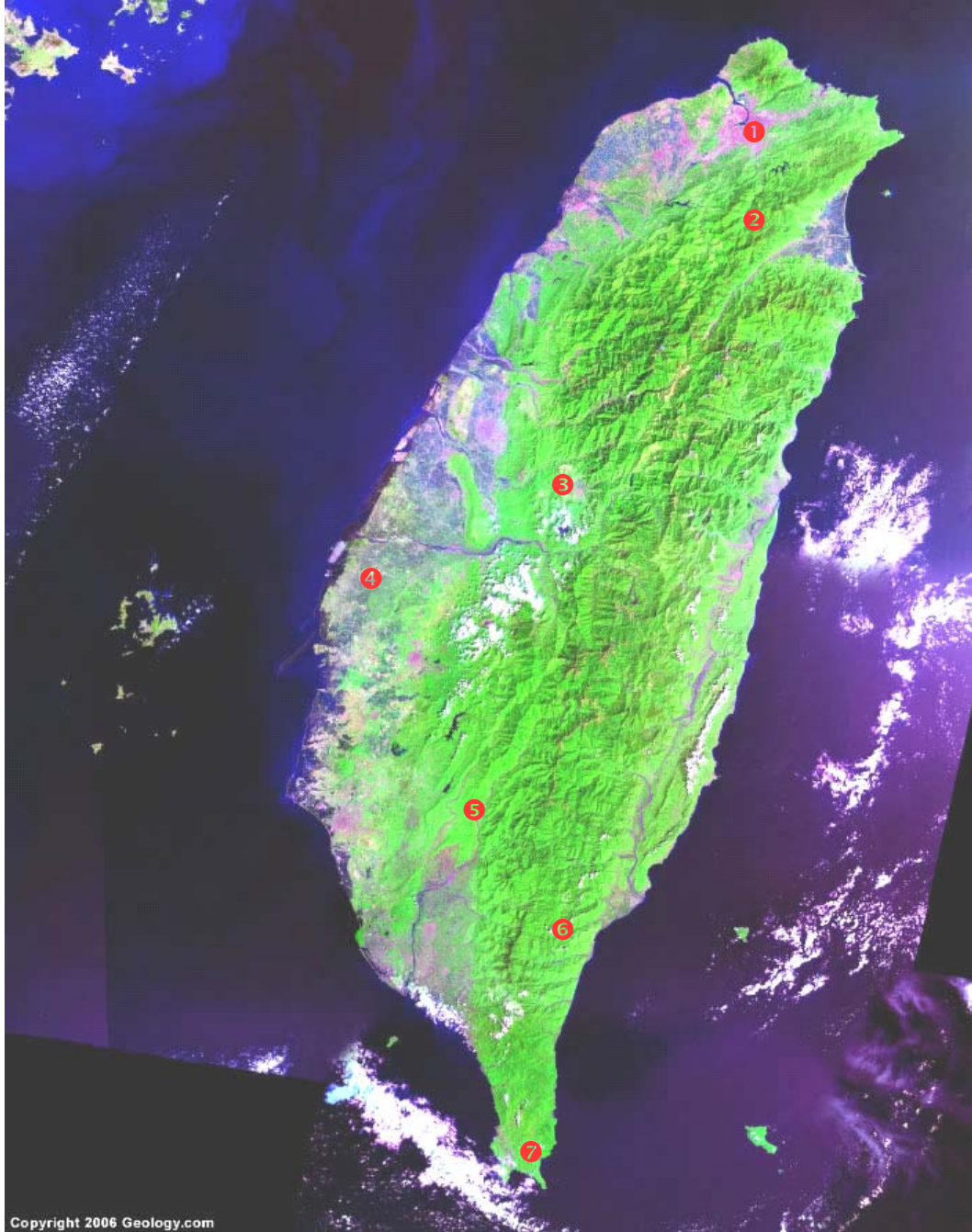


Fig. 1. Locations of tree conservation sites in Taiwan.



- ❶ Tree Seed Bank (Taipei). Plantation test of *Keteleeria davidiana*.
- ❷ Fushan subtropical forest long-term dynamic plot experiment (25 ha).
Fushan natural forest regeneration experiment.
- ❸ Clonal seed orchard and progeny tests of *Calocedrus macrolepis* var. *formosana* and *Cunninghamia lanceolata* var. *konishii*.
Clonal orchard of *Taxus mairei*.
Clonal seed orchard of *Taiwania cryptomerioides*, *Pinus morrisonicola*, and *Cinnamomum osmophloeum*.
Anti-disease orchard of *Paulownia* × *taiwaniana*.
Provenance tests of *Taiwania cryptomerioides*, *Chamaecyparis formosensis*, *Cha. obtusa* var. *formosana*, and *Cunninghamia lanceolata* (species from China).
Lienhuachih low-elevation broadleaf forest long-term dynamic plot experiment (25 ha)
- ❹ International provenance test of *Casuarina* spp.
- ❺ Tree density test of *Zelkova serrata*.
Liouguei dynamic plot experiment of *Taiwania cryptomerioides* (10 ha)
Plantation test of *Cinnamomum kanehirae* using seedlings from stem cuttings.
Plantation and management test of *Taiwania cryptomerioides*.
Progeny test of *Calocedrus macrolepis* var. *formosana*, *Chamaecyparis formosensis*, *Cha. obtusa* var. *formosana*, and *Taiwania cryptomerioides*.
Plantation test of *Taxus mairei* from various seed sources.
- ❻ Provenance test of *Cinnamomum kanehirae*.
Mixed-species test of *Cinnamomum kanehirae*, *Michelia compressa*, and *Zelkova serrata*.
Plantation test of *Pasania hancei* var. *ternaticupila*.
- ❼ Kenting uplifted coral reef forest long-term dynamic plot experiment (10 ha).
Ex situ plantation and conservation of endangered and vulnerable species of the tropical Hengchun Peninsula.

Tree seed bank and seed storage behavior

The purpose of the TFRI tree seed bank is to mainly conserve orthodox seeds of woody plants and some recalcitrant seeds collected from natural forests, managed plantations, and seed orchards. The Tree Seed Bank currently stores 51 families, 183 species of woody plants and more than 600 seed lots collected from throughout the island (Table 1). Orthodox seeds are stored at 5°C for short-term storage (~5 yr) and at -20°C for long-term storage. For example, orthodox seeds of *Chamaecyparis formosensis*, *Cha. obtusa* var. *formosana*, *Calocedrus macrolepis* var. *formosana*, *Cunninghamia konishii*, and *Taiwania cryptomerioides*, the 5 most important coniferous trees of Taiwan, have been stored at -20°C for more than 20 yr. Pinaceae seeds including *Abies kawakamii*, *Pinus armandii* var. *masteriana*, *Pin. morrisonicola*, *Pin. taiwanensis*, *Picea morrisonicola*, *Pseudotsuga wilsoniana*, and *Tsuga chinensis* var. *formosana* exhibit orthodox storage behavior and have been stored for more than 10 yr at -20°C (Wang et al. 1995). However, seeds of *Keteleeria davidiana* have recalcitrant storage behavior, and cold-stratification at 4°C for 3 mo of freshly harvested seeds increases the germination percentage and rate (Yang et al. 2006).

Table 1. Seeds of woody species stored at the Tree Seed Bank, TFRI

GYMNOSPERMAE

CUPRESSACEAE

Calocedrus macrolepis var. *formosana*

Chamaecyparis formosensis

Chamaecyparis obtusa var. *formosana*

PINACEAE

Abies kawakamii

Picea morrisonicola

Pinus armandii var. *masteriana*

Pinus massoniana

Pinus morrisonicola

Pinus taiwanensis

Pseudotsuga wilsoniana

Tsuga chinensis var. *formosana*

PODOCARPACEAE

Nageia nagi

Podocarpus costalis

Podocarpus nakaii

TAXODIACEAE

Cunninghamia konishii

Cunninghamia lanceolata

Taiwania cryptomerioides

ANGIOSPERMAE

ACERACEAE

Acer albopurpurascens

Acer kawakamii

Acer morrisonense

Acer buergerianum var. *formosanum*

Acer serrulatum

ANACARDIACEAE

Pistacia chinensis

Rhus javanica var. *roxburghiana*

Rhus succedanea

AQUIFOLIACEAE

Ilex asprella

Ilex formosana

Ilex lonicerifolia var. *matsudai*

Ilex micrococca

Ilex rotunda

ARALIACEAE

Aralia bipinnata

Dendropanax dentiger

Schefflera octophylla

Schefflera arboricola

Schefflera taiwaniana

BETULACEAE

Alnus formosana

Carpinus kawakamii

BIGNONIACEAE

Jacaranda acutifolia

Radermachia sinica

BORAGINACEAE

Ehretia longiflora

CAPRIFOLIACEAE

Sambucus chinensis

Viburnum foetidum

Viburnum luzonicum

Viburnum odoratissimum

CELASTRACEAE

Celastrus kusanoi

Euonymus laxiflorus

Euonymus spraguei

CONVOLVULACEAE

Ipomoea pes-caprae



(con't)

CORNACEAE

Benthamidia japonica var. *chinensis*

EBENACEAE

Diospyros ferrea

Diospyros morrisiana

Diospyros philippensis

ELAEOCARPACEAE

Elaeocarpus japonicus

Elaeocarpus sylvestris var. *sylvestris*

Sloanea formosana

EUPHORBIACEAE

Bischofia javanica

Glochidion rubrum

Sapium sebiferum

FAGACEAE

Castanopsis cuspidata var. *carlesii*

Castanopsis fargesii

Castanopsis subacuminata

Cyclobalanopsis gilva

Cyclobalanopsis glauca var. *glauca*

Cyclobalanopsis longinux var. *longinux*

Cyclobalanopsis morii

Cyclobalanopsis pachyloma

Cyclobalanopsis sessilifolia

Cyclobalanopsis stenophylloides

Pasania glabra

Pasania hancei var. *ternaticupula*

Pasania harlandii

Pasania konishii

Quercus spinosa

Quercus tatakaensis

Quercus variabilis

FLACOURTIACEAE

Idesia polycarpa

Scolopia oldhamii

GUTTIFERAE

Calophyllum inophyllum

HAMAMELIDACEAE

Liquidambar formosana

JUGLANDACEAE

Engelhardia roxburghiana

Juglans cathayensis

Platycarya strobilacea

LAURACEAE

Beilschmiedia erythrophloia

Beilschmiedia tsangii

Cinnamomum burmanni

Cinnamomum camphora

Cinnamomum insulari-montanum

Cinnamomum osmophloeum

Cinnamomum subavenium

Cryptocarya chinensis

Lindera erythrocarpa

Lindera megaphylla

Litsea acuminata

Litsea cubeba

Litsea hypophaea

Machilus japonica var. *japonica*

Machilus japonica var. *kusanoi*

Machilus thunbergii

Machilus zuihoensis var. *mushaensis*

Machilus zuihoensis var. *zuihoensis*

Neolitsea acuminatissima

Neolitsea konishii

Neolitsea parvigemma

Neolitsea sericea var. *sericea*

Phoebe formosana

Sassafras randaiense

LEGUMINOSAE

Acacia confusa

Adenanthera microsperma

Canavalia rosea

Gleditsia rolfei

<i>Ormosia formosana</i>	<i>Pongamia pinnata</i>
MAGNOLIACEAE	
<i>Michelia compressa</i>	
MALVACEAE	
<i>Hibiscus tiliaceus</i>	
MELIACEAE	
<i>Aglaia formosana</i>	<i>Melia azedarach</i>
<i>Swietenia macrophylla</i>	
MORACEAE	
<i>Broussonetia papyrifera</i>	
MYRICACEAE	
<i>Myrica rubra</i>	
MYRTACEAE	
<i>Syzygium formosanum</i>	
NYSSACEAE	
<i>Camptotheca acuminata</i>	
OLEACEAE	
<i>Chionanthus retusus</i>	<i>Fraxinus griffithii</i>
<i>Fraxinus insularis</i>	<i>Ligustrum sinense</i>
PALMAE (AREACACEAE)	
<i>Phoenix hanceana</i>	
PITTOSPORACEAE	
<i>Pittosporum illicioides</i> var. <i>illicioides</i>	<i>Pittosporum pentandrum</i>
PROTEACEAE	
<i>Helicia cochinchinensis</i>	<i>Helicia rengetiensis</i>
RHAMNACEAE	
<i>Rhamnus nakaharae</i>	
ROSACEAE	
<i>Eriobotrya deflexa</i>	<i>Photinia niitakayamensis</i>
<i>Photinia serratifolia</i> var. <i>serratifolia</i>	<i>Prunus buergeriana</i>
<i>Prunus campanulata</i>	<i>Prunus matuurai</i>
<i>Prunus taiwaniana</i>	<i>Prunus transarisanensis</i>
<i>Pyracantha koidzumii</i>	<i>Rhaphiolepis indica</i>
<i>Rhaphiolepis indica</i> var. <i>hiiranensis</i>	<i>Sorbus randaiensis</i>
RUBIACEAE	
<i>Morinda citrifolia</i>	<i>Tricalysia dubia</i>
RUTACEAE	
<i>Phellodendron amurense</i> var. <i>wilsonii</i>	<i>Skimmia reevesiana</i>
<i>Tetradium glabrifolium</i>	<i>Zanthoxylum ailanthoides</i>
SAPINDACEAE	
<i>Dodonaea viscosa</i>	<i>Koelreuteria henryi</i>
<i>Sapindus mukorossi</i>	
SCROPHULARIACEAE	
<i>Paulownia kawakamii</i>	<i>Paulownia</i> × <i>taiwaniana</i>
SIMAROUBACEAE	
<i>Ailanthus altissima</i> var. <i>tanakai</i>	



(con't)

STAPHYLEACEAE

Euscaphis japonica

Turpinia formosana

STERCULIACEAE

Firmiana simplex

Reevesia formosana

STYRACACEAE

Alniphyllum pterospermum

Styrax formosana var. *formosana*

Styrax suberifolia

SYMPLOCACEAE

Symplocos stellaris

THEACEAE

Adinandra formosana var. *formosana*

Camellia sinensis

Cleyera japonica var. *morii*

Eurya chinensis

Eurya glaberrima

Gordonia axillaris

Schima superba var. *kankaensis*

Schima superba var. *superba*

Ternstroemia gymnanthera

TROCHODENDRACEAE

Trochodendron aralioides

ULMACEAE

Aphananthe aspera

Celtis formosana

Celtis sinensis

Trema orientalis

Ulmus parvifolia

Ulmus uyematsui

Zelkova serrata

VERBENACEAE

Clerodendrum trichotomum

Premna microphylla

Premna serratifolia

Seed storage behaviors of broadleaf trees in Taiwan include orthodox, recalcitrant, and intermediate types. Most seeds of the Lauraceae and Fagaceae, 2 major tree families in Taiwan, are recalcitrant, and their seeds can be cold-stratified with moist sphagnum moss at 5°C for > 2 mo to 2 yr if the seeds are mature and handled quickly after harvest. For example, seeds of *Machilus* species (Lauraceae) have a shorter lifespan when they are dried to a lower moisture content, but with cold stratification at 4°C, the original germinability is retained for about ≥ 2 mo depending on the species (Lin and Chien 1995, Chien and Lin 1997). However, seeds of 2 *Helicia* (Lauraceae) species mixed with moist sphagnum moss were capable of cold-stratification at 4°C for ≥ 24 mo (Chien et al. 2004). Previous reports also found that seeds of *Cinnamomum camphora*, *Cin. osmophloeum*, *Cin. subavenium*, *Lindera megaphylla*, *Neolitsea aciculate* var. *variabilissima* and *Neo. parvigemma* exhibit intermediate storage behavior, and some of them exhibit seed dormancy. Although those seeds showed some degree of desiccation tolerance, germinability gradually decreased when stored at 4 or 15°C. Cold stratification at 4~5°C is the best practical way to preserve these seeds (Lin 1996, Chien and Lin 1999, Chen et al. 2007a).

Seeds of *Cyclobalanopsis gilva*, *Cyc. glauca*, *Cyc. Morii*, and *Quercus spinosa* (Fagaceae) cannot tolerate desiccation, and the germination decreased when the seeds were stored with reduced moisture contents. However, cold-stratified seeds at 4°C retained their germinability for at least 1 yr (Lin 1995). In addition, some species of the

Lauraceae have intermediate seed storage behavior, including *Champereia manillana* (Opiliaceae), *Daphniphyllum glaucescens* (Daphniphyllaceae), *Schefflera octophylla* (Araliaceae), *Trema cannabina*, and *Zelkova serrata* (Ulmaceae) (Chen et al. 2007b, 2008, Yang et al 2007, Chien et al. 2010). We recommend that if seeds of the Fagaceae, Lauraceae, and other families with recalcitrant and intermediate storage behavior cannot be sown immediately after collection, the best storage condition is moist stratification on sphagnum moss at 4~5°C. The advantages of sphagnum medium for seed germination and stratification were reported by Wang et al. (1998).

Tree conservation in the field for seed production, reforestation, and long-term experimentation

Ecologically, Taiwan has 58% forest cover ranging from high-elevation temperate, mid- to low-elevation subtropical, to the southern-most tropical zones. These are the most precious, important, renewable resources of this island. Certainly, we have seen and experienced some evidence of the value of forests from recent disasters, such as floods, landslides, water shortages, and even weather changes. Such trends are going to become worse with time. In the past decades, more than 60 experimental stands were established by the TFRI. These stands include seed orchards and progeny tests, ecological research of natural and managed plantations, forest dynamic plot experiments, international provenance tests, natural superior tree selection and preservation, forest fire prevention research, tree thinning tests, etc. Information compiled from these long-term experimental stands was published by TFRI in 2009 (TFRI extension series no. 199). Seeds from clonal seed orchards of *Calocedrus macrolepis* var. *formosana*, *Cunninghamia lanceolata* var. *konishii*, *Taiwania cryptomerioides*, *Pin. morrisonicola*, and *Cin. osmophloeum* are available for reforestation and storage. Forest long-term dynamic plot experiments at the Fushan (northern Taiwan), Lienhuachih (central Taiwan), Liouguei (southern Taiwan), and Kenting (southern tip of Taiwan) Experimental Stations were established (Fig. 1), and second reinvestigations were recently completed. As far as we know, long-term data collection from these experimental plots is important to understand the complexities, dynamics, and spatial and biological diversity of ecological systems. By incorporating Taiwan's forest dynamic plots (FDPs) with FDPs of the Center of Tropical Forest Science, Smithsonian Institution (Washington, DC, USA), a wide network has been built and information is being shared. We believe that these plots facilitate long-term research, multidisciplinary collaboration, integrated approaches, and information sharing.

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Timing of Seed Germination and Life History of Trees: Case Studies from Greece

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Petros Panayiotopoulos¹⁾

[Summary]

Most Mediterranean plants have adopted autumn germination which enables seedlings to take full advantage of the entire growing (wet) season before the summer drought. On the other hand, many montane and alpine species are adapted to avoid the freezing winter by timing their germination in spring, usually making use of a stratification mechanism. However, there also exist additional, alternative adaptations that result in a post-winter timing of germination. Such mechanisms include (a) a tuning shift of the reproductive cycle which ends up producing and dispersing promptly germinating seeds at the end of the winter, as exemplified by *Pinus nigra*, (b) a long exposure to freezing temperatures (usually under snow cover) leading to germination before snow melt, as exemplified by *Aesculus hippocastanum*, (c) a ‘thermoswitch’ mechanism that enables germination only after a particular lengthy period of cool temperatures, as exemplified by *Zelkova abelicea*, and (d) an extensive delay of germination by low temperatures and photoinhibition, as exemplified by *Phoenix theophrasti*.

INTRODUCTION

While the rest period of a seed is by far the most resistant stage in a plant's life cycle, the ensuing seedling phase is extremely vulnerable. Over millennia, natural selection has admirably strived to minimize the risks of the seed-to-seedling transition by fine tuning the reproductive process *sensu lato* (including seed maturation, dispersal, seed banks, and seedling establishment) and, in particular, by bequeathing a full array of germination strategies and adaptations to seeds (e.g., Fenner and Thompson 2005).

The Mediterranean climate is characterized by marked seasonality, particularly in regard to precipitation; the wet season usually begins in October and lasts until April~May followed thereafter by a prolonged period of drought. Most Mediterranean plants (and especially annual and perennial herbs) have adapted to the climatic seasonality by timing seed germination and offspring recruitment to the beginning of the rainy season (e.g., Doussi and Thanos 2002, Daskalakou and Thanos 2004). On the other hand, a considerable number of plant species (and particularly many trees) show spring

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germination. Most of the latter manage to evade the adverse temperature conditions of winter, which may prove lethal to seedlings, by incorporating a stratification requirement, often several months long, into their germination strategy (e.g., Garcia-Fayos 2001, Piotto and Di Noi 2003).

In the present work, 4 tree species naturally occurring in Greece are showcased: they are all adapted to germinate in the field at the end of winter or early spring, but their germination does not include low-temperature preconditioning.

MATERIALS AND METHODS

Plant species

Pinus nigra (black pine) is a typical, montane tree of southern Europe and the Mediterranean rim (from Spain and Morocco to Cyprus and Turkey). It is widespread throughout Greece at elevations of 700–2000 m. Black pine forests were declared a European Community priority habitat, and quite recently they are increasingly threatened by wildfires.

Aesculus hippocastanum (horse chestnut) is a Tertiary relict of the boreotropical flora. Despite its wide cultivation all over Europe, this deciduous tree is a Balkan endemic with a geographical distribution mainly restricted to northern Greece (and to a lesser extent in Albania and FYROM). Recently, it was recorded growing in almost 100 fragmented subpopulations (with a total of 1500 mature individuals) in riparian, montane habitats (at 500–1500 m); its conservation status has been assessed as ‘critically endangered’ (Tsiroukis et al. 2007).

Zelkova abelicea (Cretan zelkova) is an alpine Tertiary relict species and a unique endemic tree of the Cretan flora. It is a deciduous tree (or shrub) up to 15 m tall, growing in about 30 fragmented subpopulations on the 5 mountainous ranges of Crete (900–1700 m), and its conservation status has been assessed as ‘vulnerable’.

Phoenix theophrasti (Cretan palm) is the single member of the genus that is native to Europe, in several coastal sites in Crete (as well as in similar habitats on the southwestern coast of Turkey). It is a Tertiary relict taxon, closely related to the common date palm (*Phoenix dactylifera*) but markedly differing in seed shape from the latter; its conservation status is currently characterized as ‘vulnerable’.

Germination experiments

Seeds of *P. nigra* were extracted from cones collected from 10 different, isolated populations in Greece. Collection took place during February when cones were still closed, but the enclosed seeds had fully matured. Germination experiments were performed with 5 samples (of 20 seeds each) imbibed in Petri dishes and subjected to various constant temperatures under continuous darkness or diurnally alternating white light (12 h) and darkness (12 h). Seeds of *A. hippocastanum* were collected during the dispersal period (October) from half-opened fruits of 4 large native subpopulations in Thessaly. Germination tests were carried out as described above, and, in addition, seeds collected on Kissavos Mt. (Thessaly) were sown in the soil, in December, covered (or not) by fallen leaves, protected from predation by chicken wire, and subjected to field conditions (at an elevation of 700 m) until spring. *Zelkova abelicea* seeds were also collected in October (from one of the largest populations, on Omalos Plateau, at 1250 m)



and subsequently subjected to laboratory experimentation as described above. Finally, *P. theophrasti* fruits were collected ripe in October–December from the 2 major localities of the species' occurrence in Crete (Vai and Preveli). Germination tests took place both in the lab and under field conditions with seeds buried (in December) 5 cm below surface in the natural habitat of the palm (Preveli).

Meteorological data

During the field experiments on *A. hippocastanum* and *P. theophrasti*, temperatures at the level of the seeds were monitored every 15 or 10 min, respectively, with the use of temperature sensors/dataloggers (Tidbit, ONSET, city?, ST?, USA). For *Z. abelicea*, meteorological data (air temperature and precipitation, monitored every 10 min) were provided by the National Observatory of Athens, Greece from a nearby, fully equipped, field meteorological station.

RESULTS AND DISCUSSION

Germination tests of *P. nigra* seeds (temperature range tested: 5–25°C) showed a relatively constant pattern with only minor variations among different populations; thus data were pooled, and germination maxima were found at the 'Mediterranean' temperatures of 15 and 20°C (Fig. 1), while light application increased final germination levels by 10–20% compared to continuous darkness (data not shown). This pattern is strikingly similar to that of the 3 coastal Mediterranean pines native to Greece (*P. halepensis*, *P. brutia*, and *P. pinea*) which are well adapted for autumn germination (Skordilis and Thanos 1997). However, field germination of *P. nigra* seeds takes place early in spring with no need for a stratification requirement (as in many other montane orboreal pines), since cone and seed maturation is timed to occur at the end of winter,

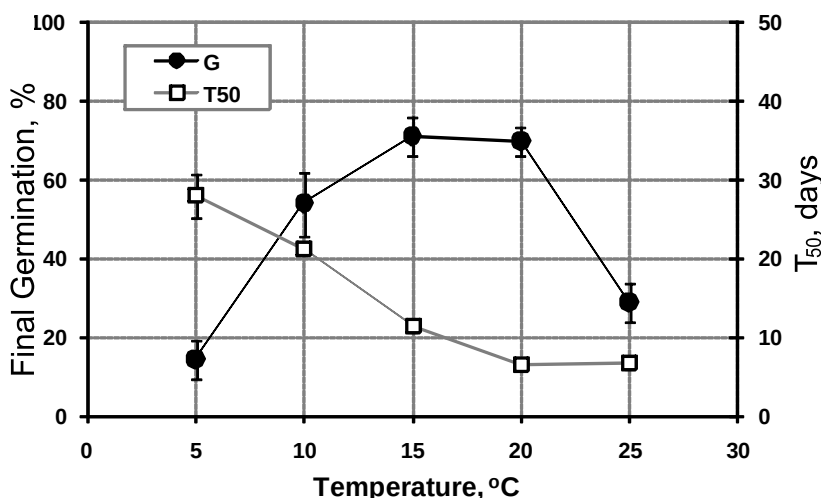


Fig. 1. Final germination of *Pinus nigra* seeds as a function of temperature, in continuous darkness. Data are mean values of 10 seed provenances from Greece. The rate of germination is illustrated by pooled T_{50} values. Vertical lines on each point represent $\pm$ SE.

exactly when mature cones burst open with the first warm spring spells to promptly disperse their seeds (Fig. 2). Moreover, the rate of germination in black pine is considerably faster than in the other 3 species, and, overall, prompt and fast spring germination makes ecological sense for a montane pine, growing in a climate with a relatively harsh winter but with only minor summer drought stress (Panayiotopoulos and Thanos 2002).

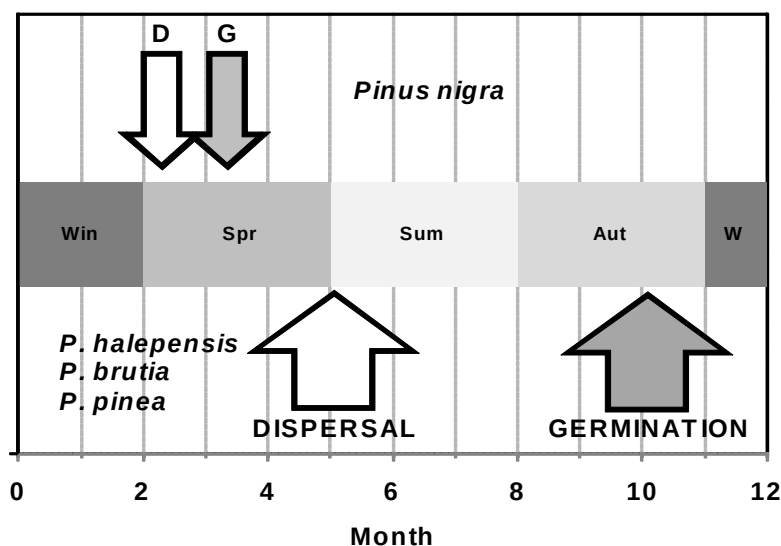


Fig. 2. Diagrammatic representation of the seasons of seed dispersal and germination for 4 Mediterranean pines, native to Greece: *Pinus halepensis*, *P. brutia*, *P. pinea* (coastal), and *P. nigra* (montane).

Aesculus hippocastanum seeds are well known for their large mass (the largest seeds in the European flora) and recalcitrant storage behavior. These traits in combination with the apparent lack of specialized animal-dispersers may also account for the current, restricted geographical distribution of the species within isolated, post-glacial refugia of riparian vegetation (Fig. 3) which experience milder winter temperatures and virtually no water stress. Horse chestnut seed germination is known to occur only at very high temperatures ($\geq 30^{\circ}\text{C}$) immediately after maturation and dispersal (Daws et al. 2004), possibly a relictual feature inherited from the boreotropical origin of the species. In addition, it is also known that a relatively long period (4~6 mo) of low temperatures will eventually remove this 'dormancy' (type 2, deep physiological dormancy according to Baskin and Baskin 2004) thus enabling germination over a wide temperature range. However, field observations and experimentation (Fig. 4) indicated field germination takes place during winter (even under the snow), i.e., under prolonged cold conditions. Therefore, this apparent (from lab experiments) stratification requirement could be viewed as an adaptation for very cold germination, which again makes sense in the ecological context: seedlings are recruited immediately after snow melt and their establishment and growth can make full use of the entire spring and favorable summer season.

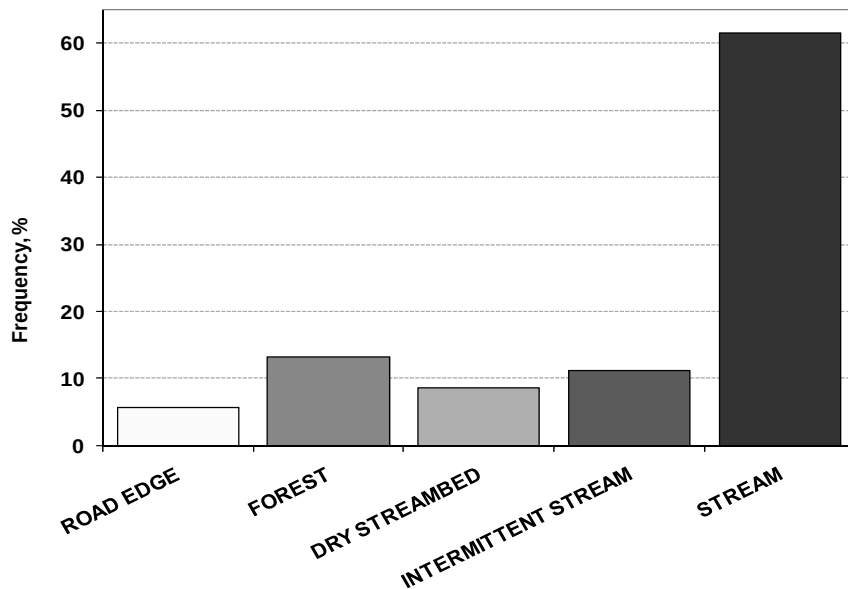


Fig. 3. Frequency histogram of habitat types for 1464 mature individuals of *Aesculus hippocastanum* native to Greece.

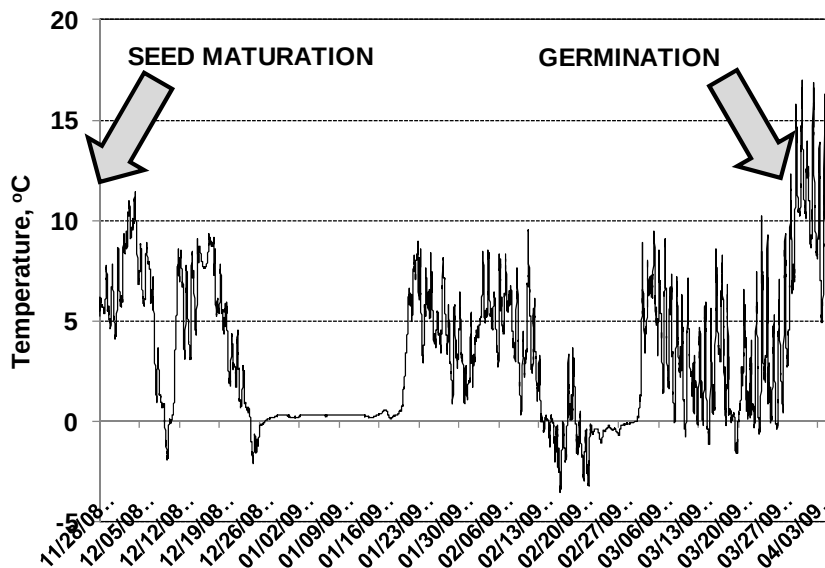


Fig. 4. Soil surface temperatures recorded (every 15 min) at Mt. Kissavos (Thessaly) from December 2008 to April 2009, in the natural habitat of *Aesculus hippocastanum* (at an elevation of 700 m). Arrows depict the seasons of horse chestnut seed dispersal (October~November) and germination (March~early April).

Germination of *Z. abelicea* seeds took place only at low temperatures of 5 and 10°C (Fig. 5). The germination rate was remarkably slow: germination was manifested 40 d after the onset of imbibition and was completed after a total period of 5 mo at 10°C light/dark, the optimal conditions for germination (Fournaraki and Thanos 2002). In addition, stratification pretreatment was completely ineffective in promoting germination at higher temperatures (data not shown). Thus, it is evident that the germination of the species has adopted a 'thermal switch' mechanism that 'turns on' germination at temperatures of $\leq 10^{\circ}\text{C}$ and 'turns it off' at $> 10^{\circ}\text{C}$. Combining lab germination results with meteorological data (Fig. 6), a remarkable adaptation to field conditions emerges. The duration of the cold period of the current climate at the natural habitat of the species is almost exactly as long as required for the completion of seed germination; thus seedlings are once again recruited at the very start of the spring growing season to take full advantage of the entire favorable season (sufficiently wet even during summertime) before the advent of the following winter.

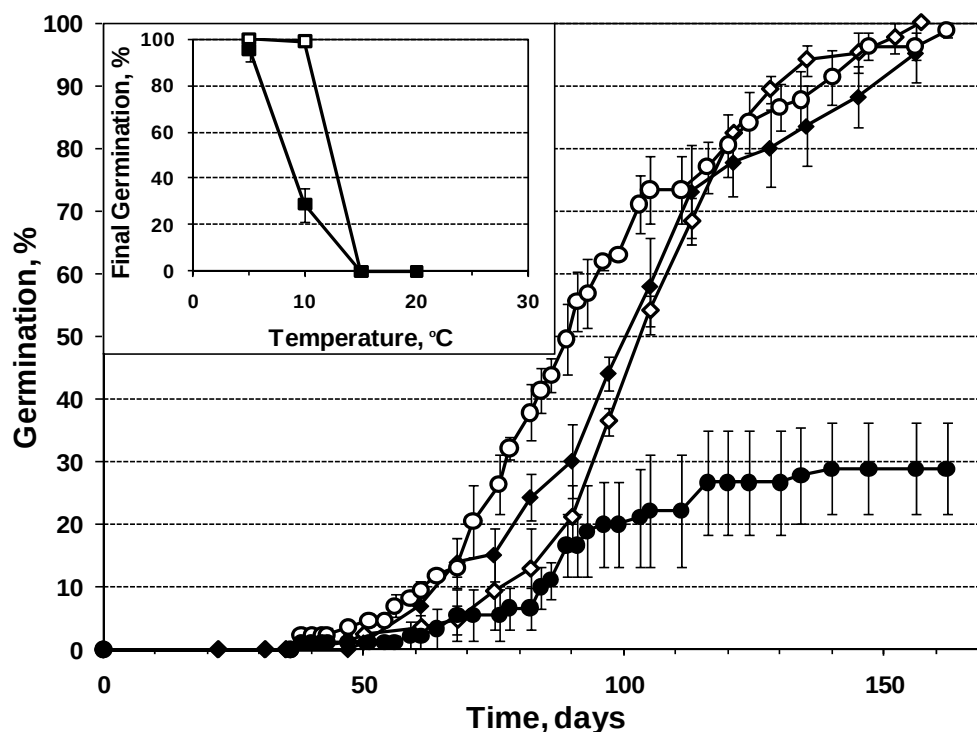


Fig. 5. Time course of *Zelkova abelicea* seed germination at 5 and 10°C (circles, 10°C; diamonds, 5°C; open symbols: 12 h of white light/12 h of darkness; solid symbols, continuous darkness). Inset diagram: final germination as a function of temperature (open and solid symbols as above). Vertical bars (over symbols) represent $\pm$ SE.



Phoenix theophrasti seed germination takes place promptly in the lab only at relatively high temperatures, 20~30°C (Fournaraki et al. 2007). However, high temperatures never coincide with water availability under the current natural conditions of the species. On the other hand, germination at low temperatures may indeed occur, albeit at a very slow rate (Fig. 7). In addition, a marked photoinhibition of germination was observed, a feature quite common for coastal Mediterranean plants, considered to be an adaptation mechanism against seedling growth and establishment at the ‘water-scanty’ surface of sandy coastal habitats (Thanos et al. 1991). Field observations and experiments showed that palm seeds buried in the soil at the end of autumn (dispersal season) do germinate at the beginning of spring (March~April) during a period when temperatures range 10~15°C (Fig. 8). This winter-avoiding strategy is not very clear on the basis of prevailing temperatures (only rarely cool enough to approach freezing), but considering the subtropical origin of the species, one could argue that Cretan palm seedlings might be cold intolerant (a postulation worth investigating).

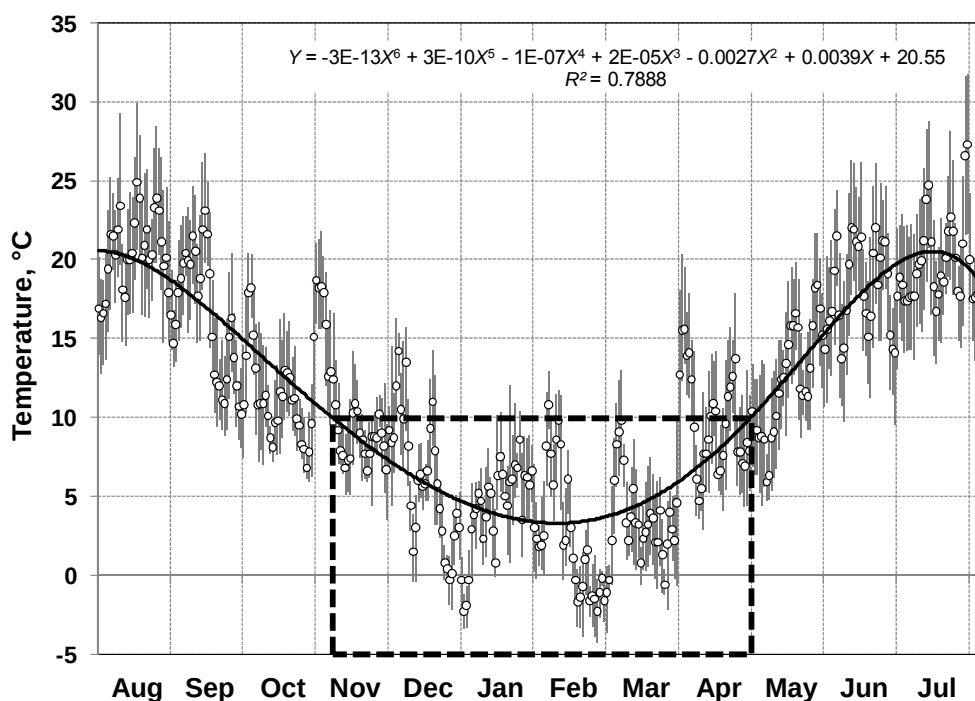


Fig. 6. Air temperature recorded on Omalos Plateau (Lefka Ori Mts.) from August 2008 to July 2009. Circles represent mean daily values, vertical lines are daily fluctuations, and the gray area corresponds to mean monthly temperature values. The solid line (a sixth degree polynomial, equation shown at the top) was the best fitting curve, regressed over the daily means. The stippled box corresponds to the period (early November to early May) with mean daily temperatures of $\leq 10^\circ\text{C}$ when seed germination of *Zelkova abelicea* takes place.

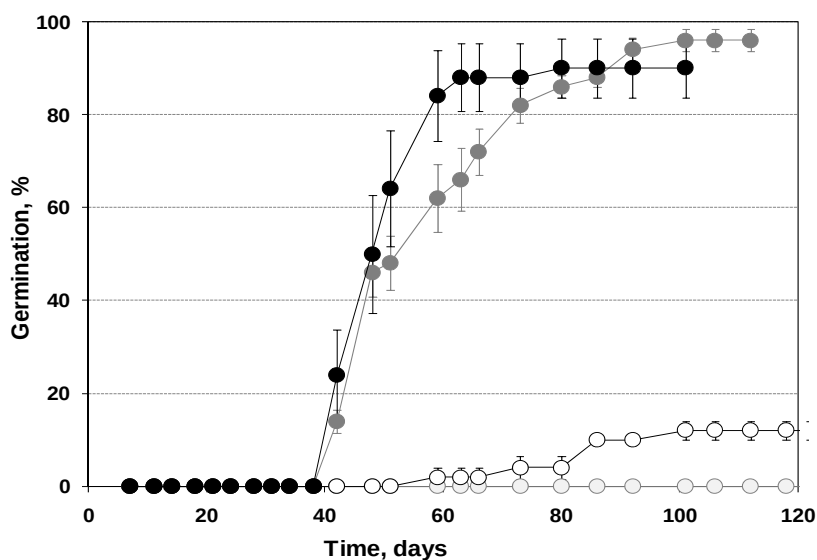


Fig. 7. Time course of *Phoenix theophrasti* seed germination. Two Cretan provenances, Vai and Preveli (black and gray circles, respectively) were investigated at diurnally alternating temperatures 20/11°C (12 h at each temperature), under either continuous darkness (solid symbols) or white light during the warm period (open symbols). Vertical lines on each point represent $\pm$ SE.

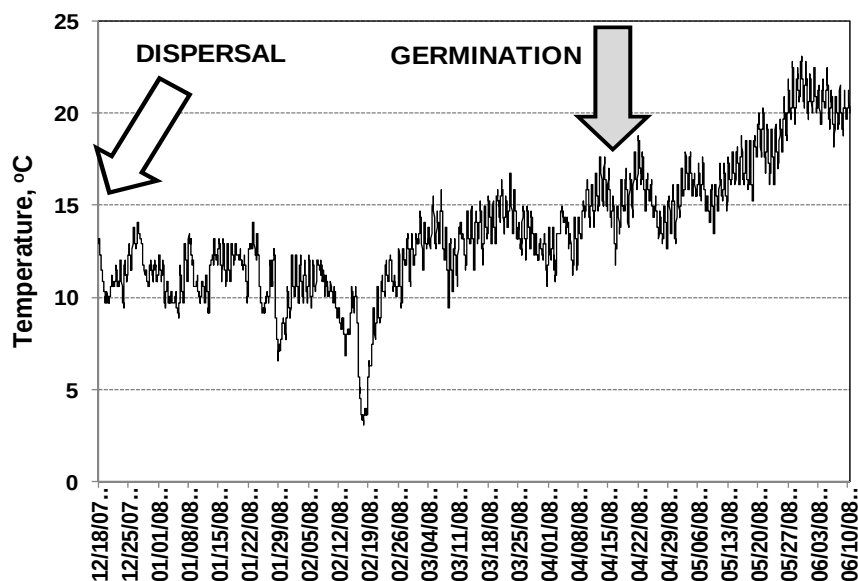


Fig. 8. Temperatures recorded (every 10 min) in the natural habitat of *Phoenix theophrasti*, at Preveli, Crete (5 cm below the soil surface). Arrows indicate the seasons of fruit dispersal (October–December) and seed germination (March–April).



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Moist Chilling and Dormancy of Eastern White Pine (*Pinus strobus* L.) Seeds

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[Summary]

We reviewed and analyzed our accumulated data on seed dormancy and germination of eastern white pine (*Pinus strobus* L.) from its natural distribution in Ontario, Quebec, and the Maritime Provinces. The evidence demonstrated that dormancy in eastern white pine varies among individual trees, and between stands (populations), seed years, latitudes, and geographic locations. Pretreatment with 28 d of moist chilling released dormancy and enhanced germination regardless of the seed source.

Key words: dormancy, germination, eastern white pine, *Pinus strobus*.

INTRODUCTION

Eastern white pine (*Pinus strobus* L.) is the most valuable coniferous tree species in North America, distributed in Canada from Newfoundland, west to southern Manitoba and in the US from Iowa and Illinois south to Georgia (Critchfield and Little 1966, Farrar 1995). The range spans 35°~50°N latitude and 55°~95°W longitude. Eastern white pine occurs on a variety of sites, from sandy soils and rocky ridges to sphagnum bogs, although its best growth is found on moist, sandy loam, usually mixed with other species (Farrar 1995).

Eastern white pine is a species used for reforestation in eastern Canada, but only comprises 3% of seedlings planted annually (Morgenstern and Wang 2001). The decline in its use for reforestation was primarily due to prevailing pest problems with the white pine weevil (*Pissodes strobi*) and white pine blister rust (*Cronartium ribicola*). However, there has been an increased interest in regenerating eastern white pine in Ontario, and attention has focused on seeds and seedling culture.

Eastern white pine seeds are reported to exhibit various degrees of embryo dormancy according to geographic location, and require different durations of moist chilling to alleviate dormancy and maximize germination (Mergen 1963, Fowler and Dwight 1964, Krugman and Jenkinson 2008). Fowler and Dwight (1964) suggested that variations in dormancy of eastern white pine seed are clinal with deeper dormancy in southern sources (latitudes 34.8°~40.8°N) to shallower dormancy in northern sources (latitudes 44.4°~46.4°N). However, other reports found that dormancy was deeper in the north (Krugman and Jenkinson 2008) or varied among individual trees within a stand, and among stands, crop years, and geographic locations (Wang unpubl. data).

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In the Upper Ottawa Valley, in Ontario, 12 seedlots of eastern white pine collected in 1966 from stands within a 50-km radius showed a range in dormancy of 2~75% (with a range in germination of 20~88%, without moist chilling) (Wang unpubl. data). For the purpose of reconciling testing rules between the Association of the Official Seed Analysts (AOSA) and the International Seed Testing Association (ISTA), the AOSA Tree and Shrub Seed Sub-Committee agreed to conduct an evaluation of testing prescriptions in 1984 (Wang and D'Eon 2003). That study involved seedlots from a northern location of the Chalk River, Ontario, Canada through Minnesota, southern Ohio to a southern source in North Carolina, USA. The results indicated that seed dormancy ranged from 48% in southern Ohio to 20% in Minnesota. All seedlots had germination of 81~86% after 28 d of moist chilling.

It seems apparent from the above evidence that there is variation in dormancy in eastern white pine seeds. In this paper, we present results from an array of sources and discuss some factors that may contribute to seed dormancy.

MATERIALS AND METHODS

The seedlots used for this review were from Ontario, Quebec, New Brunswick (NB), Nova Scotia (NS), and Prince Edward Island (PEI) and were collected in 1977~2006. Geographically, the seedlots ranged at latitude 44°39'~48°47'N, longitude 62°57'~79°25'W, and elevations of 8~400 m. Germination tests were carried out in 2009 on 63 seedlots, collected in 1990~2006, according to established standard procedures used at the National Tree Seed Centre (Simpson et al. 2004). Four, 50-seed replicates were sown on moist VersapakTM in Petawawa germination boxes. Moist chilling was carried out by placing the boxes in a cooler at 2~4°C for 28 d in the dark. At the time the boxes were removed from the cooler, a duplicate set of boxes was prepared representing non-chilled treatment, and all boxes were placed in germinators for 28 d. Germination conditions consisted of 8 h of light at 30°C and 16 h of darkness at 20°C with a constant relative humidity of 85%. Germinants were assessed every third day beginning on day 10. A seed was considered to have germinated when the cotyledons were visible, and the hypocotyl and radicle were well developed.

The degree of dormancy was calculated as the difference in germination between moist chilled seeds and non-chilled seeds.

RESULTS AND DISCUSSION

Seedlots collected from latitudes 45°N in NB in 1997, 46°N on PEI in 2004, and 44°N in NS in 2006 exhibited variations in seed dormancy and germination rates among trees at each collection site in a province and among the 3 provinces (Fig. 1). Moist chilling of the seeds promoted the greatest germination for seedlots collected at PEI and NS. The mean dormancy among seedlots from NB was 20% (0~47%) compared to 57% (15~76%) for those collected on PEI and 53% (22~72%) for seedlots from NS.

Figure 2 illustrates variations in seed dormancy among 25 eastern white pine seedlots collected in 1977 from latitudes 46° and 47°N in Ontario and tested in 1978 at the Petawawa Research Forest. Moist chilling promoted germination. The mean dormancy of the 11 seedlots at latitude 46°N was less than that for the 14 seedlots at latitude 47°N (25 vs. 37%); however the range in dormancy was greater at latitude 46°N than at latitude 47°N (4~78 vs. 11~68%).

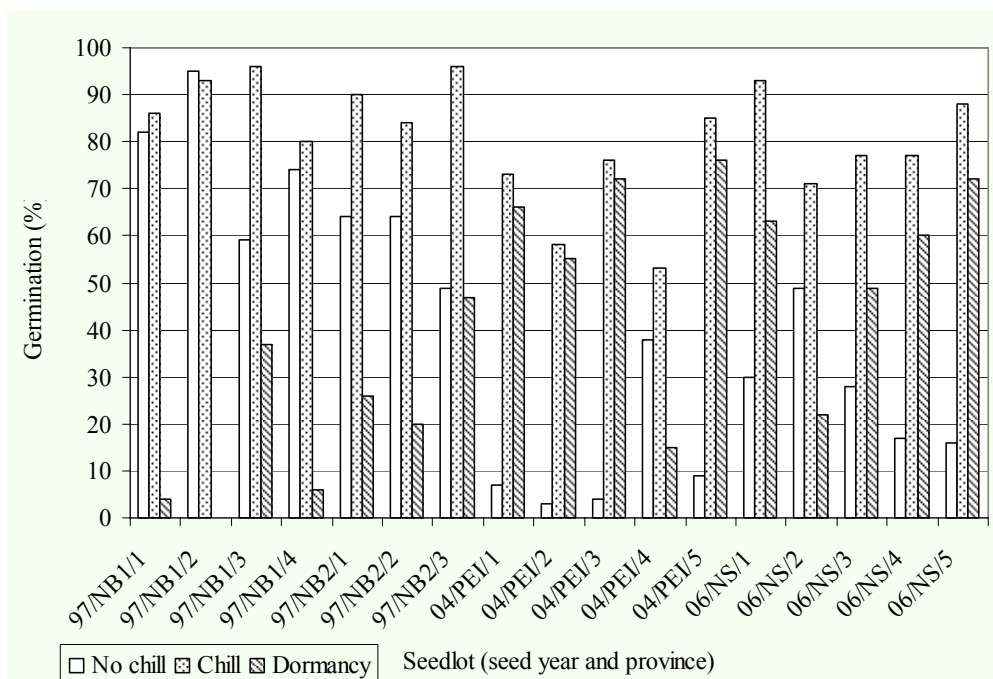


Fig. 1. Variations in germination (%) and dormancy (%) of single-tree eastern white pine seedlots collected in different years in the Maritime Provinces.

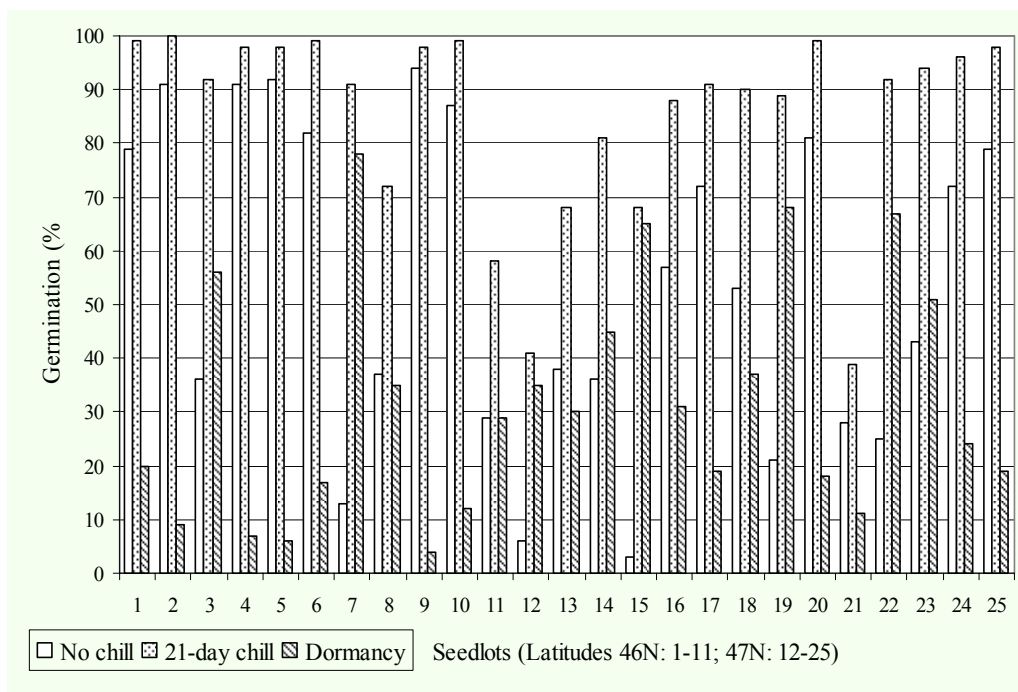


Fig. 2. Variations in germination (%) and dormancy (%) of single-tree eastern white pine seedlots collected in Ontario.

Figure 3 presents variations in seed dormancy and germination of eastern white pine populations collected from Quebec. The 23 seedlots collected in 1996~1998 revealed variations in seed dormancy and germination rates within and among seed collection years. The degree of dormancy of seedlots collected in 1996 averaged 17% (0~34%), compared to 18% (6~77%) for 1997 seedlots, and 43% (8~60%) for 1998 seedlots.

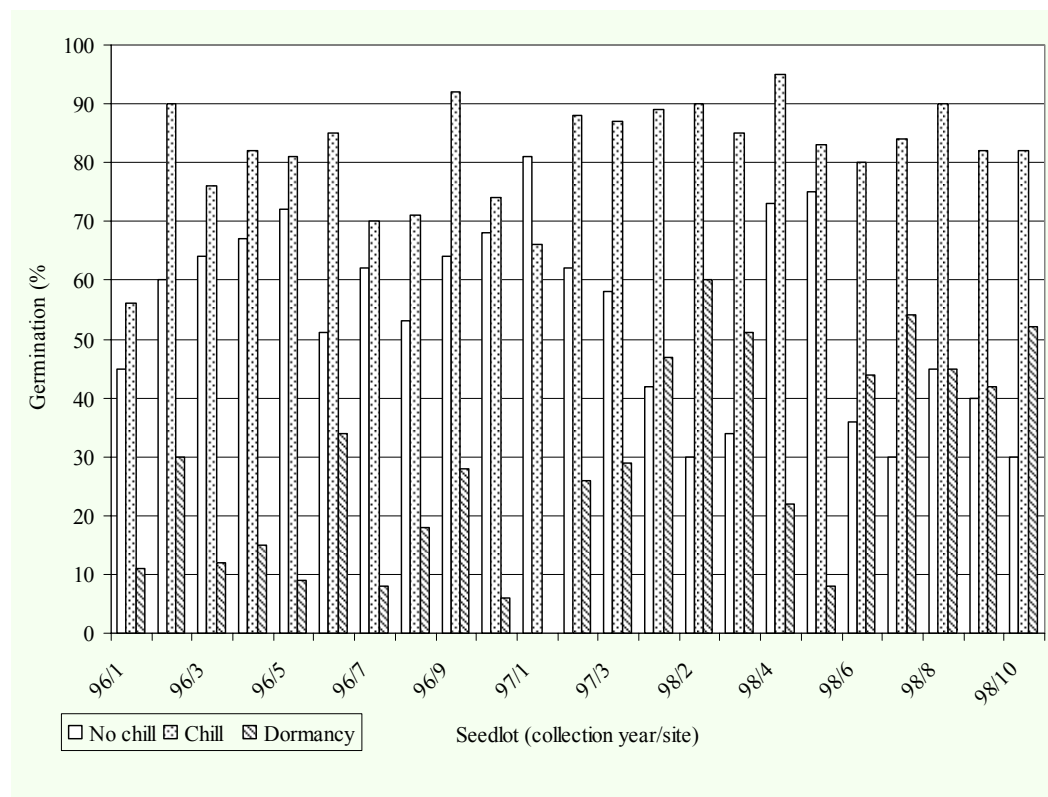


Fig. 3. Variations in germination (%) and dormancy (%) of eastern white pine seedlots collected from different populations in different years in Quebec.

Table 1 exhibits variations in seed dormancy of eastern white pine among collection years at different locations in Quebec. Seeds collected at 3 locations in 1990 were dormant at locations 1 and 3 and less dormant at location 2. Seeds collected in 1996 exhibited about the same degree of dormancy among the 3 locations. A similar trend was evident for collections made in 1998. For locations 3, 4, and 5, where seeds were collected during 2 different years, dormancy varied between collection years with seeds from the 1998 collections being more dormant. Clearly, seed dormancy varied with seed crop year within the same location and among locations.



Table 1. Variations in germination (%) and dormancy (Dorm.; %) of eastern white pine seeds collected in different years at different locations in Quebec

Location	Collection year								
	1990			1996			1998		
	No chilling	Chilling	Dorm.	No chilling	Chilling	Dorm.	No chilling	Chilling	Dorm.
1	37	58	21						
2	43	33	0						
3	45	59	14	62	70	8			
4				55	66	11	40	82	42
5				68	74	6	45	90	45

Graber (1965) studied eastern white pine seeds from southwestern Maine and reported that germination was almost complete following 30 d of moist chilling, with a moist chilling period in excess of 60 d maximizing the rate of germination. In the case of Ontario seeds, 91% of 56-d moist-chilled seeds germinated in 9 d (Mittal et al. 1987), confirming the positive impact of moist chilling on germination speed. Krugman et al. (2008) recommended 30~60 d of moist chilling for fresh seeds and 60 d for stored seeds. The increased period of moist chilling for stored seeds suggests that deeper dormancy is induced during storage, although no explanation was provided for this phenomenon. ISTA (2010) prescribes paired tests of no moist chilling and 28-d moist chilling for eastern white pine seeds as a means of evaluating dormancy. Fowler (1959) reported a rapid germination technique for eastern white pine seeds by removing the seed coat (92%) compared to 3 d of soaking in aerated water (80%), 20 d of moist chilling (85%), and no treatment (79%). However, the seedlot used in that study appeared to be only slightly dormant. Removing the seed coat is too labor-intensive for practical applications.

After our analysis of the above data on seed dormancy and germination of eastern white pine seeds collected from its range in the provinces of Ontario, Quebec, NB, NS, and PEI, we concluded that seed dormancy varies with individual trees within a stand, and among stands, latitudes, years of collection, and geographic locations. There are other factors that could also have an impact on these findings such as the timing of collection, the time period between collection and cone processing, processing methods, seed storage conditions, and the duration of storage. Moist chilling for 28 d is an effective treatment to release dormancy for laboratory testing.

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Seed Source Variations in Cone and Seed Traits in Three Himalayan Pines

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[Summary]

The Himalayas, with its unique ecology, has an extensive influence on the environment and livelihood of millions of people, and pines form a significant part of the forest vegetation in these areas. In seed source variation studies on Himalayan pines, patterns of cone and seed parameters across the natural distributional ranges of 3 important pines of India were studied.

Pinus roxburghii: The various traits of cones and seeds of 63 seed sources from around the country were investigated. Most cone characters showed north-south trends, i.e., they were correlated with latitude and revealed a significant impact of the length of the growing season on the traits. The cone size and cone weight were negatively correlated with longitude showing that cone size decreased towards the northeastern parts of India. Seeds from sources in Nafra (Arunchal Pradesh; AP) and Dhirang (AP) exhibited smaller sizes, owing to the poor nutritional quality of the highly leached soils in this zone. Considerable variation also existed in seed characteristics among different seed sources. The maximum seed length and width were recorded for the Una (Himachal Pradesh) and Shankri (Uttarakhand) seed sources, respectively, and these were correlated with neither latitude, longitude, nor elevation. Differences may be attributed to their adaptations to diverse environmental conditions. Seed viability and vigor in terms of the germination percentage, mean germination time, and germination value of different seed sources also exhibited significant variations with a north-south trend. The number of cotyledons showed no clinal pattern, but was positively correlated with other traits like the 1000-seed weight, seed size, germination percentage, and germination value.

Pinus wallichiana: Significant variations were observed in cones, seeds, and seed germination parameters of 17 seed sources. The specific gravity of cones, seed weight, and moisture content of seeds of various seed sources were also analyzed.

Pinus gerardiana: The seed and germination parameters of 5 seed sources were analyzed, and an appreciable amount of variation was observed.

Key words: pines, seed sources, cone, seed, variations, germination.

INTRODUCTION

Stretching in an arc shape, the Himalayas have great influences on environmental and climatic conditions of northern India and the people living in the Indo-Gangetic Plains. The ecology of the Himalayas is unique, and pines form a significant part of the forest vegetation in the region and have an extensive influence on the lives of these peoples. Five pine species were reported in India, namely, *Pinus roxburghii*, *P.*

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wallichiana, *P. gerardiana*, *P. kesiya*, and *P. merkusii*, each with different distribution patterns. The first 3 are found in the northwestern Himalayas, and the other 2 are indigenous to the northeastern Himalayas. Their economic importances are many-fold in the form of wood production, resin, fuelwood, needles, torchwood, etc. in addition to their secondary functions of maintaining ecosystems and environmental services. These pines have a significant place in industrial and commercial plantations of India along with *Tectona grandis*, *Gmelina arborea*, *Dalbergia sissoo*, *Acacia nilotica*, *Populus* spp., *Eucalyptus* spp., etc. The collection of good-quality seeds requires great care to have successful plantations; hence detailed seed studies were undertaken to generate valuable data.

The wide range of climatic conditions in the natural distribution of pines is expected to result in high genetic variation within different populations of a given species (Thapliyal et al. 2008). Pines are amenable to improvement by selection procedures for a wide range of traits, but the correct choice of base populations is critical. The selection of good seed sources is also an important aspect for nursery seedling production (Mc Millan 1979, Dirr and Heuser 1987). These programs have produced substantial gains in growth rates, tree form, resistance to certain diseases, and sometimes climatic adaptations of various pines in the world. In India, genetic improvement work on these pines has also been carried out, but the use of seeds cannot be fully justified to produce adequate nursery stocks of high vigor and uniform plantable sizes due to faulty and improper collection, handling, and storage, or results of low emergence due to dormancy and a lack of information on suitable methods of pretreatment.

The present research paper highlights some of the important findings on seed source variations and seed biology of *P. roxburghii*, *P. wallichiana*, and *P. gerardiana*.

Pinus roxburghii

Pinus roxburghii, commonly known as chir pine, is a large evergreen tree, one of the main coniferous species in the Himalayan region up to 2500 m with latitudinal limits of 26°N~36°N, and is of great ecological, silvicultural, and economic importance. The species mostly inhabits the subtropical and warm temperature monsoon belt, but occasionally can be found in areas where winter snow is a regular feature and also tropical climates where summer temperatures sometimes reach as high as 40°C. It is a very successful pioneer species on exposed sites, is planted on bare lands where it withstands drought, frost, and snow, and is free from serious pests and diseases. It exhibits considerable variations in growth rate, seedling, stem form, resin yields, and fiber/grain angle throughout its large natural stands associated with elevation and site (Troup 1921, Siddqui and Khan 1978, Seikh 1979).

Cone and seed characters were shown to vary among species, provenances, and genotypes in pines (Sorenson and Miles 1978, Singh et al. 1996, Roy et al. 2004). Cone size and seed size are used for intraspecific taxonomic distinctions between populations of *P. gregii* (Donahue and Upton 1996), while parameters like seed weight and length are useful for classifying provenances into high- and lowland groups in *P. caribea* (Salazar 1986). The germination responses of seeds vary according to latitude, elevation, soil moisture, soil nutrients, temperature, kind and density of cover, and the degree of habitat disturbance of a site where seeds mature. Likewise, a tree's potential for seed quality depends on associated environmental, genetic, and physiological factors. Variations in



the storage potential of seeds are also not limited to species but are also affected by provenance and seed source (Justice and Bass 1978).

Being very important species in the Himalayas, this study aimed to understand the nature, extent and pattern of variation existing in different populations of 3 species of pine from their distribution zone in the country, with respect to cone and seed characteristics.

MATERIAL AND METHODS

Mature cones were collected from 63 different sources within the natural distribution ranges of the species from the Himalayan states of Uttarakhand (UK), Himachal Pradesh (HP), and Arunachal Pradesh (AP) (Table 1). Cones were collected from randomly selected average trees located at least 100 m apart from each other at each location. Laboratory work was carried out at the Forest Tree Seed Laboratory, Forest Research Institute, Dehradun, India.

Table 1. Geographic information on seed sources

Source no.	Seed source	State	Latitude	Longitude	Elevation (m)
1	Mohand	Uttarakhand (UK)	30.18	77.17	675
2	August Muni	UK	30.23	79.30	762
3	Trisula	UK	30.12	79.15	1800
4	Nandprayag	UK	30.70	79.50	1200
5	Birahi	UK	30.90	79.90	1219
6	Gaucher	UK	30.17	79.11	729
7	Bhowali	UK	29.23	79.33	1706
8	Almora	UK	29.23	79.40	1546
9	Ranikhet	UK	29.38	79.25	1800
10	Kamlekh	UK	29.18	79.47	1500
11	Gongolihat	UK	29.40	80.02	1100
12	Askot	UK	29.46	80.19	1387
13	Ghansali	UK	30.26	78.39	954
14	Dharasu	UK	30.37	78.17	650
15	Theture	UK	30.30	78.11	1500
16	Pharsula	UK	29.49	78.44	1300
17	Barkot	UK	30.48	78.14	1524
18	Jarmola	UK	30.57	78.60	1828
19	Shankri	UK	31.70	78.50	1800
20	Thadiyar	UK	30.58	77.55	1200
21	Darmigarh	UK	30.26	77.38	913
22	Chiwa	UK	30.57	77.50	1500
23	Thalisian	UK	30.10	79.20	1500
24	Pokhara	UK	29.56	78.50	1350
25	Diba	UK	29.50	78.58	1600
26	Palampur	Himachal Pradesh (HP)	31.40	76.90	1240
27	Dharamshala	HP	32.70	76.23	1250
28	Malan	HP	32.20	76.43	1200
29	Baijnath	HP	32.13	76.39	1125
30	Narang	HP	30.20	77.25	1300
31	Rajgarh	HP	30.51	77.19	1400
32	Hamirpur	HP	32.12	76.30	850

(con't)

33	Nadaun	HP	32.20	76.60	650
34	Kiarla	HP	30.55	77.38	1200
35	Nerwa	HP	30.50	77.18	1250
36	Jamta	HP	30.36	77.18	932
37	Banethi	HP	30.38	77.16	1370
38	Dharampur	HP	30.54	77.14	1500
39	Sabathu	HP	30.58	76.59	1400
40	Suket	HP	31.23	77.60	1830
41	Mandi	HP	31.43	76.55	760
42	Nachan Gohar	HP	31.45	77.05	1292
43	Manikaran	HP	32.20	77.21	1737
44	Bhuntar	HP	32.90	77.10	1100
45	Una	HP	31.28	76.16	675
46	Danoghat	HP	32.81	76.12	1100
47	Kunihar	HP	30.52	76.36	1300
48	Kothar	HP	30.50	76.40	1150
49	Taradevi	HP	31.06	77.90	2205
50	Brindaban	HP	31.12	76.33	575
51	Chamba Tikri	HP	32.46	76.13	923
52	Bakloh	HP	32.44	75.52	1350
53	Mastgarh	HP	32.23	76.10	700
54	Jumhar	HP	32.44	76.10	1100
55	Banikhet	HP	32.40	75.40	1400
56	Bahli Taklekh	HP	31.23	77.37	1300
57	Katra	Jammu and Kashmir	32.58	74.55	520
58	Rajouri	(J&K)	33.22	74.16	668
59	Akhnoor	J&K	32.55	74.42	1225
60	Noushera	J&K	33.09	74.17	1200
61	Ramnagar	J&K	32.48	75.18	520
62	Nafra	J&K	27.19	92.23	1200
63	Dhirang	Arunachal Pradesh (AP)	27.45	92.15	1350
		AP			

Measurements were recorded for cone size (length and width), fresh weight and specific gravity of cones, and numbers of scales and seeds per cone using randomly drawn mature cones from each location. The specific gravity of a cone was calculated by a water displacement method as described by Barnett (1979). Seed length and width were measured using digital calipers. Seed weight was determined on a pure seed fraction and was expressed as the 1000-seed weight. The percent moisture content of seeds was recorded on a wet basis at 103°C for 17 h (ISTA 1993).

Seeds of all seed sources were germinated in a temperature-controlled cabinet maintained at 30°C, on the top of moist blotting paper using 4 replicates of 100 seeds each. Seeds were considered germinated when about 1 cm of the radicle had emerged through the coat. Seed germination values were recorded and quantified as percent germination, whereas the germination value (GV) was calculated as per Czabator (1962) and the mean germination time (MGT) according to Bonner (1983).

To assess the storability of seeds, these were sampled from 11 different seed sources (5 from UK, 2 from Jumma and Kashmir (J&K), 2 from HP, and 2 from AP). The initial moisture contents were determined just after extraction as per the ISTA (1993), and these ranged 7~9%. Seeds of each source were divided into 2 lots: 1 lot



stored at the initial moisture content with a combination of 3 temperatures of ambient temperature, 15, and -5°C, while the second lot was dried in the sun to reduce the moisture contents to 4% and these were stored in sealed plastic jars at same temperature combinations as for lot one. The initial germination percent was determined before putting the seeds in storage.

Data were analyzed by an analysis of variance (ANOVA), and simple correlations were calculated to correlate the various traits of cones and seeds with geographic parameters.

RESULTS AND DISCUSSION

The results of the range, mean standard deviation (SD), coefficient of variation (CV), and correlation values with respect to variations in cone and seed characters are given in Tables 2 and 3.

Table 2. Seed source variations in cone and seed parameters

	Parameter	Range	Mean	SD	CV%	CD(0.05)
1	Cone length (cm)	6.75~15.67	13.06	1.47	11.25	2.55
2	Cone width (cm)	4.88~9.87	6.53	0.72	11.25	1.14
3	Cone fresh weight (g)	115.22~228.02	169.77	26.38	15.54	53.07
4	Cone specific gravity	0.54~0.83	0.74	0.06	8.11	0.08
5	No. of scales/cone	68.44~112.70	89.90	9.28	10.32	14.93
6	No. of seeds/cone	24.50~101.25	53.79	17.51	32.55	22.16
7	1000-seed weight (g)	77.22~140.43	113.55	15.84	13.95	11.79
8	Seed length (mm)	9.34~12.71	11.23	0.85	7.56	1.85
9	Seed width (mm)	5.13~7.43	3.25	0.45	6.95	1.02
10	Moisture content (%)	4.92~10.83	7.83	1.03	13.15	1.20
11	Germination (%)	42.50~99.50	81.43	14.55	17.62	10.84
12	Germination value (GV)	10.04~126.30	55.21	27.45	50.00	16.86
13	Mean germination time	4.72~12.89	8.11	2.05	25.27	1.05
14	(MGT)	9.75~13.00	11.57	0.79	6.82	1.28
	Cotyledon number					

Table modified from Roy et al. (2004).

SD, standard deviation; CV%, coefficient of variation; CD, coefficient of determination. Cone characters revealed significant differences among seed sources at the 5% level of significance (Table 2). The maximum CV was observed for the number of seeds per cone (32.55) followed by the cone fresh weight (15.54) (Table 2). The differences recorded may pertain to different intensities of natural selection pressures acting upon these traits in natural habitats. Most of the cone characters showed a north-south trend, i.e., they were correlated with latitude as cone size often increases with geographic changes that are associated with an increase in the length of the growing season, for example decreasing elevation (Hermann 1968) and decreasing latitude (Sziklai 1969). The cone fresh weight and width were negatively correlated ($r = -0.30$ and -0.26 , respectively) with longitude; the cone width was also positively correlated ($r = 0.35$) with latitude (Table 3). These parameters in the species decreased towards the

wetter, high-rainfall parts of northeastern India. The smaller size of cones of the Nafra (AP) and Dhirang (AP) seed sources may have been due to the poor nutritional quality of the highly leached soils in this part of India (Tripathi and Barik 2001). Sorenson and Miles (1978) also reported a moderate pattern of clinal variations in cone length, width, dry weight, number of scales, and seed weight in Douglas fir (please give scientific name). A negative correlation between the latitude of the seed source and number of seeds per cone ($r = -0.44$) reflects the effect of a shorter growing period at higher latitudes. Dermeritt and Hocker (1975) also reported the same results for cone and seed characters of *P. strobus*.

Table 3. Simple correlation between studied traits and the geography of seed sources

	Parameter	Latitude	Longitude	Elevation
1	Cone fresh weight	-0.16	-0.30*	-0.15
2	Cone length	-0.08	-0.20	-0.12
3	Cone width	0.35*	-0.26*	-0.12
4	No. of scales/cone	-0.15	-0.03	0.13
5	No. of seeds/cone	-0.44**	-0.02	0.25*
6	Cone specific gravity	-0.13	-0.17	-0.06
7	1000-seed weight	0.10	0.13	-0.12
8	Seed length	0.10	-0.19	0.04
9	Seed width	0.03	-0.07	-0.02
10	Moisture content	0.33*	-0.14	-0.15
11	Germination percentage	-0.36*	0.21	0.04
12	Germination value (GV)	-0.54**	0.24	0.18
13	Mean germination time (MGT)	0.42**	-0.13	-0.04
14	Cotyledon number	-0.18	0.04	0.22

* $p < 0.05$, ** $p < 0.01$.

Table was reproduced from Roy et al. (2004).

The remaining cone traits of cone length, number of scales per cone, and specific gravity were not related to any geographical factor.

Seed characters of seed length, width, weight, and moisture content significantly varied among seed sources (Table 2). These traits showed no particular trend with latitude, longitude, or elevation. In this species, the maximum seed weight was observed for the Birahi (140.43 g) seed source in UK, which is approximately double the seed weight of Kiarla (77.40 g) in HP. Considering seed weight when delineating and understanding geographical variations has been advocated (Harper et al. 1970). Maximum values of seed length (12.71 mm) and width (7.43 mm) were recorded for the Una (HP) and Shankri populations (UK). The minimum seed length (9.34 mm) was found in Kiarla (HP), while the least width was 5.13 mm for the Bahli (HP) seed source. These variations are due to edaphic factors, such as crown exposure and genotype of the trees as illustrated by Mathur et al. (1984).

Seed vigor traits of germination, GV, and MGT significantly differed among the seed sources. These values were negatively correlated with latitude (Table 3) and were also related to an increase in the growing season at lower latitudes. Due to a longer



growing period, there was also an increment in the nutrient assimilation rate and more food material. Similar results were also reported by Khalil (1986) for *Picea glauca* and Pitcher (1984) for *Prunus serotina*. The number of cotyledons showed no clinal pattern, but was positively correlated with other traits like the 1000-seed weight, seed size, germination percentage, and GV.

Pinus wallichiana

Pinus wallichiana, commonly known as blue pine, is also an important component of mid- and high-elevation Himalayan forests and has a broad natural distribution range of 26°~36°N latitude (Troup 1921). It is a 5-needle pine and has gained worldwide attention for its resistance to blister rust among white pines. Considerable variations in morphological and anatomical characteristics of needles, cones, and seeds in natural stands exist across the natural distribution of the species, especially in mesic and xeric habitats. These variations suggested the differentiation of this species in ecotypes or varieties as reported by various authors. However, the level of genetic diversity was found to be relatively high, and the degree of genetic differentiation was low compared to other pines. The wide range of climatic condition within the natural distribution of this pine is expected to result in high genetic variation within different populations of the species. So studies of seed source variations in cone and seed characteristics across its natural distribution were carried out to determine the nature and extent of variation present in the populations in the states of HP and UK.

The present study was undertaken to evaluate the variability in the forms of cone and seed characters from various seed sources. Seeds were collected from 17 seed sources from the 2 states and analyzed for cone, seed, and germination characters. The materials and methods followed for the study were similar to those followed for *P. roxburghii*.

The variability estimates of cone and seed parameters of different seed sources are given in Table 4. Variations were observed in the cone fresh weight, length, width, and specific gravity among seed sources. The cone weight varied from 44.4 to 114 g, and a higher cone weight was recorded at higher elevations (Table 4). Such variations in cone characters of *P. wallichiana* were also observed by Singh et al. (1996).

Table 4. Variability estimates for cone, seed, and seedling traits of *Pinus wallichiana*

	Parameter	Range	Mean	SD	CV%	CD (level 0.05)
1	Cone weight (g)	44.4~114	75.59	17.82	23.57	25.84
2	Cone length (cm)	21.1~39.29	30.98	4.41	14.24	6.29
3	Cone width (cm)	12.9~22.8	18.46	2.51	13.57	3.59
4	Seed length (mm)	6.94~9.22	7.92	0.56	7.06	0.65
5	Seed width (mm)	4.43~7.73	4.90	0.76	15.47	0.49
6	1000-Seed weight (g)	35.70~69.30	48.23	9.18	19.00	4.84
7	Moisture content (%)	7.0~23.7	12.66	4.63	36.61	3.16
8	Lab germination (%)	40~85	57.71	12.12	21.01	21.20
9	Root length (cm)	3.6~7.35	4.91	0.93	18.92	0.93
10	Shoot length (cm)	4.25~7.1	5.72	0.93	16.33	1.10
11	Cotyledon number	7~12	10	0.81	7.9	1.39

SD, standard deviation; CV%, coefficient of variation; CD, coefficient of determination. Variations in seed characters of seed weight, moisture contents, and germination percentage were observed among seed sources. The germination percentage varied 40~85%, whereas the cotyledon number varied 7~12. The seed weight ranged 35.70~69.30 g for all seed sources (Table 4). These variations can be further utilized to identify the best seed sources to improve the productivity of this species in the future.

Pinus gerardiana

Pinus gerardiana, commonly known as Chilgoza pine, is found in rocky and dry regions well outside the influence of the monsoons. This pine makes little demands on the fertility of the soil and is capable of growing on excessively dry, barren hillsides with shallow soil and even on bare rocks. For this peculiar characteristic, it is described it as the 'Champion of the Rocky Mountains' (Kapoor et al. 2003). The distribution of this pine is quite sparse in the world and is confined to parts of eastern Afghanistan, Pakistan, and inner valleys of the northern Himalayas in India at elevations of 2100~3300 m (Troup 1921, Mirov 1967). In India, it mainly occurs in the district of Kinnaur, and some patches in Pangi and Bharmour of Chamba district in the state of HP. Its seeds are well known in the dry fruit trade and are high-priced and valued as an edible nut.

Because of the increasing demand for nuts in the dry-fruit market of the country, this pine has become the backbone of the livelihood of tribal inhabitants in the region. At the same time, depletion of the crop area and density is linked to no or very meager natural regeneration due to the reckless lopping of branches for the extraction of each and every possible cone even in extremely inaccessible sites. Since the right to harvest chilgoza nuts was given to local inhabitants, there is no check on extraction methods by state forestry departments. Hence, there is much room for improvement and the domestication of this important pine through various methods.

The present study was undertaken to evaluate the variability in the form of seed characters from various seed sources for further use to enhance the productivity. Seeds were collected from 5 seed sources from the state of HP, and seed and germination characters were analyzed.

The results of this study on seed traits of the species of 5 seed sources are given in Table 5. Appreciable amounts of variation were observed in seed weight, moisture content, and germination percentage of seeds from the 5 seed sources. The 1000-seed weight varied 380~460 g, whereas the germination percentage varied 18.5~56.0% among the studied seed sources. These parameters can be of use while undertaking improvement programs for this species. Singh (1993) and Negi (2002) also advocated improving the productivity of *P. gerardiana* by undertaking sustainable steps towards regeneration of the species.

Table 5. Seed parameters of different seed sources of *Pinus gerardiana*

Seed source	Seed parameters					
	Length (mm)	Width (mm)	1000-Seed weight (g)	Moisture (%)	Germination (%)	Cotyledon number
1 Jangi (HP)	20.83	7.23	380.00	28.33	42.50	9.30
2 Kalpa (HP)	21.70	7.37	440.00	29.33	20.00	9.57
3 Rispa (HP)	22.31	7.65	460.00	24.00	35.00	9.50
4 Peo (HP)	20.92	6.92	400.00	19.67	18.50	9.00
5 Koti (HP)	23.08	7.56	440.00	28.67	56.00	10.00



CONCLUSIONS

There were significant differences in cone and seed traits among the seed sources of *P. roxburghii* and *P. wallichiana* and in seed traits of *P. gerardiana*. The extent of genetic control over germination in coniferous seeds was reported to be high due to the high proportion of maternal effects in their seed structure. Variations can be tapped to select the most promising sources for further improvements and breeding programs. However, such studies need to be done on a wider scale, i.e., on all seed sources across the natural distribution range of the species and also at the stage of nursery emergence and performance. These efforts should be supported by further research on the genetic diversity in these widespread and phenotypically variable species.

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Desiccation Tolerance and Storage Response of *Bassia latifolia*

Roxb. Seeds

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[Summary]

Seeds of *Bassia latifolia* were harvested at intervals from the early maturation stage until shedding. With the progression of seed development, an increase in the seed dry weight was observed. The maximum dry weight was seen after full maturation at 70 d after anthesis (DAA). Germinability began at 49 DAA, and seeds acquired full germinability at 63 DAA after slight desiccation from 58 to 42%. The desiccation tolerance of seeds increased with the progression of seed maturity. Fully mature seeds were desiccation tolerant to a 35% moisture content. Different storage media and temperatures were used to achieve maximum viability. Seeds stored at 28°C with a 41% moisture content best retained their viability for at least 150 d. Seeds were chilling sensitive even at 15°C, which confirms their highly recalcitrant nature.

Key words: *Bassia latifolia*, seed maturation, germinability, desiccation tolerance, seed storage.

INTRODUCTION

Bassia latifolia (Synm. *Madhuca indica*) commonly known as mahua belongs to the family Sapotaceae. It is a medium to large deciduous tree distributed throughout most parts of northern and central India and Myanmar (Luna 1996). Its fleshy, pale, or dull-white musk-scented flowers appear in February to April, and the greenish fleshy fruits ripen during June and July. Seeds are either double-convex or flattened on 1 or 2 sides, pale-brown and 3.3~3.8 cm long with a moderately hard testa.

The species has immense importance for its flower, fruit, and wood, and it is preferred by forest-dwelling tribes that are keen protectors of this tree. The wood is used for building purposes as beams, doors, window frames, bridges, and wheels, and fuel wood. The flowers are edible, and are largely used to prepare a locally made liquor. The oil extract from the seed is used in cooking, for preparation of vegetable butter, as a fuel oil, and for manufacturing soap and detergent. The oil cake is used as a fertilizer and fish poison. The bark, leaves, flowers, and fruits have various medicinal uses. The tree is altogether useful to the tribal communities who consider it a holy tree.

Good seed years are expected once or twice every 3 yr. The season for seed collection is very short, and a considerable portion of the mature crop is lost in the rains (Luna 1996). Fresh seeds have a high germination capacity, but they are easily spoiled during storage (Dent 1948). Seeds are considered to be recalcitrant and difficult to store for long periods (Vergeese et al. 2002). However, previous studies on the development

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of these seeds did not identify the best time to collect seeds. The conditions for maintaining viability were also not evaluated for short- or medium-term storage of the seeds of this species.

The objective of this investigation was to assess seed quality and the possibility of harvesting seeds before natural shedding. Different methods to store seeds of this recalcitrant species to achieve maximum viability were evaluated.

MATERIALS AND METHODS

An evaluation was carried out in a natural stand of 20-yr-old plants in a forest area 10 km from Jabalpur, India. The mean annual temperature in the study area is 32°C with an average minimum of 15°C and maximum of 42°C. Rainfall is concentrated in June to September.

The flowers from 10 trees, which began flowering at about the same time, were labeled during fruit set to follow fruit development. From 35 d after anthesis (DAA), fruits were collected by hand and brought to the laboratory at 6–8-d intervals until the natural dispersal of the seed occurred. The pericarp was manually removed, and immature and mature seeds were extracted. Data on the fresh weight, dry weight, water content, and germination percentage were recorded for each sample.

To determine the fresh and dry weights of the seeds, 5 replicates of 5 seeds each were weighed on an analytical balance to a precision of 0.001 g to obtain the fresh mass. The dry mass was determined after drying at 80°C until a constant weight was achieved. To determine the water content, seeds were dried in a forced-draft oven for 17 h at 103°C (ISTA 1993). Five replicates of 5 seeds each were used at each time point. The water content is expressed on a fresh-weight basis.

A germination test was performed by placing 3 replicates of 50 seeds each on moistened paper in Petri dishes at 30±2°C with a 16: 8-h light: dark photoperiod. Germination was counted daily and continued for 28 d. Seeds were considered to have germinated when the radicle had grown at least 1 cm. After that, numbers of rotten, empty, and good seeds were determined by a cutting test. From these data, the percentage germination was calculated.

The desiccation sensitivity of seeds at each maturity level was tested. Seeds were kept over regularly regenerated silica gel in a desiccator at 28±2°C and withdrawn at intervals to determine the water content and germination percentage. Control seeds were treated with 0.2% Bavistin (fungicide) and stored over water in sealed glass containers.

To evaluate the storage potential, seeds were stored under the following conditions.

1. Seeds stored at the shedding moisture content (MC): Seeds were kept in sealed polythene bags with a shedding MC of 41±2% at 10, 15, and 28°C.
2. Seeds stored after partial drying: Seeds were stored at 38.2±1.6 and 34.5±1.5% MCs and 15 and 28°C in sealed polythene bags.
3. Seeds stored in open-mouth polythene bags: Seeds were kept in polythene bags with the mouth open and stored at 15 and 28°C.
4. Seeds stored in an open tray: Seeds were stored in an open tray at 15 and 28°C.



Seeds for all storage treatments were sampled at 15-d intervals for up to 60 d, and then sampling was done at 90 and 150 d after storage began. Viability was monitored by a germination test following the method described above.

RESULTS

The fresh weight of seeds reached its maximum level at 55 DAA, when it was about 4.82 g (Fig. 1). After this period, little reduction in the fresh weight was observed until 80 DAA when the final harvest was completed. On the other hand, the dry weight of seeds continued to increase until the seed matured at 70 DAA, when it had increased to about 2.75 g from its initial weight of 0.27 g at 35 DAA. After that, no significant change in the dry weight was observed.

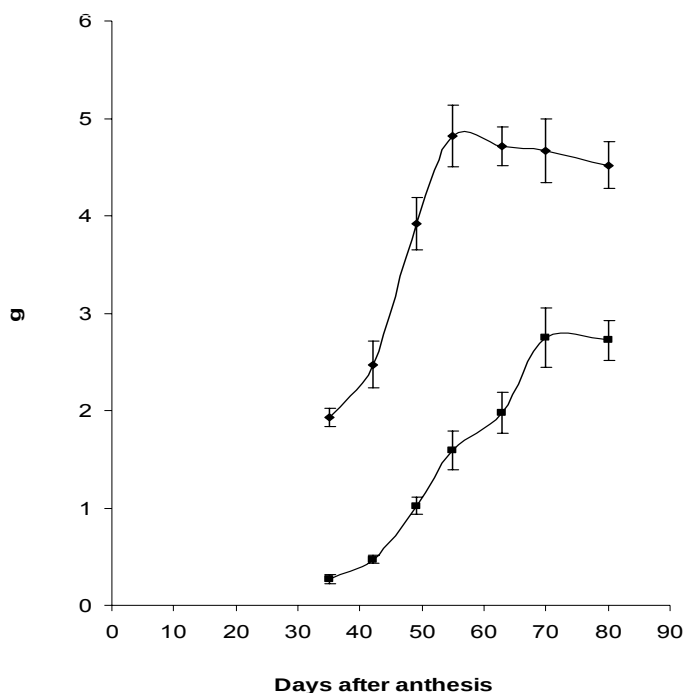


Fig. 1. Changes in the mean fresh and dry weights at different stages of maturity of *Bassia latifolia* seeds. ♦, Fresh weight; ■, dry weight. Bars represent $\pm$ standard error.

The MC of seeds began to decrease from the initial stage at 35 DAA to maturity at 70 DAA when seeds began to shed at a $41.2 \pm 2.1\%$ water content (Fig. 2).

Seeds began germinating at about 49 DAA, and about 43.26% seeds were observed to germinate at this stage (Fig. 2). However, full germinability was attained by seeds after full maturity at 70 DAA. The tolerance to drying of seeds harvested at different maturation stages was tested, and germination of seeds decreased with drying at all stages of development. However, mature seeds were found to be more desiccation tolerant than immature ones (Fig. 3). The full germination capacity was achieved by seeds at 63 DAA after slight drying, and there were no significant differences in tolerance levels among seeds harvested at 63, 70, and 80 DAA.

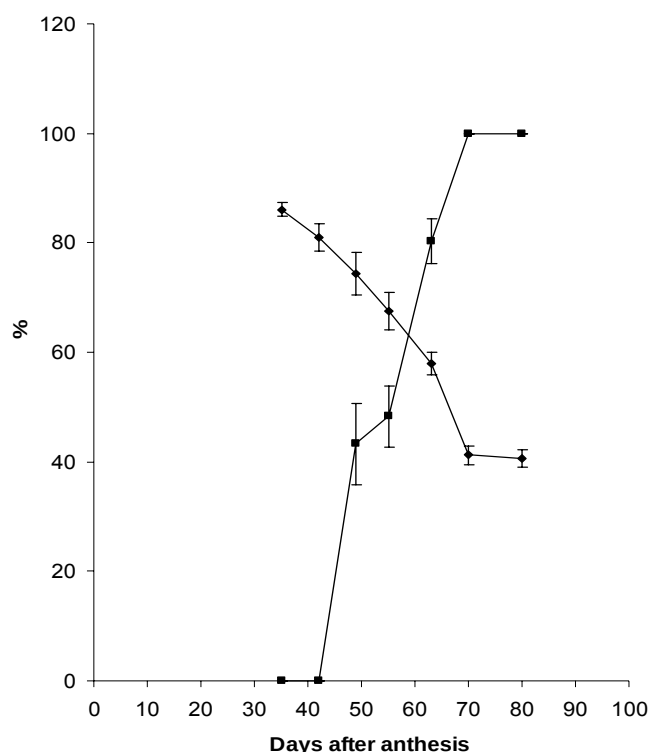


Fig. 2. Changes in the moisture content and percentage germination at harvest during different stages of maturity of *Bassia latifolia* seeds. ◆, Moisture content; ■, percentage germination. Bars represent $\pm$ standard error.

The storage temperature had a significant influence on germination, and 28°C was a more-suitable temperature for storing mahua seeds compared to 10 or 15°C irrespective of the initial MC or method of storage (Table 1). Seeds stored in a hermetic condition (a sealed polybag) at 15°C lost about 46% viability, and more than 80% seeds were unable to survive at 10°C in the same hermetic condition after 3 mo of storage.

Seeds at 28°C in sealed polythene bags retained maximum viability for up to 150 d with an 82% germination percentage, if stored with a shedding MC of 41%. A small reduction in the MC before storage (38%) decreased the longevity of seeds, and 67% of seeds were viable after 90 d of storage. A lower percentage of seeds was noted to have germinated compared to undried ones. A further reduction in the MC to 34% before storage caused a drastic reduction in the viability of seeds, and no seeds were viable after 60 d of storage.

Seeds stored in a hermetic condition began germinating at 28°C and were prone to fungal infestation unless they were treated. Storing seeds in an open tray at 28°C reduced contamination and germination, but seeds did not survive beyond 60 d, when only 47% of seeds retained their capacity to germinate.

A rapid decline in the germination percentage was also observed in seeds stored in open polybags at 28°C after 30 d of storage. Seeds stored by this method were more often infected at both 15 and 28°C.

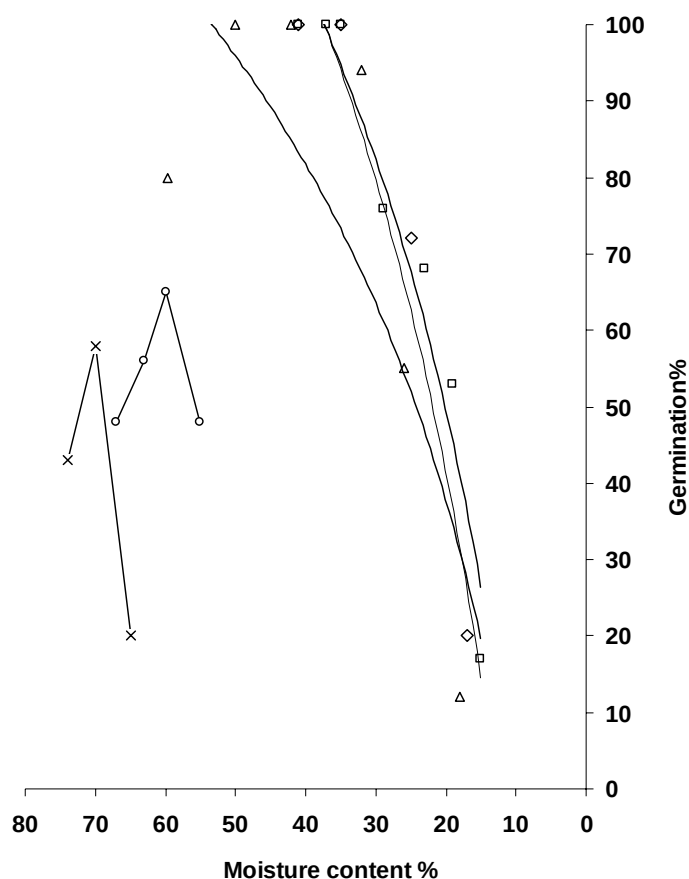


Fig. 3. Relationship between the seed ¹ and germination percentage at different developmental stages. ×, 49 d after anthesis (DAA); o, 55 DAA; Δ, 63 DAA; □, 70 DAA; ◇, 80 DAA.

Table 1. Viability (assessed as germination percentage) of seeds of *Bassia latifolia* after storage at different conditions

Storage conditions	Storage duration						
	0 day	15 days	30 days	45 days	60 days	90 days	150 days
41% ¹ , 28°C, sealed polybag	100	100	100	100	100	100	82.0±2.4
41% ¹ , 15°C, sealed polybag	100	100	54.2±4.0	30.5±5.4	22.8±3.8	0	0
41%, 10°C, sealed polybag	100	21.4±1.6	16.2±2.5	0	0	0	0
38% ¹ , 28°C, sealed polybag	100	100	100	100	100	67.2±5.2	17.5±3.0
38% ¹ , 15°C, sealed polybag	100	100	45.6±4.2	30.0±2.5	18.4±3.0	0	0
34% ¹ , 28°C, sealed polybag	100	100	100	72.5±4.5	61.3±5.5	15.8±2.4	0
34% ¹ , 15°C, sealed polybag	100	68.5±	32.6±2.5	20.8±4.1	11.4±1.6	0	0
41% ¹ , 28°C, open mouth polybag	100	100	100	20.6±3.1	0	0	0
41% ¹ , 15°C, open mouth polybag	100	100	50.6±3.6	12.8±2.8	0	0	0
Open tray, 28°C	100	100	100	66.6±4.0	47.5±3.2	0	0
Open tray, 15°C	100	78.2	50.6±4.5	33.4±1.5	0	0	0

¹ moisture content

DISCUSSION

Storage of seeds is considered to be the most effective and efficient method for the *ex situ* conservation of plant genetic resources. Although orthodox type of seeds can be stored for desirable periods, it is still a problem to store recalcitrant seeds, as these seeds are very sensitive to desiccation and freezing which are the primary conditions used for the long-term storage of seeds. Chin (1988) suggested storing recalcitrant seeds under moist conditions to avoid desiccation damage. However, a hydrated state induces metabolic activity and is responsible for germination-associated events during storage (Pammenter et al. 1994). Furthermore, storing seeds with a high moisture content at sub-ambient/ambient temperatures can result in fungal contamination and germination. Although these problems can be reduced by lowering the temperature, it is difficult to apply this in cases of recalcitrant seeds which are sensitive to temperatures below 15°C (Hor et al. 1984, Bedi and Basra 1993).

Several methods were developed by earlier workers to store desiccation-sensitive seeds, including moist storage (Thompson 1950, Ponnuswamy et al. 1991), sealed containers and perforated polythene bags (Maithani et al. 1989), cryostorage (Pence 1992), and partially dry storage (Tang and Tamari 1973). Each of these methods has its own limitations. Therefore, the best way to store recalcitrant seeds is to determine the optimum storage environment that can retard the germination process and prevent chilling injury at the lowest possible temperature. This may help retain the viability for short periods to allow enough time for transport and nursery preparation, and even to wait for favorable weather conditions.

Like other recalcitrant species, the dry weight of *B. latifolia* seeds increased until completion of their development. The fresh weight rapidly increased until the 8th week of seed development, and after that, a small decrease in the fresh weight was noted. The rapid increase in the dry weight with a concomitant decrease in the seed water content resulted in small changes in the fresh weight during the latter phase of seed development (Berjak and Pammenter 2000). Seeds began to germinate at 49 DAA when fewer than half of the seeds were filled, and the germination capacity increased with the progression of seed development. Full germination was achieved after completion of dry-mass accumulation at full maturity. Seeds of *B. latifolia* are desiccation-sensitive at all stages of development. Immature seeds were more sensitive to drying compared to mature ones. The increase in desiccation tolerance of *Quercus robusta* with advancement of the developmental stage was recorded by Finch-Savage (1992). On the other hand, Fu et al. (1994) showed that maturing seeds were more desiccation tolerant than mature ones in *Clausena lancium*. During the early stage of mahua seed development, drying induced some germination. Full germinability was achieved by seeds at 63 DAA, after the seed MC was dropped from about 58 to 42%, although the seeds had not attained mass maturity at this stage of development.

At 70 DAA, the seeds had completed dry-mass accumulation and full germinability, and the critical MC (King and Roberts 1979) was about 35%, below which the germination percentage began to decline. A significant difference in desiccation tolerance was not observed between the stages at 63 and 70 DAA, despite a difference in the dry mass. After mass maturity, desiccation tolerance did not change in mahua seeds, and the seeds began shedding. As in recalcitrant seeds, attainment of



maximum desiccation tolerance is not an indicator of maximum seed quality, as it is only achieved after completion of dry-mass accumulation (Kundu and Sett 2005). Collection of seeds as early as possible after mass maturity is advisable to maintain better viability.

Results of storage trials of mahua seeds revealed that the seeds are chilling sensitive at 15°C, although some tropical recalcitrant seeds were reported to retain short-term viability at 12~15°C. The damage from chilling may arise from the highly sensitive nature of the seeds to drying. Although hermetic storage at 28°C initiated the germination process, it can be delayed by keeping the seeds in a minimum water-stress condition that prevents the death of seeds. Germination and fungal growth are major problems of storage of seeds at relatively high MCs and temperatures. Anti-fungal treatment proved to be effective at minimizing microbial contamination (Finch-Savage et al. 2003). Storage of germinated seeds can be recommended (Corbineau and Come 1986, Krishnapally 2000), especially when the seeds are sensitive to temperatures that are too low to initiate germination.

However, the longevity of seeds after the initiation of germination event is highly species specific, as was evident from storage trials of *Hopea parviflora*, for which the viability of seeds rapidly declined within the first week of storage irrespective of temperature (Sunilkumar and Sudhakara 1998).

Storing *Bassia latifolia* seeds in polythene bags with the mouth open encouraged desiccation as well as fungal contamination resulting in loss of viability. Although storing seeds in an open tray at either temperature prevented fungal infestations, rapid desiccation was the reason for the decline in germination percentages within 60 d of storage at 28°C.

Partially dried seeds could not achieve the same longevity as undried ones at 28°C, and deterioration was positively related to the reduction in the seed MC at the time of storage within the limit of the lowest safe MC. Vergeese et al. (2002) also observed that the viability of partially dried seeds was reduced during storage at 15 and 25°C compared to undried seeds of *B. latifolia*. In contrast, Wan (2009) got better results in maintaining the viability of *Livistona chinensis* seeds by drying the seeds above the lowest safe MC level before storage. In the present study, partial drying even to a small extent did not show better results of retaining viability over the undried method, because the desiccation damage was not repaired during storage.

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Dynamics of Imbibition, Seed Germination, and Seedling Development of Austrian Pine (*Pinus nigra* Arnold) from Populations Growing in Contrasting Habitats of Southeastern Europe

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[Summary]

Seeds from Austrian pine (*Pinus nigra* Arnold) were collected from test trees in 5 Balkan provenances. Within each provenance, trees were located in either extremely harsh, rocky conditions or in a favorable (control) habitat. The dynamics of water uptake were measured (grams of water per gram of fresh seed mass) during 48 h of exposure to water. Additionally, seeds were exposed to drought conditions (simulated using a 4% sucrose solution) and compared to normal conditions (distilled water). Morphometric parameters of seedlings were measured after 21 d. Seeds collected from rocky habitats had lower water uptake than those from control habitats. However, water uptake was not correlated with germination percentages. Germination decreased in simulated drought conditions regardless of the habitat conditions. Seeds collected from rocky habitats had lower germination, with the exception of seeds from 1 provenance. In most cases (root, hypocotyl, and cotyledon lengths), seedlings grown from seeds originating from rocky habitats were larger. The data suggest some degree of adaptation to environmental conditions although the variability was very high within each provenance's habitat types.

Key words: *Pinus nigra*, seed imbibition, germination, sapling, adaptation, drought stress.

INTRODUCTION

Despite many scientific and professional papers on the genetic potential of Austrian pine, remote populations and individual trees of this species growing on Balkan Peninsula cliffs and canyons have not been adequately researched (Vidaković 1974, Mataruga et al. 1997, Bojovic et al. 2004). These individual trees and populations of Austrian pine grow in extremely harsh habitat conditions with little or no surface substrate (Gajić et al. 1992, Mataruga 2006). Moisture availability varies greatly with weather conditions and precipitation. This, combined with the steep terrain, results in limited water available for seed germination. Previous research showed significant differences in morphological parameters such as needle size, cone dimensions, and anatomical structure of needles among mature Austrian pine trees growing in various environmental conditions (Mataruga et al. 2003).

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The majority of research regarding the biochemical processes of seeds during imbibition is on agricultural species (Gidrol et al. 1994, Blum 1996, De Gara et al. 2000, Shao et al. 2009). Seed germination requires water uptake and seed swelling followed by biochemical activity, initiation of growth, and synthesis of new metabolites and tissues. In a strict sense, the time between seed imbibition and radicle emergence is the period of germination. Following initial water uptake, this phase of development is characterized by relatively little change in the seed water content until the initiation of embryo growth (Bradford et al. 2000).

There has been much research on the effects of moisture upon germination in tree species (Belcher and Perkins 1985, Bonner and Farmer 1966, Kaufmann and Eckhard 1977), into imbibition and germination of pine seeds, and the importance of the seed coat (Barnett and Pesacreta 1993, Barnett et al. 1999, Tommasi et al. 2001). However, research concerning the germinative behavior among ecotypes, provenances, or populations within the same species is very limited. Falusi et al. (1983) showed that germination energy, the germination rate, and root growth of *P. halepensis* seed under a range of moisture conditions varied among seed sources. Similarly, significant differences were found in the germination of *P. sylvestris* seeds from 6 provenances placed under varying water potential conditions with those from the southernmost provenances showing less susceptibility to water stress (Tilki 2005). Boydak et al. (2003) also found that germination rates of *P. brutia* seeds from provenances of humid regions were most susceptible to moisture stress, and this was attributed to intraspecific variations resulting from natural selection. Variations in soil moisture may result in selective pressure and adaptation by influencing the germination rate and affecting the survival of newly emerged seedlings.

Each plant species in the sapling phase is mostly and easily susceptible to elimination in both natural (stands) and artificial (nurseries) conditions. Therefore, many authors are convinced (Tucović and Isajev 2000) that it is of special significance to know more about the variabilities of morphological traits and characteristics of the earliest phases of ontogenetic development, considering the large differences that can be observed at that age, which are largely the consequence of genetic variability (polymorphism). The first and strongest natural selection actually occurs when plants are in the germination stage. Therefore, comprehensive studies have shown significant interdependence among properties of cotyledons, the hypo- and epicotyl, and roots with subsequent characteristics of adult trees (Vasiljčenko 1960).

This study was developed to address the importance of species plasticity and ecosystem function across various environmental conditions, which is a primary factor in species survival and thereby species diversity (Booy et al. 2000). The objective of the study was to determine the relationship between seed germination and water uptake of seeds collected from various provenances and habitats. These research findings are increasingly important when considering potential impacts of global temperature increases and the anticipated migration of certain species due to climate and habitat changes.



MATERIALS AND METHODS

Site selection and seed collection

Seeds collected from natural populations of Austrian pine were used in this study. Seeds collected from a total of 40 open-pollinated trees originating from 5 provenances were analyzed (Table 1, Fig. 1). Within each provenance, seeds were collected from 5 trees growing in extremely harsh habitats (rocky) and 3 trees growing in a favorable habitat (control). The harsh habitats were characterized by steep terrain, high temperatures, southerly and southeasterly exposures, and very shallow soils with an extremely or absolutely rocky surface. High average air and soil temperatures and low, sporadic precipitation during the vegetative period were recorded in these habitats (Stefanović et al. 1983). Habitat types found in the Durmitor and Teslić provenances clearly differed between the rocky and control within the provenances. However, the control sites were not as favorable as those found in the other 3 provenances (based on visual observations). During the 1998–2000 period (just prior to and during seed collection), annual precipitation recorded at 3 metrological stations in closest proximity to the collection sites ranged 750–1000 mm and varied by only 7–15% among stations. Annual temperature averaged 10–12°C with little variation among seasons or stations (minimum temperatures averaged 5.0–7.1°C and maximum temperatures averaged 16.1–19.1°C).

Table 1. Site conditions of populations where seeds were collected

Provenance	Habitat	Trees	Latitude	Longitude	Elevation (m)	Geological substrate	Soil type
Sutjeska	rocky	1–5	43°19'20"	18°42'46"	1400	limestone	no surface soil
	control	6–8	43°19'19"	18°39'33"	1300	limestone	colluvium
Višegrad	rocky	1–5	43°45'20"	19°24'04"	550	limestone	no surface soil
	control	6–8	43°51'26"	19°14'24"	475	serpentinite	eutric cambisol
Tara	rocky	1–5	43°52'50"	19°24'20"	800	limestone	no surface soil
	control	6–8	43°53'20"	19°32'40"	1050	serpentinite	eutric cambisol
Teslić	rocky	1–5	44°34'30"	17°43'28"	510	serpentinite	eutric leptosol
	control	6–8	44°34'03"	17°43'34"	470	serpentinite	albeluvisol
Durmitor	rocky	1–5	43°09'12"	19°15'53"	1240	limestone	melanosol
	control	6–8	43°00'29"	19°25'29"	1320	limestone	calco-cambisol

Cones collected from individual trees (families) were selected based on the presence of cones and accessibility in their specific habitat. Approximately 200 cones from each tree were collected. Cones were initially dried at room temperature (20°C) in wooden crates for 48–72 h. They were then placed in a drying oven (45°C) for 24–48 h. Seeds were then extracted from the cones, dewinged (by manual rubbing), and placed into sealed plastic bags in cold storage (4°C) until laboratory testing. Initial morphological characteristics (seed length and width, with or without wings) was measured and tended to be smaller for seeds from rocky sites for most provenances but largest from rocky habitats in the Durmitor provenance (data not shown; water uptake was determined on a relative basis).



Fig. 1. Distribution of provenances and habitats where seeds were collected within the Balkan Peninsula. Populations are identified by the first 3 letters of the provenance name and either 'r' for rocky habitat or 'c' for control habitat.

Water uptake experiment

In July 2001, four samples (replications) of 100 seeds from each family were tested for water uptake. Each seed sample was weighed and then placed in a Petri dish (12 cm in diameter) between layers of filter paper. Distilled water (7 ml) was added to each Petri dish. Water uptake (seed weight over time) was monitored 3 min after water was added and again after 3, 6, 12, 24, 36, and 48 h. This time period was selected based on the results of Stone (1958) which showed that all water uptake required for germination occurred during the initial 48 h. The initial seed weight varied among populations (no specific patterns, data not shown); therefore the relative water uptake was calculated as grams of water per gram of the initial seed sample fresh weight.

Germination and properties of 21-d-old seedling

Germination was recorded after 9 d (number of filled seeds with an emerged radicle) as normal germination based on ISTA standards (Isajev and Mančić 2001). The test duration was selected based on a preliminary test with the same seed sources which showed nearly 100% germination within 10 d (Mataruga 2006). Temperatures during the test period were kept constant at 21°C in a germination chamber with no supplemental lighting.



The lengths of hypocotyls, roots, and cotyledons (in millimeters), and number of cotyledons (in no. of pieces) were measured in 21-d-old seedlings.

Simulated drought experiment

This test was initiated to determine seed tolerance to drought conditions during germination. To accomplish this, seeds were placed in a 4% sucrose solution. This created a water deficit condition by lowering the water potential and thereby decreasing the water uptake capability. The percent germination under those conditions indicates to what extent these plants are able to germinate in drought conditions (Džamić et al. 1999).

In July 2001, four replicates of seed (100 seeds each) from each tree from each habitat within each provenance (Table 1) were placed in Petri dishes (12 cm in diameter). Treatments were applied by adding 7 ml of either distilled water or a 4% sucrose solution to each Petri dish. There were 320 Petri dishes in total (40 trees x 2 treatments x 4 replicates) placed randomly in the germination chamber. The number of germinated seeds was determined after 3, 5, 7, and 9 d. A germination index was calculated according to the following formula (Džamić et al. 1999):

$$GI = \frac{[A] \times [B]}{[C] \times [D]}$$

where GI is the germination index; A is the percent germinated seeds from an individual population in sucrose treatment; B is the germination mean percentage of all populations in distilled water; C is the percent of germinated seeds from an individual population in distilled water; and D is the germination mean percentage of all populations in the sucrose treatment.

Statistical analyses

Data were analyzed at multiple levels: tree, habitat, and provenance. Factorial analyses were conducted to determine significant differences among the 40 tested families as well as any differences among replicates within the same family. If findings were significant, 2-, 3-, and 4-factorial analyses were conducted to determine which factor most influenced the tested properties. Only critical factors identified from the variance analyses are presented in the results. Additionally, the coefficient of determination (r^2) was determined to show the interdependence of all tested variables. Statistica 6.0 and SPSS 12.0 software (SPSS, Chicago, IL, USA) were used for all data analyses.

RESULTS AND DISCUSSION

Water uptake experiment

There were significant differences for each analyzed factor as well as significant interactions (Table 2). Seeds collected from rocky habitats had lower water uptake than seeds from control habitats in the Sutjeska, Višegrad, and Tara provenances (Fig. 2). Seeds from control habitats in the Durmitor and Teslić provenances absorbed significantly more water than those from rocky habitats during the initial 6 and 12 h, respectively, but water uptake was similar thereafter (Fig. 2). For the first 6 h, the greatest water uptake among all populations was observed for the Teslić control habitat,

while the Sutjeska control habitat had the greatest overall water absorption during the observed time period. Seeds from the Tara and Višegrad rocky habitats had the least water uptake overall among all populations (Fig. 2). Coefficients of determination between the germination and water uptake after 3 min and 48 h of exposure to water were non-significant ($p = 0.578$ and 0.185 , respectively).

Table 2. Variance analysis of water absorption relative to the initial weight [gram water per gram seed prior to analysis]. * $p < 0.05$, ** $p < 0.01$, * $p < 0.001$**

Factor	df	Time of measurement (h)						
		0.05	3	6	12	24	36	48
Provenance(1)	4	40.83**	82.79***	101.52***	68.21***	48.77**	24.53***	22.45***
Tree (2)	7	10.93***	30.49***	33.25***	25.42***	14.56**	10.91***	11.55**
Interaction 1x2	28	6.75***	17.19***	16.86***	12.31***	7.46**	6.42**	5.51***
Population (1)	9	20.80***	52.93***	62.88***	40.37***	28.84**	20.31**	21.19***
Tree (2)	24-14	11.23***	26.54***	22.97***	23.66***	15.49***	9.83**	7.87**
Interaction 1x2		7.57***	15.27***	15.23***	11.43***	6.27***	4.53**	3.34***
Habitat (1)	1	11.43**	186.72***	257.39***	155.34***	80.14***	69.16***	69.62***
Tree (2)	24-14	10.05***	23.46***	24.95***	21.03***	14.58***	9.96**	8.58**
Interaction 1x2		13.41***	25.95***	26.55***	15.17***	8.50*	5.64***	5.00***

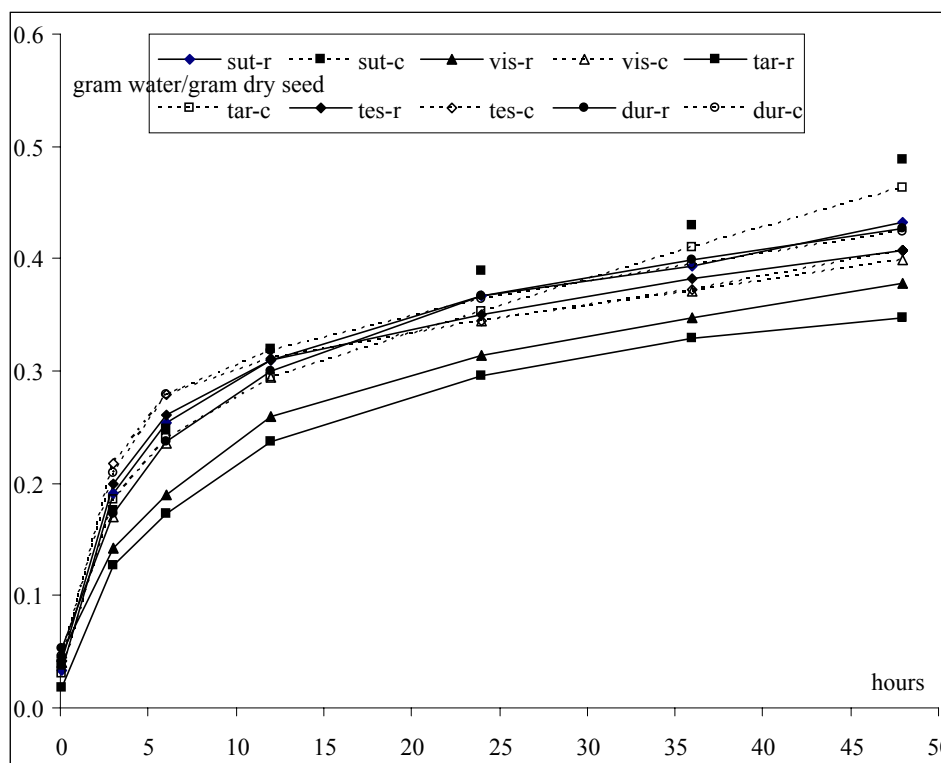


Fig. 2. Average water uptake between rocky and control sites from each provenance [g water/g seed].



The lower water uptake observed for populations from harsh habitats in some of the provenances suggests a small degree of natural selection toward survival and germination in a droughty environment. This is similar to other research (Kermode 1995, Ma et al. 2008) which observed genetic influences on the seed coat structure. Seed germination characteristics are, at least partially, under genetic control (Whittington 1973); therefore, local populations of a widely distributed species may develop adaptive variability in germination ecology (Quinn 1977). Provenance differences in the seed germination response to moisture were explained by the conditions of the site of origin for several species (Fraser 1971, Moore and Kidd 1982, Gibson and Bachealard 1987). This fact may explain the variation of differences among habitats within specific provenances.

Seed characteristics which influence water uptake can be closely related to morphological parameters of trees and depend on climatic and environmental conditions. The seed coat, in particular, influences water uptake and is affected by heritability (Barnett 1998). The seed coat structure of Scots pine (*P. sylvestris* L.) seeds from different provenances contributed to differences in water imbibition (Tillman-Sutela and Kauppi, 1995b). As much as 69% of the variation in germination speed for 5 pine species was related to the seed coat among 3 physical criteria measured (Barnett 1976). As such, the morphological structure of the seed coat, consisting of the sarcotesta, sclerotesta, and endotesta has a significant role in imbibition.

Although some differences in water uptake were observed between habitats, there was no correlation with subsequent germination indicating that this characteristic is more dependent on genotype than habitat conditions. In a study to examine germination of ponderosa pine (*P. ponderosa*) seeds from 225 sites (Weber and Sorensen 1992), results suggested that some of the geographic variation in the speed of seed germination was related to the severity of the summer drought; germination speed was greater in locations with drought-limited growing seasons. However, most variations occurred within locations. Similarly, we found a great deal of variation in germination among families within habitats. Interestingly, seeds from the Višegrad provenance were the only ones to have greater germination from the rocky habitat compared to those collected from the control habitat, whereas overall water uptake was relatively lower for seeds from that provenance.

We found that 50% of the total water uptake during a 48-h period occurred during the first 3~4 h. Terskikh et al. (2005) found similar results during the first 3~5 h of imbibition. The sarcotesta immediately becomes hydrated after the first contact of the seed with the imbibing medium. Hydration of the sclerotesta and endotesta is evident a little later, and water subsequently appears to accumulate in the region between the seed coat and megagametophyte. With time, these high-intensity signals extend to envelop the megagametophyte. At the same time, the greatest appearance of the mobile water signal in embryos occurred after about 10 h of imbibition. At 50 h, imbibition was complete, and only minor changes in the magnetic resonance image were observed thereafter (Terskikh et al. 2005).

Simulated drought experiment

Germination in water was significantly greater than germination in the 4% sucrose solution (Table 3). However, patterns among populations were similar on the 2

substrates, which shows that seeds with higher germination on distilled water, also had higher germination in the 4% sucrose solution, but rates differed in magnitude by 20~40% (Fig. 3a, b). There was a significant correlation between germination on distilled water and on the 4% sucrose solution ($r^2 = 0.5665$; $p < 0.01$) (Fig. 4). With the exception of the Višegrad provenance, seeds from control habitats had higher germination percentages than those from rocky habitats on both the 4% sucrose solution and distilled water (Fig. 3a, b).

Table 3. Variance analysis of germinated seeds on distilled water or 4% sucrose solution. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant

Factor	df	Day			
		3rd	5th	7th	9th
Solution (1)	1	108.93***	134.90***	144.06***	161.67***
Provenance (2)	4	9.10***	5.18***	5.61***	9.58***
Interaction 1 x 2	4	6.94***	3.86**	3.70**	2.42*
Solution (1)	1	152.28***	154.84***	150.71***	173.579***
Population (2)	9	12.46***	7.35***	7.39***	11.37***
Interaction 1 x 2	9	6.86***	2.78**	1.94*	1.80 ^{ns}
Solution (1)	1	114.05***	131.65***	125.64***	131.65***
Habitat (2)	1	27.63***	13.32***	4.09*	1.17 ^{ns}
Interaction 1 x 2	1	11.245**	3.26 ^{ns}	0.04 ^{ns}	0.22 ^{ns}

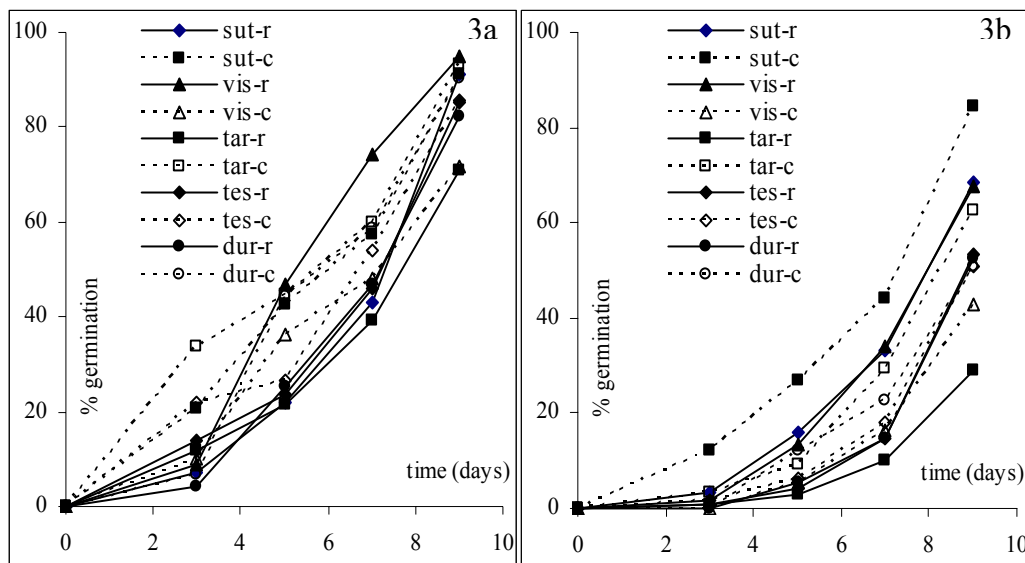


Fig. 3. Germination patterns of seeds between rocky and control sites from each provenance in distilled water (a) and a 4% sucrose solution (b).

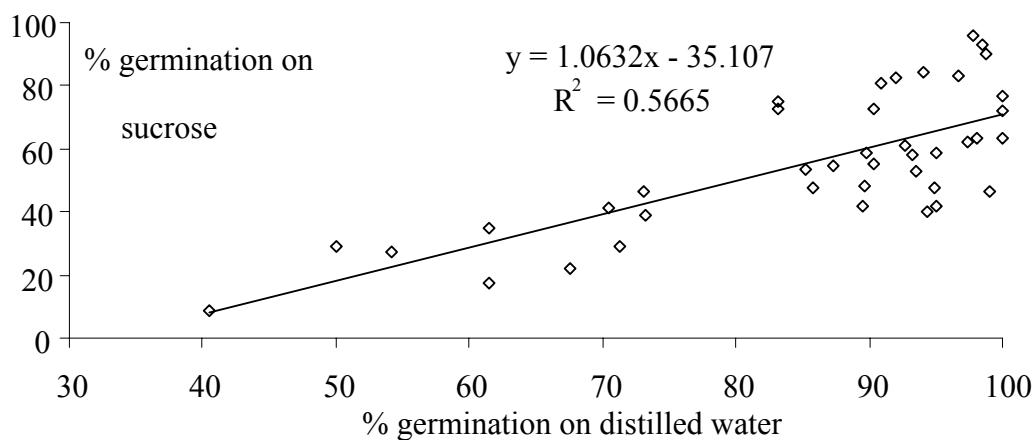


Fig. 4. Regression analyses between germination of seeds in distilled water and a 4% sucrose solution (simulated drought).

For the calculated germination index, a value of > 1.0 indicates higher drought tolerance. Values of > 1.0 were found for the Sutjeska-control, Sutjeska-rocky, Višegrad-rocky, and Tara-control populations. With the exception of the Sutjeska and Tara provenances, the germination index of seeds from rocky habitats was lower than that of seeds from control habitats (Fig. 5).

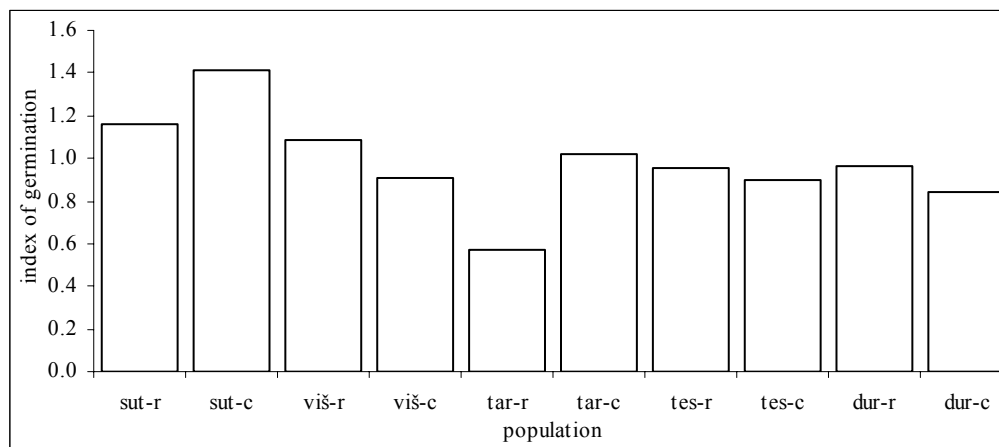


Fig. 5. Index of germination among populations indicating the relative drought tolerance.

We hypothesized that seeds from rocky and control habitats would differ in their germination responses under drought conditions as a result of adaptive evolution to their native habitats. However, there were no significant differences between the 2 habitats for any of the provenances indicating little ecotypic differentiation. Similarly, Wang and Macdonald (1992) found few differences in isozyme variations or seed germination

among populations of black spruce (*Picea mariana* Mill) on peatland and upland sites under different temperatures (15, 25, and 35°C) and drought conditions (0, -5, -10, and -15 bars). The lack of significant differences between habitats may be explained in a variety of ways. First, the proximity of trees growing in the rocky and control habitats within each provenance may be conducive to outcrossing, and thereby gene exchange between adjacent populations may have occurred, resulting in little or no ecotype variation. Second, there was a great deal of variability among individual trees within a population suggesting a large intrapopulational variability which could be greater than the interpopulational variability. This may also be attributable to variations in the age structure among populations (although all were fully mature for seed production). Third, our test was based on seed collections from “safe sites” (Harper 1977) which may vary little between habitats with respect to selection pressures and were collected in a single year (given the difficult terrain and the sporadic occurrence of cone crops, additional collections were simply not cost or time effective). Fourth, natural environmental conditions between the 2 selected habitats are very different for seed germination, whereas the test conditions were similar for each. Fifth, our test only addressed a narrow window of time for drought tolerance; tolerant genotypes would be more evident during a dry, warm summer or at other times of the year. Sixth, our test was conducted on stored seeds which may respond differently than seeds under natural conditions. Additional experimentation, beyond the scope of this paper, examined seedling development from these same seed populations grown in seedbeds covering a range of drought conditions. Growth and survival differences among provenances were evident and seedlings grown from control habitats tended to perform better than those from rocky habitats (with the exception of the Visergrad provenance; data not shown).

Although seed responses did not significantly vary between habitats, previous data indicated significant differences among morphological parameters of tested trees by site conditions (Mataruga 2003). Drought stress resulted in reduced leaf size, stem extension, and root proliferation. As a result, there was some discussion to justify separating subspecies or ecotypes within Austrian pine (Vidaković 1974, Gajić 1988, Mataruga et al. 2007). Plants display a variety of physiological and biochemical responses to drought at both the cellular and whole-plant level, thus making it a complex phenomenon (Farooq et al. 2009).

Properties of 21-d-old seedlings

The largest range of variations for all 40 tested half-sib lines was measured in root length, and the lowest was in the number of cotyledons. Almost all of the features of saplings of seed trees growing on rocks were more prominent than those of saplings of half-sib lines from the control populations (Figs. 6-10). The most significant difference was reflected in the size of the root, and also in the length of the cotyledons, where significant differences were proven.

By analyzing differences between populations in different provenances based on the variance analysis tests (Table 4), we concluded the following.

Significantly higher values were confirmed for root and cotyledon lengths, and the ratio of the lengths of the hypocotyl/root for rocky populations.

There was no significant difference in the length of the hypocotyls, although half-sib lines growing on rocks had somewhat higher values. (It should be noted that



populations from rocky provenances of Durmitor and Visegrad had significantly higher values, while in the Sutjeska provenance, significantly lower values of this population were measured, but values for Tara and Teslic were not significantly lower).

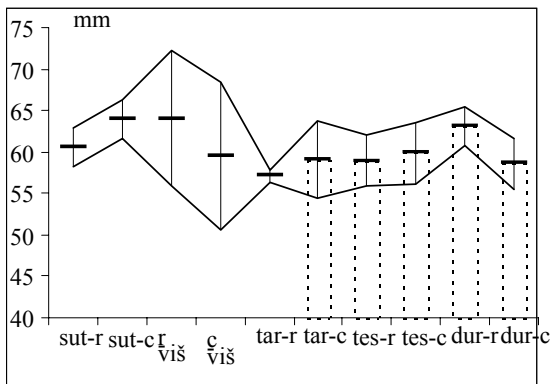


Fig. 6. Hypocotyl length (mm).

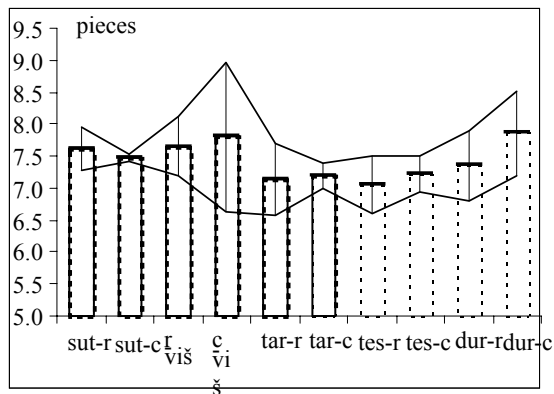


Fig. 7. Number of cotyledons (no. of pieces).

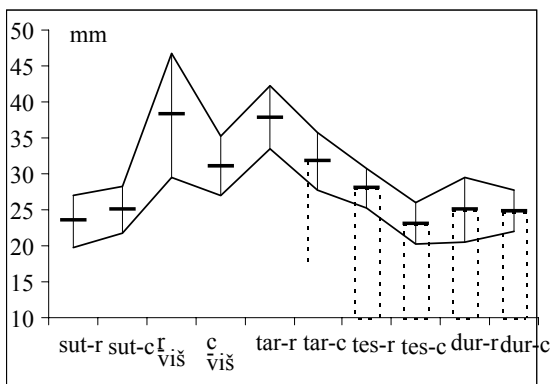


Fig. 8. Root length (mm).

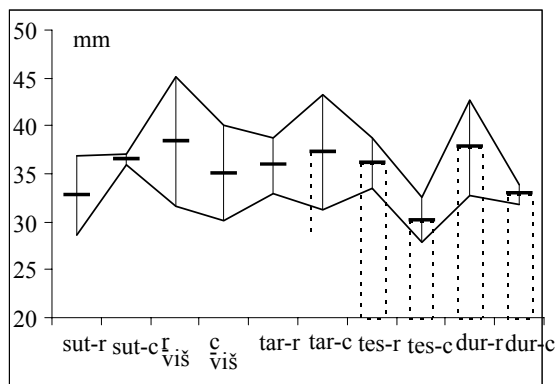


Fig. 9. Cotyledon length (mm).

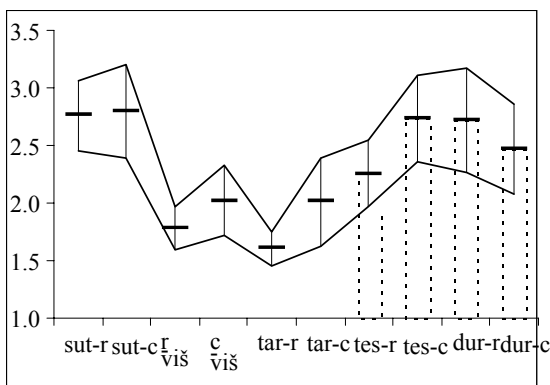


Fig. 10. Relation between hypocotyl length and cotyledon length.

Table 4. Variance analysis of traits of 21-d-old saplings. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns, not significant

Factor	df	Hypocotyl	Root	Ratio hypoc./root	Number of cotyledons	Cotyledon length
Population (1)	9	7726***	41,111***	25,513***	4449***	13,593***
Half-sib (2)	(2~4)	12,293***	15,888***	4771**	2555*	14,060***
Repetition (3)	3	769 ^{ns}	3390 ^{ns}	3599 ^{ns}	133 ^{ns}	520 ^{ns}
Interaction (1x2)		4678***	4611***	2981***	4250***	8730***
Provenance (1)	4	8167***	75,118***	50,589***	8086***	8580***
Half-sib (2)	7	7202***	13,582***	4336***	3556**	11,677***
Repetition (3)	3	3559 ^{ns}	2738 ^{ns}	3165 ^{ns}	999 ^{ns}	1111 ^{ns}
Interaction (1x2)		5362***	5300***	3433***	3750***	10,177***
Habitat (1)	1	310 ^{ns}	75,796***	29,713***	213 ^{ns}	6868**
Half-sib (2)	14~24	5859***	15,229***	9005***	3498***	10,389***
Repetition (3)	3	2294 ^{ns}	1655 ^{ns}	2088 ^{ns}	487 ^{ns}	2014 ^{ns}
Interaction (1x2)		6552***	10,614***	7367***	5372***	9686***

Differences in site conditions in which test trees were situated had the least influence on the properties of saplings and the highest influence on features of individual trees.

The length of cotyledons was distinguished as the property in which significant differences between populations in all provenances were observed (except for Tara). On the other hand, the number of cotyledons differed only between habitats within the provenance Durmitor.

CONCLUSIONS

Seeds from among the 5 Balkan provenances showed some differences in water uptake when those from harsh, arid sites were compared to those from favorable environmental conditions. However, there was no corresponding correlation with subsequent germination, even under simulated drought conditions making it difficult to consistently discern distinct ecotypic differences between habitat types.

Almost all of the features of saplings (21-d-old seedlings) from seed trees which were growing on rocks were higher than those of saplings from control populations. The most significant difference was reflected in the size of the root and also the length of the cotyledons.

In future research to predict drought tolerance and response to climate change, development of selection and breeding programs in Austrian pine should emphasize the variability of individual open-pollinated families within specific habitat types. Until that time, use of seeds from favorable habitats is recommended since seed quality is likely to be superior regardless of where seeds are sown.



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Seed Dormancy and Hydrotime Model for Seed Populations in Two Habitats of an Invasive Fabaceae Species, *Prosopis juliflora*

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[Summary]

Prosopis juliflora, a species of the Fabaceae, is native to North and South America and is invasive in several other countries including Sri Lanka. In Sri Lanka, *P. juliflora* is distributed in coastal areas of the semiarid zone. Its seed biology fosters invasion management. Thus, our study mainly focused on determining the germination characteristics of *P. juliflora*. Seeds were collected from numerous trees growing along roadsides away from the coast and trees growing near a brackish-water lagoon. Germination and imbibition characteristics of untreated and manually scarified seeds were studied. Acid-scarified seeds of the roadside and lagoon collections were separately germinated in a series of salt concentrations to develop a hydrotime model.

The mass of manually scarified seeds increased by > 140%, while that of untreated seeds increased by < 80%. In light/dark and dark conditions, 100% of manually scarified seeds germinated, whereas only 65% of untreated seeds germinated. These experiments confirmed that seeds of *P. juliflora* exhibit physical dormancy. Contrasting results were observed for NaCl and KNO₃ salts. Results for KNO₃ were not reliable because a high percentage of seeds died before 50% germination was reached. In NaCl, values of the base water potential ($\psi_{b(50)}$) of 5.86 and 5.80 were observed for the seashore and roadside seeds, respectively. To the best of our knowledge, this is the first study to develop a hydrotime model for a woody invasive species. A slight shift in the base water potential of seeds indicates the plasticity of the *P. juliflora* for invading a new habitat of roadsides with lower salinities than those of seashore habitats, where it initially invaded.

Key words: hydrotime model, invasive plants, physical dormancy, scarification.

INTRODUCTION

Exotic invasive species are organisms that colonize areas outside their normal range with or without human interference (Huverd 1993). They can become very aggressive in their new habitat if the ecological conditions are favorable for their survival (Mack et al. 2000, Kolar and Lodge 2001). Invasive species can become dominant in the new area in a relatively short period by suppressing previously established native species or plant communities. *Prosopis juliflora* is a good example of this phenomenon. It is native to North and South America (Asfaw and Thulin 1989) and has become invasive in several countries in Asia and Africa. *Prosopis juliflora* belongs to the subfamily Mimosoideae of the Fabaceae. There are 45 species in the genus, mainly found in arid

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and semiarid regions in the world (Asfaw and Thulin 1989). Currently *P. juliflora* is rapidly invading the traditional agro- and silvo-pastoral land of the Afar and Isa ethnic groups in the Afar National Regional State of Ethiopia (Shiferaw et al. 2004), as well as in Kenya (Mwangi and Swallow 2005), the Kumbhalgarh Wildlife Sanctuary (KWS) in Rajasthan, India (Cempbell et al. 2008), and Sri Lanka (Algama and Seneviratne 2000).

Prosopis juliflora was introduced as a food and fodder plant and as a shade tree for apiculture. This species is the major source of honey for bees and a source of fuel, fiber, timber, gum, resin, tannin, dyestuffs, alcohol, medicines, sand stabilizer, and shade tree in the semiarid tropics. In Sri Lanka, it is distributed in coastal semiarid zones including Hambantota District in the southern province where it has spread towards the sea and brackish-water lagoon and Puttulum District in the north-central province, where it has colonized a different habitat about 20 km inland from the sea (Algama and Seneviratne 2000). In Bundala National Park (BNP), the only wetland in Sri Lanka listed under the RAMSAR convention, *P. juliflora* is distributed around brackish-water lagoons and has now become a great threat to the native flora and fauna. Therefore, immediate actions have to be taken to control the spread of this invasive species. Since seeds are the major dispersal unit of *P. juliflora*, it is important to understand the germination ecology and seed biology of this species when planning necessary control measures.

Seed dormancy is one of the major factors that influence the timing of seed germination. There are conflicting opinions on the seed dormancy of *P. juliflora*. Germination experiments by Shiferaw et al. (2004) suggested that the high dormancy of *P. juliflora* seeds is caused by a water-impermeable seed coat (physical dormancy; Baskin and Baskin 2004). However, they did not confirm this with an imbibition test (see Baskin et al. 2006). Algama and Seneviratne (2000) agreed with this suggestion, and they claimed that physical dormancy of *P. juliflora* can be overcome by chemical scarification using H_2SO_4 . In contrast, Jayasuriya and Perera (2004 2005) claimed that *P. juliflora* seeds were non-dormant.

Prosopis juliflora is found in a wide spectrum of ecological conditions. Thus, seeds seem to have the capability of germinating in a wide range of environmental conditions. Environmental conditions determine when dormancy is broken as well as the germination of the seeds after dormancy is broken. Dormancy is an internal condition of the seed that impedes its germination under otherwise adequate hydric, thermal, and gaseous conditions (Benech-Arnold et al. 2000). A hydrotime model is an important mathematical model based on characterizing variations that occur in germination times among individual seeds in a population that can describe and quantify the water potential effect on seed dormancy, and the hydrothermal model based on both the water potential and temperature effects on seed dormancy. In 1986, Gummerson proposed the hydrotime concept. He defined the hydrotime constant (Θ_H) as

$$\Theta_H = (\psi - \psi_{b(g)}) t_g$$

where $\psi_{b(g)}$ is the base or threshold ψ that will just prevent germination of fraction 'g' of the seed population. In this model $\psi_{b(g)}$ represents the variation in threshold (ψ_b) values among seeds in the population which often can be described by a normal distribution. Since Θ_H constantly varies in t_g values among seeds, a normal distribution of $\psi_{b(g)}$ values results in a right-skewed sigmoid cumulative time course of germination events, as is generally observed for seed populations (Bradford 1997). Analogous to the



thermal time or degree-days, the time to germination is related to the magnitude of the difference between the seed water potential (ψ) and physiological base or threshold water potential for radicle emergence (ψ_b). ψ_b can be thought of as the lowest ψ at which a given seed can complete germination (Bradford 2002). In the BNP the *P. juliflora* is confined to the seashore where brackish-water lagoons are found and also away from the sea, where comparatively lower soil salinities are observed, resulting in different threshold water potential effects on germination patterns over time of *P. juliflora* seed populations in these 2 sites in the BNP.

Thus, the major focus of this study was to study the biology of seed germination of *P. juliflora* and determine the hydrotime model for germination of its seeds in the 2 habitats.

MATERIALS AND METHODS

Imbibition test

The purpose of this experiment was to determine whether *P. juliflora* seeds are physically dormant or not. Physically dormant seeds can imbibe well when an opening is created in the water-impermeable seed coat. Two samples of 15 manually scarified and untreated seeds were initially weighed using a digital chemical balance to the nearest 0.001 g. They were sown in Petri dishes on filter paper moistened with distilled water and incubated under ambient laboratory conditions (27°C temperature and 12 h of light/12 h of dark). Imbibing seeds were retrieved at 2-h intervals from the filter paper for weighing, and they were again placed on the filter paper. The procedure was repeated until all the mechanically scarified seeds had undergone imbibition.

Seed germination test

The purpose of this experiment was to check whether *P. juliflora* seeds experience dormancy or not. Dormant seeds will not germinate as do non-dormant seeds when suitable environmental conditions are provided. Two samples of 3 replicates of 15 manually scarified and untreated seeds were incubated under ambient laboratory conditions (12 h of light/12 h of dark) and dark conditions as 2 treatments. Seeds were checked at 3-d intervals for germination. Radicle emergence was the criterion for germination.

Dormancy-breaking treatment

The purpose of this experiment was to determine the best dormancy-breaking treatment for *P. juliflora* seeds to be utilized in the hydrotime model experiments. Eight samples containing 3 replicates of 10 seeds each were subjected to 8 different treatments grouped into 4 categories, prior to incubation at ambient laboratory temperature conditions: (i) no treatment (control); (ii) mechanical scarification at the chalazal side of the seeds, with about 2 mm of the hard seed coat removed to facilitate imbibition of water by the seeds; (iii) boiling water when seeds were immersed in boiling water for 1, 2, and 5 min after which they were removed and allowed to cool before sowing; and (iv) chemical scarification in which seeds were immersed in 96% H_2SO_4 for 5, 10, and 20 min. Seeds were thoroughly washed under running tap water before being sown after the treatment and prior to incubation (Laura et al. 2005). All seed samples were placed on

moist filter paper in Petri dishes during incubation. Radicle emergence was the criterion for germination. Samples were checked for germination at 2-d intervals for 15 d.

Hydrotime model

This experiment was conducted to determine the base water potential of 2 seed populations from 2 habitats to compare the plasticity of *P. juliflora*. The 2 habitats were the lagoon shore (saline) and roadside (non-saline) in Hambanthota. Three replicates of 10 chemically scarified (by immersing seeds in H_2SO_4 for 5 min and then washing them with flowing tap water) *P. juliflora* seeds were placed in Petri dishes on filter paper moistened with salt solutions (NaCl and KNO_3) with specific water potentials (of -0.1, -0.2, -0.5, 0, -1, -2, -5, and -7 MPa). Plates were incubated at ambient laboratory conditions (27°C temperature and 12 h of light/12 h of dark). The procedure was repeated with both the lagoon shore seed and roadside seed populations for the NaCl and KNO_3 salt solutions. Seeds were checked for germination at 1-d intervals. The criterion for germination was radicle emergence.

Data analysis

Data were analyzed according to a completely randomized design. Data of the imbibition and germination tests were analyzed according to a *t*-test procedure using SAS statistical software (vers. 6.12, SAS Institute, Cary, NC, USA). Dormancy-breaking treatments were analyzed using a 2-way analysis of variance (ANOVA) procedure in the SAS statistical software. Data were normalized using an arcsine transformation prior to analysis. Duncan's mean separation was used to identify differences between treatments. Data obtained from the hydrotime model were analyzed using linear and polynomial regression procedures. Data obtained from the hydrotime model conducted using KNO_3 salt were not reliable. Therefore, those data were not analyzed, and the base water potential [$\Psi_b(50\%)$] values were compared without a statistical analysis.

RESULTS

Imbibition test

The mass of manually scarified seeds of *P. juliflora* increased by > 140% during imbibition, while untreated seeds increased by only < 80% (Fig. 1). This mass increment in the untreated seed sample was caused by 47% of the untreated seeds in the sample. Thus, the mass increases of untreated seeds did not statistically significantly differ from that of mechanically scarified seeds ($t = 1.46$, $p = 0.15$). However, when the mass increase of these non-dormant seeds were excluded, the sample mass increase of untreated seeds was < 25%, and statistically differed from that of the manually scarified sample (Fig. 1).

Seed-germination test

In both light/dark and dark conditions, 100% of manually scarified seeds germinated, whereas germination of untreated seeds was significantly lower than that (65%, $R^2 = 99.6\%$, $p = 0.0001$) (Fig. 2). No statistical difference was observed between



the light/dark and dark treatments in either the untreated or manually scarified seed samples ($p = 0.1952$).

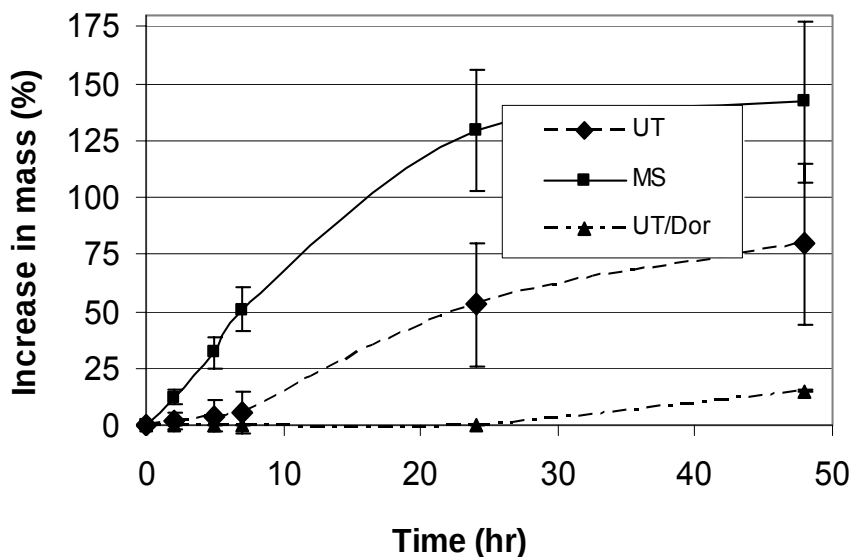


Fig. 1. Increase in mass of untreated (UT), manually scarified (MS), and the dormant portion of untreated (UT/D) *Prosopis juliflora* seeds on moist filter paper under ambient laboratory conditions. Error bars are $\pm$ SE.

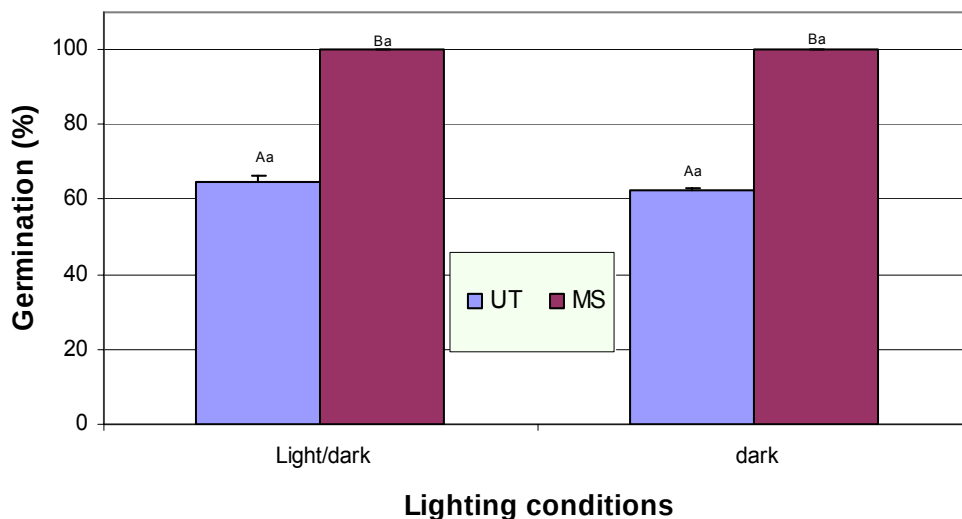


Fig. 2. Germination of untreated and manually scarified *P. juliflora* seeds under light/dark and dark-only conditions. Different upper case letters indicate a significant difference between intact and mechanically scarified seeds incubated at the same light level. Different lowercase letters indicate a significant difference between light levels and between the scarification treatments. Error bars are $\pm$ SE. Dormancy-breaking treatments

All of the manually scarified seeds and chemically scarified (H_2SO_4 treatments of 5, 10, and 20 min) germinated, whereas 73.3% of seeds treated with boiling water (immersed in boiling water for 2 and 5 min) germinated, and 60% of untreated seeds germinated (data not shown). Differences in germination percentages among treatments were statistically significant ($R^2 = 73\%$, $p = 0.002$).

Hydrotime model

The roadside seed collection showed 100% germination in the 1 MPa KNO_3 solution and 97% germination in both the 1 and 0.1 MPa NaCl solutions (Fig. 3). Germination results of the KNO_3 salt solutions were not as reliable as those of NaCl salt solutions for the statistical analysis, because seeds in the KNO_3 salt solutions died before they reached 50% germination. So the base water potential ($\psi_{b(50)}$) of KNO_3 was compared without a statistical analysis. In NaCl , the observed $\psi_{b(50\%)}$ values were -5.86 and -5.8 for the lagoon shore and roadside seed populations, respectively (Fig. 4).

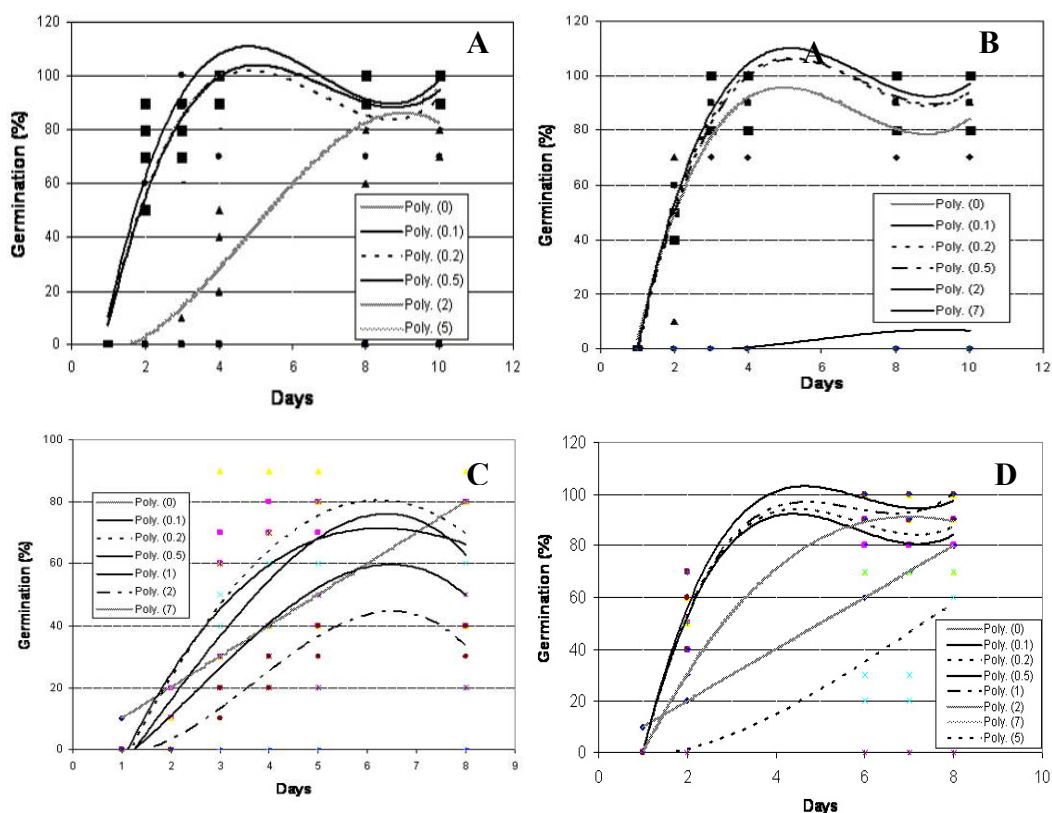


Fig. 3. Percentages of germination with time. (A) Seashore seeds in NaCl ; (B) roadside seeds in NaCl ; (C) seashore seeds in KNO_3 ; (D) roadside seeds in KNO_3 .

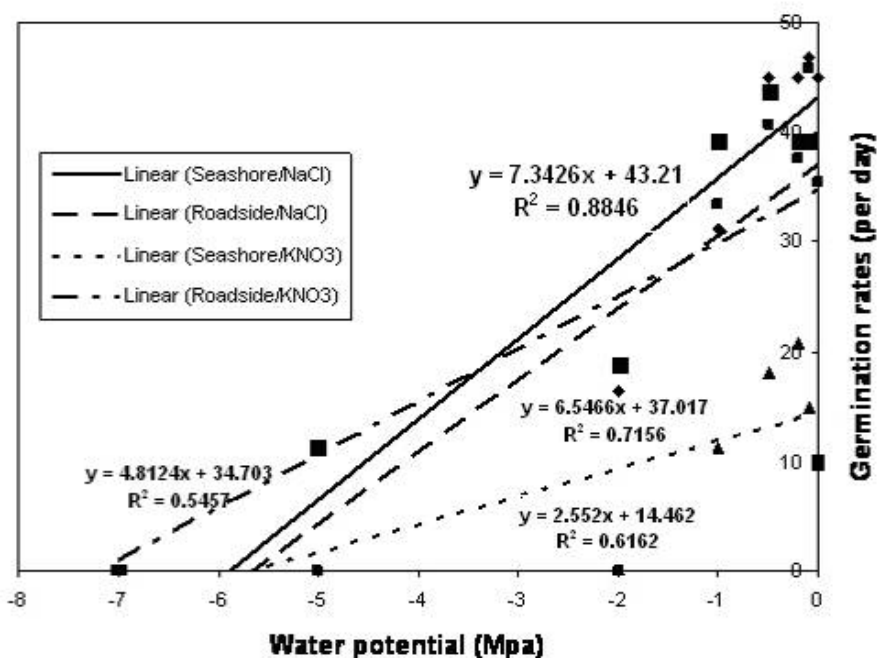


Fig. 4. Rates of germination with different water potentials.

DISCUSSION

Manually scarified seed of *P. juliflora* took up water rapidly at ambient laboratory conditions (27~28°C temperature, 12 h of light/12 h of dark). Thus, the imbibition rate was significantly higher in manually scarified seeds than in intact seeds (untreated) when the non-dormant portion of the intact seeds was removed. This suggests that at least some seeds of *P. juliflora* are physically dormant. The results of the germination test agree with this assumption, in that mechanical scarification of *P. juliflora* seeds facilitated germination with 100% of manually scarified seeds germinating, whereas only 65% of untreated ones germinated (Fig. 2). Germination tests further suggested that *P. juliflora* seeds have no physiological dormancy, as 100% of manually scarified seeds and the non-dormant imbibed portion of intact seeds germinated at a rapid rate. Therefore, from this study, we can clearly conclude that seeds of *P. juliflora* have physical dormancy. However, a portion (47~65%) of *P. juliflora* seeds were non-dormant. Results of our study do not agree with the results of Jayasuriya and Perera (2003, 2004) who claimed that seeds of *P. juliflora* showed no dormancy. The observations of our study agree with those of Shiferaw et al. (2004) who claimed that seeds of *P. juliflora* have dormancy caused by the hard seed coat. However, in contrast to Shiferaw et al. (2004), our study showed that a large amount of seeds were also non-dormant at seed dispersal. This may be an adaptation acquired by *P. juliflora* after it was introduced to Asia. The biological and ecological significance of physical dormancy was discussed by various authors (Cavanagh 1987, Baskin and Baskin 1989). Dormancy is a valuable characteristic for the survival of a species under variable environmental

conditions. However, under varying environmental conditions, partial non-dormancy of a seed population is also important. Non-dormant seeds germinate and produce seedlings as soon as they experience favorable environmental conditions, whereas dormant seeds remain a long time in soil seed banks to maintain a soil genetic bank of the species. Seed dormancy and non-dormancy cause a temporal dispersal of seedlings, and this temporal seedling distribution is important for the survival of the species (Shiferaw et al. 2004).

To develop a hydrotime model for a seed sample, the sample should be non-dormant. Otherwise the effect of dormancy will confound the effect of the water potential. The timing of seed germination and seedling emergence from a non-dormant population of seeds is mostly regulated by the soil environment, where temperatures and water contents are the 2 main factors (King and Oliver 1984). Results of our study show that some portion of *P. juliflora* seeds possesses physical dormancy. Among the dormancy-breaking treatments, the highest germination was achieved by 2 treatments: chemical scarification and mechanical scarification. Chemical scarification was used as the most suitable dormancy-breaking treatment due to its greater uniformity over mechanical scarification.

The hydrotime model which explains the germination response to the water potential was described by Gummerson (1986) and Bardford (1990). As observed in other species, germination of *P. juliflora* was markedly affected by the water potential in the seed as well as the water potential of the substrate. The hydrotime model efficiently describes this effect at a constant temperature. The calculated base water potentials for 50% germination [$\Psi_b(50\%)$] of the 2 seeds populations from *P. juliflora* trees of the roadside and seashore using KNO_3 and NaCl salt solutions markedly differed from each other. However, a high level of error can be expected in the KNO_3 salt treatments, where seeds died and rotted before they reached 50% germination. One reason for this may have been the low quality of the seed sample used for the KNO_3 experiments, or KNO_3 may be poisonous to the seeds. So the [$\Psi_b(50\%)$] value calculated with results obtained using the KNO_3 salt solutions was not reliable. Therefore, only the results obtained using the NaCl solutions are discussed below. However, to the best of our knowledge, this is the first time that the hydrotime model has been proposed for seed germination of a tree species.

Roadside seed collection had higher germination rates in both the 1 and 0.1 MPa NaCl solutions, and seashore seeds had higher germination in the 0.1 MPa solution. Calculated $\Psi_b(50\%)$ values differed in the 2 seed populations collected from the 2 habitats. The highest $\Psi_b(50\%)$ value was obtained for roadside seeds and the lowest $\Psi_b(50\%)$ value was obtained for the lagoon shore seed population. The slight increase in $\Psi_b(50\%)$ value of seeds collected from the lagoon shore compared to seeds collected from the roadside shows the plasticity of *P. juliflora* seeds. The lower $\Psi_b(50\%)$ observed in seeds collected from the lagoon shore plants facilitate the imbibition of water during germination even under saline soil conditions with a low water potential which occurs in lagoon shore soils. Salinity of the roadside soil was lower than that of the seashore soil (pers. comm. with Dr. Anoma Perera). Therefore, seed $\Psi_b(50\%)$ values need not to be as low as in the lagoon soil to germinate at a rapid rate. *Prosopis juliflora* trees invaded roadside habitats more recently (within the past 4~5 yr; pers. observ.). The shift in the $\Psi_b(50\%)$ value in seeds to a higher value seems to be an adaptation acquired by the species after the invasion of this new habitat. This shows the plasticity of the *P.*



juliflora plants which is 1 advantageous character of an invasive species enhancing its invasive ability by surviving under different habitats. *Prosopis juliflora* was initially introduced to the seashore area. Local records imply that *P. juliflora* was introduced to Sri Lanka in 1880 by the British. However it was not introduced to its present highly populated location in BNP until the 1950's. It was intentionally introduced to those southern infertile saline soils as a productive, salt-tolerant tree (Algama and Seneviratne 2000). It has now invaded roadside habitats showing its plasticity, and it can also likely invade other habitats in the dry zone of Sri Lanka. Thus, immediate action has to be taken to prevent seed dispersal to other important and more-sensitive ecosystems located in the dry zone of Sri Lanka.

CONCLUSIONS

A portion of *P. juliflora* seeds has physical dormancy, while the other portion is non-dormant, and the most appropriate treatment for breaking dormancy is chemical scarification using concentrated H_2SO_4 . *Prosopis juliflora* plants have high plasticity to change their characteristics in order to adapt to new environments, an important characteristic for invasive species.

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Seed Mycoflora, and Physicochemical and Biochemical Changes in Tree Seeds during Storage

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[Summary]

In the present investigation, the effects of different storage conditions on the seed mycoflora, seed germination, seed moisture, and protein, phenol, and sugar contents were evaluated at regular time intervals. Seeds of 4 different forest trees species, *Dendrocalamus strictus*, *Hardwickia binata*, *Phyllanthus emblica*, and *Dalbergia latifolia*, were selected for storage studies. Seeds were stored for 12 mo in different types of containers, including polythene-covered containers, cotton bags, glass containers, and paper-covered containers, at 3 different temperatures of 5°C, room temperature (RT; 26 ± 2°C), and 40°C. Seeds were removed at regular 3-mo intervals, and the incidence of different seed mycoflora and seed germination percentages were recorded. Seeds stored in pervious containers like cotton bags and paper-covered containers at room temperature showed higher incidences of mycoflora compared to seeds stored in impervious containers like polythene-covered containers and glass containers at temperatures of 5 and 40°C. Seeds stored at 5°C maintained viability for a long period. The moisture, protein, sugar, and phenol contents of seed samples stored at room temperature in different types of containers were analyzed. Fluctuations were observed in the moisture content of seeds stored in pervious containers but the protein, sugar, and phenol contents were found to have dropped at the end of the storage period.

Key words: chemical components in seeds, *Dendrocalamus strictus*, *Hardwickia binata*, *Phyllanthus emblica*, and *Dalbergia latifolia*, seed mycoflora, seed storage.

INTRODUCTION

There is an increasing awareness worldwide that unless we intensify efforts at gene conservation, reforestation, and intensive forest management, serious depletion of the world's forests will result. Although reforestation is recognized as an essential activity, an adequate supply of seeds of high quality and high genetic potential is often a limiting factor in many countries. This emphasizes the need for organized seed production and seed research to resolve many problems related to reforestation (Mittal and Mathur 2002).

Irregular and often infrequent seed production by many forest tree species necessitates seed storage. Seeds have to be stored over periods varying from a few months to several years in order to maintain regular supplies through years of low production. The duration of successful storage depends upon both the objectives and species concerned (Hong and Ellis 1996). Seed research has shown the way towards

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more-efficient seed-handling procedures for individual species; for example, in relation to storage behavior and dormancy (Sacande et al. 2004, Schmidt 2007).

Many forest tree seeds are stored improperly, which results in seed deterioration and loss of viability. Among forest tree species, the viability period, and moisture and nutrient contents of the seeds vary from species to species, according to nutrient availability, climatic conditions, seed texture, variety, place of collection, period, and conditions of storage. Under regulated storage conditions, the longevity of many seeds can be extended more than 10-fold (Barton 1961). Successful seed storage requires a sound knowledge of the characteristics of the seeds of different forest tree species, including seed longevity and factors influencing it under storage conditions such as the seed quality before storage, seed moisture content (MC), and storage temperature. Poor conditions facilitate fungal infections, the spread of decay organisms, insect infestations, and deterioration of the embryo due to fluctuations in humidity, all of which affect the quality of the seeds. Attempts were made to study the germination of seeds of forest tree species under specific storage conditions (Gupta et al. 1975), but studies on the association of seeds and viability under different storage conditions on seed germination are lacking. In the present investigation, the effects of different storage periods and conditions on the seed mycoflora, seed germination, and seed moisture, protein, phenol, and sugar contents were evaluated at regular intervals.

MATERIALS AND METHODS

Seeds selected for this study of *Dendrocalamus strictus*, *Phyllanthus emblica*, *Hardwickia binata*, and *Dalbergia latifolia* were obtained from the Seed Development Unit's Research wing, Karnataka Forest Development, Nagavala, Mysore, India. Seeds were stored in different types of containers, including cloth bags, polythene-covered containers, glass containers, and paper-covered containers, at different temperatures of 5°C, room temperature (RT; $26 \pm 2^\circ\text{C}$) and 40°C.

The seed mycoflora, germination percentage, and MC of seed samples were evaluated both before and after being stored by drawing seed samples at regular 3-mo intervals. The moisture, protein, phenolic, and sugar contents were estimated for seeds stored at room temperature in different containers.

Evaluation of the seed mycoflora

For each species, 200 seeds each in 4 replicates were subjected to a standard blotter test according to standard conditions of ISTA (2004). Fungi were identified using the following reference books: Booth (1971), Ellis (1971, 1976), Subramanian (1971), and Barnett and Hunter (1972).

Evaluation of the germination percentage

For each species, 200 seeds each in 4 replicates were subjected to the between-paper method and incubated for a period of 10 d under alternate cycles of 12/12 h of light/darkness at $22 \pm 2^\circ\text{C}$. The germination percentage was calculated on the 10th day.



Estimation of the seed moisture percentage

The MC of seeds was estimated by the AOAC dry-air oven method (Anonymous 1947). From each sample, 10 g of seeds was placed in a weighing dish of known weight and kept in a hot-air oven for 5 h at 110°C. Each sample was then cooled over calcium chloride in a desiccator, and the experiment was repeated until a constant weight was obtained. The moisture percentage was calculated using the following formula:

Seed moisture percent = [(Fresh weight of the seed – Dry weight of the seed)/Dry weight of the seed] x 100.

Biochemical studies

The protein, total sugar, and phenolic contents of the seed samples were estimated before storage and under storage conditions. Seed samples were subjected to 12 mo of storage in different containers of cotton bags, polythene-covered containers, glass containers, and paper-covered containers at RT. Seed samples were taken at 3-mo intervals, and seed samples in storage were analyzed to estimate biochemical changes.

Estimation of the protein content

The total protein of seeds was estimated by the Lowry method (Lowry et al. 1951). One gram of seeds was homogenized with 10 ml of 0.1 M phosphate buffer at pH 7 and centrifuged for 15 min at 10,000 rpm. The clear supernatant was used as the protein source. This was assayed according to Lowry's method using an alkali copper reagent and Folin Ciocalteu reagent. Absorbance was measured at 750 nm. Four replicates were used, and the average absorbance readings were recorded. The amount of protein present in the samples was calculated with a standard graph prepared using bovine serum albumin (BSA) (1 mg/5 ml) of known concentration and was expressed as mg/ml of the seed extract. The amount of total protein was also expressed in mg/g of fresh seeds.

Estimation of total sugar by the anthrone method (Dubois et al. 1951)

One milliliter of freshly prepared seed extract (5 g of seeds in 20 ml of a 60% methanol solution) was put in a test tube. Four milliliters of freshly prepared anthrone reagent was added to the test tube. Then the tube was placed in a boiling-water bath for 10 min, after which it was cooled to RT. Similarly, a blank was measured at 625 nm in a spectrophotometer. Four replicates were used, and the average readings were recorded. Then the amount of total sugars present in the seed extract was estimated as mg of glucose released/ml of extract using a standard graph of glucose (10 mg/100 ml) of known concentration. Finally the amount of total sugars was expressed in mg of glucose released/g of fresh seeds.

Estimation of the total phenolic content (Bray and Thorpe 1954)

One milliliter of a freshly prepared seed extract (2 of seed in 10 ml of a 60% methanol solution) was put in a test tube to which 1 ml of Folin Ciocalteu reagent was added. This was followed by 2 ml of a Na₂CO₃ solution. The test tube was shaken, placed in a boiling-water bath for 1 min, and then cooled under running tap water. The blue



solution was diluted to 25 ml with water. Similarly, a blank was prepared using all reagents except the seed extract. The absorbance was measured at 650 nm in a spectrophotometer. Four replicates were used, and the average readings were recorded. The amount of total phenols present in the seed extract was estimated as mg of catechol released/ml of extract using a standard graph of catechol of known concentration. Finally the amount of total phenol was expressed in mg of catechol released/g of fresh seeds.

Preparation of the seed extract

Two grams of dry ground seed samples was added to 10 ml of 60% methanol and boiled for 3 min. The extraction was repeated three times, each time adding 10 ml of 60% methanol and boiling for 3 min. The extract was filtered, and the volume of filtrate was made up to 50 ml by adding 60% methanol. The extract was used to estimate total sugars and the total phenolic content.

Statistical analysis

A completely randomized design was followed for the experiments, and the data were analyzed by analysis of variance (ANOVA). Variations among the means were compared using a post-hoc Duncan's multiple-range test at $p < 0.05$. To measure the mutual relationship between 2 parameters, correlation coefficients were calculated by subjecting the data to Pearson's product moment correlations.

RESULTS

The results obtained from stored seed samples of *Den. strictus*, *P. emblica*, *H. binata*, and *Dal. latifolia* are presented. Preliminary screening of all 4 seed samples was done. Different fungal organisms were isolated at different storage periods from different containers. Mycoflora of different seed samples are presented in Tables 1-12.

Seed mycoflora under storage conditions

Fungal species of the genera *Aspergillus*, *Fusarium*, *Chaetomium*, *Penicillium*, *Rhizopus*, *Myrothecium*, and *Dreschlera* were recorded on seeds of *Den. strictus* in varying extents under different storage conditions. Out of 4 *Aspergillus* species recorded, *A. flavus* and *A. niger* were found to be frequently associated throughout the storage period at RT. *Aspergillus nidulans* and *A. ochraceous* were found at lower percentages.

Out of the different containers tested, seeds stored in cotton bags and paper-covered containers harbored more fungi compared to polythene-covered and glass containers. The seed germination percentage also rapidly dropped in cotton bags and paper-covered containers compared to polythene-covered and glass containers. The rate of germination also decreased with an increase in the storage period. *Dendrocalamus strictus* seeds stored at 5°C maintained their viability for a long period and were less prone to fungal attack. Although minimal fungal invasion was recorded on seeds stored at 40°C, they lost their viability within 9 mo. More fungal species were recorded on seeds stored at RT.

Fifteen fungal species were recorded on seeds of *P. emblica*, including *Aspergillus*, *Fusarium*, *Chaetomium*, *Penicillium*, *Rhizopus*, *Phoma*, and *Trichothecium*. In total, 8 *Aspergillus* species were recorded, and most of these persisted throughout the



storage period. *Curvularia* and *Fusarium* spp. gradually disappeared with an increase in the storage period. *Phoma exigua*, an important seed-borne fungus, was recorded at a low percentage. Fungal invasion was greater in seeds stored at RT and in pervious containers like cotton bags and paper-covered containers than seeds stored at 5°C and 40°C and in impervious containers like polythene-covered and glass containers. *Aspergillus niger* was recorded at all storage temperatures, but was optimum at RT. With 5°C storage, growth of fungi was very slow. *Fusarium solani* was recorded even in the 12th month of storage, due to slow and reduced growth of other fungi, which made way for the growth of *Fusarium*. Loss of viability was rapid in seeds stored at 40°C followed by seeds stored at RT.

Ten fungal species were recorded on seeds of *Dal. latifolia* at RT like *A. niger*, *A. flavus*, *A. flavus-columnaris*, *A. flavus-oryzae*, *A. versicolor*, *Penicillium* sp., *Phoma* sp., *Fusarium moniliforme*, and *Trichothecium roseum*. *Aspergillus niger*, *A. flavus*, and *A. versicolor* were dominant along with *Penicillium* spp. *Phoma* and *Fusarium* were recorded in the early period but gradually disappeared with an increase in the storage period as the dominance of fungi increased. The germination percentage rapidly decreased in seeds stored at 40°C. A storage temperature of 40°C showed an adverse effect on the viability of seeds.

Eight fungal species were recorded on seeds of *H. binata*, and most of the recorded fungi were storage-related fungi except *Fusarium* and *Rhizopus*. All these storage-related fungi rapidly increased with an increase in the storage period at RT, but gradually increased at 5°C. At RT with an increase in the storage period, seeds were found to be completely colonized by these fungi and showed no germination.

Effect of storage period on the germination percentage

Germination percentages of the 4 seed samples in 4 different containers were evaluated. There was a gradual change over 12 mo of storage. This change in germination percentage varied from container to container. The results are presented in Tables 1-12.

At RT, the initial germination percentage of *Den. strictus* seeds was 55% but decreased to 20, 11, 16, and 8% in polythene-covered containers, cotton bags, glass containers, and paper-covered containers, respectively. The initial germination percentage of *P. emblica* seeds was 32% but decreased to 18, 12, 16, and 11%. In *H. binata* seeds, germination percentages decreased to 30, 20, 24, and 16% from 52%. In *Dal. latifolia* seeds, germination percentages decreased to 17, 7, 12, and 10% from 40% in polythene-covered container, cotton bags, glass containers, and paper-covered containers, respectively. In all cases, a decrease in the germination percentage with an increase in fungal numbers was observed.

Effect of the storage period on the MC of seeds

In the present investigation, seeds had high MCs in the initial period, but these gradually decreased with an increase in the storage period (Figs. 1-4). Fluctuations were observed in the MCs of seeds stored in cotton bags and paper-covered containers.

Table 1. Percentage frequency of fungi isolated from seeds of *Dendrocalamus strictus* by a standard blotter method after being stored at 5°C

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Alternaria alternata</i>	7	6	8	6	10	2	4	-	3	-	-	-	-	-	-	-	-
<i>Aspergillus flavus</i>	3	-	2	-	-	-	6	-	2	-	2	-	-	-	6	-	4
<i>A. nidulans</i>	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. niger</i>	5	-	-	-	3	-	2	-	-	-	-	-	-	-	8	-	6
<i>Chaetomium globosum</i>	2	-	9	-	-	-	-	-	-	4	11	-	8	-	-	-	6
<i>Curvularia lunata</i>	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Drechslera hawaiiensis</i>	8	4	16	11	12	-	12	2	2	-	-	-	-	-	-	-	-
<i>Fusarium moniliforme</i>	11	15	26	13	18	3	8	-	6	-	1	2	2	-	-	-	-
<i>F. oxysporum</i>	6	10	32	14	20	11	18	6	14	-	9	3	3	-	-	-	-
<i>Myrothecium roridum</i>	8	-	-	-	11	-	2	-	4	-	8	-	8	-	-	-	2
<i>Penicillium</i> sp.	-	2	-	-	-	6	4	-	-	-	-	-	-	-	-	1	3
<i>Rhizopus</i> sp.	-	-	-	-	-	-	4	1	8	2	5	-	-	-	-	-	-
<i>Trichothecium roseum</i>	-	-	-	-	-	-	-	-	2	6	18	7	16	8	22	11	18
Germination %	55	69	60	70	61	62	59	65	56	42	33	46	35	32	20	28	25

Data are based on the average of 4 replicates consisting of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.

Table 2. Percentage frequency of fungi isolated from seeds of *Dendrocalamus strictus* by a standard blotter method after being stored at 26 ± 2°C

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Alternaria alternata</i>	7	8	20	10	18	6	8	8	14	-	4	-	11	-	-	-	-
<i>Aspergillus flavus</i>	3	7	19	7	16	18	24	18	29	20	29	21	27	21	36	24	33
<i>A. nidulans</i>	-	-	-	-	-	-	3	8	-	13	17	9	20	15	29	12	24
<i>A. niger</i>	5	8	13	11	15	20	28	23	26	21	22	24	34	24	36	25	40
<i>A. ochraceous</i>	-	-	-	-	-	-	-	-	-	2	10	-	6	-	14	-	-
<i>Chaetomium cryspatum</i>	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium globosum</i>	2	-	-	-	4	2	-	-	-	-	-	-	-	-	-	-	-
<i>Curvularia lunata</i>	4	8	16	7	17	-	6	4	4	-	-	-	-	-	-	-	-
<i>Drechslera hawaiiensis</i>	8	14	19	9	21	6	12	2	10	-	-	-	-	-	-	-	-
<i>Fusarium moniliforme</i>	11	2	12	10	16	-	-	-	8	-	-	-	-	-	-	-	-
<i>F. oxysporum</i>	6	12	20	11	18	5	12	-	-	2	-	-	-	-	-	-	-
<i>Myrothecium roridum</i>	8	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Penicillium</i> sp.	-	-	-	-	-	9	2	-	14	17	22	15	26	16	32	15	29
<i>Rhizopus</i> sp.	-	-	-	-	2	6	16	-	18	-	7	-	9	-	-	-	-
<i>Trichothecium roseum</i>	-	-	-	-	-	-	10	4	12	3	15	4	11	6	16	12	21
Germination %	55	60	52	56	46	49	36	51	42	33	28	31	26	20	11	16	8

Data are based on the average of 4 replicates consisting of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.



Table 3. Percentage frequency of fungi isolated from seeds of *Dendrocalamus strictus* by a standard blotter method after being stored at 40°C

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Alternaria alternata</i>	7	2	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus flavus</i>	3	11	16	10	14	10	12	2	6	-	2	-	-	-	-	-	3
<i>A. nidulans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. niger</i>	5	6	12	11	14	13	22	-	10	-	1	-	2	-	4	-	6
<i>Chaetomium globosum</i>	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
<i>Curvularia lunata</i>	4	-	7	-	-	-	-	-	3	-	-	-	-	-	-	-	-
<i>Drechslera hawaiiensis</i>	8	4	6	2	7	-	4	-	-	-	-	-	-	-	-	-	-
<i>Fusarium moniliforme</i>	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>F. oxysporum</i>	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Myrothecium roridum</i>	8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhizopus</i> sp.	-	-	-	-	-	-	2	-	2	-	4	-	6	-	2	-	5
Germination %	55	52	40	30	33	20	17	12	13	12	10	5	9	1	-	-	-

Data are based on the average of 4 replicates of 200 seeds each. T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.

Table 4. Percentage frequency of fungi isolated from seeds of *Phyllanthus emblica* by a standard blotter method after being stored at 5°C

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Aspergillus candidus</i>	-	-	-	-	-	-	-	-	-	-	2	-	12	-	4	2	6
<i>Aspergillus flavus</i>	6	-	4	-	8	-	-	-	12	-	9	-	8	2	11	3	13
<i>A. flavus</i> pv. <i>columnaris</i>	1	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-	-
<i>A. niger</i>	4	6	8	-	2	-	-	-	3	-	-	-	1	-	4	-	2
<i>A. versicolor</i>	6	2	-	6	-	-	6	-	-	-	2	-	4	-	4	-	3
<i>Chaetomium indicum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Curvularia lunata</i>	-	4	6	-	12	18	17	2	19	6	19	6	7	-	5	-	-
<i>Drechslera hawaiiensis</i>	1	7	12	-	4	12	18	-	-	6	2	-	-	-	-	-	-
<i>Fusarium moniliforme</i>	2	6	24	12	18	14	20	6	16	3	12	2	4	-	3	-	2
<i>Phoma exigua</i>	-	2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhizopus</i> sp.	-	-	-	-	-	2	-	4	-	-	-	2	-	-	-	-	-
Germination %	32	52	56	48	60	56	50	48	51	42	36	40	32	32	26	28	20

Data are based on the average of 4 replicates of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.

Table 5. Percentage frequency of fungi isolated from seeds of *Phyllanthus emblica* by a standard blotter method after being stored at $26 \pm 2^\circ\text{C}$

Seed mycoflora	0	3 mo				6 mo				9 mo				12 mo			
	day	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Aspergillus candidus</i>	-	6	12	-	2	13	11	2	6	4	7	-	8	22	14	5	8
<i>Aspergillus flavus</i>	6	10	18	12	15	14	22	18	10	22	26	24	16	18	30	32	22
<i>A. flavus</i> pv. <i>columnaris</i>	1	-	9	-	12	3	4	6	10	14	11	8	22	12	15	12	20
<i>A. flavus</i> pv. <i>oryzae</i>	-	-	-	-	4	2	8	2	9	12	12	6	13	10	14	8	10
<i>A. fumigatus</i>	4	-	12	9	16	6	10	-	15	4	14	6	18	12	18	12	19
<i>A. nidulans</i>	-	-	-	-	-	-	-	-	8	2	5	-	12	13	8	4	17
<i>A. niger</i>	6	9	12	12	10	6	14	12	15	14	22	12	21	12	28	18	23
<i>A. versicolor</i>	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	4
<i>Chaetomium indicum</i>	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
<i>Curvularia lunata</i>	1	6	8	2	12	4	14	6	15	-	2	-	1	-	-	-	-
<i>Fusarium moniliforme</i>	2	6	5	-	8	12	18	-	12	-	-	-	-	-	-	-	-
<i>Penicillium</i> sp.	-	-	-	-	-	-	8	-	6	2	12	4	12	8	15	12	20
<i>Phoma exigua</i>	-	-	-	-	-	-	-	-	3	-	6	-	7	-	12	-	11
<i>Rhizopus</i> sp.	-	-	2	-	-	11	16	8	8	6	12	2	10	-	2	-	-
<i>Trichothecium roseum</i>	-	-	-	-	-	-	-	-	-	2	6	8	9	4	11	8	12
Germination %	32	49	42	46	52	62	52	53	60	34	27	29	32	18	12	16	11

Data are based on the average of 4 replicates of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.

Table 6. Percentage frequency of fungi isolated from seeds of *Dendrocalamus strictus* by a standard blotter method after being stored at 40°C

Seed mycoflora	0	3 mo				6 mo				9 mo				12 mo			
	day	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Aspergillus flavus</i>	6	-	12	2	10	3	6	-	16	-	10	-	16	-	-	-	-
<i>A. flavus</i> pv. <i>columnaris</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. fumigatus</i>	4	-	-	2	-	1	2	4	-	-	-	-	1	-	-	-	-
<i>A. niger</i>	6	6	4	-	11	2	8	-	4	4	2	-	-	6	3	-	2
<i>Curvularia lunata</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Fusarium moniliforme</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhizopus</i> sp.	-	5	-	3	-	2	-	4	-	-	-	-	-	-	-	-	-
Germination %	32	26	20	16	18	18	12	9	6	7	5	-	-	-	-	-	-

Data are based on the average of 4 replicates of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.



Table 7. Percentage frequency of fungi isolated from seeds of *Hardwickia binata* by a standard blotter method after being stored at 5°C

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Aspergillus flavus</i>	8	-	3	-	-	-	-	-	4	-	-	-	-	-	3	-	1
<i>Aspergillus flavus</i> pv. <i>oryzae</i>	4	-	2	-	1	-	-	-	1	-	3	-	-	-	1	-	4
<i>A. nidulans</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. niger</i>	6	3	2	11	-	-	3	-	-	-	-	-	1	-	2	-	-
<i>Fusarium moniliforme</i>	1	4	11	6	13	12	19	2	7	3	8	-	9	-	-	-	-
<i>Penicillium</i> sp.	2	-	4	1	2	-	-	-	-	-	1	-	-	-	2	2	1
Germination %	52	49	56	46	42	52	54	47	40	40	39	36	39	36	32	29	26

Data are based on the average of 4 replicates of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.

Table 8. Percentage frequency of fungi isolated from seeds of *Hardwickia binata* by a standard blotter method after being stored at 26 ± 2°C

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Aspergillus flavus</i>	8	6	8	10	13	12	16	11	17	9	21	16	6	11	28	10	15
<i>Aspergillus flavus</i> pv. <i>oryzae</i>	4	12	7	-	4	6	9	2	10	13	13	5	2	2	15	4	5
<i>A. fumigatus</i>	-	-	-	-	-	-	12	-	2	6	11	3	9	5	10	14	4
<i>A. nidulans</i>	1	-	5	2	4	-	14	-	9	4	13	9	15	12	14	13	13
<i>A. niger</i>	6	6	10	15	12	11	17	9	15	6	23	6	19	12	29	14	24
<i>Fusarium moniliforme</i>	1	16	12	2	2	-	8	4	-	-	2	-	-	-	-	-	-
<i>Penicillium</i> sp.	2	6	11	-	-	13	20	-	8	22	24	8	17	20	20	11	22
Germination %	52	50	53	50	47	46	42	38	42	40	32	36	26	30	20	24	16

Data are based on the average of 4 replicates of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.

Table 9. Percentage frequency of fungi isolated from seeds of *Hardwickia binata* by a standard blotter method after being stored at 40°C

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Aspergillus flavus</i>	8	-	-	-	-	2	3	-	-	-	4	-	3	-	2	-	-
<i>Aspergillus flavus</i> pv. <i>oryzae</i>	4	-	6	-	-	-	1	-	2	-	-	-	1	-	-	-	-
<i>A. nidulans</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. niger</i>	6	-	6	-	-	-	8	-	4	1	2	-	-	-	3	-	3
<i>Fusarium moniliforme</i>	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Penicillium</i> sp.	2	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
Germination %	52	30	36	20	22	22	20	15	10	15	10	4	8	5	2	-	-

Data based on the average of 4 replicates 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.

Table 10. Percentage frequency of fungi isolated from seeds of *Dalbergia latifolia* by a standard blotter method after being stored at 5°C

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Aspergillus flavus</i>	7	2	4	-	6	-	2	-	4	-	6	-	8	-	8	-	12
<i>A. flavus-columnaris</i>	2	-	-	-	-	-	-	-	-	-	6	-	4	-	9	-	11
<i>Aspergillus flavus</i> pv. <i>oryzae</i>	5	-	-	-	-	-	2	-	-	-	7	-	-	-	2	-	-
<i>A. niger</i>	6	2	-	4	-	-	6	2	4	-	12	4	7	2	8	1	4
<i>A. nidulans</i>	4	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-
<i>Fusarium moniliforme</i>	4	-	14	3	20	-	21	8	24	-	24	17	26	7	18	19	21
<i>Penicillium</i> sp.	1	-	-	-	2	-	-	-	-	-	12	-	14	-	8	-	15
<i>Phoma</i> sp.	5	-	-	-	4	-	2	-	6	-	11	-	14	-	13	-	17
<i>Trichothecium roseum</i>	3	-	-	-	4	-	2	-	-	-	12	-	14	-	8	-	15
Germination %	40	47	36	42	39	42	32	38	28	38	30	35	22	30	24	27	20

Data are based on the average of 4 replicates of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.



Table 11. Percentage frequency of fungi isolated from seeds of *Dalbergia latifolia* by a standard blotter method after being stored at $26 \pm 2^\circ\text{C}$

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Aspergillus flavus</i>	7	12	14	10	16	16	24	15	15	20	24	12	20	25	30	16	29
<i>A. flavus</i> pv. <i>columnaris</i>	2	-	4	-	6	-	2	-	9	2	14	4	10	5	10	5	12
<i>Aspergillus flavus-oryzae</i>	5	-	2	-	11	-	8	3	6	4	10	4	8	9	14	11	16
<i>A. niger</i>	6	10	14	12	10	11	14	12	12	14	21	18	15	18	20	23	24
<i>A. versicolor</i>	4	6	4	3	2	8	11	6	3	10	15	2	11	6	13	7	8
<i>Fusarium moniliforme</i>	4	8	13	11	10	2	6	-	3	-	-	-	-	-	-	-	-
<i>Penicillium</i> sp.	1	14	8	7	6	9	17	11	11	3	15	4	12	5	11	10	17
<i>Phoma</i> sp.	5	7	9	4	8	2	6	-	-	2	-	-	-	-	-	-	-
<i>Trichothecium roseum</i>	3	-	6	-	-	-	6	2	10	-	2	-	4	-	-	-	-
Germination %	40	36	30	32	32	32	22	27	24	26	12	19	16	17	7	12	10

Data are based on the average of 4 replicates of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.

Table 12. Percentage frequency of fungi isolated from seeds of *Dalbergia latifolia* by a standard blotter method after being stored at 40°C

Seed mycoflora	0 day	3 mo				6 mo				9 mo				12 mo			
		T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4	T1	T2	T3	T4
<i>Aspergillus flavus</i>	7	6	9	-	11	-	13	2	17	2	8	-	-	-	-	-	-
<i>A. flavus</i> pv. <i>columnaris</i>	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus flavus</i> pv. <i>oryzae</i>	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>A. niger</i>	6	2	10	4	7	-	6	-	2	-	2	-	4	-	4	-	2
<i>A. nidulans</i>	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Fusarium moniliforme</i>	4	-	1	-	8	-	-	-	-	-	-	-	-	-	-	-	-
<i>Penicillium</i> sp.	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Phoma</i> sp.	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Trichothecium roseum</i>	3	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Germination %	40	37	36	28	31	26	20	14	21	15	12	6	10	10	7	-	4

Data are based on the average of 4 replicates of 200 seeds each.

T1, polythene-covered container; T2, cotton bag; T3, glass container; T4, paper-covered container.

Fig 1: Effect of storage period on Moisture content of seeds of *Dendrocalamus strictus*

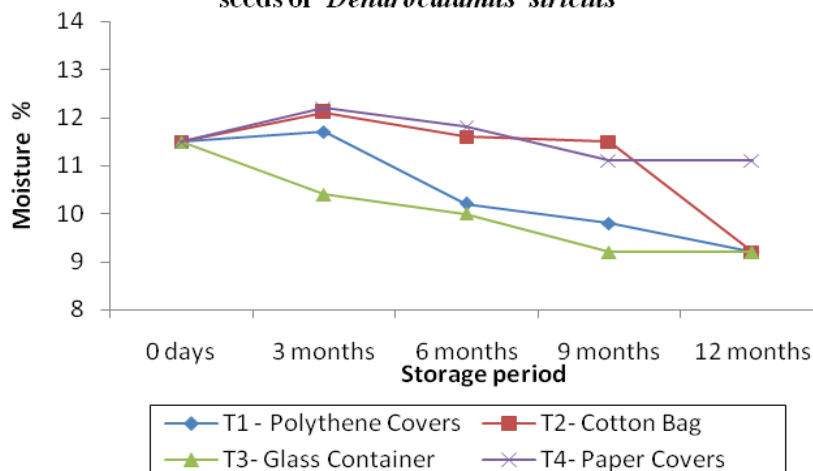


Fig 2: Effect of storage period on Moisture content of seeds of *Phyllanthus emblica*

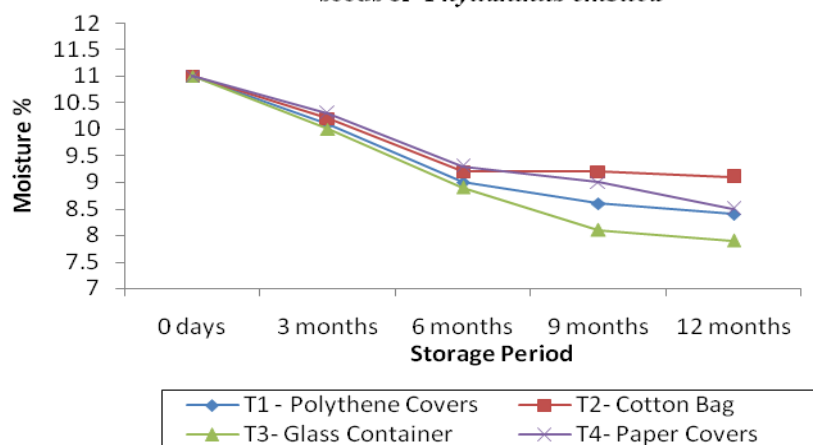


Fig 3: Effect of storage period on Moisture content of seeds of *Hardwickia binata*

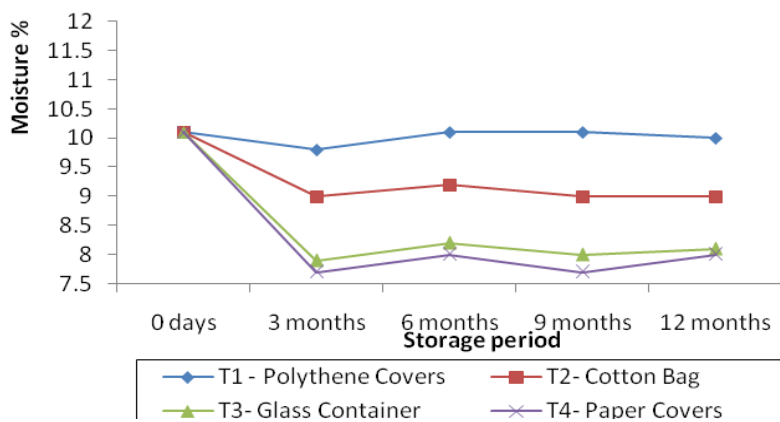
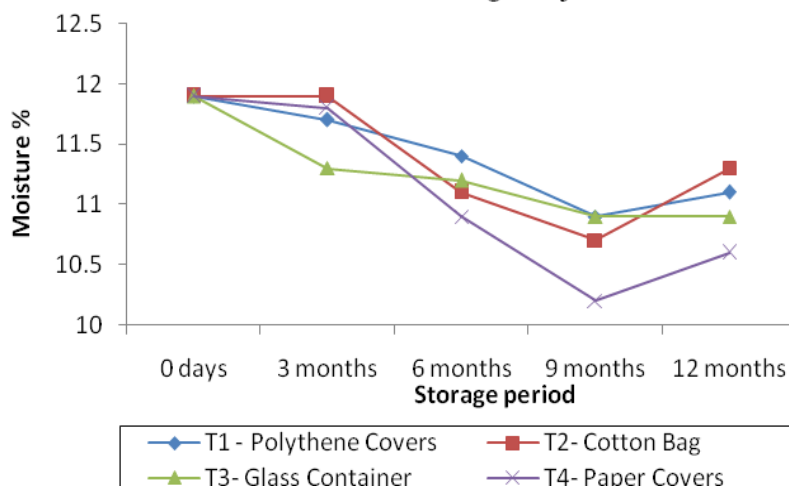




Fig 4: Effect of storage period on Moisture content of seeds of *Dalbergia latifolia*



Effect of storage period on the protein content of seeds

Protein contents of the 4 seed samples showed gradual changes during 12 mo of storage in different containers at RT (Figs. 5-8). The protein contents of *Den. strictus*, *P. emblica*, *H. binata*, and *Dal. latifolia* seed samples were recorded at 3-mo intervals. The initial protein content of *Den. strictus* was 2.00 mg/g of seeds, but this dropped to 0.50 mg/g of seeds in polythene-covered containers and to 0 mg/g of seeds in the cotton bags, glass containers, and paper-covered containers. The initial protein content of *P. emblica* seeds was 13.75 mg/g of seeds, but this dropped to 9.50, 8.50, 9.00, and 8.25 mg/g of seeds in the polythene-covered containers, cotton bags, glass containers, and paper-covered containers, respectively. The protein content of *H. binata* seeds was reduced from 33.00 mg/g of seeds to 25.00, 22.00, 24.75, and 23.75 mg/g of seeds, and the initial protein content of *D. latifolia* was 30.25 mg/g of seeds but decreased to 24.00, 22.75, 23.12, and 22.10 mg/g of seeds in polythene-covered containers, cotton bags, glass containers, and paper-covered containers, respectively. In all cases, the protein content decreased with an increase in fungal numbers.

Fig 5: Effect of storage period on Protein content of seeds of *Dendrocalamus strictus*

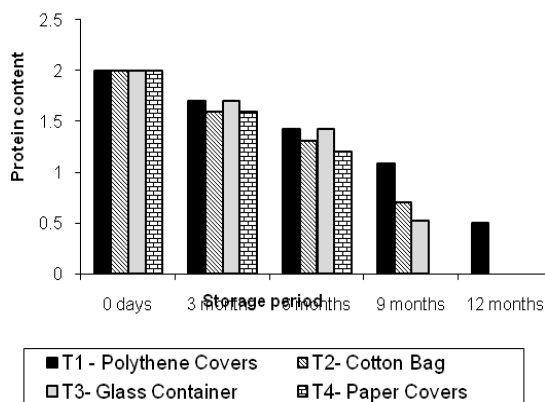


Fig 6: Effect of storage period on Protein content of seeds of *Hyllanthus emblica*

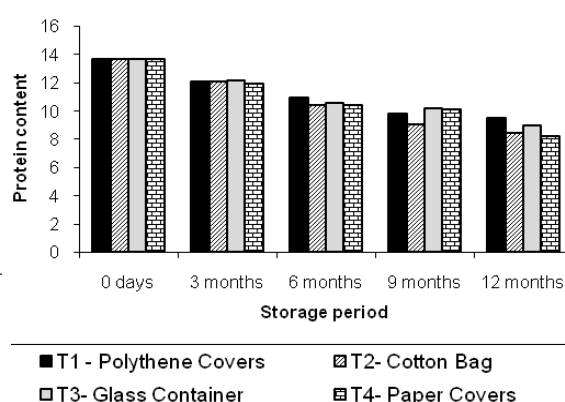




Fig 7: Effect of storage period on Protein content of seeds of *Hardwickia binata*

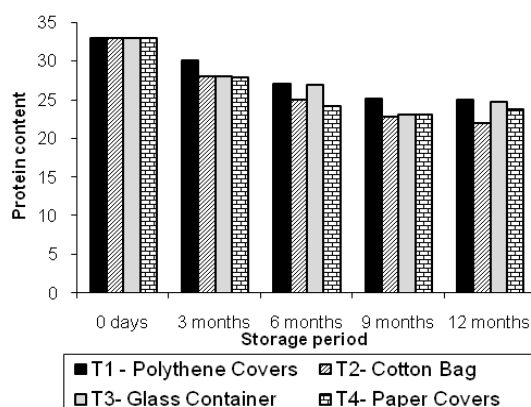
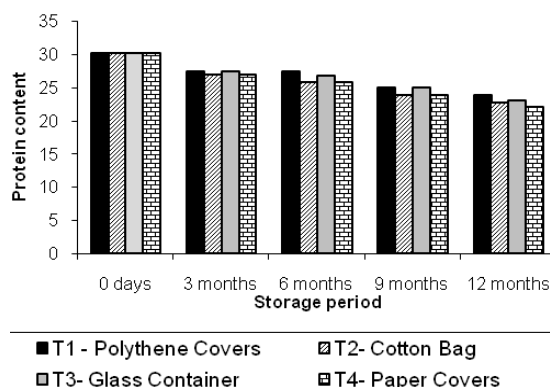


Fig 8: Effect of storage period on Protein content of seeds of *Dalbergia latifolia*



Effects of storage period on the total sugar content of seeds

The total sugar contents of the 4 seed samples in 4 different types of containers showed variations during the 12 mo of storage, and results are presented in Figs. 9-12. All seed samples showed gradual decreases in the total sugar content. The initial sugar content was calculated before and after storage at 3-mo intervals in different containers. In *Den. strictus*, the initial sugar content was 71.25 mg/g of seeds but dropped to 17.5, 12.50, 21.25, and 12.5 mg/g of seeds; in *P. emblica*, it was 62.00 mg/g of seeds but dropped to 22.00, 15.50, 21.75, and 16.25 mg/g of seeds; in *H. binata*, it was 120.00 mg/g of seeds but dropped to 45.00, 35.50, 46.00, and 32.50 mg/g seeds; and in *Dal. latifolia*, the initial sugar content was 87.50 mg/g of seeds but dropped to 42.00, 28.00, 38.00, and 32.50 mg/g of seeds in the polythene-covered containers, cotton bags, glass containers, and paper-covered containers, respectively. A decrease in the total sugar level was observed in all cases with an increase in the storage period.

Fig 9: Effect of storage period on Total sugar content of seeds of *Dendrocalamus strictus*

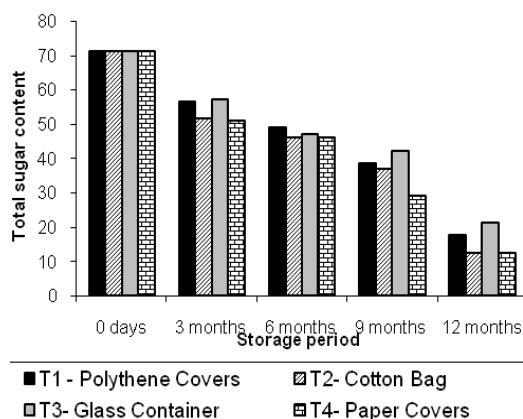


Fig 10: Effect of storage period on Total sugar content of seeds of *Phyllanthus emblica*

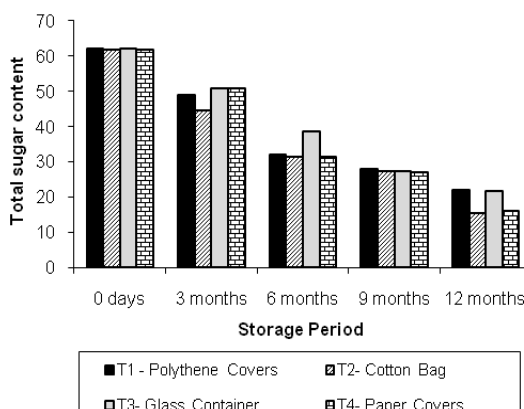




Fig 11: Effect of storage period on Total sugar content of seeds of *Hardwickia binata*

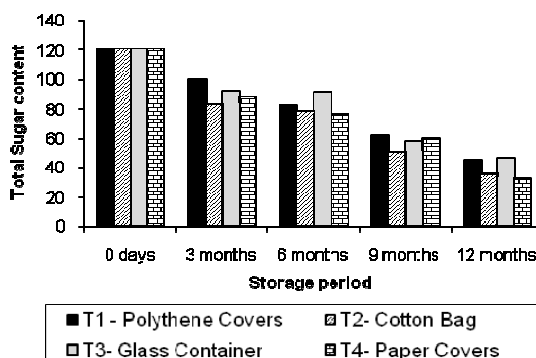
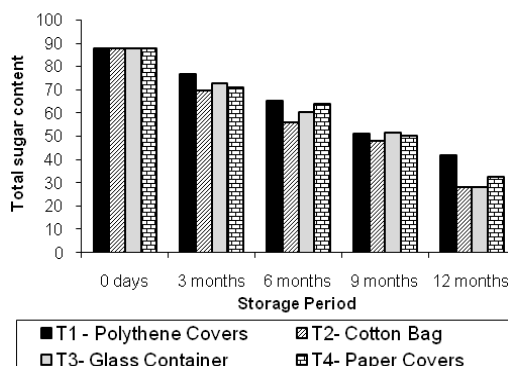


Fig 12: Effect of storage period on Total sugar content of seeds of *Dalbergia latifolia*



Effects of the storage period on the total phenolic content of seeds

Phenol contents of the 4 seed samples showed variations during 12 mo of storage. The results are presented in Figs. 13-16.

In *Den. strictus* the initial phenol content was 1.50 mg/g of seeds but this decreased to 0.25, 0.5, 0.37, and 0.25 mg/g of seeds at the end of the storage period in polythene-covered containers, cotton bags, glass containers, and paper-covered containers, respectively. In *P. emblica*, the initial phenol content was 5.25 mg/g of seeds, and this slightly increased in the initial storage period but later gradually decreased to 4.45, 4.75, 5.00, and 4.60 mg/g of seeds; in *H. binata*, the initial phenol content was 4.75 mg/g of seeds, but this decreased to 2.00, 1.87, 2.12, and 2.00 mg/g of seeds; and in *Dal. latifolia*, the initial phenol content decreased from 2.50 mg/g of seeds to 1.25, 1.00, 1.37, and 1.25 mg/g of seeds, respectively.

Fig 13: Effect of storage period on Phenolic content of seeds of *Dendrocalamus strictus*

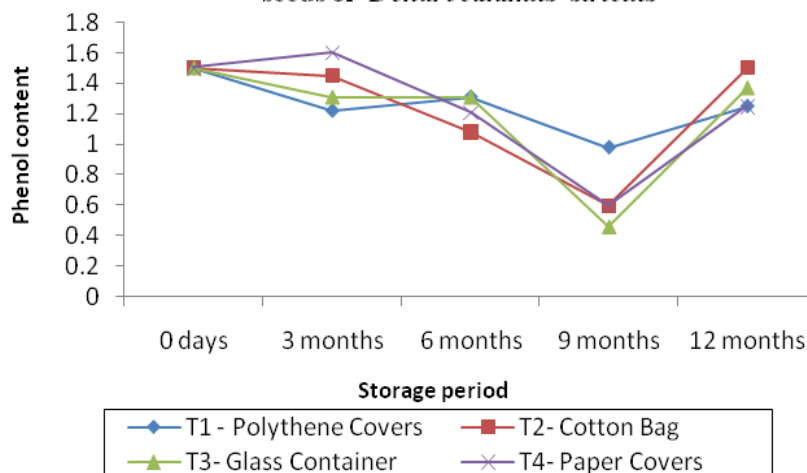


Fig 14: Effect of storage period on Phenolic content of seeds of *Phyllanthus emblica*

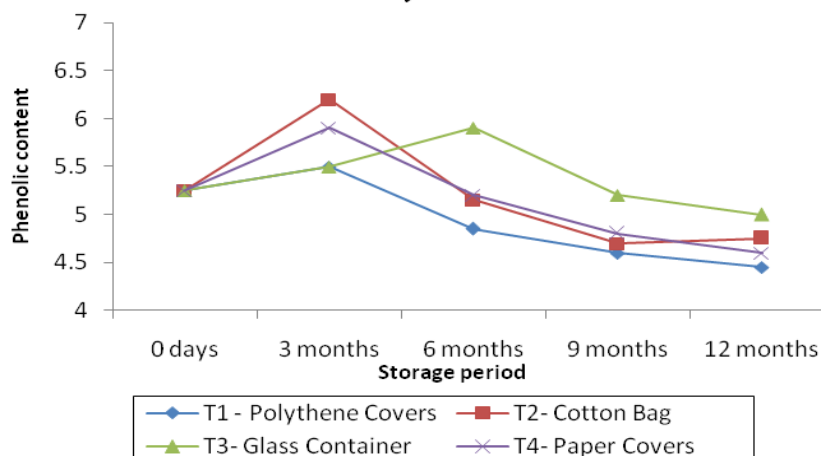


Fig 15: Effect of storage period on Phenolic content of seeds of *Hardwickia binata*

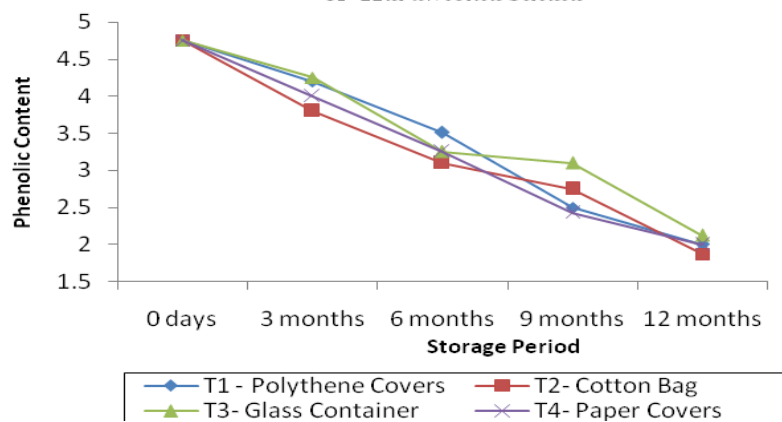
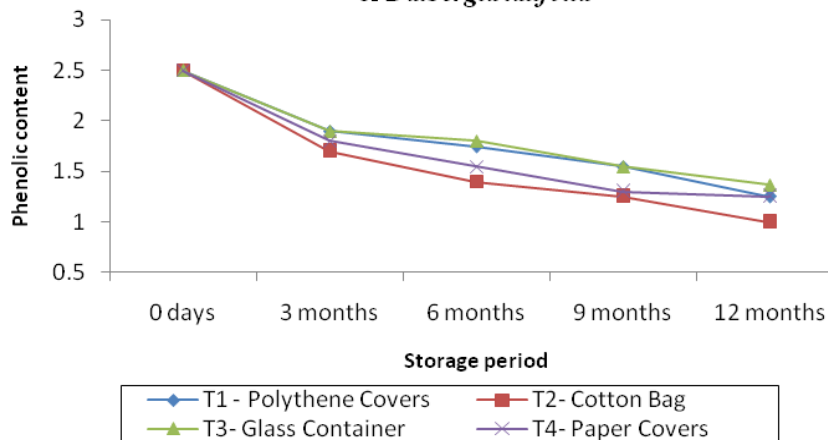


Fig 16: Effect of storage period on Phenolic content of seeds of *Dalbergia latifolia*





DISCUSSION

As seeds are major components of propagation, the study of seed mycoflora, health, and vigor is important. Biochemical studies can help in developing proper strategies for the storage of seeds. Seeds of forest trees are frequently contaminated with mycoflora, which are capable of producing mycotoxins and damaging the seeds. The microorganisms present on seeds play an important role because they cause seed decay. Fungal invasion of seeds in storage results in discoloration, reduced germinability, production of mycotoxins, mustiness, and ultimately decay (Christensen 1973).

In the present study it was interesting to note that significant differences existed in the fungal flora of the 4 seed samples tested. It was also evident from the results that *Aspergillus* spp. were the most frequently occurring fungi, as they were present in all samples. Sinnaiah et al. (1983) studied the seed mycoflora of *Azadirachta indica* during storage and found them to be highly contaminated by fungi, most of which were *Aspergillus* species. *Aspergillus niger* was commonly associated with all seed samples. Interestingly *A. niger* could thrive at all of the temperatures tested, but the extent of survival varied. This shows that it has a tolerance to a wide range of temperatures. At RT, the growth was optimum, at 5°C the growth was slow, but at 40°C it showed a lower frequency of occurrence. Among seed samples stored at RT and at 5°C, the fungal numbers increased along with the storage period, which is similar to observations made by earlier workers (Komaraiah and Reddy 1985). With a longer storage period, storage-related fungi increased with a simultaneous decrease in field-associated fungi. This however does not mean that the latter were eliminated, but it could be that storage-related fungi are more suited to those conditions and outcompeted the others.

A decrease in the germination percentage was observed during storage. Seed-borne fungi affect the germinability of seeds. At the time of harvest, seeds are considered to have the maximum potential for survival. Thereafter, most seeds undergo certain irreversible changes that reduce the survival capacity and lead to loss of vigor and germinability (Feliciano et al. 1987, Maithani et al. 1989). In the present study, variations in the reduction of seed germination in the seed samples were observed. This was because the rate of deterioration varied among the kinds of seeds, seed lots, and among individual seeds within a seed lot. A reduction in the germination percentage of seeds, as observed in the present study, can be attributed to extensive fungal growth and toxic metabolites produced by storage-related fungi. This result is similar to those for *Artocarpus heterophyllus* by Rekha et al. (2007).

In the present study, less fungal invasion with better retention of the germination rate was seen among seeds stored at 5°C in impervious containers like polythene-covered and glass containers. Similar observations were made by Bhardwaj et al. (2001). Storage of seeds in polythene-covered and glass containers at 5°C was found to be suitable to retain the germination potential for a longer period in all 4 forest tree species seeds tested. Gupta and Sood (1978) recommended storage of *Den. strictus* seeds in sealed glass bottles at 3°C in a refrigerator. Somen and Seethalakshmi (1989) suggested storage of *Bambusa arundinacea* seeds at low temperatures. However as most of the forest tree seeds are large and the seeds are collected in huge amounts, storage in refrigerators would be expensive and impractical. For warehouse storage, any of the above mentioned techniques cannot be directly applied. Therefore, the results of the present study indicate

that storage of seeds in polythene-covered containers or polylined bags may be economically feasible and practical, as these conditions reduced microbial growth and extended the viability period of the seeds considered.

The MC of seeds plays an important role in determining the longevity under different storage conditions (Mondal et al. 1981, Nandi 1982). In the present investigation, seeds had high MCs before storage, which decreased during 12 mo of storage. Similar observations were made in pulses by Dhand (1980) and in neem by Bhardwaj and Chand (1995). The decrease in the MC of stored seeds may be due to the prevailing atmospheric conditions (Vaidehi 1999).

Fungi associated with seeds are known to bring about certain biochemical changes in the seeds during storage, such as degrading the seed constituents like proteins, sugars, and phenols. During storage, changes in seed contents are influenced by existing fungi and differences in storage conditions (Mondal et al. 1981).

In the present study, samples analyzed during storage showed lower protein contents than before storage, similar to observations made by Mahadevan et al. (1982) and Nandi (1982). This decrease in protein might have been due to enzymatic degradation into simpler components, which are subsequently utilized by fungi (Mahadevan et al. 1982).

A gradual decrease in the total sugar content was observed among all the different seed samples tested. This loss of total sugars intensified with prolonged storage, which is in line with observations made by earlier workers (Nandi et al. 1988, Vaidehi 1999). With an increase in storage-related fungi, there was a significant decrease in the sugar content, which indicates that storage-related fungi are capable of catabolic activities and utilize the breakdown products for their growth and development.

Decreases in the phenolic contents were observed in seeds of *Den. strictus*, *Dal. latifolia*, and *H. binata*. This may have been due to greater invasion by fungi, as fungal numbers were found to be high. A decrease in the phenol content might have been due to enzymatic degradation into simpler components, which were subsequently utilized by fungi (Mahadevan et al. 1982). Similarly a remarkable decrease in the phenolic content of chickpea seedlings was observed when inoculated with *Botrytis cinerea*, indicating that fungal invasion decreases the phenolic content (Mitter et al. 1997). In the present study, there was an initial increase in the phenolic content in seeds of *P. emblica*. Similarly, accumulation of phenols in *Medicago sativa* against *Colletotrichum trifolii* was observed by Baker et al. (1989). This increase in the phenolic content in the initial stage might have been due to the release of phenolic compounds to resist fungal growth. But later as the fungal invasion advanced, there was a decrease in the phenolic content.

From the present studies, it was observed that seeds stored at 5°C showed less fungal invasion compared to seeds stored at RT (26 ± 2°C) and 40°C. Seeds stored in impervious containers like polythene-covered and glass containers showed less bio-deterioration and increased germination compared to seeds stored in cotton bags and paper-covered containers. Therefore, we recommend that seeds of forest trees such as *Den. strictus*, *P. emblica*, *H. binata*, and *Dal. latifolia* be stored at 5°C and in impervious containers like polythene-covered containers or glass containers.



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Effects of Light, Temperature and Applied Chemicals on Laboratory Germination of *Pellacalyx yunnanensis* Seeds

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[Summary]

Pellacalyx yunnanensis Hu is an endangered species endemic to Yunnan Province, southwestern China and is protected by the Chinese government. In this paper, the effects of temperature, light, phytohormones, and nitric oxide (NO) on the germination responses of *P. yunnanensis* seeds are reported. Germination percentages were almost 0 after 44 d of imbibition in the dark at constant (15, 20, 25, 30, 35, and 40°C) and fluctuating temperatures (daytime/nighttime, 15/10, 20/10, 25/10, 30/10, 35/10, and 40/10°C). However, germination occurred when these seeds were transferred to a constant temperature and alternating photoperiod (14 h of light at 12 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 10 h of dark) at either constant or fluctuating temperatures. Under an alternating photoperiod, the most favorable constant temperature for seed germination was about 30°C, and at fluctuating temperatures the most favorable temperatures were the 30/25, 30/20, 30/15, and 30/10°C regimes. Seeds germinated at 30/20°C with an alternating photoperiod or 30/20°C in darkness, but the germination rate of seeds was much faster at 30/20°C with an alternating photoperiod, than at 30/20°C in darkness, with mean germination times of 21.41 and 38.80 d, respectively. Seed germination was influenced by the light intensity, and the most effective light intensity was 10~15 $\mu\text{mol m}^{-2} \text{s}^{-1}$. With an alternating photoperiod of 30/20°C, seed germination was not stimulated by 0.005~0.1 mM gibberellin (GA_3), 0.001~1 mM 6-benzyladenine (6-BA), or 0.01 or 0.025 mM sodium nitroprusside (SNP, an NO donor), but was inhibited by 1 mM GA_3 , 10 mM 6-BA, and 0.05~1.0 mM SNP. In darkness at 30/20°C, the germination percentage and rate of seeds were not influenced by 0.005~0.1 mM GA_3 , increased with 0.01~1 mM 6-BA, and were significantly inhibited by 1 mM GA_3 and 10 mM 6-BA. It is interesting that the germination percentage and rate both dramatically increased with 0.01, 0.025, 0.05, and 0.1 mM SNP, but decreased with 0.5 and 1.0 mM SNP in the darkness at 30/20°C.

Key words: *Pellacalyx yunnanensis*, seed germination, light, temperature, nitric oxide, hormone.

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Efficacy of a Microbial Consortium on *Acacia nilotica* (L.) Willd. ex. Del Seeds for the Production of Quality Seedlings in the Nursery

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[Summary]

Acacia nilotica (L.) Willd. ex Del. belongs to the family Leguminosae and the subfamily Mimosoideae. Commonly known as babul or Indian gum Arabic tree, it is recognized worldwide as a multipurpose leguminous tree. It was an economically very important plant in early times as a source of tannin, gum, timber, fuel, and fodder. It is also extensively used for timber, firewood, and the production of pulp and paper. The present study was conducted to determine the efficacy of some ecofriendly microbial consortiums containing organic amendments and microbes on the growth of *Acacia nilotica* in the seedling stage. A multifactorial experiment was laid out in a completely randomized design at the nursery of the Forest Pathology Division, Tropical Forest Research Institute, MP, India. In total, 16 treatment combinations were prepared with 2 microorganisms (*Pseudomonas fluorescens* and *Trichoderma harzianum*), an AM fungal inoculum (*Acaulospora larvis*, *Glomus etunicatum*, *G. intraradices*, *G. mosseae*, and *Scutellospora pellucida*), and 2 organic amendments (farmyard manure (FYM) and vermicompost). After 7 d of treatment of seeds, sowing data were recorded as the percentage of seed germination, seedling height, collar diameter, percent seedling survival, percent root colonization, root surface area, number of root nodules, and dry biomass. The recorded data were statistically analyzed. The study revealed that application of AM fungi in combination with vermicompost and *T. harzianum* enhanced seedling height (31.87 cm), collar diameter (4.86 cm), seedling survival (91.66%), seed germination (58.66%), root surface area (176.49 cm²), the number of root nodules (14.67), and root colonization (73.33%) followed by combinations of the other 4. On the basis of the above study, application of AM fungi along with the companion fungus *T. harzianum* and an organic amendment is recommended to boost the growth of *A. nilotica* in the nursery stage.

Key words: AM fungi, microbial consortium, *Pseudomonas fluorescens*, *Trichoderma harzianum*.

INTRODUCTION

Acacia nilotica (L.) Willd. ex Del. belongs to the family Leguminosae and subfamily Mimosoideae. Commonly known as babul or Indian gum Arabic tree, it is recognized worldwide as a multipurpose leguminous tree (National Academy of Sciences 1980). It was economically very important plant in early times as source of tannin, gum,

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timber, fuel, and fodder. It is also extensively used for timber, firewood, and the production of pulp and paper (Gupta 1970, Mahgoub 1979, Nasroun 1979, New 1980). It is a pioneer species which can be easily regenerated from seed and has an irregular flowering pattern, but generally flowers between June and September; seed fall takes place from March to May. A mature tree can produce 2000~3000 pods in a good fruiting season, each with 8~16 seeds, yielding 5000~16,000 seeds/kg depending on the subspecies (Ybirk 1989). The germination time is 1~3 wk, and germination rates vary at 5~97% (Dwivedi 1993).

Several microorganisms, known as biofertilizers, are beneficial for plant growth due to different mechanisms, e.g., solubilization of insoluble phosphates, enhancement of solute uptake, antagonism to disease organisms, production of growth hormones, etc. These microbes may be bacteria, actinomycetes, or fungi. The population of microorganisms increases in the rhizosphere, where the population of mycorrhizal roots (mycorrhizosphere) is further enhanced (Ames et al. 1984), indicating their role in the growth and development of plants. The occurrence of various bacteria on the surface or in the cytoplasm of AM spores was also reported by several workers (Mosse 1962, Varma et al. 1981, Tilak et al. 1989) as well as mineralization of organic phosphorus by AM fungi (Jayachandran et al. 1992). Synergistic effects of AM fungi and diazotrophic bacteria on the nutrition and growth of plants were reported (Azcon-Aguilar et al. 1986, Bagyaraj and Menge 1978, Ho 1988, Nagrajan et al. 1989, Tilak et al. 1989). Interactions of AM fungi and phosphorus-solubilizing bacteria were also reported (Azcon-Aguilar et al. 1976, 1986, Myer and Linderman 1986). Many soil fungi help plant growth as promoters of mycorrhizal functioning (companion fungi) or competition against pathogens. *Trichoderma harzianum* is well known for producing various kinds of active compounds including antifungal and antibacterial agents like harzianic acid against the nursery disease pathogens of *Pythium irregulare*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani* (Vinale 2009).

In the present work, we studied the effect of applying organic matter in the form of farmyard manure (FYM) and vermicompost, which are very rich sources of organic carbon (C), nitrogen (N), phosphorous (P), and potassium (K) (Soil and Plant Laboratory, Canada, 2006), along with the AM fungi of *Pseudomonas fluorescens* and *Trichoderma harzianum* on the germination of seeds and growth of *A. nilotica* seedlings in a sterilized potting mixture.

MATERIALS AND METHODS

Experimental design

The experiment was multifactorial and conducted in a completely randomized design (CRD) with 3 replications. Different treatment combinations were as follows:

1. $A_0B_0C_0$: control; 2. $A_1B_0C_0$: AM fungi; 3. $A_0B_0C_1$: *Trichoderma harzianum*; 4. $A_1B_0C_1$: AM fungi+*T. harzianum*; 5. $A_0B_1C_0$: *Pseudomonas fluorescens*; 6. $A_1B_1C_0$: AM fungi+*P. fluorescens*; 7. $A_0B_1C_1$: *P. fluorescens*+*T. harzianum*; 8. $A_1B_1C_1$: AM fungi+*P. fluorescens*+*T. harzianum*; 9. $A_0B_0C_0$: FYM; 10. $A_1B_0C_0$: AM fungi+FYM; 11. $A_0B_1C_1$: FYM+*T. harzianum*; 12. $A_1B_1C_1$: AM fungi+FYM+*T. harzianum*; 13. $A_0B_0C_0$: vermicompost; 14. $A_1B_0C_0$: AM fungi+vermicompost; 15. $A_0B_1C_1$:



vermicompost+*T. harzianum*; 16. A₁O₂B₀C₁: AM fungi+vermicompost+*T. harzianum*; where A is AM fungi, O₁ is FYM, O₂ is vermicompost, B is the bacterium, *P. fluorescens*, and C is the companion fungi, *T. harzianum*.

Collection of seeds and pretreatment

Pods of *A. nilotica* were collected from different adjoining areas of Jabalpur Province in May 2008. After mechanical scarification, seeds were extracted from the pods and stored for the experiment. To break dormancy, seeds were subjected to hot-water treatment before sowing.

Raising of seedlings

Seedlings were raised from collected seeds in root trainers of 300-ml capacity in sterilized potting mixture containing soil, sand, and organic matter in a 2: 1: 2 ratio. The root trainers were kept on iron angle beds and watered with sterilized water twice a day.

Preparation of inocula of the AM fungi, *P. fluorescens* and *T. harzianum*

An inoculum of AM fungi isolated from *A. nilotica* rhizosphere soil which contained *Acaulospora larvis*, *Glomus etunicatum*, *G. intraradices*, *G. mosseae*, and *Scutellospora pellucida* was multiplied in pot cultures using Marie gold as a trap plant. Mycorrhizal roots of trap plants were harvested, washed, and sheared to get the AM inoculum. *Pseudomonas fluorescens* and *T. harzianum* were also isolated from *A. nilotica* rhizosphere soils. The companion fungus was grown in 250-ml conical flasks in potato dextrose agar (PDA) broth. Spore suspensions and mycelial slurries were prepared in saline water by diluting both fungi 6-fold in a mixer grinder.

Inoculation

AM inoculum consisted of a mixture of rhizospheric soil from the trap culture containing spores, hyphae, and mycorrhizal root fragments. Ten milliliters of sheared root AM inoculum prepared as described above, containing 86 infected propagules (Liu and Luo 1994), was inoculated per unit area of the root trainer. Ten milliliters of a microbial suspension of *T. harzianum* (874.33×10^3 colony-forming units (cfu)/ml) and *P. fluorescens* (302.33×10^3 cfu/ml) was poured on each root trainer unit containing 4 seeds.

Data recording

Data were recorded for 1 mo, from seed sowing to germination. After germination was complete, further data were recorded on seedling height and diameter, and survival percentage until 9 mo. Seedlings were harvested after 9 mo by uprooting them with a spade, and they were carefully washed with water. Feeder roots were collected and stained (Koske and Gamma 1989). A grid-line-intersect method (Giovaetti and Mosse 1980) was used to determine root colonization by AM fungi. The root surface area was measured by a method based on nitrate adsorption and desorption (Ansari et al. 1995). Harvested roots and shoots were oven-dried at 48°C for 72 h to determine the dry biomass.

Statistical analysis

The effects of treatments were analyzed using a linear-model analysis of variance (ANOVA) by NH analytical software (Statistix, PC, DOS vers. 2.0, 1987) and SPSS (Statistical Package of Social Sciences, vers. 14.0; Chicago, IL, USA). The means were

compared using Duncan's multiple-range test (DMRT) for each parameter if the F value was significant at $p = 0.05$. Correlation coefficients and linear regression equation were calculated to establish relationships of the months of 3 different seasons with seedling height and diameter, and survival at $p = 0.05$.

RESULTS

Seed germination

After 1 mo, maximum germination was obtained with AM+FYM (15.52% greater than the control) followed by *P. fluorescens*+*T. harzianum* (11.11%) and vermicompost+*T. harzianum* (9.03% than the control). The effects of different treatment combinations on the germination of seeds were not significant (CD 5% = 13.52). The interaction between the organism and organic matter was statistically significant ($p > 0.05$), and the calculated F value was 0.72 (Tables 1, 2.1).

Plant height, diameter, and survival

Application of AM fungi along with FYM or vermicompost and companion fungi showed a statistically similar effect on plant height which was highest for the AM+FYM combination (147.90% greater than the control). The effect of different treatment combinations on plant height was significant (CD 5% = 5.08). The interaction between the organism and organic matter was statistically significant ($p < 0.05$), and the calculated F value was 11.71 (Tables 1, 2.2).

The maximum diameter at the collar was obtained with AM+FYM+companion fungi (166.88% greater than the control) followed by AM+vermicompost (162.54% greater than the control). AM fungi treatment produced a statistically smaller effect (89.59% smaller than the control) (Table 2). All treatment combinations showed statistically significant effects on the diameter of seedlings, and the interaction between the organism and organic matter was statistically significant ($p < 0.05$), with a calculated F value of 8.44 (Tables 1, 2.3).

AM fungi in combination with FYM and companion fungi or vermicompost followed by FYM with companion fungi showed statistically similar high rates of survival among all treatment combinations (156.54% greater than the control). The effects of all treatment combinations on rates of survival were statistically significant (CD 5% = 12.37). The interaction between the organism and organic matter was statistically significant ($p < 0.05$), with a calculated F value of 5.20 (Tables 1, 2.4). The multiple-regression ANOVA results of diameter showed that the regression sum of squares was 1.86, the residual sum of squares was 0.18, the coefficient of determination R^2 was 0.991, and the calculated F value was 746.28. The linear regression equation for diameter was: $Y = 0.1768X + 2.0761$ (Fig. 1). ANOVA results of height showed a regression sum of squares of 83.91, a residual sum of squares of 10.69, and a coefficient for determination R^2 of 0.88. The linear regression equation for height was: $Y = 1.1822X + 15.35$ (Fig. 2). ANOVA results of seedling survival showed a regression sum of squares of 183.91, a residual sum of squares of 29.50, and a coefficient for determination R^2 of 0.86. The linear regression equation for survival was: $Y = -1.751X + 100.31$ (Fig. 3). All 3 parameters were significant at the 0.05 level of significance.

Table 1. Effects of treatment combinations on the different parameters on the *Acacia nilotica* seedlings in the nursery stage

Treatments	Seed germination (%)	Diameter (cm)	Height (cm)	Survival (%)	Fresh root weight (g)	Fresh shoot weight (g)	Dry root weight (g)	Dry shoot weight (g)	Root surface area (cm)	Root colonization (%)	Root nodules (no.)
Control	39.58 ^{a3}	2.98 ^{9,10}	21.98 ^{3,4,5}	63.88 ^{4,5}	0.15 ³	0.41 ⁵	0.10 ⁴	0.19 ²	44.73 ^{4,5}	0.00 ⁵	6.33 ^{3,4}
AM fungi	37.51 ^{2,3}	2.67 ¹⁰	18.05 ^{5,6}	50.00 ⁵	0.18 ³	0.43 ⁴⁵	0.11 ⁵	0.21 ²	36.08 ²	30.00 ^{3,4}	5.0 ^{3,4}
<i>Trichoderma harzianum</i>	38.19 ^{2,3}	2.95 ^{8,9,10}	20.14 ^{4,5,6}	72.22 ^{3,4,5}	0.46 ³	0.57 ⁴⁵	0.16 ⁵	0.26 ²	78.14 ^{4,5}	0.00 ⁵	8.00 ^{1,2,3,4}
AM+ <i>T. harzianum</i>	28.47 ³	2.84 ^{9,10}	15.64 ⁶	61.11 ^{4,5}	0.48 ³	0.38 ⁵	0.19 ⁵	0.24 ²	40.01 ^{4,5}	26.67 ^{3,4}	11.67 ^{1,2,3}
<i>Pseudomonas fluorescens</i>	43.05 ^{1,2}	3.30 ^{6,7}	22.25 ^{3,4,5}	83.33 ^{1,2}	0.55 ³	0.46 ⁴⁵	0.19 ⁵	0.26 ²	62.68 ^{4,5}	0.00 ⁵	7.00 ^{2,3,4}
AM+ <i>P. fluorescens</i>	46.53 ^{1,2}	3.56 ^{5,6}	23.33 ^{3,4}	94.44 ^{1,2}	0.42 ³	0.48 ⁴⁵	0.15 ⁵	0.21 ²	89.87 ^{3,4,5}	33.33 ³	5.33 ^{2,3,4}
<i>P. fluorescens</i> + <i>T. harzianum</i>	50.69 ^{1,2}	3.17 ^{6,7,8,9}	25.81 ^{2,3}	91.66 ^{1,2}	0.23 ³	0.41 ⁵	0.17 ⁵	0.26 ²	78.89 ^{3,4,5}	0.00 ⁵	4.00 ⁴
AM+ <i>P. fluorescens</i> + <i>T. harzianum</i>	44.45 ^{1,2}	2.98 ^{7,8,9,10}	21.27 ^{3,4,5}	75.00 ^{3,4}	0.54 ³	0.67 ⁴⁵	0.21 ^{3,4,5}	0.30 ¹	75.43 ^{3,4,5}	33.33 ³	9.00 ^{1,2,3,4}
Farmyard manure (FYM)	55.10 ¹	4.01 ⁴	32.51 ¹	75.00 ^{3,4}	1.38 ²	1.19 ³⁴	0.57 ^{1,2}	0.67 ²	91.54 ^{2,3,4,5}	0.00 ⁵	13.67 ^{1,2}
AM+FYM	43.75 ^{1,2}	3.80 ^{4,5}	29.89 ^{1,2}	100.0 ^{3,4}	1.62 ¹²	2.18 ¹²	0.59 ^{1,2}	1.02 ²	91.58 ^{2,3,4,5}	50.00 ²	6.00 ^{3,4}
FYM+ <i>T. harzianum</i>	42.36 ^{1,2}	4.99 ¹	30.16 ^{1,2}	97.22 ¹	1.21 ²	0.96 ³⁴	0.41 ^{2,3,4}	0.58 ²	96.03 ^{2,3,4,5}	0.00 ⁵	7.33 ^{3,4,5}
AM+FYM+ <i>T. harzianum</i>	46.53 ^{1,2}	4.16 ^{3,4}	31.87 ¹	100 ¹	1.18 ²	1.68 ¹²³	0.45 ^{1,2}	0.82 ²	112.3 ^{1,2,3}	20.00 ⁴	7.00 ²
Vermicompost	43.75 ^{1,2}	4.56 ²	31.00 ¹	97.22 ¹	1.53 ¹²	1.68 ¹²³	0.59 ^{1,2}	1.1.05 ²	112.5 ^{1,2,3}	0.00 ⁵	14.67 ¹
AM+Vermicompost	43.75 ^{1,2}	4.86 ^{1,2}	30.81 ¹	100 ¹	1.47 ¹²	2.03 ¹²	0.42 ^{1,2}	0.87 ²	157.3 ^{1,2,3}	46.67 ²	10.33 ^{1,2,3,4}
Vermicompost+ <i>T. harzianum</i>	48.61 ^{1,2}	4.52 ^{2,3}	31.08 ¹	97.22 ¹	1.90 ¹	2.28 ¹	0.59 ^{1,2}	1.06 ²	176.5 ¹	0.00 ⁵	10.33 ^{1,2,3,4}
AM+Vermicompost+ <i>T. harzianum</i>	45.10 ^{1,2}	3.38 ^{6,7}	22.27 ^{3,4,5}	83.33 ^{2,3}	1.90 ¹	1.45 ²³	0.63 ¹	0.93 ²	137.0 ^{1,2,3}	73.33 ¹	10.67 ^{1,2,3,4}
Standard error	1.17	0.03	0.43	1.07	0.05	0.06	0.01	0.02	6.32	1.02	0.59
CD = 5%	13.52	0.39	5.08	12.37	0.30	0.75	0.21	10.31	73.12	11.85	6.82

^a Indicates the number of statistically homogenous groups in which the value in the columns and rows comes under.



Applied to Tables 2.1~2.11: AM, AM fungi; CF, companion fungi (*Trichoderma harzianum*), BAC, bacteria (*Pseudomonas fluorescens*); CF, *P. fluorescens*+*T. harzianum*, OM₁: Farmyard manure (FYM); OM₂, vermicompost.

Table 2.1 Effects of treatment combinations on the seed germination (%) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	39.58	37.51	38.19	28.47
BAC	43.05	46.53	50.69	44.45
OM ₁	55.10	43.75	42.36	46.53
OM ₂	43.75	43.75	48.61	45.11
	Organisms (OS)	Organic matter (OM)	OS x OR	
<i>F</i> value = 0.05	3.37	0.58	0.72	
<i>p</i> value = 0.05	0.031	0.63	0.68	

Table 2.2 Effects of treatment combinations on the diameter (cm) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	2.99	2.66	2.95	2.84
BAC	3.30	3.56	3.17	2.98
OM ₁	4.01	3.80	4.99	4.10
OM ₂	4.56	4.86	4.52	3.38
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	104.45	10.26	8.44	
<i>p</i> value = 0.05	0.00	0.00	0.00	

Table 2.3 Effects of treatment combinations on the height (cm) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	21.98	18.05	20.14	15.64
BAC	22.25	23.33	25.81	21.27
OM ₁	32.51	29.89	30.16	31.87
OM ₂	31.00	30.81	31.08	22.27
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	36.54	4.48	1.69	
<i>p</i> value = 0.05	0.00	0.010	0.132	



Table 2.4 Effects of treatment combinations on the survival (%) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	63.88	50.00	72.22	61.11
BAC	83.33	94.44	91.66	75.00
OM ₁	75.00	100	97.22	100
OM ₂	97.22	100	97.22	83.33
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	41.35	6.28	5.20	
<i>p</i> value = 0.05	0.00	0.00	0.00	

Table 2.5 Effects of treatment combinations on the fresh root weight (g) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	0.15	0.18	0.46	0.48
BAC	0.55	0.42	0.23	0.54
OM ₁	1.38	1.62	1.21	1.18
OM ₂	1.53	1.47	1.90	1.90
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	34.40	0.22	0.86	
<i>p</i> value = 0.05	0.00	0.88	0.56	

Table 2.6 Effects of treatment combinations on the fresh shoot weight (g) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	0.41	0.43	0.57	0.38
BAC	0.46	0.48	0.41	0.67
OM ₁	1.19	2.18	0.96	1.68
OM ₂	1.68	2.03	2.28	1.45
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	26.97	1.25	1.60	
<i>p</i> value = 0.05	0.00	0.30	0.15	

Table 2.7 Effects of treatment combinations on the dry root weight (g) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	0.10	0.11	0.16	0.19
BAC	0.19	0.15	0.17	0.21
OM ₁	0.57	0.59	0.41	0.45
OM ₂	0.59	0.42	0.59	0.63
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	29.00	0.35	0.88	
<i>p</i> value = 0.05	0.00	0.78	0.55	

Table 2.8 Effects of treatment combinations on the dry shoot weight (g) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	0.19	0.21	0.26	0.24
BAC	0.26	0.21	0.27	0.30
OM ₁	0.67	1.02	0.58	0.82
OM ₂	1.05	0.87	1.06	0.93
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	40.22	0.21	1.11	
<i>p</i> value = 0.05	0.00	0.88	0.38	

Table 2.9 Effects of treatment combinations on the root colonization (%) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	0.00	30.00	0.00	26.67
BAC	0.09	33.33	0.00	33.33
OM ₁	0.00	50.00	0.00	20.00
OM ₂	0.00	46.67	0.00	73.33
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	11.91	120.62	9.41	
<i>p</i> value = 0.05	0.00	0.00	0.00	



Table 2.10 Effects of treatment combinations on the root surface area (cm) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	44.73	36.08	78.14	40.01
BAC	62.68	89.87	78.89	75.43
OM ₁	91.54	91.58	96.03	112.3
OM ₂	112.5	157.3	176.5	137.0
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	9.11	0.78	0.36	
<i>p</i> value = 0.05	0.00	0.50	0.94	

Table 2.11. Effects of treatment combinations on the number of root nodules (no.) of *Acacia nilotica*

Organisms (OS)→ Organic matter (OR)↓	Control	AM	CF	AM+CF
Control	6.33	5.00	8.00	11.67
BAC	7.00	5.33	4.00	9.00
OM ₁	13.67	6.00	7.33	7.00
OM ₂	14.67	10.33	10.33	10.67
	Organisms (OS)	Organic matter (OR)	OS x OR	
<i>F</i> value = 0.05	3.72	3.34	0.80	
<i>p</i> value = 0.05	0.02	0.03	0.61	

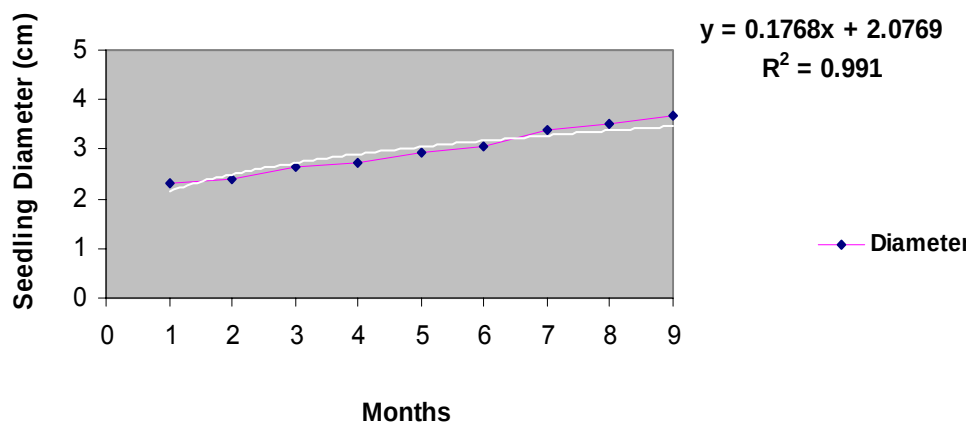


Fig. 1. Effects of different months on the diameter of the *Acacia nilotica* seedlings in the nursery stage.

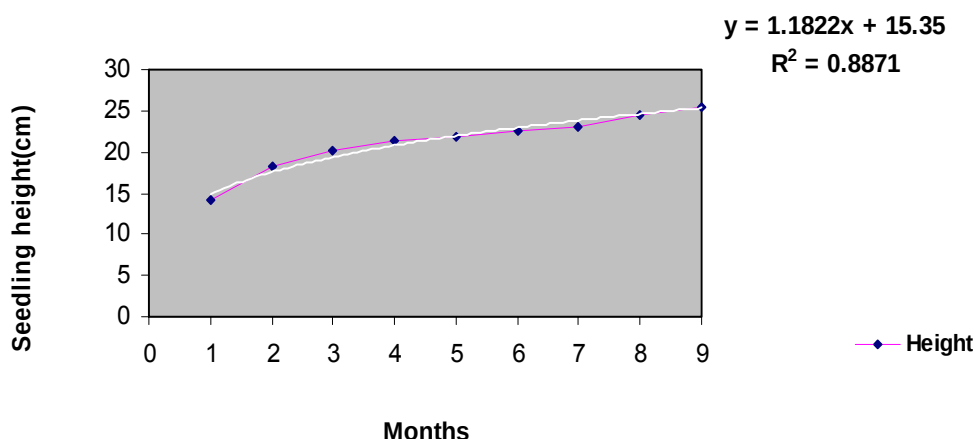


Fig. 2. Effects of different months on the height of the *Acacia nilotica* seedlings in the nursery stage.

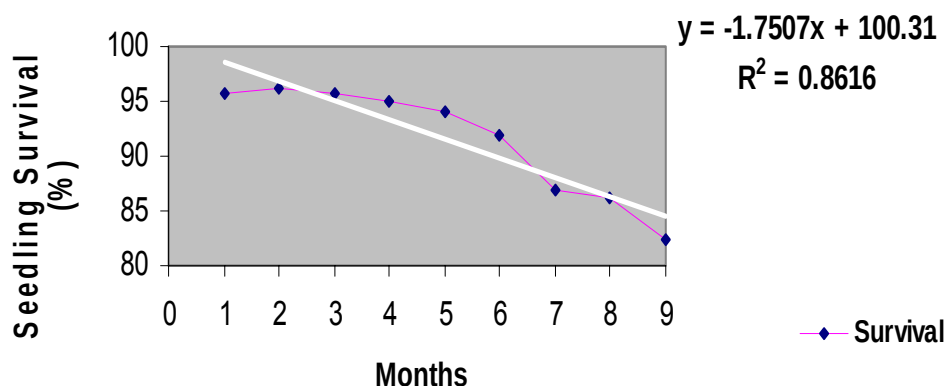


Fig. 3. Effects of different months on the survival of the *Acacia nilotica* seedlings in the nursery stage.

Fresh biomass

Trichoderma harzianum showed a statistically similar effect on fresh root weight of seedlings in combination with FYM (1266.67% greater than the control) and vermicompost (1080% greater than the control). A similar trend of the effect was found for fresh shoot weight (531.70% greater than the control.). All treatments were statistically non-significant. Interactions between organic matter and the organism were also non-significant ($p > 0.05$). The calculated F values for fresh root weight and dry shoot weight were 0.86 and 1.60, respectively (Tables 1, 2.5, 2.6).

Dry biomass

The maximum dry root weight of seedlings was found in treatment combinations containing companion fungi, vermicompost, and FYM. Statistically, a similar root



weight was found for vermicompost alone (5.9% greater than the control). All treatment combinations were statistically non-significant. The interaction between organic matter and organism was also non-significant ($p > 0.05$). The calculated F value for the dry root weight was 0.88. Among all treatment combinations, the maximum dry shoot weight of seedlings was found for companion fungi with vermicompost (557.89% greater than the control). The effects of all treatment combinations were statistically non-significant. The interaction between organic matter and the organism was also non-significant ($p > 0.05$). The calculated F value for dry shoot weight was 1.11. (Tables 1, 2.7, 2.8).

Root colonization

All treatment combinations receiving AM fungi produced significantly higher root colonization compared to any not treated with AM (73.33% greater than the control). Maximum root colonization was obtained in the treatment combination with AM+vermicompost and companion fungi. The results were statistically significant, and the interaction among organic matter and the organism was also significant ($p < 0.05$). The calculated F value for root colonization was 9.41 (Tables 1, 2.9).

Root surface area

The highest root surface area was found in the companion fungi with vermicompost treatment combination (394.58% greater than the control). The effects of all treatment combinations were statistically non-significant for the above parameter. The interaction between organic matter and the organism was significant ($p > 0.05$). The calculated F value for the root surface area was 0.36 (Tables 1, 2.10).

Number of root nodules

Vermicompost alone was more effective on the number of root nodules formed among all treatment combinations (231.75% greater than the control) followed by AM+FYM (215.95% greater than the control). All treatment combinations were statistically non-significant. The interaction between organic matter and the organism was also non-significant ($p > 0.05$). The calculated F value for the number of root nodules was 0.08.

DISCUSSION

AM symbiosis is known to promote the acquisition of mineral nutrients, especially phosphorous by host plants (Cooper and Tinker 1978, Lambert et al. 1979, Bolan 1991) as a result of additional growth of AM hyphae that enlarge the root soil interface, facilitating efficient exploration of the soil around the root (Jacobsene et al. 1992, Liu et al. 1994).

Higher root colonization facilitates improved P-uptake that results in enhanced height and collar diameter in plants with higher root colonization or those treated with companion fungi. Development of mycorrhizae in feeder roots and the spread of hyphae of AM fungi in the rhizosphere may help trap additional nutrients from the soil by mycorrhizal plants compared to the control.

Enhancement of growth of AM-treated seedlings may also be due to mineralization of organic phosphate by AM fungi (Jayachandran et al. 1992) and by *T. harzianum*. Phosphate-solubilizing bacteria also mineralize insoluble phosphorus present

in the soil and make it available to plants for their development. Maximum germination was obtained in *T. harzianum*-treatment combinations, which may have been due to production of cellulosic enzymes and plant growth-promoting hormones.

Enhancements in height, diameter, and survival of seedling treated with *T. harzianum* were possibly due to N₂ fixation by the bacterium which was utilized by *A. nilotica* seedlings. There were significant synergistic interactions between AM fungi and *T. harzianum* for both height and root colonization. *Trichoderma harzianum* can be considered a mycorrhizal helper bacterium. Many plant growth-promoting rhizobacteria are also reported to be mycorrhizal helper bacteria (Garbaye 1994).

Results of the multiple-regression analysis determined a linear positive correlation between the dependent variables of height and diameter and the independent variables of months of 3 different seasons. But the survival percentage showed negative correlations with the same independent variables. The diameter of seedlings increased by an average of 0.54 cm, and a 123.37% increment was found between the rainy and winter seasons. After 2 mo, the collar diameter had increased by 0.75 cm, adding a 126.31% increment. The maximum increment was recorded between winter and summer seasons. Seedling height also increased by an average of 7.08 cm with a 150.14% increment between the rainy and winter seasons, and 3.81 cm and a 117.97% increment between the winter and summer seasons. The percentage of seedling survival declined due to seasonal changes with a 2.53% mortality rate between rainy and winter seasons, and the maximum mortality rate was recorded between the winter and summer seasons. These trends continued with an average fall (1.67°C maximum and 10°C minimum) and increase (11.17°C maximum and 10.5°C minimum) in temperatures between the rainy and winter seasons and between the winter and summer seasons, respectively. The lower temperature during rainy and winter seasons enhanced seedling survival, height, and collar diameter (Table 3) due to the maximum availability of moisture in the soil and less transpiration, whereas the higher temperatures during winter and summer seasons may have enhanced the microbial activity in the rhizosphere soil producing the maximum supply of nutrients.

Table 3. Effects of seasonal changes on the different parameters of *Acacia nilotica*

S. no	Month	Season	¹⁾ Average temperature (°C)		Diameter (cm)	Height (cm)	Survival (%)
			Max.	Min.			
1	September	Rainy	31	23	2.31	14.12	95.83
2	October		32	19	2.41	18.27	96.09
3	November		29	13	2.63	20.21	95.75
4	December	Winter	26	9	2.74	21.4	95.05
5	January		26	9	2.93	21.87	94.1
6	February		29	12	3.06	22.46	92.01
7	March	Summer	34	16	3.37	23	86.8
8	April		39	21	3.52	24.5	86.11
9	May		42	26	3.68	25.52	82.29

¹⁾ Source: India Meteorological Department, New Delhi, India.



CONCLUSIONS

In the present study, application of AM fungi along with *Trichoderma harzianum* produced beneficial effects on plant growth, like disease resistance, drought tolerance, etc.; therefore this treatment is recommended for production of *Acacia nilotica* seedlings in commercial nurseries.

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Genetic Diversity of Mahogany for Mitigation and Adaptation to Climate Change

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[Summary]

Swietenia macrophylla (big-leaf mahogany) is one of the most important commercial species of the Neotropics and has one of the highest-valued lumber in the world. Because of that, exploitation has been very high, and inadequate management of logging and regeneration has caused the depletion of many populations. Two of the problems that the species has nowadays are that few sources still survive that can be used to adapt to climate change and there are barriers to migration to more-suitable places. We explain the methodology of reciprocal experiments to evaluate adaptations to climate change in several populations collected in Costa Rica. Results of both germination and initial growth in greenhouses are presented. Experiments were established in all localities of the provenance collection, on the same exact farm, and the trials were evaluated for survival and growth.

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The Pacific Decadal Oscillation and Weevils Influence Acorn Production and Germination in the Endemic Evergreen Oak, *Cyclobalanopsis glaucoides*

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[Summary]

Southern Yunnan Province, southwestern China is a biodiversity hotspot containing more than 18,000 species of higher plants and the animals that depend on them, but is under threat from habitat loss due to logging, burning, clearing for commercial crops, and other human activities. *Cyclobalanopsis glaucoides*, an evergreen oak, is typically the dominant tree in these diverse broadleaf forests and provides the major structural component. However, even in years of higher acorn production, there is only a small increase in the number of seedlings. For 4 yr (2006~2009) we collected acorns of *C. glaucoides* daily during the production season from a natural stand in Kunming, China. Acorns were examined for weevil (*Curculio* sp.) infestation, and samples were tested for germination. We show that the number of acorns produced by *C. glaucoides* was influenced by large-scale climatic events (the Pacific Decadal Oscillation) that affect local weather conditions, primarily spring temperatures and precipitation. The number of acorns that survived was regulated in a density-dependent manner by weevil attack. Our results indicate why an understanding of the factors that influence the oak's abundance and long-term survival is critical to the maintenance of these forests.

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Early Ovule Development of Taiwanese Yew (*Taxus sumatrana*)

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[Summary]

In this research, we attempted to establish a time table of ovule development of the Taiwanese yew (*Taxus sumatrana*), and compared it to those of temperate species to understand how the climate affects the timing of development of yews.

Taiwanese yew is a species native to Taiwan. It usually grows above 1500 m in elevation. Unlike most gymnosperms which bear compound cones, *Taxus* is characterized by a single enclosed ovule, the aril of which turns red when the seed matures. The sexual reproduction of European and Pacific yew was studied by Pennell (1987, 1988, 1989) and Anderson (1999, 2000), but related information on Asian yew species awaits further investigation.

Branches of the Taiwanese yew with female generative buds were collected once biweekly from January to June 2010 from a nursery at Pasiashan (at 1000 m in elevation). Our current results showed that Taiwanese yew's ovules formed in January, underwent the mid-nucellus stage from early to mid-February, and entered the late-nucellus stage from late February to early March. In mid- and late March, almost all samples collected were in the early gametophyte stage, while a few were in the 4-megaspore stage. In April, ovules entered the free-nuclear stage and cellular-gametophyte stage. This was followed by the archegonia stage and fertilization stage before the remaining ovules formed embryos from late May to early June. The embryos matured after June.

According to the time table, Taiwanese yew forms megaspores later than Pacific yews, but underwent subsequent stages more rapidly; that is, the early development schedule of Taiwanese yew is slightly more compact than those of Europe and Pacific species. Based on our current results, we can accumulate data at more-accurate time points in the future, so that we can apply our results to entire populations, and thus can compare our results with other yews.

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Assessment of Seed Distribution, Dissemination, and Diffusion Pathways of Priority Tree Plantation Species in the Philippines

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[Summary]

Understanding the state of the country's seeds sources, origins of priority forest tree seeds, and systems of seed distribution, dissemination, and diffusion of priority plantation trees in the Philippines are key concerns in developing strategies and programs to address forestation development. In this study, the major tree seed suppliers were characterized and analyzed in terms of their seed collection/acquisition system, and their processing, handling, storage, and distribution practices. The seed quality and quantity supplied by seed suppliers were evaluated as well as the phenotypic characteristics of the seed trees from which the seeds were collected. Based on these results, a modification of the guidelines for plus tree selection was proposed. Data were collected using document reviews, field surveys, and interviews. Results of the study revealed that a considerable number of seed sources surveyed are distributed in major tree islands of the country, located mostly the southern part of the Philippines. Access to these seed sources is relatively easy for government-based seed suppliers while generally difficult for privately owned seed suppliers. A limited number of seed sources can be considered of phenotypically good quality due to the absence of rouging. The study revealed 5 major categories of tree seed producers, distributors, and suppliers and 4 major seed pathway linkages from the origins and primary seed suppliers to various end-users. Five industrial tree plantation (ITP) species with the most number of seed sources surveyed were yemane (*Gmelina arborea* Roxb.), mahogany (*Swietenia macrophylla* King), mangium (*Acacia mangium* Willd.), bagras (*Eucalyptus deglupta* Blume.), and narra (*Pterocarpus indicus* Willd.). The origins and movement pathways of seeds of these species proved to be difficult to trace due to poor documentation or the complete absence of records of trees planted many years ago.

Key words: forest tree seed sources, seed distribution, dissemination and diffusion pathways, tree plantation specie.

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INTRODUCTION

The last 2 decades saw a sharp decline in Philippine forests after years of logging and other intensive uses. From an estimated 41×10^6 ha of primary forest at the beginning of the 20th century, it has been reduced to barely $< 5.4 \times 10^6$ ha in 2001 or about 18% of the country's total land area (DENR 2003).

In 1975, the country's total combined roundwood exports amounted to 6.84×10^6 m³, with a total value of US\$283 $\times 10^6$, which drastically declined to 4000 m³ with a total value of just US\$207,000 in 1994. In a matter of 18 years, the country slid from being the world's largest producer of tropical hardwood to being a net timber-importing nation (FMB-DENR 2003). Obviously, the contribution of this sector to the economy likewise decreased as a result of the decline in forest resources.

Despite all these downturns, the last decade saw emerging interest by people to venture into tree farming and the use of plantation woods by wood-based industries.

In the early 1990s, tree plantation establishment was at its peak. The combined area planted by the government and private sector in 1990 totaled 191,663 ha. This trend was brought about by an infusion of reforestation funds on the government side and the promise to private tree growers that tree farming would make them millionaires in just 7~8 yr after planting fast-growing tree species such as yemane, mangium, and bagras.

However, this promise resulted in frustrations due to low growth and yields of tree farms coupled with misguided marketing situations. Species planted were not compatible with the sites, and little regard was given to the quality of the planted materials. The choice of species and availability of quality planting stocks were not related to the intended goal of harvesting high-quality and required volumes of timber for specific end uses with limited investments in woodlot management, protection, and silviculture (Aggangan 2001).

The quantity and quality of forest tree seeds are recognized worldwide as among vital factors that determine the success or failure of any tree-planting activity. These 2 factors are essential biological attributes that can contribute to the success or failure of tree-planting programs (Roshetko et al. 2004).

Use of poor-quality seeds results in poor nursery stock production, and ultimately in poor field performance. Carandang and Lasco (1998) also noted that the use of poor-quality seeds is an important reason for the failure of reforestation efforts in the Philippines.

Likewise, the poor genetic quality of seed sources often results in poor form and quality of the end products (inferior wood, fruit, forage, and other products). Profound ripple effects of seed quality on the end product should be a convincing motivation for planters to use the best-quality seeds (Tolentino 2004).

The quantity and quality of planting materials are among the technical factors that contribute to the formation and transformation of secondary forests in the Philippines (Lasco et al. 2001). As such, seed quality is the composite of numerous interacting factors which can be traced from seed production and spans the entire spectrum of silviculture – from seedling production to harvesting.

In the 2003 Revised Master Plan for Forestry (MPFD)(UNFAO and FMBDENR 2003), among the major problems cited for failure of forest management is the “weak



forest science foundation”. Problems identified were related to tree plantation development considered “serious lapses” – starting from site selection, to seed procurement, nursery and plantation establishment and management, and harvesting.

The central problem that guided this study was the limited information on the state of the country’s forest tree seed sources, seed origins, and the manner in which seeds are distributed from suppliers to end-users. Likewise, the systems of how seeds are collected and processed, and the level of quality and quantity of available seeds of priority plantation species are vital contributions to the success or failure of forestation programs and ITP development in the country.

MATERIALS AND METHODS

Locations of the study

The study areas were places where seed sources, producers, suppliers, and dealers are located on the 3 major islands of the Philippines (Appendix 1). Selection of sites was based on the following criteria: a) a well-known seed source and/or seed supplier/dealer of species used for ITP, agroforestry, and forestation development during the last 5 yr; and b) seed sources and seed dealers/suppliers of subject tree species.

Prior common knowledge and information in terms of the existence of active and wide plantings of priority forestation, agroforestry, and industrial tree plantation species in the locality were the major bases of the first criterion. Seed sources, suppliers, and dealers encountered during data collection were also included in the study. They were identified during actual field visits by locals, including their roles and contributions as major sources of forest tree seeds in the locality.

Target study areas were pre-identified for the 3 major island groups of the country, namely, Luzon (north), Visayas (central), and Mindanao (south). Aside from the pre-identified seed sources, any substantial tree seed sources encountered in the course of the survey were included as study sites.

Field data gathering and laboratory and nursery seed tests

Field visits and surveys of seed supplier for evaluation were conducted for a period of 5 mo, in November 2006, and April, May, October, and November 2007.

Laboratory and nursery tests of seeds collected from the seed sources were conducted by evaluating the purity, number of seeds per unit weight test, moisture content, and germination tests in the research station immediately after each fieldwork was completed.

Research design, analysis, and interpretation

Descriptive statistics were employed to determine the relationships between the quality of tree seeds from a seed source and from a seed supplier/dealer in terms of the quality of the resulting stands in the tree seed pathways. Quality evaluation of seeds supplied and/or distributed by major seed suppliers followed standard laboratory seed testing protocols. Comparison of the stand quality of a given seed source with the resulting tree farms and reforestation projects was qualitatively evaluated in terms of the straightness of the bole or tree form, the diameter at breast height (DBH), and the merchantable height (MH).

RESULTS AND DISCUSSION

The study surveyed a total of 119 seed sources covering 14 forest tree species distributed on the 3 major islands of the country. In terms of seed source distribution, Mindanao had the highest with 69 seed sources, followed by Luzon with 29 and Visayas with 12.

The study also revealed that of the 14 tree species evaluated, the top 5 species considered a priority in order of the greatest number of seed sources surveyed were yemane (*Gmelina arborea* Roxb.) with 27 seed sources, mahogany (*Swietenia macrophylla* King) with 18, mangium (*Acacia mangium* Willd.) with 17, bagras (*Eucalyptus deglupta* Blume) with 10, and narra (*Pterocarpus indicus* Willd.) with 9 seed sources.

The total number of seed sources for the top 5 species totaled 81, and they were scattered in various parts of the country.

Result of the study also revealed that there were 4 major types or groups of seed sources, producers, and suppliers/distributors based on their management and/or type of ownership. These included the following.

- 1) Corporate-based seed sources, either privately owned or controlled corporations, in which investments and locations of plantations are purely private, and quasi or government-controlled corporations, in which investments are largely government but with private financial infusions.
- 2) Government-based seed sources, either, the Department of Environment and Natural Resources (DENR), which is the primary government agency for the conservation and management of the country's natural resources, and the academia-based seed sources, the seed sources of which are located within the land area or forest reservations of any given state college and/or university (SCU).
- 3) Smallholder tree farmer-based, with 2 types, namely, private smallholder tree farmers, whose tree farms are their seed sources located on a... (A&D) lands and outside the jurisdiction of the government, and community-based forest management (CBFM) smallholder tree farmers, whose tree farms are within government or public forestlands and are managed by community members under the government's CBFM program. These areas are former government forestation projects the management of which has already been turned over to the community.
- 4) Forest tree seed suppliers and dealers. These are individuals generally engaged in supplying/selling seeds. They do not necessarily own any tree farm but are involved in actual seed collection and processing. They source their seeds from any of the previous types of seed sources mentioned.

From among these seed sources types, smallholder tree farmers are the top seed sources/distributors of priority tree species, with 31 seed sources or 38% of the total seed sources surveyed from the 3 major islands of the country.

In terms of the seed source attributes, the average number of seed trees ha^{-1} is 73, with an average area of 7.5 ha and an average age of 21.5 yr. The average annual seed production of seed sources includes 153.9, 182.6, 20.85, 2.92, and 13.55 $\text{kg ha}^{-1} \text{yr}^{-1}$ for yemane, mahogany, mangium, bagras, and narra.

Based on the results of this study, there were at least 3 major linkage pathways in terms of movements of seeds from the origin to the various end-users, namely; a) from



the original seed source to primary seed users, referred to in this study as the “distribution” linkage; b) from the primary seed users to secondary seed users, referred to in this study as the “dissemination” linkage; and c) from secondary to tertiary seed users, referred to in this study as the “diffusion” linkage.

Yemane plantings in the country are believed to have originated from Burma through UNDP-FAO facilitation in Thailand. According to existing records and personal interviews with people previously involved in its introduction, yemane was among the species that was included in the UPLB-MNR/BFD Tree Seeds Orchards Program from the late 1970s to the early 1980s.

Introduction of mahogany occurred during the early 1900s. Based on the literature, 880 mahogany seeds were introduced to the Philippines in 1913 through a donation by the Royal Botanic Garden Sibphur of Calcutta, India (Chinte 1984). Accordingly, it was also in this Botanic Garden where Jacquin, in 1760 and over a century later by King, described the species grown from seeds which reportedly originated from British Honduras, now Belize. It is from these seedlots that plantings of mahogany in the Philippines originated.

Mangium in the Philippines originated mostly from Australia, with a few known derived provenances from New Zealand and various Asian countries, e.g., Indonesia, Malaysia, Thailand, and India. These were introduced to the Philippines through a joint project with the CSIRO/UNDP-FORTIP Program. Except for seeds used to establish the SPA/Seed Orchard project in Mindoro, the other pathways were difficult to determine.

Bagras, the only native eucalypt species, can be found in the island of Mindanao, particularly in the provinces of Surigao del Sur and Compostela Valley, where there are still considerable numbers of remaining natural stands. The species also naturally occurs in the provinces of Davao del Norte, Cotabato, and Sultan Kudarat, as well as in Bukidnon, where few remaining naturally grown trees can still be found. There are 2 major provenances of bagras existing in the Philippines, the Pasion, Bislig provenance and the New Bataan, Compostela provenance.

The seed origins and movements of narra, a native species and the “national tree” of the Philippines, is also difficult to establish due to poor documentation of seed collection and plantation development. However, even though there are existing provenance trials and evaluation studies of the species, the details of the design are difficult to access.

Access to seed sources is generally good. The majority (60%) or 34 of 57 seed sources evaluated can be reached by all forms of land vehicles since the roads leading to the seed sources are all-weather roads. Corporate-based seed sources are relatively more difficult to access, both in terms of location (more than 1 km from the main road) and the restrictive policies of the companies when entering their plantations. Government-based seed sources are easier to access due to the open-access nature of public lands where the seed sources are located.

The majority of seed collections are completed in just 1 d, except for some seed collectors who stay overnight in the collection area to maximize their seed collection activities and save on transportation costs.

The manner of seed collection also varies. Large-seeded species, such as mahogany, narra, and yemane, are collected through a combination of climbing and ground collection. Of these 3 species, only yemane seeds are collected off the ground, as

the fruits easily fall when mature. While the practice is proven to be economical, seeds collected are a mixture of seeds coming from good and bad mother trees, since the seed stands surveyed for yemane have not undergone any rouging. Thus, the quality of plantations derived from seeds collected through this process is an open question.

Seeds undergo post seed collection activities in the field to prepare them prior to actual seed processing. The time this takes and the activities involved from seed collection to post-selection and other post seed collection activities are important as they affect the seed quality and cost efficiency of seed collection and processing. The study showed that the majority (96%) of the post seed collection time lag from seed collection to post-collection activities occur on the same day as seed collection. This indicates a negligible lag time prior to seed transport for processing. This is good in terms of avoiding deterioration of seeds due to prolonged lag times from seed collection to actual processing.

Seed collection practices of seed sources revealed that all yemane and mahogany seed sources extract the seeds from the fruit immediately after seed collection. This is to avoid seed deterioration due to heating and potential attack by fungus if not immediately pre-processed. This is contrary to the post seed collection practice for mangium and bagras, where 13 of 24 seed sources stored the fruits/pods.

The heating effect of storage in sacks allows the pods/fruits to further mature. This in turn results in pods/fruits opening up for easier seed extraction during seed processing. Except for narra, the other 4 species undergo seed extraction. Being a samara type of fruit, narra seeds are not normally extracted but remain within the fruit at least until sowing (Schmidt 2000). Narra fruits are enclosed, and the seeds are smaller compared to those of the other 4 species. In addition, it is very tedious and impractical to extract narra seeds due to the seed size and difficulty with extraction. There are 2 or 3 tiny seeds in each samara fruit of narra.

All seed suppliers of mahogany practice manual seed extraction and cleaning using winnowing. Likewise, all mangium and bagras seed suppliers use manual seed extraction and cleaning. Manual cleaning involves either the use of a winnowing implement made of woven bamboo or *tahip* or by manual controlled blowing.

Seed-drying practices prior to storage vary. However, the majority of seed suppliers of the 5 species sun-dry the seeds for more than a day then air-dry them. This practice is based on experience, and it results in good germination percentages. However, the practice is not in any way directed towards attaining any particular moisture content (MC), as it is largely based on estimation. Thus, it is expected that the MC greatly varies, and this has important implications in terms of the length of storage, viability, and overall seed quality. Most seed processors are not aware whether a certain species is classified as either being orthodox or recalcitrant. Regardless of species, their established drying schedules hold true for all kinds of tree species.

The tools, facilities, and implements used in seed extraction are generally improvised, inexpensive, locally produced, and practical. These includes tools and implements such as improvised wooden mallets, depulping machines, jute and plastic sacks to separate the seeds from the pulp for yemane, improvised wooden mallets to break mahogany fruits, and for mangium and bagras, putting the fruits/pods inside a sack and either pounding it with a wooden mallet and/or thumping it with the feet to extract the seeds from the pods/fruits.



Overall, tools used by corporate-based seed sources are more sophisticated. They have separate seed processing facilities/rooms equipped with processing tables, mechanical tumblers, seed blowers, and sieving screens.

The seed-processing practices used by various seed sources showed some similarities and differences between and among species. Basic among seed-processing practices is conducting pre-processing activities aimed at facilitating seed extraction.

This study revealed the absence of concrete objectives of drying seeds to a certain MC level. However, the importance of seed cleaning to remove various undesirable materials, and deteriorated or damaged seeds to further upgrade the overall seed quality is well recognized by seed suppliers.

Despite the lack of more-sophisticated and appropriate processing tools, implements, and facilities, local seed processors are still able to produce seeds and attain their objectives of providing tree farmers with a sufficient viable supply of seeds through their ingenuity and hard work.

Storage practices by seed suppliers for the 5 priority tree species examined revealed varying periods of time and conditions. Of the 5 species studied, yemane has the shortest period of storage at 6 mo for all respondents. None of the respondents had any available information on the MC of yemane prior to storage.

In this study, all respondents cited that in the absence of cold storage, seeds are stored under ambient conditions. Differences in storage periods were observed from 6 mo or less, 1 yr or less, to > 1 yr. This is attributed to the variability in prevailing conditions during seed collection (e.g., seed maturity), drying (e.g., MC and environmental conditions), and storage.

The majority of respondents employ simple germination tests to determine the viability and overall seed quality. This is done to determine the percent germination or viability of the seeds, especially if the seeds have been stored for longer than 3 mo. A combination of cutting/breaking tests and germination are done to test the seed viability for species like yemane, mahogany, mangium, and narra.

The evaluation of seed quality from seed suppliers revealed variable results. Higher germination rates were recorded for corporate- and government-based seeds suppliers compared to the other seed supplier types. This complements the good reputation of the seed suppliers to provide good-quality seeds for forestation activities of the sector. Results of this study offer some avenues for improvement in terms of seed processing so that seed quality can further be improved, and of efficiency and longevity of seed storage.

Finally, phenotypic evaluation carried out of the plus trees of respondents' seed suppliers revealed that more than half (51%) of the 1134 seed trees sampled were evaluated as being straight trees.

Bagras and mangium registered the highest percentage of straight trees with 67% and 66.3%, respectively. Mahogany and yemane closely followed with 59.8% and 55.2%, straight trees, respectively. Narra registered the lowest percentage of straight seed trees with 39.2%.

The results suggest the need to reevaluate the process by which plus trees are selected. This is critical to ensure a good level of genetically superior seed sources and a reliable number of plus trees in a stand which can result in the same percentage of

phenotypically good trees planted by tree farmers, tree plantation development projects, and forestation and agroforestry projects.

CONCLUSIONS

Understanding the importance and the state of forest tree seed sources in the country is imperative to determining the degree of success or failure of forestation and industrial tree plantation development. Information on where and how many seeds can be acquired is critical in forestation and tree plantation development planning and management.

The difficulty in establishing seed origins of priority tree species studied also has implications for the growth and yield performances of seeds being used as it generally relates to genetic aspects. This has implications in terms of pests and diseases as well as growth and yields as related to a narrowing of the genetic base.

Thus, in terms of forest tree seed movements/pathways, seeds currently being used by tree farmers are not collected from original seed sources but from seed sources that have been established from the 4th to 5th generations with reference to the primary seed source. This again has implications in terms of a narrowing of the genetic base of species and on the quality (growth and yields) of the resulting stands/tree farms.

Based on these situations, good-quality seeds to support the forestation and tree farming efforts are a major concern. This study offers a number of interim seed sources (designated SPAs) of seeds of the 5 major ITP species in various parts of the country. The information can provide forestation planners information on what to expect in terms of prospective areas to source seeds, and what strategies to use in the event of an immediate need for large quantities of seeds for tree farming or reforestation programs in the country.

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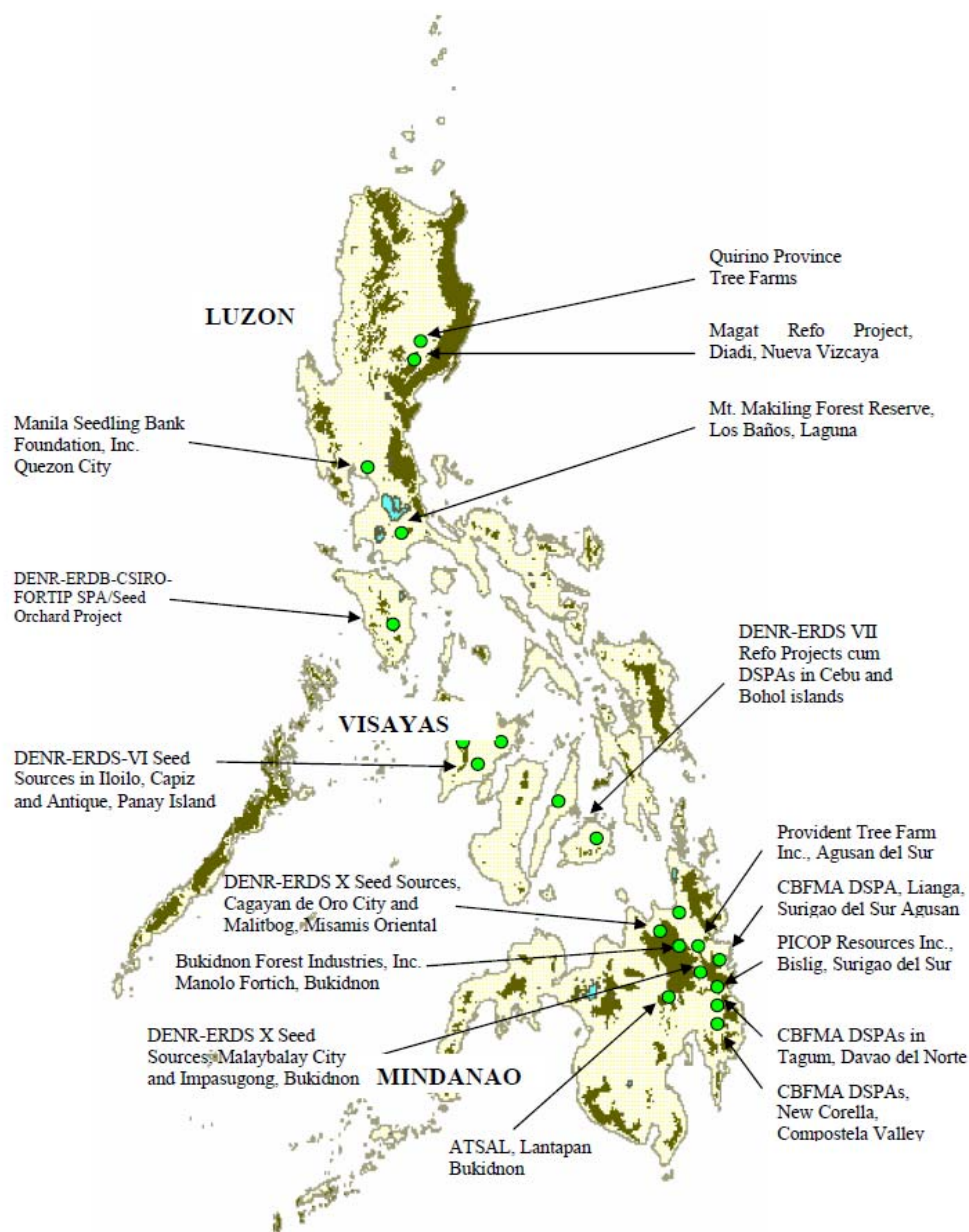
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Appendix 1. Location map showing the locations of the major study areas.





Forest Seed Science Research in India

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[Summary]

The importance of understanding various processes involved in the production and assessment of various quality parameters of forest tree seeds cannot be overemphasized. While attached to the mother plant, a developing seed has to endure the vagaries of nature like fluctuating temperature extremes, dry scorching wind, prolonged periods of rain, and pest infestations. After being collected from the tree, the seed has to pass through a series of procedures like extraction, cleaning, drying, storage, seed pretreatment, etc. Any kind of damage suffered by the seed at any of the stages during maturation and handling can result in a low seed survival rate. It is often stated that there is little point in producing genetically improved seeds at a high cost if they are killed by poor handling techniques. Raising trees and preserving their seeds are means of supporting reforestation, combating desertification, safeguarding the environment, and conserving biodiversity. However, this is an enormous challenge that requires the planting of large numbers of adapted species. This also implies a need for the careful selection of species and great demands for quality seeds.

India has varied climatic conditions due to its geographical features like the Himalayas, river and coastal plains, deserts, and Western and Eastern Ghats which support varied ecosystems that house a plethora of plant diversity. Seeds of plant species exhibit behaviors from orthodox to intermediate to recalcitrant, hence it is important to study their storage physiology before devising conservation strategies. Much research on these and other aspects of seeds has been and is being carried out by government and non-government research institutions, universities, etc. In the present paper an attempt was made to present the advances and details of seed research, and the production of and demand for quality seeds in India to emphasize the potential roles that seeds can play in the preservation and regeneration of forest genetic resources.

INTRODUCTION

India is a large developing country known for its diverse forest ecosystems and megabiodiversity and ranks 10th among the most forested nations of the world (FAO 2006). It accounts for approximately 2.5% of the world's land surface area and 1.8% of the world's forest area. The geographical features and land forms of the country are as varied as its extent: the towering Himalayas; the extensive river plains such as Ganga and Deccan in the north, center, and south; the coastal plains or ghats to the east and west; and numerous islands (Rawat and Ginwal 2009). Its botanical wealth consists of over

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15,000 species of higher plants, of which approximately 4900 species or 33% are endemic. Among the 34 biodiversity hot spots in the world, the Western Ghats and Himalayas were identified from India. Indian forests are predominantly comprised of tropical and subtropical forests, and only 11.31% consist of temperate and alpine forests (Table 1).

Table 1. Forest cover in India in different forest types (FSI, 2009)

Group	Forest type	%
Group 1	Tropical wet evergreen forest	8.75
Group 2	Tropical semi-evergreen forest	3.35
Group 3	Tropical moist deciduous forest	33.92
Group 4	Littoral and swamp forest	0.38
Group 5	Tropical dry deciduous forest	30.16
Group 6	Tropical thorn forest	5.11
Group 7	Tropical dry evergreen forest	0.29
Group 8	Subtropical broadleaf hill forest	0.38
Group 9	Subtropical pine forest	5.99
Group 10	Subtropical dry evergreen forest	0.36
Group 11	Montane wet temperate forests	3.45
Group 12	Himalayan moist temperate forests	3.79
Group 13	Himalayan dry temperate forests	0.29
Groups 14~16	Sub-alpine, moist alpine scrub, and alpine forests	3.79
Total		100.00

Forests provide a wide range of goods and services with enormous economic, social, and intangible benefits to humans, such as forest products, employment, and protection of sites of cultural value. However, deforestation, mainly conversion of forests to agricultural land, is continuing at an alarmingly high rate. The forested area, which is about 30% (4×10^9 ha) of the global total land area, decreased worldwide by 0.22% per year in the period 1990~2000 and 0.18% per year between 2000 and 2005. However, the net loss of forests is slowing as a result of the planting of new forests and of natural expansion. Forests and trees are being planted for many purposes and at increasing rates (FAO 2006).

Forests in India were considerably reduced over a period of time during the 20th century. The factors attributed to the present state of forests are: 1) human and cattle population explosion, 2) diversion of forest land to agriculture, industry, and human settlement, and 3) degradation due to illicit felling, grazing, shifting cultivation, and fire.

In recent years, India has implemented an aggressive afforestation program, and tree planting has become an intensive activity to meet the multifarious demands of timber, fuel, fodder, and non-wood forest produce besides the other associated services provided by forests. Therefore the National Forest Policy has identified a number of priority research areas. Among them, 2 important priority areas are: a) increasing the productivity of wood and other forest produce per unit of area per unit of time by applying modern scientific and technological methods, and b) effective conservation and management of existing forest resources (mainly natural forest ecosystems) through *ex situ* and *in situ* measures. While afforestation activities are progressing, the need to



conserve genetic resources is also gaining momentum, and recent figures by the FSI (2009) clearly show that there was an increase in forest and tree cover compared to 2005 (Table 2). Although industrial and commercial plantations are targeting a few indigenous and exotic species such as *Tectona grandis*, *Gmelina arborea*, *Dalbergia sissoo*, *Acacia nilotica*, *Populus*, *Eucalyptus*, and *Pinus* spp., there are numerous tree species with either timber or non-timber value such as fruits, medicines, and lumber; but development of those species has not gained momentum and they are still under-utilized due to a lack of scientific exploration and proper documentation. To utilize such species in a sustained way and to conserve their populations in natural habitats, it is imperative that cultivation techniques and conservation strategies be simultaneously developed. In propagation and conservation of a species, seeds play a very important role. In recent years owing to greater emphases on social forestry and wasteland development and to support the National Mission for a Green India as a part of the “National Action Plan on Climate Change”, the requirements for seeds have tremendously increased. Large quantities of seeds are needed every year to raise nursery stocks to propagate desired plant species. A plentiful supply of quality seeds, i.e., high in viability and vigor, is therefore one of the prerequisites to ensure that this activity is a success.

Table 2. Forest cover in India (FSI, 2009)

Class	Area (km ²)	Percent (%) of geographical area	
		2007	2005
Forest cover			
Very dense forest (canopy density > 70% of land area)	83,510	2.54	1.7
Moderately dense forest (canopy density 40~70%)	319,012	9.71	10.1
Open forests (canopy density 10~40%)	288,377	8.77	8.80
Total forest cover ^a	690,899	21.02	20.60
Non forest ^b			
Scrub (stunted trees, canopy density <10%)	41,525	1.26	1.20
Not forested	2,554,839	77.72	78.20
Total geographical area	3,287,263	100.00	

^a Includes 4639 km² of mangroves.

^b Excludes scrub and includes water bodies.

Seeds are the most suitable form to conserve germplasm and also as a means to distribute it. The quality of seeds depends on the choice of seed collection stands, seed crop abundances, time of collection, handling, and storage. The duration of the viability of the seeds in nature varies widely among species. The immense diversity of plant species found in tropical rainforests is reflected in a remarkable variety of seed characteristics. Seed traits reflect the forest diversity in aspects as distinct as season of production, volume of the seed crop, number of seeds per fruit, seed size, shape, morphology, anatomy, moisture content (MC), nature of reserves, and presence of secondary compounds (Vazquez Yanes and Rojas Arechiga 1996). Considerable

progress has been made in tropical forest seed research, but much more needs to be done. Seed collections of most species are usually done on an ad hoc basis. Presently in India, requirements for forest seeds for various program have not been properly documented, although some estimates have been made to quantify seed demands of various forestry species based on past utilization of those seeds (Ramprasad and Kandyia 1992).

Present level of production and use of genetically superior propagules in India

Although various tree species are planted every year, 90% of the plantation programs consist of *Eucalyptus*, *Acacia*, *Albizia*, *Prosopis juliflora*, *Pro. cineraria*, *D. sissoo*, *Tec. grandis*, conifers, and bamboo. During the late 1980s and early 1990s, 3×10^9 plants were planted annually. Of these, only a certain percentage of seeds were obtained from seed production areas (SPAs). Nearly 8000 ha of conifer seed stands were identified (but not SPAs). There are 24.6 ha of SPAs for *D. sissoo* available and 91 ha for eucalypts. For various other species, for which there is only limited local demand, seeds are collected from respective SPAs. An estimated 155,000 kg of teak seeds are annually available from the 3100 ha of SPAs for teak. With a germination rate of 35% and survival rate of 60%, a little over 16,000 ha can be planted with these seeds. On average, 30 kg of seeds are collected per hectare. From the 900 ha of CSOs of teak in the country, 27,000 kg of seeds are collected which are only sufficient to plant 3000 ha with 30% germination and 60% survival rates. Therefore, it was observed that CSOs produce much fewer seeds than expected per tree. For *D. sissoo*, around 300 kg of seeds can be obtained from the 24 ha of SPAs, which is sufficient to plant 9000 ha. There are 90 ha of SPAs for eucalypts providing 450 kg of seeds sufficient to plant 40,000 ha. The work done on conifers is not reliable, as most of the areas classified as seed stands are uncultured. Seed yields highly vary from tree to tree. In the case of bamboo, large amounts of seeds are collected, but these cannot be classified as superior seeds. They are collected in bulk when the entire plantation flowers, and for many years, there might not be any collections at all. The annual planting of tree seedlings in the country exceeds 3.02×10^9 seedlings with 180×10^6 seedlings originating from SPAs; the majority of planted species are teak, *D. sissoo*, and eucalypts (Katwal et al. 2003).

Future requirements for superior propagules

Being a vast country with varying climatic and edaphic conditions, India has a variety of vegetation types. Cultural diversity coupled with traditional practices has made the people highly dependent on various types of local vegetation. Therefore, preferences for different species considerably vary. This results in a dilemma when choosing species, especially when sociological aspects are taken into consideration. Various state forest departments have developed strategies to grow species by considering local requirements, in addition to other species that are required in large amounts. Future annual planting targets are expected to be little over 3×10^6 ha, consisting mainly of bamboo, *Eucalyptus*, *Acacia*, *Albizia*, *Prosopis*, *Casuarina*, *Dalbergia*, conifers, and teak. The projected annual requirements for tree seedlings is 6.16×10^9 , of which around 23.5% are expected to be raised from SPAs of certified seed sources and around 15% are expected to be raised from genetically improved sources. Fifty per cent of the teak seeds will come from



SPAs and 25% from genetically improved stock. Likewise, about 25% of future *Eucalyptus* seeds are expected to be provided as genetically improved stock. In the case of *Acacias* and *Albizia*, at least 30% of seeds will be collected from identified/certified seed sources. In the case of *Casuarina* and *D. sissoo*, 20 and 10%, respectively, will be made available from genetically improved plants. It is possible that the amounts of seeds of these 2 species available from genetically improved plants can be doubled as a result of tree improvement programs. In the case of conifers, however, only 20% of seeds will be collected from SPAs, and the supply of genetically improved seeds might not be more than 2% (Table 3). Annual seed requirements of the country were projected by Ramprasad and Kandya (1992) and Katwal et al. (2003), but a consolidated effort to determine actual requirements at the country level have to be carried out immediately because implementation of the National Mission for a Green India as part of the National Action Plan for Climate Change can further enhance the future requirements for seeds to address the mitigation potential of the forestry sector.

Table 3. Projected quantity of seeds for tree planting in India, including improved seeds (all figures in kilograms, percentages are in parentheses) (Katwal et al. 2003)

Species	Seeds from SPAs	Genetically improved seeds	Seeds by conventional practice	Total seeds
<i>Eucalyptus</i> spp.	650 (18.5%)	875 (25%)	1975 (56.5%)	3500
<i>Acacias</i>	88,800 (30%)	29,600 (10%)	177,600 (60%)	296,000
<i>Albizia</i> spp.	48,860 (30%)	15,620 (10%)	93,720 (60%)	156,200
<i>Casuarina equisetifolia</i>	114 (20%)	114 (20%)	342 (60%)	570
<i>Dalbergia sissoo</i>	412 (10%)	412 (10%)	3303 (80%)	41,276
Conifers	16,660 (20%)	1670 (2%)	64,970 (78%)	83,300
<i>Tectona grandis</i>	228,570 (50%)	114,285 (25%)	114,285 (25%)	457,140

SPAs, seed production areas.

Seed science research in India

Forestry research in India is one of the oldest in the tropical world and is looked upon as a model for other countries in the region. In India, forestry seed research is conducted by the Indian Council of Forestry Research and Education (ICFRE), an autonomous council under the Ministry of Environment and Forests of the Indian government, universities, colleges, and research wings of state forest departments. The need for production of quality seeds has been stressed at almost every silvicultural and forestry conference held in the country since the inception of scientific forestry. The National Wasteland Development Board has a country-wide target of planting a millions hectares of wastelands annually with the aim of bringing 33% of the geographical area of the country under forest/tree cover as envisaged in the National Forest Policy 1988. In order to meet this ambitious target, measures are needed to improve the overall technology and develop human skills with forest seeds. Annual expenditures on seeds and seedling production can be saved if suitable technology is adopted for better processing and maintenance of seeds in storage and by adopting improved nursery

practices. In this paper, we attempted to provide insights into important aspects of seed research carried out with particular reference to seed source variations, dormancy, and storage in relation to *ex situ* conservation in India by various researchers and also work carried out by the ICFRE, state forest departments, universities, and colleges.

Studies on seed source variations

Seed quality potential is determined by environmental factors in combination with genetic and physiological factors. To obtain quality seeds, it is imperative to document the variability that exists within a species. Seed-source studies help screen naturally available genetic variations and identify the best material for improvement, utilization, and conservation. Extensive work was carried out on seed-source variations and effects on seedling growth. In *A. nilotica*, effects of seed sources on seeds and seedling characters were studied for trees from 27 seed sources by Bagchi (1996), from 40 sources by Ubale et al. (1998), and from 12 different populations by Ginwal and Gera (2000). Variations in seeds and seedling characters were reported for seeds from 10 phenotypically superior trees of *Terminalia tomentosa* (Karoshi and Patil 2000) and 5 candidate plus trees of *Aca. mangium* (Buvaneswaran and Navamaniraj 2007). Significant seed source variations in seed morphology, seed germination, and seedling growth parameters were recorded for 8 seed sources of *Pongamia pinnata* (Shivanna, et al. 2007) and 10 seed sources of *Jatropha curcas* (Ginwal et al. 2005). Chauhan and Kanwar (2001) and Roy et al. (2004) reported seed source variations in cone and seed characters of *Pinus roxburghii* and concluded that these traits are under strong genetic control; good amounts of heritable additive genetic components can be exploited for further selection and improvement of this species. On variability in teak in India, Bedell (1989) reported variations of the effect of pretreatment on germination of seeds from 36 Indian provenances. Sivakumar et al. (2002) reported variability in drupe and germination characteristics in seeds of *Tec. grandis* collected from 30 sources covering India, Bangladesh, and Laos. Khera et al. (2004) recorded seed size variations and their impacts on germination and initial seedling growth for *Aca. catechu* Willd., *Aca. nilotica*, *Albizia lebbek*, *D. sissoo*, and *Tec. grandis* and reported that small seeds of *Aca. catechu*, *Aca. nilotica*, *Alb. lebbek*, and *D. sissoo* performed poorly in terms of germination and suggested that small seeds should be removed from the sample to achieve better and more-uniform germination and quality of seedlings. Studies on the variability of pod and seed traits of *Alb. chinensis* Thakur et al. (2002) reported high heritability coupled with high genetic advances for pod breadth, thickness, weight, and seed weight/pod. The high positive values of genotypic correlation coefficients between pod and seed characters indicated that traits are genetically controlled, and selection can be very effective in tree improvement programs. Mahadevan et al. (1999) reported that in *Casuarina equisetifolia*, identification of better seed sources or grading of seeds can be carried out based on seed weight, size, and/or shape to obtain better progenies.

In *Azadirachta indica*, Jindal et al. (1999) reported that associations among fruit, seed, and kernel weights were positive and highly significant. For the same species, Kaundal et al. (2002) evaluated seed variations from 10 different sources in India on the basis of seed traits and seed germination indices. Nawa Bahar (2007) also reported significant morphological variations in *Aza. indica* for seed traits from 14 seed sources in southern and western Haryana. In *D. sissoo*, variability was reported with reference to



pod quality for pods from different girth classes (Yadav et al. 1994), seed characters for seeds from 30 superior phenotypes (Dhillon et al. 1995), and pod and seed traits from 19 different elevational sources (Singh and Bhatt 2008). Variability in fruit and seed characters was studied in *Zizyphus mauritiana* (Bisla and Daulta 1988), *Moringa pterygosperma*, *Mor. oleifera* (Pandian et al. 1992), *Lagerstroemia speciosa* (Jamaludheen et al. 1995), and *Buchanania lanzan* genotypes (Machewad et al. 2003, Munde et al. 2004). Genetic variability among fruit characters was studied by Kumar et al. (2006) in 120 1-parent families of *Garcinia gummi-gutta* tree and in 10 genotypes of *Artocarpus heterophyllus* by Sharma et al. (2005). Nawa Bahar and Singh (2007) studied seed source variations in *Sapindus mukorossi* to identify superior seed sources for the production of quality seedlings.

Effects of provenance variations on seed and seedling characteristics were reported for morphological and physiological traits of *Grewia oppositifolia* by Uniyal et al. (2003). Annapurna et al. (2005) reported variability in seed morphological characters and germination among clones of *Santalum album*. For the same species, Jagadish et al. (2008) reported effects of seed source and collection time on germination. Based on intrapopulation variations in *Alb. procera*, Gera et al. (2001) emphasized the selection of superior trees based on germination testing in addition to tree characters like diameter at breast height for quality seed collection.

Studies on seed dormancy

Tropical trees are remarkable in their variety of systems employed to regenerate and perpetuate themselves. Under natural conditions, wild species have to adjust the timing of germination to the availability of proper temperature and light, and more importantly moisture conditions. Native species of forests are able to simultaneously sense several environmental conditions and confine their germination and emergence to particular periods of the year and habitat locations for successful establishment and survival. There are species which shed seeds at a time when moisture is not available, and in such cases seeds have to wait until conditions are conducive for germination. Such seeds have evolved dormancy mechanisms to postpone germination until favorable conditions are available. Baskin and Baskin (1998) classified dormancy as mechanical (physical), chemical, physiological, and morpho-physiological dormancy. Information on dormancy breaking and germination requirements of seeds is critical to understanding the population dynamics of a species, and also how to propagate it *in situ* and *ex situ* (Baskin and Baskin 2005).

Physical dormancy caused by an impermeable seed coat appears to be the main reason for poor germination. This can be overcome by water treatment or scarification. Improved germination by treating with cold water was reported in *Aisandra butyracea* (Tewari et al. 1996), *Pterocarpus marsupium* (Warrier et al., 2007), *Bauhinia variegata*, *Grewia disprema*, *Schleichera oleosa*, and *Ter. bellerica* (Khantwal et al., 2008). Hot-water treatment was found to be effective in overcoming dormancy in *Alb. lebbeck*, *Alb. odoratissima*, *Pro. juliflora*, *D. sissoo*, *Hardwickia binata*, *Casuarina equisetifolia*, and *Anogeissus latifolia* (Aswathanarayana et al., 1996). In *Cleistanthus collinus* (Sharma et al., 1999), *Aca. nilotica*, *Pro. cineraria*, and *Leucaena leucocephala* (Singh and Dhillon 2007), the best germination was achieved by soaking seeds in boiled water.

Thakur et al. (2002) studied the effectiveness of an aqueous solution of sulfuric acid in reducing the endocarp/seed coat dormancy in *Gre. optiva* seeds. In *Thespesia populnea*, acid scarification for 10–20 min was most effective in breaking seed dormancy (Gupta et al. 2004). Nawa Bahar (2007) recommended hot-water soaking for 24 h or sulfuric acid for 15 min for seeds of *Aca. robusta*. Physical dormancy of *Cryptocarya floribunda* caused by the high oil content in the seeds is overcome in nature by rupture under dry conditions or exposure to the sun, thereby facilitating its removal and germination (Thapliyal et al. 2004). Physical dormancy can be treated by nicking the hard seed coat in *Aca. auriculiformis*, *Aca. nilotica* (Chaturvedi and Das 2004), and *Parkia roxburghii* (Sahoo et al. 2007).

Physiological dormancy of seeds can be overcome by treatment with chemicals or stratification. *Caesalpinia sappan* seeds treated with gibberellic acid at 300 ppm showed maximum germination (71.10%), while the earliest germination was noted in etrel at 100 ppm (Channegowda et al. 2001). Gibberellic acid enhanced the *in vivo* seed germination of *Givotia rottleriformis* (Rambabu et al. 2005). *Strychnos nux-vomica* has slow and erratic germination; soaking in 500 ppm gibberellic acid (GA₃) for 24 h, incubation of seeds at 40°C for 3 d, and alternate water soaking (16 h) and drying (8 h) for 14 d significantly increased its germination (Shivakumar et al. 2006). In *Gymnacranthera canarica*, an endangered, endemic tree species of the Western Ghats, treatment with GA₃ with partial removal of the seed coat significantly enhanced germination (Tambat et al. 2006). GA₃ treatment caused an appreciable shortening of the germination period by 10 d in *Abies pindrow*, *Cupressus torulosa*, and *Picea smithiana* (Rawat et al. 2006). When prechilled to 4°C for 20 d, imbibed seeds of *Myrica esculenta* showed the best response (Bhatt 2000). Morphological dormancy due to an underdeveloped embryo was reported by Thapliyal et al. (2008) in *Dillenia indica*.

Studies on seed storage

Irregular and often infrequent seed production by forest species necessitates the storage of seeds. It is generally believed that seeds produced in a good seed year will have high genetic quality. These seeds should be maintained as sources of germplasm so that quality seeds can be supplied in lean seed years. It is from this point of view that storage under controlled conditions is considered the most convenient and commonly used short- to medium-term *ex situ* conservation strategy for forestry trees. According to Harrington (1972) of all of the *ex situ* conservation strategies, the easiest and least expensive method of preserving the world's existing plant genotypes would appear to be seed storage. Knowledge about the nature of seeds and their longevity is important for researchers and forest managers, as seeds carry genetic information that has a bearing on evolution, ecology, and physiology and thereby helps strengthen ecosystems to withstand the vagaries of nature. *Ex situ* conservation by seed storage is 1 viable option for preserving germplasm of valuable and endangered plants by prolonging seed viability in storage under optimal conditions. Based on the desiccation tolerance, seeds are classified as a) orthodox, b) recalcitrant, and c) intermediate (Ellis et al. 1991). When studying different aspects of *ex situ* conservation, various biological factors of species must be considered, and primarily important are the nature, production, size, and volume of seeds.



The seed storage temperature and seed MC are 2 critical factors affecting the viability of seeds in storage. The rate of drying significantly affects the viability of seeds. Rapid drying to lower moisture levels excludes the possibility of desiccation-induced damage and was reported in *Shorea robusta* (Varghese et al. 2004) and *Gmelina arborea* (Naithani et al. 2006). Similarly *Ter. bellerica* seeds can withstand desiccation and gradual moisture reduction which significantly improved germination (Warrier et al. 2005). Prolonging viability by storing at low temperatures was reported for *Grewia oppositifolia* (Uniyal et al. 2000), *Toona ciliata* (Sharma et al. 2002), *Pin. roxburghii* (Gautam et al., 2005), *Ter. bellerica* (Warrier et al., 2005), and *Feronia elephantum* (Sivakumar et al. 2006). A desiccation-tolerant and orthodox nature was reported for *Ailanthus excelsa* (Rawat et al. 2001), *Acer caesium*, *Ulmus wallichiana* (Phartyal et al. 2003a, b), *Gre. optiva* (Nayal et al. 2002), *Dendrocalamus membranaceus* (Rawat et al. 2003), *Den. strictus* (Sharma et al. 2005), *Buchanania lanzan*, *Diospyros melanoxylon*, *Gme. arborea* (Naithani et al. 2005), *Strychnos nux-vomica* (Sivakumar et al. 2006), and *Santalum album* (Joshi and Arun Kumar 2008). Effects of storage conditions on seed longevity were studied in *Ter. myriocarpa* (Bahuguna et al. 1987), *Tecomella undulata* (Jindal et al. 1990), *Adina cordifolia*, *Mitragyna parvifolia*, and *Hymenodictyon excelsum* (Rajput and Mishra 1996). Athaya (1985) reported that seeds of *Alb. procera*, *Cas. fistula*, *Lagerstroemia parviflora*, and *Ter. arjuna* were viable after 3 yr of storage. Khomane and Bhosale (2003) studied the storage behavior of seeds of 15 important fuelwood species *Cas. siamea*, *Cas. fistula*, *Alb. lebbbeck*, *Aca. nilotica* var. *cupressiformis*, *Peltophorum pterocarpum*, *Parkinsonia aculeata*, *Aca. auriculiformis*, *Aca. holosericea*, *Aca. ampliceps*, *Aca. mangium*, *Azadirachta indica*, *Euc. tereticornis*, *Casuarina equisetifolia*, *Pro. juliflora*, and *Gliricidia sepium*.

Tropical recalcitrant seeds are characterized by sensitivity to desiccation and low temperatures. This was reported for *Dipterocarpus retusus* (Kundu 2001), *Myristica malabarica* (Kumar et al. 2002), *Garcinia indica*, *Gar. cambogia*, *Gar. Xanthochymus* (Malik et al. 2005), and *Madhuca indica* (Varghese et al. 2006, Joshi et al. 2008). *Holoptelea integrifolia* seeds are sensitive to low temperature but are not sensitive to desiccation and can be dried to a 5% MC (Rajput et al. 2001). On the contrary *Phoebe goalparensis* seeds are sensitive to desiccation and can be stored at 5°C temperature at 33% moisture content (Kundu 2003). Sivakumar et al. (2006) reported that *Aegle marmelos* is sensitive to low temperatures. In *Quercus leucotrichophora*, seeds stored at 5°C in polybags maintained 90% germination for 9 mo (Sharma and Bharadwaj 1999). Dormancy in recalcitrant seeds was reported for *Cryptocarya floribunda* (Thapliyal et al. 2004) and *Gar. gummi-gutta* (Joshi and Arun Kumar 2007).

Extensive studies were carried out on the storage behavior of neem (*Aza. indica*) seeds (Bharadwaj et al. 1995, Singh et al. 1997, Mishra et al. 1998, Khera et al. 2000, Chauhan et al. 2002, Rajaput 2004, Kumar and Bangarwa, 2005). Nayal et al. (2002) reported neem seeds as being intermediate in nature.

Seed science research at the Indian Council of Forestry Research and Education (ICFRE)

The ICFRE, an autonomous council under the Ministry of Environment and Forest, Government of India, has the mandate to carry out forestry research and

education. One of the research priority areas is to conduct research on conservation, protection, and sustainable development of existing forests, to conserve biodiversity, and to increase productivity of existing forests and future plantations through high-quality seed production. The ICFRE has principally agreed to establish a National Bureau of Forest Genetic Resources (NBFGR) under its Genetic Resource Programme, with a wide network of regional institutes situated at various agroecological zones for germplasm collection, *ex situ* and *in situ* conservation, introduction, and evaluation. Different seed laboratories of ICFRE institutes have identified tree species (Table 4), and the main line of research is developing procedures for optimizing the germination requirements, storage, and desiccation tolerance of tropical tree species. The council is supported by various funding agencies, and most of the work on storage physiology of major species has been carried out under various externally funded programs by DANIDA (1977~1987), the World Bank (1994~2001), USDA (1996~2006), and in-house funding provided by the ICFRE. Under a World Bank-aided Forestry Research and Extension Project, various institutes of the ICFRE have established *ex situ* conservation plots for various species as sources of quality planting material (Table 5).

Seed science research by organizations other than ICFRE institutes

Realizing the necessity of quality seeds for different activities, forest departments have initiated various research activities pertaining to seeds. Research needs and perspectives on seed-related activities of various forest departments are provided in Appendix I. Presently, most of the activities of forest department are oriented towards establishing seed orchards as sources of quality seeds and as *ex situ* conservation measures (Table 6). A list of universities and colleges engaging in education and research in forestry with emphasis on seed science is provided in Appendix II.

NBPGR under the auspices of the Indian Council of Agricultural Research is primarily involved in plant genetic resource management by undertaking activities of germplasm collection (region/crop specific), characterization, evaluation, and conservation using both conventional seed-storage techniques and applying biotechnological approaches for *in vitro* conservation and cryopreservation. Preliminary studies on seed-storage behaviors of some fruit, agro-forestry, and fruit tree species have been conducted, and suitable short- and medium-term protocols were developed. Their studies revealed that seeds of *Santalum album*, *Jatropha curcas*, *Pongamia pinnata*, *Putranjiva roxburghii*, and *Phoenix dactylifera* are orthodox in nature, whereas, those of *Sapindus emarginatus* and *Embelica officinalis* are intermediate, and those of *Syzygium cumini* are strictly recalcitrant. NBPGR in its vision document envisages work on quantification of desiccation and freezing sensitivity of seeds of uninvestigated tropical, subtropical, and temperate fruits, nuts, and multipurpose tree species.



Table 4. Priority forestry species and jurisdiction states of different ICFRE Institutes

S. No.	Institute	Jurisdiction sates	Species
1	Arid Forest Research Institute, Jodhpur	Rajasthan, Gujarat and Dadra, and Nagar Haveli	<i>Acacia nilotica</i> , <i>Dalbergia sissoo</i> , <i>Prosopis cineraria</i> , <i>Eucalyptus camaldulensis</i> , <i>Holoptelia integerifolia</i> , <i>Tecomella undulata</i> , <i>Zizyphus</i> spp., <i>Terminalia</i> spp., <i>Salvadora persica</i>
2	Institute of Forest Genetics & Tree Breeding, Coimbatore	Tamil Nadu and Kerala and the Union Territories of Andaman and Nicobar Islands, Lakshadweep, and Pondicherry.	<i>Azadirachta indica</i> , <i>Tectona grandis</i> , <i>Hopea parviflora</i> , <i>Vateria indica</i> , <i>Madhuca longifolia</i> , <i>Michelia champaca</i> , <i>Oroxylum indicum</i> , <i>Pterocarpus marsupium</i> , <i>Terminalia bellerica</i> , and <i>Calophyllum inophyllum</i>
3	Forest Research Institute, Dehradun	Uttarakhand, Uttar Pradesh, Chandigarh, Haryana, Punjab, and Delhi	<i>Eucalyptus</i> spp., <i>Shorea robusta</i> , <i>Dalbergia sissoo</i> , <i>Acacia</i> sp., Poplars, <i>Leucaena leucocephala</i> , Bamboo, Himalayan pines, tropical pines, <i>Abies pindrow</i> , <i>Picea smithiana</i> , [spell out]RET species, oaks, fodder species, etc.
4	Tropical Forest Research Institute, Jabalpur	Chattisgarh, Madhya Pradesh, Maharashtra, and Orissa	<i>Pterocarpus marsupium</i> , <i>Schleichera trijuga</i> , <i>Terminalia arjuna</i> , <i>Hardwickia binnata</i> , <i>Moringa oleifera</i> , <i>Holoptelea integrifolia</i> , <i>Sapindus laurifolia</i> , <i>Terminalia chebula</i> , <i>Ablomoscus moscatius</i> , <i>Rauvolfia serpentina</i> , <i>Emblica officinalis</i> , <i>Bassia latifolia</i> (R), and <i>Mimusops elengi</i> (I)
5	Himalayan Forest Research Institute, Shimla	Himachal Pradesh, Jammu, and Kashmir	<i>Picea smithiana</i> (spruce), <i>Abies pindrow</i> (silver fir), <i>Cedrus deodara</i> (deodar), <i>Taxus baccata</i> (yew), and their broadleaf associates like <i>Quercus dilatata</i> (Mohru oak), <i>Populus ciliata</i> , <i>Prinsepia utilis</i> , <i>Aesculus indica</i> , <i>Ulmus laeviegata</i> , <i>Dodonea viscosa</i> , <i>Indigofera gerardiana</i> , <i>Woodfordia fruticosa</i> , <i>Prunus cornuta</i> , etc.
6	Institute of Wood Science & Technology, Bangalore	Karnataka, Andhra Pradesh, and Goa	<i>Santalum album</i> , <i>Tectona grandis</i> , bamboo, <i>Garcinia gummi-gutta</i> , <i>Dysoxylum malabaricum</i> , <i>Myristica fragrans</i> , <i>Madhuca indica</i> , <i>Dipterocarpus indicus</i> , and <i>Hydnocarpus pentandra</i>
7	Rain Forest Research Institute, Jorhat	Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, & Tripura,	<i>Gmelina arborea</i> , <i>Tectona grandis</i> , bamboo, <i>Acacia</i> spp., <i>Dipterocarpus</i> sp.
8	Institute of Forest Productivity, Ranchi	Bihar, Jharkhand, Sikkim, and West Bengal	eucalyptus, <i>Acacia catechu</i> , <i>Adina cordifolia</i> , tropical pines, <i>Leucaena leucocephala</i> , <i>Gmelina arborea</i> , and <i>Schleichera oleosa</i>

Table 5. *Ex situ* conservation of various species as clonal and seedling seed orchards established by different institutes of ICFRE

State	Coordinating institute of ICFRE	Clonal seed orchard	Seedling seed orchard
UP, Haryana, and Punjab	Forest Research Institute Dehra Dun	<i>Dalbergia sissoo</i> , <i>Eucalyptus tereticornis</i> , <i>Pinus roxburghii</i> (28)	<i>Dalbergia sissoo</i> , <i>Eucalyptus tereticornis</i> , <i>Pinus roxburghii</i> (25.2)
TN, Kerala, Andaman, and Nicobar	Institute of Forest Genetics and Tree Breeding, Coimbatore	<i>Eucalyptus</i> spp., <i>Casuarina</i> spp., <i>Tectona grandis</i> (27.7)	<i>Eucalyptus</i> spp., <i>Casuarina</i> spp., <i>Tectona grandis</i> (38.3)
Karnataka and Andhra Pradesh	Institute of Wood Science and Technology, Bangalore	<i>Eucalyptus</i> spp., <i>Casuarina</i> spp., <i>Tectona grandis</i> (12)	<i>Eucalyptus</i> spp., <i>Casuarina</i> spp., <i>Tectona grandis</i> (34.0)
MP, Maharashtra, and Orissa	Tropical Forest Research Institute, Jabalpur	<i>Tectona grandis</i> , <i>Casuarina</i> spp., <i>Albizia procera</i> , bamboo (41.0)	<i>Tectona grandis</i> , <i>Casuarina</i> spp., <i>Albizia procera</i> , bamboo (83.5)
Rajasthan	Arid Forest Research Institute, Jodhpur	<i>Tectona grandis</i>	<i>Dalbergia sissoo</i> , <i>Acacia nilotica</i> , <i>Eucalyptus</i> spp. (55.0)
Gujarat		<i>Dalbergia sissoo</i> , <i>Acacia nilotica</i> , <i>Eucalyptus</i> spp. (29.0)	
Jammu, and Kashmir	Himalayan Forest Research Institute	<i>Dalbergia sissoo</i> , <i>Pinus</i> spp. (12.8)	<i>Dalbergia sissoo</i> , <i>Pinus</i> spp. (6.0)
Himachal Pradesh			
UP	Centre for Social Forestry and Eco-Rehabilitation Allahabad	<i>Eucalyptus</i> spp. (8.0)	<i>Acacia</i> spp., <i>Dalbergia sissoo</i> (12.0)
Bihar, Orissa	Institute of Forest Productivity, Ranchi	<i>Eucalyptus</i> spp. (3.0)	<i>Acacia</i> spp., <i>Eucalyptus</i> spp., <i>Dalbergia sissoo</i> , <i>Gmelina arborea</i> (30.5)
W.B.			

(Values within parenthesis are area in hectares).

Future perspectives

Future lines of work on seed research and *ex situ* conservation by ICFRE will primarily aim for a holistic strategy for conservation using all available methods and approaches. To strengthen forestry conservation programs in India, the council has taken the initiative to establish the National Bureau of Forest Genetic Resources. When identifying a species for conservation and utilization, it is necessary to understand the variability it harbors, its genetic makeup, its silvicultural response, its immediate use, and the probable threats it faces. Although seed research work has been carried out on a number of species, a complete package of practices for collection, handling, and storage is still lacking. Therefore, there is an urgent need for systematic and focused studies on understanding seed development, maturation, dormancy, conditions required to break dormancy, promoting germination of non-dormant seeds, storage behavior including



desiccation and chilling tolerance of orthodox and recalcitrant seeds, use of cryopreservation techniques, and understanding the roles of microorganisms on seed viability. With advances in science and technology, the future looks bright for *ex situ* conservation. Conservation studies and strategies should ultimately aim at complimenting *ex situ* and *in situ* methods for the unified purpose of conservation and sustained utilization. We are of the opinion that seed research per se from conservation perspectives has crossed from the exclusive domain of seed technologists to a cross-disciplinary venture of botanists, ecologists, biochemists, geneticists, and biotechnologists.

Table 6. Seed orchards established by different state forest departments in India

States	Species (area in hectares)
Arunachal Pradesh	<i>Bombax ceiba</i> (4), <i>Chukrasia tabularis</i> (1), <i>Duabanga grandiflora</i> (1), <i>Gmelina arborea</i> (3), <i>Michelia champaca</i> (1), <i>Phoebe goalparensis</i> (1), <i>Tectona grandis</i> (17), <i>Terminalia myriocarpa</i> (2)
Bihar	<i>Dalbergia sissoo</i> (2), <i>Tectona grandis</i> (134)
Chhattisgarh	<i>Emblica officinalis</i> (20), <i>Eucalyptus</i> (15), <i>Gmelina arborea</i> (39), <i>Tectona grandis</i> (98)
Haryana	<i>Dalbergia sissoo</i> , <i>Tectona grandis</i> , <i>Azadirachta indica</i> , <i>Ficus benghalensis</i> , <i>F. religiosa</i> , <i>Eucalyptus</i> spp., <i>Populus deltoides</i> , <i>Acacia nilotica</i> and <i>Melia azedarach</i> (total area 43), <i>Euc. tereticornis</i> (12)
Jharkhand	<i>Acacia catechu</i> , <i>Cassia siamea</i> , <i>Tectona grandis</i> , <i>Dalbergia sissoo</i> (total 60)
Karnataka	<i>Eucalyptus</i> (18), <i>Tectona grandis</i> (110)
Kerala	<i>Tectona grandis</i> (51)
Madhya Pradesh	<i>Tectona grandis</i> (113)
Maharashtra	<i>Dalbergia sissoo</i> (1), <i>Tectona grandis</i> (235)
Manipur	<i>Pinus kesiya</i> (0.5), <i>Tectona grandis</i> (0.3)
Orissa	<i>Tectona grandis</i> (12)
Punjab	<i>Dalbergia sissoo</i> (4)
Tamil Nadu	<i>Anacardium occidentale</i> (12), <i>Casuarina equisetifolia</i> (5), <i>Eucalyptus tereticornis</i> (2), <i>Pterocarpus marsupium</i> (2), <i>Santalum album</i> (2), <i>Tectona grandis</i> (19), <i>Terminalia</i> sp. (6)
Tripura	<i>Gmelina arborea</i> (5), <i>Tectona grandis</i> (5)
Uttarakhand	<i>Cedrus deodara</i> , <i>Pinus roxburghii</i> , <i>P. wallichiana</i> , <i>Ficus micrantha</i> , <i>Juglans regia</i> and <i>Abies pindrow</i> (total 216)
Uttar Pradesh	<i>Acacia nilotica</i> (6), <i>Bombax ceiba</i> (7), <i>D. sissoo</i> (95), <i>Tectona grandis</i> (3)

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Appendix I. Research activities carried out by some of the forest departments in India

Sl. No.	Forest department	Research activity
1.	Arunachal Pradesh	It has the only State Forest Research Institute in northeast India. It has established one of the largest clonal seed orchards of teak with over 132 clones. Clonal seed orchards were established for <i>Acrocarpus fraxinifolius</i> , <i>Chukrasia tabularis</i> , <i>Tectona grandis</i> , <i>Terminalia myriocarpa</i> , <i>Gmelina arborea</i> , <i>Michelia champaca</i> , <i>Phoebe goalparensis</i> , <i>Bombax ceiba</i> , <i>Altingia excelsa</i> , <i>Morus laviegata</i> , <i>Anthocephalus chinensis</i> , and <i>Duabanga grandiflora</i>
2.	Chattisgarh	With the objective of conservation and development, the department proposed establishing a Forest Research Institute. It has also established seedling and seed[?] orchards on 193 ha and clonal seed orchards o 184.5 ha for important species.
3.	Haryana	The department has taken the initiative by carrying out germination studies and viability studies for different species, and established clonal orchards for <i>Dalbergia sissoo</i> and <i>Eucalyptus</i> spp. from which quality seeds are being collected.
4.	Jammu and Kashmir	One of the priority research activities of the State Forest Research Institute is seed collection, storage, and testing of important species. As an <i>ex situ</i> conservation measure, established seedling seed orchards of <i>Acacia catechu</i> and clonal seed orchards of <i>Dalbergia sissoo</i> were established.
5.	Karnataka	As a part of <i>ex situ</i> conservation, seedling (752.11 ha, 55 species), clonal seed orchard (1642.79 ha, 27 species), and germplasm banks of important species were established. The department has set aside the following mandates for activities related to seed research: • Collection and processing of quality seeds from identified superior sources such as seed stands, clonal seed orchards and seedling seed orchards • Distribution of these seeds to various forest divisions of the state • Carrying out trials with respect to germination percentage of various species
6.	Kerala	The Kerala Forest Seed Centre established jointly by the Kerala Forest Research Institute (KFRI) and the Kerala Forest Department (KFD), supplies certified seeds indicating the source, purity, germination percentage and number of seeds per kilogram.
7.	Madhya Pradesh	The first state-level forest research institute in the country, was established in 1963. The activities of this institute include collecting quality seeds from identified superior genetic sources, seed storage, seed certification, and conducting research on seed biology, pollination biology, physiology, and biochemistry. An area of 495 ha of a seedling seed orchard and 365.50 ha of a clonal seed orchard of commercially important species were established.



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| 8. | Maharashtra | Maharashtra Van Sanshodhan Sanstha (MVSS) was established in 1968. It has the largest gene pool of teak in Asia comprising of 260 clones from all over India. The institute is recognized for developing a prototype machine for teak seed treatment which has helped reduce the germination time of teak seeds. Presently there are 424.64 ha of teak seed orchards and 8.5 ha of seedling seed orchards distributed in various agro-climatic zones of Maharashtra. |
| 9. | Manipur | As an <i>ex situ</i> conservation measure, the department established a seedling seed orchard of <i>Gmelina arborea</i> and clonal seedling seed orchards of <i>Tectona grandis</i> and <i>Gmelina arborea</i> . |
| 10. | Meghalaya | The department has initiated research work related to conservation of germplasm by establishing conservation plots for rare and endangered species such as <i>Taxus baccata</i> , <i>Podocarpus nerifolia</i> , <i>Rhododendron arboreum</i> , <i>Acquilaria agallocha</i> , and some medicinally important species such as <i>Azadirachta indica</i> , <i>Terminalia chebula</i> , and <i>Terminalia bellerica</i> . |
| 11. | Rajasthan | The Forest Department's state policy clearly mentions that in order to enhance productivity, seedling seed orchards and clonal seed orchards shall be established in different regions so as to ensure certified seeds of various species. |
| 12. | Tamil Nadu | India's first 'Forest Seed Centre' was established by Tamil Nadu Forest Department in 1974 which mandated itself to collect quality seeds from seed orchards, seed production areas, seed stands etc., for the then economically important tree species like Teak, Wattle, <i>Eucalyptus</i> , <i>Acacia species</i> , <i>Ailanthus</i> , <i>Pinus patula</i> , and other species. The seeds thus collected from genetically superior stock were cleaned, graded, tested for germination status, stored in appropriate containers, and then distributed to users, mostly to forest departments of the southern states based on their intent. The world's first clonal seed orchard of <i>Casuarina equisetifolia</i> was established at Neyveli Research Centre. The department also standardized seed quality, pretreatment methods, and viability periods for various species such as <i>Acacia concinna</i> , <i>Aca. elata</i> , <i>Aca. nilotica</i> , <i>Adenanthera pavoniana</i> , <i>Albizia chinensis</i> , <i>Alb. lucida</i> , <i>Bixa orellana</i> , <i>Cassia elata</i> , <i>Cas. fistula</i> , <i>Cas. javanica</i> , <i>Chloroxylon switenia</i> , <i>Crotalaria Linnaeus</i> , <i>Dendrocalamus giganteous</i> , <i>Derris indica</i> , <i>Emblica officinalis</i> , <i>Erythrina stricta</i> , <i>Garcinia indica</i> , <i>Glyricidia maculate</i> , <i>Gmelina arborea</i> , <i>Jatropha curcas</i> , <i>Khaya nasica</i> , <i>Melia dubia</i> , <i>Peltophorum pterocarpum</i> , <i>Phyllanthus amarus</i> , <i>Pinus patula</i> , <i>Podocarpus wallichiana</i> , <i>Pterocarpus marsupium</i> , <i>Puteranjiva roxburgiana</i> , <i>Robinia pseudocacia</i> , <i>Santalum album</i> , <i>Simaruba glauca</i> , <i>Spondias pinnata</i> , <i>Syzigium cumini</i> , <i>Vatica roxburgiana</i> , <i>Zizyphus trinerva</i> , and <i>Z. xylopyrus</i> . |
| 13. | Uttar Pradesh | State Forest Research Laboratory was established and the department took over responsibility of carrying out scientific research on varied aspects of forestry. One of the main objectives is to supply quality seeds to the department as well as to other entities. |

Appendix II. Universities and colleges with educational programs and carrying out research on seed science

Sl No.	Name of the university or college
1	Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan
2	College of Forestry, Ponnampet (University of Agricultural Sciences, Bangalore)
3	College of Forestry, Sirsi (University of Agricultural Sciences, Dharwar)
4	College of Forestry, Vellanikkara (Kerala Agricultural University, Thrissur)
5	College of Forestry, Mettupalyam, (Tamil Nadu Agricultural University, Coimbatore)
6	Aspee College of Horticulture and Forestry (Navasari Agricultural University)
7	College of Forestry and Hill Agriculture, (Govind Ballabh Pant University of Agriculture and Technology, Ranichauri, Uttaranchal)
8	College of Horticulture and Forestry, Pasighat (Central Agriculture University, Imphal, Manipur)
9	College of Horticulture and Forestry (Maharana Pratap University of Agriculture and Technology, Jhalwar, Rajasthan)
10	College of Forestry (Birsa Agriculture University, Ranchi, Jharkhand)
11	College of Agriculture (CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh)
12	Department of Forestry (Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli)
13	Department of Forestry (Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Jammu and Kashmir)
14	Department of Forestry (North Eastern Regional Institute of Science and Technology, Nirjuli, Arunachal Pradesh),
15	Department of Forestry, College of Agriculture (Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur)
16	Department of Forestry (Orissa University of Agriculture and Technology, Bhubaneswar)
17	Department of Forestry (Chaudhary Charan Singh Haryana Agriculture University, Hisar, Haryana)
18	Department of Forestry (Chandra Shekhar Azad University of Agriculture and Technology, Kanpur, Uttar Pradesh)
19	Department of Forestry and Natural Resources, College of Agriculture, Ludhiana (Punjab Agricultural University, Ludhiana)
20	Department of Forestry, Wild life, Environmental Science and Eco Development (Guru Ghasidas Vishwavidyalaya, Bilaspur, Chattisgarh)
21	Kumaun University, Nainital, Uttarakhand
22	H.N.B. Garhwal University, Srinagar, Pauri Garhwal, Uttarakhand
23	Uttar Banga Krishi Vishwavidyalaya, Cooch Behar, West Bengal
24	Forest Research Institute Deemed University, Dehradun



Pretreatment to Enhance Germination of Seeds of *Diospyros melanoxylon* Roxb.

Geeta Joshi,¹⁾ Arun Kumar¹⁾

[Summary]

Diospyros melanoxylon, an endemic tree of India, occurs in dry tropical habitats and has multiples uses. The wood is popularly called Indian ebony and is used for carving, and making boxes, combs, plows, and beams. The bark and seeds have medicinal properties. The leaves of this plant constitute one of the most important raw materials of the “bidi” (Indian cheap smoke) industry. The fruits are edible, and seeds have low germination. To enhance the germination percentage of *D. melanoxylon*, seeds were pretreated by manual scarification, and by soaking in gibberellic acid, boiled water, hydrogen peroxide, calcium hypochlorite (bleaching powder) and various combinations, consisting of 13 treatments including a control. Untreated seeds had 40% germination while manual scarification resulted in a reduction in the germination percentage. Treatment with gibberellic acid did not enhance germination. Seeds soaked in bleaching powder for 15 min had 56% germination. Germination was further enhanced to 72% by combination treatment of bleaching powder and gibberellic acid (500 ppm for 16 h). The study showed that *D. melanoxylon* has a combination of physical and physiological dormancy.

Key words: bleaching powder, gibberellic acid, seed dormancy, seed germination, seed pretreatment.

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Advances in Precision Seed Quality Assessment in Conifer Nurseries

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[Summary]

Conventional practice in many North American forest nurseries dictates over-sowing of conifer seeds when the germination capacity of seed lots is low. Results of experiments at the University of Idaho Center for Forest Nursery and Seedling Research suggest that this practice may have adverse effects. First, empty and poorly formed conifer seeds may be more likely to serve as vectors for seed-borne fungus. Second, over-sowing of seeds based on low mean germination capacity as determined in standardized test procedures (e.g., International Seed Testing Association) tends to under-utilize valuable, high quality seed. Although over-compensation for mean germination capacity successfully results in filled container cells, there are trade-offs: many good seeds are lost during the subsequent thinning of emerged seedlings that occur in multiples. New statistical sampling strategies, coupled with digital X-radiographic seed quality assessment, make it possible to screen and filter seed such that only very high quality individuals with particular attributes are sown. Because this strategy both minimizes the transfer of fungal contamination and maximizes use of high-value seed, seed conservation is optimized. Advances in computer simulation further allow particular seed physiological attributes of interest to be targeted during seed cleaning, grading and sowing.

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Seed Longevity and Deterioration in Orthodox Seeds: A Perspective Based on Structural Stability of Visco-Elastic Materials

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[Summary]

Longevity of orthodox seeds during storage under controlled conditions can be estimated by mathematical models describing general temperature and moisture responses and accounting for variation within species by the initial seed quality. Despite the well-known trends, longevity of a particular seed lot is hard to reliably predict. Poor predictive power may result from our inability to adequately define initial seed quality and identify the traits associated with it. Or, reliable predictions may be limited because models do not fully account for anomalies in temperature and moisture response or interactions among factors. This paper presents seed deterioration as a problem of maintaining structural stability within the cytoplasmic matrix of seed cells as they dry and while they are stored. This perspective challenges a number of assumptions of the models used to define orthodox seed behavior. Concepts about visco-elastic behavior build from earlier concepts about the role of molecular glasses in seed longevity and provide a means to explain anomalous response to storage environment that distinguish orthodox from recalcitrant and intermediate behaviors.

INTRODUCTION

Orthodox and recalcitrant seed categories have long been used to classify seed viability response to moisture, temperature and storage time (Roberts 1973). In modern usage, orthodoxy usually refers to seeds' increasing longevity with drying, and recalcitrance refers to seeds' decreasing longevity (immediate mortality, really) with drying. The main use of the seed storage categories is to guide management decisions that prolong seed lifespans. Longevity of orthodox seeds typically follow temperature and moisture responses described by empirical models such as Harrington's Thumb Rules from the 1950s and Roberts and Ellis' Viability Equations improved in the 1980s (Ellis and Roberts 1981) and modified again for temperature in the 1990s (Dickie et al. 1990). Recalcitrant seeds, on the other hand, do not, and so are considered problematic to store. A category called intermediate seeds was introduced in recognition that moisture or temperature responses in some seed species partially adhere to longevity models but show unexplained anomalies in response to temperature or moisture (Ellis et al. 1990a). The orthodox-recalcitrant paradigm is largely based on observations using cultivated species. As the storage physiology of more and more species has been documented, the incidence of seeds classified with intermediate storage behavior has increased and the nominal categories of seed storage behavior appear somewhat arbitrary (Berjak and Pammenter 2008). Moreover, the continued problem of being unable to accurately

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predict seed longevity despite models, suggest that unknown factors play a role in the kinetics of seed deterioration. This paper presents the argument that those unaccounted for factors are hidden within the assumptions of longevity models and that a different perspective on seed aging that uses concepts of molecular mobility within the dried structure of seeds may help to elucidate other factors that control seed longevity and ultimately extend seed lifespans or reliably predict when they will end.

Longevity Models and Unexplained Variation in Seed Storage Behavior

Longevity models mathematically describe seed viability responses to time, temperature, moisture and initial quality and have provided the essential framework for hypothesis testing on critical seed storage questions for the past 30 years. These models inform about species characteristics on average and identify seeds that tend toward short or long lifespans within the orthodox seed storage category (e.g. Priestley 1986, Simpson et al. 2004, Walters et al. 2005, Probert et al. 2009). Viability models are less effective at describing potential longevity of a particular seed lot or explaining variation in measured longevity among seed lots.

A closer examination of the assumptions of longevity models may reveal the basis for the unexplained variation. For orthodox seeds, we generally assume that responses are continuous; discontinuities (e.g., anomalous responses) may signal an intermediate physiology. The most famous longevity model, the Viability Equations (Ellis and Roberts 1981), also makes the assumption that explanatory factors of seed longevity – moisture, temperature and initial seed quality – are independent and hence their effects are additive. Other models account for interaction of factors, for example Harrington's '100s Rule' which states that suitable conditions for 5 year storage can be accomplished if the sum of relative humidity (RH) and temperature (in degrees F) is less than 100. The Viability Equations provide species constants to describe moisture and temperature response. The assumption that response to environmental conditions is uniform within a species leads to the additional assumption that variation among seed lots is explained by the initial seed quality which, as already stated, is assumed to behave independently of temperature or moisture.

Many of the assumptions of longevity models have not held up to scrutiny. For example, early on it was discovered that response to moisture could only be modeled within a restricted moisture range, and that discontinuities in the form of a limited benefit of drying occurred at critical water contents (Ellis et al. 199b, Walters 1998). Because most orthodox species exhibited moisture anomalies; the discontinuous behavior was not treated as a universal exception to the model and debate usually focused only on the value of the critical water content (or relative humidity) and whether there were detrimental effects associated with drying beyond some critical value (Walters 1998). Later, discontinuities in temperature responses were discovered (Walters 2004, Walters et al. 2005, Ellis and Hong, 2006), which explain the limited benefit of low temperature that were already accounted for in Ellis and Roberts' Viability Equations (Ellis and Roberts 1981) and its modification (Dickie et al. 1990). Experimental demonstration that the primary factors explaining seed longevity are interacting factors requires a complete factorial experimental design which is rare because the experiment is technically difficult and time consuming (Niedzielski et al. 2009). Interactions of moisture and temperature are implied by the established involvement of seed glasses in seed longevity (Buitink and



Leprince 2004). The interacting effects of these factors in seed aging suggest that species constants are actually averages with associated variation. Moreover, the seed quality factor measured using challenges of high moisture–high temperature treatments in so-called accelerated aging or controlled seed deterioration tests (assuming that it is conducted within the upper limit of inference for the longevity models) reveal some aspects of seed quality, but cannot reveal interacting effects of seed quality and the moisture/temperature environment.

The Essential Question: How do we know when seeds will die in storage?

The seed aging time course resembles an accumulated risk model with threshold representing catastrophic losses in seed viability (Fig. 1). Viability monitoring tests were used to discover that aging followed this type of kinetic; however, individual monitor tests can only reveal whether aging in a seed population is still asymptomatic or at a stage where rapid losses in viability are now detectable. A monitor test during the asymptomatic phase cannot reveal when the threshold marking catastrophic viability loss will occur. This is a substantial problem for seed companies and seed banks where unscheduled mortality of stored seed can cost millions of dollars (McDonald 1999) or lead to irretrievable losses of precious germplasm. Seed banks need to reliably predict when the threshold of rapid viability loss will occur so that regenerations can be scheduled efficiently. This is especially true for tree species because regeneration is so costly.

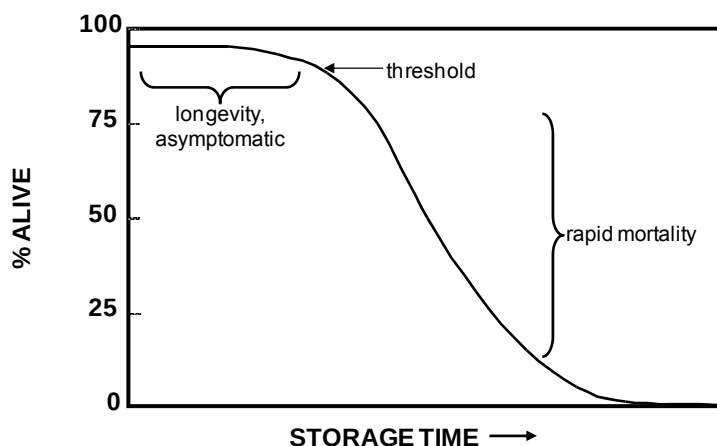


Fig. 1. Schematic diagram of seed deterioration time course showing asymptomatic and rapid viability loss phases (from Walters et al. 2010).

We know that there is considerable unexplained variation in longevity within-species. For example, despite high initial germination in 19 seed lots of *Picea glauca*, germination ranged from 0 to 89 percent (average germination was about 40%) for seeds stored for 24 years at 4°C and water contents ranging from 5.5 to 6.4%, and initial % germination or storage water content were not significant explanatory factors in the range of germination measured Simpson et al. (2004). Other examples of unexplained high variation abound, and one example for a genebank of crop species and their wild relatives is illustrated by Walters et al. (2005).



The seed aging time course in Figure 1 depicts a reaction in which sufficient product must accumulate in order for the reaction can proceed rapidly (Walters et al. 2010). These types of reactions are called ‘cooperative reactions.’ Cooperative reactions are a defining feature of visco-elastic materials. Visco-elastic materials can be viewed as something in the “gray area” between a fluid and a solid. Typical visco-elastic substances are engineered plastics; drying seeds also enter into this “gray area” as water is removed and a glass is formed (Walters et al. 2010, Walters and Koster 2007, Buitink and Leprince 2004). The tendency for motion as a fluid, the viscosity, causes irreversibly losses to structure. The tendency for motion as a solid, elasticity describes the ability to deform and recover and is quantified by the elastic modulus. The study of motion in solids is really the study of structural stability and the elastic modulus and other properties such as brittleness, toughness, and yield force (when the solid begins to act like a fluid) describe how a visco-elastic material will behave through time and in response to environmental conditions (Walters et al. 2010). Visco-elastic properties are affected by a multitude of interacting factors including moisture, temperature, composition and processing steps, and these effects in engineered materials is a well-developed scientific discipline (Walters et al. 2010). Our working hypothesis is that understanding motion and structure in seed glasses will lead to reliable predictions of seed longevity.

Structural changes associated with desiccation, the creation of cellular glasses and the orthodox-recalcitrant dichotomy

The structure and stability of seed cells is highly impacted by drying. Cells shrink as water that once occupied cell volume is removed (Fig. 2a). The more that cells are loaded with dry matter reserves, the less they shrink (Fig. 2b). Eventually, molecules cannot compress further and a matrix with voids forms. A glass is formed when the matrix including void spaces moves so slowly that it can support its own weight (Walters et al. 2010).

The change in structure during drying is a critical feature describing desiccation tolerance of the cells. A 50% loss in volume or surface area (reviewed by Walters and Koster 2007) is considered lethal, and this volume change occurs in immature orthodox embryos and mature recalcitrant embryos that are dried from water potentials *in planta* to water potentials associated with glass formation (Walters and Koster, 2007; Perez and Walters, unpublished). Orthodox embryos do not experience such large volume changes because they are loaded with dry matter. Perhaps, seeds that exhibit intermediate storage behavior come close to the lethal limits of volume change when they are dried to moisture levels typically used in conventional seed storage.

Further drying of orthodox seeds within the glass phase stabilizes structure as viscous properties become less apparent and elastic properties predominate (Fig. 3). Drying likely has a limited effect on viscous properties of the glass, and extreme drying may even compromise the solid structure by allowing greater compression or inducing brittleness. The model of visco-elastic matrix within orthodox seed cells conforms nicely to observed changes in aging rates, the limit to the beneficial effects of drying, and the possible detrimental effects of overdrying.

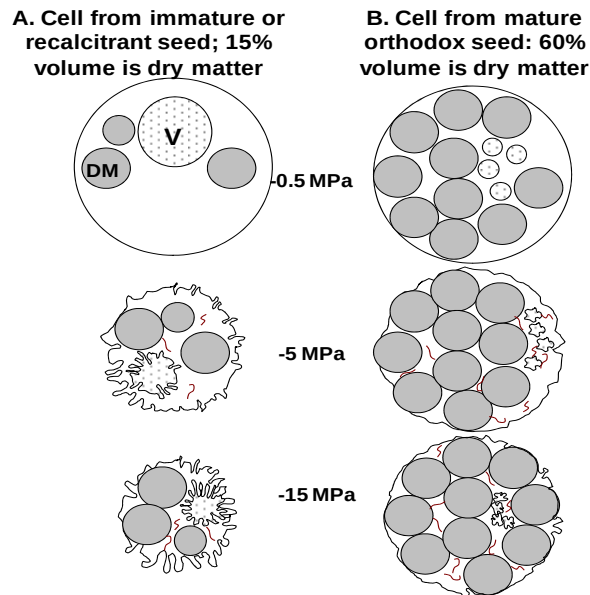


Fig. 2. Scaled diagram of mechanical shrinkage when immature or recalcitrant embryos (A) or mature orthodox embryos (B) are desiccated from *in planta* water potentials to -15MPa. The difference in shrinkage is purely a function of the portion of dry matter that occupies the cell. Volume loss is lethal in A and tolerated in B. (from Walters and Koster 2007) .

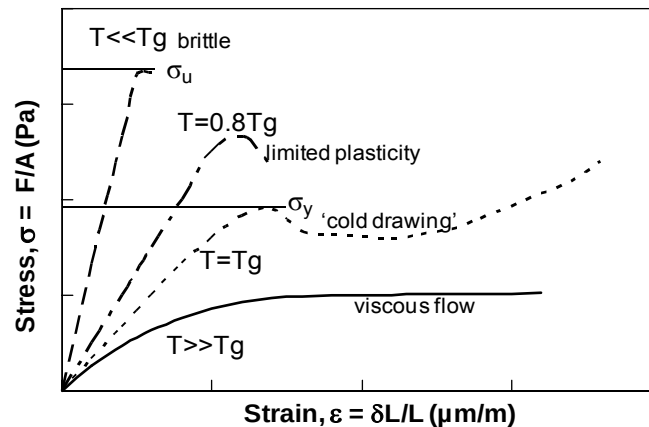


Fig. 3. Typical stress strain relationships of a visco-elastic material as a function of temperature above and below the glass transition temperature T_g . Stress is the force applied to the material to induce deformation (the strain). The slope of the linear portion is the elastic modulus and σ_u and σ_y refer to the force causing the material to break or yield, respectively. 'toughness is the area under the stress-strain relationship. Moisture has similar plasticization effects on visco-elastic materials with the solid curve representing stress-strain relationships of materials above the glass transition and the upper most curve representing extremely dry materials. (from Walters et al. 2010).

Physical changes associated with low temperature and the intermediate seed storage category

Temperature effects are classically modeled by Arrhenius behavior, which may show some curvature for complex reactions (Walters 1998). Near the glass transition temperature, an even greater temperature response is expected, as the fluid structure solidifies. The elastic modulus and brittleness of the glass increase with decreasing temperature (Fig. 3). At sufficiently low temperature, the fluid properties of the glass are overwhelmed by the solid properties, and molecular mobility resumes Arrhenius behavior but with a lower temperature coefficient (Walters 2004). The effect of temperature on glass properties is consistent with the observed response of seed aging to temperature, in which there is a diminishing benefit of low temperature on seed longevity as temperature decreases below about -20 to -40°C (Dickie et al. 1990; Walters et al. 2004).

Temperature also affects structural properties of lipids, causing changes from fluid to metastable solid to crystallized solid. Seeds with lipids that crystallize at temperatures greater than -10°C do not store well in the freezer relative to refrigerated storage (Crane et al. 2006, Pritchard and Seaton 1993, Hamilton et al. 2009) and exhibit anomalous response to models of temperature effects on seed aging rate. Because lipids crystallize slowly, these anomalous effects are not immediately apparent but correlate with the rate of lipid crystallization (Crane et al. 2006). How lipid crystallization negatively impacts seed viability is not understood, but may be related to the associated reduction in volume in oil bodies that may promote movement, rearrangement and additional compression of the aqueous cell matrix to the lethal limit (described in previous section). Cooling seeds to liquid nitrogen temperatures and cryogenic storage has proven effective in preventing lipids from crystallizing and assuring long-term stability of seeds with this temperature anomaly (Crane and Walters unpublished). Preliminary evidence suggests that seeds containing lipids that crystallize at temperatures as low as -25°C may also be susceptible to relatively faster aging in the freezer, and NCGRP may be revising its storage protocols to address this contingency.

Anomalies to temperature or moisture responses modeled for seed longevity are the hallmark of intermediate seeds. The phase behavior of seed storage lipids seems to contribute to anomalies. The observation brings up two questions: 1) Are all seeds containing lipids susceptible to temperature anomalies at storage temperatures where their lipids can crystallize? 2) Does susceptibility to aging increase with lipid content when seeds are stored at increasingly lower temperature? If the presence of storage lipids predisposes seeds to temperature anomalies, then we would have to conclude that most oil-rich seeds fit criteria for intermediate storage behavior but it will not be detected unless conventional storage temperature are reduced to less than -20°C.

Seed quality factors

Preventing change in cooperative-type reactions that typify seed ageing involves chemical or structural stabilizers, or both. Many of the chemical stabilizers, for example antioxidants or repair enzymes (e.g., Sattler et al. 2004, Ogé et al. 2008), will be effective at the full range of water contents for which aging reactions are relevant or will exert effects after aging has occurred. Hence these components of seed quality may exhibit



effects independent of moisture and temperature. Other components of seed quality will affect the structural stability of the cell matrix – for example, total dry matter reserves which affect volume changes during drying or lipids that expand or contract with temperature. These elements of seed quality will have interacting effects with temperature and moisture. Numerous gene products may serve as glass stabilizers, and it is intriguing to hypothesize this role for heat shock proteins, LEA-like proteins and raffinose oligosaccharides, which have previously been implicated in desiccation tolerance and seed longevity (Berjak and Pammenter 2008).

Sometimes apparently desiccation-tolerant seeds age fast and storage moisture or temperature appear to have limited effects on prolonging lifespan (e.g., Chien et al. 2010, species; species from Hawaii studied by Walters et al. unpublished). These seeds also typify the intermediate storage category because their non-response to moisture and temperature is indeed anomalous to seed aging models. However, discontinuities in modeled responses used to classify other seeds as intermediate are sometimes not observed in this type of storage behavior (*D. glaucescens* described by Chien et al. 2010 exhibits a rather severe response to temperature that may indicate additional lipid factors). The super-fast aging, with virtually no asymptomatic phase, suggests perhaps that the seeds are “preaged” at harvest and are very near the threshold marking catastrophic losses in viability (Fig. 1). As seeds approach the threshold marking rapid viability loss, temperature has diminishing effect on aging kinetics (Walters et al. 2004). One can only speculate as to the basis of this aging kinetic, the seed quality factors that cause it, and procedures that can be applied to prevent deterioration. Seeds with this physiology may be as problematic to store as recalcitrant seeds.

Interacting factors and limits to beneficial effects of drying or cooling

The aging kinetics of both orthodox and intermediate seeds exhibit departure from modeled effects of temperature and moisture within narrow temperature and moisture ranges. These temperature and moisture ranges appear to be higher in intermediate seeds compared to orthodox seeds, but both classes of seeds show limited benefit of temperature and moisture at some point. Moreover, there is some evidence of detrimental effects of drying below a critical water content in orthodox seeds (Walters 1998), and there is modeled detrimental effects of cooling below about -40°C in seed aging models (Ellis and Roberts 1981) that is partially supported by long-term storage data of seeds stored cryogenically (Walters unpublished). These anomalous effects in temperature and moisture coefficients can be rationalized by the visco-elastic model of seeds presented in this paper. Essentially, drying and cooling promote solid state (elastic) behavior at the expense of fluid (viscous) behavior to a limit after which there is no further effect, or the solid structure becomes increasingly fragile.

What happens if the limited benefit of drying is over-laid with the interacting effect of temperature? A thought experiment leads to the conclusion that critical water contents increase with decreasing temperature (Walters 1998). Moreover, eventually the increasing value of critical water contents with decreasing temperature leads to a limited benefit of low temperature (Dickie et al. 1990, Walters et al. 2004). If detrimental effects occur below the critical water content, we arrive at the conclusion that low temperature may also have detrimental effects. The overall conclusion is that the lifespan of a seed is finite and there is a temperature-moisture content combination that will give the



maximum longevity. The environmental conditions and the achieved longevity will be a product of factors intrinsic to the seed.

CONCLUSIONS

Longevity of orthodox seeds generally increases as moisture and temperature for storage decrease. Despite this overall and common response, there is considerable variation within and among seed species that is unexplained by models of longevity and that preclude reliable prediction of when a particular seed lot will die. Currently, the large and unexplained variation in seed longevity is mostly accounted for within the initial seed quality factor. Yet there are no compelling traits known to provide large, consistent protective effects against aging. An added complication are the limited benefits of drying or low temperature on longevity that are exhibited in most orthodox seeds but unexplained by current models. A perspective that presents seeds as visco-elastic materials, and considers aging as a reaction that inextricably links structural and chemical stability, accounts for apparent temperature and moisture anomalies. A major consequence of this perspective is that moisture, temperature and seed quality interact to control the rate at which seeds age. The interaction of these factors may explain the wide variation in seed longevity observed within and among species and ultimately lead to a mechanistic model that explains why, and accurately predicts when, seeds die during storage.

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