THE INFLUENCE OF SEED SIZE AND SEED PRE-TREATMENT ON GERMINATION, EARLY SEEDLING GROWTH, FIELD SURVIVAL AND GROWTH OF *PINUS ELLIOTTII*

by

Owen Piston Petersen

Submitted in partial fulfilment of the requirements for the degree
MSc (Forestry Science)
in the Faculty of Natural and Agricultural Sciences
University of Pretoria

Supervisor: Professor Paxie Chirwa
Co-Supervisor: Professor Terry Aveling

October 2022

ABSTRACT

Successful tree species establishment depends on the quality of nursery-raised seedlings and good silvicultural practices such as appropriate site x species matching, site preparation, tending and tree protection. Commercial nurseries depend on quality seed, which has a high germination and will germinate rapidly, uniformly, and consistently. *Pinus elliottii* seed germination has been described as unpredictable, where one seed lot may result in 90% germination while another lot may only germinate at 30%. This phenomenon forces nursery managers to buy more seed and double sow to reach production targets. However, this has a negative influence on nursery profitability — hence the main objective of this research study, which is to investigate the impact of seed size, pre-treatment, and their interaction on germination, early seedling growth, field survival, and growth of *Pinus elliottii* in South Africa. Seed sizes included: small (3.1–4 mm), large (5.1–6 mm), and mixed (a mixture of 88% large and 12% small seed) seeds. The pre-treatments included hydrogen peroxide, hydro-priming, kelp-p-max, stratification, hydrogen peroxide + hydro-priming, hydrogen peroxide + kelp-p-max and a control. Germination trials were done under laboratory (controlled environmental conditions in a growth chamber) and nursery (uncontrolled) conditions. Other germination parameters used to measure seed quality included: Time to 50% Germination (T_{50}), Mean Germination Time (MGT), Germination Value (GV), Germination Rate Index (GRI), and Coefficient of Velocity of Germination (CVG). Seedlings were kept in the nursery to measure, after 5 months, early seedling growth (seedling height, root collar diameter and sturdiness ratios) and then planted infield, where growth (seedling height, ground line diameter and biomass index) and survival assessments were done 12 months after planting.

Large seed had the highest germination (79.1%) followed by mixed seed (76.9%), while the small seed had the worst performance (60.7%) under laboratory conditions. The composition of the mixed seed (a mixture of 88% large and 12% small seed) caused it to have a similar germination to that of large seed. The hydrogen peroxide + kelp-p-max_large interaction treatment had the highest germination (92.3%), while the control_small treatment (60.8%) was the worst under nursery conditions.

Large seed had the highest seed quality by having the best T_{50} (6.2 days), MGT (8.4 days), GV (21.7), GRI (11.5%), GI (1507.3), and CVG (12.2) values, with the small seed having the worst T_{50} (8.6 days), MGT (10 days), GV (10.1), GRI (7.6), GI (1041.8) and CVG (10.2) values, in

the laboratory. T_{50} (15.4 days), MGT (19.0 days) and GRI (5.4%) for large seed and T_{50} (11.9 days), MGT (15.7 days) and GRI (6.6%) for the stratification pre-treatment demonstrated the highest germination speed with the best values in the nursery. The combination of final germination and germination speed was best for the stratification_large interaction treatment by showing the best GV (11.3), GI (3106), and CVG (7.3) values under nursery conditions. This indicates that large seed (in laboratory and nursery trials) was significantly more vigorous than small seed.

Seedling height and sturdiness ratio was highest for large seed (33.2 cm and 8.7, respectively), while small seed had the lowest (22.4 cm and 6.7, respectively). RCD was highest for mixed seed (3.9 mm), while small seed had the lowest (3.4 mm). Seedling height and RCD (29.3 cm and 3.9 mm, respectively) were the highest for the stratification pre-treatment, while the hydrogen peroxide + kelp-p-max (8.1) pre-treatment had the highest sturdiness ratio. The hydrogen peroxide + hydro-prime pre-treatment had the shortest seedlings and smallest RCDs (26.1 cm and 3.5 mm, respectively), while the control had the lowest sturdiness ratio (7.0).

Seedling height, ground line diameter, and biomass index were the highest for seedlings from large seeds (800.6 mm, 26.7 mm, and 634 454, respectively). Seedlings from the kelp-p-max pre-treatment were the tallest (749.6 mm) and were significantly taller than the seedlings from the hydrogen peroxide + hydro-prime pre-treatment (699.9 mm). Survival in the trial was high and survival for seedlings from the control_large, control_small, hydrogen peroxide_large, hydrogen peroxide_mixed, hydrogen peroxide + hydro-prime_small, hydrogen peroxide + kelp-p-max_large, hydro-prime_large, kelp-p-max_small treatments (100%) was significantly higher than for the kelp-p-max_mixed treatment (83.3%). Thus, the interaction between seed size and seed pre-treatment influenced germination under nursery conditions, while under laboratory conditions germination was dependent on seed size. Seed size was important for early seedling growth in the nursery, with large seed producing the tallest seedlings. Infield growth 12 months after planting was influenced by seed size and pre-treatment, while survival depended on the interaction between seed size and pre-treatment.

Key words: Stratification, sturdiness ratio, germination rate index, seedling height and ground line diameter.

DECLARATION

I, Owen Petersen, hereby declare that this thesis is my own work, except where otherwise indicated, and has not been submitted at any other tertiary institution.

Signature_____ Date: October 2022

ACKNOWLEDGEMENTS

First, I would like to thank the Lord for giving me the grace, strength, and patience to complete this project. Then, would like to express my sincere thanks and gratitude to the following persons and organisations:

Mondi Pty (Ltd), who offered me the opportunity, time, and resources to conduct this study.

The Seed Team at Mountain Home Nursery—Bandile, Ntombenhle, Nomakhosi, Nomgidi, Alfred, Impi, Clearance and Sthembiso—for their assistance in setting up, measuring, and maintaining the trials.

Sunshine Seedlings, in particular Mr. Bryn Pollard, for making available their facilities to conduct the nursery trial.

My colleagues, Dr Kitt Payn, Dr Marius du Plessis, Dr Mmoledi Mphahlele, Phillip Ascough, Tracy Newmarch, Morris Chauke, Nicci Edwards, Mnqobi Zuma and Nokukhanya Maplanka for their help and encouragement.

Professor Keith Little for his part in and assistance with the statistical analysis.

Ullrich Hecter, Renaan Thompson and Edgar Mangwende for their assistance and help.

Jacqui and Karen for their assistance in completing this project.

Prof. Paxie Chirwa and late Prof. Terry Aveling for their patience, support, and advice whilst supervising this project.

Ps Kay Govender and the Bethel Ministries leadership for their prayers and spiritual support.

Finally, I would like to acknowledge my family for their moral support and prayers.

DEDICATION

This work is dedicated to my parents (Edward and Hendrina Petersen), wife (Lynette) and children (Terri-Leigh, Abigail, Lucas, and Layla) for their unconditional love, patience, and support. Thank you for believing in me.

Table of Contents

LIST OF ABBREVIATIONS

DAS:	Days after sowing
T_{50}:	Time to 50% germination
MGT:	Mean germination time
GV:	Germination value
GRI:	Germination rate index
GI:	Germination index
CVG:	Coefficient of velocity of germination
FG:	Final germination
RCD:	Root collar diameter
GLD:	Ground line diameter
BI:	Biomass index
EC:	Electrical conductivity
ANOVA:	Analysis of variance
MAT:	Mean annual temperature
MAP:	Mean annual precipitation
ERD:	Effective root depth
ISTA:	International Seed Testing Association
CI:	Confidence interval

GLOSSARY

Cold test: A germination test that is used to evaluate the emergence of a seed lot in cold wet soils

Desiccation: The process of drying seed

Families: A group of closely related genotypes

Germination speed: The rate of germination in terms of the total number of seeds that germinate in a time interval

Gravity separation: The process whereby full and empty seeds are separated based on their weight, using air

Hybrids: Plants produced by crossing two different species, e.g., *P. elliottii* crossed with *P. caribaea*

Orthodox seed: Seed that can be dried to a moisture content as low as 5% without injury and can tolerate freezing

Realised gain: The expected change in the average breeding value of a population over at least one cycle of selection for a particular trait or index of traits

Recalcitrant seed: Seed that cannot be dried to a moisture content below 20% without injury and that are unable to tolerate freezing

Rogue: Remove inferior or defective plants or seedlings from a crop

Seed dormancy: The state or a condition in which seeds are prevented from germinating even under favourable environmental conditions

Seed germination: The initial step in the life cycle of plants, which begins when the inactive dry seed imbibes water and is completed with the protrusion of the radicle from the seed coat; the development of a plant from a seed or spore after a period of dormancy.

Seed lot: Seed collected from a specific orchard with similar genetic make-up

Seed vigour: Those seed properties that determine the potential for rapid uniform emergence and development of normal seedling under a wide range of field conditions

Seed purity test: Determines the percentage by weight of pure seed, other crop seeds, inert matter, and weed seeds in a test sample

Stratification: The placing of seeds close together in layers in moist sand or peat to preserve them or to help them germinate

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Commercial timber plantations play an essential role in the South African economy. In 2015, the forestry industry contributed R31.1 billion to the gross domestic product (GDP) and generated R25.8 billion by exporting forest products (FSA, 2017). Currently, 57% of all timber plantations in South Africa are managed for pulpwood, 38% for sawlogs, 2% for mining timber and 3% for other products (DAFF, 2017/2018).

Exotic tree species, consisting of fast-growing softwoods and hardwoods, are widely used when establishing commercial timber plantations. In South Africa, softwood species cover 49% of commercial timber plantation areas, while hardwood species (represented mainly by various eucalypts and *Acacia mearnsii*) make up the other 51% (DAFF, 2017/2018). *Pinus patula* Schiede ex Schltdl. & Cham covers 286 017 hectares or 48.9% of the total softwood area and occurs mainly in northern and southern Mpumalanga, KwaZulu-Natal, and the Eastern Cape (Table 1.1). *Pinus elliottii* Engelm covers 29% of the softwood plantation area and is found in all commercial timber-growing regions in South Africa. (DAFF, 2017/2018).

Table 1.1. Soft wood species distribution in South Africa (planted as seedlings).

Species	**Area (ha)**	**Contribution (%)**
Pinus patula	286 017	48.9
Pinus elliottii	169 296	29.0
Pinus taeda L.	18 500	3.2
Pinus radiata	45 135	7.7
Pinus pinaster Aiton	282	0.1
Other	65 107	11.1
Total (ha)	584 337	100

Successful tree species establishment depends on the quality of nursery-raised seedlings and good silvicultural practices such as appropriate site x species matching, site preparation, tending and tree protection (MacLennan & Fennessy, 2006; Pinto *et al*., 2011). Qualities such as physical appearance and genetic make-up influence seedling quality (Landis *et al*., 2010). Physical appearance includes seedling height, root collar diameter, root-shoot ratio, root form and seedling health, while genetic make-up refers to the seed source (unimproved seed or improved seed) where the seed from which the seedlings were grown was collected (Landis *et*

al., 2010). High-quality seedlings, when compared to poor-quality seedlings have the following traits:

1. Seedlings are propagated from seed collected from genetically and phenotypically superior mother trees from seed orchards;
2. Healthy and disease-free seedlings;
3. Larger root collar diameter;
4. Root systems free from deformities (j-roots), dense with many fine fibrous hairs with white root tips; and
5. Balanced shoot-to-root ratio (2:1 or less) – sturdy seedlings (Haase, 2007).

High-quality seedlings have a better chance of survival in forest plantations than poor-quality seedlings because of their superior physical and genetic qualities. Moreover, they are more tolerant to pests and diseases, mainly when tree breeding selection criteria include pest and disease tolerance (Haase, 2007; Gregorio *et al*., 2010; Grotta *et al*., 2019).

Profit margins are constantly under pressure in the nursery environment, and any improvement in the seedling production chain will alleviate losses (Karrfalt, 2011). For nurseries to produce quality seedlings, they depend on cultural practices (correct choice and use of growing media, sustainable supply of quality water, nutrient supply) and quality seed (seed with high germination and subsequent growth vigour) (Karrfalt, 2011). Quality seed is harvested from genetically improved seed orchards and shows characteristics of high vigour and germination (>85%). Quality seed germinates rapidly (high germination rate), uniformly and consistently (Barnett, 2002; Karrfalt, 2011).

Producing quality *Pinus elliottii* seed is dependent on genetics, site productivity, the climate under which the cones are formed and developed, the maturity of the harvested cones, the processing of seed without injury, and the sizing and treating of the seed before sowing (Edwards, 1981; Barnett, 1996; Karrfalt, 2011). Improved germination and subsequent growth are functions of seed sizing and pre-treatment (Sulewska *et al*., 2014). Seed size influences germination: larger seeds generally perform better than smaller seeds (Naidu & Jones, 2007; Kolawole *et al*., 2011). This could be advantageous with *Pinus elliottii* seed because it has a wide or considerable variation in seed size (between 2 mm and 7 mm) (Lohrey & Kossuth, 1990; Kral, 1993). The seed can thus be graded to ensure that the grade giving the best

germination is selected. In addition, the larger seeds contains more endosperm reserves to initiate, stimulate and sustain germination than the lower food reserves occurring in the smaller seed (Couvillon, 2002; Naidu & Jones, 2007; Hojjat, 2011; Owoh *et al.*, 2011; Sadeghi *et al.*, 2011; Ahirwar, 2012; Missanjo *et al.*, 2013; Attri *et al.*, 2017; Fornah *et al.*, 2017; Leishangthem and Rana, 2017; Attri *et al.*, 2018). As a southern pine, *P. elliottii* occurs in the upper areas (in terms of altitude) of the South Atlantic states of the United States of America, across the southern states to Texas and Oklahoma (Gaby, 1985). Based on past studies, southern pines react positively to seed pre-treatments designed to break dormancy (Barnett, 2008).

Pinus elliottii seed germination has been described as variable by nursery managers in South Africa. For example, germination between seed lots may vary between 30 – 90% (Forrest, 1964; Bonner, 1987; Mackellar, 2008; Asary, 2017; B. Pollard pers.comm., Sunshine Seedlings, 12 March 2019; A. Changing-Pearce pers.comm., 12 March 2019; S. Biggs pers.comm., Sutherland Seedlings, 14 March 2019). This variability in germination makes planning difficult, for example, how much seed, fertiliser, and growing media need to be purchased and whether extra labour would be needed. Therefore, nursery managers must make alternative plans to mitigate problems associated with variable germination such as:

1. Purchasing seedlings from another nursery; or
2. Purchasing extra seed from the same genetic source.

Most nursery managers opt to purchase more seed as this gives them the option of double sowing, where two seeds are sown per insert, which increases the chances of one of the two seeds germinating. However, double sowing potentially leads to an increased usage of growing media, fertiliser, and water, increasing input costs due to the purchase of more seed, and the need to remove one seedling per insert where both survive (Karrfalt, 2011).

Seed sizing and seed pre-treatment are essential functions of germination and could assist in better understanding their influence on early growth and infield survival. Currently, *P. elliottii* seed is not sized in South Africa, and the opportunity exists firstly, to grade the seed into different size classes, and secondly to use different pre-treatments to investigate the influence of the different seed sizes, pre-treatment and their interaction on germination, early seedling growth and field survival and growth.

1.1. RESEARCH OBJECTIVES

This study aims to investigate the effects of *Pinus elliottii* seed size, pre-treatment, and their interaction on the germination, early seedling growth (in the nursery), and field survival and growth following out-planting.

The specific objectives and associated hypothesis were as follows:

1. The first objective was to determine the effect of seed size, pre-treatment, and their interaction on germination and early seedling growth of *P. elliottii* in the laboratory and nursery. It was hypothesised that seed size, seed pre-treatment and their interaction would have no influence on germination (FG, T_{50}, MGT, GV, GRI, GI and CVG) in the laboratory and nursery. It was further hypothesised that seed size and seed pre-treatment will have no influence on early seedling growth (seedling height, root collar diameter and sturdiness ratio) in the nursery.
2. The second objective was to determine the effect of seed size, pre-treatment, and their interaction on field survival and growth (seedling height, ground line diameter and biomass index), 12 months after planting *P. elliottii*. It was hypothesised that seed size, seed pre-treatment and their interaction would have no influence on infield growth and field survival.

1.2. STUDY OUTCOMES

The outcomes of this study are expected to contribute to the decision support system for nursery managers and seed producers when dealing with *Pinus elliottii* seed in terms of:

1. whether seed size, pre-treatment, or their interaction positively influence *P. elliottii* seed germination and early seedling growth in the nursery environment; and
2. whether the benefits (improved germination and early seedling growth) of seed size, seed pre-treatment or their interaction are carried over in the field once planted (survival and growth).

1.3. THESIS STRUCTURE

The report is divided into six chapters. The first chapter covers the study's general introduction, objectives, and outcomes. Chapter 2 covers the literature review, while materials and methods are covered in Chapter 3. Chapter 4 deals with the results, followed by a detailed discussion of

the results in Chapter 5. Chapter 6 includes the conclusion and recommendations of the research.

CHAPTER 2: LITERATURE REVIEW

South Africa has a total land mass of 122.1 million hectares, of which 0.97% (1 191 638 ha) is under commercial timber plantations (DAFF, 2017/2018). Some significant hardwood pure species planted in South Africa include *Eucalyptus grandis* Hill, *E. dunnii* Maiden., *E. benthamii* Maiden & Cambage, *E. nitens* Deane & Maiden, *E. MacArthur* Deane & Maiden and *E. smithii* Baker (Hettasch *et al.*, 2009). In contrast, the significant softwood pure species produced from seedlings include *P. patula*, *P. elliottii*, *P. taeda*, *P. radiata* and *P. pinaster* (DAFF, 2017/2018). *Pinus elliottii* is the most versatile of the softwood species due to its ability to grow under different site conditions, including well-drained and poorly drained soils, shallow soils, dry sites, and hydromorphic soils (Schutz, 1994). It tolerates wet soil conditions and produces higher yields than *P. patula* and *P. taeda* when planted in shallow, stony soils but is outperformed by *P. patula* on good sites (deep, well-drained soils) (Schutz, 1994).

Pine and eucalypt hybrids have been developed in South Africa for the purpose of combining various traits of economic importance, including growth, disease tolerance, pulp properties, rooting ability, drought, and cold tolerance (Hettasch *et* al., 2009). Some of the important hybrids include *E. grandis* x *E. nitens* (combining the growth potential of *E. grandis* with the cold tolerance and rooting ability of *E. nitens*), *E. grandis* x *E. urophylla* (combining the growth potential of *E. grandis* with the stem canker tolerance of *E. urophylla*) and *E. grandis* x *E. camaldulensis* (combining the growth potential of *E. grandis* with the drought tolerance of *E. camaldulensis*) (Hettasch *et* al., 2009). Some popular pine hybrid species include *P. elliottii* x *P. caribaea* (combining higher density of *P. elliottii* with the growth potential of *P. caribaea*). *P. patula* x *P. tecunumanii* (combining the desirable pulp- and paper-making properties and frost tolerance of *P. patula* with the high growth potential and pitch canker tolerance of *P. tecunumanii*) hybrids (Kanzler *et al.*, 2012).

Seed is the simplest and most cost-effective form of propagation when compared to vegetative propagation methods, which require more knowledge, skill, and time. The annual pine seed requirements in South Africa (DAFF, 2017/2018) indicate that the demand for *P. elliottii* seed is highest (414.57 kg), with *P. patula* (193.04 kg) and for *P. taeda* (34.5 kg) lower (Table 2.1).

Table 2.1. Annual seed requirements for some pine species in South Africa (DAFF, 2017/2018).

Species	Annual replant area (ha)	Seeds per kg	Annual seed requirement in kg at 70% germination	Total plants per annum
P. patula	14 702	110 000	193.04[b]	16 333 922[a]
P. elliottii	8611	30 000	414.57	9 566 821
P. taeda	953	40 000	34.5	1 058 783

[a]**Total plants 16 333 922 = 14 702 (ha) x 1 111 (stems per hectare);** [b]**Seed 193.1 kg = 16 333 922 / 84 615.38 (70% germination)**

2.1 PINUS ELLIOTTII

2.1.1 Taxonomy

Pinus elliottii was first identified by the botanist Stephen Elliott, with George Engelmann naming the species in his (Stephen Elliott) honour in 1880 (Coder, 2017). Commonly, *Pinus elliottii* is known as slash pine, yellow slash pine, yellow pine, southern pine, Hondurus pine, Cuban pine, swamp pine or pith pine (Coder, 2017).

2.1.2 Origin and history in South Africa

Slash pine consists of two varieties: Pinus *elliottii* var. *elliottii* and *Pinus elliottii* var. *densa* (Poynton, 1977; Lohrey & Kossuth, 1990; Coder, 2017). Due to its rapid early growth, excellent fibre, lumber, and poles, *P. elliottii* was introduced into many countries, including Brazil, Australia, and South Africa (Dickens *et al*., 2004; Nilsson, 2014). The first *P. elliottii* var. *elliottii* seed was imported into South Africa in 1918 under the name *Pinus caribaea* and was of unknown origin (Darrow, 1984). From the unknown seed lots, approximately 45 kg were used to establish the first *P. elliottii* commercial stands in Zululand. These stands were then used as a seed source from 1928 to supply the local forestry industry. In 1936, seed was sourced from six locations within its natural range (from southern South Carolina westwards to south-eastern Louisiana and southwards to the Florida Keys) to establish provenance trials in the southern Cape, KwaZulu-Natal, and Mpumalanga (Darrow, 1984). Since 1939, local seed sources have been adequate to meet local demand, with improved *P. elliottii* seed (from local tree breeding programs) available from the early 1970s following selections from plus trees (superior in growth) (Poynton, 1977; Darrow, 1984).

2.1.3 Reproduction, tree, cone, and seed description

Pinus elliottii is monoecious and is wind pollinated (Lohrey & Kossuth, 1990). Under optimal conditions, seed production can start from ten-year of age, and cones mature after 20 months (Poynton, 1977; Lohrey & Kossuth, 1990; Kral, 1993; Coder, 2017). *Pinus elliottii* trees can grow up to 36 m and reach a diameter of 0.9 m (Poynton, 1977; Kral, 1993). The needles are dark green, 18–25 cm in length, and in fascicles of 2 or 3 (Barnett & Sheffield, 2004; Coder, 2017). *Pinus elliottii* develops egg-shaped cones which are 10–15 cm in length (Figure 2.3A) (Kral, 1993; Barnett & Sheffield, 2004). These cones are reddish-brown, with small sharp prickles/spines on the ends of the scales. *Pinus elliottii* seeds are between 6 and 7 mm long (Figure 2.3C) and are mottled grey or black with thin wings of 25 mm long attached (Figure 2.3B) (Kral, 1993; Barnett & Sheffield, 2004; Coder, 2017). As *P. elliottii* mature seed can be dried to a low moisture content of 5–10% without damaging the seed (orthodox nature), these can be stored for long periods (>25 years) (Bonner, 1990; Donald & Jacobs, 1994).

Figure 2.1. Cone size (A), Winged seed (B) and Seed size (C) for Pinus elliottii.

2.2 SEED QUALITY

Ferguson *et al*. (2004) described seed quality as the potential performance (germination) of seed lots. Deneke and Landis (1978) associated seed quality with terms such as: “clean (free from empty and damaged seed), high viability (above 85%), and vigorous seedlings”. Santos (2010) included additional aspects, such as seed health and moisture content, in the definition of seed quality. Quality seed is vital in producing nursery plants that meet management goals and perform well infield (Barnett, 2008). Traits of seed quality are genetic, physical, and physiological.

2.2.1 Genetic quality

Genetic quality encompasses important characteristics of tree performance such as vigour and fast growth, and tolerance against biotic (e.g., pests and disease) and abiotic (e.g., cold and drought) factors. Growing trees from genetically improved seed can improve forest productivity (Mulawarman *et al*., 2003), thereby increasing investment returns (Verryn *et al*., 2009). Unimproved seeds (seeds collected from stands that were not rogued) may germinate in the nursery but perform poorly infield, resulting in potential financial losses (Barnett, 2002; Karrfalt, 2011). Li *et al.* (1999) showed that improved *P. taeda* seed from unrogued 1st generation seed orchards resulted in a 7% increase in tree performance over unimproved seed, while seed from rogued (trees with undesirable traits having been removed from the orchard) 1st generation orchards further improved yields by 12% per ha. Higher tree volume was obtained for rogued 2nd generation orchard seed (30%) compared to unrogued 2nd generation orchard seed (17%). Trees grown from improved seed also resulted in higher disease resistance and improved stem form over trees from unimproved seed. Trees from the seed of rogued 2nd generation orchards showed volume gains of 14–23% over trees from 1st generation seed. According to McKeand (2015), gains in *P. taeda* volume production have shown an increase of 5–10% over three successive generations. Gains of 15–22% for stem diameter and 5–18% for stem straightness were predicted for *Picea sitchensis* (Bong.) Carr. (Sitka spruce) after the 1st generation (Hubert & Lee, 2005). Verryn *et al*. (2009) also reported genetic gains of 13% on average for tree volume over three generations in *E. grandis*. In genetic gain trials done by Verryn *et al*. (2009), the average realised (actual) gains were 14% per generation. Realised gains are the expected change in the average breeding value of a population over at least one cycle of selection for a particular trait or index of traits (Rutkoski, 2019a). Moreover, they are obtained from experiments or trials that plant improved varieties next to unimproved varieties in randomized, replicated trials across several sites (they are retrospective because results are obtained several years after planting those trials). Van Wyk and van der Sijde (1983) also predicted that tree breeding could lead to improvements of between 5 (for pines) and 30% (for eucalyptus) in volume growth of South African commercially planted trees.

2.2.2 Physical quality

This refers to the physical appearance of the seed, including seed size, colour, seed coat condition (crack-free) and diseases present on the seed coat, with only seed size discussed further.

2.2.2.1 Seed size

There are conflicting reports as to the effect of seed size on germination and early seedling growth, both between and within specific species. According to Sulewska *et al*. (2014), plant yields for maize (*Zea mays* L.) are a function of seed size, as indicated by their three-year field experimental data. Yield increased with a decrease in seed size, and vigour tests (warm and cold tests) also showed that germination was significantly higher for smaller seeds than for larger ones. Germination of large seeds in a cold test (germination test that simulates cold and wet conditions) were 6.7% lower than small seeds. Higher amylase activities recorded within smaller seeds were responsible for higher germination and vigour shown among smaller seeds. Hojjat (2011) found that germination parameters were significantly related to lentil seed size (*Lens culinaris* Medik). Under laboratory conditions, larger seeds germinated earlier and showed better germination than smaller seeds. A final germination of 77.7% for larger seeds was recorded, while smaller seeds only achieved 63.2% (14.5% difference between the two sizes). Seed size also affected seedling growth, with larger seeds producing larger seedlings. The study by Singh *et al*. (2009) on the effect of seed size on quality within seed lots of pea (*Pisum sativum* L.) showed that smaller seeds germinated faster than larger seeds because smaller seed imbibe water faster than larger seeds. Germination was higher in smaller seeds (38%) than in large seeds (32%). When a vigour test (accelerated ageing test) was conducted, the medium seed grade had the highest mean germination (36%) compared to small and large seeds (28% and 26%, respectively). Small and medium seed grade seeds also produced the tallest seedlings (both were 6.4 cm) compared to seedlings from large seeds (5 cm). Under field conditions, however, large, and medium seeds showed higher survival (36.5% and 33.5%, respectively) and growth rates compared to small seeds (25.5%), indicating that standard laboratory germination tests cannot always be used to predict infield results.

Kolawole *et al*. (2011) found in their study on the shea nut tree (*Vitellaria paradoxa* C.F Gaertn.) that seed size can influence germination, emergence, and yield. Their study showed that the nutrient contents of N, P, K, Ca, and Mg increased with an increase in seed size. Larger

seeds also had a higher germination rate (80%) compared to medium (70%) and small seeds (65%). It took 78 days for the last seedling to emerge from a large seed, while it took 98 days in medium and small seeds. Seedlings produced from large seeds were significantly taller than those from medium and small seeds. Missanjo and Maya (2015) recorded no significant difference for *Albizia lebbeck* (L) Benth. when comparing germination between small (45.7%), medium (46.5%) and large seed (48.5%). However, significant differences in seedling height, root collar diameter, root length, seedling length, root-shoot ratio, number of leaves, leaf area and dry weight occurred, with large > medium > small for these variates. Survival in-field was also higher for seedlings from large seed (89.6%), compared to medium (65.2%) and small seed (46.1%).

Naidu and Jones (2007) reported higher germination rates and final germination for both *E. grandis* and *E. smithii* with differences of between 40% and 50% between large and small seeds. Although the small seed size produced the shortest seedlings, no consistent trend was observed for root collar diameter, except for *E. smithii* where the small seed had a larger root collar diameter compared to the larger seed size. Infield survival was good for all seed sizes for both species. Dunlap and Barnett (1983) investigated the influence of seed size on germination and early development of loblolly pine (*P. taeda*) germinants. Their study found that large seeds-initiated germination quicker than medium and small-sized seeds. After six days, large seed germination was double that of smaller seeds, however final germination was lower for larger seeds than for medium and small seeds. Larger seeds produced larger seedlings, and the mean root and shoot length was found to increase with seed size under laboratory conditions after 28 days. Similar tests were also completed under greenhouse conditions. Larger seeds were the first to germinate, while medium and small seeds took longer. Larger seeds also produced taller seedlings than medium and small seeds. Germination speed was higher for larger and smaller seeds under laboratory and greenhouse conditions. Germination speed was identified as the main predictor for nursery seedling performance in *P. taeda*, where seed germinating first produced the tallest seedlings (Barnett & McLemore, 1984). Sluder (1979) also found that larger loblolly pine (*P. taeda*) seeds produced taller trees on average than small seeds. This was established at the end of the third growing season in his study on the effects of seed and seedling size on the survival and growth of *P. taeda*. In their study on sizing *P. elliottii* seeds as a nursery procedure, Belcher *et al*. (1984) found that lighter seeds (in terms of mass) had lower laboratory germination than heavier seeds. Small seeds also

had lower survival rates in the nursery, producing shorter seedlings than larger ones. Large seeds also produced seedlings with a more extensive root collar diameter and heavier seedling weight. Sluder (1991), in his study on seed and seedling size grading of *P. elliottii*, found no significant differences in seedling height between the different seed size classes for this species, with only a slight increase in height as seed size increased. Seed size also did not affect tree height at plantation ages three, ten or fifteen. Seed size, however, did affect survival and thus volume at age fifteen, with trees from medium-sized seeds performing the worst.

2.3 PHYSIOLOGICAL QUALITY

Several important attributes influence the number of seedlings germinating from a seed lot, and include seed purity, weight, moisture content, germination, and vigour. All the above attributes can be measured by seed testing or analysis (Kumar *et al*., 2009; Mbora *et al*., 2009; Karrfalt, 2011).

2.3.1 Seed purity analysis

The purpose of purity testing is to determine the percentage (based on weight) within a seed sample of the pure seed (seed with the identical genetic make-up) of the species being tested. This is because a proportion of seeds from other crops, weed seeds and inert matter, which includes leaves and cone scales, could be present in the sample (ISTA, 2016). “Pure seed” describes the species being tested and includes both viable and non-viable seed and seed that is damaged, undersized, immature, and germinated (Table 2.2). A damaged seed with more than half of the seed remaining is regarded as pure seed, whereas a seed without a seed coat is regarded as inert matter or “impure seed”. Seeds of other seed crops include seeds from all species except the species under investigation, and weed seeds are the seeds of identified weeds. Inert matter, or impure seed, includes seeds of less than half the original seed size, wings, seeds without seed coats, leaves, twigs, stones, and soil (Table 2.2) (Mbora *et al*., 2009; ISTA, 2016).

Table 2.2. Criteria used during seed purity testing.

Pure seed	Impure seed
Mature seed Undamaged seed Undersized seed Pieces of seed more than half the seed’s original size Shrivelled seed Immature seed Germinated seed	Seed of other species Stones, leaves, twigs, soil Seed wings Pieces of seed less than half the seed’s original size Legume seed without seed coats

The interpretation of a purity analysis is essential as a low purity result indicates the presence of much other material in the seed sample, which means further cleaning is needed. Purity for pine species in South Africa can range from 86% to 100% (Table 2.3) (Bayley *et al.*, 2000). Purity affects the number of pure seeds per kilogram and indicates the quantity needed for sowing. High purity (above 95%) indicates that the seed lot is clean without much other material, which will also increase sowing efficiencies because of the absence of other materials in the seed (Barnett, 2002; Barnett & Varela, 2004; Karrfalt, 2011).

Table 2.3. Purity for pine species in Southern Africa.

Species	Purity (% and range)
Pinus patula	98.8 (96.7 – 99.8)
Pinus elliottii	99.4 (96.6 – 100)
Pinus taeda	99.3 (97.3 – 100)
Pinus radiata	97.5 (86.2 – 99.7)
Pinus pinaster	98.8 (98.8 – 100)

2.3.2 Seed weight

Seed weight is typically expressed as 1 000 pure seed weight and is required for calculating sowing rates in the nursery, in addition for the determination of the number of seeds per gram or kilogram. *Pinus patula* has 116 700 seeds per kg^{-1}, *P. taeda* 42 400 seeds per kg^{-1} and *P. elliottii* 31 300 seeds per kg^{-1} (Table 2.4) (Bayley *et al.*, 2000).

Seed weight is a function of:

1. Moisture content, where a higher moisture content increases the seed weight while lower moisture content will decrease seed weight;
2. Seed size, where a larger seed weighs more than a smaller seed as it contains more food reserves and is likely to germinate better and produce more vigorous seedlings; and
3. The proportion of whole seed in each seed lot, where full seeds within a seed lot will germinate at a higher rate than partially filled or empty seeds (Tanaka, 1984; ISTA, 2016).

Table 2.4. Seed per kilogram for certain pine species.

Species	Seed per kg (range) x 10^3
Pinus patula	116.7 (94.2 – 148.7)
Pinus elliottii	31.3 (20.1 – 39.2)
Pinus taeda	42.4 (25.6 – 60.1)
Pinus radiata	40.4 (31.0 – 59.3)
Pinus pinaster	19.1 (14.4 – 27.0)

2.3.3 Seed moisture content

Seed moisture content is expressed as the percentage of moisture in the seed (Mulawarman *et al*., 2003; ISTA, 2016). Seed moisture is crucial for seed longevity and to prevent seed deterioration (Bonner, 2008). A seed is typically classified as recalcitrant, orthodox, or intermediate based on the amount of desiccation a mature seed can tolerate (Yang *et al.*, 2007; Pammenter & Berjak, 2014). Recalcitrant seeds are those that cannot tolerate low moisture content levels, and viability will drop when dried (Wen, 2009). Recalcitrant seed has moisture content levels ranging between 30% and 70% and include species such as cocoa seeds (*Theobroma cacao* L.), which germinate rapidly when sown fresh, but are sensitive to desiccation and freezing (Mng'omba *et al.*, 2007; Bonner, 2008; Dussert *et al.*, 2018). Recalcitrant seed is difficult to store and can die below moisture content levels of 26% or below temperatures of 15°C (Mng'omba *et al.*, 2007). A study conducted by Chin *et al.* (1989) indicated that *Havea brasiliensis* Mull. Arg. (rubber) seeds perish when stored at 15–20%, while *Theobroma cacao* seeds perish at 26% moisture content. *Havea brasiliensis* and *Theobroma cacao* have short shelf lives, ranging from a few weeks to a few months. Wen (2009) demonstrated in his case study with the Chinese fan palm (*Livistona chinensis* (Jacq) R.Br. ex Mart.), which is recalcitrant, that the seeds were susceptible to drying and freezing and failed to survive sub-zero temperatures.

Orthodox seed can be dried without any damage due to keeping metabolic activities to the minimum. Lower moisture leads to reduced respiration, decreases seed deterioration and ranges between 5% and 14% (Mng'omba *et al.*, 2007). Shelf life for orthodox seed can be short-term (2 years) to long-term (50 years) without influencing viability (Bonner, 2008). Donald and Jacobs (1994) tested the long-term storage of *P. elliottii, P. taeda, P. patula* and *P. radiata* over 25 years. This study found that seed of the above species were still viable and gave rise to perfectly normal seedlings after 25 years under storage conditions of -16°C and moisture content below 10%. Germination capacity was highest for *P. elliottii* (92.5%) and *P. taeda* (91.2), with *P. radiata* at 75.2% and *P. patula* at 60.4%. The moisture content of the orthodox seed is essential for medium to long-term storage (Bonner, 2008). Seeds can be injured at a moisture content below 5%, while optimum storage conditions range from 5–7% (Table 2.5). Fungal growth occurs when the moisture content is between 10% and 18%, while germination is initiated when the moisture content is above 30% (Table 2.5).

Table 2.5. Potential effect of seed moisture content.

Moisture content (%)	Potential effect
>30	Germination initiated
10–18	Fungal growth
8–9	Insect activity minimized
5–7	Optimum range for sealed seed storage
<5	Drying injury possible in certain species

Intermediate seed can tolerate some desiccation, but not as much as orthodox seed. However, intermediate seed is more tolerant to desiccation than the recalcitrant seed. Coffee seed (*Coffea canephora* Pierre ex A. Froehner) is included within this category and can be stored for 2–5 years (Mng'omba *et al.*, 2007). Yang (2007), in his study on the intermediate seed behaviour of Japanese Zelkova (*Zelkova serrata* (Thunb) Makino), found that seed can be stored for up to two years at the optimum temperature of 15°C and with a moisture content of 10% (Bonner, 2008).

2.3.4 Germination

Germination includes the emergence from the seed and the development of essential structures such as the root, shoot, cotyledons, and coleoptiles, producing a normal plant under favourable conditions (ISTA, 2016; Bewley, 1997). Germination tests are conducted to identify the capacity or potential of a specific seed lot to produce healthy, vigorous plants under controlled conditions. Germination is an indication of the ability of the seed to emerge from the soil and produce a normal seedling under normal environmental circumstances (Mbora *et al*., 2009).

High levels of germination (above 85%), vigour and purity are important indicators of successful germination as they ensure the development of quality seedlings in the nursery and infield (Barnett and Varela, 2004; Barnett, 2008). These factors (germination and seed vigour) will affect seed sowing efficiency, transplanting, uniformity, and the number of empty cavities per tray in the nursery (Barnett, 2002; Barnett & Varela, 2004; Karrfalt, 2011). Uniformity in the production of pine seedlings primarily depends on rapid and uniform germination, early seedling development and a variety of nursery management practices (irrigation, fertigation, and disease management) applied to seedlings (Barnett, 2008). Seedling costs potentially increase as germination decreases (Figure 2.2), with low germination (less than 80%) reducing nursery profitability whilst making it difficult for the nursery manager to reach production targets (Barnett, 2002; Karrfalt, 2011). This makes planning critical in the nursery, and double

sowing has become a common practice to compensate for poor quality, low germinating seed. However, double sowing can also impact negatively in various ways, including:

1) Costs;
2) Double the amount of seed needs to be bought (increased seed cost);
3) Increased sowing time (more the time needed for sowing);
4) Double handling due to pricking out of seedlings;
5) Transplanting seedlings into empty cavities could potentially lead to J-rooted seedlings;
6) Damage to roots can lead to dying seedlings; and
7) Infield, J roots normally lead to stunted growth and windfalls (Edwards, 1981; Karrfalt, 2011).

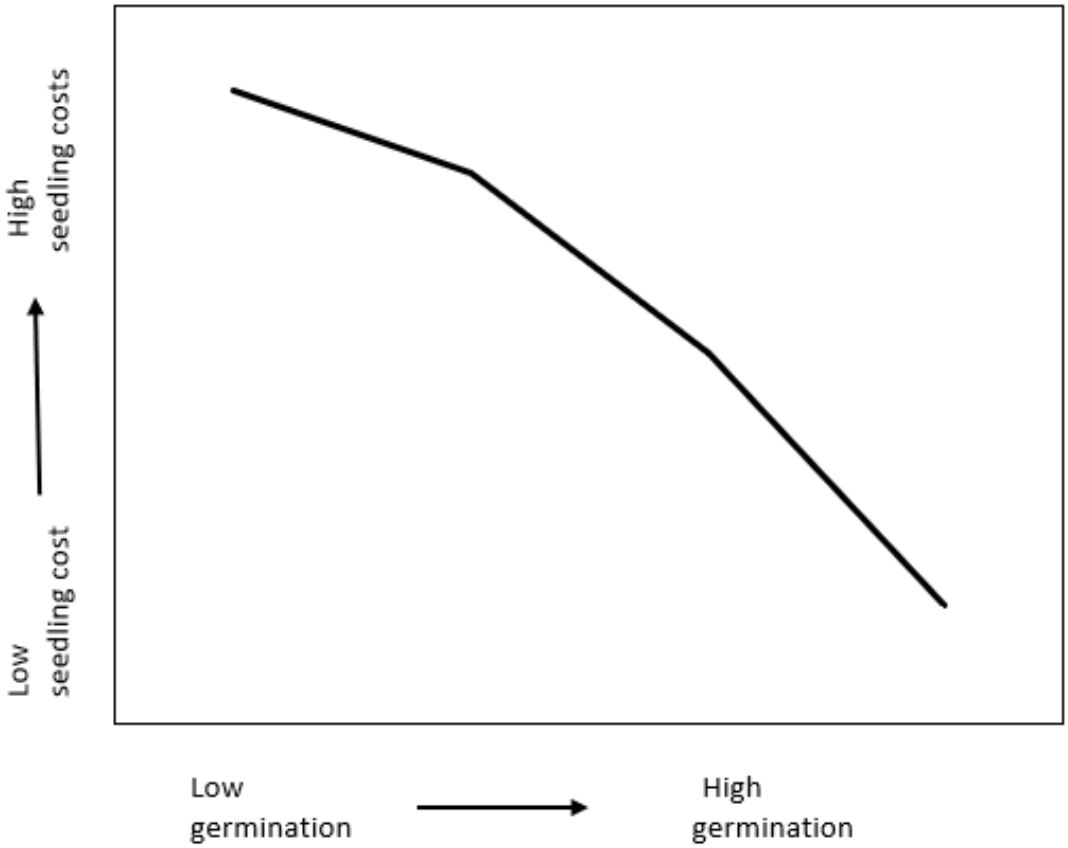

Figure 2.2. Impact of germination on seedling cost (Karrfalt, 2011).

2.3.4 Vigour

Within a seed lot, seed vigour is the ability for rapid and uniform emergence under a wide range of environmental conditions (ISTA, 2016; Sharma, 2018). Seed age, together with storage conditions, has a significant influence on seed vigour. Thus, seed vigour decreases over time and under sub-optimal storage conditions (Shaban, 2013). Over time seed generally loses

vigour faster than it loses viability or germination (Marcos-Filho, 2015). Vigour can be enhanced by sizing seed and using seed treatments, which includes the priming of seed (Rajjou *et al*., 2012).

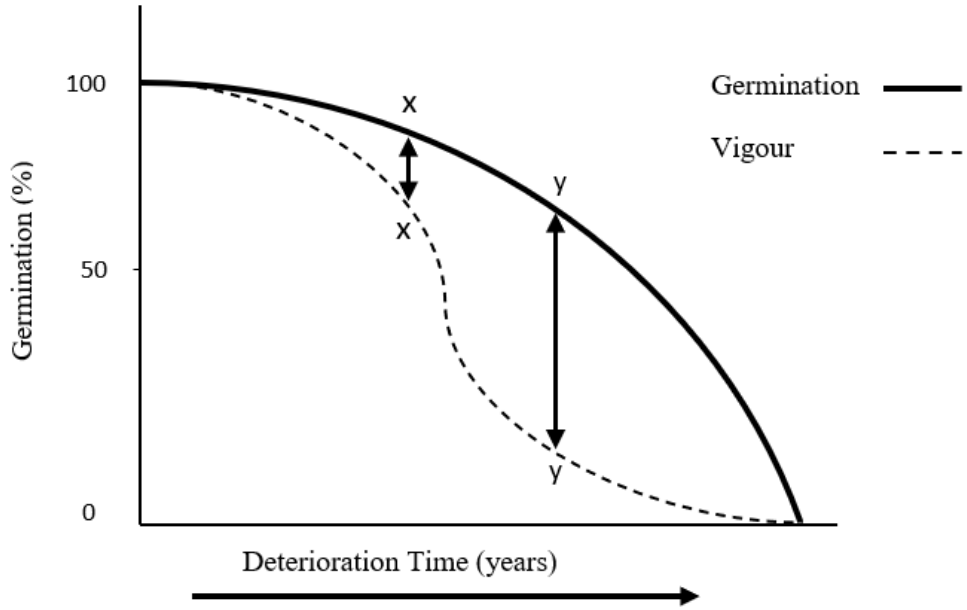

Figure 2.3. Comparison between seed viability and vigour over time (Marcos-Filho, 2015).

Seed size is one of the main factors that influence seed quality in terms of germination, vigour, and yield (Singh *et al.*, 2009). Large seed is generally assumed to be better than smaller seed when it comes to germination and seedling vigour. This is because larger seed contains more food to initiate and sustain rapid germination (Ndor *et al.*, 2012).

Singh *et al*. (2009) found in pea (*Pisum sativum*) seed that medium-sized seed had 90% germination compared to 71% for small seed and 65% for large seed, during an accelerated aging vigour test (a test that exposes seed to extreme environments such as high temperatures to accelerate the aging process within the seed). They concluded that, when left-over seed is used from the previous season, medium seed should be used for planting. Couvillon (2002) found, with *Cercis Canadensis* L. seed, that larger seed had higher germination values than smaller seed, which was an indication that larger seed was more vigorous than smaller seed because of its rapid germination and uniform seedling growth. Seed treatment, which involves treating the seed to initiate germination and includes treatments such as stratification, priming, scarification and boiling, also plays an important role in improving seed vigour. Calvardo *et al*. (2013) found that seaweed extract (*A. nodosum* (L.) Le Jolis) improves the seed vigour of

dry bean (*Phaseolus vulgaris* L.) seed when immersed for 15 minutes. Priming is a process whereby germination is initiated without the emergence of the radicle. Seed priming techniques are also used to re-invigorate seeds that show signs of low vigour. Artola *et al.* (2003) also found that hydro-priming increased seed vigour for *Lotus corniculatus* L. (birdsfoot trefoil) by 16 to 145%, over the un-primed control. In their study they found that there was no difference in germination and emergence, but time to 50% germination (T_{50}) was between 21 and 36 hours quicker, while time to 30% emergence (T_{30}) was between 15 and 30 hours quicker for primed seed over the un-primed control.

2.4 PRODUCING QUALITY SEED

Producing quality seed that consistently performs well in nurseries and, subsequently, infield can be achieved through processes such as cone handling (for pines), seed handling, seed sizing and seed treatments. Seed viability can be reduced by 20–30% by improper cone and seed handling and incorrect storage (Barnett, 1976; Edwards, 1981; Barnett, 1996; Barnett & McGilvray, 2002; Marwanto, 2004; Shelar, 2008; Barnett & Varela, 2003; Karrfalt, 2011; Andrews, 1965).

2.4.1 Cone handling

Time of harvest and storage of cones can significantly influence the yield and germination of *P. elliottii* seed. The number of seeds per cone is generally influenced by the ripeness of the cone (with riper cones yielding more seeds per cone compared to immature cones). Seed yields increase as cone ripeness and storage time increase (Lohrey & Kossuth, 1990; Barnett & McGilvray, 2002; Barnett & Varela, 2003). A five-week storage time after cone collection gives immature seed the opportunity to mature, while mature seed has been shown to improve in viability. Cones are fully mature (the specific gravity of cones with mature seeds is about 0.9 and they float in SAE 20 motor oil) when they open easily after harvest (Lohrey & Kossuth, 1990). To achieve maximum germination, *P. elliottii* cones must be fully matured and stored before further processing (Barnett & McGilvray, 2002; Barnett & Varela, 2003). *Pinus taeda* seed matures before the cones, and as soon as the cones open the seed is fully mature and will germinate at the maximum level. *Pinus palustris* cone harvesting needs to happen when the cones are completely mature (Barnett & McGilvray, 2002; Barnett & Varela, 2003).

2.4.2 Seed handling

Seed handling includes activities such as oven drying (at temperatures between 30 and 40.5ºC) to fully open the closed cones for seed extraction (Barnett & Varela, 2003). Drying at temperatures that are too high will be detrimental to the seed inside the cones. "Tumbling" is the process by which winged seed is extracted from open cones. A de-winging operation follows the tumbling process, where the wings are removed from the seeds. After de-winging, empty and full seeds are separated from each other through a process of gravity separation. Seeds can easily be damaged during these processes, causing a decrease in seed viability (Barnett & Varela, 2003; Karrfalt, 2008).

2.4.3 Seed sizing

"Sizing" is the process whereby seeds are sorted into different sizes, with the main objective being the efficient use of seed in the nursery (Karrfalt, 2008). Seed can be sized based on weight or diameter. Sizing based on diameter requires the seed to pass over different screens with holes of different sizes. Gravity separation is used to grade seed based on weight (Karrfalt, (2008). Grading seed into different size categories will ensure:

1. The generation of a uniform product (seedlings) from the start.
2. The ability of the nursery manager to improve sowing efficiencies through the use of a uniform product (seed will be the same size).
3. Uniform germination, within a seed grade.
4. Easier and more efficient management, as more efficient and effective watering and fertilizing schedules can be implemented if the seed is uniform.
5. Improved planning and scheduling in the nursery as the nurseryman, knowing how long each size class will take to germinate and how long each seedling from a specific size class will stay in the nursery, can plan the daily production more accurately.
6. Improved ability to turn every seed into a saleable seedling.
7. Fewer cullings because nursery managers can control germination speed and seedling growth using seed that is of the same class, thus reducing seedling costs (Barnett, 1996; Karrfalt, 2011).

2.4.4 Seed pre-treatments

Seed pre-treatments are applied prior to sowing with the aim of improving germination and seedling growth, and examples includes priming, stratification, and soaking (Barnett, 2008).

Germination, or the lack thereof, in a seed lot can be a result of dormancy, hard seed coats, diseased seed coats and/or environmental stresses (e.g., water, heat or salt). Some seed species need fire (e.g., fynbos) or require a hot water treatment (e.g., *A. mearnsii* De Wild.) for germination to be initiated (Willan, 1986; Bayley *et al.*, 2000). If required, the treating of seed before sowing is critical to ensure early and uniform germination (Barnett, 2008). Some seed treatments include scarification, stratification, priming, pre-soaking, light regimes, growth regulator applications, thermal shock, coating with pesticides, sterilising, or the use of seaweed liquid fertilizers. Of these, stratification, priming, sterilising and seaweed liquid fertilizers are widely used pre-treatments, and are discussed in more detail below (Barnett, 2008; Luna *et al.*, 2009).

2.4.4.1 Stratification

Depending on species, "stratification" is a process whereby seed is exposed to low temperatures and moist layers to increase rapid and uniform germination by overcoming seed dormancy. Its success depends on sufficient moisture, low temperatures, and adequate ventilation. The seed is soaked in water for 24 to 48 hours, drained, then placed between moist layers of cotton wool, peat moss or sand and then stored at low temperatures of 2–5^{o}C for several weeks (Barnett, 2008). Seed can be stratified between moist layers of peat moss in a 210 L drum (Figure 2.4) (Willan, 1986). This process simulates the cold, moist environmental conditions the seed is exposed to in its natural environment before germination. In its natural environment, the seed is normally shed in autumn and early winter, where it can take up water before dormancy and low temperatures prevent germination. After winter, the dormancy is over and an increase in temperature in spring initiates germination. Stratification removes metabolic blocks and weakens the seed coats to promote germination capacity and rate (Willan, 1986).

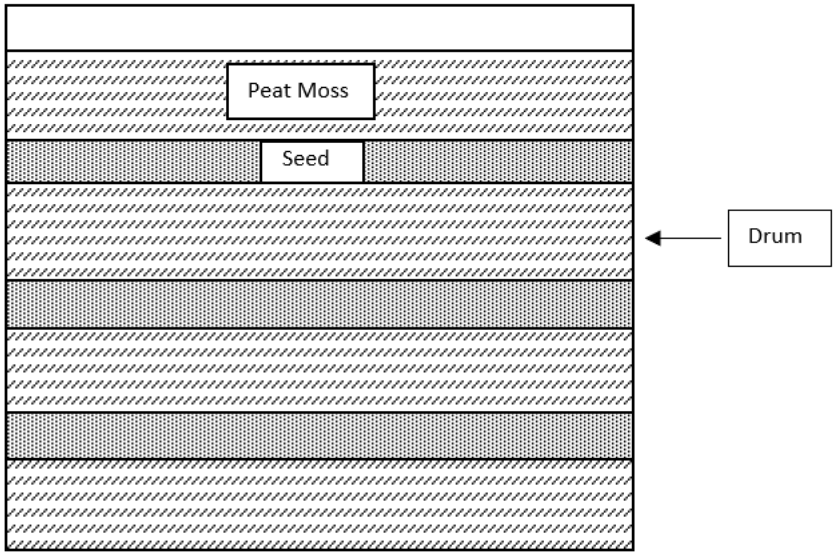

Figure 2.4. Seed being stratified between layers of moist peat moss in a drum (Willan, 1986).

The stratification period depends on the degree of dormancy experienced by the seed. Normally, seed with deeper dormancies needs longer periods of stratification, while seed with mild or lighter dormancies requires shorter periods of stratification. Stratification can vary from two weeks to up to a few months (Barnett & McLemore, 1967; Barnett & McLemore, 1984; Gansel, 1987; Jones & Gosling, 1994; Leadem, 1997; Mackellar, 2008). A 90-day stratification period improved germination capacity for *P. albicaulis*, while ISTA (2016) recommends 14 days for *P. elliottii*, 28 days for *P. taeda* and between six and nine months for *Tilia cordata*.

Stratification is widely used in southern pine species such as *P. taeda* and *P. elliottii* where seed dormancy is expected (Swofford, 1958). In western white pine species, a combination of warm stratification and cold stratification improved the germination of poor germinators (Gansel, 1987). Germination was also improved in temperate pine species, such as *Abies balsamea* (L.) Mill, *P. strobus* L., *P. resinosa* Sol. Ex Aiton and *P. radiata*, when seeds were treated with fungicide before stratification (Connolly *et al.*, 2017; Menzies *et al*., 1991). Germination speed and uniformity were also increased in species such as *P. radiata* (Menzies *et al*., 1991), *P. ponderosa* Douglas ex C. Lawson (Weber & Sorensen, 1990) and *P. albicaulis* Engelm. (Robertson *et al*., 2013). Stratification has also been shown to have a positive impact on early *P. taeda, P. radiata* and *Magnolia grandifola* L. seedling growth (Barnett & McLemore, 1967; Menzies *et al*., 1991; Fetouh & Hassan, 2014).

Seed that is sown in soils with low temperatures normally needs to be stratified for longer periods to ensure rapid emergence. Longer periods of stratification could, however, lead to some seed producing mould or germinating prematurely, thus causing damage to the small radicles (Barnett & McLemore, 1967). If the seed is stratified for too short a period, seed dormancy may not be broken and the seed will take longer to germinate, if at all. Damaged seeds should also be avoided during stratification as this will further reduced seed quality (Jones & Gosling, 1994; Leadem, 1997).

2.4.4.2 Sterilising seed with hydrogen peroxide

Sterilisation can be achieved by physical, chemical, and physio-chemical means. The seed coats of southern pine seed can contain seed-borne pathogens which may cause seed and seedling mortality (Barnett, 1976). These pathogens reduce seed vigour and germination, and cause diseases in the nursery such as damping off and root rot. To reduce these pathogens, seeds are treated with fungicides or sterilised. Depending on the fungicide, some may be phytotoxic, reducing seed germination (Barnett, 1976). Hydrogen peroxide, which has been found to kill pathogens, also stimulates germination, and is successfully used on the seed of many tree species (Barnett, 1976).

Barnett *et al.*, (1999) used hydrogen peroxide to reduce seed-borne pathogens to improve germination of *P. palustris*. The successful application of hydrogen peroxide depends on the concentration and on the time the seed is exposed to the hydrogen peroxide solution. *Pinus elliottii* and *P. palustris* obtained 85% and 77% germination with 0% infestation (meaning all pathogens were removed from the seed coat) when soaked in a 30% hydrogen peroxide solution for 30 minutes and one hour, respectively (Table 2.6). *Pinus taeda* and *P. etchinata* achieved 94% and 82% germination at 2% and 0% infestation, after being treated with a 3% hydrogen peroxide solution for 48 and four hours, respectively (Table 2.6) (Barnett & Varela, 2004).

Table 2.6. Hydrogen peroxide concentration application for some pine species (Barnett and Varela, 2004).

Species	Hydrogen peroxide concentration (%)	Time soaked (Hours)	Infestation (%)	Germination (%)
P. taeda	3	48	2	94
P. elliottii	30	0.5	0	85
P. palustris	30	1	0	77
P. etchinata	3	4	0	82

Hydrogen peroxide has also been used in combination with other treatments, such as stratification, to increase germination and seedling growth (Barnett & McLemore, 1967). Cavuslo & Glabar (2010) investigated the impact of hydrogen peroxide application on barley seed under high temperatures and salt conditions which improved germination and early seedling development. In a review of chemical treatments to improve *P. palustris*, Barnett & Varela (2004) found that hydrogen peroxide application/exposure also reduced the need to apply fungicide in early seedling growth. When applied to *Pseudotsuga menziesii* (Mayr) Franco and *P. roxburghii* Sarg. seed, hydrogen peroxide not only improved the mean germination, but also increased germination speed (Ching, 1959; Dumroese *et al*., 1988; Sharma & Gairola, 2009; Cavusoglu & Kabar, 2010). *Fusarium circinatum* Nirenberg & O'Donnell is the most common pathogen found on *P. elliottii*, *P. taeda*, *P. patula* and *P. radiata* seed (Table 2.7) and causes damping-off and shoot die-back of seedlings, while *Diplodia pinea* is associated with seed damage, and *Fusarium proliferatum* (Matsush.) Nirenberg ex Gerlach & Nirenberg causes damping-off amongst *P. elliottii* seedlings.

Table 2.7. Seed-borne pathogens of North American forest tree species (Cram & Fraedrich, 2010).

Pathogen	Host(s)	Disease
Caloscypha fulgens	*Abies grandis, Picea glauca, Pinus contorta, Pinus sylvestris, Pinus stroba*	Seed disease
Fusarium spp.	Conifers	Seed, cotyledon blight, damping-off
Fusarium circinatum	*Pinus elliottii var. elliottii, Pinus taeda, Pinus palustris, Pinus radiata.*	Seed, damping-off, shoot die back, cankers
Fusarium oxysporum	*Pseudotsuga menziesii*	Root rot, seed, damping-off
Fusarium proliferatum	*Pinus elliottii var. elliottii*	Damping-off
Lasiodiplodia theobromae	*Pinus elliottii var. elliottii*	Seed
Diplodia pinea	*Pinus elliottii var. elliottii*	Associated with seed damage

2.4.4.3 Priming

"Priming" is a seed treatment that initiates the germination process without the emergence of the radicle (Lutts *et al*., 2016). Germination normally consists of three phases, beginning with imbibition or water uptake in phase one. This triggers an increase in metabolic activity in phase two, resulting in radicle protrusion in phase three. Seed priming requires that the seed be soaked in water and then re-dried or dehydrated back to its original moisture content before sowing (Lutts *et al*., 2016). The purpose of priming is to achieve rapid and synchronised germination that will give rise to more vigorous seedlings able to withstand more stressful

conditions compared to seedlings from un-primed seed (Farahani *et al*., 2011; Uche *et al*., 2016). The more common forms of priming are discussed in more detail.

2.4.4.3.1 Osmo-priming

Osmo-priming relies on soaking seed in an osmotic solution with low water potential, which allows for a gradual and controlled imbibition process without radicle emergence. The most common chemical used in this process is polyethylene glycol (PEG), while others include mannitol, sorbitol, and glycerol (Lutts *et al*., 2016).

2.4.4.3.2 Halo-priming

Halo-priming involves seeds being soaked in salt solutions such as NaCl, KCl, KNO_3, K_3PO_4, $MgSO_4$ and $CaCl_2$ (Lutts *et al*., 2016).

2.4.4.3.3 Solid matrix priming (SMP)

Seed is first mixed and incubated in a wet solid water carrier (matrix), after which the seed is removed from the matrix, rinsed, and dried back to its original moisture content before sowing. Solid water carriers include peat moss, vermiculite, charcoal, sand, and clay (Lutts *et al*., 2016).

2.4.4.3.4 Hormo-priming

Seed imbibition takes place in the presence of growth hormones. Growth hormones commonly used include abscisic acid, auxins, gibberellins, kinetic, ethylene, polyamines, and salicylic acid (Lutts *et al*., 2016).

2.4.4.3.5 Bio-priming

Bio-priming involves imbibing seed in a bacterial solution to improve seed health and promote rapid and uniform germination. Rhizobacteria are commonly used during bio-priming (Lutts *et al*., 2016).

2.4.4.3.6 Hydro-priming

Hydro-priming involves soaking the seed in water and then re-drying them to their original moisture content before sowing (Lutts *et al*., 2016). Hydro-priming is a chemical-free, low cost, environmentally friendly method used to improve the speed and uniformity of germination (Farahani *et al*., 2011; Matsushima & Sakagami, 2013; Zulueta-Rodriguez *et al*., 2015; Lutts *et al*., 2016; Khafagy *et al*., 2017; Sepehri & Rouhi, 2017). Hydro-priming has been used extensively in vegetables such as basil (*Ocimum basilicum* L.), green bell pepper (*Capsicum annum cv*. Goliath), Chinese cabbage (*Brassica rapa* subsp. *Pekinensis* L.), barley

(*Hordeum vulgare* sbsp. *vulgare* L.), groundnut (*Arachis hypogaea* L.) and tomato (*Solanum lycopersicum* L.). Hydro-priming improves germination, emergence and seedling vigour in basil, green bell pepper, barley, and tomato.

Hydro-priming further improved shoot elongation, which assisted rice seedlings under stressful soil moisture conditions. Low vigour groundnut seed was hydro-primed and the final germination, germination rate and vigour index were improved over the control (Farahani *et al*., 2011; Uche *et al*., 2016; Yan, 2015; Khafagy *et al*., 2017; Sepehri & Rouhi, 2017; Matsushima & Sakagami, 2013; Patel *et al*., 2017).

Hydro-priming in combination with other priming methods such as bio-priming demonstrated positive effects on germination and seedling growth (Patel *et al*., 2017; Zulueta-Rodriguez, *et al*., 2015). In timber, species such as *Abies hickelii* Flous & Gaussen, *Abies religiosa* (Kunth) Schltdl. & Cham. and *Parkia nitida* showed improved germination and infield survival when hydro-priming was combined with bio-priming (Zulueta-Rodriguez *et al*., 2015; Moraes *et al*., 2015). Hydro-priming demonstrated improved seedling emergence, emergence uniformity and seedling vigour when seed was germinated under drought stresses (Yan, 2015; Ghassemi-Golezani *et al*., 2014; Matsushima & Sakagami, 2013). Similar results were found for immature seed (Moraes *et al*., 2015) and poor-quality (low germination) seed (Artola *et al*., 2003; Sepehri & Rouhi, 2017). When seeds of different sizes were tested, the biggest impact was on the smaller seed, which were of lower quality than the larger seed (Noorbakhshian *et al*., 2011).

2.4.4.4 Seaweed Liquid Fertilizer (Kelp-p-max)

Seaweed is used as a bio-fertilizer within the agriculture and horticulture industries (Sujatha *et al*., 2015; Elgubbi *et al*., 2019). Brown, red, and green seaweed extracts are used to enhance seedling vigour and growth. Kelp-p-max, a brown seaweed extract is made from *Ascophyllum nodosum* kelp and is used extensively as a fertilizer, and is bio-degradable, non-toxic, non-polluting, and non-hazardous to plant and animal life (Sujatha *et al*., 2015; Elgubbi *et al*., 2019). Seaweed-derived fertilizers are beneficial to seeds and plants as they also contain plant growth hormones such as auxins, cytokinins and betaines and micro- and macro-nutrients (Sujatha *et al*., 2015; Elgubbi *et al*., 2019). Besides being cheaper to produce than chemical fertilizers, they improve seed germination, root, and shoot development, leaf quality and seedling vigour, and increases a seed or plant's resistance to pathogens (Sujatha *et al*., 2015;

Elgubbi *et al*., 2019; Sasikala *et al*., 2016; Kavipriya *et al*., 2011; Dilavarnaik *et al*., 2017; Rao & Chatterjee, 2014).

Seaweed extracts have been widely tested on vegetables such as tomatoes (*Solanum lycopersicum* L.), green gram (*Vigna radiate* L.), sunflower (*Helianthus annus* L.) and beans (*Phaseolus vulgaris* L.). Benefits that were reported included:

1. Improved germination and root development;
2. Rapid and uniform seedling emergence;
3. Recovery from problems associated with stress;
4. Reduced fungal attacks;
5. Increased yields; and
6. Increased uptake of minerals from the soil by plants (Carvalho *et al*., 2013; Sivritepe, 2008; Demir *et al*., 2006; Elgubbi *et al*., 2019; Sasikala *et al*., 2016; El-Din, 2015; Popescu, 2016; Salma, *et al*., 2014).

2.5 SEEDLING QUALITY

Quality seedlings can be described as "those plants that are able to survive, grow and thrive under a wide variety of infield conditions" (Landis *et al.*, 2010). Although seed size and seed pre-treatments are important determinants of quality seedlings, seedling quality can also be determined by measuring the physical/morphological and physiological parameters of seedlings before planting (Haase, 2008; Jacobs *et al.*, 2004).

2.5.1 Physical parameters

These characteristics are visible and can be easily measured or calculated. Examples include seedling height, root collar diameter, sturdiness ratio, shoot-to-root ratio, the fresh weight and dry weight of shoots and roots and colour (Haase, 2008; Jacobs *et al.*, 2004; Landis *et al.*, 2010).

2.5.1.1 Seedling height

Seedling height is an indication of photosynthetic capacity and transpiration area. Taller seedlings may be an indication of improved genetics and may compete better against weeds, but they are more prone to water loss due to a greater transpiration area. Plants that are too tall are difficult to plant and may be subject to wind damage (Haase, 2008; Landis *et al.*, 2010).

2.5.1.2 Root collar diameter

Root collar or stem diameter is viewed as the best indicator of infield survival and growth. Larger root collar diameters are correlated with higher infield survival. It also is an indication of increased root volume, which will improve field survival due to increased water and nutrient uptake (Haase, 2008; Landis *et al.*, 2010).

2.5.1.3 Sturdiness ratio

The sturdiness ratio refers to the ratio between shoot height and root collar diameter. High ratios indicate tall, thin seedlings, while low ratios indicate shorter, robust plants. Infield survival is lower for taller, thinner seedlings (i.e., those with high ratios) because they have smaller root systems which are unable to support the plant above the ground (Haase, 2008).

2.5.1.4 Shoot mass (wet and dry weight)

A high shoot mass indicates a higher photosynthetic potential. Disproportionate shoot and root masses may have a negative impact on seedling survival. Where the shoot mass is higher than the root mass, this will create a situation where the plant will transpire quicker than it can absorb water, causing water stress, especially on drier sites (Haase, 2008; Landis *et al.*, 2010).

2.5.1.5 Shoot-to-root ratio

The shoot-to-root ratio indicates the balance between the shoots and roots. This relates to the balance between the area of transpiration and water uptake. For containerised seedlings, the ratio should not be less than 2:1 (Haase, 2008; Landis *et al.*, 2010).

2.5.1.6 Colour

Seedling colour is a good indicator of plant stress such as nutrient deficiency or the presence of diseases (Haase, 2008; Landis *et al.*, 2010).

2.5.2 Physiological parameters

Unlike physical or morphological indicators, physiological indicators are not visible and are measured with laboratory equipment. Physiological parameters measured include cold hardiness, root growth potential, stomatal conductance, chlorophyll fluorescence and plant moisture stress (Haase, 2008).

2.5.2.1 Cold hardiness

Cold hardiness is influenced by seed source, environment and nursery culture and indicates stress resistance (Haase, 2008; Landis *et al.*, 2010).

2.5.2.2 Root growth potential

Root growth potential is a good indicator of seedling vigour and determines the ability of the seedling to develop new root growth, which is critical for survival after infield planting (Haase, 2008; Landis *et al.*, 2010).

2.5.2.3 Plant moisture stress

Plant moisture stress relates to increased water stress levels in plants resulting in stomata closure. This will lead to a decrease in photosynthesis and plant growth and can cause mortality (Haase, 2008; Landis *et al.*, 2010).

2.5.2.4 Chlorophyll fluorescence

Chlorophyll fluorescence indicates the photosynthetic activity, with poor quality seedlings expressing low levels of fluorescence. This indicates a decrease in photosynthetic activity (Haase, 2008; Landis *et al.*, 2010).

2.6 SUMMARY

Foresters require quality seedlings that will not only survive infield, but also give better yields than the previous crop (Barnett, 2008). Nursery managers want to ensure that they propagate high quality seedlings by using the best seed available to achieve this goal (Barnett, 2002; Karrfalt, 2011). Seed producers can guarantee that quality seed reach nurseries by ensuring that the processes and systems used to get the seed from the seed orchard to the nursery are of the highest standard. Therefore, for *P. elliottii,* cones need to be harvested when ripe (mature) and must be stored under the right conditions (moisture content of between 5 and 10%, processing the seed without injury, and maintaining the right storage temperatures (2 to 5°C) until required (Barnett & McGilvray, 2002; Barnett & Varela, 2003).

Seed quality can potentially be improved further by sizing and treating the seed before sowing (Barnett, 1976; Edwards, 1981; Barnett, 1996; Barnett & McGilvray, 2002; Marwanto, 2004; Shelar, 2008; Barnett & Varela, 2003; Karrfalt, 2011; Andrews, 1965). *Pinus elliottii* seed vary in size, which provides an opportunity to sort seed into different sizes and takes advantage of the variation. Research in many species has shown that seed size influences germination and early seedling development (Mtambalika *et al.*, 2014). Large seed is superior to smaller seed in terms of higher and more rapid germination and increased seed and seedling vigour (Gomez, 2004). Studies on *P. taeda* clearly demonstrate that larger seed germinated faster than smaller seed and that the seedlings produced from larger seeds were taller than the seedlings produced

from smaller seeds (Dunlap & Barnett, 1983). Other benefits associated with sized seed include uniform product, synchronised sowing, and uniform germination (Barnett, 1996; Karrfalt, 2011). Treating seed before sowing has been researched all over the world, and these studies have shown the benefits of finding the best treatment for a specific species to improve germination. Treatments include seed priming, stratification, scarification, sterilising, imbibing seed and treating seed with hot water (Mackellar, 2008). Stratification has been successfully used to improve final germination and germination speed in species such as *P. taeda* (Swofford, 1958). Priming seed has been used for decades as a method to improve seed and seedling vigour (Farahani *et al*., 2011; Matsushima & Sakagami, 2013; Zulueta-Rodriguez *et al*., 2015; Lutts *et al*., 2016; Khafagy *et al*., 2017; Sepehri & Rouhi, 2017). Seed size and seed pre-treatments are procedures used by the nursery manager to improve seed germination and seedling quality. Quality seedlings from the nursery can potentially translate into increased seedling survival and growth infield.

CHAPTER 3: MATERIALS AND METHODS

3.1 STUDY AREAS

Trials were planted across three locations:

1) **Laboratory**

The first germination trial was conducted in the laboratory located at the Mondi Seedex facility in Hilton, KwaZulu-Natal (29°34' S and 30°16' E).

2) **Nursery**

A second germination trial was conducted at the Sunshine Seedlings Nursery (29°3' S and 30°28' E) outside Pietermaritzburg.

3) **Field**

A field trial was established on the Mondi Montigny farm in Kwambonambi (28°35' S and 32°12' E), KwaZulu-Natal.

A summary of the site description can be found in Table 3.1.

Table 3.1. Summary of the Montigny farm site description.

CLIMATIC_ZONE	Sub-tropical
MEAN ALTITUDE	58.3 m
MEAN ANNUAL MAP	1210.6 mm
MEAN ANNUAL MAT	22.2°C
SOIL_TYPE	Fernwood 1110
ERD	>151 cm
TEXTURE	Medium Sand
LITHOLOGY	Clastic sediments
CARBON_CONTENT	Low (<0.3% organic carbon)
LANDFORM	Plain

3.2 SEED SOURCE

The seed used for all three trials were obtained from *Pinus elliottii* cones harvested during March 2018 from a 2nd generation clonal seed orchard on the Mondi Montigny farm in Kwambonambi (28°35' S and 32°12' E).

3.3 SEED PROCESSING

Cones were air dried (cones were left outside in the sun to dry in trays for four weeks), tumbled (the process where winged seed are separated from the cones), de-winged (the process where wings are separated from seed using air and water), sized (seeds were grouped into different

sizes using sieves of different sizes ranging from 3.1 to 5 mm) and gravity separated (the process where empty and full seed are separated from each other by air, based on seed weight) (Karrfalt, 2008).

3.3.1 Seed sizing/grading

Seed were graded into two size classes (small and large), with seed with a diameter of between 3.1 and 4 mm classified as "small", while seed with a diameter of between 4.1 and 5 mm classified as "large". "Mixed" (a combination of large and small seed) seed consisted of 88% large and 12% small seed (Table 3.1). Seed was graded using sieves of different sizes, where seed was moved slowly over the sieve and seed with a diameter of 3.5 mm would fall through the sieve sized between 3.1 and 4 mm, while the seed with a diameter of 4.5 mm would continue until it found a sieve size between 4.1 and 5 mm to fall through. Any seed smaller than 3.1 mm and bigger than 5 mm was discarded as they were most likely particles of cone that had broken off during the cleaning process and found their way into the grading process.

3.3.2 Seed analysis

After processing, a moisture content test, the purity test and the 1 000 seed weight test were conducted. The aim of the moisture content test was to determine the moisture content within the seed for storage purposes. Moisture tests are carried out by oven-drying seed at 103°C for 17 hours (Bonner, 1981), and was calculated as follows:

$$\text{Moisture content} = \frac{\text{original weight} - \text{oven dry weight}}{\text{original weight}} \text{ x } 100 \text{ (ISTA, 2016)}$$

The 1 000 seed weight test was used to determine the number of seeds per gram or kilogram and was calculated as follows:

$$\text{1 000 seed weight} = \frac{\text{1 000 x 1 000}}{\text{mass of seed}} \text{ (ISTA, 2016)}$$

The purpose of purity testing was to determine what percentage within a seed sample, based on weight, was made up of the pure seed of the species being tested and was calculated as follows:

$$\text{Purity} = \frac{\text{weight of "pure" seed}}{\text{total weight of original sample}} \text{ x } 100 \text{ (ISTA, 2016)}$$

3.4 LABORATORY TRIAL

The laboratory trial was conducted to determine the influence, under controlled conditions of seed size, pre-treatment, and their interaction on germination. The purpose of the trial was to determine final germination (FG), time to 50% germination (T_{50}), mean germination time (MGT), germination index (GI), germination rate index (GRI), germination value (GV), and coefficient of velocity of germination (CVG).

3.4.1 Trial design and layout

The seed treatment trial consisted of a 3 x 7 factorial arrangement of 21 treatments. Factor 1 = Seed size (large, mixed, and small); factor 2 = Seed pre-treatment (hydrogen peroxide, hydro-priming, kelp-p-max, stratification, hydrogen peroxide + hydro-priming, hydrogen peroxide + kelp-p-max and a control). Seed size consisted of large seed (seed with a diameter between 4.1 and 5 mm), small seed (seed with a diameter between 3.1 and 4 mm), while mixed seed was made up of a mixture of small and large seed with small seed contributing 12% to the mix and large seed 88%. Germination trials were carried out based on the individual family's weighted contribution towards the total harvest. This was done to:

1) Include all families that were harvested (to ensure that genetic diversity is maintained).
2) Include all families based on their contribution to the total harvest.
3) Seed lots were prepared similar to normal seed production protocols, where not all families and seed sizes within families contribute equally to the harvest (e.g., if a family consisted of 1 kg where 0.7 kg were large seed and 0.3 kg were small seed then a 0.7 to 0.3 ratio was used in a germination test). The objective was to ensure that the probability of a constituent being present is determined only by its level of occurrence in the seed lot (ISTA, 2016).

For this study, large seed (from all families) contributed 88% and small seed (from all families) 12% towards the total harvest. The twenty-one treatments were replicated four times and laid out in randomised complete blocks design (RCBD). Due to the lack of space in the germination chamber (48 containers), the trial was sown over 2 cycles, with two replications per cycle. Each treatment plot consisted of four hundred seeds which were divided into four replications of one hundred seeds per plot (ISTA, 2016). A total of 8 400 seeds were hand sown directly onto cotton wool in plastic containers (lunch boxes).

3.4.2 Containers

Seed were sown into plastic containers (Figure 3.1) that were 151 x 151 x 43 mm (volume of 600 ml). These containers were used instead of petri-dishes because they were bigger and deeper which allowed for the seed to germinate, and they provided enough space for the seedlings to fully develop their roots and shoots without constriction.

Figure 3.1. Plastic container used in the laboratory germination trial.

3.4.3 Growing medium

Cotton wool was used as the growing medium with seed was sown on top of the cotton wool (ISTA, 2016). The cotton wool was moistened (35 ml) with municipal tap water and placed inside the plastic containers before sowing. Cotton wool (2.97 grams per container) was used in preference over germination paper as it would stay moist for the duration of the trial (28 days) without re-watering required.

3.4.4 Seed lot make-up

"Seed lots" are seed of a specific quantity and are uniquely identifiable, while "families" are trees that are genetically closely related (Hettasch *et al.*, 2009). Families were kept separate during the processing phase to determine individual family contributions towards the total seed harvest. The seed lot was made up based on the individual family's weighted contribution towards the total harvest with large seed contributing 88% of the total harvest and small seed 12%. In this study, mixed seed consisted of a combination of large and small seed. In a total of 400 seeds there would be 352 (88%) large seeds and 48 (12%) small seeds. This was done to approximate the seed lot make-up under normal seed production conditions (where all seed is needed to meet seed demand). It also provides more accurate germination for the seed lot (representative sample size) (ISTA, 2016). A total of 15 families was included (Table 3.2)

across the different seed grades, except for the mixed seed grade where some sizes for some families were excluded due to their weighted contribution which was too low (less than 1 seed).

Table 3.2. Number of seeds per family per size class per plot to demonstrate the ratio between small (12%) and large (88%) seed.

Family	Small	Large	Mixed	
			Small (12%)	Large (88%)
F1[a]	5	3	1	3
F2	6	2	1	2
F3	3	2	0	2
F4	4	17	1	15
F5	1	2	0	2
F6	18	11	2	10
F7	21	14	2	12
F8	3	15	0	13
F9	1	2	0	2
F10	3	17	0	13
F11	1	6	0	6
F12	13	1	2	1
F13	11	5	1	4
F14	4	2	1	2
F15	6	1	1	1
Total	**100**	**100**	**12**	**88**
			100	

[a]F1 = Family 1

3.4.5 Seed pre-treatments

3.4.5.1 Treatment 1: Stratification

Stratification is a process whereby seed is exposed to low temperatures (between 2 and 5ºC) between moist layers (such cotton wool, sand, or vermiculite) to increase rapid and uniform germination by overcoming seed dormancy (seed that do not germinate when conditions are favourable for germination) and is used as a pre-treatment for *P. elliottii* and *P. taeda* where seed dormancy is expected (Swofford, 1958; ISTA, 2016). In this study the seed was imbibed (allowed to take up water to initiate the metabolic processes in the seed) for 24 hours, after which the excess water was drained, and the seed placed in moist cotton wool in 50 ml plastic bottles. Municipal tap water (35 ml) was used to moisten the cotton wool. Each bottle contained 100 seeds, which was one of 4 replications (4 bottles were used per treatment, with each bottle containing one plot's worth of seeds, that is 100 seeds). These bottles were then placed into a

cold room with a temperature of 4 ± 2°C for 14 days (ISTA, 2016). After the 14 days, the seed were removed from the cold room and sown.

3.4.5.2 Treatment 2: Hydro-Priming

Hydro-priming was selected as it is a low-cost pre-treatment method that involves the soaking of seed in water and then re-drying them to their original moisture content before sowing (Lutts *et al*., 2016). Besides hydro-priming being ecologically sound it is also used by farmers as an economically viable option for the germination of vegetable seed (Farahani *et al*., 2011; Matsushima & Sakagami, 2013; Zulueta-Rodriguez *et al*., 2015; Lutts *et al*., 2016; Khafagy *et al*., 2017; Sepehri & Rouhi, 2017). In this study, seeds of each category (small, mixed, and large) were randomly selected (100 seeds per plot), weighed to determine their dry weight and placed into 50 ml bottles. Each bottle contained 100 seeds, which was one of 4 replications (4 bottles were used per treatment, with each bottle containing seed for one plot). Seeds were soaked in municipal tap water (35 ml) and kept for 12 hours in the cold room at a temperature of 4 ± 2°C. Seeds were removed from the cold room after 12 hours and the water was drained. The seed was then dried in the sun and weighed at one-hour intervals until they had regained their initial dry weight, after which they were kept in the cold room until sowing.

3.4.5.3 Treatment 3: Hydrogen peroxide

Barnett and Varela (2004) used hydrogen peroxide as a pre-treatment on *P. elliottii* seed to remove any pathogens that may be present on the seed coat and to improve germination. In this study, seeds of each category (small, mixed, and large) were randomly selected (100 seeds per plot) and soaked in a 30% hydrogen peroxide solution for one hour in 50 ml plastic bottles. Each bottle contained 100 seeds, which was one of 4 replications (4 bottles were used per treatment, with each bottle containing seed for one plot). The hydrogen peroxide solution was then drained, and seeds rinsed with municipal tap water for five minutes before sowing. Seeds were sown directly after rinsing as no storage was required.

3.4.5.4 Treatment 4: Kelp-P-Max

Kelp-P-Max is a liquid fertilizer used in nurseries as a foliar application to stimulate root and leaf/needle development. It contains nitrogen in the form of ammonium (energy rich proteins), phosphorous (essential for energy releasing reactions), amino acids (building blocks of protein and a growth promotor) and micronutrients (regulate growth processes). This product was included as it also contains natural growth hormones (auxins and cytokinins) that stimulate cell division and root development (Sujatha *et al*., 2015; Elgubbi *et al*., 2019). In this study, seed for each plot (100 seeds per plot) of small, large, and mixed seed were soaked for 24 hours in

a five-millilitre kelp-p-max solution (which was diluted with one litre of municipal tap water to obtain a 0.005 concentration) in 50 ml plastic bottles and kept in the cold room at a temperature of 4 ± 2^{o}C. Each bottle contained 100 seeds, which was one of 4 replications (4 bottles were used per treatment, with each bottle containing seed for one plot). After 24 hours the seed was removed from the cold room, the kelp-p-max solution drained, and the seeds sown.

3.4.5.5 Treatment 5: Hydrogen peroxide + Hydro-priming

Hydrogen peroxide (removing pathogens on the seed coat and promoting germination) in combination with hydro-priming (a chemical-free, low cost, environmentally-friendly method used to improve the speed and uniformity of germination) was used to determine their combined effects on germination (Farahani *et al*., 2011; Matsushima & Sakagami, 2013; Zulueta-Rodriguez *et al*., 2015; Lutts *et al*., 2016; Khafagy *et al*., 2017; Sepehri & Rouhi, 2017), to see if the result from the combination would be better than each on its own. Seed for each replication (100 seeds per plot with 4 replications in total) of small, mixed, and large seed were first treated as for 3.4.3.3 (hydrogen peroxide) and were thereafter treated as for 3.4.3.2 (hydro-priming) before being sown.

3.4.5.6 Treatment 6: Hydrogen peroxide + Kelp-P-Max solution

Hydrogen peroxide (removing pathogens on the seed coat and promoting germination) in combination with kelp-p-max (contains natural auxins and cytokinins that stimulate cell division and root development) was used to determine their combined effects on germination. Seed for each replication (100 seeds per plot with 4 replications in total) of small, large, and mixed seed were first treated as for 3.4.3.3 (hydrogen peroxide) and were thereafter treated as for 3.4.3.4 (Kelp-P-Max) before being sown.

3.4.5.7 Treatment 7: Control

Seed for each replication (100 seeds per plot with 4 replications in total) of small, mixed, and large seed were placed into a 50 ml bottle. No pre-treatments were applied. Each bottle contained 100 seeds, which was one of 4 replications (4 bottles were used per treatment, with each bottle containing seed for one plot). These bottles were kept in the cold room with a temperature of 4 ± 2^{o}C until sowing.

3.4.6 Treatment combinations

Treatment combinations were prepared in such a manner that any interactions between the two main factors (Factor 1 = Seed size and Factor 2 = Seed pre-treatment) could be obtained (Table 3.3).

Table 3.3. Treatment combinations for germination trials.

Treatment no.	Factor 1: Seed size	Factor 2: Seed pre-treatments
T1	Small	No treatment
T2	Small	Seed stratified for 14 days
T3	Small	Seed hydro-primed for 12 hours
T4	Small	Seed soaked in kelp-p-max for 24 hours
T5	Small	Seed soaked in hydrogen peroxide (30%) for 1 hour
T6	Small	Seed soaked in hydrogen peroxide (30%) (1 hour) and soaked in kelp-p-max (for 24 hours)
T7	Small	Seed soaked in hydrogen peroxide (30%) (for 1 hour) and hydro-primed (for 12 hours)
T8	Mixed	No treatment
T9	Mixed	Seed stratified for 14 days
T10	Mixed	Seed hydro-primed for 12 hours
T11	Mixed	Seed soaked in kelp-p-max for 24 hours
T12	Mixed	Seed soaked in hydrogen peroxide (30%) for 1 hour
T13	Mixed	Seed soaked in hydrogen peroxide (30%) (1 hour) and soaked in kelp-p-max (for 24 hours)
T14	Mixed	Seed soaked in hydrogen peroxide (30%) (for 1 hour) and hydro-primed (for 12 hours)
T15	Large	No treatment
T16	Large	Seed stratified for 14 days
T17	Large	Seed hydro-primed for 12 hours
T18	Large	Seed soaked in kelp-p-max for 24 hours
T19	Large	Seed soaked in hydrogen peroxide (30%) for 1 hour
T20	Large	Seed soaked in hydrogen peroxide (30%) (1 hour) and soaked in kelp-p-max (for 24 hours)
T21	Large	Seed soaked in hydrogen peroxide (30%) (for 1 hour) and hydro-primed (for 12 hours)

3.4.7 Growth Chamber

A growth chamber (Figure 3.2) was used for the germination trial. The temperature was set at 25°C with continuous lighting (Fluorescent F18W/GRO) and kept at 100% humidity for the duration of the trial (28 days).

Figure 3.2. Growth chamber used for laboratory germination trial.

3.4.8 Storage

Seed were kept in the cold room at the Mondi Seedex facility in Hilton (Figure 3.3), at a temperature of 4 ± 2°C and humidity of 30% until ready for sowing. Seed was kept at a moisture

content of 6% to inhibit germination, and in airtight containers to prevent moisture ingress and thus the occurrence of any metabolic activity.

Figure 3.3. Cold room where seed was kept until sowing.

3.4.9 Seed sowing

As discussed, due to a lack of space in the germination chamber the trial was implemented in two cycles of two replications. Replications 1 and 2 (cycle 1) were sown on 25 January 2019 and replications 3 and 4 (cycle 2) were sown on 25 February 2019.

Figure 3.4. Plastic germination containers inside growth chamber during germination.

Seed was recorded as germinated as soon as the radicle emerged from the seed coat (Figure 3.5).

Figure 3.5. Germinating seed on cotton wool inside plastic container.

3.4.10 Data collection

The trial lasted 28 days, with germination recorded on 7, 14, 21 and 28 days after sowing (DAS) (ISTA, 2016). These weekly recordings were carried out to investigate germination over time, from which cumulative germination (expressed as a percentage) could be determined. Seed germination parameters measured included Final Germination (FG), Time to 50% Germination (T_{50}), Mean Germination Time (MGT), Germination Index (GI), Germination Rate Index (GRI), Germination Value (GV), and Coefficient of Velocity of Germination (CVG) (Table 3.4). These germination parameters were selected to further investigate the quality of seed germination as final germination only gives an indication of the germination capacity of a seed lot and does not give any indication of germination speed, synchrony or spread of germination (Kader, 2005). For example, although two seed lots may both have 80% germination, they may have different rates or spreads of germination, with those reaching 80% sooner more preferable to those that germinate later (5 versus 10 days)

Table 3.4. Formulas for germination parameter calculations.

Germination parameter	Formula	Description	Reference
Final Germination (FG)	FG = (NT/N) x 100	NT = total number of seeds germinated. N = total number of seeds sown	The higher the FG value, the greater the germination (ISTA, 2016)
Mean Germination Time (MGT)	MGT = Σ *fini* / N	*fi* = day during germination period *ni* = the number of germinated seed germinated on day *fi* N = total number of germinated seed	The lower the MGT, the faster the seeds have germinated (Kader, 2005)
Time to 50% Germination (T_{50})	T_{50} = ti + (N/2 – ni) (tj – ti) / (nj – ni)	N is the final number of germinating seeds. nj and ni are the cumulative number of seeds germinated at times t j and t i, respectively, when ni<N/2<ni.	The lower the T_{50}, the faster the seeds have germinated (Thomson and El-Kassaby, 1993)
Germination Value (GV)	GV = MDG x PV	Where mean daily germination (MDG) calculated as the full seed germinated at the end of the test divided by the number of days to the end of the test. PV is peak value, which is the maximum quotient derived from all the cumulative full seed germination percentages on any day divided by the number of days to reach these percentages.	GV is a combination of complete germination and germination speed. Higher values indicate high germination over a short period (Czabator, 1962)
Germination Index (GI)	GI = (10×n1) + (9×n2) + · · · + (1×n10)	n1, n2 . . . n10 = No. of germinated seeds on the first, second and subsequent days until the 10th day; 10, 9 . . . and 1 are weights given to the number of germinated seeds on the first, second and subsequent days, respectively	Higher GI value denotes a higher percentage and rate of germination (Kader, 2005)
Germination Rate Index (GRI)	GRI = G1/1 + G2/2 +· · ·+ Gx/x	G1 = Germination percentage × 100 at the first day after sowing, G2 = Germination percentage × 100 at the second day after sowing	The GRI reflects the percentage of germination on each day of the germination period (Kader, 2005)
Coefficient of Velocity of Germination (CVG)	CVG = N1 + N2 + · · · + Nx/100 × N1T1 + · · · + NxTx	N = No. of seeds germinated each day, T = No. of days from seeding corresponding to N	Places emphasis on the time required for reaching final germination (Kader 2005)

3.4.11 Data analysis

Data was subjected to generalised analysis of variance (ANOVA) using VSN International (2022). GenStat *for Windows* 22nd Edition. VSN International, Hemel Hempstead, UK. Only if the overall *F*-probability were significant for any factor or interactions between factors, were the differences between treatment means further investigated using the "Students least significant difference" statistic (LSD). The data (including graphs) was managed using MS Excel 2016.

3.5 NURSERY TRIAL

The nursery trial was conducted to determine the influence of seed size, pre-treatment, and their interaction on germination under commercial nursery (uncontrolled) conditions. The

purpose of the trial was to determine final germination (FG), time to 50% germination (T_{50}), mean germination time (MGT), germination index (GI), germination rate index (GRI), germination value (GV), and coefficient of velocity of germination (CVG).

3.5.1 Trial design and layout

The trial design and layout were the same as for the laboratory trial (see section 3.4.1), except that all the seed were sown at the same time (not in cycles) in polystyrene trays (not in containers/lunch boxes).

3.5.2 Seed lot make-up

See section 3.4.2 under laboratory trial.

3.5.3 Seed pre-treatments

See section 3.4.3 under laboratory trial.

3.5.4 Treatment combinations

See section 3.4.4 under laboratory trial.

3.5.5 Containers

Polystyrene containers of 128 cavities each (Figure 3.6) were used for the sowing study. Each cavity has a volume of 52 ml and depth of 90 mm. They are also easier to handle than the plastic containers (Unigrow's) with loose inserts (the Unigrow containers are also heavier than the polystyrene containers). As the polystyrene containers were used, they were dipped in copper oxychloride before sowing to prevent the roots from growing into the side walls of the container.

Figure 3.6. A 128 Polystyrene tray used during nursery trial.

3.5.6 Growing medium

A coir, bark, peat, and vermiculite blend with a ratio of 3:2:1:1 was used as the growing medium (Figure 3.7). Coir and peat have a high water-holding capacity, excellent drainage, cation exchange capacity and electric conductivity, while composted bark also has a high water-holding capacity and provides nutrients during further decay. Vermiculite is important for its good aeration capacity and high retention of nutrients and water (Landis, 1990; Stayton & Portie, 1992).

Figure 3.7. Growing medium consisting of a coir, bark, peat, and vermiculite blend.

3.5.7 Growth Chamber

A germination chamber (Figure 3.8) was used for the initiation of germination. The trays were stacked on top of each other (to maintain high humidity) with empty trays/plastic placed on the top row of trays to prevent the trays immediately underneath from drying out. Trays were placed into the germination chamber for 7 days, where conditions (25°C and >90% humidity) favour the initiation of germination before being moved to the grow-out section of the nursery.

Figure 3.8. Germination chamber where seed was kept to initiate germination.

3.5.8 Storage

See section 3.4.8 under laboratory trial.

3.5.9 Seed sowing

One seed was sown by hand into each of one hundred of the 128 cavities, with one tray representing one plot. All twenty-one treatments (84 trays in total) were sown at on 26 October 2018 (over a 6-hour period) at the Sunshine Seedlings Nursery, Pietermaritzburg, KwaZulu-Natal. After sowing, the seeds were covered with a thin layer of vermiculite (approximately 1 mm) and irrigated before being placed in the germination chamber. The vermiculite covering was used as it is light and does not inhibit the development of the plumule following germination.

3.5.10 Grow-out section

The grow-out section provides an opportunity for continued germination, irrigation, fertigation and weeding of germinants. Trays were placed on wire beds, 0.6 m above ground level. A hail net (30% shade net) was used as overhead covering (Figure 3.9).

Figure 3.9. Trial in grow-out section at the Sunshine Seedlings Nursery, Pietermaritzburg, KwaZulu-Natal.

Germinants were irrigated for 10 minutes daily, where a scale of one to five (Figure 3.10) was used to determine if irrigation was needed or not, where:

1 = growing media is completely dry and may separate away from the insert walls, and the plant may not recover if wilted;

2 = growing media is almost dry, and plants may begin to wilt;

3 = growing media is drying due to plant uptake and evaporation;

4 = maximum holding capacity; and

5 = medium is saturated (Figure 3.10).

Irrigation occurred at level two up till level 4 was reached, with levels one (dry) and five (over watering) avoided (Fisher *et al.*, 2019).

Figure 3.10. Dryness scale showing the different dryness levels from one to five (Fisher *et al.*, 2019).

On hot days irrigation was increased, with less on cooler days and no irrigation on rainy days. Once a week, fertilizer (2:3:2) in liquid form was applied at an Electro Conductivity (EC) of 1000 uc. The EC measures the salt content in the water, with an EC of between 700 and 1200 preferred to ensure sufficient nutrient uptake (Landis & Dumroese, 2006). Weeds that germinated in the trays were weeded by hand as required. At 3 months, the seedlings trays were moved to the Mondi Mountain Home nursery, with the management regime being maintained for a further four months before planting.

3.5.11 Data collection

Data collection was similar to that of the laboratory trial except that the trial was conducted over 42 days, with germination recorded at 7, 14, 21, 28, 35 and 42 days (Figure 3.11) after sowing (ISTA, 2016). Seed was recorded as germinated when the seed coat was visible above the growth medium.

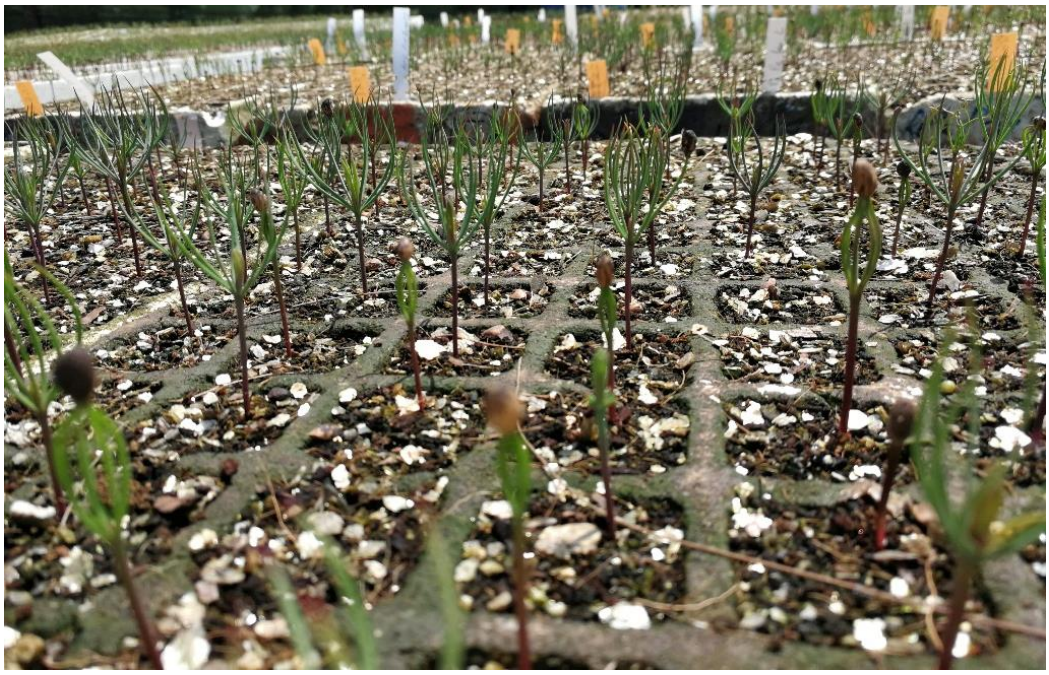

Figure 3.11. Germinants in the grow-out section, recorded weekly.

3.5.12 Data analysis

Data was subjected to generalised analysis of variance (ANOVA) using VSN International (2022). GenStat *for Windows* 22nd Edition. VSN International, Hemel Hempstead, UK. Only if the overall *F*-probability were significant for any factor or interactions between factors, were the differences between treatment means further investigated using the "Students least

significant difference" statistic (LSD). The data (including graphs) was managed using MS Excel 2016.

3.6 SEEDLING GROWTH

3.6.1 Data collection

To determine the effects of seed size and pre-treatments on seedling growth, seedling height was measured with a ruler while root collar diameter was measured (at five months in the nursery) using a digital Vernier calliper (Figure 3.12). Seedling height and root collar diameter was measured using twenty randomly selected, healthy seedlings from all treatments in the nursery. The sturdiness ratio of each seedling was determined by dividing the seedling height (mm) by the root collar diameter (mm). A total of 420 seedlings, aged 5 months, were measured to investigate the effect of seed size and seed pre-treatment on seedling height, root collar diameter and sturdiness ratio.

Figure 3.12. Measuring seedling height and root collar diameter using a calliper.

3.6.2 Data analysis

Seedling height, root collar diameter and the sturdiness ratio of seedling growth were analysed using descriptive statistics and displayed graphically (using Microsoft Excel 2016). The 95% confidence interval level was used to show treatment differences and variation between means.

3.7 FIELD TRIAL

3.7.1 Trial design and layout

The trial consisted of a 3 x 7 factorial arrangement of 21 treatments. Factor 1 = Seed size (large, mixed, and small); factor 2 = Seed pre-treatment (hydrogen peroxide, hydro-priming, kelp-p-max, stratification, hydrogen peroxide + hydro-priming and hydrogen peroxide + kelp-p-max and a control (seed was not pre-treated). The twenty-one treatments were replicated six times with six tree line plots and laid out in randomised complete blocks design (RCBD). A total number of 756 seedlings were planted in the field trial.

3.7.2 Seedling selection, site preparation and planting

Prior to planting, seedlings for each treatment were selected based on the average height of seedlings per size class (Figure 3.13). No pre-plant chemical spray was applied to the site, with the site mowed every second month during summer to keep the fuel load as low as possible. The site was prepared by first mowing it, after which it was marked for pitting (between-row spacing of 3 m, and within-row of 2 m). Seedlings were well-watered before being transported in-field and kept moist until planting on 31 July 2019 (Mondi Montigny farm, Kwambonambi). Each seedling was planted with 700 ml of hydrogel (3 g hydrogel in 1 L water per pit) (Figure 3.14A and B), with no fertilizer applied following planting. A 60 cm ring clean around each pit was carried out as required so as to reduced competition.

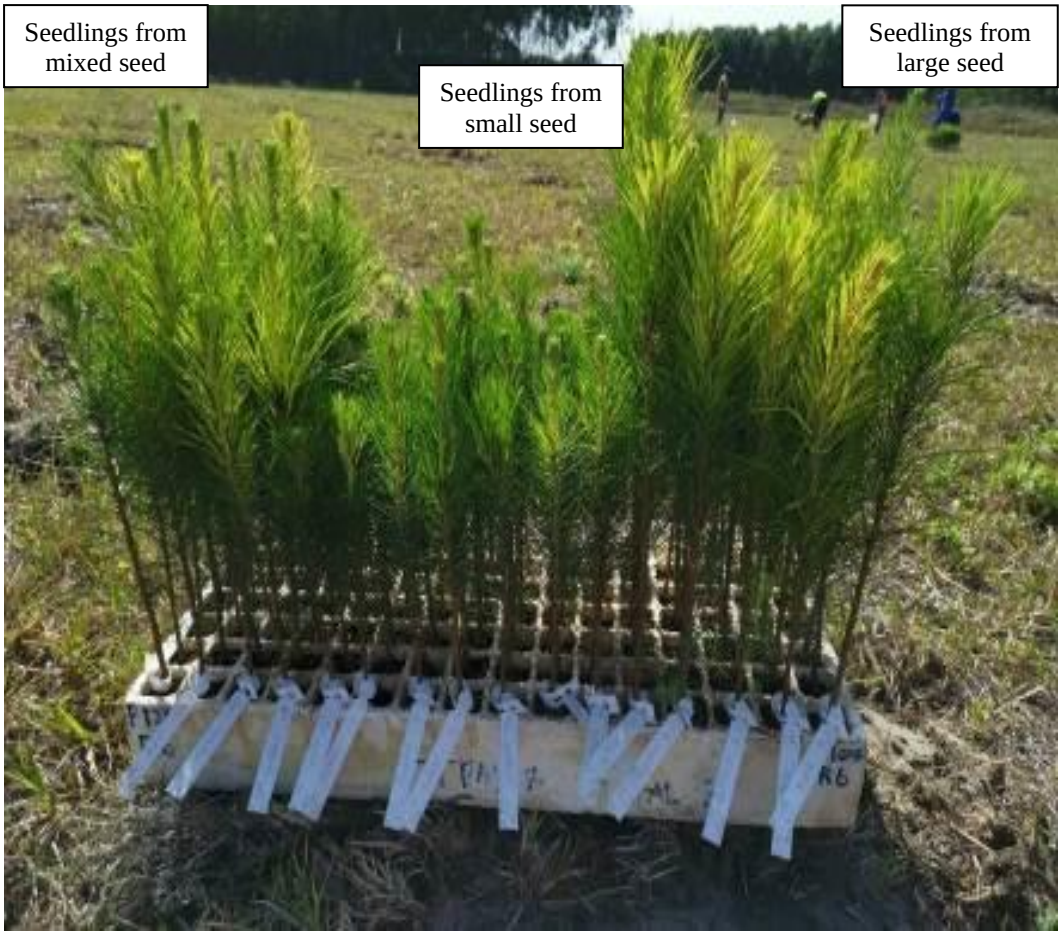

Figure 3.13. Seedlings from mixed, small, and large seed.

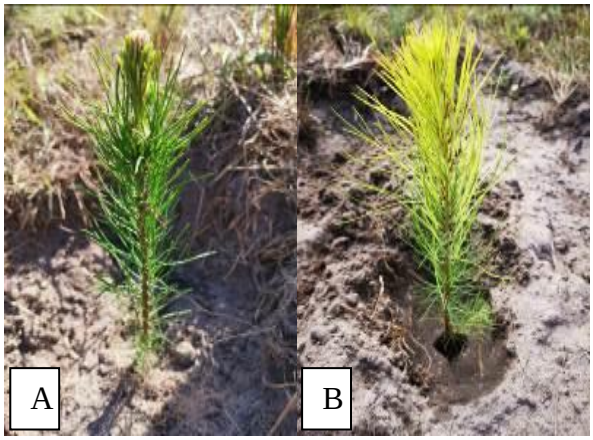

Figure 3.14. (A) Planted seedling and (B) Hydrogel in planting hole.

3.7.3 Data collection

Survival, height, and ground line diameters were assessed at 3, 6, 9 and 12 months. Tree height was measured from the ground to the tip of the tree using an aluminium ruler, while the ground line diameters were measured using digital Vernier callipers. From this the following were

calculated: survival (%), mean height (mm), mean diameter (mm), and the biomass index after twelve months. The biomass index was calculated as follows:

Biomass index = (ground line diameter)^2 x tree height (Rolando & Allan, 2004).

3.7.4 Data analysis

Seedling height and ground line diameter data were subjected to generalised analysis of variance (ANOVA) using VSN International (2022). GenStat *for Windows* 22nd Edition. VSN International, Hemel Hempstead, UK. LSD (least significant difference) was used to determine differences between treatment means. Angular transformation (arcsine transformation) was applied in all survival and biomass index data prior to analysis. Data was then subjected to generalised analysis of variance (ANOVA) using VSN International (2022). GenStat *for Windows* 22nd Edition. VSN International, Hemel Hempstead, UK. LSD (least significant difference) was used to determine differences between treatment means. Data (including graphs) was managed using MS Excel 2016.

3.8 ASSUMPTIONS AND PRESCRIPTIONS

A) All seedlings planted with hydrogel, which is standard practice for Mondi during planting.

B) No fertilizer was applied (fertilizer would be an additional variable in the study).

C) No blanking was carried out.

CHAPTER 4: RESULTS

This chapter covers the results of the laboratory, nursery, and field trials. The study reports the effects of seed size, pre-treatment and their interaction on germination, early seedling growth, field survival and growth of *P. elliottii.*

4.1 LABORATORY TRIAL

4.1.1 Final germination (FG)

Only seed size was significantly different ($p < 0.001$) for final germination, while no significance was observed for pre-treatment and their interaction 28 days after sowing (Table 4.1). This indicates that germination was dependent on seed size when seed was sown under controlled conditions in the laboratory.

Table 4.1. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on final germination.

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Cycle	1	253.76	253.76	1.55	
Cycle.Rep	2	327.05	163.52	3.74	
Seed size (A)	2	5623.14	2811.57	64.3	<0.001
Pre-treatment (B)	6	478.31	79.72	1.82	0.110
Seed size.Pre-treatment	12	628.19	52.35	1.2	0.306
Error (residual)	60	2623.69	43.73		
Total	83	9934.14			

B) Seed size

Final germination at 28 DAS was significantly ($p < 0.05$) higher for large (79.1%) and mixed seed (76.9%) than for small seed (60.7%) (Figure 4.1). The composition of the mixed seed was not a 50% large, 50% small make-up, but 88% large and 12% small make-up, which is why the germination of the mixed seed is similar (just slightly lower) to that of the large seed (Figure 4.1). Large seed had 18.4% and 2.2% better germination than small and mixed seed, respectively. Germination increased as seed size increased, but it was lower than the expected germination (85%) level for all seed sizes (Barnett, 2002; Barnett & Varela, 2004; Barnett, 2008).

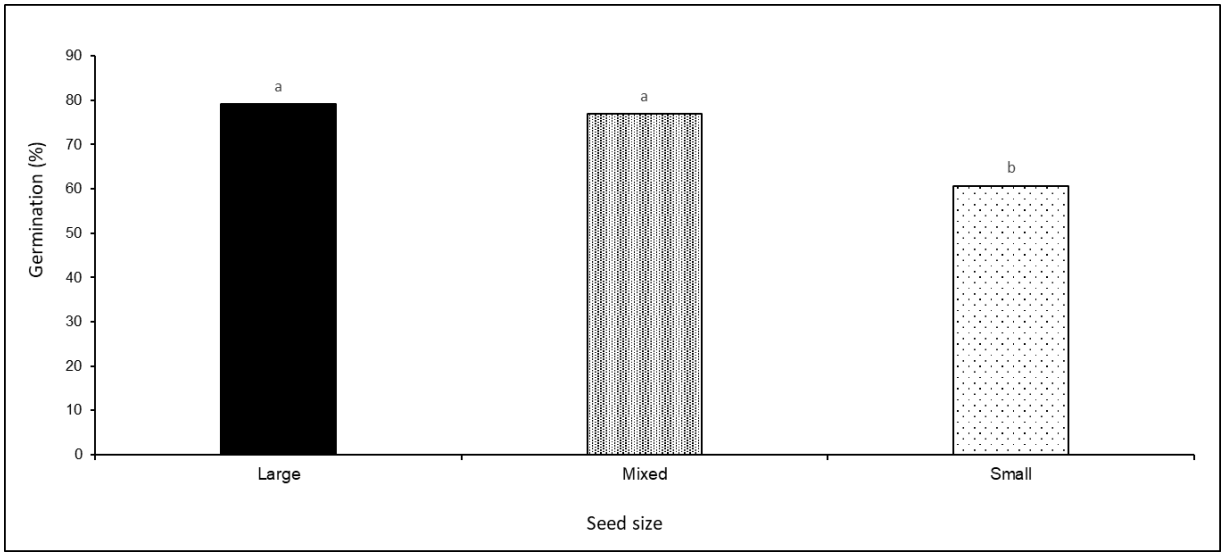

Figure 4.1. Final germination for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

Cumulative germination for large and mixed seed was significantly higher than for small seed. This is likely due to the composition of the mixed seed (88% large and 12% small seed), which was closer to the large seed than mid-way between the small and large (50% large and 50% small seed) (Figure 4.2). Germination for all seed sizes peaked at 14 days, with little additional germination occurring between days 21 and 28 (Figure 4.2).

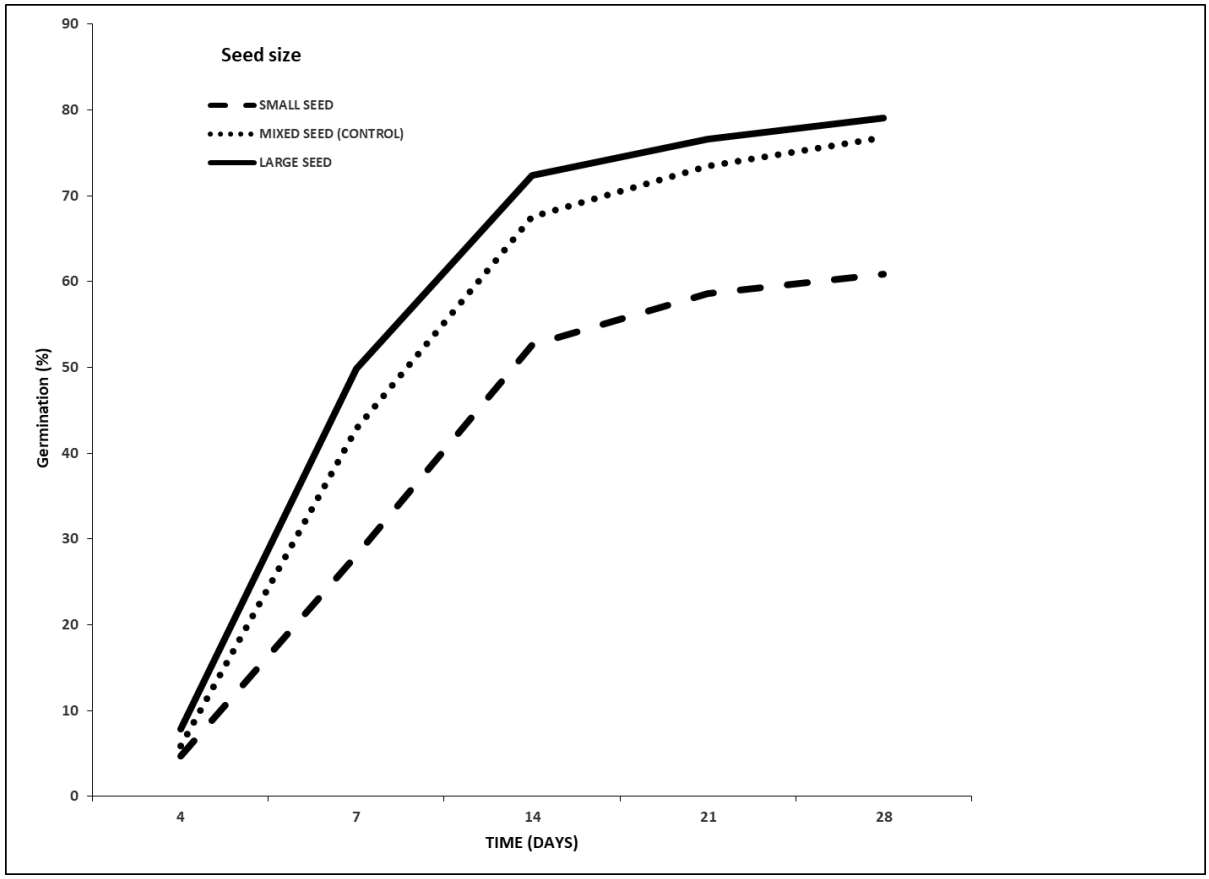

Figure 4.2. Cumulative germination for small, mixed, and large seed.

Daily germination for large and mixed seed was much higher compared to small seed, with all seed classes peaking at 7 days (Figure 4.3). Daily germination was low between days 15 and 28.

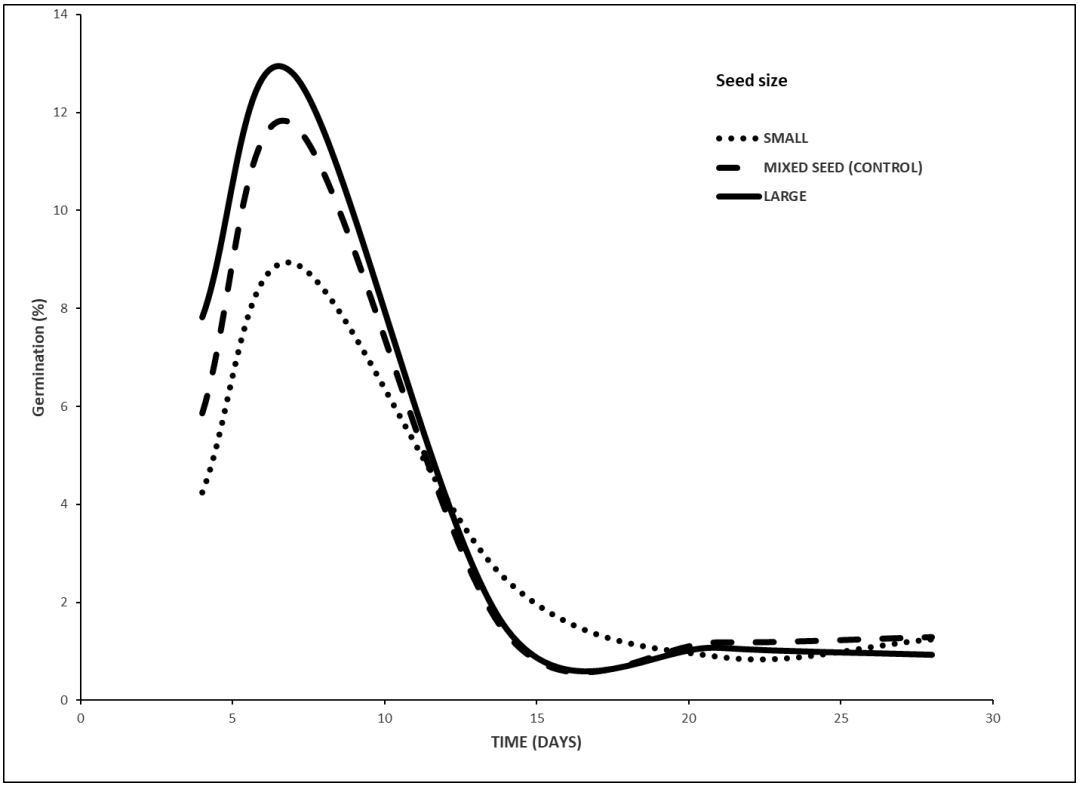

Figure 4.3. Daily germination for small, mixed, and large seed.

4.1.2 Time to 50% germination (T_{50})

T_{50} was significantly different for seed size (p = 0.002) and pre-treatment (p = 0.004), while no significant differences for their interaction were observed (Table 4.2).

Table 4.2. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on time to 50% germination (T_{50}).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Cycle	1	2.99	2.99	1.57	
Cycle.Rep	2	3.827	1.914	0.34	
Seed size (A)	2	80.301	40.151	7.21	0.002
Pre-treatment (B)	6	120.352	20.059	3.6	0.004
Seed size.Pre-treatment	12	113.036	9.420	1.69	0.092
Error (residual)	60	334.325	5.572		
Total	83	654.831			

A) Seed size

T_{50} is the time taken to reach 50% germination (Kader, 2005), with lower values indicating shorter times and higher values longer time (lower values/shorter times being better). T_{50} was

significantly ($p < 0.05$) lower for large (6.2 days) and mixed (6.9 days) seed than for small seed (8.6 days). Large seed reached T_{50} in 2.4 and 0.7 days quicker than small and mixed seed, respectively (Figure 4.4). There was an increase in the T_{50} as seed size decreased, which is an indication that germination speed for larger seed is higher than for small seed.

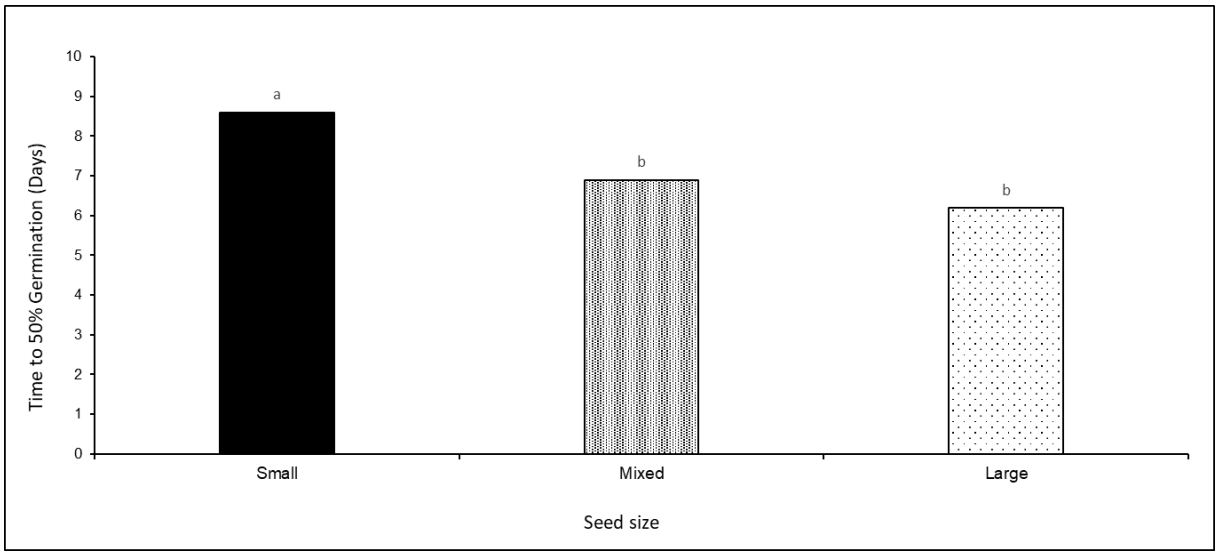

Figure 4.4. Time to 50% germination for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

B) Pre-treatment

T_{50} for the stratification (5.5 days) pre-treatment was significantly ($p < 0.05$) lower than for the hydrogen peroxide + hydro-prime (9.5 days) and kelp-p-max (7.9 days) pre-treatments (Figure 4.5). This indicates that the stratification pre-treatment reached 50% germination fastest, while the hydrogen peroxide + hydro-prime pre-treatment took the longest. T_{50} for the control (7.4 days) was not significantly ($p < 0.05$) different from the hydro-prime (7.2 days), kelp-p-max (8 days), hydrogen peroxide (7.1 days), hydrogen peroxide + kelp-p-max (6 days) and stratification pre-treatments (Figure 4.5). The stratification pre-treatment reached T_{50} 1.85 days quicker than the control.

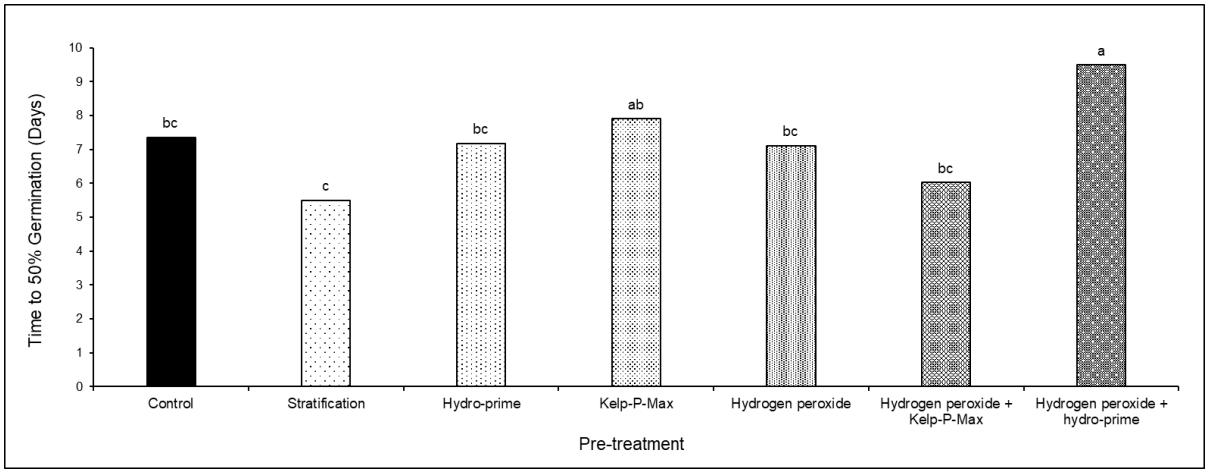

Figure 4.5. Time to 50% germination for pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.1.3 Mean Germination Time (MGT)

Mean germination time (MGT) was significantly different for seed size ($p < 0.001$) and pre-treatment ($p < 0.001$), while no significant differences for their interaction were observed (Table 4.3).

Table 4.3. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on mean germination time (MGT).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Cycle	1	11.247	11.247	10.81	
Cycle.Rep	2	2.081	1.040	0.57	
Seed size (A)	2	36.435	18.218	9.96	<0.001
Pre-treatment (B)	6	76.069	12.678	6.93	<0.001
Seed size.Pre-treatment	12	9.915	0.826	0.45	0.934
Error (residual)	60	109.715	1.829		
Total	83	245.462			

A) Seed size

Mean germination time is the average time taken to complete germination (Kader, 2005), with lower values indicating shorter average times and higher values longer average times (lower values/shorter times being better). MGT was significantly ($p < 0.05$) lower for large (8.4 days) seed than for mixed (9.2 days) and small (10.0 days) seed, with mixed seed being significantly ($p < 0.05$) different to small seed. Large seed reached MGT in 1.6 and 0.8 days quicker than small and mixed seed, respectively. There was an increase in the MGT as seed size decreased (Figure 4.6), indicating germination speed was higher for large compared to mixed and small seed.

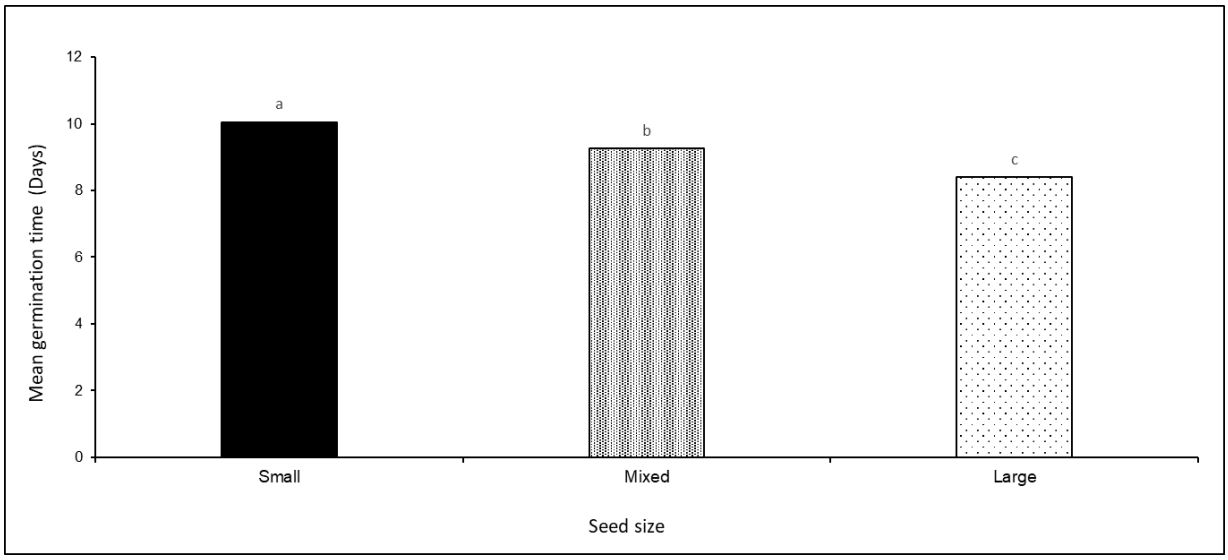

Figure 4.6. Mean germination time for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

B) Pre-treatment

The stratification (7.6 days) pre-treatment had the lowest MGT value and together with the hydrogen peroxide + kelp-p-max pre-treatment (8 days) was significantly ($p < 0.05$) lower than the control (10.1 days), hydro-prime (9.4 days), kelp-p-max (9.9 days), hydrogen peroxide (9.6 days), and hydrogen peroxide + hydro-prime (10 days) pre-treatments (Figure 4.7). Mean germination time (average time taken to complete germination) was thus quickest for the stratification and hydrogen peroxide + kelp-p-max pre-treatments, with the control and hydrogen peroxide + hydro-prime being the slowest.

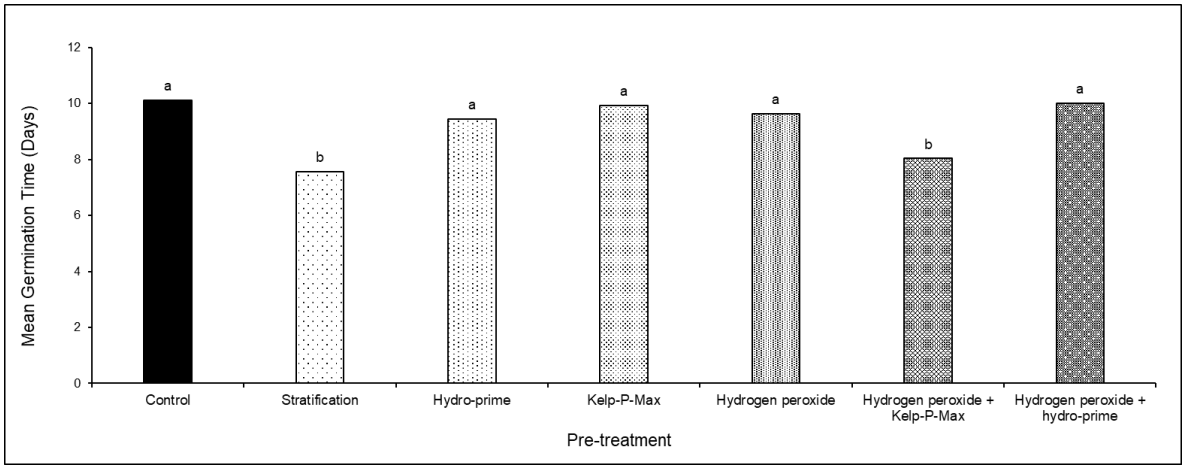

Figure 4.7. Mean germination time for pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.1.4 Germination Value (GV)

GV was significantly different for seed size ($p < 0.001$), while no significant differences for pre-treatment and their interaction were observed (Table 4.4).

Table 4.4. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on germination value (GV).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Cycle	1	50.30	50.30		
Cycle.Rep	2	125.74	62.87	2.72	
Seed size (A)	2	2065.79	1032.89	44.66	<0.001
Pre-treatment (B)	6	202.29	33.71	1.46	0.208
Seed size x Pre-treatment	12	214.21	17.85	0.77	0.676
Error (residual)	60	1387.71	23.13		
Total	83	4046.04			

A) Seed size

The Germination Value (GV), which is a combination of final germination and germination speed (Czabator, 1962), with higher values indicating higher and faster germination (with higher values being better). GV was significantly ($p < 0.05$) higher for large (21.7) seed than mixed seed (18.9) and small seed (10.1), with mixed seed being significantly ($p < 0.05$) higher than small seed (Figure 4.8). The GV for large seed was 11.6 and 2.8 higher than small and mixed seed, respectively. Germination value increased as seed size increased, with large seed demonstrating high and fast germination (Figure 4.8).

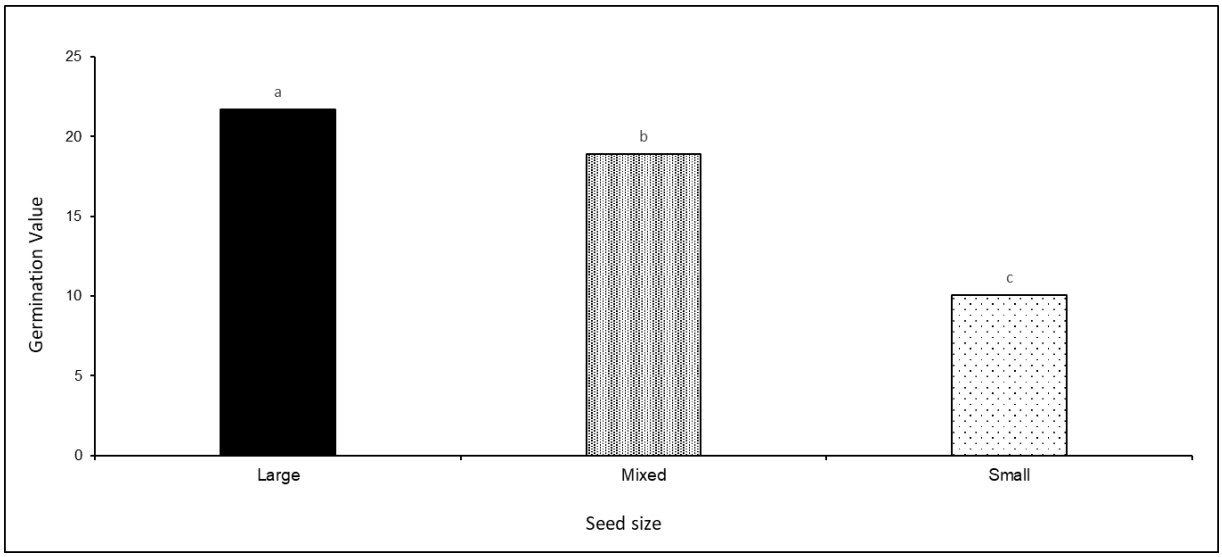

Figure 4.8. Germination value for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

4.1.5 Germination Rate Index (GRI)

GRI was significantly different for seed size ($p < 0.001$) and pre-treatment ($p < 0.001$), while no significant differences for their interaction were observed (Table 4.5).

Table 4.5. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on germination rate index (GRI).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Cycle	1	3.527	3.527	0.48	
Cycle.Rep	2	14.818	7.409	2.76	
Seed size (A)	2	230.352	115.176	42.93	<0.001
Pre-treatment (B)	6	70.607	11.768	4.39	<0.001
Seed size.Pre-treatment	12	18.314	1.526	0.57	0.858
Error (residual)	60	160.987	2.683		
Total	83	498.605			

A) Seed size

Germination rate index reflects the percentage of germination on each day of the germination period, with higher values indicating higher daily germination (with higher being better) (Kader, 2005). The germination rate index (GRI) for large (11.5 %) seed was significantly ($p < 0.05$) higher than for mixed and (10.5 %) small seed (7.6 %), with mixed seed being significantly ($p < 0.05$) higher than small seed (Figure 4.9). GRI for large seed was 3.9 and 1.0% more per day than small and mixed seed, respectively. GRI increased as seed size increased, indicating that the germination speed for large seed was significantly higher than for mixed and small seed (mixed seed being significantly higher than small seed).

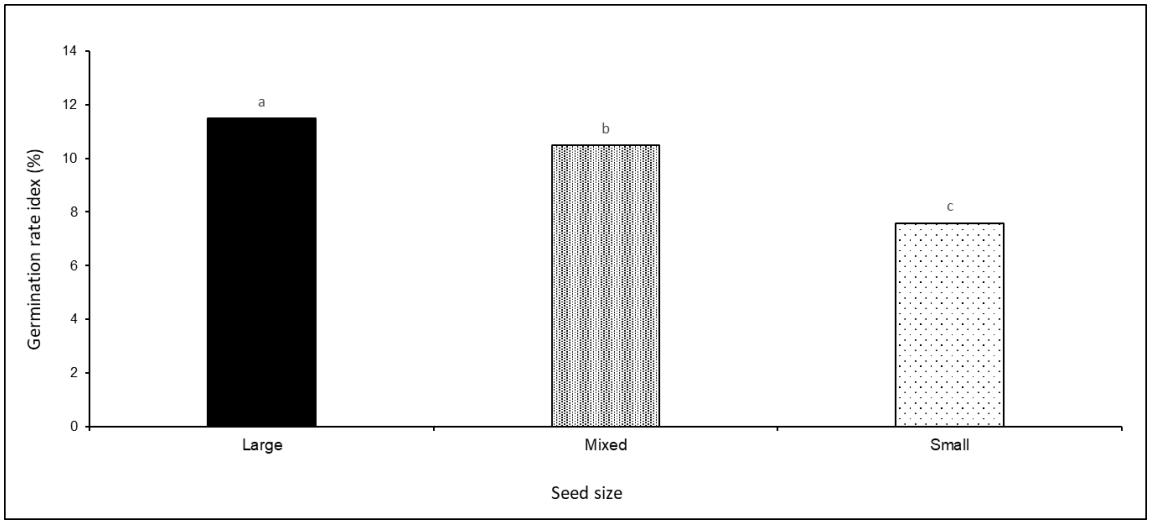

Figure 4.9. Germination rate index for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

B) Pre-treatment

GRI was significantly ($p < 0.05$) higher for the stratification (11.4 %) pre-treatment than for the hydrogen peroxide (9.8%), hydro-prime (9.5%), control (9.2%), hydrogen peroxide + hydro-prime (9.1%) and kelp-p-max (9.0 %) pre-treatments (Figure 4.10). This indicates that the daily rate at which germination took place was the highest for the stratification pre-treatment, with the kelp-p-max pre-treatment having the lowest daily germination. Stratification and hydrogen peroxide + kelp-p-max (11.0%) were the only pre-treatments with a significantly ($p < 0.05$) higher GRI than the control. This indicates that germination speed was highest for the stratification pre-treatment whilst it was the lowest for the kelp-p-max pre-treatment.

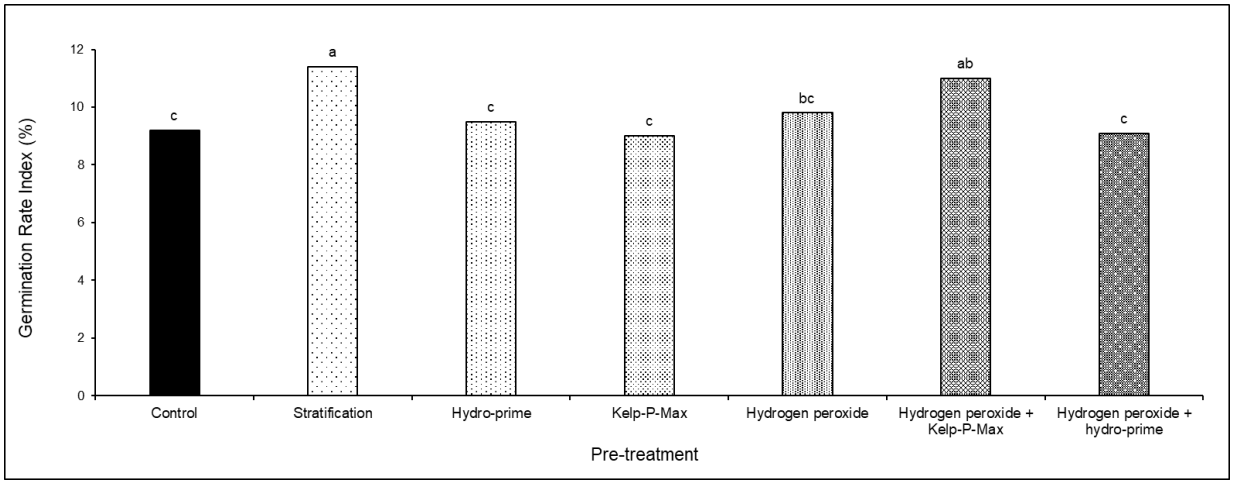

Figure 4.10. Germination rate index for pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.1.6 Germination Index (GI)

GI was significantly different for seed size ($p < 0.001$) and pre-treatment ($p = 0.037$), while no significant differences for their interaction were observed (Table 4.6).

Table 4.6. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on germination index (GI).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Cycle	1	184 523	184 523	1.6	
Cycle.Rep	2	230 321	115 160	3.5	
Seed size (A)	2	3 342 481	1 671 240	50.73	<0.001
Pre-treatment (B)	6	477 537	79 589	2.42	0.037
Seed size.Pre-treatment	12	320 990	26 749	0.81	0.637
Error (residual)	60	1 976 487	32 941		
Total	83	6 532 339			

A) Seed size

Higher GI value denotes a higher percentage and rate of germination (with higher values being better) (Kader, 2005). The Germination Index (GI) for large (1507.3) seed was significantly ($p < 0.05$) higher than for mixed (1403.0) and small seed (1041.8), with mixed seed being significantly ($p < 0.05$) higher than small seed (Figure 4.11). The GI values for large seed was 465.5 and 104.3 higher than for small and mixed, respectively. GI values increased as seed size increased (Figure 4.11). This indicates that germination speed was highest for large seed while it was lowest for small seed.

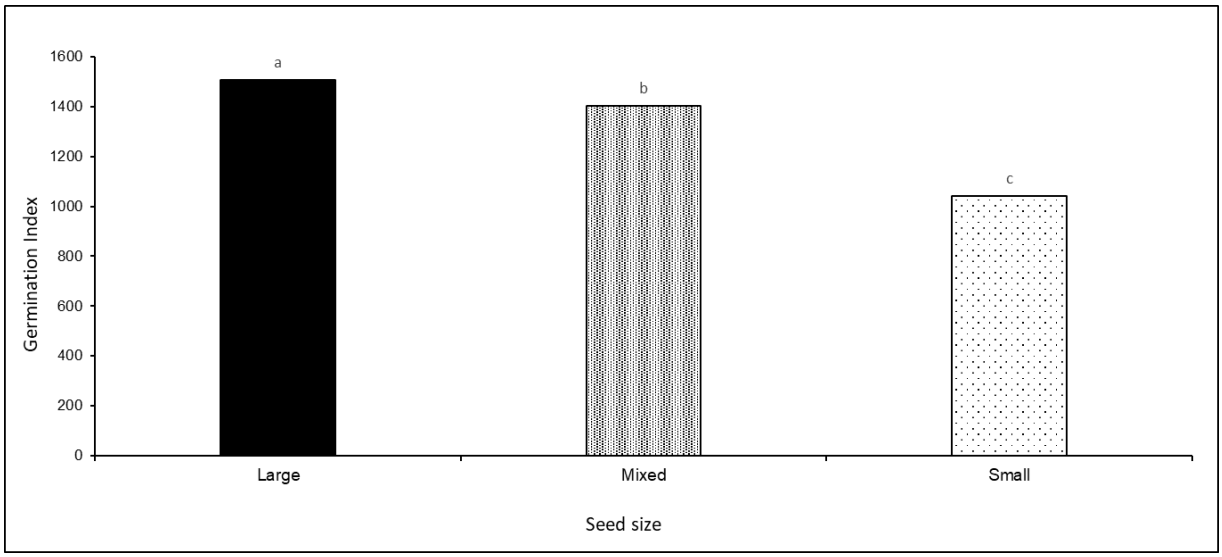

Figure 4.11. Germination index for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

B) Pre-treatment

The stratification (1436) pre-treatment had the highest GI value and the stratification together with the hydrogen peroxide + kelp-p-max pre-treatment (1409) had a significantly ($p < 0.05$) higher GI value than the control (1259), hydrogen peroxide + hydro-prime (1248) and kelp-p-max (1222) pre-treatments (Figure 4.12). This indicates that germination was high and quick for the stratification and hydrogen peroxide + kelp-p-max pre-treatments, while it was low and slow for the kelp-p-max, hydrogen peroxide + hydro-prime and the control pre-treatments.

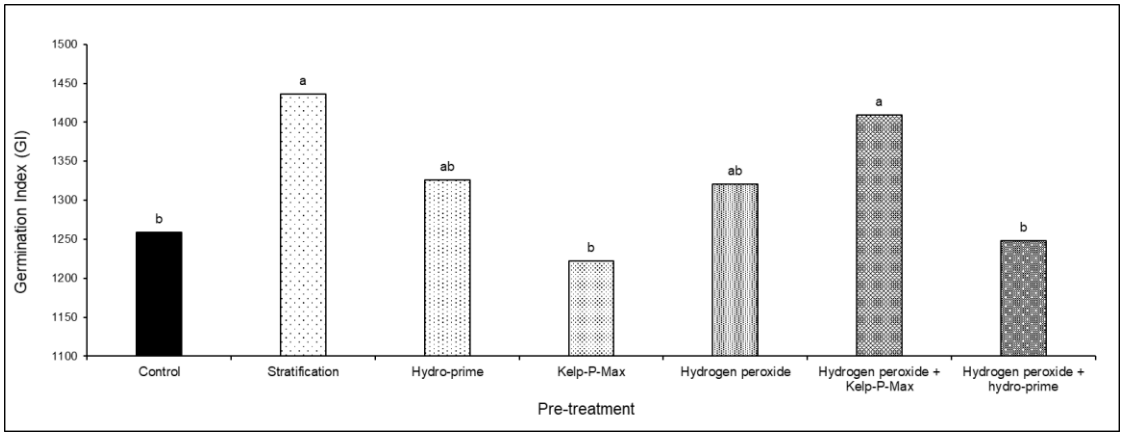

Figure 4.12. Germination index for different pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.1.7 Coefficient of Velocity of Germination (CVG)

CVG was significantly different for seed size ($p < 0.001$) and pre-treatment ($p < 0.001$), while no significant differences for their interaction were observed (Table 4.7).

Table 4.7. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on coefficient of velocity of germination (CVG).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Cycle	1	10.376	10.376	3.29	
Cycle.Rep	2	6.307	3.154	1.34	
Seed size (A)	2	53.733	26.867	11.42	<0.001
Pre-treatment (B)	6	135.846	22.641	9.62	<0.001
Seed size.Pre-treatment	12	6.468	0.539	0.23	0.996
Error (residual)	60	141.206	2.353		
Total	83	353.936			

A) Seed size

The CVG is an indication of the final germination and speed of germination (Kader, 2005). It increases when a high number of seeds germinate over a short period of time. The CVG places emphasis on the time required to reach final germination, with higher values indicating high germination over a short time (Kader, 2005). The coefficient of velocity of germination (CVG) was significantly ($p < 0.05$) higher for large seed (12.2) than for mixed (11.2) and small seed (10.2) (Figure 4.13). There was an increase in the CVG as seed size increased, indicating that germination was high and quick for large seed, while it was low and slow for small seed.

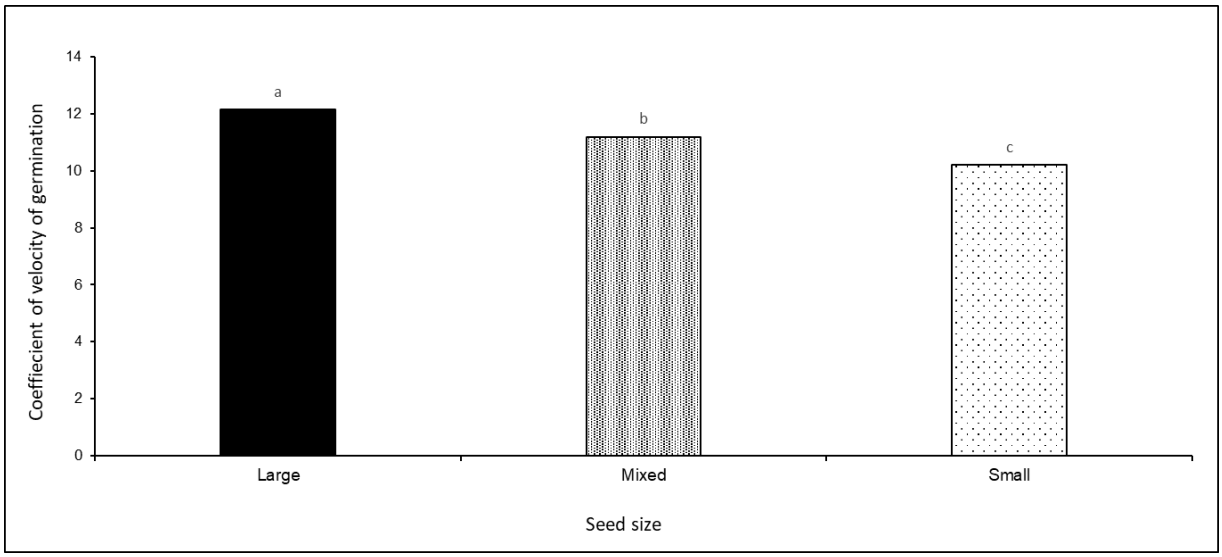

Figure 4.13. Coefficient of velocity of germination for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

B) Pre-treatment

The stratification (13.6) pre-treatment had the highest CVG value and the CVG value for stratification together with the hydrogen peroxide + kelp-p-max pre-treatment (12.69) was significantly ($p < 0.05$) higher than for the control (10.0), hydrogen peroxide + hydro-prime (10.2), kelp-p-max (10.5), hydrogen peroxide (10.6) and hydro-prime (10.7) pre-treatments (Figure 4.14). Although the stratification pre-treatment had a higher CVG value than the hydrogen peroxide + kelp-p-max pre-treatment, no significant ($p < 0.05$) differences were observed between them. This indicates that germination was high and more rapid for the stratification pre-treatment, while it was low and slow for the control (Figure 4.14).

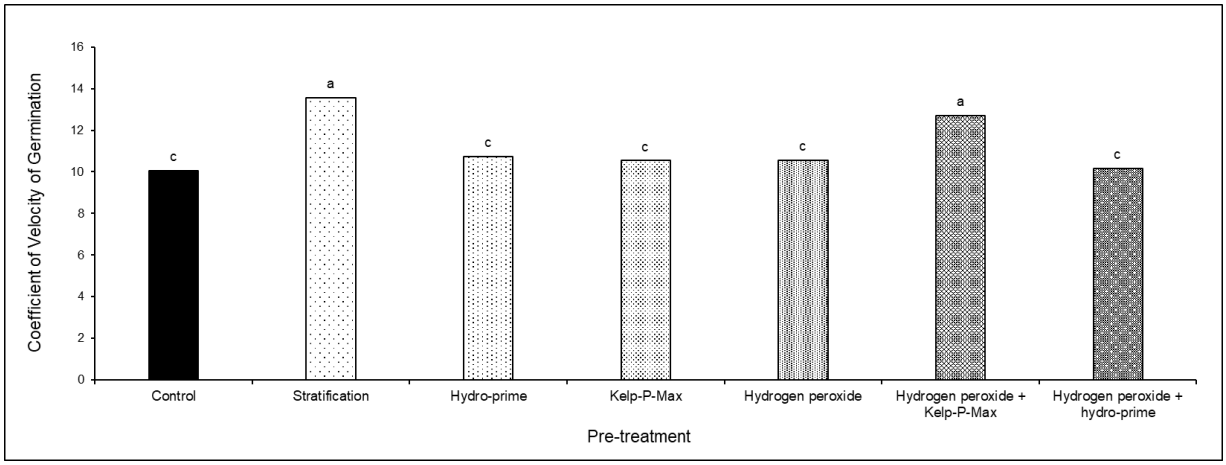

Figure 4.14. Coefficient of velocity of germination for pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.2 NURSERY TRIAL

4.2.1 Final germination

The interaction between seed size and pre-treatment demonstrated a significant ($p < 0.001$) impact on final germination, although seed size and pre-treatment (main effects) also showed significant differences ($p < 0.001$) at the end of the trial (42 DAS) (Table 4.8).

Table 4.8. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and its interaction on germination.

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	3	29.27	9.76	0.66	
Seed size (A)	2	2558.86	1279.43	86.50	<0.001
Pre-treatment (B)	6	2275.57	379.26	25.64	<0.001
Seed size.Pre-treatment	12	591.14	49.26	3.33	<0.001
Error (residual)	60	887.48	14.79		
Total	83	6342.32			

A) Interaction between seed size and pre-treatment

The interaction between hydrogen peroxide + kelp-p-max and the large seed had the highest germination (92.3%) in the trial (Figure 4.15), followed by the stratification_mix (91.0%) and stratification_large (88.0%) treatments (although there was no significant difference between the hydrogen peroxide + kelp-p-max_large, stratification_mix and stratification_large treatments). The control_large (84.3%) demonstrated significantly ($p < 0.05$) lower germination than the hydrogen peroxide + kelp-p-max_large and stratification_mix treatments

while it was significantly ($p < 0.05$) better than the hydrogen peroxide + hydro-prime mixed (78.8), hydro-prime_large (78.5%), hydrogen peroxide_mixed (76.3%), hydro-prime_mixed (76.0%), hydrogen peroxide + kelp-p-max_small (75.8%), control_mixed (75%), hydrogen peroxide_small (72.3%), hydrogen peroxide + hydro-prime_small (71.3%), kelp-p-max_small (70.8%) and hydro-prime_small (65.8%) and control_small (60.8%) treatments.

The hydrogen peroxide + kelp-p-max_large (92.3%), stratification_mix (91.0%), stratification_large (88.0%), hydrogen peroxide + kelp-p-max_mixed (86.5%), kelp-p-max_large (86.5%) and kelp-p-max_mix (85.8%) treatments were the only treatments where germination was higher than the acceptable 85% germination level (Barnett, 2002; Barnett & Varela, 2004; Barnett, 2008) for commercial nurseries. The control_small treatment was the worst performer in the trial but was not significantly different to the hydro-prime_small treatment. Germination for the interaction between the pre-treatments and large seed size class was generally higher than the interaction between the pre-treatments and mixed and small seed, except in the case where the stratification_large treatment was lower than the stratification_mixed treatment (although they were not significantly different to each other) (Figure 4.15). The stratification_small (84.8%) treatment had the highest germination for interaction between the pre-treatments and small seed size class and was slightly higher than the control_large (84.3%) with no significant difference between the two treatments. Interactions between pre-treatments and the small seed size had the lowest germination (except for the stratification_small and hydrogen peroxide + kelp-p-max_small treatments). Germination seemed to increase with an increase in seed size with the interaction between large seed and pre-treatment having higher values than mixed and small seed (except where the stratification_mixed treatment was higher than the stratification_large treatment) (Figure 4.15). The stratification_small treatment (84.8%) was the only treatment (where the pre-treatments interacted with the small seed size) that had germination above 80% (Figure 4.15). The hydrogen peroxide + kelp-p-max_large treatment had the highest germination where pre-treatments interacted with large seed and were 8% better than the control_large treatment. The stratification_mixed treatment had the highest germination where pre-treatments interacted with mixed seed and were 16% better than the control_mixed treatment. The stratification_small treatment had the highest germination when pre-treatments interacted with small seed and were 24% better than the control_small treatment. Although it is slightly below the accepted germination of 85%, the stratification_small demonstrated the highest improvement, (twice as high as the stratification_mixed and three time better than the hydrogen

peroxide + kelp-p-max_large), when pre-treatments interacted with seed size compared to their controls within the same seed size. It was only for the interaction between the large seed and pre-treatments that the control_large treatment did not have the lowest germination (the hydrogen peroxide_large, hydrogen peroxide + hydro-prime_large had lower germination while the hydro-prime_large treatment was significantly lower); while the control_mixed had the lowest germination when the mixed seed interacted with the pre-treatments and the control_small treatment had the lowest when small seed interacted with the pre-treatments.

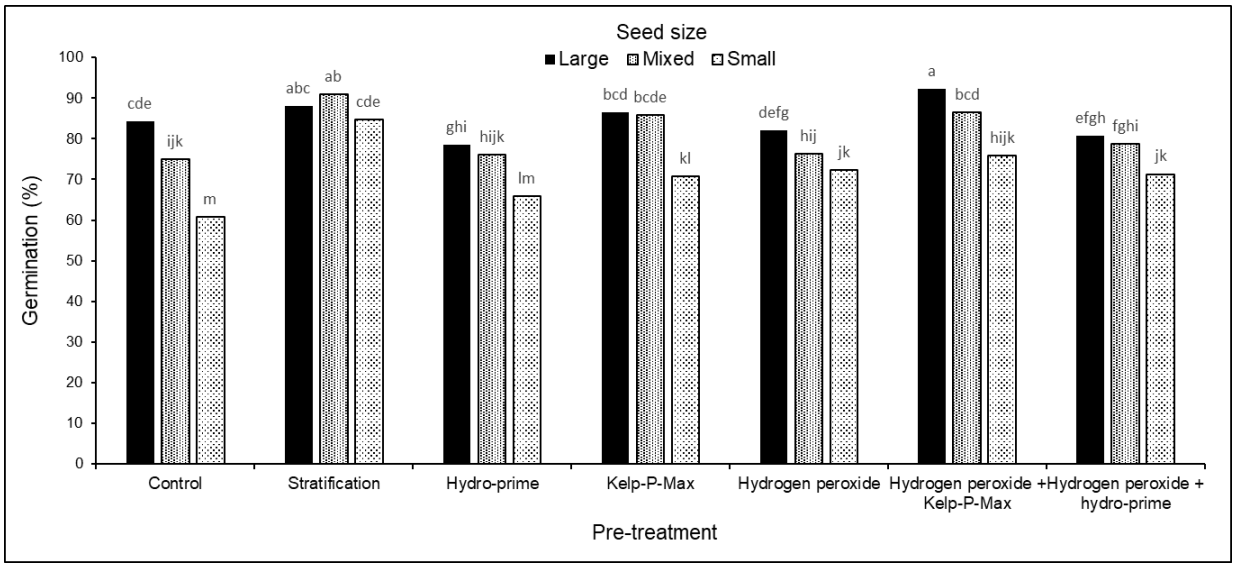

Figure 4.15. Interaction between seed size and pre-treatment. Different letters indicate a significant difference at $p < 0.05$.

4.2.2 Time to 50% germination (T_{50})

T_{50} was significantly different for seed size ($p < 0.001$) and pre-treatment ($p < 0.001$), with no significant differences for their interactions observed (Table 4.9).

Table 4.9. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on T_{50}.

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	3	9.188	3.063	2.56	
Seed size (A)	2	421.005	210.503	176.28	<0.001
Pre-treatment (B)	6	911.358	151.893	127.20	<0.001
Seed size.Pre-treatment	12	14.133	1.178	0.99	0.472
Error (residual)	60	71.649	1.194		
Total	83	1427.333			

A) Seed size

T_{50} is the time taken to reach 50% germination (Kader, 2005), with lower values indicating shorter times and higher values longer times to reach 50% germination (lower values/shorter times being better). T_{50} was significantly ($p < 0.05$) lower for large seed (15.4 days) than both mixed (16.8 days) and small (20.7 days) seed, with mixed seed being significantly ($p < 0.05$) lower than small seed (Figure 4.16). Large seed reached T_{50}, in 5.3 and 1.4 days quicker than small and mixed seed, respectively. This indicates that large seed had the highest germination speed while small seed was the slowest.

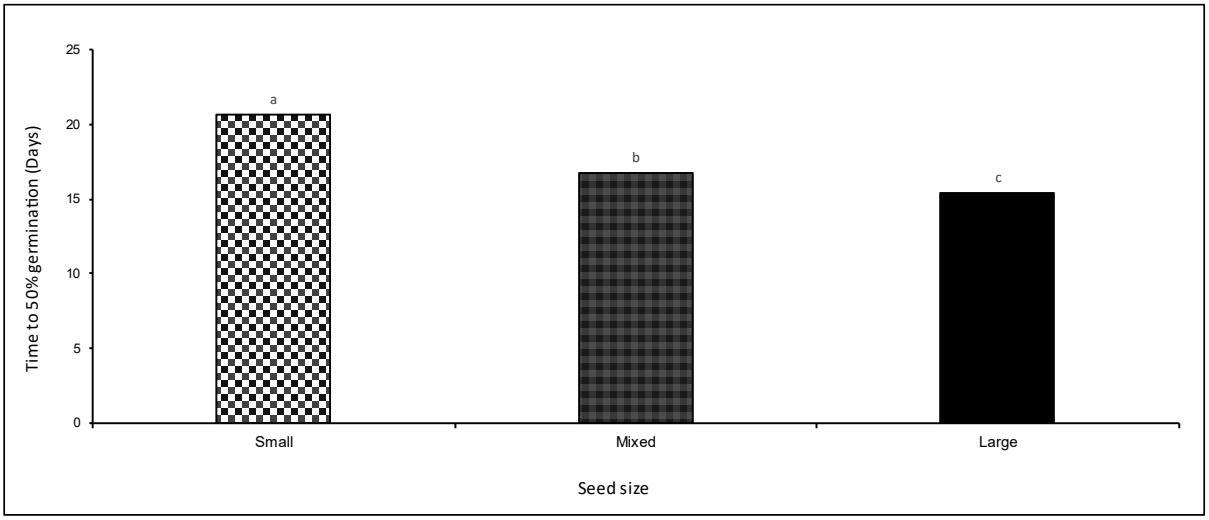

Figure 4.16. T_{50} for small, mixed and large seed. Different letters indicate a significant difference at $p < 0.05$.

B) Pre-treatment

The T_{50} for the stratification pre-treatment was significantly ($p < 0.05$) lower (11.9 days) than for all the other pre-treatments (Figure 4.17). This indicates that the stratification pre-treatment

reached 50% germination the quickest, with the hydro-prime pre-treatment (21.3 days) being the slowest. T_{50} values for the stratification, hydrogen peroxide + kelp-p-max (15.2 days), kelp-p-max (15 days), hydrogen peroxide + hydro-prime (19 days) and hydrogen peroxide (20 days) pre-treatments were all significantly ($p < 0.05$) lower than the control (20.8 days). The control displayed similar (not significantly different from each other) values to hydro-prime pre-treatment (21.3 days).

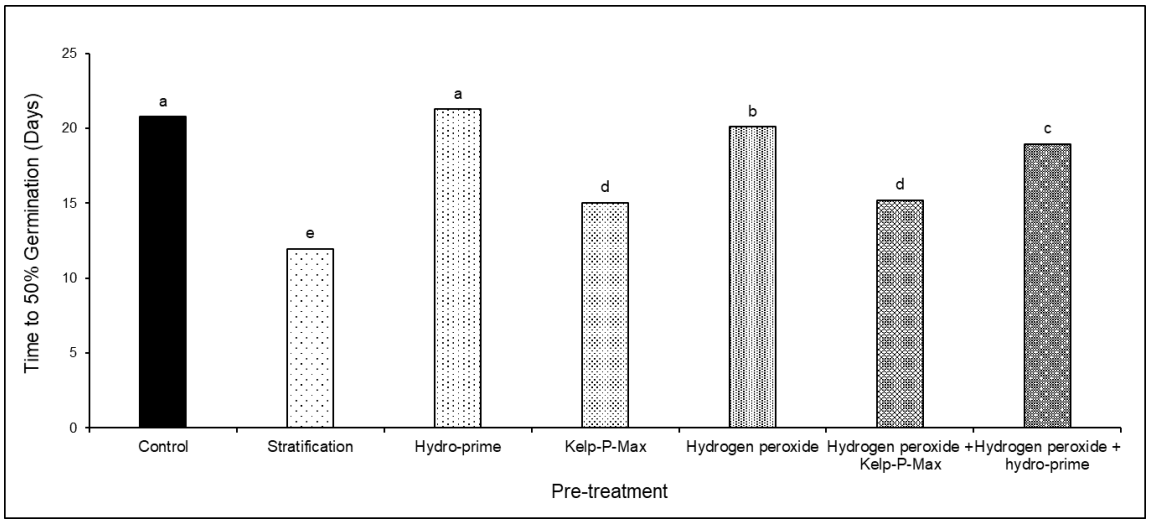

Figure 4.17. T_{50} for the different seed pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.2.3 Mean Germination Time (MGT)

MGT was significantly different for seed size ($p < 0.001$) and pre-treatment ($p < 0.001$), while no significant differences for their interaction were observed (Table 4.10).

Table 4.10. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on mean germination time (MGT).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	3	7.319	2.440	2.35	
Seed size (A)	2	368.898	184.449	177.78	<0.001
Pre-treatment (B)	6	711.693	118.616	114.32	<0.001
Seed size.Pre-treatment	12	10.842	0.903	0.87	0.580
Error (residual)	60	62.252	1.038		
Total	83	1161.004			

A) Seed size

Mean germination time is the average time taken to complete germination (Kader, 2005), with lower values indicating shorter average times and higher values indicating longer average times (with lower values/shorter times being better). The MGT was significantly ($p < 0.05$) lower for large seed (19.0 days) than for both mixed (20.4 days) and small (24 days) seed, with mixed seed being significantly ($p < 0.05$) lower than small seed (Figure 4.18). MGT for large seed was 5 and 1.4 days quicker than for small and mixed seed, respectively. This is an indication that the mean germination time (average time taken to complete germination) was the quickest for large seed, with small seed being the slowest.

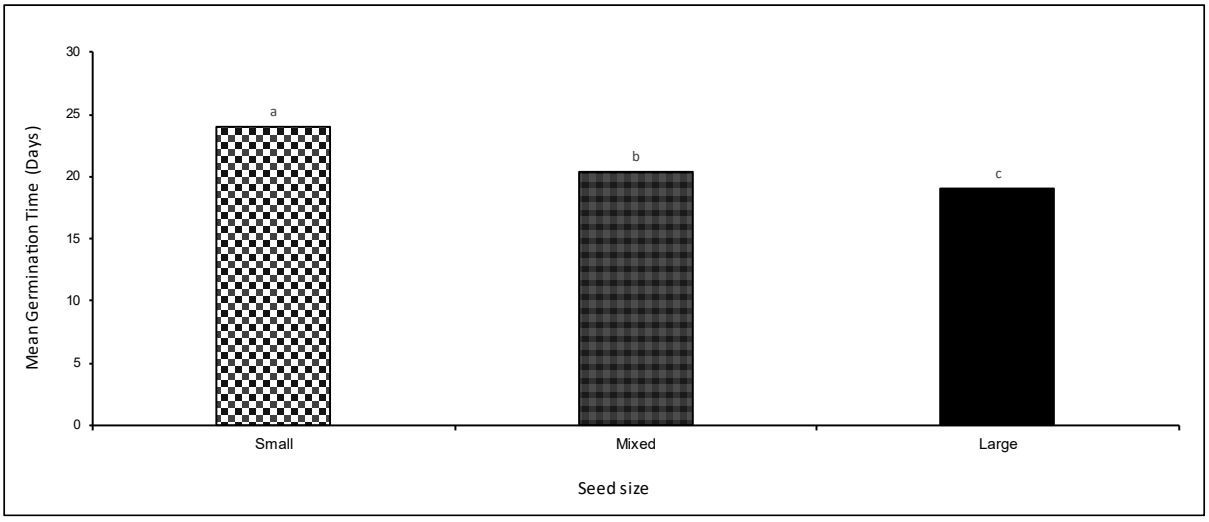

Figure 4.18. MGT for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

B) Pre-treatment

MGT for the stratification pre-treatment was significantly ($p < 0.05$) lower (15.7 days) than all the other pre-treatments (Figure 4.19), which indicates that mean germination time (time taken to complete germination) was the quickest for the stratification pre-treatment, with the hydro-prime treatment being the slowest (24.4 days). MGT for the stratification, hydrogen peroxide + kelp-p-max (19.3 days), kelp-p-max (19.3 days) and hydrogen peroxide + hydro-prime (22.2 days) pre-treatments were all significantly lower than the control (23.7 days). The control

displayed similar (not significantly different from each other) values to hydro-prime (24.4 days) pre-treatment.

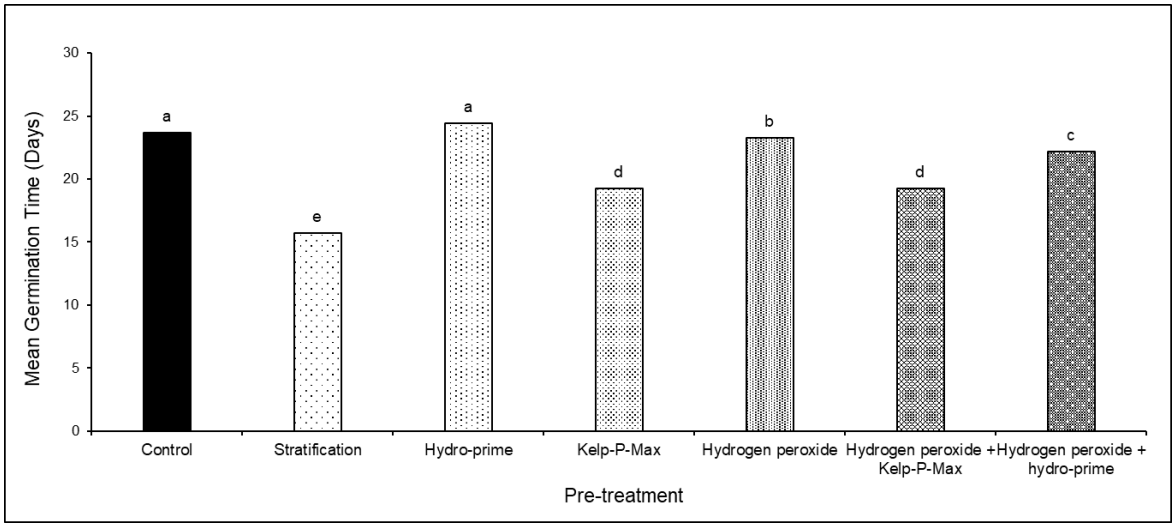

Figure 4.19. MGT for the different seed pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.2.4 Germination rate index (GRI)

GRI was significantly different for seed size ($p < 0.001$) and pre-treatment ($p < 0.001$), while no significant differences for their interaction were observed (Table 4.11).

Table 4.11. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on germination rate index (GRI).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	3	0.9576	0.3192	1.54	
Seed size (A)	2	52.6426	26.3213	127.31	<0.001
Pre-treatment (B)	6	94.4211	15.7368	76.12	<0.001
Seed size.Pre-treatment	12	2.8788	0.2399	1.16	0.332
Error (residual)	60	12.4050	0.2067		
Total	83	163.3051			

A) Seed size

Germination rate index reflects the percentage of germination on each day of the germination period, with higher values indicating higher daily germination (with higher being better) (Kader, 2005). The GRI was significantly ($p < 0.05$) higher for large seed (5.4%) than for both mixed (4.9%) and small (3.6%) seed, with mixed seed being significantly ($p < 0.05$) higher

than small seed (Figure 4.20). The GRI for large seed was 1.8 and 0.5 % more per day than for small and mixed seed, respectively. GRI increased as seed size increased (Figure 4.20). This indicates that germination speed was highest for large seed and lowest for small seed.

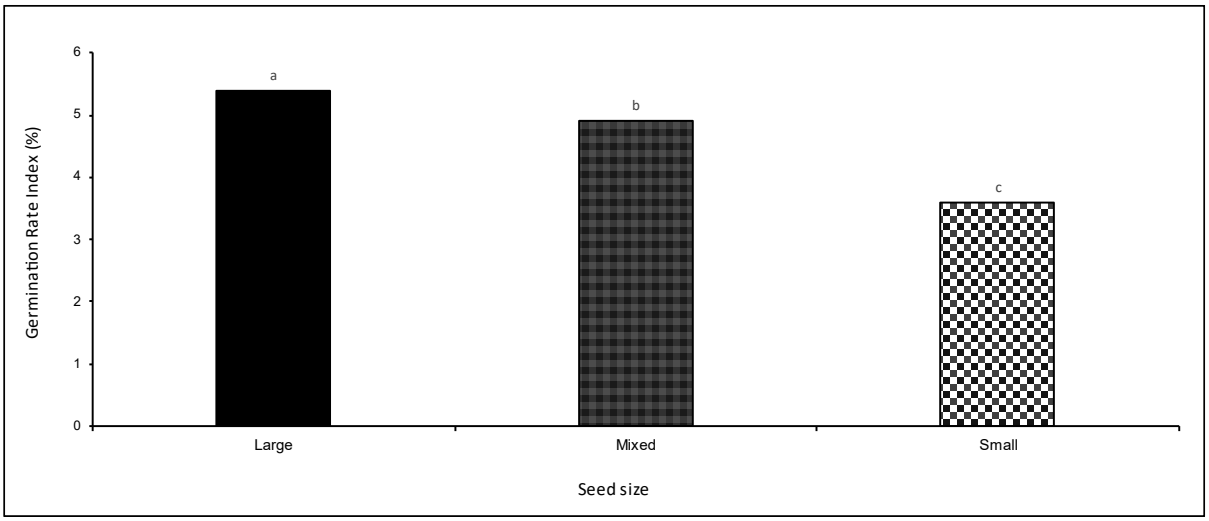

Figure 4.20. GRI for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

B) Pre-treatment

GRI for the stratification pre-treatment was significantly ($p < 0.05$) higher (6.6%) than for all the other pre-treatments (Figure 4.21), which indicates that the GRI was quickest for the stratification pre-treatment, with the hydro-prime treatment being the slowest (3.5%). GRI for the stratification, hydrogen peroxide + kelp-p-max (5.4%), kelp-p-max (5.2%) and hydrogen peroxide + hydro-prime (4.1%) pre-treatments were all significantly higher than for the control (3.6%). The control displayed similar values to hydro-prime and hydrogen peroxide (3.9%) pre-treatments (not significantly different from each other).

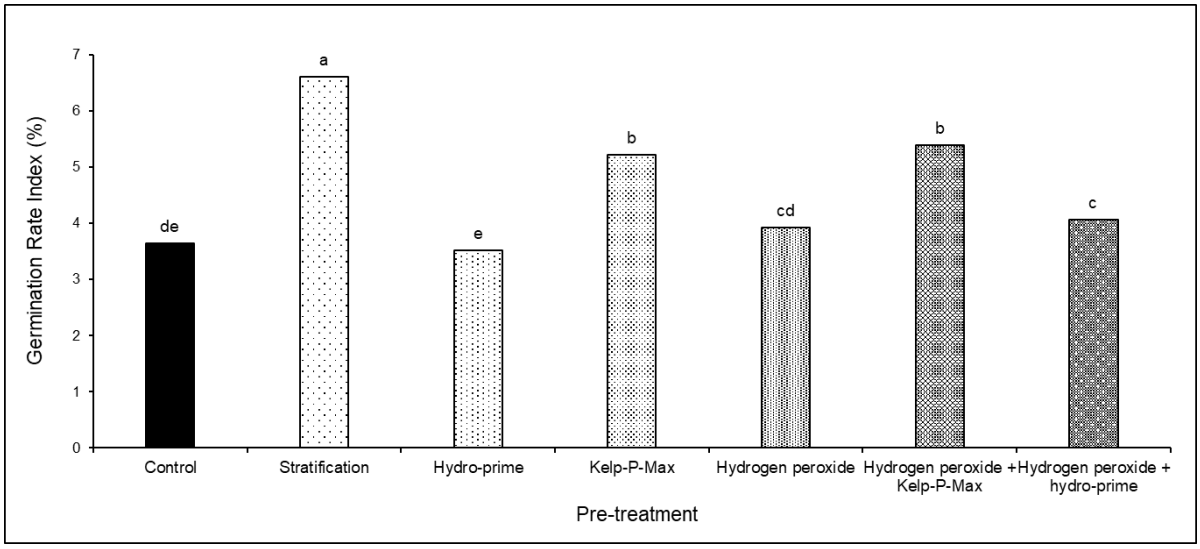

Figure 4.21. GRI for the different seed pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.2.5 Germination value (GV)

GV was significantly different for the interaction between seed size and pre-treatment ($p < 0.001$), while the main effects (seed size and pre-treatment) also demonstrated significant differences ($p < 0.001$) (Table 4.12).

Table 4.12. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on germination value (GV).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	3	1.7675	0.5892	1.02	
Seed size (A)	2	141.7037	70.8519	122.79	<0.001
Pre-treatment (B)	6	262.0166	43.6694	75.68	<0.001
Seed size.Pre-treatment	12	22.8948	1.9079	3.31	0.001
Error (residual)	60	34.6221	0.5770		
Total	83	463.0047			

A) Interaction between seed size and pre-treatment

Germination value is a combination of germination (%) and germination speed with higher values indicating higher seed quality. The interaction between the stratification pre-treatment and large seed (stratification_large) had the largest value (11.3) in the trial (indicating that germination was high and quick), with the control_small having the lowest value (2.6). Germination values for the stratification_large, stratification_mixed (10.4), hydrogen peroxide + kelp-p-max_large (8.4) and kelp-p-max_large (8.0) interaction treatments were all

significantly ($p < 0.05$) higher than the control_large (5.9) (Figure 4.22). The hydrogen peroxide + kelp-p-max_mix (6.9), kelp-p-max_mixed (6.8), stratification_small (6.6), hydrogen peroxide + hydro-prime_large (5.7), hydrogen peroxide_large (5.4), hydro-prime_large (5.0) and hydrogen peroxide + hydro-prime_mixed (4.90) were not significantly ($p < 0.05$) different from the control_large interaction treatment, while all other interaction treatments were significantly ($p < 0.05$) lower. Germination values for the interactions between pre-treatment and large seed were generally higher compared to the interactions between pre-treatment with mixed and small seed (with interactions between pre-treatment and mixed seed higher than interactions between pre-treatment and small seed) (Figure 4.22).

Interactions between pre-treatments and the small seed size class (except the stratification_small and hydrogen peroxide + kelp-p-max_small treatments) had the lowest GVs. This includes the kelp-p-max_small (3.8), hydrogen peroxide_small (3.7), hydrogen peroxide + hydro-prime_small (3.8) and hydro-prime_small (2.8) treatments. No significant ($p < 0.05$) differences existed between the kelp-p-max_small, hydrogen peroxide_small and hydrogen peroxide + hydro-prime_small interaction treatments but all of them were significantly ($p < 0.05$) different from the control_small interaction treatment. This indicates that germination for these interaction treatments (kelp-p-max_small, hydrogen peroxide_small, hydrogen peroxide + hydro-prime_small and hydro-prime_small) was low and slow with the control_small treatment being the lowest and slowest.

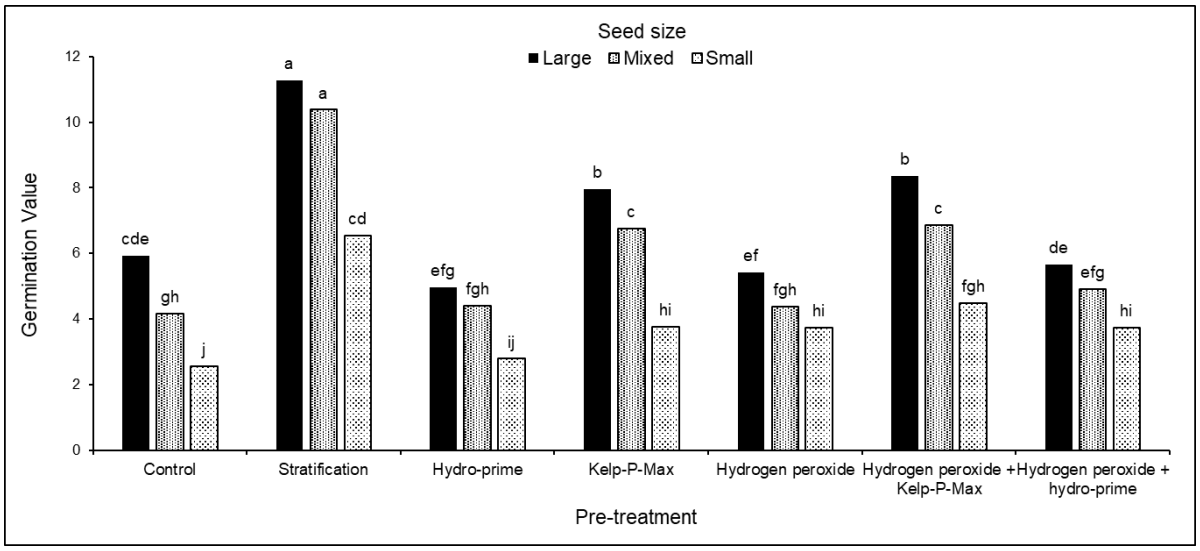

Figure 4.22. Interactions between seed size and pre-treatments for germination value. Different letters indicate a significant difference at $p < 0.05$.

Germination Values (GVs) for the interaction between the pre-treatments and large seed size class was higher than the interaction between the pre-treatments and small seed (Figure 4.22). Due to the nature of the composition of the mixed seed (not a 50% large and 50% small make-up, but 88% large and 12% small make-up), its germination values were closer to the values for the interactions between the pre-treatment and large seed (Figure 4.22), except for the hydrogen peroxide_mixed interaction treatment (which was closer to the hydrogen peroxide_small interaction treatment). The stratification_small interaction treatment was the only interaction treatment involving small seed that was higher than the control_large treatment, although there was no significant difference between the two. Germination values were lowest where pre-treatments interacted with the control within mixed and small seed sizes (control_mixed were the lowest when pre-treatment interacted with mixed seed, while the same was true for the control_small when pre-treatment interacted with small seed).

4.2.6 Germination index (GI)

GI was significantly different for the interaction between seed size and pre-treatment (p = 0.037), while the main effects (seed size and pre-treatment) also demonstrated significant differences ($p < 0.001$) (Table 4.13).

Table 4.13. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on germination index (GI).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	3	111137.	37046.	1.59	
Seed size (A)	2	8138312.	4069156.	174.27	<0.001
Pre-treatment (B)	6	119965203.	1994200.	85.40	<0.001
Seed size.Pre-treatment	12	567387.	47282.	2.02	0.037
Error (residual)	60	1401003.	23350		
Total	83	22183042.			

B) Interaction between seed size and pre-treatment

Germination Index (GI) is an indication of germination (%) and germination speed; higher values indicate higher seed quality, while lower GI values indicate lower seed quality. The interaction between the stratification pre-treatment and large seed (stratification_large) had the largest GI value (3106) (indicating that germination was high and quick), with the

control_small (1353) having the lowest value (indicating that germination was low and slow) (Figure 4.23).

Germination Index (GI) for the stratification_large (3106), stratification_mixed (3098), hydrogen peroxide + kelp-p-max_large (2914), hydrogen peroxide + kelp-p-max_mixed (2641), kelp-p-max_large (2762), kelp-p-max_mixed (2614) interaction treatments were all significantly ($p < 0.05$) higher than for the control_large (2387) (Figure 4.23). This indicates that germination for these interaction treatments (stratification_large, stratification_mixed, hydrogen peroxide + kelp-p-max_large, hydrogen peroxide + kelp-p-max_mixed, kelp-p-max_large, kelp-p-max_mixed) was significantly ($p < 0.05$) higher and quicker than the control_large treatment. The GIs for stratification_small (2580), hydrogen peroxide_large (2254), hydrogen peroxide + hydro-prime_large (2310), hydrogen peroxide + hydro-prime_mixed (2175) were not significantly ($p < 0.05$) different from the control_large interaction treatment, while the values for all other interaction treatments were significantly ($p < 0.05$) lower. Germination index values for the interactions between pre-treatment and large seed were higher compared to the interactions between pre-treatment with mixed and small seed (with values for interactions between pre-treatment and mixed seed higher than for interactions between pre-treatment and small seed) (Figure 4.23).

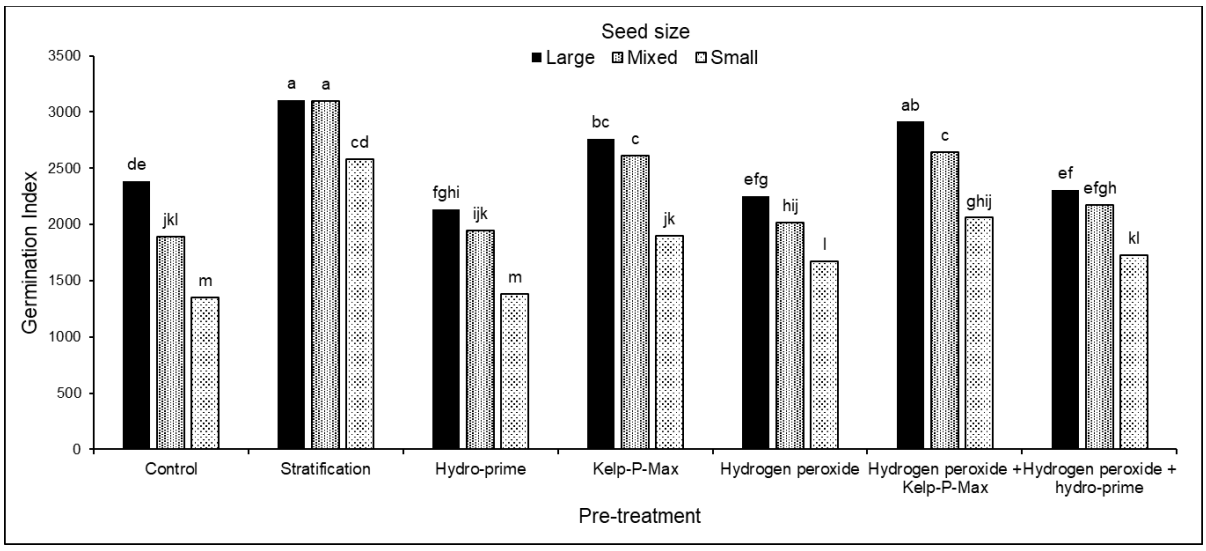

Figure 4.23. Interactions between seed size and pre-treatment for germination index. Different letters indicate a significant difference at $p < 0.05$.

4.2.7 Coefficient of Velocity of Germination (CVG)

CVG was significantly different for the interaction between seed size and pre-treatment (p = 0.008), while the main effects (seed size and pre-treatment) also demonstrated significant differences ($p < 0.001$) (Table 4.14).

Table 4.14. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on coefficient of velocity of germination (CVG).

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	3	0.42744	0.14248	2.17	
Seed size (A)	2	19.82253	9.91127	151.26	<0.001
Pre-treatment (B)	6	49.567784	8.26131	126.08	<0.001
Seed size.Pre-treatment	12	2.04186	0.17015	2.60	0.008
Error (residual)	60	3.93144	0.06552		
Total	83	75.79111			

A) Interaction between seed size and pre-treatment

Coefficient of Velocity of Germination Index (CVG) refers to the time required to reach final germination, with higher values implying higher seed quality. The interaction between the stratification pre-treatment and large seed (stratification_large) had the largest CVG value (7.3) (indicating that this treatment reached final germination first amongst all the treatments), with the hydro-prime_small (3.6) having the lowest value (indicating that this treatment reached final germination last). Coefficient of velocity of germination index for the stratification_large (7.3), stratification_mixed (6.7), stratification_small (5.4), kelp-p-max_large (5.9), kelp-p-max_mixed (5.4), hydrogen peroxide + kelp-p-max_large (5.7) and hydrogen peroxide + kelp-p-max_mixed (5.4), interaction treatments were all significantly ($p < 0.05$) higher than for the control_large (4.9) (Figure 4.24). This indicates that final germination for these interaction treatments (stratification_large, stratification_mixed, stratification_small, kelp-p-max_large, kelp-p-max_mixed, hydrogen peroxide + kelp-p-max_large and hydrogen peroxide + kelp-p-max_mixed) was significantly ($p < 0.05$) shorter than for the control_large treatment.

The hydro-prime_large (4.6), kelp-p-max_small (4.5), hydrogen peroxide_large (4.7), hydrogen peroxide + kelp-p-max_small (4.6), hydrogen peroxide + hydro-prime_large (4.9) and hydrogen peroxide + hydro-prime_mixed (4.7) interaction treatments were not significantly ($p < 0.05$) different from the control_large interaction treatment (indicating that

final germination was reached at similar times), while all other interaction treatments were significantly ($p < 0.05$) lower (indicating that final germination for these treatments took significantly ($p < 0.05$) longer compared to the control_large treatment). The CVG values for the interactions between pre-treatment and large seed were higher than for the interactions between pre-treatment with mixed and small seed (with interactions between pre-treatment and values for mixed seed higher than interactions between pre-treatment and small seed) (Figure 4.24).

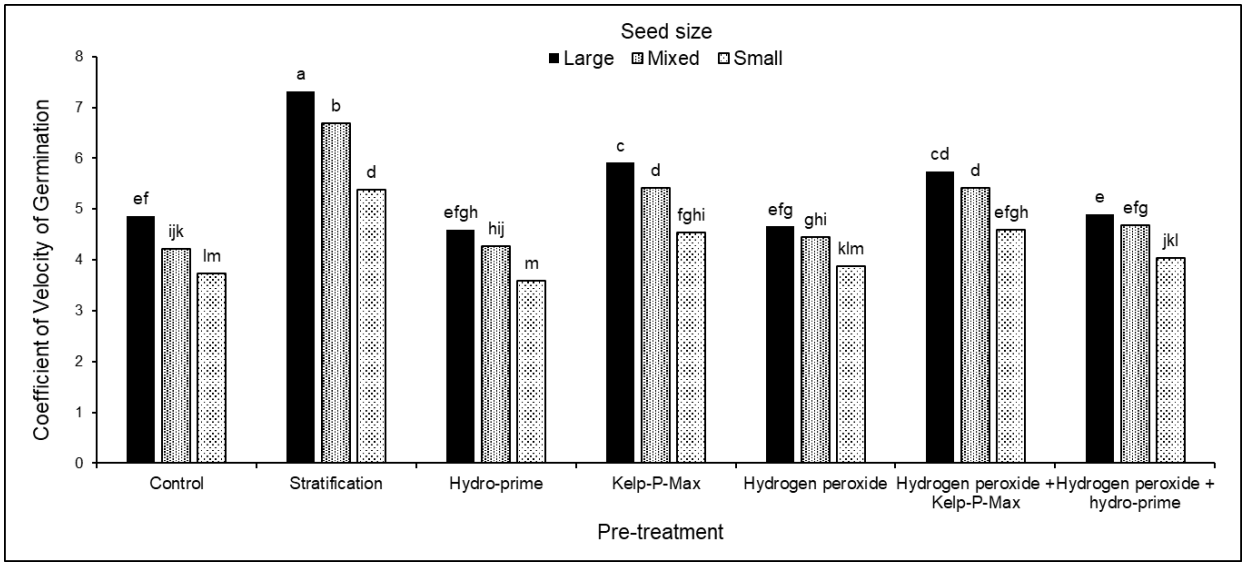

Figure 4.24. Interactions between seed size and pre-treatment for the CVG parameter. Different letters indicate a significant difference at $p < 0.05$.

4.3 SEEDLING GROWTH

4.3.1 Seedling height

A) Seed size

Seedlings from large seed (33.2 cm) were significantly ($p < 0.05$) taller than seedlings from mixed (28.1 cm) and small (22.4 cm) seed (Figure 4.25), indicating that seedling height increased as seed size increased. Variation was highest for seedlings from large seed and decreased as seed size decreased, with seedlings from large, mixed, and small seed having a range of 20.1 cm, 17 cm, and 9 cm, respectively.

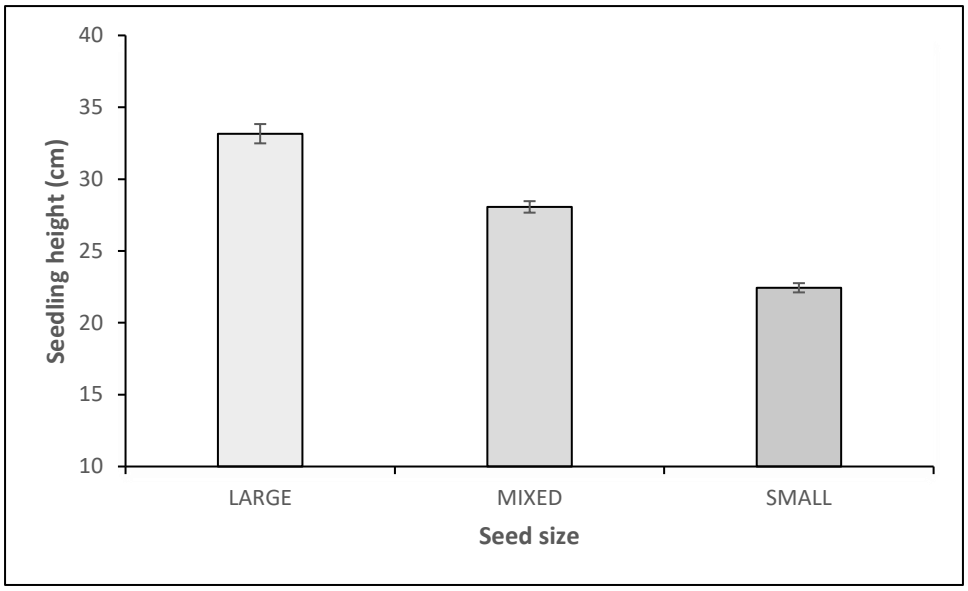

Figure 4.25. Seedling height for seedlings from small, mixed, and large seed. Error bars represent confidence interval (CI).

A) Pre-treatment

Seed from the stratification pre-treatment produced the tallest seedlings (29.3 cm), while seeds from the hydrogen peroxide + hydro-prime pre-treatment produced the shortest (26.1 cm) (Figure 4.26). Seedlings from the stratification and the hydrogen peroxide + kelp-p-max (28.7 cm) pre-treatments were significantly ($p < 0.05$) taller than seedlings from the hydrogen peroxide + hydro-prime pre-treatment, while none of the seedlings from the pre-treatments was significantly ($p < 0.05$) taller than the control (27.3 cm). Seedlings from the control were only taller than seedlings from the hydrogen peroxide + hydro-prime pre-treatment, while they were shorter than all the other pre-treatments (Figure 4.28). Variation in seedling height was highest for seedlings from the hydrogen peroxide (range = 26 cm), stratification (range = 22 cm) and hydrogen peroxide + kelp-p-max (range = 20.5 cm) pre-treatments, while it was lowest for seedlings from the kelp-p-max (range = 17 cm) and hydrogen peroxide + hydro-prime (14.7 cm) pre-treatments.

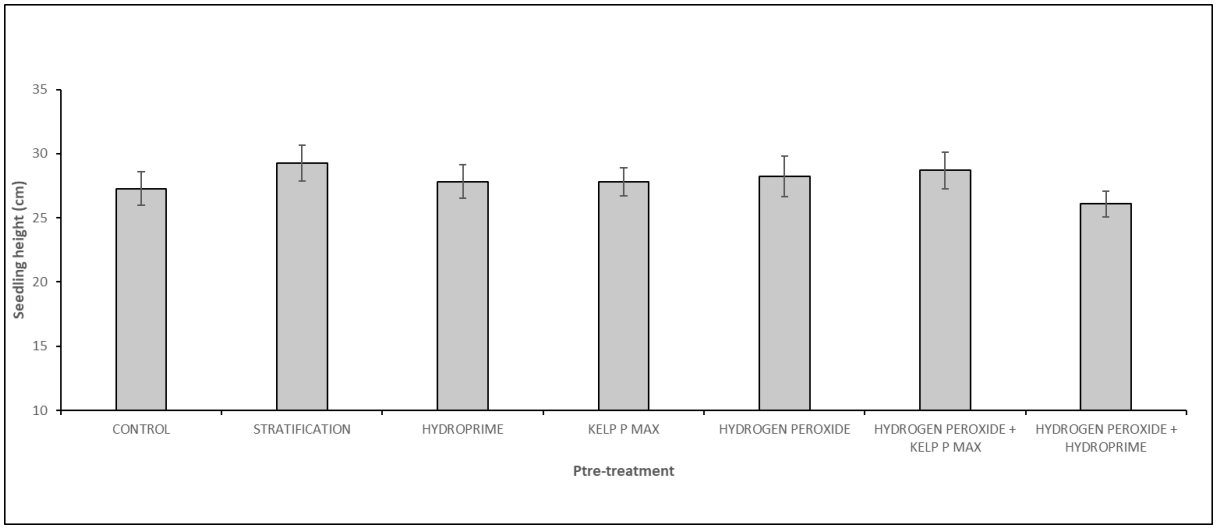

Figure 4.26. Seedling height for seedlings from different seed pre-treatments. Error bars represent CI.

4.3.2 Root collar diameter (RCD)

A) Seed size

RCD for seedlings from large (3.86 mm) and mixed (3.89 mm) seed was significantly ($p < 0.05$) bigger than that of seedlings from small seed (3.4 mm) (Figure 4.26). This demonstrates that RCD increased as seed size increased. Variation in RCD was highest for seedlings from large seed and was lowest for seedlings from small seed.

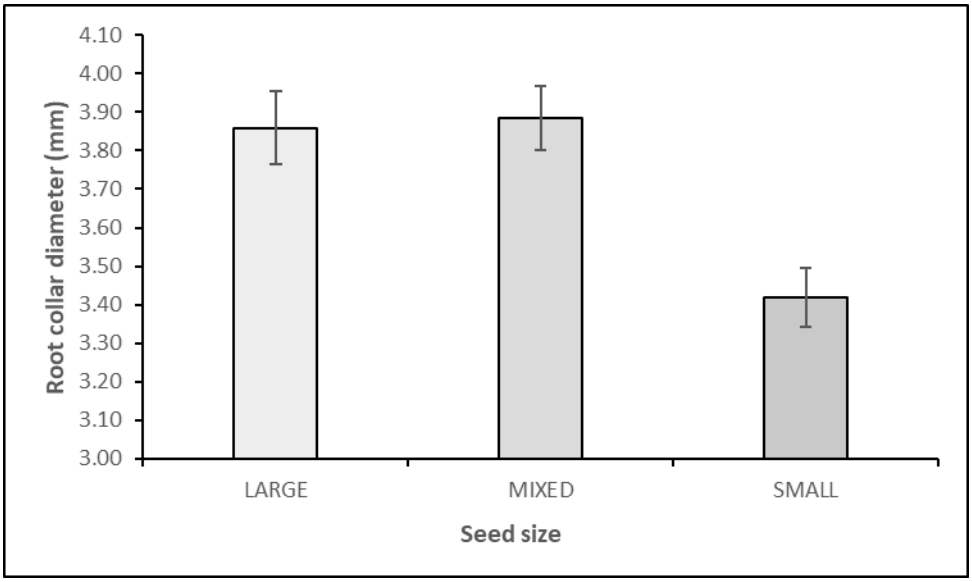

Figure 4.26: Root collar diameter for seedlings from small, mixed, and large seed. Error bars represent CI.

B) Pre-treatment

Seeds from the stratification pre-treatment produced seedlings with the biggest root collar diameters (3.92 mm) followed by the control (3.90 mm), while seedlings from the hydrogen peroxide + hydro-prime (3.54 mm) produced the smallest RCDs (Figure 4.27). RCDs for seedlings from the stratification pre-treatment and the control were significantly ($p < 0.05$) bigger than the RCDs for seedlings from the hydrogen peroxide + hydro-prime (3.54 mm), hydrogen peroxide + kelp-p-max (3.58 mm) and hydro-prime (3.61 mm), while none of the pre-treatments were significantly bigger than the control.

Variation in root collar diameter was highest for seedlings from the hydrogen peroxide + kelp-p-max (range = 3.7 mm), while it was lowest for the hydrogen peroxide (range = 2.1 mm) pre-treatment and the control (1.97 mm) (Figure 4.27).

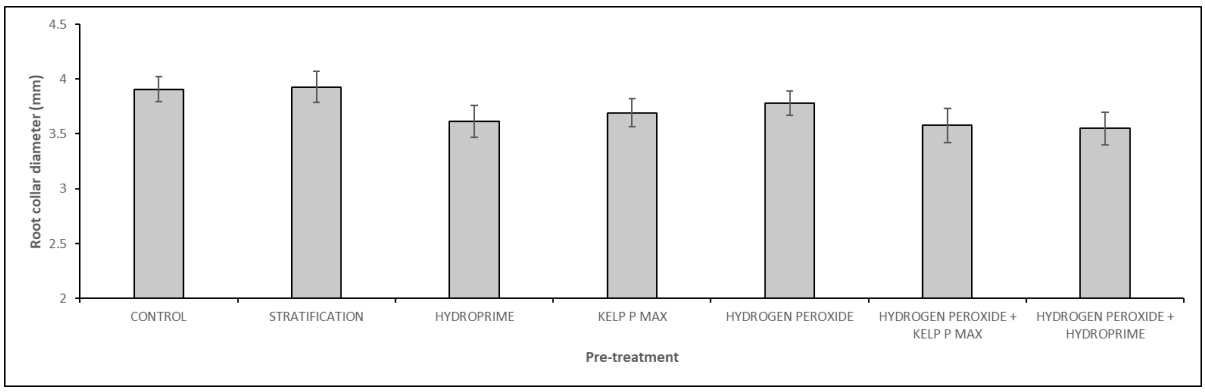

Figure 4.27. Root collar diameter for seedlings from different seed pre-treatments. Error bars represent CI.

4.3.3 Sturdiness Ratio

A) Seed size

Sturdiness ratio for large seed (8.7) was significantly ($p < 0.05$) higher than mixed (7.3) and small seed (6.7), with mixed seed significantly ($p < 0.05$) higher than small seed (Figure 4.28), demonstrating that sturdiness ratio increased as seed size increased (Figure 4.28). Variation in sturdiness ratio was highest for seedlings from large seed and was lowest for seedlings from mixed seed, with sturdiness ratios from large, mixed, and small seed having ranges of 7.6, 5.6 and 6.8, respectively.

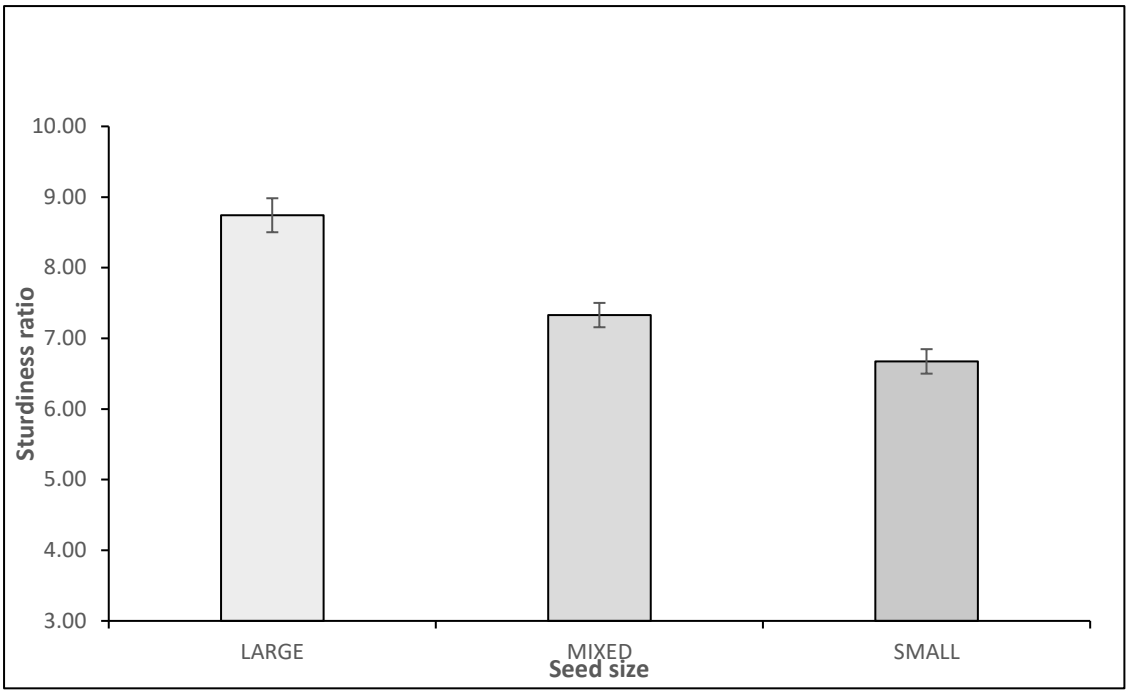

Figure 4.28. Sturdiness ratios for seedlings from small, mixed, and large seed. Error bars represent CI.

B) Pre-treatment

The sturdiness ratio refers to the ratio between shoot height and root collar diameter. High ratios indicate tall, thin seedlings, while low ratios indicate shorter, more robust plants. Infield survival is lower for taller, thinner seedlings (i.e., those with high ratios) because they have smaller root systems which are unable to support the plant above the ground (Haase, 2008). Sturdiness ratio for the control (7.0) was the lowest and was highest for the hydrogen peroxide + kelp-p-max (8.1) pre-treatment. This indicates that the seedlings of the control were more robust than the seedlings from the hydrogen peroxide + kelp-p-max pre-treatment, with an opportunity of good infield survival. The control was significantly ($p < 0.05$) lower than hydrogen peroxide + kelp-p-max (8.1) and hydro-prime (7.77) pre-treatments (Figure 4.29). Sturdiness ratio for the control was similar to the kelp-p-max (7.67), hydrogen peroxide (7.51), stratification (7.50) and hydrogen peroxide + hydro-prime (7.43) pre-treatments. Variation in sturdiness ratio was highest for seedlings from the hydrogen peroxide pre-treatment with a range of 8.2, while the range for seedlings from the hydrogen peroxide + hydro-prime treatment (4.2) was the lowest (Figure 4.29).

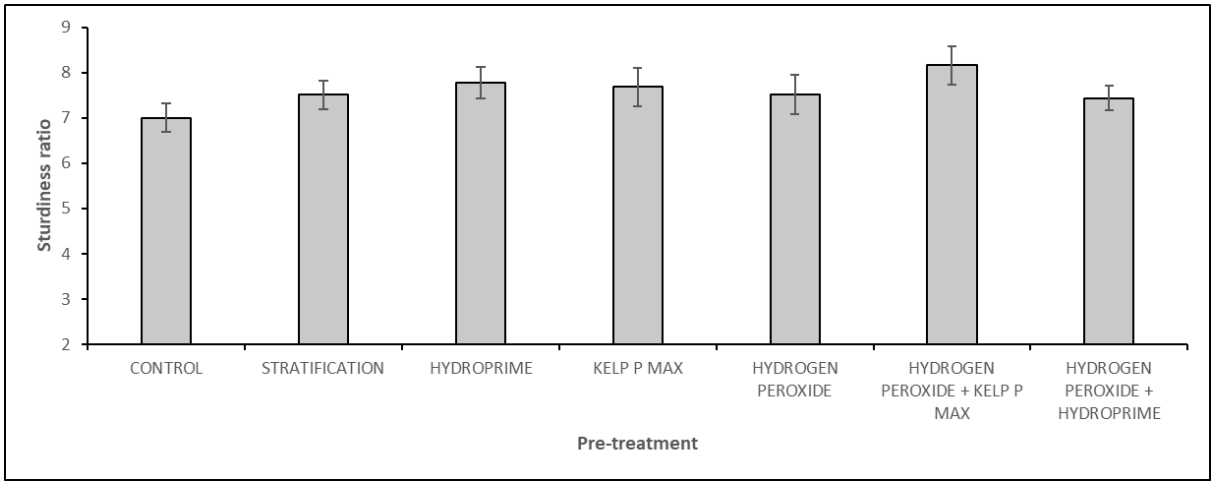

Figure 4.29. Sturdiness ratios for seedlings from different seed pre-treatments. Error bars represent CI.

4.4 FIELD TRIAL

4.4.1 Seedling height

Seedling height was significantly different for seed size ($p < 0.001$) and pre-treatment ($p = 0.036$), while no significant differences for their interaction were observed (Table 4.15).

Table 4.15. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on seedling height.

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	5	576550.	115310.	33.60	
Seed size (A)	2	592247.	296123.	86.29	<0.001
Pre-treatment (B)	6	48493.	8082.	2.36	0.036
Seed size.Pre-treatment	12	30696.	2558	0.75	0.704
Error (residual)	100	343172	3432		
Total	125	1591158.			

A) Seed size

Seedlings from large seed (800.6 mm) were significantly ($p < 0.05$) taller than seedlings from mixed (710.6 mm) and small (632.8 mm) seed, with seedlings from mixed seed significantly taller than seedlings from small seed (Figure 4.30). Seedling height for large seed were 92 mm and 167.8 mm taller than mixed and seed, respectively, indicating that seedling height increased as seed size increased (Figure 4.30).

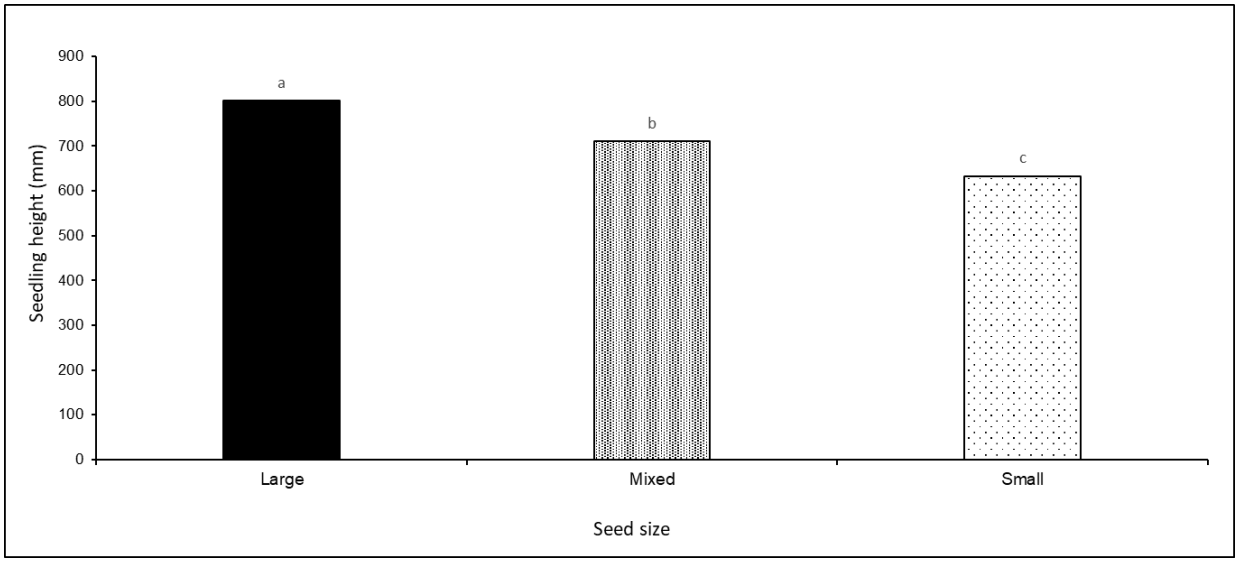

Figure 4.30. Seedling height for small, mixed, and large seed 12 months after planting. Different letters indicate a significant difference at $p < 0.05$.

B) Pre-treatment

Seed from the kelp-p-max pre-treatment produced the tallest seedlings (749.6 mm), while seeds from the hydrogen peroxide + hydro-prime pre-treatment produced the shortest (687.9 mm) (Figure 4.31). Seedlings from the kelp-p-max pre-treatment were significantly ($p < 0.05$) taller than seedlings from the hydrogen peroxide + hydro-prime pre-treatment, hydro-prime (699.9 mm), control (702.4 mm) and stratification (709.8 mm) pre-treatments.

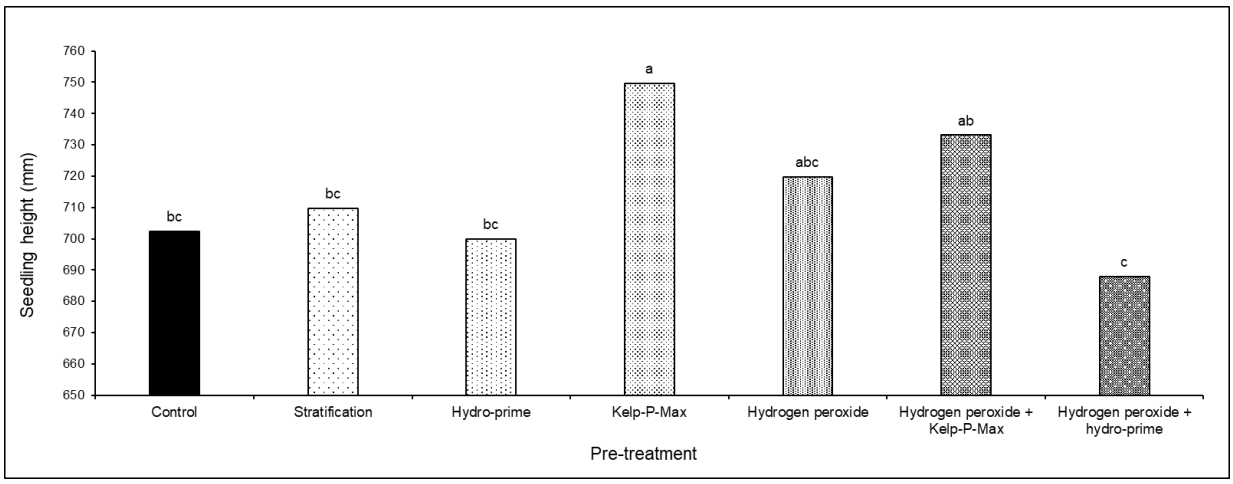

Figure 4.31. Seedling height for pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.4.2 Ground line diameter

Ground line diameter was significantly different for seed size ($p < 0.001$), while no significant differences for pre-treatment and their interaction were observed (Table 4.16).

Table 4.16. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on ground line diameter.

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	5	506.645	101.329	13.96	
Seed size (A)	2	661.180	330.590	45.56	<0.001
Pre-treatment (B)	6	52.226	8.704	1.20	0.313
Seed size.Pre-treatment	12	96.076	8.006	1.10	0.366
Error (residual)	100	725.676	7.257		
Total	125	2041.803			

A) Seed size

Ground line diameter for seedlings from large seed (26.7 mm) was significantly ($p < 0.05$) bigger than mixed (23.2 mm) and small seed (21.2 mm), with seedlings from mixed seed having significantly ($p < 0.05$) bigger ground line diameters than seedlings from small seed (Figure 4.32). Ground line diameter for large seed were 3.5 mm and 5.5 mm bigger than mixed and seed, respectively. Ground line diameter increased as seed size increased.

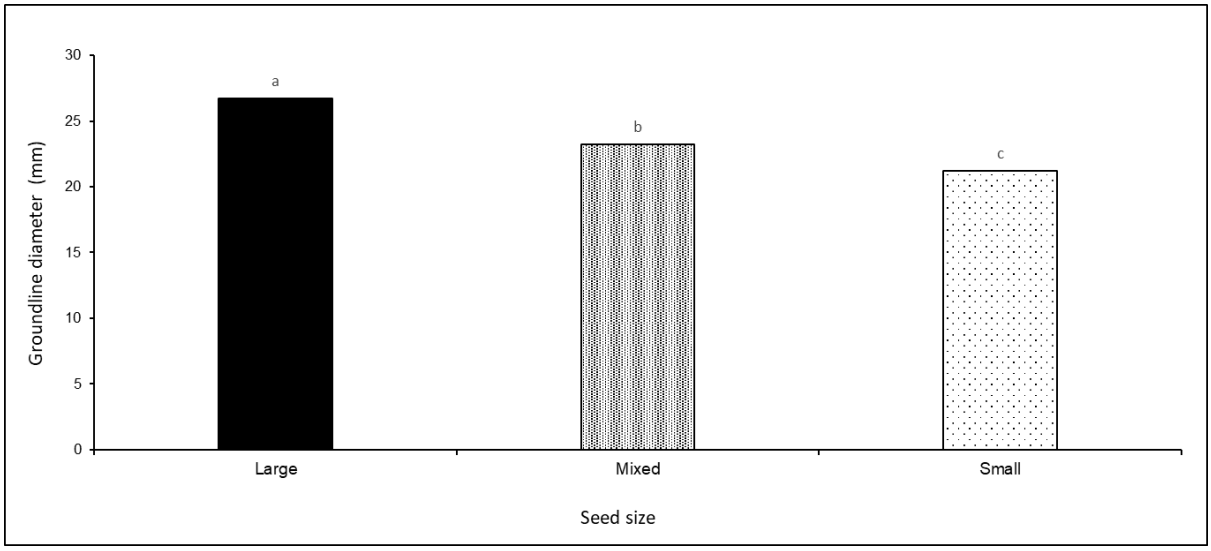

Figure 4.32. Ground line diameter for small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

4.4.3 Biomass index

Biomass index was significantly different for seed size ($p < 0.001$), while no significant differences for pre-treatment and the interaction between seed size and pre-treatment were observed (Table 4.17).

Table 4.17. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on biomass index.

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	5	860086	172017	20.31	
Seed size (A)	2	1017371	508685	60.07	<0.001
Pre-treatment (B)	6	77808	12968	1.53	0.176
Seed size.Pre-treatment	12	91501	7625	0.90	0.549
Error (residual)	100	846877	8469		
Total	125	2893641			

A) Seed size

Biomass index for seedlings from large seed was significantly ($p < 0.05$) higher than mixed and small seed, with seedlings from mixed seed having significantly ($p < 0.05$) higher biomass than seedlings from small seed (Figure 4.33). Biomass index increased as seed size increased.

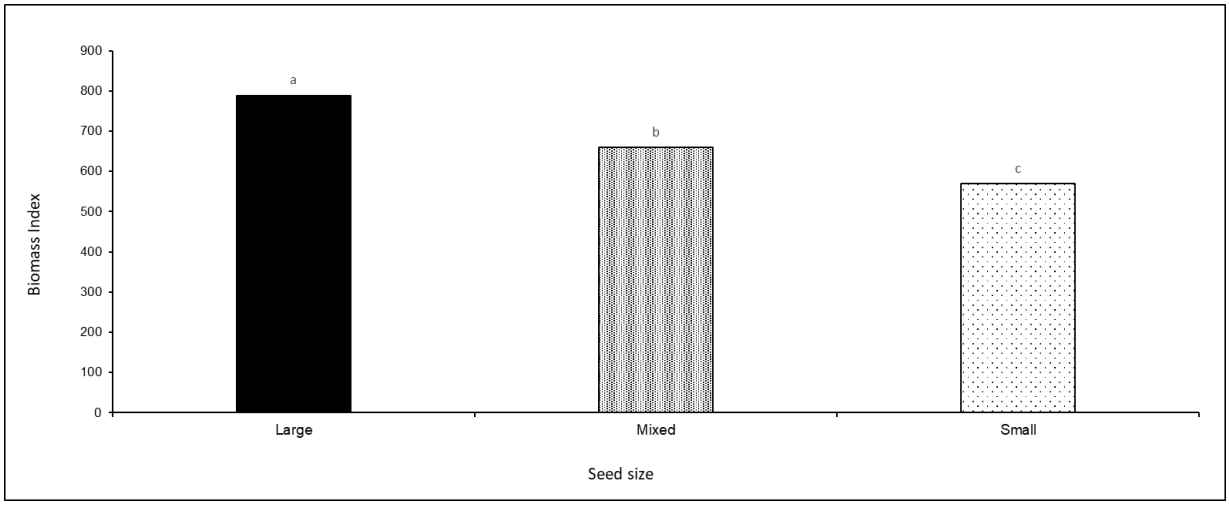

Figure 4.33. Biomass index for seedlings produced from small, mixed, and large seed. Different letters indicate a significant difference at $p < 0.05$.

4.4.4 Survival

Survival was significantly different for the interaction between seed size and pre-treatment ($p = 0.031$), while no significant differences for seed size and pre-treatment were observed (Table 4.18).

Table 4.18. Two-way ANOVA analysis of the effects of seed size, pre-treatment, and their interaction on survival.

Source of variation	Degrees of freedom	SS	MS	*F*-prob	Significance
Rep stratum	5	0.3524	0.0705	0.46	
Seed size (A)	2	0.5887	0.2944	1.93	0.151
Pre-treatment (B)	6	1.1523	0.1921	1.26	0.283
Seed size.Pre-treatment	12	3.6798	0.3066	2.01	0.031
Error (residual)	100	15.2600	0.1526		
Total	125	21.0332			

A) Interaction between seed size and pre-treatment

Survival was high (97.1%) in the trial, with seedlings from the control_large, control_small, hydrogen peroxide_large, hydrogen peroxide_mixed, hydrogen peroxide + hydro-prime_small, hydrogen peroxide + kelp-p-max large, hydro-prime_large, kelp-p-max_small interaction treatments having 100% survival which were significantly ($p < 0.05$) higher than the kelp-p-max_mixed treatment (83.3%) (Figure 4.34). The kelp-p-max_mixed interaction pre-treatment had the lowest survival in the trial (Figure 4.35). Survival for seedlings that were

produced from the interaction between the control and the different seed sizes (small, mixed, and large) were high with only the control_mixed (97.22%) seed having a survival lower than 100%. The interaction between the hydrogen peroxide pre-treatment and the different seed sizes (small, mixed, and large) yielded the same results with only the hydrogen peroxide_small (97.22%) interaction treatment having a survival of lower than 100% (Figure 4.34).

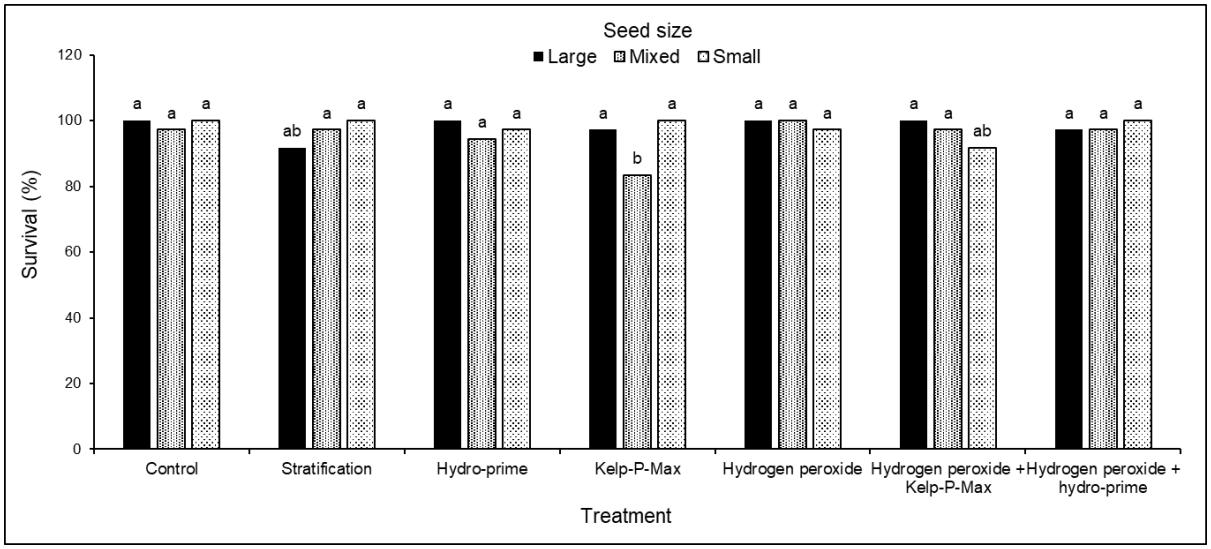

Figure 4.34. Infield survival for pre-treatments. Different letters indicate a significant difference at $p < 0.05$.

4.5 GENERAL SUMMARY RESULTS

The objective of this study was to investigate the influence of seed size, pre-treatment and their interaction on the germination, early seedling growth and infield seedling survival and growth of *P. elliottii*. The first trial was established in the laboratory while a second trial was established in the nursery to investigate the influence of seed size, pre-treatment and their interaction on final germination, time to 50% germination (T_{50}), mean germination time (MGT), germination value (GV), germination rate index (GRI), germination index (GI) and coefficient of velocity (CVG). After five months in the nursery, physical traits such as seedling height and root collar diameter were measured while the sturdiness ratio was calculated to determine the influence of seed size and pre-treatment on early seedling growth in the nursery. A field trial was established to investigate the influence of seed size, pre-treatment and their interaction on seedling survival and growth. The results were as follows:

4.5.1 Laboratory trial

Final germination, time to 50% germination (T_{50}), mean germination time (MGT), germination value (GV), germination rate index (GRI), germination index (GI) and coefficient of velocity (CVG) were all significantly ($p < 0.05$) influenced by seed size, while pre-treatment was only significant ($p < 0.05$) for T_{50}, MGT, GRI, GI and CVG. Large seed was significantly ($p < 0.05$) better than mixed and small seed for MGT, GV, GRI, GI and CVG. None of the pre-treatments were significantly better than the control for T_{50}, but the stratification pre-treatment was significantly ($p < 0.05$) better than the hydrogen peroxide + hydro-prime pre-treatment. The stratification pre-treatment had the best MGT, GRI, GI and CVG values and, together with the hydrogen peroxide + kelp-p-max pre-treatment, was significantly ($p < 0.05$) better than the control. No significant differences ($p < 0.05$) for the interaction between seed size and pre-treatment were observed.

4.5.2 Nursery trial

Final germination, GV, GI and CVG were significantly ($p < 0.05$) influenced by the interaction between seed size and pre-treatment. The hydrogen peroxide + kelp-p-max_large and stratification_mix treatments were significantly ($p < 0.05$) higher than control_large. The hydrogen peroxide + kelp-p-max_large, stratification_mix, stratification_large, hydrogen peroxide + kelp-p-max_mixed, kelp-p-max_large and kelp-p-max_mix treatments were the only treatments where final germination was higher than the acceptable 85% germination level for commercial nurseries. Germination values for the stratification_large, stratification_mixed, hydrogen peroxide + kelp-p-max_large and kelp-p-max_large interaction treatments were all significantly ($p < 0.05$) higher than the control_large. Germination Index (GI) for the stratification_large, stratification_mixed, hydrogen peroxide + kelp-p-max_large, hydrogen peroxide + kelp-p-max_mixed, kelp-p-max_large and kelp-p-max_mixed interaction treatments were all significantly ($p < 0.05$) higher than the control_large. Coefficient of velocity of germination index for the stratification_large, stratification_mixed, stratification_small, kelp-p-max_large, kelp-p-max_mixed, hydrogen peroxide + kelp-p-max_large and hydrogen peroxide + kelp-p-max_mixed interaction treatments were all significantly ($p < 0.05$) higher than for the control_large.

T_{50}, MGT and GRI were significantly ($p < 0.05$) different for seed size and pre-treatment, with large seed being significantly ($p < 0.05$) better than the mixed and small seed. T_{50}, MGT and GRI values for the stratification, hydrogen peroxide + kelp-p-max, kelp-p-max, hydrogen

peroxide + hydro-prime pre-treatments were generally significantly ($p < 0.05$) better than the control. This was also the case for T_{50} and MGT for the hydrogen peroxide treatment.

Seedling height for seedlings from large seed were significantly ($p < 0.05$) taller than seedlings from mixed and small seed. Root collar diameter for seedlings from mixed and large seed were significantly bigger than seedlings from small seed, while seedlings from small seed were significantly ($p < 0.05$) sturdier than seedlings from mixed and large seed. The stratification pre-treatment produced seedlings that were the tallest with the biggest root collar diameters, with seedlings from the control being the sturdiest. No pre-treatments were significantly ($p < 0.05$) better than the control seedling height, root collar diameter or sturdiness ratio.

4.5.3 Field trial

Seedling height and ground line diameter for seedlings from large seed were significantly ($p < 0.05$) higher than for seedlings from mixed and small seed, while seedlings were tallest for the kelp-p-max pre-treatment. BI was significantly ($p < 0.05$) higher for seedlings from large seeds than for seedlings from mixed and small seeds. Interaction between seed size and pre-treatment was important for survival and the survival of control_large, control_small, hydrogen peroxide_large, hydrogen peroxide_mixed, hydrogen peroxide + hydro-prime_small, hydrogen peroxide + kelp-p-max large, hydro-prime_large, kelp-p-max_small treatments (100%) was significantly ($p < 0.05$) higher than for the kelp-p-max_mixed treatment (83.3%).

CHAPTER 5: DISCUSSION

The objective of this study was to investigate the influence of seed size and seed pre-treatment and their interaction on germination, early seedling growth and infield seedling survival and growth of *P. elliottii*. This chapter thus discusses the results of germination, early seedling growth and infield survival as it relates to seed size, pre-treatment, and their interaction.

5.1 THE EFFECTS OF SEED SIZE, PRE-TREATMENT, AND THEIR INTERACTION ON GERMINATION

Seed germination is influenced by many internal and external factors, with seed size and pre-treatment being two of these factors. Seed size and pre-treatment play a vital role in the germination, growth and biomass of nursery seedlings and future crops (Leishangthem & Rana, 2017; Attri *et al.*, 2018). This study investigated the influence of seed size, pre-treatment, and their interaction on germination of *P. elliottii* under nursery and laboratory conditions. It was hypothesized that seed size and pre-treatment would have no significant influence on germination.

5.1.1 Final germination

In this study, germination was significantly ($p < 0.05$) influenced by seed size under laboratory conditions (28 DAS), while it was significantly ($p < 0.05$) influenced by the interaction between seed size and pre-treatment under nursery conditions (42 DAS). This is an indication that germination was dependent on seed size in the laboratory (when sown under optimal environmental/favourable conditions for germination), while it benefitted from the interaction between seed size and pre-treatment in the nursery (when seed was sown under stressful environmental conditions). These findings are contradictory to the hypothesis statement.

A) Seed size

Seed size is an important morphological trait and is a good indicator of seed quality as it affects seed vigour (Okonkwo *et al.*, 2020). This study found that seed size had a significant effect on germination under laboratory conditions (28 DAS). An increase in germination was observed as seed size increased, with large and mixed seed being significantly ($p < 0.05$) better than small seed. The mixed seed was a combination of large and small seed with large seed contributing 88% and small seed 12% towards the mix, which explained why mixed seed was similar to large seed (not significantly different). Seed size reflects the nutrient pool and energy of a seed, which affect future growth. Increased germination for large seed is ascribed to the

larger amount of endosperm reserves that are available to initiate, stimulate and sustain germination, compared to the lower food reserves in smaller seed (Couvillon, 2002; Naidu & Jones, 2007; Hojjat, 2011; Owoh *et al.*, 2011; Sadeghi *et al.*, 2011; Ahirwar, 2012; Missanjo *et al.*, 2013; Attri *et al.*, 2015; Fornah *et al.*, 2017; Leishangthem & Rana, 2017; Attri *et al.*, 2018).

Results of this study agree with studies conducted by Chacon (1998) on *Cryptocarya alba*, where germination was significantly higher for large (86.7%) seed than small (43.3%) seed. Couvillon (2002) also reported that germination for large seed was significantly higher (91%) than that of small seed (63%) for *Cercis canadensis* L. seed. Similarly, Mirgal *et al.* (2016) reported that larger *Saraca asoca* seed produced a germination of 86.7% compared to 45.0% for small seed.

Other studies have reported results that differ from this study, reporting that, although large seed germinated better than small seed, no significant differences between seed size classes could be found. This could be because all seed size classes used were of high physiological quality, traits which include plumpness, high purity, being disease-free and demonstrating optimum moisture content (Missanjo *et al.*, 2013; Mtambalika *et al.*, 2014). Non-dormant seeds sown under optimal germination conditions also tend not to be influenced by seed size (Missanjo *et al.*, 2013; Mtambalika *et al.*, 2014). Mtambalika *et al.*, (2014) recorded no significant differences between seed sizes for *Afzelia quanzensis*, where the germination was 94.9% and 88.4% for large and small seed, respectively. Missanjo *et al.*, (2013) reported similar results for *Albizia lebbeck*, where no significant differences were found between large (48.5%) and small (45.7%) seed.

Souza and Fagundes (2014) observed conflicting results for *Copaifera langsdorffii* compared to this study, finding that the germination for small seed (80%) was significantly better than that of large seed (64.4%). The germination for small seed was 15.6% higher than large seed. Thinner seed coats of small *C. langsdorfii* seed cause it to imbibe water more effectively than large seed, thus promoting germination (Souza & Fagundes, 2014). According to Sulewska *et al*. (2014), higher amylase activities were recorded within smaller seed than large seed, which were responsible for higher germination in smaller seed.

B) Interaction between seed size and pre-treatment

This study found that the interaction between seed size and pre-treatment had a significant effect on germination (although seed size and pre-treatment was also each significant on its own). In this study it was observed that the hydrogen peroxide + kelp-p-max_large treatment was the best and together with the stratification_mixed treatment was significantly ($p < 0.05$) better than the control_large treatment under nursery conditions (42 DAS). The hydrogen peroxide + kelp-p-max_large, stratification_mix, stratification_large, hydrogen peroxide + kelp-p-max_mixed, kelp-p-max_large and kelp-p-max_mix treatments were the only treatments where germination was higher than the acceptable 85% germination level for commercial nurseries. The hydrogen peroxide + kelp-p-max pre-treatment interacting with the large and mixed seed resulted in high germination (92.3 and 86.5%, respectively), with the kelp-p-max pre-treatment interacting with the large and mixed seed yielding similar results (86.5 and 85.8%, respectively). The above combinations were unique to this study and have not been tested in previous studies, but the reason for their high germination could be explained as follows:

1) The combined benefits of the hydrogen peroxide and kelp-p-max, where the hydrogen peroxide acted as a sterilizer that removes pathogens on the seed coat and assists with germination (Barnett & Varela, 2004), while the kelp-p-max contained natural hormones auxins and cytokinins that stimulate cell division and root development (Sujatha *et al*., 2015; Elgubbi *et al*., 2019); and

2) Combining the hydrogen peroxide + kelp-p-max and kelp-p-max pre-treatments with the large and mixed (88% large and 12% small seed) seed continued to benefit germination because large seed contains more nutrients to initiate and sustain germination (Swofford, 1958; Willan, 1986; Couvillon, 2002).

3) The benefits of a sterilizer (removing pathogens from the seed coat and assisting with germination) combined with a liquid fertilizer (containing auxins and cytokinins that stimulates cell division and root development) further interacting with large seed (containing nutrients to initiate and sustain germination) could have been responsible for the high germination.

The stratification pre-treatment interacting with all seed sizes generally demonstrated high germination, especially its interaction with the small seed size. The stratification_mixed (91%) and stratification_large (88%) treatments were also combinations unique (because of mixed

seed composition, 88% large and 12% small seed) to this study and have not been done in any previous studies. The possible explanation for the high germination could be that the combined benefits of the stratification pre-treatment (stratification removes metabolic blocks and weakens the seed coats to promote germination and breaks dormancy, Willan, 1986) and mixed (made up of 88% large seed and only 12% small) seed (with the big amount large seed in the mixture containing more nutrients to initiate and sustain germination, Swofford, 1958; Willan, 1986; Couvillon, 2002) enhanced germination. *Pinus elliottii* are known for dormant seed and are stratified to completely break dormancy and to enhance rapid and uniform germination (Swofford, 1958; Willan, 1986). This was further demonstrated in this study, in that the stratification_small treatment was also better (though not significantly better) than the control_large treatment and showed the highest improvement (24%) in the trial, because stratification is associated with breaking dormancy and small *P. elliottii* seed is regarded as more dormant than large seed, (Swofford, 1958; Willan, 1986).

5.1.2 T_{50}, MGT, GV, GRI. GI and CVG

Time to 50% Germination (T_{50}), Mean Germination Time (MGT), Germination Value (GV), Germination Rate Index (GRI), Germination Index (GI) and Coefficient of Velocity of Germination (CVG), were used to further investigate seed quality. These parameters are essential and have a direct impact on germination speed and early seedling growth (Javanmaer *et al.*, 2017). This study investigated the influence of seed size and pre-treatment on six germination parameters for *P. elliottii* seed under nursery and laboratory conditions.

This study found that T_{50}, MGT, GV, GRI, GI and CVG were significantly ($p < 0.05$) influenced by seed size, while T_{50}, MGT, GRI, GI and CVG were significantly ($p < 0.05$) influenced by pre-treatment, under laboratory conditions (28 DAS). It was further observed that T_{50}, MGT and GRI were significantly ($p < 0.05$) influenced by seed size and pre-treatment, while GV, GI and CVG were significantly ($p < 0.05$) influenced by the interaction between seed size and pre-treatment, under nursery conditions (42 DAS). These findings are in contrast with the hypothesis statement that seed size, pre-treatment and their interaction would not influence germination (T_{50}, MGT, GV, GRI, GI and CVG).

A) Seed size

T_{50}, MGT, GV, GRI, GI and CVG parameters are all indicators of germination and germination speed and give a good indication of seed vigour or seed quality (Kader, 2005). This study found

that large seed were generally significantly ($p < 0.05$) better (MGT, GV, GRI, GI and CVG) than mixed and small seed (except for T_{50}, which was not significantly different for large and mixed seed) under laboratory conditions. Under nursery conditions, T_{50}, MGT and GRI were also significantly better for large seed, for than mixed and small seed. This study found that germination speed (T_{50}, MGT and GRI) and the combination of final germination and germination speed (GV, GI and CVG) for large seed were significantly ($p < 0.05$) better than for small seed, which is an indication that seed quality for large seed was significantly better than mixed and small seed. The reason for this could be that large seed contains large amounts of endosperm reserves that are available to initiate, stimulate and sustain germination, compared to the lower food reserves in smaller seed (Couvillon, 2002; Naidu & Jones, 2007; Hojjat, 2011; Owoh *et al.*, 2011; Sadeghi *et al.*, 2011; Ahirwar, 2012; Missanjo *et al.*, 2013; Attri *et al.*, 2015; Fornah *et al.*, 2017; Leishangthem & Rana, 2017; Attri *et al.*, 2018).

T_{50} and MGT were significantly reduced for large seed compared to small seed in studies by Tanveer *et al.*, (2013) (T_{50}) on *Convolvulus*, and Mut and Akay (2010) on *Avena sativa*. GV, GRI and CVG parameters were significantly higher for larger seed than small seed in studies by Couvillon (2002) on *Cercis canadensis*, Wang (2005) on *Kraschenninikovia lanata*, Mandal *et al.* (2008) on *Hyptis suaveolus* (Lamiaceae). These results were contradictory to Mosavian & Eshraghi-Nejad (2013) and Okonkwo *et al.* (2020), who observed no significant difference for T_{50} between small, medium and large seed for wheat (*Triticum aestivum* var *Chamran*) and *Artocarpus altilis* seeds, while Souza and Fagundes (2014) reported that MGT for small seed (29 days) was significantly less than for large seed (41 days) for *C. langsdorffii*, because small seed, with its thinner seed coat and higher relative surface, is more water-permeable and thus germinates quicker than large seed.

B) Pre-treatment

Seed pre-treatments are among many techniques used by nursery managers to improve seed performance (Karrfalt, 2011). This study found that the stratification pre-treatment performed the best for the T_{50}, MGT, GRI, GI and CVG germination parameters and together with the hydrogen peroxide + kelp-p-max pre-treatment were significantly better for MGT, GRI, GI and CVG than the control, (but no pre-treatment was significantly better than the control for T_{50}) under laboratory conditions. The stratification pre-treatment was best for the T_{50}, MGT and GRI values and together with the hydrogen peroxide + kelp-p-max, kelp-p-max, hydrogen peroxide + hydro-prime pre-treatments (hydrogen peroxide also for T_{50}) were significantly (p

< 0.05) better than the control, under nursery conditions. This also contradicts the hypothesis statement that pre-treatment would not affect germination. This study found that germination speed (T_{50}, MGT and GRI) was best for the stratification pre-treatment than all the other pre-treatments, which is an indication of its positive impact on germination and thus seed quality/vigour.

Fetouh and Hassan (2014), in their study on seed germination criteria and seedling characteristics of *Magnolia grandiflora* trees, also observed that stratification treatments significantly reduced MGT, while GV and GI values were also significantly higher compared to the control. Skordilis and Thanos (1995) recorded a decrease in T_{50} for stratified *P. brutia* and *P. halepensis* seed when compared to the control. Graber (1965) also reported a significant decrease in time to 33% germination for eastern white pine seed, while the control could not reach 33% germination. Similarly, Kaur *et al.* (2016) reported that stratification had a significant effect on T_{50} for *Alnus viridis* subsp. *crispa* seed. Improvements caused by the stratification pre-treatment could be because stratification is a process that is used to increase rapid and uniform germination by overcoming seed dormancy. Stratification is commonly used in species such as *P. taeda* and *P. elliottii* where seed dormancy is anticipated (Swofford, 1958). It removes the metabolic blocks and weakens the seed coats to promote germination capacity and rate (Willan, 1986).

C) Interaction between seed size and pre-treatment

This study found that GV, GI and CVG (combination of final germination and germination speed) was significantly ($p < 0.05$) influenced by the interaction between seed size and pre-treatment, under nursery conditions (42 DAS). The stratification_large and stratification_mixed treatments were generally the best (significantly better than all other interaction combinations, except for GI where it was the highest but not significantly better than hydrogen peroxide + kelp-p-max_large) for GV, GI and CVG. This study found that the combination of GV, GI and CVG, is best for the stratification_large and stratification_mixed treatments and is an indication of its positive influence on germination and seed quality. This is a confirmation that germination for stratified seed that interacted with large and mixed seed was not only high but also quick/fast.

As discussed, (provide section number), for the interaction between seed size and pre-treatment (nursery), the stratification_large and stratification_mixed treatments were unique (because of

mixed seed composition, 88% large and 12% small seed) to this study and have not been tested in previous studies. The possible explanation for the high germination could be that the combined benefits of the stratification pre-treatment and seed size enhanced germination, in that:

1) stratification removes metabolic blocks and weakens the seed coats to promote germination and breaks dormancy (Willan, 1986); and
2) large and mixed (88% large, 12% small) seed contain more nutrients to initiate and sustain germination) (Swofford, 1958; Willan, 1986; Couvillon, 2002)

5.2 THE EFFECT OF SEED SIZE AND PRE-TREATMENT ON SEEDLING GROWTH

Seed size and pre-treatments play important roles in seed germination and early seedling growth (Mwase & Mvula, 2011; Missanjo *et al.*, 2013). This study investigated the influence of seed size and pre-treatment on early seedling growth of *P. elliottii*. It was hypothesized that seed size and pre-treatment would have no significant improvement on early seedling growth (shoot length, root collar diameter and root sturdiness). In this study, seedling growth (seedling height, root collar diameter and sturdiness ratio) was significantly influenced by seed size, while none of pre-treatments were better than the control, which is contrary to the hypothesis statement (for seed size).

5.2.1 Seedling height

A) Seed size

This study found that seedling height for seedlings from large seed was significantly ($p < 0.05$) greater than for seedlings from mixed and small seed, while seedling height for mixed seed was significantly ($p < 0.05$) greater than for seedlings from small seed. Thus, seedling height increased as seed size increased, with large seed producing taller seedlings than seedlings from small seed (Dunlop & Barnett, 1983). This agrees with Mtambalika *et al.* (2014), who observed that seedlings from large seed were 36% and 25% taller than those from small and medium seed respectively in *Afzelia quanzensis*. Large seed also resulted in taller seedlings for *Bauhinia thonningii* compared to seedlings from small seed (Mwase & Mvula, 2011). Langdon (1958) studied the effects of cone and seed size of South Florida slash pine (*Pinus elliottii* var. *densa*) on seedling size and survival and found that 59% of seedlings from small seed were graded as small seedlings, while only 27% from large seed were graded as small seedlings. Similarly,

Chacon *et al.* (1998) studied the effect of seed size on germination and seedling growth of *Cryptocarya alba* and observed that seedling height for large seed was significantly greater than for small seed. Similar results were also found by Naidu and Jones, 2007; Dunlop and Barnett, 1983; Bernard and Toft, 2007; Kolawola *et al.*, 2011 and Burgar (1964) with *Eucalyptus*, *P. taeda*, *E. nauseosa*, *V. paradoxa* and *P. glauca* (white spruce) seed, respectively. These differences in seedling height between large, mixed, and small seed could be attributed to the differences in food reserves. Larger seeds store greater reserves of carbohydrates in their endosperm than small seeds. These food reserves stimulate and sustain early seedling growth in the absence of photosynthesis (Missanjo *et al.*, 2013; Mtambalika *et al*., 2014). These differences could also be a function of the timing at which germination takes place, which is influenced by seed size, where seed that germinated first have taller seedlings than those that germinated later (Dunlop & Barnett, 1983; Naidu & Jones, 2007).

B) Pre-treatment

This study found that seedlings from the stratification pre-treatment produced the tallest seedlings. None of the seedlings from the pre-treatments was significantly taller than seedlings from the control. The reason for the taller seedlings from the stratification pre-treatment could be the increased germination speed caused by stratification pre-treatment (Barnett & McLemore, 1984). In other words, stratified seeds germinated quicker, and these seedlings were taller because they had had more time to grow. Another reason for this could be that stratification increases the solubility of fats and sugars while also increasing the gibberellic synthesis to boost growth (Hassan, 2014).

5.2.2 Root Collar Diameter (RCD)

A) Seed size

This study found that root collar diameter (RCD) for mixed and large seed was significantly bigger than in small seed. RCD increased as seed size increased, with seedlings from mixed and large seed having the largest RCD and seedlings from small seed having the smallest. RCD is a good indicator of infield survival, with seedlings with larger RCDs having a better chance of survival than seedlings with smaller RCDs. Larger RCDs are positively correlated with larger root volumes, thus improving infield survival due to increased root-to-soil contact and improved water and nutrient uptake (Haase, 2007). Mtambalika *et al.* (2014), also observed that RCD for seedlings from large seed (0.8 mm) was significantly bigger than small (0.5 mm)

seed for *Afzelia quanzensis*. Naidu and Jones (2007) reported in their study on the effect of seed size on field survival and growth of *Eucalyptus* in KwaZulu-Natal, South Africa, that the RCD for small seed was bigger than RCD for large seed. This was ascribed to low germination leading to a lack of competition and allowing resources to increase RCD growth rather than height growth, while seedlings that are exposed to more light also experienced increased diameter growth (Naidu & Jones, 2007).

B) Pre-treatment

This study found that root collar diameter was the biggest for the stratification pre-treatment. None of the seedlings from the pre-treatments had significantly bigger root collar diameters than seedlings from the control. The reason for this could be that stratification pre-treatment improved seed vigour, which led to rapid germination and improved seedling vigour, in turn resulting in larger diameter growth. Seedlings from stratified seed thus had more time to develop compared to seedlings from other pre-treated seed (Dunlap & Barnett, 1984).

5.2.3 Sturdiness ratio

A) Seed size

This study found that the sturdiness ratio for large seed was significantly higher than for mixed and small seed. The sturdiness ratio increased as seed size increased, with large seed having the highest ratio and small seed the lowest. Higher values indicate tall, spindly seedlings, while low values indicate more robust seedlings. Robust seedlings normally have higher infield survival rates (Haase, 2007). This agrees with Naidu and Jones, 2007, who observed that small seed produced sturdier seedlings for *E. smithii*, because of more open spaces caused by delayed and lower germination. Lower germination made more light available to small seed seedlings, promoting diameter growth that resulted in sturdier plants (Naidu & Jones, 2007).

B) Pre-treatment

Sturdiness ratio was lowest for the control, meaning that the control produced very robust seedlings with a good potential of survival infield (Haase, 2007). The reason for the control having the lowest sturdiness ratio could be that the lower densities in the trays (because of lower germination) were allowing more light and space for seedlings. This encouraged diameter growth, resulting in sturdier seedlings (Naidu & Jones, 2007).

5.3 THE EFFECT OF SEED SIZE, PRE-TREATMENT AND THEIR INTERACTION ON INFIELD GROWTH AND SURVIVAL

Seedling quality cannot be described at the nursery level alone but is also determined by out-planting performance and how this meets economic and management goals **(**Jacobs *et al.*, 2004; Haase, 2007**).** This study investigated the influence of seed size, pre-treatment and their interaction on the survival and infield growth of *P. elliottii*. It was hypothesized that seed size and pre-treatment would have no significant improvement on infield growth (seedling height, ground line diameter, biomass index) and survival, 12 months after planting.

In this study seed size and pre-treatment influenced seedling growth, while there was interaction observed between size and pre-treatment for survival 12 months after planting, which is contrary to the hypothesis statement.

5.3.1 Seedling height

A) Seed size

This study found that seedlings from large seed were significantly taller than seedlings from mixed and small seed. Seedlings from large seed maintained their height advantage over seedlings from mixed and small seed observed in the nursery. The reason for this could be that taller seedlings had larger photosynthetic and larger transpiration areas which is beneficial for growth. It could also be that taller seedlings compete better for water and nutrients than smaller seedlings do on sites with severe weed competition (Haase, 2007). These results are consistent with Burgar (1964), who reported that seedlings from large seed were significantly taller than seedlings from small and medium seed for *P. glauca* after the first growing season. However, these results are inconsistent with Langdon (1958), who reported that seed size did not significantly affect total height at one year after planting for *P. elliottii* var *densa*. Sluder (1991) also observed different results compared to this study where no significant differences for seedling height were found for seedlings from small, medium, and large seed for *P. elliottii* at 1, 3, 10 and 15 years after planting. Naidu and Jones (2007) also found no significant differences between seedling heights for *E. grandis* and *E. smithii* 12 months after planting. Naidu and Jones (2007) found that seed source or improved genetic material proved to be more important in predicting infield growth than seed size.

B) Pre-treatment

This study found that seedlings raised from the kelp-p-max pre-treatment were the tallest and were significantly ($p < 0.05$) taller than seedlings from the control. The reason for this could be that seed that was pre-treated with kelp-p-max had the tallest seedlings but had the lowest survival. The lower survival could have provided the seedlings with less competition for sunlight, water, and nutrient uptake than the other treatments, causing them to be the taller and bigger (Naidu and Jones, 2007). Sorensen (1980) reported that growth differences between seedlings only lasted for one growing season and were no longer significant after the second year for *P. menziesii* (Douglas-fir).

5.3.2 Ground line diameter

A) Seed size

This study found that the ground line diameter for seedlings from large seed was significantly bigger than that of seedlings from mixed and small seed, 12 months after planting. The reason for this could be that seedlings from large seed have larger root systems and stem volume, which aid with diameter growth (Haase, 2007). These results, however, disagree with Naidu and Jones (2007), who reported no significant differences between ground line diameter for *E. grandis* and *E. smithii* 12 months after planting, because genetics was a much better infield growth predictor than seed size.

5.3.3 Biomass index

A) Seed size

This study found that biomass index for seedlings from large seed was significantly higher than seedlings from mixed and small seed, 12 months after planting. This shows that yield increased as seed size increased. The reason for this could be that seedlings from large seed were the tallest and had the largest ground line diameters and tree volume is a function of height and diameter at time of planting (Haase, 2007). These results, however, disagree with Naidu and Jones (2007) who reported no significant differences for volume index between *E. grandis* and *E. smithii,* 12 months after planting, which was ascribed to the importance of genetics and not seed size.

5.3.4 Survival

A) Interaction between seed size and pre-treatment

Interaction between seed size and pre-treatment was important for survival, and the survival in control_large, control_small, hydrogen peroxide_large, hydrogen peroxide_mixed, hydrogen peroxide + hydro-prime_small, hydrogen peroxide + kelp-p-max large, hydro-prime_large, kelp-p-max_small treatments (100%) were significantly ($p < 0.05$) higher than the kelp-p-max_mixed treatment (83.3%). Interaction combinations were unique to this trial and reasons for high survival could be that:

Sturdiness ratio for the control was the lowest, while RCD for the control was also second biggest, indicating that robust seedlings had increased chances of survival infield (Haase, 2008). Interaction that included the control (control_large and control_small) could have benefitted from the low sturdiness ratio increasing chances of infield survival. Hydrogen peroxide, kelp-p-max pre-treatments, and hydrogen peroxide + hydro-prime pre-treatments had similar sturdiness ratios as the control, and interaction with these treatments could be similar to the control in that they could have benefitted from the low sturdiness ratio increasing chances of infield survival.

CHAPTER 6: CONCLUSIONS AND RESEARCH RECOMMENDATIONS

The objectives of this study were to investigate the influence of seed size, pre-treatment and their interaction on germination, early seedling growth (in the nursery), and infield survival and growth of *P. elliottii*. This chapter presents conclusions based on the research objectives, and recommendations.

6.1 CONCLUSIONS

6.1.1 The influence of seed size, pre-treatment, and their interaction on the germination.

6.1.1.1 Laboratory trial

Seed germination is influenced by many internal and external factors, with seed size and pre-treatment important in terms of germination, growth and biomass of nursery seedlings and future. Germination was significantly ($p < 0.05$) influenced by seed size under laboratory conditions (28 DAS), with germination being dependent on seed size in the laboratory (when sown under optimal environmental/favourable conditions for germination). Germination increased as seed size increased, where large and mixed seed were significantly ($p < 0.05$) better than small seed. The similar performance of the mixed and large seed sizes was most likely due to the high contribution of large (88%) seed within the mixed seed (small = 12%).

Six germination parameters, which have a direct impact on germination speed and early seedling growth were assessed and included the Time to 50% Germination (T_{50}), Mean Germination Time (MGT), Germination Value (GV), Germination Rate Index (GRI), Germination Index (GI) and Coefficient of Velocity of Germination (CVG), were used to further investigate seed quality. Large seed were significantly ($p < 0.05$) better (MGT, GV, GRI, GI and CVG) than mixed and small seed (except for T_{50}, where large and mixed were not significantly different). This indicates that large seed were more vigorous than small seed.

The stratification pre-treatment performed the best for the T_{50}, MGT, GRI, GI and CVG germination parameters and together with the hydrogen peroxide + kelp-p-max pre-treatment were significantly better for MGT, GRI, GI and CVG than the control, (but no pre-treatment was significantly better than the control for T_{50}). This indicates that seed from the stratification and hydrogen peroxide + kelp-p-max pre-treatments were more vigorous than those from the control.

6.1.1.2 Nursery trial

Germination was significantly ($p < 0.05$) influenced by the interaction between seed size and pre-treatment (42 DAS). The hydrogen peroxide + kelp-p-max_large treatment was the best and together with the stratification_mixed treatment was significantly ($p < 0.05$) better than the control_large treatment. T_{50}, MGT and GRI were significantly ($p < 0.05$) influenced by seed size and pre-treatment, while GV, GI and CVG were significantly ($p < 0.05$) influenced by the interaction between seed size and pre-treatment (42 DAS). Large seed was significantly ($p < 0.05$) better than mixed and small seed for T_{50}, MGT and GRI. The stratification pre-treatment was significantly ($p < 0.05$) better than all other pre-treatments for T_{50}, MGT and GRI. The stratification_large together with the stratification_mixed were the best interaction treatments for GV, GI and CVG, indicating that large seed together with the stratification pre-treatment and the stratification_large interaction treatment were more vigorous than small seed, control, and control_large treatments, respectively.

6.1.2 The influence of seed size and pre-treatment on early seedling growth

Seed size and pre-treatments play important roles in seed germination and early seedling growth. Seedlings from large seed were significantly ($p < 0.05$) taller than seedlings from mixed and small seed, while seedlings from mixed seed were significantly ($p < 0.05$) taller than seedlings from small seed. Thus, seedling height increased as seed size increased, with large seed producing taller seedlings than seedlings from small seed. Root Collar Diameter (RCD) for large and mixed seed was significantly bigger than small seed. RCD increased as seed size increased, with seedlings from mixed seed having the largest RCD and seedlings from small seed having the smallest. Sturdiness ratio for large seed was significantly higher compared to mixed and small seed. The sturdiness ratio increased as seed size increased, with large seed having the largest ratio and small seed the lowest. Seedlings from the stratification pre-treatment produced the tallest seedlings. None of the seedlings from the pre-treatments were significantly taller than seedlings from the control. Root collar diameter was the biggest for the stratification pre-treatment. None of the seedlings from the pre-treatments had significantly bigger root collar diameters than seedlings from the control. Seedlings from the control were the sturdiest, having the lowest sturdiness ratios.

6.1.3 The influence of seed size, pre-treatment and their interaction on infield survival and growth, 12 months after planting.

Seedlings from large seed were significantly taller than seedlings from mixed and small seed. Seedlings from large seed maintained their height advantage over seedlings from mixed and small seed that was observed in the nursery. Seedlings from the kelp-p-max pre-treatment were the tallest and were significantly taller than the control. Ground line diameter for seedlings from large seed was significantly bigger than that of seedlings from mixed and small seed, 12 months after planting. Biomass index for seedlings from large seed was significantly higher than seedlings from mixed and small seed, 12 months after planting.

Interaction between seed size and pre-treatment was important for survival and survival in the control_large, control_small, hydrogen peroxide_large, hydrogen peroxide_mixed, hydrogen peroxide + hydro-prime_small, hydrogen peroxide + kelp-p-max large, hydro-prime_large, kelp-p-max_small treatments (100%) were significantly ($p < 0.05$) higher than in the kelp-p-max_mixed treatment (83.3%).

Based on the outcomes of this study, it is concluded that seed size, pre-treatment and their interaction had a positive influence *P. elliottii* seed germination and early seedling growth. This study demonstrated that germination in the laboratory was dependent on seed size, while it benefitted significantly from the interaction between seed size and pre-treatment in the nursery. Large and mixed seed outperformed small seed in the laboratory, while the hydrogen peroxide + kelp-p-max_large, stratification_mixed, stratification_large, hydrogen peroxide + kelp-p-max_mixed, kelp-p-max_large and kelp-p-max_mix treatments produced better results than the acceptable 85% germination levels needed in commercial nurseries. If small seed are used for sowing due to a seed shortage, it is recommended that the seed be stratified because the stratified_small (84.8%) treatment was better than the control_large treatment (84.3%). Early seedling growth was positively influenced by seed size, with large seed producing taller seedlings, bigger root collar diameters and higher sturdiness ratios than seedlings from small seed. Seedlings from large seed maintained their height advantage 12 months after planting over seedlings from small seed that was observed in the nursery. Ground line diameter and biomass index were positively influenced by seed size, with large seed producing higher values than seedlings from mixed and small seed. Survival was high (97.1%) in the trial with survival for seedlings from the kelp-p-max_mixed treatment being significantly lower than for seedlings from the control_large, control_mixed and control_small treatments.

6.2 RESEARCH SHORTCOMINGS

The study was limited to six pre-treatments and an untreated control to investigate the influence of these six pre-treatments on germination, early seedling growth and field survival and growth, while future research should focus on different pre-treatments (halo-priming with NaCl, KCl, KNO_3, K_3PO_4, $MgSO_4$ and $CaCl_2$, warm stratification, and scarification).

The study compared small, mixed, and large seed, with the mixed seed having a weighted contribution between small and large seed, with large having the largest contribution (88% contribution) towards the mixed seed size. It is thus suggested that the mixed seed size have a 50:50 contribution for future studies.

The study only combined hydrogen peroxide + kelp-p-max, hydrogen peroxide + hydro-prime while future studies should include more combinations (hydrogen peroxide + stratification, kelp-p-max + stratification, hydro-prime + stratification, hydro-prime + kelp-p-max and hydrogen peroxide + kelp-p-max + stratification).

Finally, this study focussed only on one species, namely *P. elliottii*, while future studies should investigate the influence of seed size and pre-treatments on other pine hybrids (*P. patula* x *P. tecunumanii*, *P. patula* x *P. greggii* and *P. elliottii* x *P. caribaea*).

6.3 RESEARCH RECOMMENDATIONS

Based on the results obtained in this study the following recommendations are suggested:

- Large and mixed seed to be used during sowing.
- Hydrogen peroxide + kelp-p-max, stratification and kelp-p-max treatments used on the above seed sizes before sowing.
- Small seed should be avoided unless it is stratified before sowing.

Future research should focus on techniques and technologies that can assist in increasing the production of large seed in seed orchards in order to improve germination.

7. REFERENCES

Adrews, C.H. (1965) Mechanical injury in seeds. In: *Short Course For Seedsmen*. Proceedings: Mississippi State University Seed Technology Laboratory, 1965 pp. 125–130.

Ahirwar, J.R. (2012) Effect of seed size and weight on seed germination of *Alangium lamarckii*, Akola, India. *Research Journal of Recent Sciences*, 1, pp. 320–322.

Artola, A., Carrillo-Castaneda, G. and De Los Santos, G.G. (2003) Hydro priming: A strategy to *increase Lotus corniculatus* L. seed vigor. *Seed Science and Technology, 31*(2), pp. 455–463.

Asary, M. (2017) *An investigation into the poor emergence* of Pinus elliottii and Pinus taeda s*eeds*. Doctoral dissertation, University of Cape Town.

Attri, V., Pant, K.S., Dhiman, R., Lal, C. and Sarvade, S. (2015) Effect of seed size and pre-sowing treatments on growth parameters and biomass of *Sapindus mukorossi* (Gaertn) seedlings under nursery condition. *Environment & Ecology*, *33*(1), pp. 46–49.

Attri, V., Pant, K.S. and Tiwari, P. (2018) Effect of seed size and pre-sowing treatment on germination of some important trees: A review. *Research Journal of Chemical and Environmental Sciences*, *6*(1), pp. 104–113.

Barnett, J.P. (1976) *Cone and seed maturation of southern pines*. Vol. 122. Department of Agriculture, Forest Service, Southern Forest Experiment Station.

Barnett, J.P. (1976) Sterilizing southern pine seeds with hydrogen peroxide. *Tree Planters' Notes 27*(3), pp. 17–19.

Barnett, J.P. 1(996) How seed orchard culture affects seed quality: Experience with the southern pines. *The Forestry Chronicle, 72*(5*)*, pp. 469–473.

Barnett, J.P. (2002) Activities that increase germination and establishment of longleaf pine seedlings in containers. In: Moorhead D.J. (Ed.) *Proceedings of workshops on growing longleaf pine in containers. General Techical Report*. Southern Research Station, U.S. Department of Agriculture, Forest Service, Southern Research Station, Asheville, 56, pp. 21–23.

Barnett, J.P. (2008) Relating seed treatments to nursery performance: Experience with southern pines. In: Dumroese, R.K. and Riley, L.E. (Tech. coords). *National Proceedings: Forest and Conservation Nursery Associations-2007. Proc. RMRS-P-57.* Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station, 57, pp. 27–37.

Barnett, J. P. and J. M. McGilvray, J.M. (1971) Stratification of short leaf pine seeds. *Tree Planters' Notes 22*(3), pp. 19–21.

Barnett, J.P., McGilvray, J.M. (2002) *Guidelines for producing quality longleaf pine seeds General Techical Report SRS-52.* Asheville (NC): U.S. Department of Agriculture, Forest Service, Southern Research Station, 21.

Barnett, J.P., McLemore, B.F. (1967) Germination of loblolly pine seed hastened by soakings in aerated cold water. *Tree Planters' Notes, 18*(2), pp. 24–25.

Barnett, J.P. and McLemore, B.F. (1984) Germination speed as a predictor of nursery seedling performance. *Southern Journal of Applied Forestry*, *8*(3*)*, pp. 157–162.

Barnett, J.P., Sheffield, R.M. (2004) Slash pine: Characteristics, history, status and trends. In. E.D. Dickens, J.P. Barnett, W.G. Hubbard and E.L. Jokela (Eds.) *Slash Pine: Still Growing and Growing! Proceedings of the Slash Pine Symposium*. pp. 1–6.

Barnett, J.P. and Varela, S. (2003) Producing high-quality slash pine seeds. In: Riley L.E., Dumroese, R.K. and Landis, T.L. (Tech. coords). *Proceedings of the national forest and conservation nursery associations—2002.* Ogden (UT): USDA Forest Service, Rocky Mountain Re-search Station. Proceedings RMRS-P-28. pp. 52–56.

Barnett, J.P. and Varela, S. (2004) A review of chemical treatments to improve germination of longleaf pine seeds. *Native Plants Journal, 5*(1), pp. 18–24.

Barnett, J.P., Pickens, B., Karrfalt, R. (1999) Longleaf presowing treatments: Effects on germination and nursery establishment. In: Landis, T.D. and Barnett, J.P. (Tech. coords). *National proceedings: Forest and conservation nursery associations – 1998.* General Tech. Report SRS-25, Asheville, NC: USDA Forest Service, Southern Research Station, pp. 43–46.

Bayley, A.D., Nixon, K., Smith, M. (2000) Storage and pre-treatment of exotic and indigenous tree seed. In: Owen, D.L. (Ed.) *South African Forestry Handbook.* Vol. 1. Pretoria: V & R Printers, pp. 53–60.

Belcher, E.W., Leach, G.N. and Gresham, H.H. (1984) Sizing salash pine seeds as a nursery procedure. *Tree Planters' Notes 35*(2), pp. 5–10.

Belcher, E.W. (1995) Effect of seed condition, stratification and germination temperature on the laboratory germination of loblolly pine seed. *Tree Planters' Notes*, *46*(4), pp. 138–142.

Benard, R.B. and Toft, C.A. (2007) Effect of seed size on seedling performance in a long-lived desert perennial shrub (*Ericameria nauseosa*: Asteraceae). *International Journal of Plant Sciences*, *168*(7), pp. 1027–1033.

Bewley, J.D. (1997) Seed germination and dormancy. *The Plant Cell*, *9*, pp. 1055–1066.

Bonner, F.T. (1981) *Measurement and management of tree seed moisture*. Vol. 177. US Department of Agriculture, Forest Service, Southern Forest Experiment Station.

Bonner, F.T. (1987) Importance of seed size in germination and seedling growth. In: S.K. Kamra, R.D. Ayling (Eds.) *Proceedings of IUFRO International Symposium on Forest Seed Problems in Africa*. Department of Forest Genetics and Plant Physiolology, Uppsala, pp. 53–61.

Bonner, F.T. (1990) Storage of seeds: Potential and limitations for germplasm conservation. *Forest Ecology and Management*, *35*(1-2), pp. 35–43.

Bonner, F.T. (2008) Storage of seeds. In: F.T Bonner and R.G. Nisley (Eds.). *Woody plant seed manual, Agriculture handbook 727*. USDA Forest Service, pp. 85–96.

Botai, C. M., Botai, J. O. and Adeola, A. M. (2018) Spatial distribution of temporal precipitation contrasts in South Africa. *South African Journal of Science 114(7)*:1–9.

Burgar, R.J. (1964) The effect of seed size on germination, survival and initial growth in white spruce. *The Forestry Chronicle*, *40*(1), pp. 93–97.

Carvalho, M.E.A., Castro, P.R.C., Novembre, A.D.C., and Chamma, H.M.C.P. (2013) Seaweed extract improves the vigor and provides the rapid emergence of dry bean seeds. *American-Eurasian Journal of Agriculture & Environmental Sciences, 13*(8), pp. 1104–1107.

Castro, J. (1999) Seed mass versus seedling performance in Scots pine: A maternally dependant trait. *New Phytologist*, *144*(1), pp. 153–161.

Cavusoglu, K. and Kabar, K. (2010) Effects of hydrogen peroxide on the germination and early seedling growth of barley under NaCl and high temperature stresses. *EurAsian Journal of BioScience*, *4*, pp. 70–79.

Chacón, P.A., Bustamante, R.O. and Henríquez, C.A. (1998) The effect of seed size on germination and seedling growth of *Cryptocarya alba* (Lauraceae) in Chile. *Revista Chilena de Historia Natural*, *71*(2), pp.189–197.

Chaisurisri, K., Edwards, D.G.W., El-Kassaby, Y.A. (1992) Genetic control of seed size and germination in Sitka spruce. *Silvae Genetica*, *41*, pp. 348–355.

Chin, H.F., Krishnapilly, B., Stanwood, P.C. (1989) Seed moisture: Recalcitrant vs. orthodox seeds. In: Stanwood, P.C. and Mcdonald, M.B. (eds). *Seed Moisture*. Madison: Crop Science Society of America, 14, pp. 15–22.

Ching, T.M. (1959) Activation of germination in Douglas fir seed by hydrogen peroxide. *Plant Physiology*, *34*(5), pp. 557–563.

Coder, K. D. (2017) Pinus elliottii *slash pine*. Warnell School of Forestry & Natural Resources, University of Georgia, Outreach Publication WSFR-17WMD, 5. https://www.warnell.uga.edu/sites/default/files/publications/WSFNR-17-24%20Coder.pdf

Connolly, B.M., Agnew, L.K. and Orrock, J.L. (2017) Interactive effects of contact fungicide and cold stratification on the germination rate for five dominant temperate tree species. *Forest Science*, *63*(3), pp. 303–309.

Couvillon, G.A. (2002) *Cercis canadensis* L. seed size influences germination rate, seedling dry matter and seedling leaf area. *HortScience 37*(1), pp. 206–207.

Cram, M. and Fraedrich, S. (2010) Seed diseases and seedborne pathogens of North America. *Tree Planters' Notes 53*(2), pp. 35–44.

Czabator, F.J. (1962) Germination value: An index combining speed and completeness of pine seed germination. *Forest Science*, *8*(4), pp. 386–396.

Darrow, W.K. (1984) The origin of imported *Pinus elliotii* seedlots planted in South Africa: A historical investigation. *South African Forestry Journal*, *131*(1), pp. 25–27.

Davis, A.S. and Jacobs, D.F. (2005) Quantifying root system quality of nursery seedlings and relationship to outplanting performance. *New Forests*, *30*(2-3), pp. 295–311.

Demir, N., Dural, B. and Yildirim, K. (2006) Effect of seaweed suspensions on seed germination of tomato, pepper and aubergine. *Jounal of Biological Sciences*, *6*(6), pp. 1130–1133.

Deneke, F.J. and Landis, T.D. (1978) *The value of quality seed in forest tree nursery operations and reforestation programs*. Combined Nurseryman's Conference and Workshop, pp. D27–D34. California: Intermountain Nurseryman's Association. https://rngr.net/publications/proceedings/1978/PDF.2004-02-17.1133

Department of Agriculture, Forestry and Fisheries. Directorate of Forestry Regulations and Oversight (2017/2018) *Report on the commercial timber resources and primary roundwood processing in South Africa.* Forestry Economic Services CC.

Dilavarnaik, S., Basavaraja, P.K., Yogendra, N.D., and Ghosh, A. (2017) Influence of seaweed saps on germination, growth and yield of hybrid maize under Cauvery command of Karmataka, India. *International Journal of Current Microbiology Applied Science*, *6*(9), pp. 1047–1056.

Dickens, E.D., Barnett, J.P., Hubbard, W.G. and Jokela, E.J. (2004). *Slash pine: Still growing and growing!* Proceedings of the slash pine symposium. Gen. Tech. Rep. SRS-76. Asheville, NC: US Department of Agriculture, Forest Service, Southern Research Station, 145 p. 76.

Domin, M., Kluza, F., Góral, D., Nazarewicz, S., Kozłowicz, K., Szmigielski, M. and Ślaska-Grzywna, B. (2019) Germination energy and capacity of maize seeds following low-temperature short storage. *Sustainability*, *12*(1), p. 46.

Donald, D.G.M. and Jacobs, C.B. (1994). Treatment and statistics of exotic tree seed. In: Van der Sijde, H.A. (Eds.) *Southern African Forestry Handbook 1994.* Pretoria: Aurora Printers, pp. 49–66.

Dumroese, R.K., James, R.L., Wenny, D.L. and Gilligan, C.J. (1988) Douglas-fir seed treatments: Effects on seed germination and seedborne organims. In: Shearer, R.C. (Ed.) *Proceedings of the combined meeting of the western forest nursery associations, 8–11 August 1988*. Fort

Collins, CO: USDA Forest Service, Rocky Mountain Forest and Range Experiment Station, pp. 155–160.

Dunlap, J.R. and Barnett, J.P. (1983) Influence of seed size on germination and early development of loblolly pine (*Pinus taeda* L.) germinants. *Canandian Journal of Forest Research, 13*(1), pp. 40–44.

Dussert, S., Serret, J., Bastos-Siqueira, A., Morcillo, F., Dechamp, E., Fofidal, V., Lashermes, P, Etienne, H. and Joët, T. (2018) Integrative analysis of the late maturation programme and desiccation tolerance mechanisms in intermediate coffee seeds. *Journal of Experimental Botany, 69*(7), pp. 1583–1597.

Edwards, D.G.W. (1981) *Impact of seed quality on costs of nursery stock.* Proceedings of a Workshop on High-quality Collection and Production of Conifer Seed, held November 14, 1979, in Edmonton, Alberta (Vol. 235, p. 79). Northern Forest Research Centre.

Edwards, D.G.W. and El-Kassaby, Y.A. (1996) The effect of stratification and artificial light on the germination of mountain hemlock seeds. *Seed Science and Technology*, *24*, pp. 225–236.

El-Din, S.M.M. (2015) Utilization of seaweed extracts as bio-fertilizers to stimulate the growth of wheat seedlings. *The Egyptian Journal of Experimental Biology (Botany), 11*(1), pp. 31–39.

Elgubbi, H., Zrmoh, M., Alzarride, A., Adam, M. and El-Zaidy, M. (2019) Enhanced growth and yield parameters of tomato (*Solanum lycopersicum* L.) var. peto 111 plant using Cladophoropsis gerloffii aqueous extract foliar. *EC Nutrition*, *14*, pp. 60–67.

Farahani, H.A., Moaveni, P. and Maroufi, K. (2011) Effect of hydro priming on seedling growth of basil (*Ocimum basilicum* L.). *Advances in Environmental Biology*, pp. 2258–2264.

Farooq, M., Basra, S.M.A., Afzal, I. and Khaliq, A. (2006) Optimization of hydro-priming techniques for rice seed invigoration. *Seed Science and Technology*, *34*(2), pp. 507–512.

Ferguson, J.M., Keys, R.D., Laughlin, M.C. and Warren, J.M. (2004) Seed Quality: A guide for seed producers, farmers and home gardeners. *Journal of Agriculture and Biology 3*, 519–520.

Fetouh, M.A. and Hassan, F.A. (2014) Seed germination criteria and seedling characteristics of Magnolia grandiflora L. trees after cold stratification treatments. *International Journal of Current Microbiology and Applied Sciences*, *3*(3), pp. 235–241.

Fisher, P.R., Yafuso, E.J. and Huang, J. (2019) *Water and air balance in plant propagation cells.* Proceedings of III International Symposium on Growing Media, Composting and Substrate Analysis 1305, June 2019, pp. 477–484.

Forestry South Africa. (2019) *Abstract of South Africa forestry facts for the year 2017/2018. Forestry South Africa*. Department of Agriculture, Forestry and Fisheries. https://www.forestrysouthafrica.co.za/wp-content/uploads/2019/12/SA-Forestry-Forest-Products-Industry-2018.pdf

Fornah, Y., Mattia, S.B., Otesile, A.A. and Kamara, E.G. (2017) Effects of provenance and seed size on germination, seedling growth and physiological traits of *Gmelina arborea*, Roxb. *International Journal of Agriculture and Forestry*, *7*(1), pp. 28–34.

Forrest, W.G. (1964) *The effect of pre-treatment on germination of Slash Pine seed*. Forestry Commmission of NSW.

Gaby, Louis I. (1985) *Southern pines: Loblolly pine (*Pinus taeda *L.), longleaf pine (*Pinus palustris *Mill.), shortleaf pine (*Pinus echinata *Mill.), slash pine (*Pinus elliottii *Engelm.).* FS-256

Gansel, C.R. (1987) *Comparison of seed stratification methods for western white pine.* Proceedings, Combined Western Forest Nursery Council and Intermountain Nursery Association Meeting: August 12-15, 1986, Tumwater, Washington. Vol. 137. p. 19. US Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station.

Ghassemi-Golezani, K., Hassanpour-Bourkheili, S., Bandeh-Hagh, A. and Abriz, S.F. (2014) Seed hydro priming, a simple way of improving mungbean performance under water stress. *International Journal of Bioscience*, *4*, pp. 12–18.

Gomez, J.M. (2004) Bigger is not always better: Conflicting selective pressures on seed size in *Quercus ilex*. *Evolution*, *(58)*, pp. 71–80.

Gonzalez, E.J. (1993) Effect of seed size on germination and seedling vigor of *Virola koschnyi* Warb. *Forest Ecology and Management*, *57*(1–4), pp. 275–281.

Graber, R.E. (1965) *Germination of eastern white pine seed as influenced by stratification.* North Eastern Forest Experiment Station, 36, Forest Service, US Department of Agriculture.

Grotta, A.T., Ahrens, G.R. and Bennett, M. (2019) *Selecting and buying quality tree seedlings.* Oregon State University Extension Service.

Gunaga, R.P. and Vasudeva, D.R. (2011) Influence of seed size on germination and seedling growth in *Mammea suriga*. *Karnataka Journal of Agricultural Sciences*, *24*(3), pp. 415–416.

Haase, D.L. (2007) Morphological and physiological evaluations of seedling quality. In: Riley, L.E., Dumroese, R.K. and Landis, T.D. (Eds.) *National Proceedings: Forest and Conservation Nursery Associations-2006. Proceedings RMRS-P-50.* Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station. pp. 3–8.

Haase, D.L. (2008) Understanding forest seedling quality: Measurements and interpretation. *Tree Planters' Notes, 52*(2), pp. 24–30.

Hettasch, M.H., Eatwell, K.A., Fossey, A., Pierce, B.T., Snedden, C.L., Steyn, D.J., Venter, H.M. and Verryn, S.D. (2009) *Natural resources and the environment: Tree breeding course manual.* Pretoria: CSIR.

Hojjat, S.S. (2011) Effects of seed size, germination and seedling growth of some lentil genotypes (Lens culinaris Medik.). *International Journal of Agriculture and Crop Sciences*, *3*(1), pp. 1–5.

Hubert, J. and Lee, S. (2005) A review of the relative roles of silviculture and tree breeding in tree improvement: The example of Sitka spruce in Britain and possible lessons for hardwood breeding. *Forestry, 78*(2), pp. 109–120.

ISTA (2016) *International rules for seed testing*. Zurich.

Jacobs, D.F., Wilson, B.C. and Davis, A.S. (2004) Recent Trends in Hardwood Seedling Quality Assessment. In: Riley LE, Dumroese RK and Landis TD (Tech coords), *National Proceedings: Forest and Conservation Nursery Associations-2003. USDA Forest Service Rocky Mountain Research Station Proceedings RMRS-P-33.*USDA Forest Service Proceedings RMRS-P-33., pp. 140-144.

Javanmaer, Z. and Tabari Kouchaksaraei, M. (2017) Effect of pre-sowing seed treatments on germination traits and early seedling growth of eldar pine. *Ecopersia*, *5*(4), pp. 1969–1980.

Jones, S.K. and Gosling, P.G. (1994) "Target moisture content" prechill overcomes the dormancy of temperate conifer seeds. *New Forests*, *8*(4), pp. 309–321.

Kader, M.A. (2005) A comparison of seed germination calculation formulae and the associated interpretation of resulting data. *Journal and Proceedings of the Royal Society of New South Wales, (139)*, pp. 65–75.

Kanzler, A., Payn, K. and Nel, A. (2012) Performance of two *Pinus patula* hybrids in southern Africa. *Southern Forests: A Journal of Forest Science*, *74*(1), pp. 19–25.

Karrfalt, R.P. (2008) Seed harvesting and conditioning. In: F.T Bonner and R.G. Nisley (Eds.) *Woody plant seed manual: Agriculture handbook 727.* USDA Forest Service, pp. 57–84.

Karrfalt, R.P. (2008). Seed testing. In: F.T Bonner and R.G. Nisley (Eds.) *Woody plant seed manual: Agriculture handbook 727.* USDA Forest Service, pp. 97–116.

Karrfalt, R.P. (2011). Producing the target seed: seed collection, treatment and storage. In: Riley, L.E., Haase, D.L., Pinto, J.R. (Tech. coords.) *National Proceedings: Forest and Conservation Nursery Associations-2010.* Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station, 65, pp. 67–73.

Kaur, J., Schoonmaker, A.L. and Sobze, J.M. (2016) Length of cold stratification period affects germination in green alder (*Alnus viridis* (Chaix) DC. subsp. crispa (Aiton) Turrill) seed collected from north western Alberta. *Native Plants Journal*, *17*(2), pp. 95–102.

Kavipriya, R., Dhanalakshmi, P.K., Jayashree, S. and Thangaraju, N. (2011) Seaweed extract as a biostimulant for legume crop, green gram. *Journal of Ecobiotechnology*, *3*, pp. 16–19.

Khafagy, M.A., Mohamed, Z.A., Farouk, S. and Amrajaa, H.K. (2017) Effect of pre-treatment of barley grain on germination and seedling growth under drought stress. *Advances in Applied Sciences. 2*(3), pp. 33–42.

Kolawole, G.O., Adebayo, K.A. and Adebayo, M.A. (2011) Effect of seed sizes on the growth and establishment of seedlings of sheanut tree (*Vitellaria paradoxa*). *Journal of Agriculture and Science Technology*, *5*(4), pp. 428–432.

Kral, R. (1993) Pinus. In: Flora of North America Editorial Committee (Eds.) *Flora of North America North of Mexico, Vol. 2.* Oxford: Oxford University Press. http://www.efloras.org.

Kumar, G.N.M., Larsen, F.E. and Schekel, K.A. (2009) *Propagating plants from seed*. Washington State University Extension, U.S.A, pp. 5-12.

Landis, T.D. *Containers and growing media.* Vol. 2. *The container nursery manual, Agric. Handbook*, 674. Washington, DC: U.S. Department of agriculture, Forest service, pp. 41–85.

Landis, T.D. and Dumroese, R.K. (2006) Monitoring electrical conductivity in soils and growing media. *Forest Nursery Notes, Summer 2006*, pp.1–10.

Landis, T.D., Dumroese, R.K. and Haase, D.L. (2010) *The Container Tree Nursery Manual*. Vol. 7. *Seedling Processing, Storage, and Outplanting. Agriculture Handbkook, 674*. Washington, DC: U.S. Department of Agriculture Forest Service, pp. 199.

Langdon, O.G. (1958) Cone and seed size of South Florida slash pine and their effects on seedling size and survival. *Journal of Forestry, 56*(2), pp. 122–127.

Leadem, C.L. (1997) *Dormancy-unlocking seed secrets*. National Proceedings, Forest and Conservation Nursery Associations. Gen. Tech. Rep. PNW-G TR-419. Portland, OR: US Department of Agriculture, Forest Service, Pacific Northwest Research Station, pp.43-52. US Department of Agriculture, Forest Service, pp. 43-52.

Leishangthem, C. and Rana, A. (2017) Seed size and pre-sowing treatment effect on germination of some tropical and sub-tropical trees: A review. *International Journal of Current Microbiology and Applied Sciences*. *6*(7), pp. 2637–2645.

Li, B., McKeand, S. and Weir, R. (1999) Tree improvement and sustainable forestry: Impact of two cycles of loblolly pine breeding in the USA. *Forest Genetics 6*(4), pp. 229–234.

Lohrey, R.E. and Kossuth, S.V. (1990) *Pinus elliottii* Engelm. Slash pine in: Burns, R.M. and Honkala, B.H. (Eds.) *Silvics of North America.* Vol. 1. *Conifers, Agriculture Handbook 654.* Washington, DC, U.S. Department of Agriculture, pp. 338–347.

Luna, T., Wilkinson, K.M. and Dumroese, R.K., 1949. Seed germination and sowing options. Nursery manual for native plants: A guide for tribal nurseries, 1, pp.133-151.

Luna, T., Wilkinson, K. and Dumroese, R. (2009). Seed germination and sowing options. In: Dumroese, R., Kasten; Luna, T. and Landis, T.D. (Eds.) *Nursery manual for native plants: A guide for tribal nurseries.*Vol. 1: *Nursery management, Agriculture handbook 730.* Washington, DC: U.S. Department of Agriculture, Forest Service, pp. 133–151.

Lutts, S., Benincasa, P., Wojtyla, L., Kubala, S., Pace, R., Lechowska, K. and Garnczarska, M. (2016). Seed priming: New comprehensive approaches for an old empirical technique. New challenges in seed biology- Basic and translational research driving seed technology. *InTechOpen,* pp. 1–46.

Mackellar, R.D. (2008) *Effect of smoke solution on performance of Pinus elliottii and P. taeda seed*. Master's dissertation, University of KwaZulu-Natal.

MacLennan, L. and Fennessy, J. (2006) *Plant quality: A key to success in forest establishment.* Proceedings of the COFORD Conference, Mount Wolseley Hotel, Tullow, Co Carlow, Irish Republic, 20-21 September 2005. National Council for Forest Research and Development (COFORD).

Mandal, S.M., Dipjyoti, C. and Kajal, G. (2010) Seed size variation: Influence on germination and subsequent seedling performance in *Hyptis suaveolens* (Lamiaceae). *Research Journal of Seed Science*, *3*(3), pp. 185–192.

Marcos-Filho, J. (2015) Seed vigor testing: An overview of the past, present and future perspective. *Scientia Agricola, 72*(4), pp. 363–374.

Marwanto, M. (2004) Seed coat colour and quantity losses in cowpea seeds during delayed harvest. *Jurnul Ilmu Pertanian Indonesia, 6*(1), pp. 1–7.

Matsushima, K.I. and Sakagami, J.I. (2013) Effects of seed hydro priming on germination and seedling vigor during emergence of rice under different soil moisture conditions. *American Journal of Plant Sciences*, *4*(8), pp. 1584–1593.

Mbora, A., Schmidt, L., Angaine, P., Meso, M., Omondi, W., Ahenda, J. and Jamnadass, R. (2009) Tree seed quality guide.
https://www.infonet- biovision.org/sites/default/files/pdf/tree_seed_qualitz_guide.pdf

McKeand, S. (2015) The success of tree breeding in the southern US. *BioResources*, *10*(1), pp. 1–2.

Menzies, M.I., Bond, S.M.C. and Dibley, M. (1991) Factors affecting radiata pine seed germination. In: *Seed Symposium: Seed development and germination.* Special Publication (No.9).

Mikola, J. (1980) The effect of seed size and duration of growth on the height of Scots pine (*Pinus sylvestris*) and Norway spruce (*Picea abies* (L) Karst.) provenances and progenies at the nursery stage. *Silva Fenn*, *14*, pp. 84–94.

Mirgal, A.B., Gunaga, R.P. and Salunkhe, C.B. (2016) Seed size and its influence on germination, seedling growth and biomass in *Saraca asoca* (Roxb). De Wilde, critically endangered tree species of Western ghats, India. *Journal of Applied and Natural Science*, *8*(3), pp. 1599–1602.

Missanjo, E., Maya, C., Kapira, D., Banda, H. and Kamanga-Thole, G. (2013) Effect of seed size and pre-treatment methods on germination of *Albizia lebbeck*. *International Scholarly Research Notices*, *2013*, pp.1–4.

Missanjo, E. and Maya, C. (2015) Survival and growth response of seedlings to seed size size variation of *Albizia lebbeck* (L.) Benth. *Journal of Agriculture and Ecology Research International*, pp. 67–74.

Mng'omba, S.A., du Toit, E.S. and Akinnifesi, F.K. (2007) Germination Characteristics of Tree Seeds: Spotlight on Southern African Tree Species. *Tree and Forestry Science and Biotechnology*, *1*(1), pp.81–88.

Moles, A.T., Ackerly, D.D., Webb, C.O., Tweddle, J.C., Dickie, J.B. and Westoby, M. (2005) A brief history of seed size. *Science*, *307*, pp. 576–580.

Moosavi, S.A., Siadat, S.A., Koochekzadeh, A., Parmoon, G. and Kiani, S. (2022) Effect of seed color and size on cardinal temperatures of castor bean (*Ricinus communis* L.) seed germination. *Agrotechniques in Industrial Crops*, *2*(1), pp.1–10.

Moraes, G.J.P., Ferraz, I.D.K. and Procopio, L. (2015) Physiological immaturity and hydro priming of *Parkia nitida* Mig. seeds with physical dormancy. *Ciencia Florestal*, *25*(4), pp. 1053–1059.

Mosavian, S.N. and Eshraghi-Nejad, M., (2013) The effects of seed size and salinity on seed germination characteristic in wheat (var. Chamran). *International Journal of Farming and Allied Sciences*, *2*(2), pp. 1379–1383.

Mtambalika, K., Munthali, C., Gondwe, D. and Missanjo, E. (2014) Effect of seed size of *Afzelia quanzensis* on germination and seedling growth. *International Journal of Forestry Research, 2014,* Article ID 384565.

Mulawarman, Roshetko, J., Sasongko, S. and Irianto, D. (2003) Tree seed management: Seed sources, seed collection and seed handling. *A field manual for field workers and farmers.* TFRI extension series no. 152. Bogor, Indonesia: International Centre for Research in Agroforestry (ICRAF) and Winrock International.

Mut, Z. and Akay, H. (2010) Effect of seed size and drought stress on germination and seedling growth of naked oat (*Avena sativa* L.). *Bulgarian Journal of Agricultural Science*, *16*(4), pp. 459–467.

Mwase, W.F. and Mvula, T. (2011) Effect of seed size and pre-treatment methods of *Bauhinia thonningii* Schum. on germination and seedling growth. *African Journal of Biotechnology*, *10*(26), pp. 5143–5148.

Naidu, R.D. and Jones, N.B. (2007) Effect of seed size on field survival and growth of Eucalyptus in KwaZulu-Natal. *Southern Hemisphere Forestry Journal*, *69*, pp. 19–26.

Ndor, E., Dauda, N.S. and Chammang, H.B. (2012) Effect of germination media and seed size on germination and seedling vigour of fluted pumpkin (*Telferia occidentalis*) Hook. F. *International Journal of Agricultural Sciences, 2*(3), pp. 113–115.

Nilsson, O.N.B. (2014) *Growth and modulus of elasticity of selected pine species and hybrids three years after planting*. Master's thesis no. 222, Swedish University of Agricultural Sciences.

Noorbakhshian, S.J., Nabipour, M., Meskarbashee, M. and Amooaghaie, R. (2011) Optimization of hydro and osmo priming in different seed size of sainfoin (*Onobrychis visiifolia* Scop). *Australian Journal of Basic and Applied Sciences, 5*(11), pp. 1236–1244.

Okonkwo, H.O., Oyediran, R.I. and Dan, P.H. (2020) Effect of seed size and storage on the germination of *Artocarpus altilis* (Parkinson: Forsberg). *Journal of Research in Forestry, Wildlife and Environment*, *12*(2), pp. 53–58.

Owoh, P.W., Offiong, M.O, Udofia, S.I. and Ekanem, V.U. (2011) Effects of seed size on germination and early morphological and physiological characteristics of *Gmelina arborea*, Roxb. *An International Multidisciplinary Journal*, *5*(6), pp. 422–433.

Pammenter, N.W. and Berjak, P. (2014) Physiology of desiccation-sensitive (recalcitrant) seeds and the implications for cryopreservation. *International Journal of Plant Science*, *175*(1), pp. 21–28.

Patel, R., Pandya, K., Jasrai, R. and Brahmbhatt, N. (2017) Effect of hydro priming and biopriming of brinjal and tomato seed. *Research Journal of Agriculture and Forestry Sciences*, *5*(6), pp. 1–14.

Pinto, J.R., Marshall, J.D., Dumroese, R.K., Davis, A.S. and Cobos, D.R. (2011) Establishment and growth of container seedlings for reforestation: A function of stocktype and edaphic conditions. *Forest Ecology Management, 261*(11*)*, pp. 1876–1884.

Pliszko, A. and Kostrakiewicz-Gierałt, K. (2018) Effect of cold stratification on seed germination in *Solidago× niederederi* (Asteraceae) and its parental species. *Biologia*, *73*(10), pp. 945–950.

Popescu, M. (2016) Comparative studies of seaweed extract on seed germination of tomato. *Current Trends in Natural Sciences*, *5*(10), pp. 117–122.

Poynton, R.J. (1977) *Tree planting in Southern Africa: The Pines, Vol 1*. Pretoria: Department of Forestry, South Africa.

Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Julia Bally, J., Job, C. and Job, D. (2012) Seed germination and vigor. *Annual Review Plant Biology*, *63*, pp. 507–533.

Rao, G.N. and Chatterjee, R. (2014) Effect of seaweed liquid fertilizer from *Gracilaria texttorii* and *Hypnea musciformis* on seed germination and productivity of some vegetable crops. *Universal Journal of Plant Science*, *2*(7), pp. 115–120.

Robertson, N., Eggelston, K., Overton, E. and McLaughlin, M. (2013) Lengthened cold stratification improves bulk white bark pine germination. In: Haase, D.L., Pinto, J.R., Wilkinson, K.M. (Tech. coords) *National Proceedings: Forest and Conservation Nursery Associations-2012.* Proceedings RMRS-P-69. Fort Collins, CO: US Department of Agriculture, Forest Service, Rocky Mountain Research Station, 69. p. 72–76.

Rolando, C. and Allan, R., (2004) Survival and growth of *Pinus patula* at two years in response to harvest residue and pest management, in Mpumalanga, South Africa. *The Southern African Forestry Journal*, *200*(1), pp.19–26.

Rutkoski, J.E. (2019) Estimation of realized rates of genetic gain and indicators for breeding program assessment. *Crop Science*, *59*(3), pp.981–993.

Sadeghi, H., Khazaei, F., Sheidaei, S. and Yari, L. (2011) Effect of seed size on seed germination behaviour of Safflower (*Carthamus tinctorius* L.). *ARPN Journal of Agriculture and Biological Science*, *6*(4), pp. 5–8.

Salma, L., Aymen, E.M., Maher, S., Hassen, A., Cherif, H., Halima, C. and Mimoun, E. (2014) Effect of seaweed extract of Sargassum vulgare on germination behavior of two bean cultivars (*Phaseolus vulgaris* L.) under salt stress. *IOSR Journal of Agriculture and Veterinary Science*, *7*, pp. 116–120.

Santos, B. (2010) *Seed and Seed Quality*. Institude of Food and Agricultural Sciences. http://www.fao.org/fileadmin/templates/tc/tce/pdf/Appendix_14_Seed_and_Seed_Quality_for_Emg.pdf

Sasikala, M., Indunathi, E., Radhika, S. and Sasireka, R. (2016) Effect of seaweed extract (*Sargassum tenerrimum*) on seed germination and growth of tomato plant. *International Journal of ChemTech Research*, *9*(9), pp. 285–293.

Schutz, C.J. (1994) Site requirements for pine species. In: Van der Sijde, H.A. (Ed.) *Southern African Forestry Handbook 1994*. Pretoria: Aurora Printers, pp. 125–137.

Schulze, R., and Maharaj, M. (2007) *Pinus elliottii* growth areas and yield estimation. In: Schulze, R.E. (Ed). *South African Atlas of Climatology and Agrohydrology*. WRC Report 1489/1/06, Section 18.6. Pretoria: Water Research Commision.

http://sarva2.dirisa.org/resources/documents/beeh/Section%2018.6%20Pinus%20elliottii.pdf

Sepehri, A., Rouhi, H.R. (2017). Effect of hydro priming on morphological and physiological performance of aged groundnut (*Arachis hypogaea* L.) seeds. *Iranian Journal of Field Crop Science*, *Special Issue*, pp. 43–53. https://www.researchgate.net/profile/Hossein_Rouhi/publication/320705844_Effect_of_hydropriming_on_morphological_and_physiological_performance_of_aged_groundnut_Arachis_hypogaea_L_seeds/links/59f65bb60f7e9b553ebd29ec/Effect-of-hydropriming-on-morphological-and-physiological-performance-of-aged-groundnut-Arachis-hypogaea-L-seeds.pdf

Shaban, M. (2013) Study on some aspects of seed viability and vigor. *International Journal of Advanced Biological and Biomedical Research, 1*(12), pp. 1692–1697.

Sharma, S. (2018) Seed vigour testing: Principles and methods. *AGROBIOS NEWSLETTER, 17(2),* pp. 80–82. https://www.researchgate.net/publication/326255175_Seed_Vigour_Testing_Principles_and_Methods/link/5b414e32a6fdccbcf90a897c/download

Sharma, S.G.C. and Gairola, S. (2009) Environmental variation in seed and seedling characteristics of Pinus roxburghii Sag. from Uttarakhand, India. *Applied Ecology and Environmental Research, 7*(2*)*, pp. 121–129. http://www.epa.hu/02500/02583/00015/pdf/EPA02583_applied_ecology_2009_02_121-129.pdf

Shelar, V.R. (2008) Role of mechanical damage in deterioration of soybean seed quality during storage: A review. *Agricultural Reviews, 29*(3), pp. 177–184.

Singh, N.I., Ali, S. and Chauhan, J.S. (2009) Effect of seed size on quality within seed lot of pea and correlation of standard germination and vigour with field emergence test. *Nature Sci. 7*(4), pp. 72–78.

Sivritepe, N. (2008) Organic priming with seaweed extract (*Ascophyllum nodosum*) affects viability of pepper seeds. *Asian Journal of Chemistry*, *20*(7), pp. 5689–5694.

Skordilis, A. and Thanos, C.A. (1995) Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *Pinus halepensis*. *Seed Science Research, 5*, pp. 151–160.

Sluder, E.R. (1979) The effects of seed and seedling size on survival and growth of loblolly pine. *Tree Planters' Notes, 30*(4), pp. 25–28.

Sluder, E.R. (1991) Seed and seedling size grading of slash pine has little effect on long term growth of trees. *Tree Planter's Notes, 42*(3), pp. 23–27.

Sorensen, F.C. (1980) *Effect of date of cone collection and stratification period on germination and growth of Douglas-fir seeds and seedlings.* Research Note PNW-RN-346. Portland, OR: US Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station, 11, p. 346.

Souza, M.L. and Fagundes, M. (2014) Seed size as key factor in germination and seedling development of *Copaifera langsdorffii* (Fabaceae). *American Journal of Plant Sciences, 2014*.

Stayton, C.L. and Portie, D. (1972) *Pine bark growing medium increases hothouse tomato production.* Leaflet/Texas Agricultural Extension Service, no. 1032.

Sujatha, K., Vijayalakshmai, V. and Suganthi, A. (2015) Comparative efficacy of brown, red and green seaweed extracts on low vigour sunflower (*Helianthus annus* L.) var TN (SUF) 7 seeds. *African Journal of Agricultural Research, 10*(20), pp. 2165–2169.

Sulewska, H., Smiatacz, K., Szymanska, G., Panasiewicz, K., Bandurska, H. and Glowicka-Woloszyn, R. (2014) Seed size effect on yield quantity and quality of maize (*Zea mays* L.) cultivated in South East Baltic region. *Zemdirbyste-Agriculture 101*(1), pp. 35–40.

Swofford, T.F. (1958) Stratification to some loblolly and slash pine seed. *Tree Planter's Notes, 32*, pp. 5–6.

Tanaka, Y. (1984) Assuring seed quality for seedling production: Cone collection and seed processing, testing, storage, and stratification. In: Duryea M.L., Landis T.D., Perry, C.R. (Eds.*) Forestry nursery manual: Production of bareroot seedlings. Forestry sciences, 11*. Dordrecht: Springer, pp. 27–39,

Tanveer, A., Tasneem, M., Khaliq, A., Javaid, M.M. and Chaudhry, M.N. (2013) Influence of seed size and ecological factors on the germination and emergence of field bindweed (*Convolvulus arvensis*). *Planta Daninha*, 31*(1)*, pp. 39–51.

Thomson, A.J. and El-Kassaby, Y.A. (1993) Interpretation of seed-germination parameters. *New Forests*, *7*(2), pp.123–132.

Tian, Y., Guan, B., Zhou, D., Yu, J., Li, G. and Lou, Y. (2014) Responses of seed germination, seedling growth, and seed yield traits to seed pre-treatment in maize (*Zea mays* L.). *The Scientific World Journal*, *2014*, pp.1–8.

Uche, O.J., Adinde, J.O., Omije, T.E., Ager, C.J. andAnieke, U.J. (2016) Influence of hydro priming on germination and seedling emergence of green bell pepper (*Capsicum annuum* cv. Goliath). *International Journal of Science and Nature*, *7(*1), pp. 70–75.

United States Department of Agriculture, Natural Resource Conservation Service. Plant guide, *Pinus elliottii*. Retrieved from https://plants.usda.gov/plantguide/pdf/pg_piel.pdf on 21 st January 2019.

Van Wyk, G. and Van der Sijde, H.A. (1983) The economic benefits of forest tree breeding. *South African Forestry Journal*, *126*(1), pp. 48–54.

Verryn, S.D., Snedden, C.L. and Eatwell, K.A. (2009) A comparison of deterministically predicted genetic gains with those realised in a South African grandis breeding program. *Southern Forests: A Journal of Forest Science*, *71*(2), pp. 141–146.

Wang, R. (2005) *Modeling seed germination and seedling emergence in winterfat (*Krascheninnikovia lanata *(Pursh) ADJ Meeuse & Smit): Physiological mechanisms and ecological relevance*. Doctoral dissertation, University of Saskatchewan.

Weber, J.C. and Sorensen, F.C. (1990) *Effects of stratification and temperature on seed germination speed and uniformity in central Oregon ponderosa pine (*Pinus ponderosa *Dougl. ex Laws).* Research Paper PNW-RP-429, USDA, Forest Service.

Wen, B. (2009) Storage of recalcitrant seeds: A case study of the Chinese fan palm, *Livistona chinensis*. *Seed Science and Technology*, *37*(1), pp. 167–179.

Willan, R.L. (1986) *A guide to forest seed handling with particular reference to the tropics.* FAO Forestry Paper 20/2. http://www.fao.org/3/ad232e/ad232e00.htm

Yan, M. (2015) Seed priming stimulate germination and early seedling growth of Chinese cabbage under drought stress. *South African Journal of Botany, 99*, pp. 88–92.

Yang, J.C., Kuo, S.R. and Lin, T.P. (2007) Intermediate storage behaviour and the effect of prechilling on germination of Japanese Zelkova (*Zelkova serrata*) seeds. *Seed Science and Technology*, *35*(1), pp. 99–110.

Zareian, A., Hamidi, A., Sadeghi, H. and Jazaeri, M.R. (2013) Effect of seed size on some germination characteristics, seedling emergence percentage and yield of three wheat (*Triticum aestivum* L.) cultivars in laboratory and field. *Middle-East Journal of Scientific Research*, *13*(8), pp. 1126–1131.

Zulueta-Rodriguez, R., Hernandez-Montiel, L.G., Murillo-Amador, B., Rueda-Puente, E.O., Capistran, L.L., Troyo-Dieguez, E. and Cordoba-Matson, M.V. (2015) Effect of hydro-priming and bio priming on seed germination and growth of two Mexican fir tree species in danger of extinction. *Forests*, *6*(9), pp. 3109–3122.