A COMPARATIVE STUDY OF TWO *EUCALYPTUS* HYBRID BREEDING STRATEGIES AND THE GENETIC GAINS OF THESE STRATEGIES

by

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DECLARATION

I, GERHARDUS JOHANNES VAN DEN BERG, Student Number:14450888, declare that the dissertation, which I hereby submit for the degree of PhD (Forest Science) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution. The specific contributions of each co-author as listed in the published journal articles are as follow:

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SUMMARY

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by

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Keywords: *E. grandis*, *E. urophylla*, hybrid, breeding strategies, genetic gains

The hybrid combination between *Eucalyptus grandis* and *Eucalyptus urophylla* plays a significant role in the production of pulp in the South African Forestry Industry. Superior clones of this hybrid combination have been bred using a conventional hybrid breeding strategy and planted successfully in the subtropical coastal region of South Africa, namely Zululand. Although the conventional hybrid breeding strategy of these species has delivered superior clones for commercial production, it is a time consuming strategy to follow. One of the constraints with the conventional hybrid breeding strategy is the time required to first test the hybrid material as seedlings before clonal testing commence. In order to address this, an accelerated version of the conventional hybrid breeding strategy is being investigated with the main focus on reducing the testing time of *E. grandis* × *E. urophylla* hybrid material as seedlings.

With this in mind, this study was set out with two main aims, firstly to review the conventional hybrid breeding strategy, and secondly to do a comparative study between the conventional hybrid breeding strategy and the accelerated hybrid breeding strategy. In order to review the conventional hybrid breeding strategy, as well as to identify the impact of accelerating this strategy has, information on the genetic control of the traits of interest is needed for the *E. grandis* and *E. urophylla* pure species populations, as well as hybrid

seedling and clonal populations. Additional information such as the correlation between parents' performance in pure species and hybrid combination, as well as the ortet-ramet correlation of the hybrids is also essential for developing an effective hybrid breeding strategy.

The main objectives of this study were, therefore, to first estimate the genetic parameters and indentify selections of *E. grandis* and *E. urophylla* pure species populations in Zululand; secondly to estimate genetic parameters of *E. grandis* × *E. urophylla* hybrid seedling and clonal populations; thirdly to investigate the correlation between *E. grandis* and *E. urophylla* parental General Combining Ability (GCA) and their General Hybridising Ability (GHA); fourthly to determine the correlation between *E. grandis* × *E. urophylla* hybrid seedling ortets and their ramets and lastly to do a comparative study between the conventional hybrid breeding strategy and the accelerated version of this strategy.

Results of the *E. grandis* and *E. urophylla* pure species populations indicated that genotype by environment interaction (G×E) effects were present, but would be practically negligible for growth in Zululand and a single breeding population will therefore be appropriate. In general, volume was under low to moderate genetic control, with narrow-sense heritabilities ranging between 0.14 and 0.48 for *E. urophylla*, and between 0.16 and 0.23 for *E. grandis*. Best linear unbiased predictor (BLUP) estimates indicated that elite selections could produce genetic gains of approximately 60% and 30% over the *E. urophylla* and *E. grandis* population means respectively.

With regards to the *E. grandis* × *E. urophylla* hybrid populations, results indicated that non-additive genetic variation explained the majority of the total genetic variation and ranged between 68% (seedling population) and 88% (clonal population from accelerated strategy). For the *E. grandis* × *E. urophylla* seedling population, most of the additive genetic variance was contributed by the *E. urophylla* parents (h^2_{male} = 0.23) and a correlation of 0.58 ($p<0.007$) was detected between the GCA and GHA values of the *E. urophylla* parents. However, for the *E. grandis* × *E. urophylla* clonal populations, narrow-sense heritabilties were low and ranged between zero (female) and 0.08 (male). Majority of the non-additive genetic variation was explained by the proportion of dominance variance (d^2=0.16), and less by the clone within family effect (c^2=0.12).

Accelerating the conventional hybrid breeding strategy by shortening the testing time of *E. grandis* × *E. urophylla* seedlings resulted in an increase in percentage realised volume

gains per year (from 1.9% to 3.7%) when compared to the *E. grandis* × *E. urophylla* commercial clone.

PUBLICATIONS AND PRESENTATIONS FROM THIS THESIS

Peer-reviewed publications

Van den Berg GJ, Verryn SD, Chirwa PW, Van Deventer F. 2016. Genetic parameters and genotype by environment interaction of *Eucalyptus grandis* populations used in intraspecific hybrid production in South Africa. *Southern Forests: a Journal of Forest Science.* DOI 10.2989/20702620.2016.1254900.

Van den Berg GJ, Verryn SD, Chirwa PW, Van Deventer F. 2016. Estimates of genetic parameters and genetic gains for growth traits of two *Eucalyptus urophylla* populations in Zululand, South Africa. *Southern Forest: a Journal of Forest Science* 78(3): 209 – 216. DOI 10.2989/20702620.2016.1162616.

Van den Berg GJ, Verryn SD, Chirwa PW, Van Deventer F. 2015. Genetic parameters of interspecific hybrids of *Eucalyptus grandis* and *E. urophylla* seedlings and cuttings. *Silvae Genetica* 64(5 – 6): 291 – 308.

Van den Berg GJ, Verryn SD, Chirwa PW, Van Deventer F. 2017. Realised genetic gains and estimated genetic parameters of two *Eucalyptus grandis* × *E. urophylla* hybrid breeding strategies. *Southern Forests: a Journal of Forest Science.* DOI 10.2989/20702620.2016.1263010

Conference and field day presentations

Van den Berg GJ, Verryn SD, Chirwa PW, Van Deventer F. 2014. A comparison between two hybrid breeding strategies employed in the production of *Eucalyptus grandis* × *Eucalyptus urophylla* in South Africa. *IUFRO Forest Tree Breeding Conference,* 25 – 29 August 2014, Prague, Czech Republic.

Van den Berg GJ, Verryn SD, Chirwa PW, Van Deventer F. 2014. A comparison between two hybrid breeding strategies employed in the production of *Eucalyptus grandis* × *Eucalyptus urophylla* in South Africa. *ICFR KZN Midlands Regional Interest Group Field Day,* 20 November 2014, Wartburg, South Africa.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 INTRODUCTION

1.1 BACKGROUND

The global demand for wood is increasing due to trends such as an increasing world population, decreasing availability of wood, economic growth in emerging economies, globalisation of forest product market, climate change and the demand for high quality value added wood products (FAO 2010, 2011). Promoting planted forests is the only way to address the wood scarcity problem and to avoid illegal logging of natural forests (Fenning and Gershenzon 2002). Eucalypt plantations are an excellent alternative due to their fast growth, suitability of wood properties to many products, huge variability and suitability to vegetative propagation (Rezende et al. 2014). The biggest gains in plantation forestry of the last two decades have been in the clonal deployment of eucalypts hybrid genetic material (Griffin et al. 2000). Eucalypt hybrid clonal forestry can go a long way to fulfil present and future global wood needs. However, specific breeding and deployment strategies are required to optimise gains in productivity and wood quality in this context (Rezende et al. 2014).

Eucalyptus hybrids hold the potential to produce genotypes with special combinations of properties that may increase the value of the genetic resources, e.g. specific timber properties, disease resistance and greater vigour on specific sites compared to the combinations of the same properties in the pure species (Hettasch et al. 2005). Interspecific hybrids between *Eucalyptus urophylla* and *Eucalyptus grandis* in particular, are becoming increasingly important for enhancing yields on some types of sites and for improving disease resistance (White et al. 2007). The hybrid combination between *E. grandis* and *E. urophylla* has been planted successfully as clones in the subtropical coastal region of South Africa, and plays a significant role in the production of pulp in the South African Forestry Industry (Retief and Stanger 2009).

The *E. grandis* × *E. urophylla* hybrid combination in South Africa has been bred by mainly using a conventional hybrid breeding strategy (CHBS). The CHBS is firstly to maintain breeding populations of parental species (**Figure 1.1**). Elite individuals are then selected from these populations for interspecific hybrid crosses. Elite selections (backwards or forwards) are based on the parents' performance as a pure species. The elite parents are used

for interspecific hybrid crosses, and hybrid material derived from these crosses is tested as seedlings in seedling progeny trials. Ortets are then selected from the seedling populations and ramets are tested as clones. One of the underlying assumptions of the CHBS is that the parents' pure-hybrid correlation is high. This correlation is a useful indicator of the consistency of parental performance when used as a hybrid parent compared to when the same selections are used as pure species parents. The second underlying assumption of the CHBS is that the performance of the seedling ortet is a good predictor of its ramets' performance.

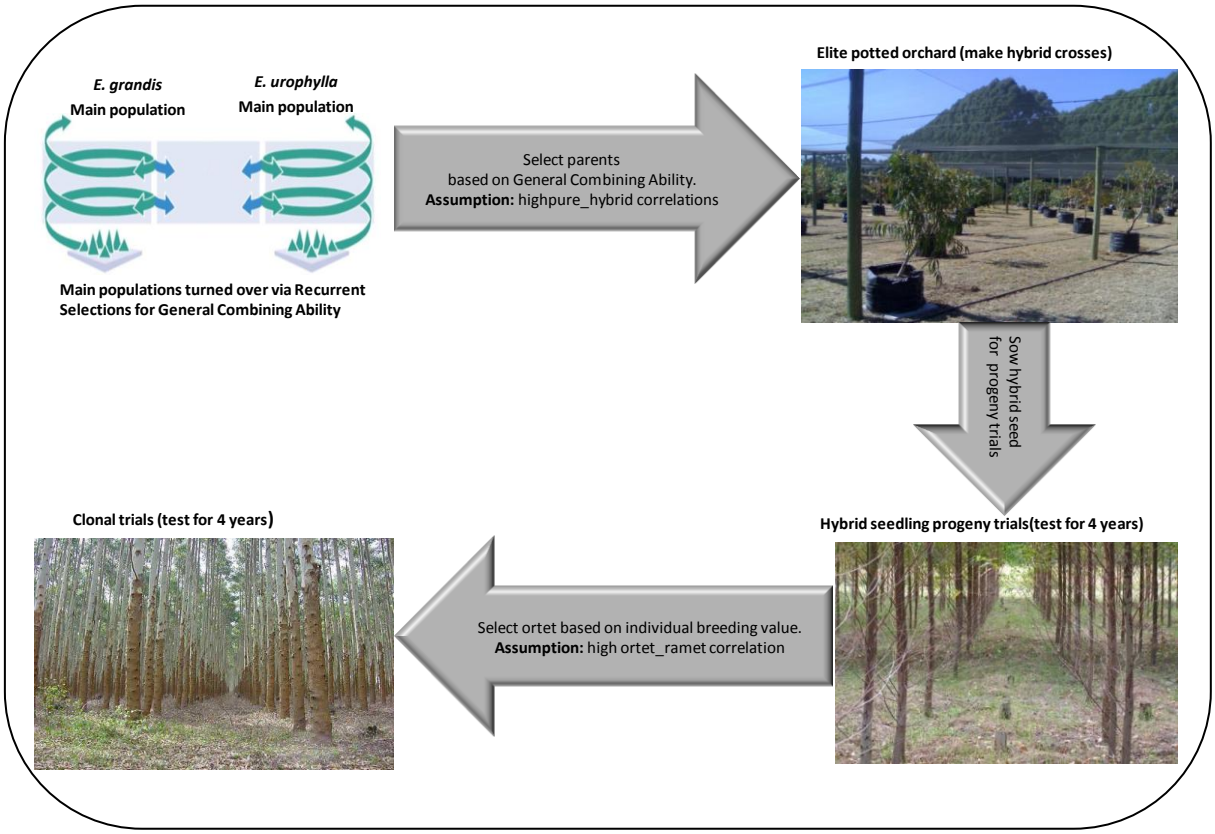

Figure 1.1 Diagram indicating the process followed for the conventional hybrid breeding strategy.

Although the CHBS has delivered superior clones with genetic gains of up to 50% (Gardner 2001), it is a time consuming and expensive strategy to follow and the need to investigate more cost-effective strategies has been identified. With this in mind, an accelerated version of the CHBS was investigated in this study. The main difference between

the strategies lies in the screening of *E. grandis* × *E. urophylla* seedlings as the first phase of testing hybrid material (**Figure 1.2**). In the accelerated strategy, the *E. grandis* × *E. urophylla* seedlings were tested over a shorter time period (1.5 years) and at a minimum cost. The hybrid seedlings are planted in a "hybrid seedling selection block" (HSSB) at the nursery instead of hybrid seedling progeny trials across several sites. At 1.5 years of age, the best individuals within each family are selected based on growth and resistance to pests and diseases. No measurements are taken at this stage and all selections are performed visually in order to save costs. Cuttings are then produced from the selected ortets and tested in clonal trials. The underlying assumption of the accelerated strategy is that bigger gains per unit time will be achieved due to shortening the testing time of the hybrid seedlings.

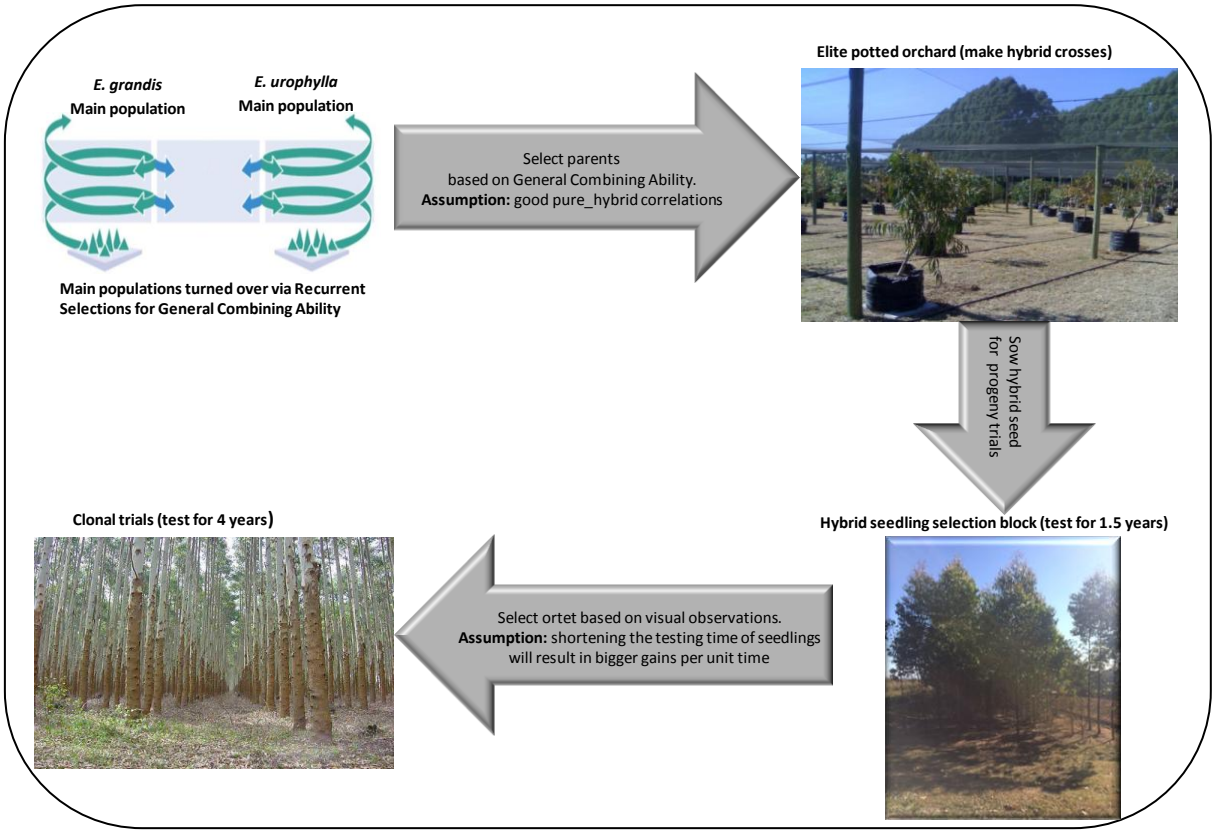

Figure 1.2 Diagram indicating the process followed for the accelerated version of the conventional hybrid breeding strategy.

In order to shed some light on this discourse and the underlying assumptions of both strategies, this study was conducted to firstly review the conventional hybrid breeding strategy, and secondly to investigate the accelerated version of the CHBS. To review the conventional hybrid breeding strategy, information on the genetic control of tree growth of the various populations involved (*E. urophylla* and *E. grandis* pure species populations and *E. grandis* × *E. urophylla* seedling and clonal hybrid populations), is needed. Additional information such as the parents' pure-hybrid correlation, as well as the ortet-ramet correlation of the hybrids is also essential for developing a good hybrid breeding strategy.

1.2 SIGNIFICANCE OF THE STUDY

Given the increasing demand for wood products globally, there is a requirement to optimise gains in productivity of planted forests. Specific breeding and deployment strategies are required to achieve this (Rezende et al. 2014). In this context, it is of utmost importance to review current breeding strategies and investigate alternative accelerated strategies. Reviewing the CHBS will shed some light on this discourse and will highlight key areas for tree breeders to focus on when constructing an alternative strategy.

In addition, genetic gains need to be achieved as rapidly as possible in order to justify expenditures associated with tree improvement. This has led to the concept of maximizing genetic gains per unit time instead of per cycle of breeding (White et al. 2007). Although the CHBS has delivered genotypes with significant gains, shortening the breeding cycle could have a significant impact on the gains per unit time, as well as associated costs. It is therefore important to test alternative accelerated strategies and identify the impact that such strategies may have on the gains per unit time. An accelerated strategy will also enable forestry companies to react quicker to the upward trend of pest and disease introductions.

1.3 OBJECTIVE OF THE STUDY

The aims of the study were to review the CHBS and to investigate an accelerated version of this strategy. To do this, the following objectives were identified:

- Estimate the genetic parameters for growth of *E. grandis* and *E. urophylla* pure species populations.
- Estimate the degree of genotype by environment interaction (G×E) of *E. grandis* and *E. urophylla* pure species population.
- Identify and calculate breeding values of elite *E. grandis* and *E. urophylla* parents to use for interspecific crosses.
- Estimate genetic parameters of *E. grandis* × *E. urophylla* hybrid seedling and clonal populations.
- Investigate the pure-hybrid correlation between *E. grandis* and *E. urophylla* parents and their hybrid seedlings and clones.
- Estimate the ortet-ramet correlation of selected *E. grandis* × *E. urophylla* hybrid individuals.
- Obtain genetic parameters of *E. grandis* × *E. urophylla* hybrid clonal populations derived from conventional and accelerated hybrid breeding strategies.
- Quantify the realised genetic gains per unit time for *E. grandis* × *E. urophylla* clones derived from conventional and accelerated hybrid breeding strategies.

1.4 STRUCTURE OF THE THESIS

This dissertation is composed of seven chapters. Chapter 1 is an Introduction chapter and Chapter 2 is a Literature Review that covers the relevant literature.

Chapters 3 – 6 are in the form of papers and have been peer reviewed and accepted as publications. Chapters 3, 4 and 5 were set out to shed light on the first aim of this study, namely to review the CHBS. Chapter 6 focuses on the second aim of this study and investigates the gains per unit time for the CHBS and the accelerated version of this strategy. **Table 1.1** Summarises the populations used for the analysis in each chapter.

Table 1.1 Summary of populations analysed to review the conventional hybrid breeding strategy and to investigate the accelerated strategy.

	Review of conventional hybrid breeding strategy				Compare conventional to accelerated strategy	
	Chapter 3	Chapter 4	Chapter 5		Chapter 6	
Species	*E. grandis*	*E. urophylla*	*E. grandis* × *E. urophylla*	*E. grandis* × *E. urophylla*	*E. grandis* × *E. urophylla*	*E. grandis* × *E. urophylla*
Trial type	Seedling progeny	Seedling provenance /progeny	Seedling progeny	Clonal	Clonal	Clonal
No. of trials and region	2 × Zululand 1 × Midlands	5 × Zululand	7 × Zululand	20 × Zululand	20 × Zululand	17 × Zululand
Treatments	116 full-sib families	219 half-sib families	108 full-sib families	148 clones	148 clones	211 clones

Chapters 3 and 4 use the results of improved full-sib *E. grandis* progeny trials and unimproved *E. urophylla* trials to characterise these populations from a genetic prospective. Estimates of variances and narrow-sense heritabilities are calculated to determine the breeding potential of the breeding population under consideration, to inform the breeding strategy and for use in selection of superior families and individuals for further intra-and interspecific breeding.

In chapter 5 estimates of genetic parameters are used to characterise *E. grandis* × *E. urophylla* hybrid seedling and clonal trials of the CHBS. The parents' pure-hybrid correlation, as well as the ortet-ramet correlation of the hybrids is explored in this chapter.

Chapter 6 is the last of the empirical chapters and describes two *E. grandis* × *E. urophylla* hybrid clonal populations, one derived from the CHBS, and the other from the accelerated version of this strategy. Genetic parameters and realised genetic gains of both clonal populations are quantified in this chapter.

Chapter 7 is a conclusion of the results of the thesis and suggestions for future studies are highlighted.

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CHAPTER 2 LITERATURE STUDY

2.1 BACKGROUND

Tree plantations have become an important renewable resource due to the increasing pressure on native forests from a growing global population (Kien et al. 2009). The *Eucalyptus* genus is the most common hardwood that is being planted in order to keep up with the increasing demand for timber products (Turnball 1999). *Eucalyptus* species are mainly native to Australia and belong to a diverse genus with more than 700 species adapted to a wide range of Australia's climatic conditions (Pryor and Johnson 1971). This genus occurs naturally at a wide latitudinal range from 7^{o}N to 43^{o}S and this could explain its ability to adapt to a range of climatic conditions, site types, management systems and product uses (Eldridge et al. 1993).

In South Africa, commercial forestry contributes a significant amount (ZAR 7.0 billion per annum) to the country's gross domestic product (GDP), whilst utilising only 1.1% of the total area of the country (Department of Agriculture, Forestry and Fisheries [DAFF] 2011). The total commercial timber plantation area in 2010 was approximately 1 271 300 hectares, of which *Eucalyptus* covers 41%. A total of 56% of the plantation area is managed for pulp production, 36% for saw logs, and 4% for mining timber with the remaining 4% for other purposes (DAFF 2011). Currently, *Eucalyptus grandis* is the most common eucalypt species planted as a pure species, or as a hybrid parent.

2.2 *EUCALYPTUS GRANDIS*

Eucalyptus grandis belongs to the subgenus *Symphyomyrtus* which consists of species with two operculums (Brooker and Kleinig 1990). Further taxonomic subdivision allows us to divide the subgenus into sections and series. *Eucalyptus grandis* falls into the section *Latoangulatae* and the series *Transversaria*. In its natural habitat, *E. grandis* mainly occurs on the coastal areas of New South Wales and Queensland, stretching from Newcastle to Bundaberg, and at altitudes ranging from sea level to 600 metres above sea level (m.a.s.l.). Scattered populations of *E. grandis* are also present in the wet tropics of northern Queensland where it grows at altitudes of up to 1100 m.a.s.l. (Boland et al. 2006, Slee et al. 2006). This

species generally occurs in frost-free areas and prefers deep moist, but well drained, loamy, alluvial or volcanic soils. *E. grandis* naturally grows in areas with temperatures ranging between mean minimum monthly temperatures of 2^{o}C and mean maximum monthly temperatures of 29^{o}C. The mean annual rainfall in its natural habitat varies between 725 mm and 3750 mm.

Due to its fast growth and adaptability, *E. grandis* has been planted on a wide variety of sites in South Africa (Van Wyk 1985). By 1981 approximately 78% of the total eucalypt forestry area in South Africa was planted to *E. grandis* (Directorate of Forestry 1981). Over and above its fast growth, *E. grandis* can also be utilised for a range of timber products, hence the large demand for this species (Van Wyk 1990). Due to the popularity of this species, numerous tree breeding studies have been conducted on this species in order to domesticate and improve the yields and the quality of its products. Hence, information on the genetic parameters of *E. grandis* populations across the world from various tree breeding programmes is available and is described below.

In Brazil for instance, Miranda et al. (2015) estimated narrow-sense heritability (h_i^2) to be between 0.30 and 0.50 for volume per hectare for open pollinated *E. grandis* seedlings at four different sites. However, they reported a significant genotype by environment interaction (G×E) and across site h_i^2 of 0.09. Another study conducted in Brazil by dos Santos et al. (2004) on open pollinated *E. grandis* seedlings indicated the potential to breed for various wood properties with h_i^2 ranging between 0.34 for basic density and 0.61 for specific gravity.

In Colombia, Osorio et al. (2003) conducted a study to investigate age-age and trait-trait correlations for *E. grandis* clonal populations and reported a broad-sense heritability (H_i^2) of 0.22 for mean annual increment (MAI) at six years of age. The high genetic correlation between three years and six years for wood density and MAI reported in their study, favours selection at age three. They also reported a G×E effect (r_B = 0.64) for *E. grandis* clones across three environments in Colombia.

In South Africa, *E. grandis* tree improvement started in 1962 (Van Wyk 1990). The genetic variation in growth performance and various wood quality characteristics of earlier *E. grandis* breeding populations in South Africa has been well described by Van Wyk (1976, 1980, 1985, 1990). Results from these studies indicated that there is enough additive variation

present for growth and wood quality traits to improve *E. grandis* through selection and sexual breeding efforts. Snedden et al. (2007) reported that additive variation explained 84% of the genetic variations in an *E. grandis* population consisting of 177 open pollinated families. In contrast, studies on controlled pollinated *E. grandis* populations showed that a higher proportion of the genetic variation can be explained by non-additive genetic variation and hence, support vegetative propagation of this species. For instance, relatively high dominance variance in *E. grandis* full-sib populations in South Africa was reported by Van Wyk (1990) and Retief and Stanger (2009a). Van Wyk (1990) suggested that additional genetic gains could be captured through repeat-controlled pollinations of such families and the vegetative propagation of its offspring. Another example of increasing genetic gains through vegetative propagation for *E. grandis* is by cloning a breeding population (Snedden and Verryn 2004). They reported a substantial increase in total genetic gains from 7.17% to 9.82% compared to a non-cloned open pollinated breeding population with the same number of families and individuals per family. However, none of these studies reported on the performance of *E. grandis* across various site classes in South Africa. One tree breeding study on the G×E of *E. grandis* in South Africa has been conducted by Pierce (2000). The author tested 27 *E. grandis* clones across 31 sites in South Africa in order to determine G×E and recorded no significant changes in clone rankings. It must be borne in mind that they only used 27 *E. grandis* elite clones for their study, and not a seedling population.

Although *E. grandis* has historically been the dominant hardwood pure species for South Africa's forestry industry, it has succumbed to the pressures of fungal diseases such as *Crysoporthe austroafricana* and *Coniothyrium* sp. cankers in the subtropical region of South Africa, namely Zululand (Retief and Stanger 2009a). Lately, *E. grandis* has also been infested by a gall wasp, namely *Leptocybe invasa*. In order to overcome challenges such as these, a hybrid breeding programme with *E. urophylla* was started in the early 1990's in Zululand. *Eucalyptus urophylla* showed to have more tolerance for diseases and insects and is therefore used as a hybrid partner with *E. grandis* (Retief and Stanger 2009a). The role of *E. grandis* is therefore shifting towards that of a hybrid partner, and breeding programmes in South Africa should focus on developing a breeding strategy to fill this niche. Information such as the correlation between general combining ability (GCA) and general hybridising ability (GHA) of *E. grandis* needs to be determined with a view to develop a better hybrid breeding strategy.

2.3 *EUCALYPTUS UROPHYLLA*

Eucalyptus urophylla belongs to the series *Transversaria* as is the case with *E. grandis*. This is important as the viability of hybrids between two species tends to increase the closer the relationship between two taxa. *Eucalyptus urophylla* is one of only two species that are not indigenous to Australia (Pryor et al. 1995), and naturally occurs on seven Indonesian islands, namely Flores, Adonara, Lembata, Pantar, Alor, Wetar and Timor. It occurs predominantly between 300 and 1100 m.a.s.l., except at Timor where it grows at an altitude of up to 2960 m.a.s.l. Mean monthly maximum temperatures vary from 27 – 30°C at 400 m.a.s.l. and decrease to between 17 and 21°C at 1900 m.a.s.l. across the seven islands. The dry season varies from two to eight months and the rainfall from 600 to 2500 mm per year (Martin and Cossalter 1976).

Eucalyptus urophylla has been planted as a pure species or as a hybrid partner by a number of organisations since the 1970's (Wright and Osorio 1996). *Eucalyptus urophylla* is especially popular in humid and sub-humid tropical climates (Eldridge et al. 1993). *Eucalyptus urophylla* is mainly deployed as a hybrid partner with other eucalypt species and is known to produce progeny with remarkable hybrid vigour for growth (Hodge and Dvorak 2015). Due to the increasing demand for this species, various tree breeding studies have been conducted for *E. urophylla* across the world and genetic information on the different breeding populations is available.

Historically, the majority of *E. urophylla* plantations have been established in Brazil (Wright and Osorio 1996). Studies on *E. urophylla* in Brazil generally indicate a large family variation for this species. For instance, Mori et al. (1988), Luz et al. (1996) and De Souza et al. (2011) reported family heritabilities of 0.50, 0.44 and 0.48 respectively. In the latter a significant G×E was detected between four tests and a loss of 26.7% in genetic gain due to G×E was calculated. A similar trend in G×E was detected for *E. urophylla* in Colombia (Wright and Osorio 1996) and in Indonesia (Nirsatmanto et al. 1996). However, even though the type B correlation was 0.49 for the two sites in Indonesia, the expected genetic gains of a selection index that incorporated across-site selections were slightly higher than that by the direct selection at each location (Nirsatmanto et al. 1996). In the case of Colombia, the

variation between 13 *E. urophylla* provenances at each site was significant and provides an opportunity to improve this species through breeding and selection (Wright and Osorio 1996). *E. urophylla* was however significantly outperformed by *E. grandis* in their study, but was still identified as a candidate species to undertake interspecific hybridization with *E. grandis* due to the greater disease resistance of *E. urophylla* × *E. grandis* grown in Colombia (Wright and Osorio 1996).

In contrast, low G×E levels were reported for South East China (Wei and Borralho 1997, 1998) and Northern Vietnam (Kien et al. 2009). Wei and Borralho (1997, 1998) conducted several studies on an *E. urophylla* population that were established across five sites in South East China that included 16 *E. urophylla* Indonesian provenances. They reported that G×E was unimportant for growth and wood property traits (Wei and Borralho 1997, 1998). Overall, growth traits were under moderate genetic control (h_i^2= 0.41 for diameter) and high for basic density (h_i^2= 0.71) and Pilodyn penetration (h_i^2 = 0.64). Even though wood property traits were under more genetic control, they concluded that volume is economically the more important trait for pulp production (Wei and Borralho 1999).

In Northern Vietnam, Kien et al. (2009) tested 144 open-pollinated *E. urophylla* families from nine natural provenances. In their study, G×E did not have a significant impact on the growth of *E. urophylla* across two sites and the genetic correlation between overbark diameter at 1.3 m (DBH) and height became stable after age three years. Lewotobi was the best performing provenance in terms of growth and this agreed with other reports on *E. urophylla* provenance performances in Vietnam (Kha et al. 2003). Family breeding values indicated that among the top 50 families, 18 come from Lewtobi and 13 from Egon. The pooled site h_i^2 for DBH was estimated at 0.24 at age nine years.

Lewotobi was also one of the top performing *E. urophylla* provenances tested in Malawi (Ngulube, 1989). Other provenances that performed well in terms of growth, stem form and basic wood density were West Alor, North East Pantar and Mandiri. The other eight provenances that were tested at Malawi were reported to be unsuitable for this area.

Reports describing *E. urophylla* populations in South Africa are sparse, even though some of the first *E. urophylla* imports date back as far as 1969. Darrow and Roeder (1983) reported on the performance of these early imports and concluded that wood mass production

(mean tree volume per hectare × weighted basic density) of *E. urophylla* was slightly better than *E. grandis* due to its higher wood density. The volume production of *E. grandis* however, was higher than the volume production of *E. urophylla*. Genetic parameters could not be calculated in their study due to the small number of treatments (14 seedlots).

One of the more comprehensive studies on *E. urophylla* populations in South Africa and in other countries were reported by Hodge and Dvorak (2015). They conducted a study on *E. urophylla* provenance variation in five countries and across 125 trial sites. A total of 62 provenances from the seven Indonesian islands were included in their study. A large variation between provenances was observed in their study with the best performing provenances showing up to 30% more volume than the mean. Within-provenance h_i^2 for volume at age three years ranged between 0.09 (at Venezuela) and 0.18 (at South Africa). Although the average between-country genetic correlation for growth traits was 0.72, they concluded that there is no one provenance or one island that is always superior over the others and that good and poor sources can be obtained from provenances such as Mt. Egon and Mt. Lewotobi. However, the relatively large provenance and family variation reported in their study set the stage for gains through selection and breeding.

Although these studies have indicated the potential to breed with *E. urophylla* as a pure species, it must be borne in mind that *E. urophylla* is mainly deployed as a hybrid partner with *E. grandis* for the pulp industry in South Africa. Genetic information on *E. urophylla* as a hybrid parent is therefore important to develop a good hybrid breeding strategy.

2.4 *EUCALYPTUS* HYBRIDS

Historically, *Eucalyptus* tree breeding programmes have focussed on the improvement of pure species (Eldridge et al. 1993). However, the biggest gains in plantation forestry of the last two decades have been in the clonal deployment of eucalypts hybrid genetic material (Griffin et al. 2000). There are many reports on the superiority of interspecific hybrids (Denison and Kietzka 1992, de Assis 2000, Kha and Cuong 2000, Potts et al. 2000, Verryn 2000, Vigneron and Bouvet 2000, Potts and Dungey 2004, Bison et al. 2006).

Various eucalypt hybrid combinations have been tested using one of the two species as a hybrid parent. Examples are interspecific hybrid combinations between either *E. grandis* or

E. urophylla with *E. camaldulensis* (de Assis 2000, Kha and Cuong 2000, Shen 2000), *E. exserta* (Kha and Cuong 2000), *E. glubulus* (Griffin et al. 2000), *E. dunnii* (Griffin et al. 2000), *E. pellita* (de Assis 2000, Vigneron and Bouvet 2000) and *E. tereticornis* (Shen 2000, Vigneron and Bouvet 2000, He et al. 2012). However, interspecific hybrids of *E. urophylla* and *E. grandis* in particular, have been successfully deployed in tropical and subtropical forestry plantations for a while, especially in Brazil (Ikemori 1984, Bison et al. 2006) and in Congo (Vigneron and Bouvet 2000). Sizeable eucalypt hybrid plantations also occur in other parts of South America and in countries such as South Africa, China and Indonesia (Dungey and Nikles 2000).

The superiority of hybrids may arise through heterosis or trait complementarity (Nikles and Griffin 1992). Heterosis is when the first generation hybrid progeny grows with more vigour than the best of both parents, whereas complementarity is the combination of characteristics which cannot easily be obtained in a pure species (Hettasch et al. 2005). Literature suggests that complementarity is the most common reason for eucalypt hybrid breeding, and enable tree breeders to identify suitable genotypes for areas which are marginal for the parental species (Namkoong et al. 1988, Denison and Kietzka 1992, Pots and Dungey 2004). A classic example of complementarity is the use of *E. urophylla* × *E. grandis* in Congo (Vigneron and Bouvet 2000) and Brazil (Campinhos and Ikemori 1989). Although *E. grandis* is favourable for its fast growth, it does suffer from canker and leaf fungi in these areas. *Eucalyptus urophylla* on the other hand, have good disease resistance and confers it in the hybrid combination with *E. grandis* (Pots and Dungey 2004).

Due to its popularity, various tree breeding studies on interspecific hybrids between *E. grandis* and *E. urophylla* have been conducted around the world. For example, Bison et al. (2006) compared the performance of open pollinated progenies and hybrid progenies of *E. grandis* and *E. urophylla* across three sites in Brazil. At age two years, the average hybrid progeny performance was 38.7% higher for growth than both the pure species' progeny performance. However, part of the heterosis in relation to parental means could be explained by inbreeding depression due to selfing that occurred in the open pollinated progenies of the pure species.

In Congo, the variance structure in *E. urophylla* × *E. grandis* hybrid populations has been well described by Bouvet and Vigneron (1996) and Bouvet et al. (2009). In the first

study, 14 males and 16 females were used in a factorial to generate 94 hybrid families. The additive variance explained 80% of the total genetic variance for diameter and tree volume, of which the male (*E. grandis*) additive variance only explained 30% and the rest by the female (*E. urophylla*) additive variance. This was mainly due to the reduced variability in the male population as a result of higher selection intensity (Bouvet and Vigneron 1996). They also reported a significant pure-hybrid correlation between the parent trees. However, in a later study when a substantially bigger population (88 females and 107 males representing 684 families) was evaluated, non-additive genetic variation explained 55% of the total genetic variation for growth (Bouvet et al. 2009). A potential explanation for this discrepancy is that parents used in later studies were more homogeneous due to higher selection intensity and could explain the decrease in additive variance (Bouvet et al. 2009).

In South Africa, Retief and Stanger (2009a and b) reported on genetic parameters estimates of *E. urophylla* and *E. grandis* intra- and interspecific hybrid populations. In the hybrid population, the non-additive genetic effect accounted for 60% of the total genetic variance for DBH (Retief and Stanger 2009a). The specific hybridising ability (SHA) estimates had a wider range (-23.0% to + 16.8%) in comparison with the specific combining ability (SCA) of the parental species. In terms of additive variance, the heritability estimates for DBH of the female and male parents from the *E. urophylla* × *E. grandis* factorial progeny trial was 0.14 and 0.21 respectively and as a result had a GHA ranging from -7.3% to +8.2%. However, the GCA estimates of the parents in the pure species dialles were all zero. Hence, they concluded that the mean performance of a parent as a pure species is not a good indicator of the parents' mean performance as a hybrid partner for growth (Retief and Stanger 2009a).

Several other studies on eucalypts also presented dominance to additive variance ratios of higher than one for growth (Vaillancourt et al. 1995, Hodge et al. 1996, Hardner and Tibbits 1998, Volker et al. 2008, Retief and Stanger 2009a). However, the results of the majority of latter studies were based on pure species populations.

In contrast to the growth, literature suggests that wood quality traits such as wood density, is under more additive genetic control when hybridising two *Eucalyptus* species (de Assis 2000, Bison et al. 2007). For instance, in South Africa, Retief and Stanger (2009b) and Malan (1993, 2000) found that the mean basic wood density of the *E. grandis* × *E. urophylla* hybrid were intermediate to the means of the parental species. The heritability estimates for

wood density of the female and male parents were 0.06 and 0.39 respectively (Retief and Stanger 2009b). The benefits of complementarity traits such as wood density should be borne in mind when constructing a hybrid breeding strategy. My study only focussed on growth traits and results and recommendations did not consider traits such as wood density.

Although many studies report on the superiority of interspecific first generation (F_1) hybrids, intraspecific controls are often absent or unrelated and makes it difficult to explain the differences between the hybrid and parental species (Potts and Dungey 2004). In addition, many of the vigorous F_1 hybrid combinations reported have been selected from a highly variable pool of F_1's that contain a high proportion of poor performing individuals (Potts and Dungey 2004). The inviability of hybrids may be expressed at germination, in the nursery or in field (Potts and Dungey 2004). Even though the germination of F_1 eucalypt hybrids has been successful (Tibbits 1988, Ellis 1991), hybrid inviability can be manifested thereafter in the nursery (Tibbits 1988, Harbard et al. 2000). Discarding abnormal seedlings in the nursery prior to planting may not eliminate all the abnormal phenotypes (Griffin et al. 2000, Lopez et al. 2000). For instance, De Assis (2000) noted that plants that looked normal at planting became abnormal up to two years after planting. Griffin et al. 2000 also reported that only 0.15% of a F_1 *E. grandis* × *E. globulus* population produced "normal" looking plants after two years of growth. However, De Assis (2000) reported that all F_1 factorials that included either *E. grandis* or *E. urophylla* (*E. grandis* × *E. camaldulensis*, *E. urophylla* × *E. camaldulensis* and *E. urophylla* × *E. pellita*) had high performing families and individuals when compared to various parental controls, even though the average growth of the F_1 hybrids was not better.

Hence, the success of most eucalypt interspecific breeding programmes relies on clonal propagation in order to test selected superior individuals and cost efficiency of the propagation process is key to their exploitation (Potts and Dungey 2004). It is therefore important to estimate the genetic parameters of hybrid clonal populations in order to develop a suitable hybrid breeding strategy, and key issues such the ortet-ramet correlation need consideration.

One of the key steps in the conventional hybrid breeding strategy is to make selections in the hybrid seedling progeny trial, vegetative propagate the selections and test them as clones. The underlying assumption of this step is that the performance of the seedling ortet is a good predictor of its ramets' performance. Literature with regards to ortet-ramet correlations

of *E. grandis* × *E. urophylla* is sparse. A study conducted by Reis et al. (2011) investigated the correlation between selected trees in a family test and their respective clones in a clonal test. The genetic correlations between ortets and ramets in their study were between 0 and 0.35 according to the estimator presented by Bernardo (2002). A simulation study conducted by Borralho and Kanowski (1995) concured that correlation between ortets and their ramets is expected to be low.

A potential reason that could influence the ortet-ramet correlation is the fundamental differences in the structure of their root system (Hartmann et al. 1990). For instance, Sasse and Sands (1997) reported that *E. globulus* seedlings had strongly gravitropic tap-roots, with two types of primary roots from which secondary roots emerged. Clones had no tap roots, but it had adventitious roots that were formed during propagation. Grossnickle and Russell (1990) found that cuttings of *Chamaecyparis nootkatensis* produced less new root area than seedlings over 21 days. Fuller and Little (2007) also reported that *E. grandis* seedlings had significantly longer roots than micro-cuttings, as well as a better distribution around the plug. However, no significance in growth was reported in their study. Gaspar et al. (2005) also reported that there was no significant difference between *E. globulus* cuttings and seedlings for growth and wood density. Sasse and Sands (1996) conducted a study to test the responses of *E. globulus* cuttings and seedlings to water stress and reported that the seedlings had greater water use than cuttings in the water stress treatments. Majority of these results were based on pure species and cuttings were produced from different genotypes than the seedlings. No direct comparison of ortets and their ramets have been reported on.

Other effects associated with cloning such as rooting ability of different individuals and C effects could also have an effect on ramet performance and hence the ortet-ramet correlations. C effects are related to non-genetic factors such as the age or environment of the original ortet that could inflate between-clone variances and upwardly bias epistatic genetic variance estimates (Libby and Jund 1962, Burdon and Shelbourne 1974, Costa e Silva et al. 2004). Ontogenic and morphological factors such as cutting position and size may also arise with cloning and could affect growth performance (Costa e Silva et al. 2004).

Majority of the results on F_1 hybrid populations in the literature are based on the performance of seedling populations and not clonal populations. In most countries, interspecific hybrids of *Eucalyptus* are commercially deployed as clones. Hence genetic

information on *Eucalyptus* clonal populations needs to be explored. Some literature on the performance of *Eucalyptus* clonal populations as pure species is available.

Costa e Silva et al. (2013) reported that the propagule type had no significant impact on genetic effects across clonal and seedling populations of the same twenty open-pollinated *E. globulus* families. The genetic correlation between seedlings and clones was 0.93 for DBH at age four years. The narrow-sense heritability for DBH based on clones (h_i^2=0.45) were higher than for seedlings (h_i^2=0.30). Another study performed on *E. globulus* indicated that the broad-sense heritability (H_i^2=0.16) of the cloned parent trees was similar to the narrow-sense heritability (h_i^2=0.18) of the progeny of those parent trees for DBH at age four years (Gaspar et al. 2005). When the heritability was obtained by using parent-offspring regression, it was slightly better (h_i^2=0.21). The improvement on accuracy of genetic parameter estimates by including cloned *E. globulus* parents into full-sib progeny trials was also demonstrated by Araujo et al. (1996). For instance, the accuracy of narrow-sense heritability improved when the parents and progeny data was combined (h_i^2=0.19±0.04) in comparison to only the progeny data (h_i^2=0.13±0.11). However, all of these studies were based on pure species clonal populations which did not go through an intense selection process as in the case of interspecific hybrid populations. Very few reports on the genetic parameter estimates of interspecific hybrid clonal populations could be found.

One study by Bouvet and Bailleres (1995) investigated the expression of growth and wood property traits among *E. urophylla* × *E. grandis* clones in the Congo. They reported differences in broad-sense heritability estimates for volume (H_i^2=0.78), percentage bark (H_i^2=0.83) and basic wood density (H_i^2=0.95). However, as noted in their study, the small size (31 clones) of the clonal population did not permit drawing of definitive conclusions and that a study on the efficiency of clone value prediction with ortet measurement is necessary (Bouvet and Bailleres 1995).

In general, there seems to be a gap in our knowledge in order to develop the best interspecific hybrid breeding strategy, especially in understanding the genetic parameters of interpsecific hybrid clonal populations. Nevertheless, studies on various hybrid breeding strategies have been conducted and reported on.

2.5 *EUCALYPTUS* HYBRID BREEDING STRATEGIES

"A breeding strategy is an overview or philosophy of the management of genetic improvement of a tree species used in man-made forests. A well-planned strategy makes one aware that current activities can have a great influence on future opportunities for genetic gains, and can help avoid cost and embarrassment of having to scrap an existing programme and start again" (Eldridge et al. 1993). A good breeding strategy should have calculations of predicted gains, costs and genetic diversity (Shelbourne, 1991). These figures can help tree breeders to make informed decisions with regards to the implementation and consequences of a breeding plan (Hettasch et al. 2005).

Classic examples of F_1 hybrid breeding strategies are reciprocal recurrent selection (RRS, Comstock et al. 1949), reciprocal recurrent selection with forward selection (RRS-SF, Nikles 1992), recurrent selection for general combining ability (RS-GCA, Jenkins 1940) and the development and crossing of inbred lines (Retief and Stanger 2009a). The majority of these breeding strategies have primarily been developed for use in agriculture crops. In forestry practices, hybrid breeding strategies are mostly an adaption of RRS, or use RS-GCA in the parent species (Dungey et al. 1999). Factors that could influence the predictability of which parents will produce the best hybrid combinations should be taken into account when deciding on which hybrid breeding strategy to use. The pure-hybrid correlation (GCA versus GHA) of parents, as well as the magnitude of specific hybridising ability (SHA), is the two most important factors to consider (Volker 2002). The various breeding strategies in the context of tree improvement are described by (Hettasch et al. 2005) as follows:

RS-GCA is a method of backwards selection. All the potential parents are mated to a heterozygous set of testers and the progenies resulting from these matings are established in field trials. Based on the progeny trials results, parents with good GCA are then selected to intermate and subsequently creating a new population. Selections for the next generation will be carried out in the latter population. In the case of hybrid breeding, the selected parents will also be used for interspecific crosses. However, in the case of unimproved populations or natural stands, results on progeny performance are not available and backwards selections are not possible. A Simple Recurrent Selection (SRS.) strategy could then be applied. This is a method of forward selection where parents are selected based on their phenotypic values. The

selected parents intermate and their progenies will be the source for individual selection in the following generation.

In the RRS strategy, two unrelated populations are chosen for breeding. These populations may consist of two species, such as *E. grandis* and *E. urophylla*. A random number of trees are selected from each population and crossed with each other. The progeny of these crosses are then tested in field trials. Parents are selected from each population based on the hybrid progeny performance. The parents intermate within each population and two new populations result. These two populations will be the source for the next cycle of selection. This strategy allows simultaneous selection for both GHA and SHA.

In the RRS-SF strategy, the backwards selection step is omitted and produces pure species and hybrid families concurrently (Nikles 1992). The RRS-SF therefore has a much shorter cycle than the RRS strategy and could affect the gains per unit time.

Alternatively to F_1 hybrid breeding strategies, advanced-generation hybrid breeding strategies can also be explored. Examples are introgression breeding, three-way or four-way crosses and the development of synthetic species (Retief and Stanger, 2009a).

In order to shed some light on different hybrid breeding strategies for tree breeding, Kerr et al. (2004) investigated the efficiency of various hybrid breeding strategies through a simulation study. Four strategies were considered in their simulation namely: RRS, RRS-SF, the development of a synthetic species (SYN) and pure species selection (PSS). In the PPS strategy, intraspecific crosses are completed and superior individuals are selected (forwards and/or backwards) based on their pure species performance. First generation hybrids are also bred with each cycle. Each strategy was simulated using various pure-hybrid correlations, additive and non-additive ratios and heritabilities. Results from their study indicated that SYN is the best strategy for improving hybrid forest trees when dominant variance is less than additive variance and the pure-hybrid correlations are positive. However, if one of the parental species exhibits mostly dominance variance, and the pure-hybrid correlations in both species are negative, then RRS-SF is the most profitable strategy (Kerr et al. 2004).

Alternatively, RS-GCA could also be a different strategy to follow if the correlation between GCA and GHA is good. The selected parents can be used to generate hybrid progeny either through controlled pollinations or by planting them in a mixed clonal orchard and collect open pollinated hybrid seed. The success of the latter will depend on the level of

outcrossing and inbreeding effect of the open pollinated seed. Campinhos et al. (1998) investigated these effects of open pollinated seed from an *E. grandis* and *E. urophylla* mixed clonal orchard in Brazil. Each *E. grandis* tree was surrounded by seven *E. urophylla* trees, and open pollinated seed was only collected from the *E. grandis* trees. The average outcrossing rates were estimated at 70.2% and the growth performance of inbred progeny was 30% lower than that of hybrid progeny.

Majority of these strategies focused on the performance of pure and hybrid seedling populations. However, in practise majority of eucalypt hybrid plantations are established with superior clones. The hybrid seedling populations merely provide a source where superior individuals are selected from and are tested as clones. Hence, our understanding of pure-hybrid correlations, and the magnitude of dominance and additive variance of the hybrid clonal populations is key in order to develop the best hybrid breeding strategy. The ortet-ramet correlation will also have a significant impact on developing a cost effective breeding strategy and needs investigation.

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CHAPTER 3 GENETIC PARAMETERS AND GENOTYPE BY ENVIRONMENT INTERACTION OF *EUCALYPTUS GRANDIS* POPULATIONS USED IN INTRASPECIFIC HYBRID PRODUCTION IN SOUTH AFRICA

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Gert J van den Berg, Steven D Verryn, Paxie W Chirwa and Francois van Deventer (2016):Genetic parameters and genotype by environment interaction of *Eucalyptus grandis* populations used in intraspecific hybrid production in South Africa, *Southern Forests: a Journal of Forest Science*, DOI: 10.2989/20702620.2016.1254900.

3.1 ABSTRACT

In South Africa, *Eucalyptus grandis* (*E. grandis*) is an important species due to its fast growth and general suitability of its timber for a range of products. However, *E. grandis* is susceptible to fungal diseases such as *Crysoporthe austroafricana* and *Coniothyrium* sp. cankers in the sub-tropical region of Zululand and is therefore mainly planted as a parental species in a hybrid combination with *Eucalyptus urophylla* (*E. urophylla*) in this region.

The current strategy is to maintain large breeding populations of both parental species in order to provide improved elite selections for hybrid crosses. In order to develop the best interspecific hybrid breeding strategy for *E. grandis*, it is important to first determine estimates of genetic parameters of the pure species parents. Estimating the genotype by environment interaction (G×E) is also necessary in proposing the basis for setting up breeding populations and selecting environmentally stable genotypes. With this in mind, two *E. grandis* full-sib progeny trials were planted in Zululand and one in the KwaZulu-Natal Midlands region.

The overall aim of this study was to determine the genetic parameters of these trials in order to help construct inter- and intraspecific breeding strategies for this species, as well as to predict genetic gains. In order to achieve this, the objectives were firstly to determine the magnitude of G×E of *E. grandis* across the three sites; secondly to estimate the genetic parameters for growth of the *E. grandis* parents selected for intraspecific crosses; and lastly to identify the best parents to use for intra- and interspecific crosses in future hybrid breeding programmes.

Results of our study indicated that G×E would be practically negligible for growth in Zululand and one group of elite parents can be used for hybrid crosses in this region. In general, growth traits were under low to moderate genetic control, and the variation in additive genetics enabled us to identify *E. grandis* parents that could be utilised for intraspecific crosses and deliver progeny with genetic gains of 28.4%. Our study also highlighted that a relatively large portion of the genetic variation was explained by dominance genetic variation and a strategy to capture this non-additive variation needs investigation.

Although our study as achieved the stated aims, it must be kept in mind that *E. grandis* is mainly used as a hybrid parent with *E. urophylla* in Zululand. A study to investigate

whether the parents with good general combining ability (GCA) values from our study are also good general combiners in interspecific hybrid combinations with *E. urophylla* needs to be conducted.

Keywords

E. grandis, diallel, general combining ability, specific combining ability, heritability, additive variance, non-additive variance

3.2 INTRODUCTION

In South Africa, approximately 520 000 ha are planted to the *Eucalyptus* genus (DAFF 2011). *Eucalyptus grandis* Hill ex Maiden (*E. grandis*) is one of the main species planted either as a pure species crop, or as an interspecific hybrid crop. The fast growth of *E. grandis* and the general suitability of its timber for a range of products are the main reasons for the large demand for *E. grandis* (Van Wyk 1990). However, *E. grandis* is susceptible to fungal diseases such as *Crysoporthe austroafricana* and *Coniothyrium* sp. cankers (Boerboom and Maas 1970, Foekel et al. 1976, Barnard et al. 1987, Conradie et al. 1990, Van Zyl and Wingfield 1999, Van Heerden and Wingfield 2002), especially in the sub-tropical regions such as Zululand in South Africa (Retief and Stanger 2009). Lately, *E. grandis* is also succumbing to infestation by the gall wasp *Leptocybe invasa*. *Eucalyptus urophylla* on the other hand has shown more tolerance for diseases and insects in Zululand and is therefore used as a hybrid partner with *E. grandis* (Retief and Stanger 2009). The role of *E. grandis* is therefore shifting towards that of a hybrid partner, and breeding programmes in South Africa should focus on developing a breeding strategy to support this switch.

In order to develop the best interspecific hybrid breeding strategy for *E. grandis*, it is important to determine estimates of genetic parameters such as general combining ability (GCA) and general hybridising ability (GHA) of the pure species and hybrid populations respectively. General combining ability is a measure of the genetic worth of a parent based on the average performance of the progeny from a particular parent, whereas GHA is a measure of the genetic worth of a hybrid parent based on the average performance of the hybrid progeny from the parent when crossed with various parents of a different species (Nikles and

Newton 1991, Hettasch et al. 2005). This information can be used to investigate to what extent good general combiners in pure species *E. grandis* are also good general combiners in interspecific hybrid populations. Estimating genotype by environment interaction (G×E) is also necessary in proposing the basis for setting up breeding populations and selecting environmentally stable genotypes. Some information on genetic parameters of *E. grandis* populations in South Africa is available (van Wyk 1990, Pierce 2000, Snedden and Verryn 2004, Snedden et al. 2007, Retief and Stanger 2009). However, all the studies that included *E. grandis* full-sib families were conducted on single sites and information on G×E is lacking.

The aim of this study was therefore to determine the genetic parameters *E. grandis* full-sib populations in order to help contruct a breeding strategy and predict genetic gains. The specific objectives were to (1) estimate the genetic parameters for growth of the *E. grandis* parents selected for intraspecific crosses (2) determine the magnitude of G×E of an *E. grandis* full-sib population and (3) identify the best *E. grandis* parents to use for intra- and interspecific crosses in future hybrid breeding programmes.

3.3 MATERIALS AND METHODS

3.3.1 Breeding material

The mating design consisted of a partial diallel with 46 *E. grandis* parents (**Figure 3.1**). The parents were selected from 37 unrelated families (second generation) in a series of four progeny trials (**Table 3.1**). All the parents were selected on their mature age phenotypes for growth, tree form and disease resistance. The basic assumption that parents were randomly selected from the population of interest when conducting a diallel mating design was therefore violated.

A total of 116 full-sib families were produced. Although only 12% of the cells of the diallel mating were completed, 40 out of 46 parents were crossed between four and 15 times.

MALE PARENTS	P3	P10	P12	P13	P18	P23	P24	P25	P26	P27	P28	P29	P30	P32	P33	P34	P35	P36	P37	P38	P39	P41	P42	P43	P45
P1							A,B,C																		
P2						A,B,C																			
P4														A,B,C											
P5					A,B,C		A,B,C							A,B,C											
P6			A,B,C		A,B,C		A												A,B,C				A		
P7	A	A				A,B,C		A,B,C				A,B,C		A,B,C				A,B,C				A,B,C			
P8			A,B,C			B,C	A,B,C		A,B			A,B		A,B,C				A,B,C							
P9	A																								
P10							A		A,B,C					A,B											
P11					A																				
P12													A												
P13																									
P14										A															
P15														A,B,C										A,B,C	A
P16						A	A		A,B,C																
P17				A,B	A,B,C		A,B				A,B,C					A,B									A,B
P18			A			A						A,B,C													
P19									A,B,C																
P20														A,B,C											
P21			A,B			A,B,C			A,B,C				A						A,B						A,B,C
P22					A																				
P24					A,B,C								A			A,B			A,B,C						
P25																					A				
P26					A,B,C	A,B	A						A												A,B
P27									A,B,C				A,B,C					A,B,C	A,B,C						
P28			A,B,C				A,B,C		A																
P29																	A,B,C								
P31						A,B,C				A,B,C							A	A	A,B,C						A,B,C
P32			A,B,C						A,B,C			A,B	A,B				A,B,C	A,B,C							
P35																					A				
P37												A,B,C						A,B,C			A,B,C				
P39															A			A,B,C							
P40										A															
P41										A		A,B													
P42					A,B,C		A,B,C											A,B,C			A,B,C				
P43												A,B,C					A	A,B,C	A		A,B,C				
P44																		A	A	A					
P45					A							A,B,C	A,B,C								A,B,C				
P46					A,B,C		A,B,C			A			A												

FEMALE PARENTS

Figure 3.1 Diagram indicating crosses in the mating design of the partial diallel. The sites at where the full-sib families were established are designated by A, B or C.

Table 3.1 Pedigree information of the *E. grandis* parents used in the diallel.

	Origin			
Parent	Trial	Family	Plot	Tree
P1	PROG_B	13	23	4
P2	PROG_B	14	63	6
P3	PROG_B	1	92	7
P4	PROG_B	17	62	1
P5	PROG_B	16	39	1
P6	PROG_B	2	96	3
P7	PROG_B	14	63	9
P8	PROG_B	3	45	1
P9	PROG_B	3	45	6
P10	PROG_B	2	96	6
P11	PROG_B	15	11	6
P12	PROG_C	21	45	6
P13	PROG_C	21	45	3
P14	PROG_C	21	45	4
P15	PROG_B	6	85	9
P16	PROG_C	20	10	1
P17	PROG_B	12	76	6
P18	PROG_C	18	1	9
P19	PROG_C	23	98	9
P20	PROG_C	22	47	6
P21	PROG_A	10	93	3
P22	PROG_A	10	48	4
P23	PROG_A	9	116	4
P24	PROG_A	4	122	3
P25	PROG_A	5	74	1
P26	PROG_A	8	196	3
P27	PROG_D	29	50	1
P28	PROG_A	7	132	2
P29	PROG_A	5	212	4
P30	PROG_D	33	63	2
P31	PROG_D	35	81	1
P32	PROG_D	26	40	2
P33	PROG_D	31	59	2
P34	PROG_D	36	84	5
P35	PROG_D	24	5	5
P36	PROG_D	30	57	7
P37	PROG_D	28	48	8
P38	PROG_D	37	90	9
P39	PROG_C	19	2	3
P40	PROG_D	24	5	5
P41	PROG_D	27	42	1
P42	PROG_D	25	7	7
P43	PROG_D	34	78	6
P44	PROG_D	32	60	7
P45	PROG_D	34	78	8
P46	PROG_A	11	148	1

3.3.2 Trial establishment and measurements

One *E. grandis* full-sub progeny trial (B) was established at the KwaZulu-Natal Midlands region and two (A and C) at Zululand. A detailed description of each site is presented in **Table 3.2**. Each trial was planted in a randomised complete block design (RCB) and replicated five times across each site. Each family was planted in a six tree line plot and at various spacing as indicated in **Table 3.2**. Trial measurements were done when the trees were seven years old, the rotation age. Growth traits height in metres and overbark diameter at 1.3 m (DBH, in centimetres), were measured. Tree volume was calculated according to the models for *E. grandis* developed by Bredenkamp and Loveday (1984).

Table 3.2 Site and trial information of *E. grandis* full-sib progeny trials. M.A.P. = mean annual precipitation, M.A.T. = mean annual temperature.

	A	B	C
		KwaZulu-Natal	
District	Zululand	Midlands	Zululand
Plantation	Nseleni	Melmoth	Nyalazi
Longitude	32° 03’ E	31° 18’ E	32° 16’ E
Latitude	28° 39’ S	28° 33’ S	28° 16’ S
M.A.P. (mm)	1070	941	961
M.A.T. (°C)	21	17	21
Altitude (m)	24	964	39
Major soil type	FW1210	Hu1200	FW1100
Effective rooting depth (m)	1.51	1.51	1.51
Planting date	03/10/1994	04/11/1994	07/10/1994
Site preparation	Rip and pit	Pit	Rip and pit
Espacement	3m × 3m	3m × 2m	3m × 3m
Number of families	116	81	66

3.3.3 Statistical analysis

3.3.3.1 Standardization of data

Forest tree growth traits often display a strong relationship between the mean of the trait and its phenotypic and genetic variances, such that field tests with bigger trees will have larger phenotypic and genetic variances than field tests with smaller trees (Hodge and Dvorak 2012). In order to deal with these different phenotypic and genetic variances across sites, White et al. (2007) recommend data standardization prior to analysis of variance, variance component analysis, or multi-site mixed model analysis. The variances that are used together in the linear model were therefore homogenized through standardization. In this way any spurious G×E was also eliminated (Burdon 1977, Eisen and Saxon 1983, Hill 1984). The data for this study was standardised to a mean of 100 as described by Hodge and Dvorak (2012). PROC STANDARD in SAS (SAS Institute 2002) was used for the standardizing process. The population mean for the growth trait was therefore interpreted as 100%, and predicted breeding values and all variance components were thus directly interpreted as percentage gain (above or below 100%) without back-transformation or rescaling.

3.3.3.2 Single site analysis of the diallel

The statistical model used for the diallel at each site was as follows:

$$y_{ijkl} = \mu + R_i + \text{gca}_j + \text{gca}_k + sca_{jk} + e_{ijkl}$$

Where,

y_{ijkl} = the l^{th} observation of the i^{th} replication for the jk^{th} family,

μ = overall mean,

R_i = fixed effect of the i^{th} replication,

gca_j or gca_k = the random GCA effect for the j^{th} female or the k^{th} male,

sca_{jk} = random specific combining ability (SCA) effect of the j^{th} and k^{th} parents,

e_{ijkl} = random within plot error term.

All effects, except the overall mean and replication effect, were assumed to be random and independently distributed. A diallel mating design is difficult to analyse with standard statistical programs due to its unique feature of a single observation with two levels of the same main effect, namely GCA. In order to overcome this challenge, a SAS program developed by Xiang and Li (2001) was used to analyse the data. Xiang and Li (2001) first constructed dummy variables for GCA effects with SAS PROC IML (SAS Institute 2002), then used PROC MIXED (SAS Institute 2002) to estimate variance components and to obtain a best linear unbiased prediction of random genetic effects (GCA and SCA) simultaneously. Some modifications were done to adapt it for single site analysis.

The relationship between variance components and the quantitative genetic model was used to estimate the additive and dominance variance (Falconer 1981).

$\hat{\sigma}_{\mathrm{a}}^2 = 4\hat{\sigma}_{\mathrm{gca}}^2$ is the additive variance due to the GCA effect,

$\hat{\sigma}_{\mathrm{d}}^2 = 4\hat{\sigma}_{\mathrm{sca}}^2$ is the dominance variance,

$\hat{\sigma}_{\mathrm{g}}^2 = \hat{\sigma}_{\mathrm{a}}^2 + \hat{\sigma}_{\mathrm{d}}^2$ is the total genetic variance,

$\hat{\sigma}_{\mathrm{total}}^2 = \hat{\sigma}_{\mathrm{g}}^2 + \hat{\sigma}_{\mathrm{e}}^2$ is the total phenotypic variance.

Heritabilities were estimated as:

$h_{\mathrm{i}}^2 = \frac{\hat{\sigma}_{\mathrm{a}}^2}{\hat{\sigma}_{\mathrm{total}}^2}$ is the narrow-sense heritability for the additive genetic effect,

$d^2 = \frac{\hat{\sigma}_{\mathrm{d}}^2}{\hat{\sigma}_{\mathrm{total}}^2}$ is the ratio of dominance variance to total individual phenotypic variance,

$H_{\mathrm{i}}^2 = \frac{\hat{\sigma}_{\mathrm{g}}^2}{\hat{\sigma}_{\mathrm{total}}^2}$ is the broad-sense heritability on an individual basis,

Standard errors of heritabilities were calculated by Dickerson's approximation (Dickerson 1969).

3.3.3.3 Combined and paired site analysis of the diallel

The statistical model used for the diallel to do the combined and paired site analysis was as follows:

$$y_{ijklm} = \mu + S_i + R_{j(i)} + gca_k + gca_l + sca_{kl} + S^*gca_{ik} + S^*gca_{il} + S^*sca_{ikl} + e_{ijklm}$$

Where,

y_{ijklm} = the m^{th} observation of the j^{th} replication for the kl^{th} family at the i^{th} site,

μ = overall mean,

S_i = fixed effect of the i^{th} site,

$R_{j(i)}$ = fixed effect of the j^{th} replication within the i^{th} site,

gca_k or gca_l = the random GCA effect for the k^{th} female or the l^{th} male,

sca_{kl} = random SCA effect of the k^{th} and l^{th} parents,

S^*gca_{ik} or gca^*m_{il} = the random GCA by Site Interaction,

S^*sca_{ikl} = random SCA by Site Interaction,

e_{ijklm} = random within plot error term,

The same SAS program that was written by Xiang and Li (2001) was used to estimate the GCA and SCA effects for all of the sites combined, as well as for each site pair. All genetic parameters were estimated the same way as described for the single site analysis. The predicted family means (or Breeding Values) were calculated as follows after all the GCA estimates were obtained (Van Wyk 1990):

$$BV_{kl} = GCA_k + GCA_l$$

In order to determine the genetic (GCA and SCA) correlations of the same trait expressed across sites as described by Burdon (1977), type B genetic (r_{Bgca} and r_{Bsca}) correlations were estimated as follow:

$$r_{\text{Bgca}} = \frac{\hat{\sigma}^2_{gca}}{\hat{\sigma}^2_{gca} + \hat{\sigma}^2_{s*gca}}$$

$$r_{\text{Bsca}} = \frac{\hat{\sigma}^2_{\text{sca}}}{\hat{\sigma}^2_{\text{sca}} + \hat{\sigma}^2_{\text{s*sca}}}$$

Type B correlation measures G×E that is due to rank changes across environments. This correlation over multiple sites can range between zero and one. An $r_B = 1$ indicates a perfect correlation between performance in different environments.

3.4 RESULTS

3.4.1 Single site analysis

Mean DBH, height, volume per tree and survival for each site are presented in **Table 3.3**. The differences between the family means were statistically significant ($p < 0.05$) for all the measurements at all the sites. Tree growth (DBH, height and volume) at the two Zululand sites (A and C) was similar with a mean DBH of 18.18 cm and 18.14 cm, respectively. Tree growth at the KwaZulu-Natal Midlands site was less with a mean DBH of 16.87 cm. The survival at all three sites (A, B and C) was good with mean survival rates of 89.9%, 88.4% and 87.0%, respectively. Site A had the biggest range in family means (DBH ranging from 14.2 cm to 22.7 cm). The range in family means was similar for sites B and C (DBH ranging from 12.9 cm to 19.6 cm at site B, and between 14.4 cm and 20.9 cm at site C). It must be borne in mind that more families (116) were established at site A than at site B (81 families) and site C (66 families), hence the bigger variation in family means.

Table 3.3 Means and ranges from the *E. grandis* partial diallel for diameter at breast height (DBH), height, tree volume and survival for the progeny trials at sites A, B and C.

Site		DBH(cm)	Height(m)	Volume(m^3)	Family survival
A	Number of trees	3340	3340	3340	3720
	Range of family means	14.2 – 22.7	19.6 – 27.5	0.1433 – 0.4570	50 – 100
	Range of individual tree values	7.6 – 28.0	9.0 – 37.6	0.0181 – 0.9057	0 – 100
	Mean	18.18	23.98	0.27	89.84
	SD	3.65	3.90	0.13	30.22
B	Number of trees	2255	2255	2255	2550
	Range of family means	12.9 – 19.6	19.9 – 25.1	0.1126 – 0.3087	66.67 – 100
	Range of individual tree values	7.9 – 24.5	15.9 – 28.8	0.0307 – 0.5313	0 – 100
	Mean	16.87	22.99	0.22	88.43
	SD	3.24	2.48	0.10	31.99
C	Number of trees	1906	1906	1906	2190
	Range of family means	14.4 – 20.9	21.1 – 26.0	0.1433 – 0.3618	50 – 100
	Range of individual tree values	9.0 – 24.8	16.9 – 29.0	0.0419 – 0.5487	0 – 100
	Mean	18.14	23.96	0.26	87.03
	SD	3.02	2.31	0.10	33.60

The GCA estimates for the *E. grandis* parents were the lowest for trial B (14.47, 3.97 and 75.35) and the highest for trial A (24.12, 10.79 and 213.78) for DBH, height and volume, respectively (**Table 3.4**). Hence, $\hat{\sigma}_a^2$ and h_i^2 followed the same trend as indicated in **Table 3.5**. Overall, h_i^2 was the highest for DBH (0.24, 0.19 and 0.24), second highest for volume (0.23, 0.16 and 0.23) and lowest for height (0.16, 0.15 and 0.21) for trials A, B and C, respectively.

Table 3.4 Variance components from the *E. grandis* partial diallel for diameter at breast height (DBH), height and tree volume for the progeny trials at trial sites A, B and C. GCA = general combining abilities, and SCA = specific combining abilities.

	Trial	GCA	SCA	Error
DBH	A	24.12±4.3	26.59±4.9	328.80±8.9
	B	14.47±6.9	19.83±6.5	256.15±7.9
	C	18.88±7.9	10.73±5.7	262.49±9.1
Height	A	10.79±1.8	14.46±3.2	224.34±4.5
	B	3.97±2.3	5.81±2.2	88.05±2.7
	C	5.49±2.7	2.68±1.9	90.20±3.1
Volume	A	213.78±21.3	249.48±32.3	2960.72±63.2
	B	75.35±37.6	128.69±40.8	1597.27±49.8
	C	111.66±47	64.30±34.8	1632.74±56.7

In contrast to the GCA values, SCA estimates (10.7, 2.7 and 64.3) were lowest at site C for DBH, height and volume respectively. Hence, $\hat{\sigma}_{\mathrm{d}}^2$ (0.14, 0.10 and 0.13) were also the lowest at this site **Table 3.5**. This is most likely due to the fact that the least amount of families (66) were established at site C. At sites A and B, $\hat{\sigma}_{\mathrm{d}}^2$ (106.37 and 79.3 for DBH) were higher than $\hat{\sigma}_{\mathrm{a}}^2$ (96.5 and 57.9 for DBH), and d^2 (0.26 and 0.26 for DBH) were therefore also higher than h_{i}^2 (0.24 and 0.19 for DBH). However, at trial C $\hat{\sigma}_{\mathrm{a}}^2$ (75.6 for DBH) was higher than $\hat{\sigma}_{\mathrm{d}}^2$ (42.9 for DBH) for all three growth traits. Total genetic variation was highest at site A (H_{i}^2 = 0.38) and lowest at site C (H_{i}^2 = 0.30). This is not surprising as site A had the most amount of families (116) and site C the least amount of families (66).

Table 3.5 Genetic parameter for diameter at breast height (DBH), height and tree volume for three *E. grandis* full-sib progeny trials established at sites A, B and C.

	DBH			Height			Volume		
	A	B	C	A	B	C	A	B	C
$\hat{\sigma}_a^2$	96.50±34.4	57.90±27.8	75.56±31.9	43.17±17.29	15.88±9.5	21.97±10.9	852.71±309.6	301.43±150.6	446.67±188.2
h_i^2	0.24±0.08	0.19±0.09	0.24±0.1	0.16±0.07	0.15±0.09	0.21±0.1	0.23±0.09	0.16±0.08	0.23±0.1
$\hat{\sigma}_d^2$	106.37±26.5	79.32±26.2	42.95±23	57.86±15.69	23.24±9	10.72±7.9	997.91±245.63	514.79±163.5	257.24±139.3
d^2	0.26±0.07	0.26±0.09	0.14±0.07	0.22±0.06	0.23±0.09	0.10±0.07	0.27±0.07	0.27±0.09	0.13±0.07
$\hat{\sigma}_g^2$	202.87	137.22	118.51	101.03	39.12	32.69	1850.62	816.22	703.91
H_i^2	0.38	0.35	0.31	0.31	0.31	0.27	0.38	0.34	0.30
$\hat{\sigma}_e^2$	328.80±8.9	256.15±7.9	262.49±9.1	224.34±4.5	88.05±2.7	90.20±3.1	2960.72±63.2	1597.27±49.8	1632.74±56.7
$\hat{\sigma}_{total}^2$	531.67	393.37	381.00	325.37	127.17	122.89	4811.34	2413.49	2336.65

3.4.2 Combined and paired site analysis

Variance components for each site pair and all the sites combined (A&B, A&C, B&C and A&B&C) are presented in **Table 3.6**. Only values for volume are presented as height and DBH are represented by volume production.

In contrast to the single site analysis, GCA estimates (37.43, 116.91, 24.15 and 76.12) were higher than the SCA estimates (0, 64.57, 0 and 0) for all the site pairs (A&B, A&C and B&C) and sites combined (A&B&C). Additive genetic variances (149.8, 467.6, 96.6 and 304.50) were therefore also higher than $\hat{\sigma}_{\mathrm{d}}^2$ (0, 258.3, 0 and 0), and h_{i}^2 (0.07, 0.24, 0.05 and 0.21) higher than d^2 (0, 0.13, 0 and 0) for the above mentioned site pairs and combined sites (**Table 3.7**). A potential reason for the discrepancy between the single and paired/combined site analysis, could be due to the fact that the site by GCA interaction estimates (77.67, 0, 61.28 and 37.09) were lower than the site by SCA estimates (146.8, 40.2, 101.1 and 117.89) for site pairs and all sites combined. This is an indication that GCA estimates were more stable than SCA estimates across sites, hence the higher values. The difference in the number of parents crossed with each other in the mating design could also have an effect on GCA and SCA estimates and might bias SCA estimates on some sites. Non-genetic effects such as C-effects and other nursery effects could also influence family performance, and hence have an impact on SCA estimates.

Table 3.6 Paired and combined site variance components for tree volume of *E. grandis* full-sib progeny trials established at sites A, B and C. GCA = general combining abilities, and SCA = specific combining abilities.

Trial pairs	A&B	A&C	B&C	A&B&C
GCA	37.43±31.71	116.91±38.81	24.15±27.21	76.12±27.82
SCA	0	64.57±25.82	0	0
Site*GCA	77.67±33.12	0	61.28±33.53	37.09±15.31
Site*SCA	146.80±28.61	40.16±17.38	101.14±27.32	117.89±20.71
Error	1806.49±35.91	1635.71±48.32	1561.16±36.25	1165.20±28.73

Overall, the Zululand site pair (A&C) had the highest GCA (116.91) and SCA (64.57) estimates for volume and had a higher broad-sense heritability (H_i^2 =0.31) than the site pairs that included the KwaZulu-Natal Midlands site (B). A potential reason for this could be due to the climatic similarities between the Zululand sites (A&C) when compared to the KwaZulu-Natal Midlands site (**Table 3.2**).

Table 3.7 Paired and combined site genetic parameter for tree volume for three *E. grandis* full-sib progeny trials established sites A, B and C.

Trial pairs	A&B	A&C	B&C	A&B&C
$\hat{\sigma}_a^2$	149.75±126.81	467.64±155.21	96.58±108.12	304.50±111.52
h_i^2	0.07±0.05	0.24±0.08	0.05±0.06	0.21±0.07
$\hat{\sigma}_d^2$	0	258.29±103.32	0	0
d^2	0	0.13±0.05	0	0
$\hat{\sigma}_g^2$	149.75	725.93	96.58	304.50
H_i^2	0.07	0.31	0.05	0.21
r_{Bgca}	0.32±0.28	1±0	0.28±0.32	0.67±0.16
r_{Bsca}	0	0.62±0.16	0	0
$\hat{\sigma}_e^2$	1806.49±35.90	1635.71±48.32	1561.16±36.23	1165.20±28.71
$\hat{\sigma}_{total}^2$	1956.24	2361.64	1657.74	1469.70

This result was also noticeable when G×E for GCA values was estimated, and the r_{Bgca} was equal to 1 for the Zululand site pair A&C (**Table 3.7**). This is an indication that there is no G×E between these two sites with regards to the growth performance of progeny from particular parents. However, G×E was detected for GCA estimates between the KwaZulu-Natal Midlands site (B) and the Zululand sites (A and C), with r_{Bgca} ranging from 0.32

(between sites A and B) to 0.28 (between sites B and C). The overall r_{Bgca} for all the sites combined was estimated at 0.67. G×E was also detected at the family level ($r_{Bsca} = 0$) for the combined sites (A&B&C) and the site pairs that included the KwaZulu-Natal Midlands site (A&B and B&C), but less so for the Zululand site pair ($r_{Bsca} = 0.62$).

The results above suggest that two separate *E. grandis* populations should be managed, one for Zululand and the other for the KwaZulu-Natal Midlands area. However, it must be borne in mind that only one trial was established on a KwaZulu-Natal midland site and that this trial was established at a different spacing and at different site conditions (**Table 3.2**). Additional factors such as C-effects, germination and other nursery effects could also influence SCA estimates and should be investigated in future studies. Further G×E studies regarding this discourse therefore needs to be conducted. For this reason, only the results of the Zululand sites will be further discussed in this paper.

Based on the multiple site analysis, a strategy to select for additive gene effects for Zululand will lead to genetic gains. Overall, GCA ranged from -22.9% to +17.3% and SCA from -12.7% to +14.6% across the two Zululand sites. As the large number of variables precludes presentation of all data, only GCA estimates and BV for volume of the top five parents for the two Zululand sites combined is given in **Table 3.8**. In a scenario where the five best parents (P5, P42, P8, P12 and P25), based on their GCA values, are crossed with each other (including reciprocals but excluding selfs), the improvement over the trial mean will be 28.4% according to their BV (**Table 3.8**). The latter gain estimate excludes potential gains or losses due to SCA effects.

Table 3.8 General combining abilities (GCA) and predicted breeding values (BV) of the five best *E. grandis* parents identified at the Zululand site pair (A&C). BV = female GCA + male GCA.

Female		Male		
Parent	GCA	Parent	GCA	BV
P5	17.3	P8	15.6	32.9
P5	17.3	P12	10.6	27.8
P5	17.3	P25	10.5	27.8
P5	17.3	P42	17.0	34.3
P8	15.6	P5	17.3	32.9
P8	15.6	P12	10.6	26.2
P8	15.6	P25	10.5	26.2
P8	15.6	P42	17.0	32.6
P12	10.6	P5	17.3	27.8
P12	10.6	P8	15.6	26.2
P12	10.6	P25	10.5	21.1
P12	10.6	P42	17.0	27.6
P25	10.5	P5	17.3	27.8
P25	10.5	P8	15.6	26.2
P25	10.5	P12	10.6	21.1
P25	10.5	P42	17.0	27.5
P42	17.0	P5	17.3	34.3
P42	17.0	P8	15.6	32.6
P42	17.0	P12	10.6	27.6
P42	17.0	P25	10.5	27.5
Average gain (%)				28.4

3.5 DISCUSSION

Results of our study indicated that managing one *E. grandis* breeding population for additive and non-additive genetic effects for Zululand should be sufficient due to the low G×E detected in this region. Although our study indicated that G×E did occur between the Zululand and KwaZulu-Natal Midlands sites, results should be treated with caution as only one trial was established in the KwaZulu-Natal Midlands and at a different spacing and climatic conditions than the Zululand trials. Another factor that could contribute to the G×E is the severity of diseases at the different sites. For instance, Van Heerden and Wingfield (1999) indicated a significant G×E effect when various *Eucalyptus* clones were inoculated with *Chryphonectria cubensis* at different localities. Although the *E. grandis* population in our study was not scored for disease tolerance, it is well known in the South African Forestry

Industry that this species has succumb to the pressures of fungal diseases in the Zululand region (Retief and Stanger 2009).

In contrast with our findings, Pierce (2000) recorded no significant changes in clone rankings when *E. grandis* clones were tested across 31 sites in South Africa (including Zululand and KwaZulu-Natal Midlands sites). It must be borne in mind that the author only used 27 *E. grandis* clones in his/her study, and not a seedling population as used in our study. Osorio et al. (2003) also reported a relatively low G×E effect (r_B = 0.64) for *E. grandis* clones across three environments in Colombia. One exception was the study performed by Miranda et al. (2015). They have reported significant differences in G×E effect for an open pollination *E. grandis* seedling population across four sites in Brazil. Despite this divergence in the literature, it is clear from our study that the combined site analysis for the two Zululand sites is sufficient to determine accurate genetic parameters of the selected *E. grandis* parents in order to develop the best strategy for an inter- and intraspecific hybrid breeding programme.

Moderate genetic control for all three growth traits was evident in this study. The heritabilities calculated in this study is consistent with those reported by others for *E. grandis*. For instance, Snedden et al. (2007) reported h_i^2 of 0.19 and H_{i}^2 of 0.21 for tree volume of *E. grandis* clones in Zululand. Miranda et al. (2015) estimated h_{i}^2 of volume per hectare to be between 0.30 and 0.50 for open pollinated *E. grandis* seedlings at four different sites in Brazil, and h_{i}^2= 0.09 across all four sites. Osorio et al. (2003) reported H_{i}^2 of mean annual increment to be between 0.21 and 0.52 for *E. grandis* clones across three environments. However, higher h_{i}^2 (0.53) for an *E. grandis* full-sib population in South Africa was reported by Van Wyk (1990).

Based on the multiple site analysis, a strategy to select for additive gene effects for the Zululand coastal region will lead to genetic gains. The relatively good h_{i}^2 indicates that individual tree selection should be practiced to obtain the best parent trees for further breeding work and for a seed production population. In forests tree breeding, the best full-sib families would often be expected from the parents with the highest general combining abilities (Cotteril et al. 1987). Van Wyk (1990) reported a good correlation between BV and observed genotypic values for an *E. grandis* full-sib population. Cotteril et al. (1987) reported similar results for full-sib pine trials in South Africa. This implies that a genetic gain (based on BV)

of 28.4% over the trial mean could be achieved if the five parents with the highest GCA values are crossed.

There is however, a high degree of relatedness among the offspring in this trial series and the selection of parents for further breeding purposes is restricted to avoid inbreeding. Inbreeding could be detrimental to growth performance in *E. grandis* as reported by Van Wyk (1981). Deviations from the predicted relatedness among offspring could also have an impact on the genetic parameter estimates and interpreting the estimates should be done with caution. For instance, SCA estimates could be bias where the mating design has only four parents crossed together. Nevertheless, the information from this trial series is useful to construct a production population with parents with high GCA values.

Additional gains could also be achieved by vegetative propagating selected parents to enrich the breeding population with superior genotypes. For instance, Snedden and Verryn (2004) reported that cloning an *E. grandis* breeding population can substantially increase the total genetic gains from 7.17% to 9.82% compared to a non-cloned open pollinated breeding population with the same number of families and individuals per family. Other studies executed by Matheson and Lindgren (1985), Park and Flower (1987) and Shelbourne (1992) also produced enhanced genetic gains by cloning individuals in a breeding population of various crops. However, the difference in costs to produce cutting versus seedlings should also be considered when conducting a cost-benefit analysis to determine the best strategy.

The relatively high dominance variance present in our study also provides an opportunity to increase genetic gains by propagating families with superior growth vegetatively (Van Wyk 1990). The phenomenon of relatively high dominance variance in an *E. grandis* full-sib population in South Africa was also reported in the studies conducted by Van Wyk (1990) and Retief and Stanger (2009). Such families could be reproduced through repeat-controlled pollinations and resultant seedlings can be vegetatively propagated for commercial production.

It must be kept in mind though that *E. grandis* is susceptible to fungal diseases such as *Crysoporthe austroafricana* and *Coniothyrium* sp. cankers and is therefore not grown as a pure species in the Zululand coastal region (Retief and Stanger 2009). It is mainly used as a hybrid parent with *E. urophylla*, and superior individuals of resultant *E. grandis* × *E.*

urophylla progeny are commercially deployed vegetatively. A study to investigate whether the parents with good GCA values from our study are also good general combiners in interspecific hybrid combinations with *E. urophylla* needs to be conducted in order to develop the best interspecific hybrid breeding strategy.

3.6 CONCLUSIONS

The study was set out with the objectives to investigate the magnitude of G×E of *E. grandis* across three sites, as well as to estimate the genetic parameters for growth of *E. grandis* parents selected for intraspecific crosses, which will also be used for interspecific crosses in future hybrid breeding programmes.

It was evident from our study that a single *E. grandis* population will be sufficient for the Zululand breeding programme. The variation in additive genetics enabled us to identify *E. grandis* parents with high GCA values that could be utilised for intraspecific crosses and deliver progeny with genetic gains of 28.4%. Our study also highlighted that a relatively large portion of the genetic variation was explained by dominance genetic variation, and that a strategy to capture this non-additive genetic variation could lead to additional genetic gains.

Although our study has offered an evaluative perspective on G×E and genetic parameter estimates of *E. grandis* full-sib populations planted in the Zululand region, a number of limitations was encountered, which need to be considered. Firstly, only one trial was established in the KwaZulu-Natal Midlands region and at a different spacing. Result with regards to G×E between the KwaZulu-Natal Midlands and Zululand regions should therefore be interpreted with caution. Secondly, the severity of diseases at the different sites was not assessed in our study and could potentially explain some of the G×E that occurred between the Zululand and KwaZulu-Natal Midlands sites.

In spite of the limitations of this study, it has attained its three primary objectives namely: to determine the magnitude of G×E of *E. grandis* full-sib populations planted in the Zululand region, to estimate the genetic parameters for growth of the observed *E. grandis* populations, and to identify the best *E. grandis* parents to use for intra- and interspecific crosses in future hybrid breeding programmes.

Although results from our study showed the potential to select for additive gene effects in *E. grandis* populations grown in Zululand, it must be kept in mind that *E. grandis* is mainly used as a hybrid parent with *E. urophylla* in this region. A study to investigate whether the parents with good GCA values from our study are also good general combiners in interspecific hybrid combinations with *E. urophylla* needs to be conducted.

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CHAPTER 4 ESTIMATES OF GENETIC PARAMETERS AND GENETIC GAINS FOR GROWTH TRAITS OF TWO *EUCALYPTUS UROPHYLLA* POPULATIONS IN ZULULAND, SOUTH AFRICA

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4.1 ABSTRACT

In South Africa, *Eucalyptus urophylla* is an important species due to its disease tolerance to fungal diseases such as *Crysoporthe austroafricana* and the *Coniothyrium* sp. cankers. It is mainly planted as a parental species in a hybrid combination with *E. grandis*. Generally, the *E. grandis* × *E. urophylla* hybrid has better disease tolerance and higher wood density than pure *E. grandis.* The current strategy is to maintain large breeding populations of both parental species in order to provide improved elite selections for hybrid crosses on a regular basis.

With this in mind, two *E. urophylla* populations, consisting of five provenance/progeny trials, were established in the subtropical region of Zululand. The overall aims of this study were to determine genetic parameters for *E. urophylla* in Zululand in order to contruct a suitable breeding strategy and predict genetic gains. To achieve this, the objectives were set to firstly determine the magnitude of genotype × environment interaction of *E. urophylla* in Zululand; secondly to estimate genetic parameter and correlations for DBH, height and volume; and lastly to identify selections to advance the current breeding population as well as to hybridise with *E. grandis*.

Results indicated a moderate degree of genotype by environment (G×E) interaction effects. In general, all growth traits were under low to moderate genetic control, with narrow-sense heritabilities ranging between 0.14 and 0.48 for volume. The genetic correlations between growth traits were high (0.98 and 0.99 for DBH-volume). This is an indication that DBH is a sufficient growth measure to use in *E. urophylla* breeding programmes. BLUP estimates indicated that a selection scenario of 200 individuals will generate genetic gains of 44.7% over the population mean. The estimated gains for the top 50 individuals that could potentially be used as hybrid parents to cross with *E. grandis* was 59.8% over the population mean.

Keywords

E. urophylla, genetic parameters, genetic correlations, genetic gains

4.2 INTRODUCTION

The *Eucalyptus* genus is now being planted worldwide in order to keep up with the demand for wood and fibre. *Eucalyptus urophylla* is one of the eucalypt species that has increased in popularity, especially in humid and sub-humid tropical climates of Africa, Latin America, Southern China and South-East Asia (Eldridge et al. 1993). In some countries *E. urophylla* is deployed as a pure species, but it is better known as an important hybrid partner and is commonly crossed with other eucalypt species to produce progeny that often exhibit remarkable hybrid vigour for growth (Hodge and Dvorak 2015).

In the subtropical regions of South Africa, namely Zululand, *E. urophylla* serves as a very important parental species in a hybrid combination with *E. grandis* Hill ex Maiden to produce fast-growing clonal plantations for the pulp industry. *Eucalyptus urophylla* is especially more tolerant to fungal diseases such as *Crysoporthe austroafricana* and the *Coniothyrium* sp than pure *E. grandis* in Zululand (Retief and Stanger 2009). Generally, the *E. grandis* × *E. urophylla* hybrid has better disease tolerance and higher wood density than pure *E. grandis* (Retief and Stanger 2009, Hodge and Dvorak 2015). The current breeding strategy is to maintain and improve large breeding populations of both parental species in order to provide elite selections for hybrid crosses on a regular basis. With this in mind, two *E. urophylla* populations were established in Zululand. A total of five trials were established and included a total of 219 families from 17 provenances.

In order to develop the best hybrid breeding strategy for these *E. urophylla* populations in Zululand, it is important to determine the genetic correlation, the magnitude of genotype by environment interaction (G×E) and estimates of genetic parameters of economically important traits. In this case, tree growth (height, diameter at 1.3 m [DBH] and volume) was identified as the most important trait. Estimating genetic correlations between the three growth traits could provide information to improve the cost effectiveness of the breeding programme. A good correlation between traits will provide the opportunity to measure fewer traits and make the programme more cost effective.

Various levels of G×E have been reported for *E. urophylla* (Mori et al. 1988, Wei and Borralho 1998, Hodge and Dvorak 2015). Mori et al. (1988) for example, have reported losses in volume of 26.7% due to G×E. It is therefore important to determine the magnitude of G×E for the two *E. urophylla* populations in Zululand in order to set up a breeding population and selecting environmentally stable genotypes.

Some information on genetic parameters for growth traits of *E. urophylla* is available. In general, growth of *E. urophylla* has been reported to be under low to moderate genetic control with narrow-sense heritabilities ranging between 0.11 and 0.41 in China (Wei and Borralho 1998), 0.1 and 0.31 in Vietnam (Kien et al. 2009), and 0.15 on average across five countries (Hodge and Dvorak 2015). However, the population structure and environmental factors of these studies are different to the ones in our study, and it is important to investigate the genetic parameters of *E. urophylla* in Zululand for hybrid breeding using this species.

The aims of this study were therefore to determine genetic parameters for *E. urophylla* in Zululand in order to contruct a suitable breeding strategy and predict genetic gains. The specific objectives were to (1) determine the magnitude of genotype × environment interaction of *E. urophylla* for the Zululand region, (2) estimate genetic parameters and correlations for DBH, height and survival, and (3) identify selections to advance the current breeding population as well as to hybridise with *E. grandis*.

4.3 MATERIALS AND METHODS

4.3.1 Breeding material

The first *E. urophylla* breeding material was imported during 1988 – 1990. Open-pollinated seed was collected from randomly selected trees located in 17 provenances in Indonesian islands. A total of 219 families from these provenances were imported and established in two populations (PE023 and PV042) across five sites. The parent trees of these families were situated at an altitude range between 175 – 1050 metres above sea level

(m.a.s.l.). **Table 4.1** gives a breakdown of all the *E. urophylla* provenances that were established in trials.

Table 4.1 Provenance data of two *Eucalyptus urophylla* populations (PE023 and PV042) established in five provenance/progeny trials in Zululand.

Provenance	No. of families	Year of import	Lat. S	Long. E	Alt. (m.a.s.l.)	Trial series
Egon	18	1988	8° 38’	122° 35’	515	PE023
Lewotobi	16	1988	8° 31’	122° 45’	398	PE023
Mandiri	5	1988	8° 15’	122° 45’	415	PE023
Wuko	4	1988	8° 33’	122° 33’	830	PE023
Ilwaki1	4	1990	7° 54’	126° 26’	490	PV042
Ilwaki2	28	1990	7° 52’	126° 27’	515	PV042
Mareke Arnau	28	1990	7° 49’	126° 10’	300	PV042
Telemar	6	1990	7° 54’	125° 58’	200	PV042
Carbutu	8	1990	7° 56’	125° 53’	175	PV042
Rotus Uhak	37	1990	7° 39’	126° 29’	350	PV042
Old Uhak	6	1990	7° 36’	126° 37’	215	PV042
Lalikki	9	1990	7° 42’	126° 21’	220	PV042
Apui	10	1990	8° 17’	124° 40’	1050	PV042
Wai Kui	10	1990	8° 14’	124° 44’	580	PV042
Pintumas	15	1990	8° 19’	124° 31’	550	PV042
Dalaki	6	1990	8° 31’	124° 05’	430	PV042
Baubillatung	9	1990	8° 20’	124° 02’	285	PV042

4.3.2 Trial establishment and measurements

Two *E. urophylla* populations, consisting of two and three trials respectively (PE023A&B and PV042A&B&C), were established in Zululand. The sites generally have deep sandy soils. Mean annual temperature (M.A.T.) is 21^{o}C at all sites, and the mean annual precipitation (M.A.P.) ranged from 852 mm to 1128 mm (**Table 4.2**). Each trial was planted in a randomised complete block design (R.C.B.), replicated between four and eight times across each site. Each family was planted in a line plot where the families of the same provenance had been grouped together within each replication (“sets within replications”). Tree planting spacing was 3 m × 2.5 m in all trials. Trial measurements were scheduled at mid-rotation (four years) and at rotation age (seven years). Measurements were not available

at all ages for all trials. Growth traits namely: height in metres and DBH in centimetres were taken and under bark tree volume was calculated using the following equation as described by Zhou and Liang (1991):

$$\text{Volume} = 0.00002618(\text{DBH})^2(\text{height})$$

Table 4.2 Site and trial information of five Eucalyptus urophylla provenance/progeny trials in Zululand.

	PE023A	PE023B	PV042A	PV042B	PV042C
Longitude	32° 13’ E	32° 21’ E	32° 04’ E	32° 17’ E	31° 58’ E
Latitude	28° 31’ S	28° 15’ S	28° 35’ S	28° 01’ S	28° 42’ S
M.A.P. (mm)	1128	995	984	852	1103
Altitude (m.a.s.l.)	24	47	87	37	61
Major soil type	Fw35	Fw1210	Hu2200	Ct1100	Ct1100
Effective rooting depth (m)	1.2	1.51	1.51	1.51	1.0
Planting date	11/1988	11/1988	03/12/1991	26/01/1992	26/03/1992
Replications	4	4	8	8	6
Plot size	1×8 tree line	1×8 tree line	1×5 tree line	1×5 tree line	1×5 tree line
Number of families	43	20	130	68	162

4.3.3 Statistical analysis

4.3.3.1 Standardisation of data

Forest trees often display heterogeneous variances for growth traits where a strong relationship between the mean of the trait and its phenotypic and genetic variances are seen. This relationship is such that the field trials with bigger trees will have larger phenotypic and genetic variances than the field trials with smaller trees even if the trials are at the same age (Hodge and Dvorak, 2012). In order to deal with this situation, White et al. (2007) recommend data standardization prior to ANOVA, variance component analysis, or multi-site mixed model analysis. Standardization of data will homogenize variances that will be used

together in the linear model. It will also eliminate any spurious G×E (Burdon 1977, Eisen and Saxon 1983).

The standardization for the analysis of this paper was performed as described by Hodge and Dvorak (2012). First, the coefficient of variation was calculated for each replication for each growth trait. The mean coefficient of variances (CV_y) for each family-site-trait combination was also calculated. The phenotypic observations were then standardised in each replication to a mean = 100, and standard deviation = 100 × CV_y using PROC STANDARD in SAS (SAS Institute 2002). This is equal to dividing all observations by the phenotypic standard deviation (SD), as recommended by White et al. (2007), followed by adding a constant (100) and multiplying by a constant (100 × CV_y). The population mean for the growth trait can therefore be interpreted as 100%, and the associated variances and SD are the same size relative to mean as in the raw data. Predicted breeding values and all variance components can thus be directly interpreted as percentage gain (above or below 100%) without back-transformation or rescaling.

4.3.3.2 Family-site variance components and genetic parameters

Variance components analyses were done using SAS (SAS Institute 2002). The following variance components analyses were conducted for each family and each site where it was tested:

- Single-trait analyses for the three growth traits (height, DBH, and volume).
- Multiple-trait analyses for the three growth traits at a single-age were used to estimate genetic parameters for each trait, and genetic correlations among traits.

The linear model for all the analyses was the same as described by Hodge and Dvorak (2012):

$$y_{ijklm} = \mu + E_i + R(E)_{ij} + P_k + PE_{jk} + F(P)_{kl} + F(P)E_{jkl} + e_{ijklm}$$

Where,

y_{ijklm} = phenotypic observation of the $ijklm^{th}$ tree

μ = overall mean

E_i = fixed effect of the i^{th} trial

$R(E)_{ij}$ = fixed effect of the j^{th} replication nested in the i^{th} trial

P_k = random effect of the k^{th} provenance

$E[P_k] = 0$

$Var[P_k] = \sigma^2_{prov}$

PE_{jk} = random interaction of the k^{th} provenance and the i^{th} trial

$E[PE_{jk}] = 0$

$Var[PE_{jk}] = \sigma^2_{pe}$

$F(P)_{kl}$= random effect across sites of the l^{th} family in the k^{th} provenance

$E[F(P)_{kl}] = 0$

$Var[F(P)_{kl}] = \sigma^2_{f(p)}$

$F(P)E_{jkl}$= random interaction of the l^{th} family in the k^{th} provenance and the i^{th} trial

$E[F(P)E_{jkl}] = 0$

$Var[F(P)E_{jkl}] = \sigma^2_{f(p)e}$

e_{ijklm} = random error associated with the $ijklm^{th}$ tree

$E[e_{ijklm}] = 0$

$Var[e_{ijklm}] = \sigma^2_e$

Phenotypic variance within-provenance (σ^2_{phen}) was estimated as follows:

$$\hat{\sigma}^2_{phen} = \hat{\sigma}^2_{f(p)} + \hat{\sigma}^2_{f(p)e} + \hat{\sigma}^2_e$$

Narrow-sense heritability within provenance ($h^2_{f(p)}$) was estimated as:

$$h^2_{f(p)} = \frac{3\hat{\sigma}^2_{f(p)}}{\hat{\sigma}^2_{phen}}$$

In addition, narrow-sense heritability for family (excluding provenance effect) was estimated as:

$$h_f^2 = \frac{3\hat{\sigma}_f^2}{\hat{\sigma}_{fam\ phen}^2}$$

Where,

$\hat{\sigma}_{fam\ phen}^2 = \hat{\sigma}_f^2 + \hat{\sigma}_{fe}^2 + \hat{\sigma}_e^2$

A certain amount of inbreeding and/or percentage full-sibs does occur among open-pollinated families. The covariance among open-pollinated families would therefore typically be higher than one-quarter of additive genetic variance (Squillace 1974). Thus a coefficient of three instead of four was multiplied by the family variance in the calculation of heritability. Dieters et al. (1995) found that using three as a coefficient gives better agreement between parameter estimates from open and controlled pollinated populations of the same genetic material.

The amount of provenance variation was estimated as follows:

$$P^2 = \frac{\hat{\sigma}_{prov}^2}{\hat{\sigma}_{phen}^2}$$

This way, provenance variation (P^2) can directly be compared to additive genetic variation ($h_{f(p)}^2$ and h_f^2) (Hodge 2012). Standard errors of P^2 and of $h_{f(p)}^2$ and h_f^2 were estimated using the standard errors of $\hat{\sigma}_{prov}^2$, $\hat{\sigma}_{f(p)}^2$ and h_f^2 respectively, and treating $\hat{\sigma}_{phen}^2$ as a constant according to Dickerson's approximation (Dickerson 1969).

In order to determine the genetic (excluding provenance effect and within provenance) or provenance correlations of the same trait expressed on two sites as described by Burdon (1977), type B genetic (r_{Bg} and $r_{Bg(p)}$) and provenance (r_{Bprov}) correlations were estimated as follows:

$$r_{Bg} = \frac{\hat{\sigma}_f^2}{(\hat{\sigma}_f^2 + \hat{\sigma}_{fe}^2)}$$

$$r_{Bg(p)} = \frac{\hat{\sigma}^2_{f(p)}}{(\hat{\sigma}^2_{f(p)} + \hat{\sigma}^2_{f(p)e})}$$

$$r_{Bprov} = \frac{\hat{\sigma}^2_{p}}{(\hat{\sigma}^2_{p} + \hat{\sigma}^2_{pe})}$$

Type B correlation measures G×E that is due to rank changes across environments. This correlation over multiple sites can range between zero and one. An $r_b = 1$ indicates a perfect correlation between performance in different environments. A genetic coefficient of variation (GCV) ignoring the provenance effect, and secondly within provenance was calculated as follows:

$$GCV_f = 100^* \frac{\hat{\sigma}_f}{\bar{x}}$$

$$GCV_{f(p)} = 100^* \frac{\hat{\sigma}_{f(p)}}{\bar{x}}$$

Where $\bar{x}$ = the trait mean. The GCV expresses the additive genetic standard deviation in terms of percent, and gives an estimation of how much genetic improvement could be made in a trait (Hodge and Dvorak 2012).

Genetic correlations between traits (DBH-height, DBH-volume and height-volume) were also calculated using SAS at the family, family within provenance and provenance levels respectively.

4.3.3.3 Across-site variance components and provenance BLUPs

An across-site analysis was performed for each trial series using the variable volume. Proc mixed in SAS (SAS Institute 2002) was used to conduct the multiple-trait analysis. Fixed and random effects in the models were the same as defined above. Site-site correlations at the family and provenance level (and standard errors) were estimated directly from the SAS

output. These analyses were also used to produce provenance, family and individual estimates using best linear unbiased predictions (BLUPs) for volume at each trial series.

4.4 RESULTS

4.4.1 Growth results

Mean DBH, height, volume per tree and survival for each provenance in each trial series are presented in **Table 4.3**. Provenance and families within each provenance effects were significant ($p<0.05$) in all the trial series in terms of standardised tree volume (**Table 4.4**). Although significant, the difference in mean DBH for provenances in the PE023 trial series was small. The top performing provenance in the PE023 trial series was Lewotobi (mean DBH = 13.8 cm), and the worst performing provenance was Mandiri (mean DBH = 13.3 cm). A bigger difference between provenances was detected in the PV042 trial series where Apui and Wai Kui provenances performed the best (mean DBH = 16.3 cm and 16.0 cm respectively) and Old Uhak the worst (mean DBH = 12.6 cm). Overall, the survival rates of all provenances were relatively good (between 72.9% and 93.6%).

Table 4.3 Mean growth (DBH, height and volume) and survival of two *Eucalyptus urophylla* populations (PE023 and PV042) established in Zululand.

Trial series	Provenance	Age (years)	Trials	DBH (cm)	Height (m)	Volume (m^3)	Survival (%)
PE023	Wuko	4	2	13.7±2.8	13.9±1.8	0.0863±0.042	78.5±41.0
	Egon		2	13.7±3.3	14.0±2.2	0.0893±0.047	88.1±30.5
	Lewotobi		2	13.8±2.9	14.6±2.0	0.0925±0.043	87.0±31.8
	Mandiri		2	13.3±2.8	14.2±1.9	0.0822±0.039	90.9±33.7
PV042	Baubillatung	7	3	14.5±3.4	16.5±3.3	0.1196±0.068	75.1±44.4
	Dalaki		3	14.9±3.6	17.2±3.2	0.1323±0.076	81.8±38.5
	Lalikki		3	14.4±3.3	17.0±3.2	0.1218±0.068	81.7±38.9
	Mareke Arnau		3	12.9±3.3	16.2±3.5	0.0952±0.064	75.8±43.5
	Wai Kui		3	16.0±3.9	17.5±3.3	0.1548±0.090	84.5±38.1
	Apui		3	16.3±4.0	17.5±3.6	0.1626±0.092	72.9±44.6
	Ilwaki1		2	13.5±3.4	16.1±3.8	0.1056±0.057	93.6±25.4
	Ilwaki2		3	14.1±3.5	16.4±3.6	0.1145±0.071	79.7±39.8
	Pintumas		3	14.7±3.8	16.4±3.6	0.1253±0.081	81.0±38.8
	Telemar		3	14.3±3.1	17.1±3.4	0.1191±0.069	79.5±40.1
	Carbutu		3	14.7±3.6	17.3±4.1	0.1289±0.074	75.1±43.6
	Rotus Uhak		3	14.8±3.3	17.2±3.2	0.1278±0.069	85.2±36.6
	Old Uhak		3	12.6±3.2	15.6±3.1	0.0888±0.058	78.3±43.1

Table 4.4 Analysis of variance table for standardised tree volume of *Eucalyptus urophylla* provenances and families within provenances in two populations (PE023 and PV042) at four and seven years of age respectively.

PE023 trial series					
Source	DF	Type III SS	Mean Square	F Value	Pr > F
Trial	1	10821.19	10821.19	6.28	0.0123
Rep(trial)	6	5239.15	873.19	0.51	0.8036
Provenance	3	19646.41	6548.80	3.80	0.0099
Family(provenance)	39	249255.43	6391.16	3.71	<.0001
Trial*provenance	3	21842.96	7280.99	4.23	0.0055
Trial*family(provenance)	16	36572.60	2285.79	1.33	0.1717
PV042 trial series					
Trial	2	22252.84	11126.42	4.33	0.0132
Rep(trial)	19	8859.23	466.28	0.18	1
Provenance	13	1411968.58	108612.97	42.25	<.0001
Family(provenance)	190	2403861.12	12651.90	4.92	<.0001
Trial*provenance	20	279734.16	13986.71	5.44	<.0001
Trial*family(provenance)	167	1522573.01	9117.20	3.55	<.0001

4.4.2 Growth trait correlations and genetic parameters

In order to examine the provenance and genetic correlations between the three different growth traits (height, DBH and volume), genetic parameter analyses were performed. The analysis was done for individual trials and each trial series combined. These results are presented in **Table 4.5**.

Overall, there were very strong genetic correlations between the various growth traits. The best provenance and genetic correlations were detected between DBH and volume and ranged from 0.97 to 1.00 for all the analyses performed. The genetic correlation between height and DBH ranged from 0.71 to 1.00, and between height and volume from 0.78 to 0.99. Provenance correlations between all three growth traits were very similar to genetic correlations at the PV042 trial series. However, provenance correlations between the three growth traits could in most cases not be calculated for the PE023 trial series as the estimates were bounded to the theoretical limit of zero.

Table 4.5 Provenance and genetic correlations between three different growth traits (height, DBH and volume) for two *Eucalyptus urophylla* populations (PE023 and PV042) established in Zululand. Error values are the SE. na = SE of a provenance or genetic correlation could not be calculated as the estimate was bounded at the theoretical limit of zero or one.

			Provenance correlations (r_{prov})		Family (Provenance) correlations ($r_{g(p)}$)		Family correlations (r_g)	
Trial	Age (years)	Trait	with DBH	with volume	with DBH	with volume	with DBH	with volume
		height	na	na	0.71±0.129	0.78±0.063	0.71±0.132	0.78±0.064
PE023A	4	DBH		na		0.98±0.004		0.98±0.004
		height	1.00(na)	0.99±0.004	0.83±0.073	0.89±0.030	0.83±0.060	0.91±0.025
PE023B	4	DBH		1.00(na)		0.99±0.004		0.98±0.006
		height	na	na	0.83±0.097	0.89±0.067	0.74±0.132	0.89±0.062
PE023A&B	4	DBH		na		0.98±0.012		0.98±0.012
		height	0.88±0.074	0.88±0.075	0.87±0.036	0.88±0.033	0.85±0.032	0.86±0.030
PV042A	7	DBH		0.99±0.001		0.99±0.002		0.99±0.001
		height	0.90±0.070	0.90±0.066	0.92±0.031	0.92±0.031	0.90±0.030	0.89±0.033
PV042B	7	DBH		0.99±0.001		0.99±0.003		0.99±0.001
		height	0.87±0.176	0.91±0.066	0.97±0.007	0.95±0.011	0.92±0.015	0.93±0.015
PV042C	7	DBH		0.98±0.013		0.98±0.005		0.97±0.005
		height	0.95±0.027	0.96±0.021	0.93±0.039	0.91±0.053	0.89±0.031	0.88±0.015
PV042A&B&C	7	DBH		0.99±0.003		0.98±0.015		0.98±0.002

Overall, growth was under weak to moderate genetic control in both the *E. urophylla* populations. At four years, heritability (h_f^2) for height, DBH and volume was calculated as

0.20, 0.12 and 0.14, respectively (**Table 4.6**). Higher heritabilities for the three growth traits were calculated at seven years for the PV042 trial series.

The across site heritability (h_f^2) for height, DBH and volume in the PV042 trial series was estimated at 0.17, 0.30 and 0.26 respectively. As expected, the genetic parameter estimates for heritability (h_f^2) and type B genetic correlation (r_{Bg}) for family without the provenance effect were higher than the heritability ($h_{f(p)}^2$) and type B genetic correlations ($r_{Bg(p)}$) for family within provenance for all three growth traits. This difference was especially evident as P^2 increases. For instance, at the PV042 trial series $h_{f(p)}^2$ was lower than h_f^2 for DBH (0.07 and 0.30) and volume (0.06 and 0.26) across the three sites. Four year results of the PE023 trial series showed that h_f^2 was 0.20 and $h_{f(p)}^2$ was 0.17 for height. Where P^2 could not be calculated for DBH and height, h_f^2 and $h_{f(p)}^2$ are reported as the same (0.12 for DBH and 0.14 for height).

The type B genetic correlation for height was higher for the family without provenance effect (r_{Bg} = 0.89) than for the family within provenance effect ($r_{Bg(p)}$= 0.86), but the same for DBH and volume (0.61) in the PE023 trial series. However, in most cases provenance variation (P^2) and type B provenance correlation (r_{Bprov}) could not be calculated for the PE023 trial series due to the insignificance of the provenance effect at the PE023B trial site. Provenance variance was calculated for the PV042 trial series at seven years of age and was lowest for height (0.03) and the same for DBH and volume (0.09) across the three trials. The r_{Bprov} followed a similar trend with height being the lowest (0.59) and DBH and volume displaying similar r_{Bprov} of 0.77 and 0.76 respectively. The provenance type B correlations were much higher than the type B genetic correlations at this trial series. The $r_{Bg(p)}$ for height, DBH and volume were 0.27, 0.20 and 0.14. When the provenance effect was excluded from the analysis, the type B genetic correlation (r_{Bg}) for height, DBH and volume increased to 0.44, 0.53 and 0.46 respectively. This is an indication that the provenance effect stayed fairly stable across sites and that a combined site analysis can be performed.

When the genetic coefficient of variation (GCV) was investigated, it was evident that the GCV was lowest for height, intermediate for DBH and highest for volume in all cases. The $GCV_{f(p)}$ for height, DBH and volume was 3.2%, 4.1% and 9.2% respectively in the

PE023 trial series. At the PV042 trial series, the $GCV_{f(p)}$ for height, DBH and volume was 2.9%, 3.5% and 7.4% respectively. The $GCV_{f(p)}$ expresses the additive genetic standard deviation (within provenance) in terms of percent. In other words, those trees in the population that have breeding values of one genetic standard deviation above the mean will have 9.2% (in the PE023 trial series) and 7.4% (in the PV042 trial series) more volume growth than the provenance mean (Cornelius 1994). It is clear that there are tremendous opportunities to make genetic gains in this *E. urophylla* population. For instance, if it is possible to identify the trees in the PV042 trial series that have breeding values of two genetic standard deviations above the mean, this would represent 14.8% additional gain above the provenance mean (Hodge and Dvorak 2012).

Table 4.6 Provenance and genetic parameters for three different growth traits (height, DBH and volume) for two *Eucalyptus urophylla* populations (PE023 and PV042) established in Zululand. Error values are the SE. na = SE of a provenance or genetic correlation could not be calculated as the estimate was bounded at the theoretical limit of zero or one.

Trial	Age (years)	Trait	Provenance		Family(provenance)				Family			
			P^2	r_{Bprov}	$h^2_{f(p)}$	$r_{Bg(p)}$	$GCV_{f(p)}$	V_{phen}	h^2_f	r_{Bg}	GCV_f	V_{phen}
PE023A	4	height	na		0.13±0.05		2.85	181.35	0.13±0.06		2.76	180.47
		DBH	na		0.17±0.06		4.99	435.41	0.17±0.06		4.97	435.3
		volume	na		0.18±0.07		10.65	1861.50	0.18±0.07		10.51	1859.02
PE023B	4	height	0.07±0.08		0.17±0.13		3.24	182.29	0.32±0.16		4.53	192.52
		DBH	0.01±0.02		0.18±0.11		5.16	454.64	0.18±0.11		5.23	455.49
		volume	0.03±0.05		0.18±0.12		10.40	1827.44	0.22±0.13		11.71	1857.24
PE023A&B	4	height	0.01±0.01	na	0.17±0.07	0.86±0.09	3.22	183.23	0.20±0.07	0.89±0.07	3.49	184.87
		DBH	na	na	0.12±0.06	0.61±0.20	4.14	442.56	0.12±0.06	0.61±0.21	4.14	442.57
		volume	na	na	0.14±0.07	0.61±0.22	9.21	1865.52	0.14±0.07	0.61±0.21	9.21	1865.52
PV042A	7	height	0.04±0.02		0.14±0.03		3.90	316.98	0.22±0.04		4.91	325.49
		DBH	0.09±0.04		0.18±0.04		5.65	536.14	0.38±0.06		8.58	576.89
		volume	0.09±0.04		0.20±0.04		13.92	2961.96	0.39±0.06		20.25	3171.98
PV042B	7	height	0.07±0.04		0.16±0.05		4.13	314.78	0.31±0.08		5.79	329.42
		DBH	0.18±0.09		0.28±0.08		6.85	497.06	0.70±0.13		11.69	584.67
		volume	0.2±0,09		0.26±0.07		15.30	2745.42	0.67±0.13		26.65	3198.78
PV042C	7	height	0.03±0.02		0.41±0.06		6.61	322.97	0.45±0.07		6.98	327.23
		DBH	0.07±0.03		0.44±0.07		9.01	548.03	0.58±0.08		10.61	579.26
		volume	0.07±0.03		0.48±0.07		22.15	3036.84	0.60±0.08		25.30	3181.83
PV042A&B&C	7	height	0.03±0.01	0.59±0.29	0.08±0.03	0.27±0.11	2.95	318.57	0.17±0.04	0.44±0.09	4.26	327.19
		DBH	0.09±0.04	0.77±0.13	0.07±0.03	0.20±0.12	3.55	535.65	0.30±0.06	0.53±0.09	7.57	579.02
		volume	0.09±0.04	0.76±0.13	0.06±0.03	0.14±0.11	7.43	2954.13	0.26±0.01	0.46±0.09	16.75	3182.93

4.4.3 BLUP and genetic gains

Best linear unbiased predictions were made for provenance (G_{prov}), families within provenance ($G_{fam(p)}$) and individual trees (G_i). Individual tree breeding values (BV) is equal to the sum of the above mentioned predictions. The predictions are expressed in units of percentage gain above the unimproved population mean for volume. For the purpose of this article, only G_{prov} and the range in $G_{fam(p)}$ for the two *E. urophylla* populations are displayed in **Table 4.7**.

Estimations of provenance predictions were calculated to be a theoretical zero at the PE023 trial series. It should be borne in mind that this trial series represents a limited number of only four provenances and the amount of families within provenances ranged between four and eighteen. In contrast to the PE023 trial series, a big difference in provenance predictions was estimated for the PV042 trial series. Predicted gains for Apui and Wai Kui provenances were the biggest at 27.8% and 24.8% respectively. Predicted gains for Old Uhak provenance were the lowest at -25.3%.

In a scenario where the top 200 individuals are selected based on their BV, the average predicted gains are 54.6% more than the population mean. However, such a scenario will drastically reduce the genetic diversity in the population and will only include individuals from 61 families and seven provenances. An alternative selection strategy would be to select the top 40 individuals from the PE023 population and the top 160 individuals from the PV042 population, but not more than two individuals per family. The estimated gains (44.7%) of this scenario will be less than the first scenario, but a reasonable genetic base (200 individuals from 100 families and 14 provenances) will remain. The genetic diversity in such a breeding population should be sufficient for future breeding, especially if one takes into account that *E. urophylla* is only utilised as a hybrid parent in making crosses with *E. grandis* in Zululand.

A scenario to construct an elite population, could be to select the top two individuals (based on BV) from the top five families (based on $G_{fam(p)}$) of the five best provenances (based on G_{prov}). This scenario would result in 50 selections with an average BV of 59.8% above the population mean.

Table 4.7 Predicted gains (%) for provenances (G_{prov}) and families within provenances ($G_{fam(p)}$) of two *Eucalyptus urophylla* populations (PE023 and PV042) in Zululand.

Trial series	Provenance	No. of families	G_{prov}	Range in $G_{fam(p)}$
PE023	Wuko	4	na	-10.7 – 3.4
	Egon	18	na	-12.4 – 19.7
	Lewotobi	16	na	-8.8 – 18.6
	Mandiri	5	na	-12.8 – 9.2
PV042	Baubillatung	9	0.38	-3.1 – 3.6
	Dalaki	6	8.95	-5.4 – 13.2
	Lalikki	9	-0.31	-8.1 – 10.2
	Mareke Arnau	28	-19.9	-9.9 – 6.9
	Wai Kui	10	24.78	-4.5 – 8.1
	Apui	10	27.81	-6.7 – 10.2
	Ilwaki1	4	-14.23	-2.9 – 0
	Ilwaki2	28	-5.89	-7.6 – 7.7
	Pintumas	15	2.1	-12.4 – 7.2
	Telemar	6	-1.51	-1.9 – 1.6
	Carbutu	8	-0.38	-7.5 – 6.6
	Rotus Uhak	37	3.51	-8.7 – 7.4
	Old Uhak	6	-25.31	-6.2 – 2.5

4.5 DISCUSSION

Results of our study indicated that G×E effects were moderate. Our results coincide with type B correlations (above 0.55) reported by Wei and Borralho (1998), and Hodge and Dvorak (2015) for *E. urophylla*. Nirsatmanto et al. (1996) reported moderate (0.49 for DBH) type B correlations between two sites in Indonesia, but indicated that predicted gains of the selection index across the sites were still greater than those of the indices at each site. One exception was the study executed by Mori et al. (1988). They have reported losses in volume of up to 26.7% due to G×E. However, their testing sites were very different from each other in terms of altitude (ranging from 50 to 820 m.a.s.l.) and M.A.T. (ranging from 21^{o}C to 23.6^{o}C). In our study, the Zululand sites are very similar and situated at altitudes between 24 and 87 m.a.s.l. and a M.A.T of 21^{o}C at all sites. However, a more comprehensive study with more trials in this region is required to understand G×E. Even though G×E effects were noticed in our study, it is recommended that only one *E. urophylla* breeding population should be

managed for Zululand, especially considering that this species will only be used as a hybrid parent.

The strong genetic correlation between the various growth traits for *E. urophylla* that was detected in this study was also noted by Hodge and Dvorak (2015). The results of our study confirm that DBH is a sufficient growth measure to use in *E. urophylla* breeding programmes. Diameter is easier and quicker to measure than heights and will make the measurements of breeding trials more efficient.

Low to moderate genetic control for all three growth traits was evident in this study. The heritabilities calculated in this study is consistent with those reported by others. Narrow-sense heritabilities of 0.11 – 0.41 was reported for China (Wei and Borralho 1998), 0.10 – 0.31 for Vietnam (Kien et al. 2009), 0.10 for Brazil (de Souza et al. 2011) and 0.15 across five countries (Hodge and Dvorak 2015). Other authors have reported that wood quality traits are under more genetic control than growth, and should be considered in the selection process in future. For instance, Greaves et al. (1996) and Wei and Borralho (1997) reported narrow-sense heritabilities for wood basic density of 0.60 and 0.71 respectively. Darrow and Roeder (1983) also reported that *E. urophylla* has higher basic wood density than pure *E. grandis* in South Africa. However, even though growth is under less genetic control than wood quality traits, volume was still identified as the dominant trait in determining the economic benefits in short-rotation species for pulp production and should therefore not be neglected (Wei and Borralho 1999).

At a provenance level, the good growth performance of the Lewotobi and/or Wai Kui provenances in this study agreed with results reported by others (Ngulube 1989, Zhou and Liang 1991, Luz et al. 1996, Wei and Borralho 1998, Kien et al. 2009). One exception was the good growth performance (27.8% gain) of the Apui provenance in our study relative to the study performed by Wei and Borralho (1998). Hodge and Dvorak (2015) reported the same trend for the Apui provenance across countries with genetic gains ranging from -13.9% in Brazil to 3.7% in South Africa. The poor growth performance of the Mandiri provenance was evident in this study, as well as in studies performed by Wei and Borralho (1998) and Kien et al. (2009), but not in the study performed by Ngulube (1989). Ngulube (1989) reported that

Mandiri was one of the four best provenances tested in Malawi. The differences in provenance performance at different countries could be due to the large variation in growth between sources from the same provenance (Hodge and Dvorak 2015). This points to the need for intensive provenance sampling and testing in *E. urophylla* to locate productive sources (Hodge and Dvorak 2015).

Overall, the relatively large provenance and family variation in the two *E. urophylla* populations in Zululand provides opportunities for impressive gains through selection and breeding. In order to conserve the genetic diversity of the main *E. urophylla* breeding population, a selection criteria of 200 selections is recommended. The number of selections from each population (PE023 and PV042) should proportionally be the same as the size of each population relative to the size of the two populations combined. In other words, approximately 20% of the selections should come from the PE023 population and 80% from the PV042 population. A maximum of two trees per family is recommended. The estimated gains for this scenario will be 44.7% over the population mean.

In order to construct an elite population for hybrid breeding, the selection of the top two individuals from the top five families of the five best provenances is recommended. This scenario would result in 50 selections with an estimated gain of 59.8%. Elite selections could be used to undertake intra- and interspecific controlled crosses. Progeny from intraspecific crosses could be infused into the breeding population to enhance the genetic pool with superior genotypes. Superior progeny from the interspecific crosses with *E. grandis* should be incorporated into an *E. grandis* × *E. urophylla* clonal testing programme in Zululand.

4.6 CONCLUSIONS

The relatively large provenance and family variation detected for the two *E. urophylla* populations in our study provides an adequate source to select for genetic gains and to maintain genetic diversity for hybrid and pure species breeding. We also conclude from this study that a single *E. urophylla* breeding population should be sufficient for Zululand due to the relatively low levels of G×E and that only DBH can be used as a growth measure in *E. urophylla* breeding programmes.

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CHAPTER 5 GENETIC PARAMETERS OF INTERSPECIFIC HYBRIDS OF *EUCALYPTUS GRANDIS* AND *E. UROPHYLLA* SEEDLINGS AND CUTTINGS

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5.1 ABSTRACT

The current *Eucalyptus grandis* × *E. urophylla* hybrid breeding strategy of South Africa's Forestry Industry is to maintain large breeding populations of both parental species in which parents are selected based on their general combining ability (GCA) estimates or predicted individual tree breeding values and are used for interspecific hybrid crosses. The hybrid material is first screened in seedling progeny trials after which superior individuals are selected and tested as clones. Although this strategy has delivered superior clones for commercial production in South Africa, it is a time consuming strategy to follow and more cost effective strategies are being investigated.

In order to review the current hybrid breeding strategy, information on the genetic control of the traits of interest is needed for *E. grandis* × *E. urophylla* seedling and clonal populations. The aim of this study was therefore to determine genetic parameters of *E. grandis* × *E. urophylla* seedling and clonal populations. The specific objectives were to firstly estimate genetic parameters for *E. grandis* × *E. urophylla* hybrid seedling and clonal populations, secondly to investigate the correlation between *E. grandis* and *E. urophylla* parental (GCA) or individual breeding values and their general hybridising ability (GHA), and lastly to determine the correlation between *E. grandis* × *E. urophylla* hybrid seedling ortets and their ramets.

Results of our study indicated that non-additive genetic variation explained the majority of the total genetic variation in *E. grandis* × *E. urophylla* seedling and clonal populations. Due to the pre-eminence of non-additive variance, the pure-hybrid correlations were weak, especially for clonal populations. It would therefore seem that GCA or predicted individual breeding values are not good predictors of GHA for growth performance in the observed populations. Our study also indicated a weak coefficient of correlation between the growth performance of seedling ortets and their ramets.

These results suggest that: firstly a hybrid breeding strategy to capture non-additive genetic variation should be adopted, and secondly that the first phase of screening *E. grandis* × *E. urophylla* hybrid material as seedlings should be revisited.

Keywords

Eucalyptus grandis × *E. urophylla*, genetic parameters, seedlings and cuttings, pearson correlations.

5.2 INTRODUCTION

The deployment of interspecific hybrids in commercial forest tree planting is prevalent worldwide (Kerr et al. 2004). There are many reports on the superiority of *Eucalyptus* interspecific hybrids (Denison and Kietzka 1992, De Assis 2000, Kha and Cuong 2000, Potts et al. 2000, Verryn 2000, Vigneron et al. 2000, Potts and Dungey 2004, Bison et al. 2006). Interspecific hybrids of *Eucalyptus urophylla* and *E. grandis* in particular, have been used in tropical and subtropical forestry for a while, especially in Brazil (Ikemori 1984, Bison et al. 2006), Congo (Vigneron and Bouvet 2000) and South Africa (Retief and Stanger 2009). *Eucalyptus grandis* suffers from fungal diseases, in particular, *Crysoporthe austroafricana* and *Coniothyrium* sp. cankers in these tropical and subtropical regions. However, the interspecific hybrids with *E. urophylla* have shown to be more resistant to the diseases of concern.

The current *E. grandis* × *E. urophylla* hybrid breeding strategy of South Africa's Forestry Industry is an adaption to the classic hybrid breeding strategy namely "Recurrent Selection for General Combining Ability (RS-GCA, Jenkins 1940). Large breeding populations of both parental species are maintained and elite selections are made based on either the parents GCA estimates (backward selection) or predicted individual tree breeding values (forward selection). General combining ability is a measure of the genetic worth of a parent based on the average performance of the progeny from a particular parent, whereas predicted individual breeding values is an estimation of the genetic worth of individuals based on their own performance, and that of their sibs (Hettasch et al. 2005). Selected parents are used for interspecific hybrid crosses, and the hybrid material is first tested as seedlings in seedling progeny trials. Superior individuals are selected at mid-rotation from the seedling populations and ramets of the ortets are then tested as clones in clonal trials.

The underlying research hypothesis of this strategy is firstly that GCA or predicted individual tree breeding values are good predictors of GHA. General hybridising ability is a measure of the genetic worth of a hybrid parent based on the average performance of the hybrid progeny from the parent when crossed with various parents of a different species (Hettasch et al. 2005). The correlation between GCA and GHA is a useful indicator of the consistency of parental performance when used as a hybrid parent compared to when the same selections are used as pure species parents. If this hypothesis is false, then a hybrid breeding strategy such as Reciprocal Recurrent Selection (RRS, Comstock et al. 1949) has obvious advantages (Vigneron and Bouvet 2000).

Although some authors indicated a poor correlation between GCA and GHA (Retief and Stanger 2009, Volker et al. 2008), literature related to this topic is sparse and more information regarding this is needed. For instance, results of the latter studies were based on the performance of seedling populations and not clonal populations. In most countries, interspecific hybrids of *E. grandis* × *E. urophylla* are commercially deployed as clones. Hence genetic information on *E. grandis* × *E. urophylla* clonal populations needs to be determined.

The second underlying hypothesis of the current breeding strategy is that the performance of the seedling ortet is a good predictor of its ramets' performance. Although some authors (Fuller and Little 2007, Gaspar et al. 2005, Sasse and Sands 1996) reported that there was no significant difference between eucalypt cuttings and seedlings for growth, no direct comparison of ortets and their ramets have been reported on. All of these studies produced cuttings from different individuals with a similar genetic makeup than that of the seedlings. Hence, the general theoretical literature on this subject and specifically in the context of *E. grandis* × *E. urophylla* hybrid populations is inconclusive and needs further investigation.

In addition, the magnitude of specific combining effects in the hybrid crosses (SHA) is also one of the most important factors to consider when developing a hybrid breeding strategy (Volker 2002) and needs investigation. With this in mind, our study was set out to address the following scientific questions:

- What is the correlation between the parents' performance as a pure species (GCA or individual tree breeding values) and in a hybrid combination (GHA) in seedling and clonal populations?
- What is the magnitude of specific combining effects in the hybrid seedling and clonal populations?
- Is the performance of the seedling ortet a good predictor of the ramets' performance?

Answers to these questions will provide valuable information to hybrid tree breeders around the world and will assist to construct suitable *Eucalyptus* hybrid breeding strategies. The objectives of this study were therefore to:

- Determine the Pearson correlation between *E. grandis* and *E. urophylla* parents (GCA or predicted individual tree breeding values) and their hybrid (GHA) progeny (seedlings and clones).
- Estimate genetic parameters for *E. grandis* × *E. urophylla* hybrid seedling and clonal populations.

Calculate the Pearson correlation between *E. grandis* × *E. urophylla* hybrid seedling ortets (predicted individual tree breeding values) and their ramets (mean best linear unbiased predictions [BLUP]).

5.3 MATERIALS AND METHODS

5.3.1 Breeding material

5.3.1.1 Seedling population

The mating design consisted of a partial factorial with 30 *E. grandis* parents (female) and 27 *E. urophylla* parents (male) (**Figure 5.1**). A total of 108 *E. grandis* × *E. urophylla* families were produced and established in progeny seedling trials. Although only 13% of the cells of the factorial mating were completed, 15 *E. grandis* and 15 *E. urophylla* parents were crossed between 3 and 14 times. Sixty nine of the 108 *E. grandis* × *E. urophylla* families were planted at two or more sites. All the parents were selected on their mature age phenotypes for growth and tree form. The *E. grandis* parents are cloned selections made in a second generation (F2) progeny trial series from the South Africa Forestry Research Institute programme (as it was called at the time). The *E. urophylla* parents used in this study are all cloned selections from an unimproved (P0) provenance/progeny trial series of open-pollinated seed collected from different provenances on Indonesian Islands.

E. grandis FEMALE PARENTS	U1	U2	U3	U4	U5	U6	U7	U8	U9	U10	U11	U12	U13	U14	U15	U16	U17	U18	U19	U20	U21	U22	U23	U24	U25	U26	U27
G1												2															
G2																					1						
G3							1										2		3								
G4			2	2		5		2				2		2		5		2	2								
G5																	3		1								
G6	2	2	1			1		3	2			1	2	3		3	3	1							1		2
G7					2			2		2	1		3		1	2		2	2	1	1	2	1	1			
G8						2		1					2				2										
G9								1																			
G10			1			3			2									1			2					1	
G11				1		1											1										
G12																		1									
G13			1					3																			
G14			3			3								2	2												
G15			2			1		2	1																		
G16														3				1									
G17						1																					
G18		2		3												3											
G19						2											2										
G20														2													
G21						1	2	2				1		1	2	2		2									
G22		1																									
G23		2											2				2	1									
G24													1				1	2									
G25				1										2	3												
G26													2														
G27				3																							
G28				1		1							5	1					2								
G29		2																									
G30			2			2		2																			

E. urophylla MALE PARENTS

Figure 5.1 Diagram indicating *Eucalyptus grandis* × *E. urophylla* crosses in the mating design of the partial factorial. The number of sites where the hybrid families were established as seedlings are designated by the number in the cell.

5.3.1.2 Clonal population

A total of 148 selections were made from 63 families (**Table 5.1**) from the above mentioned *E. grandis* × *E. urophylla* seedling population. All trees were selected on their mature age phenotypes for growth and tree form. Multiple cuttings were produced from each selection and were established in a series of clonal trials at a minimum of six sites. There was no evidence of maturation in any of the clones causing reduced rooting and coppice ability.

Table 5.1 Number of selections made from *E. grandis* × *E. urophylla* families and the number of clonal trials established with clonal material from each family.

Family	No. of selections	No. of clonal trials	Family	No. of selections	No. of clonal trials
G1×U12	1	6	G10×U3	2	8
G3×U17	3	6	G10×U6	2	6
G3×U19	1	6	G10×U21	1	8
G4×U3	3	6	G10×U26	1	6
G4×U6	1	6	G11×U6	1	6
G4×U8	9	6	G13×U3	1	6
G4×U12	4	6	G13×U8	2	6
G4×U16	4	6	G14×U6	1	6
G4×U18	7	6	G14×U14	2	6
G4×U19	1	6	G14×U15	2	6
G5×U17	2	6	G15×U8	5	6
G6×U1	5	6	G15×U9	1	8
G6×U8	2	6	G16×U14	7	6
G6×U9	1	8	G18×U16	1	6
G6×U13	1	8	G19×U6	1	6
G6×U14	2	6	G20×U14	3	6
G6×U16	1	6	G21×U14	2	6
G6×U17	1	6	G21×U15	2	6
G6×U18	2	6	G21×U16	1	6
G6×U27	5	6	G21×U18	2	6
G7×U5	4	6	G23×U2	4	6
G7×U8	7	6	G23×U13	1	6
G7×U10	1	8	G23×U17	2	6
G7×U13	3	6	G23×U18	1	6
G7×U18	2	8	G24×U18	1	6
G7×U19	3	6	G25×U14	4	6
G7×U20	1	6	G27×U4	1	6
G7×U21	1	8	G28×U13	1	6
G7×U22	1	8	G28×U19	5	6
G7×U23	1	8	G29×U2	1	6
G8×U13	1	6	G30×U8	1	8
G8×U17	5	6			

5.3.2 Trial establishment and measurements

5.3.2.1 Seedling population

Three series of trials (PE062A&B, PE80A&B&C and PE109A&B) were established in the subtropical region of South Africa, namely Zululand. Site and trial information for each trial is presented in **Table 5.2**. Each trial was planted in a randomised complete block design (RCB). Trial measurements were scheduled at mid-rotation (four years) and at rotation age (seven years). Growth traits namely: height in metres and diameter at breast height, (DBH, in centimetres) were taken and tree volume was calculated using the following equation as described by Max and Burkhart (1976):

Volume =($\frac{\pi}{40000}$)*k*DBH^2*Height

Where,

k=($\frac{\beta_1}{3}$)+($\frac{\beta_0}{2}$)-(β_0+β_1)+($\frac{\beta_2}{3}$)* a_1^3 +($\frac{\beta_3}{3}$)* a_2^3

β_0 = -2.72108

β_1 = 1.18891

β_2 = -0.90650

β_3 = 95.42845

a_1^3 = 0.83117

a_2^3 = 0.059583

Functions used in the equation were developed internally by Mondi Limited (Kotze and Fletcher, unpublished data).

Table 5.2 Site and trial information of *Eucalyptus grandis* × *E. urophylla* hybrid seedling trials.

	PE062A	PE062B	PE080A	PE080B	PE080C	PE109A	PE109B
Longitude	32° 12' E	31° 42' E	32° 11' E	31° 42' E	32° 20' E	32° 06' E	32° 06' E
Latitude	28° 34' S	29° 00' S	28° 35' S	28° 59' S	28° 16' S	28° 38' S	28° 38' S
M.A.P. (mm)	1156	1273	1271	1201	1050	1079	1079
M.A.T. (°C)	21.2	21.4	21.6	21	21.5	21.3	21.6
Altitude (m)	40	40	40	40	20	55	55
Major soil type	Fw 1110	Hu 26	Fw 1110	Hu 26	Fw 2110	Ct 2100	Ct 2100
Effective rooting depth (m)	1.51	1.51	1.51	1.51	1.51	1.51	1.51
Planting date	22/07/94	28/07/94	03/10/96	11/10/96	03/10/96	25/06/98	25/06/98
Espacement	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m
Fertiliser	4:1:1 @ 200g/tree	4:1:1 @ 200g/tree	4:2:0 @ 200g/tree	5:2:0 @ 70g/tree	No fertiliser	No fertiliser	No fertiliser
Replications	5	5	6	6	1	12	12
Plot size	1×6 tree line	1×6 tree line	1×6 tree line	1×6 tree line	Single tree	Single tree	Single tree
Number of families	39	36	48	34	29	33	19

5.3.2.2 Clonal population

Three *E. grandis* × *E. urophylla* clonal trial series (IC358A – F, IC361A – F and IC365A – H) were established across various sites at Zululand. Site and trial information for each trial site is presented in **Table 5.3**. Each trial was planted in a RCB design. Height and DBH were measured at four and seven years of age. Only four year data were available for the IC365 trial series. Tree volume was calculated using the same equation described for the seedling population.

Table 5.3 Site and trial information of *E. grandis* × *E. urophylla* hybrid clonal trials.

IC358 CLONAL TRIAL SERIES						
Trial	IC358A	IC358B	IC358C	IC358D	IC358E	IC358F
Longitude	32° 06’ E	32° 03’ E	31° 42’ E	31° 43’ E	31° 53’ E	31° 50’ E
Latitude	28° 31’ S	28° 37’ S	28° 59’ S	28° 59’ S	28° 52’ S	28° 53’ S
M.A.P. (mm)	1084 mm	1051 mm	1293 mm	1295 mm	1379mm	1476 mm
M.A.T. (°C)	21.6 °C	21.6°C	21.1 °C	21.1 °C	21.3°C	21.1 °C
Altitude (m)	55 m	63 m	24 m	47 m	63 m	95 m
Major soil type	Kd2000	Fw1210	Hu 27	Hu 26	Hu 2200	Hu 2200
Effective rooting depth (m)	1 – 1.4m	1.51	1.21	1.21	1.51	1.51
Planting date	2005/04/29	2005/05/09	2005/06/17	2005/08/24	2005/06/13	2005/07/05
Espacement	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m
Fertiliser	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree
Replications	20	20	20	20	20	20
Plot size	Single tree	Single tree	Single tree	Single tree	Single tree	Single tree
Number of clones	68	68	68	68	68	68
IC361 CLONAL TRIAL SERIES						
Trial	IC361A	IC361B	IC361C	IC361D	IC361E	IC361F
Longitude	32° 12’ E	32° 08’ E	31° 58’ E	31° 49’ E	31° 41’ E	31° 42’ E
Latitude	28° 36’ S	28° 39’ S	28° 44’ S	28° 54’ S	29° 00’ S	28° 59’ S
M.A.P. (mm)	1201	1116	1198	1486	1273	1259
M.A.T. (°C)	21.6	21.5	21.3	21.1	21	21.1
Altitude (m)	55	71	76	79	63	47
Major soil type	Fw1110	Vf 2110	Hu 26	Hu 2200	Hu 26	Fw32
Effective rooting depth (m)	1.51	1.51	1.21	1.51	1.51	0.5 – 0.8

Planting date	2006/08/23	2006/09/27	2006/09/15	2006/10/26	2006/08/17	2006/08/18		
Espacement	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m		
Fertiliser	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree		
Replications	20	20	20	20	20	20		
Plot size	Single tree	Single tree	Single tree	Single tree	Single tree	Single tree		
Number of clones	85	85	85	85	85	85		
IC365 CLONAL TRIAL SERIES								
Trial	IC365A	IC365B	IC365C	IC365D	IC365E	IC365F	IC365G	IC365H
Longitude	32^{o} 09’ E	32^{o} 08’ E	32^{o} 18’ E	31^{o} 50’ E	31^{o} 43’ E	31^{o} 52’ E	31^{o} 09’ E	31^{o} 15’ E
Latitude	28^{o} 30’ S	28^{o} 33’ S	28^{o} 18’ S	28^{o} 53’ S	28^{o} 58’ S	28^{o} 51’ S	28^{o} 40’ S	28^{o} 21’ S
M.A.P. (mm)	917	1008	1029	1524	1266	1370	1471	1038
M.A.T. (°C)	21.8	21.6	21	21.5	21.1	21.6	21.5	21.9
Altitude (m)	51	71	55	47	47	95	52	48
Major soil type	Fw1110	Fw1210	Fw1110	Fw1210	Ka10	Hu2100	Vf2110	Fw1110
Effective rooting depth (m)	151	151	151	151	50	151	151	151
Planting date	2009/05/30	2009/06/18	2009/06/03	2009/06/30	2009/07/22	2009/09/09	2009/09/21	2009/09/22
Espacement	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m	3m × 2m
Fertiliser	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)
Replications	15	15	15	15	15	15	15	15
Plot size	Single tree	Single tree	Single tree	Single tree	Single tree	Single tree	Single tree	Single tree
Number of clones	134	134	134	134	134	134	134	134

5.3.3 Statistical analysis

5.3.3.1 Descriptive statistics

In order to compute the descriptive statistics of each trial site for all the growth traits, PROC MEANS in SAS (SAS Institute 2002) was used. The descriptive analysis was executed prior to the standardisation of the data.

5.3.3.2 Standardisation of data

Forest trees often display heterogeneous variances for growth traits where a strong relationship between the mean of the trait and its phenotypic and genetic variances are seen. This relationship is such that the field trials with bigger trees will have larger phenotypic and genetic variances than the field trials with smaller trees even if the trials are at the same age (Hodge and Dvorak, 2012). In order to deal with these differences in scale, White et al. (2007) recommend data standardisation prior to analysis of variances, variance component analysis, or multi-site mixed model analysis. The standardisation of data homogenised variances that were used together in the linear model and eliminated any spurious genotype × environment interaction (Burdon 1977, Eisen and Saxon 1983, Hill 1984).

The standardisation for the analysis of this paper was performed as described by Hodge and Dvorak (2012). First, the coefficient of variation (CV) was calculated for each growth trait for each replication at each site. The mean coefficient of variances (CV_y) for each family-site-trait combination was also calculated. The phenotypic observations were then standardised with PROC STANDARD in SAS (SAS Institute 2002) for each replication at each site to a mean = 100, and with a standard deviation of = 100 × CV_y. This is equal to dividing all observations by the phenotypic standard deviation (SD), as recommended by White et al. (2007), followed by adding a constant (100) and multiplying by a constant (100 × CV_y). The population mean for the growth trait can therefore be interpreted as 100%, and the associated variances and SD are the same size relative to mean as in the raw data. Predicted breeding values and all variance components can thus be directly interpreted as percentage gain (above or below 100%) without back-transformation or rescaling.

5.3.3.3 Analysis of the *E. grandis* × *E. urophylla* seedling population

The statistical model used for the factorial was as follows:

$y_{ijklm} = \mu + S_i + R_{j(i)} + f_k + m_l + fm_{kl} + S_i*fm_{ikl} + e_{ijklm}$

Where,

y_{ijklm} = the m^{th} observation of the j^{th} replication for the kl^{th} family at the i^{th} site,

μ = overall mean,

S_i = fixed effect of the i^{th} site,

$R_{j(i)}$ = fixed effect of the j^{th} replication within the i^{th} site,

f_k or m_l = the random GHA effect for the k^{th} female or the l^{th} male,

fm_{kl} = random specific hybridising ability (SHA) effect of the k^{th} and l^{th} parents,

S_i*fm_{jkl} = random SHA by Site Interaction,

e_{ijklm} = random within plot error term,

All effects, except the overall mean, site and replication effect, were assumed to be random and independently distributed. A mixed model using PROC MIXED (SAS Institute 2002) in SAS was used to estimate variance components and to obtain BLUP estimates of random genetic effects (GHA and SHA) simultaneously. Restricted maximum likelihood (REML) was used in order to maximise the likelihood of the sample residuals. Wald test was used to determine the significance of the random effects.

The relationship between variance components derived from the quantitative genetic model was used to estimate the additive and dominance variance (Falconer 1981).

$\hat{\sigma}_f^2$ = variance due to the female effect,

$\hat{\sigma}_{Af}^2 = 4\hat{\sigma}_f^2$ is the additive variance due to the female effect,

$\hat{\sigma}_m^2$ = variance due to the male effect,

$\hat{\sigma}_{Am}^2 = 4\hat{\sigma}_m^2$ is the additive variance due to the male effect,

$\hat{\sigma}_A^2 = 1/2(\hat{\sigma}_{Af}^2 + \hat{\sigma}_{Am}^2)$ is the additive variance combining the female and male effect,

$\hat{\sigma}^2_{fm} =$ variance due to the family effect,

$\hat{\sigma}^2_D = 4\hat{\sigma}^2_{fm}$ is the dominance variance,

$\hat{\sigma}^2_G = \hat{\sigma}^2_A + \hat{\sigma}^2_D$ is the total genetic variance,

$\hat{\sigma}^2_{total} = \hat{\sigma}^2_G + \hat{\sigma}^2_e$ is the total phenotypic variance.

Heritabilities were estimated as:

$h^2_f = \frac{\hat{\sigma}^2_{Af}}{\hat{\sigma}^2_{total}}$ is the narrow-sense heritability for the female half-sibs,

$h^2_m = \frac{\hat{\sigma}^2_{Am}}{\hat{\sigma}^2_{total}}$ is the narrow-sense heritability for the male half-sibs,

$h^2_i = \frac{\hat{\sigma}^2_A}{\hat{\sigma}^2_{total}}$ is the narrow-sense heritability for the combined female and male hybrid parents,

$d^2 = \frac{\hat{\sigma}^2_D}{\hat{\sigma}^2_{total}}$ is the ratio of dominance variance to total individual phenotypic variance,

$H^2_i = \frac{\hat{\sigma}^2_G}{\hat{\sigma}^2_{total}}$ is the broad-sense heritability on an individual basis,

Standard errors of heritabilities were calculated by Dickerson's approximation (Dickerson 1969).

In order to determine the genetic correlations of the same trait expressed on multiple sites as described by Burdon (1977), type B genetic correlations were estimated as:

$r_{Bfm} = \frac{\hat{\sigma}^2_{fm}}{\hat{\sigma}^2_{fm} + \hat{\sigma}^2_{s*fm}}$ is the type B genetic correlation for the full-sib families.

Where,

$\hat{\sigma}^2_{fm}$ = variance due to the family effect,

$\hat{\sigma}^2_{s*fm}$ = variance due to the family by site interaction effect.

Type B correlation estimates the magnitude of genotype by environment interaction (G×E) that is due to rank changes across environments. This correlation over multiple sites can range between zero and one. An $r_B = 1$ predicts a perfect correlation between performance in different environments. In tree breeding, the generally accepted type B correlation estimate is equal to 0.7 (Hettasch et al. 2005).

The Pearson correlation coefficient between the GCA of *E. grandis* parents and the GHA of their interspecific hybrid progeny was estimated by using PROC CORR (SAS Institute 2002) in SAS. In the case of *E. urophylla* parents however, intraspecific progeny were not available to calculate the GCA values of the *E. urophylla* parents. Instead, individual tree breeding values were used for estimating the correlation coefficient. The individual tree breeding values of the *E. urophylla* pure species parents were estimated using BLUP analysis (Van den Berg et al. 2016a).

5.3.3.4 Analysis of the *E. grandis* × *E. urophylla* clonal population

The statistical model used for the factorial was as follows:

$y_{ijklmn} = \mu + S_i + R_{j(i)} + f_k + m_l + fm_{kl} + c_m + S_i * fm_{ikl} + S_i * c_{im} + e_{ijklmn}$

Where,

y_{ijklmn} = the n^{th} observation of the j^{th} replication for the kl^{th} family for the m^{th} clone at the i^{th} site,

μ = overall mean,

S_i = fixed effect of the i^{th} site,

$R_{j(i)}$ = fixed effect of the j^{th} replication within the i^{th} site,

f_k or m_l = the random GHA effect for the k^{th} female or the l^{th} male,

fm_{kl} = random SHA effect of the k^{th} and l^{th} parents,

C_m = random effect of the m^{th} Clone,

$S_i * fm_{jkl}$ = random SHA by Site Interaction,

$S_i * c_m$ = random Clone by Site Interaction,

e_{ijklm} = random within plot error term,

All effects, except the overall mean, site and replication effect, were assumed to be random and independently distributed. As with the seedling population, PROC MIXED (SAS Institute 2002) in SAS was used to estimate variance components and to obtain BLUP of random genetic effects (GHA, SHA and clone) simultaneously. Additive genetic variance,

dominance genetic variance and heritabilities were calculated the same way as for the hybrid seedling population with the following exceptions:

$\hat{\sigma}^2_{NA} = \hat{\sigma}^2_D + \hat{\sigma}^2_C$ is the total non-additive genetic variance,

$\hat{\sigma}^2_G = \hat{\sigma}^2_A + \hat{\sigma}^2_{NA}$ is the total genetic variance,

The ratio of clone variance to total individual phenotypic variance was estimated as:

$$c^2 = \frac{\hat{\sigma}^2_c}{\hat{\sigma}^2_{total}}$$

The type B genetic correlation for the clone effect was estimated as:

$$r_{Bc} = \frac{\hat{\sigma}^2_c}{\hat{\sigma}^2_c + \hat{\sigma}^2_{s*c}}$$

Where,

$\hat{\sigma}^2_c$ = variance due to the clone effect,

$\hat{\sigma}^2_{s*c}$ = variance due to the clone by site interaction effect.

PROC CORR (SAS Institute 2002) in SAS was used to estimate the Pearson correlation coefficients for volume between selected *E. grandis* × *E. urophylla* ortets and their ramets.

5.4 RESULTS

5.4.1 Descriptive statistics of *Eucalyptus grandis* × *E. urophylla* seedling and clonal trials

Descriptive statistics for DBH, height, tree volume and survival of the *E. grandis* × *E. urophylla* seedling progeny trials and clonal trials are presented in **Table 5.4** and **Table 5.5** respectively. The survival rates ranged between 79.4% and 92.3% for the seedling progeny trials and between 75.7% and 95.5% for the clonal trials. The mean DBH (age = seven years) for the seedling and clonal trials with the best growth rates was 24.5 cm (SD=4.65) and 17.38 (SD=3.7) respectively. The lowest mean DBH was 17.6 cm (SD=5.61) for the seedling progeny trials and 12.4 cm (SD=2.22) for the clonal trials. Overall, the standard deviations were higher for the seedling progeny trials (between 4.65 and 5.61) than for the clonal trials (between 2.22 and 3.82) for DBH at seven years of age. A similar pattern was seen for height and tree volume.

Table 5.4 Means and ranges from the *E. grandis* × *E. urophylla* seedling progeny trials for diameter at breast height (DBH in cm), height (m) and tree volume (m^3).

Site	N	Age	DBH	SD	Range	Height	SD	Range	Volume	SD	Range	Survival	SD
PE062A	1290	7	19.9	5.07	5.7 – 33.5	25.1	6.26	9.7 – 36.9	0.369	0.247	0.011 – 1.273	80.8	39.42
PE062B	1080	7	24.5	4.65	9.2 – 36.4	20.9	5.29	9.7 – 34.0	0.44	0.231	0.029 – 1.181	86.5	34.21
PE080A	1728	4	14.6	2.51	5.3 – 20.4	17.1	2.03	7.3 – 20.9	0.121	0.044	0.007 – 0.260	92.3	26.61
PE080B	1224	4	13.6	2.05	6.2 – 18.9	13.9	1.23	6.6 – 18.1	0.085	0.028	0.009 – 0.184	79.4	40.45
PE080C	894	4	11.2	2.05	5.2 – 16.5	13.2	1.73	5.8 – 18.1	0.056	0.023	0.005 – 0.128	88.8	31.51
PE109A	396	7	17.6	5.61	7.1 – 29.9	24.1	6.03	10.1 – 35.6	0.293	0.216	0.016 – 0.904	88.9	31.41
PE109B	228	7	17.9	5.59	7.5 – 29.2	23.2	5.34	10.5 – 32.7	0.288	0.211	0.018 – 0.842	88.2	32.42

Table 5.5 Means and ranges of the *E. grandis* × *E. urophylla* clonal trials for diameter at breast height (DBH in cm), height (m), tree volume (m^3) and survival (%).

Site	N	Age	DBH	SD	Range	Height	SD	Range	Volume	SD	Range	Survival	SD
IC358AKWA	1228	4	13.83	2.62	6.1 – 21.3	18.67	2.08	8.68 – 22.5	0.12	0.05	0.011 – 0.314	90.29	29.61
	1222	7	15.54	3.57	6.2 – 27.1							89.85	30.21
IC358BKWA	1224	4	13.19	2.20	5.6 – 19.2	17.26	1.81	8.12 – 21.9	0.10	0.04	0.009 – 0.236	90.22	29.71
	1196	7	15.00	3.17	5.8 – 25							87.94	32.58
IC358CMTZ	1233	4	12.19	1.66	6 – 16.8	14.64	1.16	8.4 – 17.5	0.07	0.02	0.009 – 0.151	90.66	29.11
	1173	7	13.99	2.48	6 – 20.5							86.25	34.45
IC358DMTZ	1114	4	12.56	2.11	5.5 – 19.5	15.26	1.48	7.7 – 19.9	0.08	0.03	0.007 – 0.238	81.91	38.51
	1030	7	14.68	3.20	5.5 – 24.2							75.74	42.88
IC358EPDF	1078	4	13.85	3.00	5.1 – 20.7	16.46	2.20	9 – 21.7	0.11	0.05	0.008 – 0.285	79.26	40.56
	1032	7	15.35	3.63	6.3 – 25.4							75.88	42.80

IC358FPDF	1090	4	13.72	2.84	5.1 – 20.8	15.93	2.12	8.26 – 21.4	0.10	0.05	0.008 – 0.286	80.15	39.90
	1046	7	15.89	3.82	5.9 – 26.7							76.91	42.16
IC361AKWA	1605	4	14.05	2.51	6 – 20.6	18.78	2.09	9.8 – 22.7	0.12	0.05	0.012 – 0.295	94.47	22.86
	1601	7	15.92	3.69	6.2 – 27.1							94.18	23.43
IC361BKWA	1623	4	14.96	2.60	5.5 – 21.5	19.30	1.99	11 – 22.9	0.14	0.06	0.01 – 0.332	95.47	20.80
	1602	7	16.98	3.73	5.5 – 27							94.20	23.38
IC361CPDF	1592	4	10.33	1.48	5.4 – 15.4	11.93	0.88	8 – 15.2	0.04	0.01	0.009 – 0.109	93.65	24.40
	1537	7	12.44	2.22	5.6 – 19.9							90.36	29.53
IC361DPDF	1351	4	14.87	2.59	5.3 – 21.4	17.31	1.69	9 – 21.9	0.13	0.05	0.009 – 0.29	79.41	40.45
	1338	7	17.38	3.72	5.3 – 27							78.71	40.95
IC361EMTZ	1575	4	12.37	2.30	5.6 – 20.5	15.72	1.52	9.2 – 19.6	0.08	0.03	0.01 – 0.259	92.71	26.01
	1528	7	14.99	3.57	5.6 – 28							89.88	30.17
IC361FMTZ	1609	4	12.51	2.27	5.3 – 18.5	16.29	1.97	7.8 – 20.1	0.09	0.04	0.008 – 0.215	94.65	22.52
	1532	7	14.46	3.22	5.5 – 22.6							90.06	29.93
IC365ATEZ	439	4	10.75	1.96	5.4 – 15.9	14.48	1.61	10.1 – 18.7	0.06	0.02	0.009 – 0.149	91.46	27.98
IC365BFCN	452	4	12.67	2.53	5.5 – 19	16.07	2.09	10.2 – 21.3	0.09	0.04	0.009 – 0.242	94.17	23.46
IC365CFNW	442	4	11.20	2.04	5.9 – 16.5	14.85	1.68	10.5 – 19.2	0.06	0.03	0.012 – 0.165	92.05	27.08
IC365DPDF	393	4	16.06	3.64	6.1 – 24.7	18.86	3.00	10.7 – 25.9	0.17	0.09	0.012 – 0.499	81.88	38.56
IC365EMTZ	401	4	13.45	2.46	5.2 – 19.1	16.71	2.02	9.9 – 21.4	0.10	0.04	0.008 – 0.246	83.54	37.12
IC365FPDF	419	4	15.65	2.86	5.8 – 23.3	18.52	2.36	10.4 – 24.8	0.15	0.07	0.011 – 0.424	87.29	33.34
IC365GKWA	407	4	15.26	3.24	5.7 – 23.2	18.20	2.67	10.3 – 24.7	0.15	0.07	0.011 – 0.419	84.79	35.95
IC365HDUK	438	4	11.74	2.35	5.6 – 18.3	15.30	1.93	10.2 – 20.7	0.07	0.03	0.01 – 0.218	91.25	28.29

5.4.2 Variance component estimates and genetic parameters of *Eucalyptus grandis* × *E. urophylla* seedling and clonal populations

Variance component estimates and genetic parameters are presented in **Tables 5.6** and 5.**7** for the *E. grandis* × *E. urophylla* seedling population, and in **Tables 5.8** and 5.**9** for the clonal population. The female variance component estimates ($\hat{\sigma}_f^2$) for the *E. grandis* hybrid parents were insignificant for the seedling (p<0.409 for DBH) and clonal (p<0.188 for DBH) populations (**Tables 5.6** and 5.**8**). The estimates of the female variance components ranged between zero (height and volume) and 0.76 (DBH) for the seedling population, and between 0.43 (height) and 26.55 (volume) for the clonal population. Consequently, the additive genetic variance and narrow-sense heritability were also low for the *E. grandis* hybrid parents in the seedling population ($\hat{\sigma}_{Af}^2$=3.04 and h_f^2=0.005 for DBH) and the clonal population ($\hat{\sigma}_{Af}^2$=15.12 and h_f^2=0.05 for DBH) as presented in **Tables 5.7** and 5.**9**.

The GCA estimates of the same *E. grandis* parents used in this study were between -9% and 12% and narrow-sense heritability 0.24 for volume in a full-sib pure species population (Van den Berg et al. 2016b). This is an indication that selecting for additive gene effects based on the *E. grandis* pure species parent performance may not necessary lead to genetic gains in growth if the same *E. grandis* parents are used as hybrid partners with *E. urophylla*. However, it must be born in mind that the *E. grandis* parents were selected from a second generation improved population and could allow for a more homogeneous population than the *E. urophylla* parents.

The genetic contribution to growth from the *E. urophylla* hybrid parents was significant (p<0.05 for volume) for the seedling population, but not for the clonal population (**Tables 5.6** and 5.**8**). Narrow-sense heritability for the *E. urophylla* hybrid parents in the seedling population was 0.24 for tree volume (**Table 5.7**) and 0.07 in the clonal population (**Table 5.9**). On average however, additive genetic variance ($\hat{\sigma}_A^2$=388.48) only explained 32% of the total genetic variation ($\hat{\sigma}_G^2$=1212.34) in the seedling population and narrow-sense heritability using the combined female and male additive effect was estimated at 0.12 for volume (**Table 5.7**). In the case of the clonal population, additive genetic variation ($\hat{\sigma}_A^2$=109.56) only

explained 29% of the total genetic variance ($\hat{\sigma}_G^2$=379.40) for volume at four years (**Table 5.9**). However, when the additive genetic variance was investigated in the clonal population, the progeny of *E. grandis* and *E. urophylla* hybrid parents performed similar with narrow-sense heritabilities estimated at 0.06 and 0.07 respectively for volume at four years.

A potential reason for the relatively low overall additive genetic variation could be due to the reduced genetic base of the parent populations. Parents were selected for growth, good form and resistance to parasites. This selection process allowed for a more homogeneous population for growth traits of the *E. grandis* and *E. urophylla* parents, and as a result could have reduced the additive variation in the hybrid populations. Future studies should include a wider range of hybrid parents to shed some light on this topic.

The genetic control of full-sib *E. grandis* × *E. urophylla* families was highly significant (p<0.001) for all growth traits in the seedling population (**Table 5.6**). The ratio of dominance variance to total variance (d^2) were estimated at 0.20 for DBH, 0.24 for height and 0.25 for tree volume (**Table 5.7**). Dominance variance ($\hat{\sigma}_D^2$=823.86) accounted for 68% of the total genetic variance ($\hat{\sigma}_G^2$=1212.34) for volume. The full-sib hybrid family growth performance in the seedling population was relatively stable across sites and little G×E was detected (r_{Bfm} = 0.74 for DBH, 0.75 for height and 0.84 for volume).

In the case of *E. grandis* × *E. urophylla* clonal population, non-additive genetic variation ($\hat{\sigma}_{NA}^2$=269.84) explained 71% of the total genetic variation ($\hat{\sigma}_G^2$=379.40) of the four year volume data (**Table 5.9**). Only 21.6% of the non-additive variation ($\hat{\sigma}_{NA}^2$=269.84) could be explained by the dominance variance ($\hat{\sigma}_D^2$= 58.39) and the full-sib hybrid family effect was insignificant as indicated in **Table 5.8**. The rest of the non-additive genetic variation was explained by the clone effect ($\hat{\sigma}_c^2$=211.46 for volume) and was highly significant ($p<0.001$) for the three growth traits (**Tables 5.8** and **5.9**). However, it must be borne in mind that, in many cases, only one or two individuals were selected from a specific cross to test as clones. Hence, the data from this clonal population might be inadequate to partition the non-additive genetic variance into dominance and epistatic genetic variance. This biased sample of the clonal population could also have a significant impact on the genetic parameter estimates. For instance, the portion of non-additive variance could be inflated in a case where only a few

individuals were selected from a certain family. Hence, interpreting the results from the clonal population should be done cautiously.

In order to try and shed some light onto the nature of non-additive and additive genetic effects, the random clonal effect was dropped from the linear model. It is apparent from **Table 5.9** that when clone effects were dropped from the model, the ratio explained by the dominance variance increased substantially from 0.04 to 0.27 for volume. The narrow-sense heritabilities for the *E. grandis* and the *E. urophylla* hybrid parents stayed low at 0.07 each. This is an indication that effects which the model previously allocated to remaining genetic effects among clones within a family are now mostly being absorbed by the inferred dominance genetic component of variation. This result points to a strong confounding effect between dominance and other epistatic terms present in the clone effect. Nevertheless, results from both the *E. grandis* × *E. urophylla* seedling and hybrid population indicated that a breeding strategy to capture non-additive genetic effects will be the most appropriate strategy to follow.

Table 5.6 Variance components of the random effects of the *E. grandis* × *E. urophylla* hybrid seedling population for diameter at breast height (DBH), height and tree volume.

	Effect	Estimate	SE	ZValue	ProbZ
DBH	female	0.76	3.29	0.23	0.409
	male	32.61	21.07	1.55	0.061
	full-sib hybrid families	30.93	9.75	3.17	0.001
	site*full-sib hybrid families	10.72	4.73	2.27	0.012
	error	533.64	11.15	47.86	<0.001
Height	female	0			
	male	16.56	10.86	1.52	0.064
	full-sib hybrid families	21.75	5.89	3.69	<0.001
	site*full-sib hybrid families	7.06	2.94	2.40	0.008
	error	312.53	6.53	47.87	<0.001
Volume	female	5.32E-17			
	male	194.24	114.46	1.70	0.045
	full-sib hybrid families	205.97	54.90	3.75	<0.001
	site*full-sib hybrid families	40.32	22.63	1.78	0.037
	error	2797.50	58.46	47.86	<0.001

Table 5.7 Genetic parameters of the *E. grandis* × *E. urophylla* hybrid seedling population for diameter at breast height (DBH), height and tree volume. $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{Af}}$ or $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{Am}}$ = additive genetic variance due to the female or male effect, $\boldsymbol{h}^{\boldsymbol{2}}_{\boldsymbol{f}}$ or $\boldsymbol{h}^{\boldsymbol{2}}_{\boldsymbol{m}}$ = narrow-sense heritability for female or male half-sibs, $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{D}}$ = dominance genetic variance, $\boldsymbol{d}^{\boldsymbol{2}}$ = ration of dominance variance to total phenotypic variance, $\boldsymbol{r}_{\boldsymbol{Bfm}}$ = type B correlation for the full-sib families × site interaction $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{A}}$ = additive genetic variance combining the female and male effect, $\boldsymbol{h}^{\boldsymbol{2}}_{\boldsymbol{i}}$ = narrow-sense heritability for the combined female and male effects, $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{G}}$ = total genetic variance and $\boldsymbol{H}^{\boldsymbol{2}}_{\boldsymbol{i}}$ = broad-sense heritability.

female (*E. grandis*)	DBH	Height	Volume
$\hat{\sigma}^2_{Af}$	3.04±13.27	0	0
h^2_f	0.005±0.02	0	0
male (*E. urophylla*)			
$\hat{\sigma}^2_{Am}$	130.46±84.29	66.22±43.45	776.97±457.83
h^2_m	0.21±0.14	0.19±0.12	0.24±0.14
full-sib hybrid families			
$\hat{\sigma}^2_D$	123.71±38.98	86.98±23.67	823.86±219.59
d^2	0.20±0.06	0.24±0.07	0.25±0.07
r_{Bfm}	0.74	0.75	0.84
$\hat{\sigma}^2_A$	66.75	33.11	388.48
h^2_i	0.11	0.09	0.12
$\hat{\sigma}^2_G$	190.45	120.09	1212.34
H^2_i	0.31	0.34	0.37
$\hat{\sigma}^2_{total}$	608.67	357.89	3238.03

Table 5.8 Variance components of the random effects of the *E. grandis* × *E. urophylla* hybrid clonal population for diameter at breast height (DBH), height and tree volume.

	Effect	Estimate	StdErr	ZValue	ProbZ
DBH	female	3.78	4.27	0.89	0.188
	male	5.42	4.37	1.24	0.108
	full-sib hybrid families	2.13	5.57	0.38	0.351
	clone	36.33	5.89	6.16	<0.001
	site*full-sib hybrid families	1.32	1.37	0.96	0.167
	site*clone	18.92	2.16	8.76	<0.001
	error	251.49	2.94	85.46	<0.001
Height	female	0.43	1.28	0.34	0.367
	male	0.83	1.25	0.66	0.254
	full-sib hybrid families	2.43	2.05	1.18	0.118
	clone	11.87	1.94	6.13	<0.001
	site*full-sib hybrid families	0.36	0.54	0.68	0.250
	site*clone	5.76	0.78	7.37	<0.001
	error	97.91	1.15	85.44	<0.001
Volume	female	26.55	27.62	0.96	0.168
	male	28.23	23.70	1.19	0.117
	full-sib hybrid families	14.60	31.56	0.46	0.322
	clone	211.46	33.71	6.27	<0.001
	site*full-sib hybrid families	4.57	7.02	0.65	0.257
	site*clone	103.13	11.38	9.07	<0.001
	error	1283.82	15.02	85.48	<0.001

Table 5.9 Genetic parameters of the *E. grandis* × *E. urophylla* hybrid clonal population for diameter at breast height (DBH), height and tree volume. $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{Af}}$ or $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{Am}}$ = additive genetic variance due to the female or male effect, $\boldsymbol{h}^{\boldsymbol{2}}_{\boldsymbol{f}}$ or $\boldsymbol{h}^{\boldsymbol{2}}_{\boldsymbol{m}}$ = narrow-sense heritability for female or male half-sibs, $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{D}}$ = dominance genetic variance, $\boldsymbol{d}^{\boldsymbol{2}}$ = ration of dominance variance to total phenotypic variance, $\boldsymbol{r}_{\boldsymbol{Bfm}}$ = type B correlation for the full-sib families × site interaction, $\boldsymbol{c}^{\boldsymbol{2}}$ = ration of clone variance to total phenotypic variance, $\boldsymbol{r}_{\boldsymbol{Bc}}$ = type B correlation for the clone × site interaction, $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{A}}$ = additive genetic variance combining the female and male effect, $\boldsymbol{h}^{\boldsymbol{2}}_{\boldsymbol{i}}$ = narrow-sense heritability for the combined female and male effects, $\widehat{\boldsymbol{\sigma}}^{\boldsymbol{2}}_{\boldsymbol{G}}$ = total genetic variance and $\boldsymbol{H}^{\boldsymbol{2}}_{\boldsymbol{i}}$ = broad-sense heritability.

	With clone			Without clone		
female (*E. grandis*)	DBH	Height	Volume	DBH	Height	Volume
$\hat{\sigma}^2_{Af}$	15.12±17.08	1.73±5.12	106.20±110.48	15.99±16.74	3.65±5.12	117.56±108.26
h^2_f	0.05±0.05	0.01±0.04	0.06±0.07	0.05±0.05	0.03±0.04	0.07±0.06
male (*E. urophylla*)						
$\hat{\sigma}^2_{Am}$	21.66±17.48	3.32±5.00	112.91±94.82	20.86±16.56	3.75±4.63	118.85±93.97
h^2_m	0.07±0.06	0.03±0.04	0.07±0.06	0.064±0.05	0.03±0.038	0.07±0.05
full-sib hybrid families						
$\hat{\sigma}^2_D$	8.51±22.28	9.72±8.21	58.39±126.24	79.08±23.62	28.07±8.21	452.74±135.17
d^2	0.03±0.07	0.08±0.07	0.04±0.08	0.24±0.07	0.23±0.068	0.27±0.08
r_{Bfm}	0.62	0.87	0.76	0.58	0.58	0.59
clone						
c^2	0.12±0.07	0.1±0.07	0.13±0.08			
r_{Bc}	0.66	0.67	0.67			
$\hat{\sigma}^2_A$	18.39	2.53	109.56	26.42	3.7	118.21
$\hat{\sigma}^2_{NA}$	44.84	21.59	269.84	79.08	28.07	452.74
$\hat{\sigma}^2_G$	63.24	24.12	379.40	105.5	31.77	570.95
H^2_i	0.20	0.20	0.23			
$\hat{\sigma}^2_{total}$	314.78	118.96	1644.96	324.59	121.05	1703.94

5.4.3 Correlation between pure species parents and hybrid parents growth performance

The above results indicated that when selecting for additive gene effects, *E. urophylla* hybrid parents played a bigger role than the *E. grandis* hybrid parents when the growth performance of *E. grandis* × *E. urophylla* hybrid seedlings was compared. The success of the current hybrid breeding strategy will however still depend on the correlation between the breeding values of the selected *E. urophylla* pure species parents and the GHA estimates of the *E. urophylla* hybrid parents.

In our study, the Pearson correlation between predicted individual breeding values of the *E. urophylla* pure species parents and the GHA of the *E. urophylla* hybrid parents was estimated at 0.58 (*p<0.007*) as indicated in **Table 5.10**. The individual breeding values of the selected *E. urophylla* pure species parents ranged between -3.5% to 21.6% gain (Van den Berg et al. 2016a). However, the correlation between family breeding values of the *E. urophylla* pure species families and the GHA estimates of the *E. urophylla* hybrid parents were lower (0.02, *p<0.943*), even though the range of the *E. urophylla* family breeding values (-7.8% to 19.7%) were similar to that of the individual breeding values as estimated by Van den Berg et al. (2016a).

Table 5.10 Pearson correlation coefficients between *E. urophylla* pure species parent family and individual breeding values (BV) and general hybridizing ability (GHA) of the *E. urophylla* hybrid parents. Prob > lrl under H0: Rho = 0.

Variable	N	Mean	Range	Correlation with GHA	p-value
GHA	20	1.89±9.45	-17.52 – 16.08	1	
Family BV	20	5.30±8.84	-7.75 – 19.69	0.017	0.943
Individual BV	20	12.20±5.74	-3.45 – 21.60	0.581	0.007

This result suggests that individual breeding values of the *E. urophylla* pure species are relatively good indicators of *E. urophylla* parent performance as hybrid partners with *E. grandis* if tree volume is the trait of interest. Hence, the selection for additive gene effects of

E. urophylla pure species parents will lead to genetic gains in growth traits of *E. grandis* × *E. urophylla* hybrid seedlings derived from the selected *E. urophylla* parents. However, these results are based on the performance of the *E. grandis* × *E. urophylla* hybrid seedling population. In most countries, interspecific hybrids of *E. grandis* × *E. urophylla* are commercially deployed as clones, and an important factor that should be kept in mind when deciding on the best *E. grandis* × *E. urophylla* hybrid breeding strategy is the correlation between the selected ortet and the ramets of the ortet.

5.4.4 Correlation between the standardised volumes and BLUPs of *E. grandis* × *E. urophylla* ortet and ramets

The individual tree volume of 126 *E. grandis* × *E. urophylla* seedling ortets and the mean tree volume of their ramets were used to estimate the phenotypic correlation between ortets and their ramets (**Table 5.11**). Standardised data were used to estimate the Pearson correlation coefficients. The percentage gain in individual tree volume of the selected seedling ortets that were used in calculating the correlation coefficients ranged between 1% and 197% over the *E. grandis* × *E. urophylla* hybrid seedling population mean. The tree volume means of their ramets ranged between -46% and 54% over the clonal population mean. The phenotypic correlation between the individual tree volumes of the ortets and the mean tree volumes of their ramets was positive and estimated at 0.3174 ($p<0.0003$).

In addition to the phenotypic correlations, the family and individual breeding values of the *E. grandis* × *E. urophylla* seedling ortets were correlated (Pearson) to the mean clonal BLUP estimates of their ramets. Clonal individual and family breeding values could not be calculated due to the insignificance ($p<0.322$) of the full-sib hybrid family effect in the clonal population. For the same reason, a reliable genetic correlation between *E. grandis* × *E. urophylla* hybrid ortets and ramets could not be calculated.

The family breeding values of the seedling population ranged between -25% and 39.5%, and between -8% and 61% for the individual breeding values (**Table 5.11**). The mean clonal BLUP estimates for the clonal population ranged between -31% and 38%. A correlation coefficient of 0.1522 ($p<0.0901$) was detected between the ortet family breeding values and

the mean BLUP estimates of their ramets. In the case of individual breeding values, a higher correlation coefficient of 0.2481 ($p<0.0053$) was estimated between the ortet individual breeding values and the mean BLUP estimates of the clones. The best correlation coefficient (0.3355, $p<0.0001$) however, was detected between the individual breeding values of the ortets and the mean tree volumes of their ramets.

In general, the correlation between seedling ortets and their ramets was relatively weak for all the variables used and is an indicator that the best seedling does not necessary produce the best clone. The feasibility of selection at seedling level therefore needs to be investigated.

Table 5.11 Pearson correlation coefficients between *E. grandis* × *E. urophylla* seedling ortets and their ramets for tree volume. BV = breeding values, std = standardised data, blup = best linear unbiased prediction estimates. N=126, Prob > lrl under H0: Rho = 0.

		Clonal ramets	
		mean std volume (Range: -46% – 54%)	clonal blups (Range: -31% – 38%)
Seedling ortets	individual std volume (Range: 1% – 197%)	0.3174 (*p<0.0003*)	0.2673 (*p<0.0026*)
	family bv (Range: -25% – 39.5%)	0.22566 (*p<0.0114*)	0.1522 (*p<0.0901*)
	individual bv (Range: -8% – 61%)	0.3355 (*p<0.0001*)	0.2481 (*p<0.0053*)

5.5 DISCUSSION

The results from our study indicated that non-additive genetic variation explained majority of the genetic variation present in *E. grandis* × *E. urophylla* seedling and clonal populations. The same phenomenon was found by other authors for *E. grandis* × *E. urophylla* hybrid seedlings (Bouvet et al. 2009, Retief and Stanger 2009, Rezende and de Resende 2000, Vigneron et al. 2000). Retief and Stanger (2009) reported that dominance genetic effects accounted for nearly 60% of the total genetic variance in the *E. grandis* × *E. urophylla* hybrid factorial of their study in Zululand. Bouvet et al. (2009) reported an average $\hat{\sigma}_D^2/\hat{\sigma}_A^2$ ratio of 1.2 for a relatively large *E. grandis* × *E. urophylla* hybrid seedling population (684 families).

One explanation of the high dominance variance could be due to the nature of dominance variance. The dominance effect between alleles and their frequency will determine the magnitude of the dominance variance (Lynch and Walsh 1998). Dominance variance exceeds additive variance in the case of overdominance and/or in the case of total dominance when frequencies of alleles are different when a model of one locus and two alleles are used (Lynch and Walsh 1998). Although the case of overdominance must be considered with caution (Birchle et al. 2006), it may explain some of the relatively high dominance variance present in perennial plants such as *Eucalyptus* (Bouvet et al. 2009). Nevertheless, these results indicate that a breeding strategy to capture non-additive genetic effects in the hybrid will be the most appropriate strategy to follow.

Three strategies that could potentially exploit non-additive variance are RRS (Comstock et al. 1949), reciprocal recurrent selection with forward selection (RRS-SF, Nikles 1992) and the development and crossing of inbred lines. The implications of these strategies for tree improvement have been discussed by various authors (Dungey et al. 2000, Hettasch et al. 2005, Nikles 1992, Shelbourne 2000, Vigneron 1991). Kerr et al. (2004) did a simulation study comparing RRS, RRS-SF, recurrent selection for general combining ability (RS-GCA, Jenkins 1940) and the hybrid swarm strategy over five cycles of breeding. Results from this study suggest that the RRS-SF strategy yielded the highest genetic gains per year in cases where non-additive variance is higher than additive variance and when the pure-hybrid correlations are negative or close to zero. However, the latter was not cost efficient at all.

Another important factor to consider when developing an interspecific hybrid breeding strategy is the weak correlations that were detected between *E. grandis* × *E. urophylla* hybrid seedling ortets and their ramets in our study. One possible reason for the weak correlation is the degree of environmental influence that was present in the *E. grandis* × *E. urophylla* hybrid seedling population. The H_i^2 was estimated as 0.37 and the h_i^2 as 0.12 in the seedling population. This is an indication that majority of the variance in the *E. grandis* × *E. urophylla* hybrid seedling population was explained by environmental noise and could have an impact on the ortet-ramet phenotypic correlation. Although we did investigate the correlation between family and individual breeding values of the *E. grandis* × *E. urophylla* hybrid seedling ortets and the clonal BLUP estimates of their ramets, a more detailed study is required to investigate the genetic correlation between eucalypts hybrid seedling ortets and their ramets.

Another fundamental difference between seedlings and clones that could potentially influence the correlation between ortet and ramets is the structure of their root system (Hartmann et al. 1990). Sasse and Sands (1997) reported that *E. globulus* seedlings had strongly gravitropic tap-roots, with two types of primary roots from which secondary roots emerged. Clones had no tap roots, but it had adventitious roots that were formed during propagation. Grossnickle and Russell (1990) found that cuttings of *Chamaecyparis nootkatensis* produced less new root area than seedlings over 21 days. Fuller and Little (2007)

also reported that *E. grandis* seedlings had significantly longer roots than micro-cuttings, as well as a better distribution around the plug. However, no significance in growth was reported in their study. Gaspar et al. (2005) also reported that there was no significant difference between *E. globulus* cuttings and seedlings for growth and wood density. Sasse and Sands (1996) conducted a study to test the responses of *E. globulus* cuttings and seedlings to water stress and reported that the seedlings had greater water use than cuttings in the water stress treatments. However, in all these studies cuttings were produced from different individuals with a similar genetic makeup than the seedlings of a pure species population. No direct comparison of ortets and their ramets have been reported on.

Other effects associated with cloning such as rooting ability of different individuals and C effects could also contribute to the difference in growth performance between *E. grandis* × *E. urophylla* seedling ortets and their ramets. C effects are related to non-genetic sources of covariance between ramets of the same clone and may be due to factors such as the age or the environment of the original ortet (Costa e Silva et al. 2004). When C effects are present, it may inflate estimates of between-clone variances (Libby and Jund 1962, Burdon and Shelbourne 1974), and may upwardly bias epistatic genetic variance estimates (Costa e Silva et al. 2004). Inequalities among propagules within clones due to ontogenic factors such as cutting position on the ortet or morphological factors such as cutting size may also arise with cloning and could affect growth performance (Costa e Silva et al. 2004). Nevertheless, if the correlation between the selected *E. grandis* × *E. urophylla* seedling ortet and their ramets are weak as suggested in our study, then the feasibility of selection at seedling level needs to be investigated.

5.6 CONCLUSIONS

The study was set out to review the current *E. grandis* and *E. urophyla* hybrid breeding strategy used in South Africa and has identified key areas to investigate.

Information on genetic parameters of *E. grandis* × *E. urophylla* hybrid seedling and clonal populations needed quantification. The pure-hybrid and ortet-ramet correlation values are essential to develop a suitable hybrid breeding strategy and were explored in this study. The general theoretical literature on this subject and specifically in the context of *E. grandis* × *E. urophylla* hybrid clonal populations is inconclusive on several vital questions within the hybrid breeding discourse. The study sought to answer some of these questions.

Results from our study indicated that non-additive variance plays a major role in determining the growth performance of *E. grandis* × *E. urophylla* hybrid seedlings and clones. Due to the pre-eminence of non-additive variance, the pure-hybrid correlations were weak, especially for clonal populations. It would therefore seem that GCA or individual breeding values are not good predictors of GHA for growth performance in the observed populations. Results from this study also indicated a weak ortet-ramet correlation for *E. grandis* × *E. urophylla* hybrids. This suggests that the current strategy to first screen *E. grandis* × *E. urophylla* seedlings in progeny trials should be revisited.

Although our study has offered an evaluative perspective on *Eucalyptus* hybrid breeding, it encountered a number of limitations, which need to be considered. Firstly, all the *E. grandis* and *E. urophylla* parents used for interspecific crossing were selected on their mature age phenotypes for growth and tree form. This selection process might explain the lack of additive variance present in the observed *E. grandis* × *E. urophylla* hybrid populations. Secondly, in view of the selected nature of *E. grandis* × *E. urophylla* ortets and the limited numbers of individuals per family, results on the *E. grandis* × *E. urophylla* clonal populations must be interpreted cautiously. Due to this limitation, the family effect of clonal populations was insignificant, and ortet-ramet correlations were restricted to phenotypic correlations.

The scale of this debate is therefore extensive and to develop a suitable and cost effective eucalypt hybrid breeding strategy, there is a need for more case studies to allow further assessments of this subject.

In spite of the limitations of this study, it has attained its primary objective namely: to review the current hybrid breeding strategy. From the results of the study, the overall recommendation is to adopt a hybrid breeding strategy that captures non-additive genetic effects in combination with a strategy that minimises the testing time of *E. grandis* × *E. urophylla* hybrid material as seedlings.

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CHAPTER 6 REALISED GENETIC GAINS AND ESTIMATED GENETIC PARAMETERS OF TWO *EUCALYPTUS GRANDIS* × *EUCALYPTUS UROPHYLLA* HYBRID BREEDING STRATEGIES

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6.1 ABSTRACT

Conventionally, *Eucalyptus grandis* × *E. urophylla* (GU) hybrid material has first been tested as seedlings in progeny trials for at least four years before ortets were selected and ramets of the selected ortets were propagated to test in clonal trials. The primary constraint with this "conventional hybrid breeding strategy" (CHBS) is the time required to first test the hybrid material as seedlings. In order to address this, an "accelerated hybrid breeding strategy" (AHBS) was investigated to reduce the time spend on testing GU hybrid material as seedlings. However, it is of utmost importance to quantify the impact the AHBS might have on genetic gains and genetic information.

With this in mind, two clonal populations have been established with genetic material that derived from the CHBS and the AHBS. The aim of this study is therefore to do a comparative study between the CHBS and AHBS. The specific objectives were set to firstly quantify the genetic gains per unit time for GU hybrid clonal populations that have been derived from the CHBS and AHBS respectively and secondly to obtain genetic parameters such as heritabilities, the ratio of dominance, clonal within family variance and the proportion of additive and non-additive genetic variance.

The results of our study indicated that the percentage realised volume gains per year was higher for the AHBS (3.7%) than for the CHBS (1.9%) when compared to the GU commercial clone. Thus, shortening the testing time of GU seedlings had a positive impact on volume gains per year.

With regards to genetic parameters, both the AHBS and CHBS clonal populations indicated that non-additive genetic variation explained majority (88% and 71% respectively) of the genetic variation. Due to the pre-eminence of non-additive genetic variation, the narrow-sense heritabilties for the female and male effects were negligible for both clonal populations. Overall, the majority of the non-additive genetic variation was explained by the proportion of dominance variance, and less by the clone within family effect. These results suggest that: firstly the time spend on testing GU hybrid material as seedlings should be minimised, and secondly a hybrid breeding strategy to capture non-additive genetic variation should be adopted.

Keywords

Eucalyptus grandis × *E. urophylla* clonal populations, hybrid breeding strategies, genetic parameters, realised genetic gains

6.2 INTRODUCTION

Eucalypts have the ability for interspecific hybridisation (Griffin et al. 1988, Potts and Wiltshire 1997). Its hybrids hold the potential to produce genotypes with special combinations, e.g. specific timber properties, disease resistance and greater vigour compared to the pure species (Hettasch et al. 2005). Hybrids with *Eucalyptus grandis* are important in countries such as Brazil, China, Colombia, Congo, South Africa and Venezuela (Denison and Kietzka 1992, Endo and Lambeth 1992, Nikles 1992, Ferreira and Santos, 1997, Wright 1997, de Assis 2000, Retief and Clarke 2000, Verryn 2000). Its hybrids with various species, especially *Eucalyptus urophylla*, are becoming increasingly important for enhancing yields and disease resistance (White et al. 2007). A good example of where *E. grandis* cannot be planted as a pure species due to its susceptibility to fungal diseases, but are grown successfully as a hybrid partner with *E. urophylla* is in the sub-tropical coastal region of South Africa, namely Zululand (Retief and Stanger 2009). Superior clones for pulp production in Zululand have been obtained from this interspecific hybrid population and have produced volume gains of up to 50% over the *E. grandis* seedling controls (Gardner 2001).

However, a primary constraint in the management of hybrid breeding when compared to pure species breeding is the additional time required to test the hybrid material. With the CHBS, GU hybrid material is first tested as seedlings in progeny trials across sites for at least four years. Ortets are then selected and ramets of the selected ortets are thereafter propagated to test in clonal trials. The feasibility of testing GU hybrid material as seedlings to such an extent is questionable considering the weak phenotypic correlation that was detected between ortets and ramets (Van den Berg et al. 2016). In addition, genetic gains also need to be achieved as rapidly as possible in order to justify expenditures associated with tree

improvement. This has led to the concept of maximizing genetic gains per unit time instead of per cycle of breeding (White et al. 2007).

With this in mind, an AHBS was developed and tested in order to reduce the time and money spent on testing GU hybrid material as seedlings. The impact of the AHBS on realised genetic gains needs to be quantified in order to justify the implementation of this strategy. Realised gains are obtained by comparing unimproved varieties to improved varieties (or with varieties having varying levels of improvement) in the same experiment (Zobel and Talbart 1984, White et al. 2007). In this study, five controls at various levels of improvement were used in all the experiments.

In addition, genetic parameters such as heritabilities and the proportion of additive and non-additive genetic variance of both the GU clonal populations need to be determined in order to design the best interspecific hybrid breeding strategy. A comparative study between the CHBS and the AHBS was therefore conducted with the following objectives:

- Obtain genetic parameters for GU hybrid clonal populations that were derived from the CHBS and AHBS.
- Quantify the realised genetic gains per unit time for the GU clones that were generated from the CHBS and AHBS.

6.3 MATERIALS AND METHODS

6.3.1 Hybrid breeding strategies

The breeding strategy preceding the production of GU hybrid seed was the same for the CHBS and AHBS. All the parents were selected based on their mature age phenotypes for growth and tree form. The *E. grandis* parents are cloned selections made in a second generation progeny trial series from the South Africa Forestry Research Institute programme. The *E. urophylla* parents are cloned selections from an unimproved provenance/progeny trial series of open-pollinated seed collected from different provenances on Indonesian Islands. The selected *E. grandis* and the *E. urophylla* parents were established in an elite potted orchard where controlled pollinations commenced. The controlled pollinations between the *E.*

grandis and *E. urophylla* parents were mainly driven by flowering. Full-sib GU hybrid families from the first two controlled pollination seasons were assigned to the CHBS and thereafter to the AHBS.

The main difference between the CHBS and AHBS lies in the screening of GU seedlings as the first phase of testing hybrid material (**Figure 6.1**). In the CHBS, GU hybrid seedlings were tested in a series of progeny trials across seven sites. These trials were established at the recommended commercial spacing (3m × 2m). Each GU hybrid family was planted in a 1 × 6 tree line plot and replicated between six and 12 times across each site. Tree growth was measured when the trees were four years of age, and the best performing families and individuals within families were then assessed for pest and disease resistance as well as tree form. Growth results of these GU hybrid progeny trials were described by Van den Berg et al. (2016). Based on the four year results, best individuals were then selected and used to produce cuttings for clonal testing.

In the AHBS, the GU seedlings were tested over a shorter time period and at a minimum cost. The GU hybrid seedlings were planted in a "hybrid seedling selection block" (HSSB). Each GU family was planted in a single plot of 10 × 10 trees at a single site. The site was selected at the nursery in order to reduce costs associated with establishing field trials at various sites away from the nursery. The seedlings were planted at a narrow spacing of 1 m × 1 m in order to minimize the space used for testing, as well as to force earlier onset of competition. At 1.5 years of age, the best individuals within each family were selected based on growth and resistance to pests and diseases. No measurements were taken at this stage and all selections were performed visually in order to save costs. Cuttings were then produced from the selected ortets and tested in clonal trials. The main purpose of the AHBS is to investigate what the impact will be on clonal performance if the screening of GU hybrid seedlings is done more cost effectively and over a shorter time period. With this in mind, the clones derived from both strategies were tested in clonal trials with the same design, as described in **Table 6.2**.

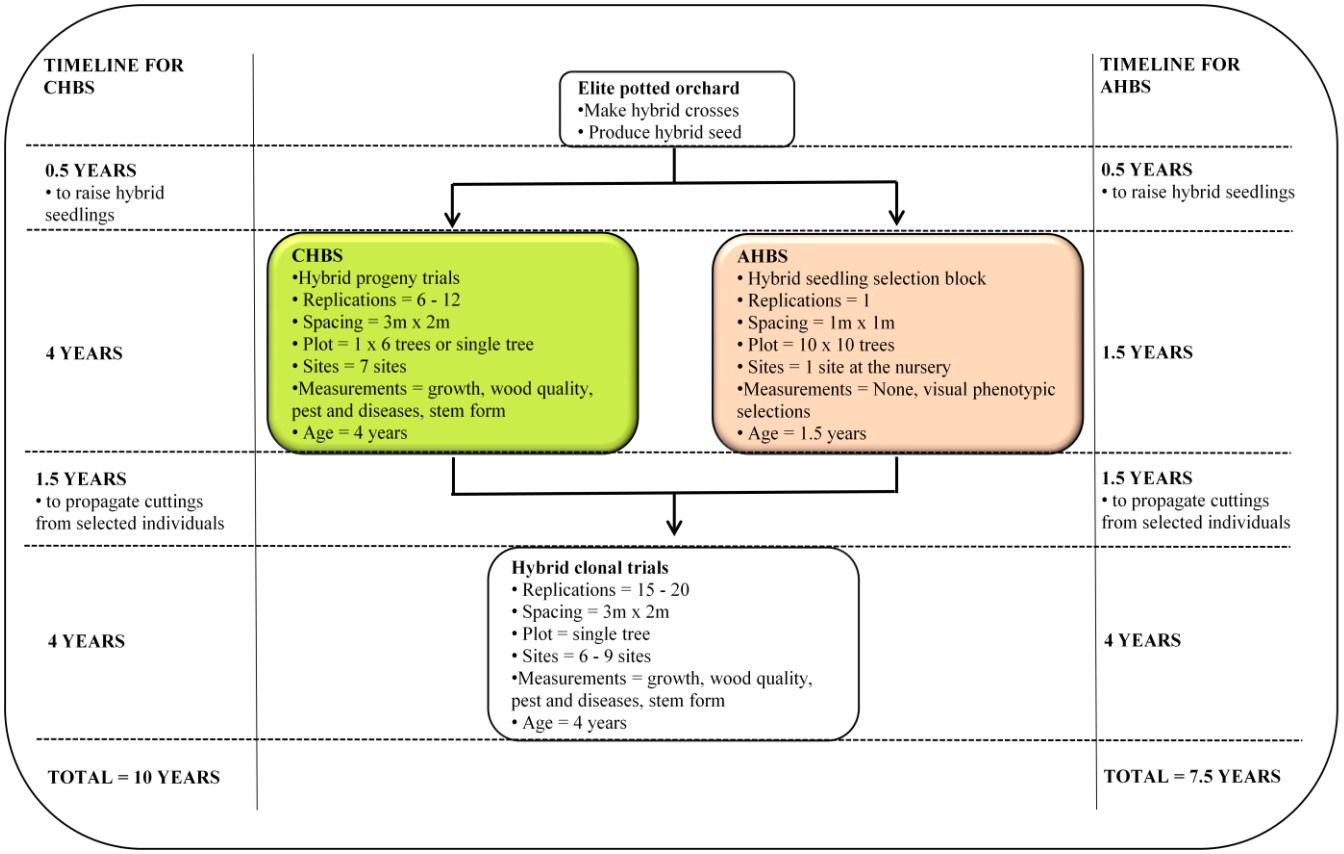

Figure 6.1 Timelines and procedures for the conventional and accelerated hybrid breeding strategies.

6.3.2 Breeding material

The clonal population of the CHBS consists of 148 selections (**Table 6.1**) that derived from a series of GU seedling progeny trials described by Van den Berg et al. (2016). A total of 24 *E. grandis* and 23 *E. urophylla* selected parents were used in various combinations to produce 108 GU families for the progeny trials. The fundamental Hardy-Weinberg assumption that crosses are made from parents selected randomly from the population of interest, has therefore been violated. The 148 GU ortets selections were made from 63 families in the progeny trials. This equates to a selection intensity of 58% (63 from108 families) at the family level and 2% (148 from 6840 individuals) at the population level. All ortets were selected between four and seven years of age for growth, pest and disease resistance as well as tree form. Between 90 and 180 ramets were produced from each ortet and were established in a series of clonal trials at a minimum of six sites.

The clonal population of the AHBS consists of 211 selections (**Table 6.1**) from 38 GU families in the HSSB. A total of 11 *E. grandis* and 18 *E. urophylla* parents were used to produce the 38 GU families (**Table 6.1**). Individual selections were made from all 38 families. All ortets were visually selected at 1.5 years of age for growth and pest and disease resistance. The selection intensity for the population was 5.5% (211 from 3900 individuals). Between 90 and 180 ramets were produced from each ortet and were established in a series of clonal trials at nine sites.

In order to link the trials and to calculate realised gains, five common commercial controls were established in all the trials namely:

- One GU current commercial clone
- One *E. grandis* × *E. camaldulensis* (GC) current commercial clone
- Two *E. grandis* post commercial clones
- Improved *E. grandis* seedlings

The two current commercial hybrid clones are those that were planted commercially at the time when the trials were established. The two *E. grandis* post commercial clones are those that were planted commercially in Zululand in the early 1990's, and the *E. grandis* seedling control is a third generation bulk seedlot.

Table 6.1 Number of selections made from each *E. grandis* × *E. urophylla* full-sib hybrid family and the number of clonal trials established for the conventional and accelerated hybrid breeding strategies.

Clonal material of conventional hybrid breeding strategy			Clonal material of accelerated hybrid breeding strategy		
Fam	**Selections**	**Trials**	**Fam**	**Selections**	**Trials**
G1×U12	1	6	G12×U28	5	9
G3×U17	3	6	G12×U29	4	9
G3×U19	1	6	G12×U38	4	9
G4×U3	3	6	G12×U40	3	9
G4×U6	1	6	G12×U42	1	9
G4×U8	9	6	G13×U39	13	9
G4×U12	4	6	G14×U2	1	9
G4×U16	4	6	G15×U32	6	9
G4×U18	7	6	G15×U33	6	9
G4×U19	1	6	G15×U34	5	9
G5×U17	2	6	G15×U41	3	9
G6×U1	5	6	G16×U34	8	9
G6×U8	2	6	G16×U35	5	9
G6×U9	1	8	G16×U43	1	9
G6×U13	1	8	G17×U30	2	9
G6×U14	2	6	G17×U33	9	9
G6×U16	1	6	G17×U34	7	9
G6×U17	1	6	G17×U35	7	9
G6×U18	2	6	G17×U36	7	9
G6×U27	5	6	G17×U39	7	9
G7×U5	4	6	G29×U31	1	9
G7×U8	7	6	G31×U36	9	9
G7×U10	1	8	G3×U28	1	9
G7×U13	3	6	G3×U29	18	9
G7×U18	2	8	G3×U32	1	9
G7×U19	3	6	G3×U35	9	9
G7×U20	1	6	G3×U36	13	9
G7×U21	1	8	G3×U37	9	9
G7×U22	1	8	G3×U40	9	9
G7×U23	1	8	G3×U41	5	9
G8×U13	1	6	G3×U43	11	9
G8×U17	5	6	G7×U28	4	9
G10×U3	2	8	G7×U29	3	9
G10×U6	2	6	G7×U40	5	9
G10×U21	1	8	G7×U42	2	9
G10×U26	1	6	G7×U44	1	9
G11×U6	1	6	G8×U28	2	9
G13×U3	1	6	G8×U30	4	9
G13×U8	2	6			
G14×U6	1	6			
G14×U14	2	6			

G14×U15	2	6	
G15×U8	5	6	
G15×U9	1	8	
G16×U14	7	6	
G18×U16	1	6	
G19×U6	1	6	
G20×U14	3	6	
G21×U14	2	6	
G21×U15	2	6	
G21×U16	1	6	
G21×U18	2	6	
G23×U2	4	6	
G23×U13	1	6	
G23×U17	2	6	
G23×U18	1	6	
G24×U18	1	6	
G25×U14	4	6	
G27×U4	1	6	
G28×U13	1	6	
G28×U19	5	6	
G29×U2	1	6	
G30×U8	1	8	

6.3.3 Trial establishment and measurements

The trial sites generally have deep sandy soils and have mean annual temperatures (M.A.T.) of 21^{o}C – 21.9^{o}C (**Table 6.2**). Mean annual precipitation (M.A.P.) range between 862 mm – 1524 mm. Three GU clonal trial series (IC358A – F, IC361A – F and IC365A – H) were established with ramets that were derived from the GU progeny trials and two trial series (IC363A – I and IC365A – H) with ramets that were derived from the HSSB. One clonal trial series (IC365A – H) consists of ramets from both strategies. Between six and nine clonal trials were established for each series. Each trial was planted in a randomized complete block (RCB) design. The clones were planted in single tree plots and replicated (reps) between 15 and 20 times across each site. Spacing was 3 m × 2 m in all trials. Growth traits namely: height (in metres) and diameter at 1.3 m, (DBH, in centimetres) were taken at four and seven years. Tree volume was calculated using the following equation as described by Max and Burkhart (1976):

Volume =($\frac{\pi}{40000}$)*k*DBH^2*HT

Where,

k=($\frac{\beta_1}{3}$)+($\frac{\beta_0}{2}$)-(β_0+β_1)+($\frac{\beta_2}{3}$)*${a_1}^3$+($\frac{\beta_3}{3}$)*${a_2}^3$

Functions used to calculate k were developed internally by Mondi Limited (Kotze and Fletcher, unpublished data).

A detailed description of each trial site is presented in **Table 6.2**.

Table 6.2 Site and trial information of *E. grandis* × *E. urophylla* hybrid clonal trials established with clonal material derived following the conventional and/or the accelerated hybrid breeding strategy.

IC358 Clonal trial series (Conventional hybrid breeding strategy)						
Trial	IC358A	IC358B	IC358C	IC358D	IC358E	IC358F
Longitude	32° 06' E	32° 03' E	31° 42' E	31° 43' E	31° 53' E	31° 50' E
Latitude	28° 31' S	28° 37' S	28° 59' S	28° 59' S	28° 52' S	28° 53' S
M.A.P. (mm)	1084 mm	1051 mm	1293 mm	1295 mm	1379mm	1476 mm
M.A.T. (°C)	21.6 °C	21.6°C	21.1 °C	21.1 °C	21.3°C	21.1 °C
Altitude (m)	55 m	63 m	24 m	47 m	63 m	95 m
Soil type	Kd2000	FERNWOOD1210	HUTTON 27	HUTTON 26	HUTTON 2200	HUTTON 2200
E.R.D. (m)	1 – 1.4m	1.51	1.21	1.21	1.51	1.51
Planting date	2005/04/29	2005/05/09	2005/06/17	2005/08/24	2005/06/13	2005/07/05
Fertiliser	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree
No. of reps	20	20	20	20	20	20
No. of clones	68	68	68	68	68	68
IC361 Clonal trial series (Conventional hybrid breeding strategy)						
Trial	IC361A	IC361B	IC361C	IC361D	IC361E	IC361F
Longitude	32° 12' E	32° 08' E	31° 58' E	31° 49' E	31° 41' E	31° 42' E
Latitude	28° 36' S	28° 39' S	28° 44' S	28° 54' S	29° 00' S	28° 59' S
M.A.P. (mm)	1201	1116	1198	1486	1273	1259
M.A.T. (°C)	21.6	21.5	21.3	21.1	21	21.1
Altitude (m)	55	71	76	79	63	47
Soil type	FERNWOOD 1110	VILAFONTES 2110	HUTTON 26	HUTTON 2200	HUTTON 26	Fw32 + We13
E.R.D. (m)	1.51	1.51	1.21	1.51	1.51	0.5 – 0.8
Planting date	2006/08/23	2006/09/27	2006/09/15	2006/10/26	2006/08/17	2006/08/18
Fertiliser	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree	DAP @ 60g/tree
No. of reps	20	20	20	20	20	20
No. of clones	85	85	85	85	85	85
IC363 Clonal trial series (Accelerated hybrid breeding strategy)						

Trial	IC363A	IC363B	IC363C	IC363D	IC363E	IC363F	IC363G	IC363H	IC363I
Longitude	31° 40' E	31° 44' E	32° 12' E	32° 22' E	32° 03' E	31° 49' E	32° 25' E	32° 11' E	31° 52' E
Latitude	29° 02' S	28° 59' S	28° 25' S	28° 13' S	28° 39' S	28° 54' S	28° 07' S	28° 37' S	28° 53' S
M.A.P. (mm)	1247	1291	881	999	1058	1467	862	1211	1427
M.A.T. (°C)	21.0	21.2	21.9	21.8	21.7	21.2	21.9	21.6	21.3
Altitude (m)	66	16	57	47	39	63	35	60	32
Soil type	HUTTON 2200	FERNWOOD 11	FERNWOOD 11	FERNWOOD 1110	FERNWOOD 1210	FERNWOOD 1210	LONGLANDS 1000	FERNWOOD 1210	KROONSTAD 1000
E.R.D. (m)	1.51	1.2	1.2	1.51	1.51	1.51	0.8	1.51	0.9
Planting date	2008/03/27	2008/05/27	2008/06/26	2008/08/14	2008/09/05	2008/10/01	2008/10/22	2008/10/21	2008/10/22
Fertiliser	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)
No. of reps	15	15	15	15	15	15	15	15	15
No. of clones	115	115	115	115	115	115	115	115	115

IC365 Clonal trial series (Conventional and Accelerated hybrid breeding strategies)

Trial	IC365A	IC365B	IC365C	IC365D	IC365E	IC365F	IC365G	IC365H
Longitude	32° 09' E	32° 08' E	32° 18' E	31° 50' E	31° 43' E	31° 52' E	31° 09' E	31° 15' E
Latitude	28° 30' S	28° 33' S	28° 18' S	28° 53' S	28° 58' S	28° 51' S	28° 40' S	28° 21' S
M.A.P. (mm)	917	1008	1029	1524	1266	1370	1471	1038
M.A.T. (°C)	21.8	21.6	21	21.5	21.1	21.5	21.5	21.9
Altitude (m)	51	71	55	47	47	95		
Soil type	FW1110	FW1210	FW1110	FW1210	Ka10	Hu2100	Vf2110	Fw1110
E.R.D. (m)	151	151	151	151	50	151	151	151
Planting date	2009/05/30	2009/06/18	2009/06/03	2009/06/30	2009/07/22	2009/09/09	2009/09/21	2009/09/22
Fertiliser	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)	LAN (28)
No. of reps	15	15	15	15	15	15	15	15
No. of clones	134	134	134	134	134	134	134	134

6.3.4 Statistical analysis

6.3.4.1 Standardisation of data

The standardisation of data prior to analysis of variances, variance component analysis, or multi-site mixed model analysis is important for three reasons: (1) To homogenized variances that were used together, (2) To eliminate statistically significant interaction for genotype × environment interaction due to scale effects, and (3) To facilitate bias-free back transformations to predict genetic gains in the units of measure in various environments (Hill 1984, Visscher et al. 1991, Jarvis et al. 1995, White et al. 2007).

The standardisation for the analysis of this paper was performed as described by Hodge and Dvorak (2012). PROC STANDARD in SAS (SAS Institute 2002) was used to standardised phenotypic observations in each replication to a mean = 100. The population mean for the growth trait can therefore be interpreted as 100%, and the associated variances and SD are the same size relative to mean as in the raw data. All variance components can thus be directly interpreted as percentage gain (above or below 100%) without back-transformation or rescaling.

6.3.4.2 Analysis of the *E. grandis* × *E. urophylla* clonal populations

The statistical model used was as follows:

$$y_{ijklmn} = \mu + S_i + R_{j(i)} + f_k + m_l + fm_{kl} + c_{m(kl)} + e_{ijklmn}$$

Where,

y_{ijklmn} = the n^{th} observation of the j^{th} replication for the kl^{th} family for the m^{th} clone at the i^{th} site,

μ = overall mean,

S_i = fixed effect of the i^{th} site,

$R_{j(i)}$ = fixed effect of the j^{th} replication within the i^{th} site,

f_k or m_l = the random general hybridising ability (GHA) effect for the k^{th} female or the l^{th} male,

fm_{kl} = random specific hybridising ability (SHA) or full-sib hybrid family effect of the k^{th} and l^{th} parents,

$c_{m(kl)}$ = random effect of the m^{th} clone within the full-sib hybrid family of the k^{th} and l^{th} parents ,

e_{ijklm} = random within plot error term,

The origin of clonal material, i.e. either from the GU progeny trials for the CHBS, or from the HSSB for the AHBS, was included in the model when a combined analysis of the two clonal populations was conducted. All effects, except the overall mean, site and replication effect, were assumed to be random and independently distributed. PROC MIXED (SAS Institute 2002) in SAS was used to estimate variance components and to obtain best linear unbiased predictions (BLUP) of random genetic effects (GHA, SHA and clone) simultaneously. The relationship between variance components and the quantitative genetic model was used to estimate the additive and dominance variance (Falconer 1981), with the assumptions that the inbreeding coefficient of the parents was zero and that epistatic effects are negligible. Variance components used consist of effects due to female, male, full-sib hybrid family and clone. Relationships were calculated as follow:

$\hat{\sigma}^2_{\mathrm{f}}$ = variance due to female (*E. grandis*) effect,

$\hat{\sigma}^2_{\mathrm{Af}} = 4\hat{\sigma}^2_{\mathrm{f}}$ is the additive variance due to the female effect,

$\hat{\sigma}^2_{\mathrm{m}}$ = variance due to male (*E. urophylla*) effect,

$\hat{\sigma}^2_{\mathrm{Am}} = 4\hat{\sigma}^2_{\mathrm{m}}$ is the additive variance due to the male effect,

$\hat{\sigma}^2_{\mathrm{A}} = 1/2(\hat{\sigma}^2_{\mathrm{Af}} + \hat{\sigma}^2_{\mathrm{Am}})$ is the additive variance combining the female and male effect,

$\hat{\sigma}^2_{\mathrm{fm}}$ = variance due to full-sib hybrid family effect,

$\hat{\sigma}^2_{\mathrm{D}} = 4\hat{\sigma}^2_{\mathrm{fm}}$ is the dominance variance,

$\hat{\sigma}^2_{\mathrm{c(fm)}}$ = variance due to clone within full-sib hybrid family effect,

$\hat{\sigma}^2_{\mathrm{NA}} = \hat{\sigma}^2_{\mathrm{D}} + \hat{\sigma}^2_{\mathrm{c(fm)}}$ is the total non-additive genetic variance,

$\hat{\sigma}^2_{\mathrm{G}} = \hat{\sigma}^2_{\mathrm{A}} + \hat{\sigma}^2_{\mathrm{NA}}$ is the total genetic variance,

$\hat{\sigma}^2_{\text{total}} = \hat{\sigma}^2_{\text{G}} + \hat{\sigma}^2_{\text{e}}$ is the total phenotypic variance.

Heritabilities were estimated as:

$h^2_{\text{f}} = \frac{\hat{\sigma}^2_{\text{Af}}}{\hat{\sigma}^2_{\text{total}}}$ is the narrow-sense heritability for the female half sibs,

$h^2_{\text{m}} = \frac{\hat{\sigma}^2_{\text{Am}}}{\hat{\sigma}^2_{\text{total}}}$ is the narrow-sense heritability for the male half sibs,

$h^2_{\text{i}} = \frac{\hat{\sigma}^2_{\text{A}}}{\hat{\sigma}^2_{\text{total}}}$ is the narrow-sense heritability for the combined female and male hybrid parents,

$d^2 = \frac{\hat{\sigma}^2_{\text{D}}}{\hat{\sigma}^2_{\text{total}}}$ is the ratio of dominance variance to total individual phenotypic variance,

$c^2 = \frac{\hat{\sigma}^2_{\text{c(fm)}}}{\hat{\sigma}^2_{\text{total}}}$ is the ration of clone within full-sib hybrid family variance to total individual phenotypic variance,

$H^2_{\text{i}} = \frac{\hat{\sigma}^2_{\text{G}}}{\hat{\sigma}^2_{\text{total}}}$ is the broad-sense heritability on an individual basis,

Standard errors of heritabilities were calculated by Dickerson's approximation (Dickerson 1969).

The analysis was first conducted separately for the two clonal populations of the CHBS and the AHBS in order to estimate genetic parameters and realised genetic gains of the different strategies. The realised genetic gain for each clone was calculated by adding the BLUP estimates of the full-sib hybrid family and the clone within the full-sib hybrid family together. An analysis combining the clonal populations of the two strategies was then performed to estimate the overall genetic parameters of GU clones in Zululand.

6.4 RESULTS

6.4.1 Means, variance component and genetic parameters of two *E. grandis* × *E. urophylla* clonal populations derived from a conventional and accelerated hybrid breeding strategy.

Mean DBH, height, volume per tree and survival for the CHBS and AHBS clonal populations are presented in **Table 6.3**. Means were calculated using the pooled data for each of the clonal populations and before the data was standardised. Mean tree volume was slightly higher for the AHBS clonal population (0.1029 m^3) than for the CHBS clonal population (0.1009 m^3) (**Table 6.3**), but not significant (*p<0.462*) as indicated in **Table 6. 4**. The overall survival was also similar for the CHBS clonal population (88.5%) and the AHBS clonal populations (86.1%) (**Table 6.3**).

Table 6.3 Means and ranges for growth traits of two *E. grandis* × *E. urophylla* clonal populations derived from a conventional and an accelerated hybrid breeding strategy.

		Conventional	Accelerated
DBH	N	18000	24120
	Mean	13.2	13.4
	Standard deviation	2.8	3.1
	Range	5.1 – 24.7	5.0 – 24.6
Height	Mean	16.5	16.5
	Standard deviation	2.7	2.6
	Range	7.7 – 26.0	9.8 – 25.9
Volume	Mean	0.1009	0.1029
	Standard deviation	0.0538	0.0605
	Range	0.0073 – 0.49998	0.0077 – 0.4938
Survival	Mean	88.5	86.1
	Standard deviation	31.9	34.6

Table 6.4 Variance components for volume of the random effects of two *E. grandis* × *E. urophylla* clonal populations derived from a conventional and an accelerated hybrid breeding strategy.

		Effect	Estimate	SE	ZValue	ProbZ
Without clone	Conventional	female	29.89	28.23	1.06	0.145
		male	33.76	25.50	1.32	0.093
		full-sib hybrid families	132.25	35.70	3.70	<0.001
		error	1614.63	18.34	88.06	<0.001
	Accelerated	female	0			
		male	68.19	60.48	1.13	0.129
		full-sib hybrid families	163.00	54.62	2.98	<0.001
		error	1613.05	15.93	101.23	<0.001
	All	strategy	0.00	.	.	.
		female	11.45	18.75	0.61	0.271
		male	30.56	23.54	1.30	0.097
		full-sib hybrid families	164.38	34.37	4.78	<0.001
		error	1615.99	12.02	134.39	<0.001
With clone	Conventional	female	28.49	29.80	0.96	0.169
		male	30.41	25.48	1.20	0.116
		full-sib hybrid families	15.08	33.72	0.45	0.327
		clone(fam)	224.84	36.20	6.76	<0.001
		error	1464.49	16.67	87.82	<0.001
	Accelerated	female	0.00	.	.	.
		male	47.90	54.58	0.88	0.19
		full-sib hybrid families	113.40	54.97	2.60	0.019
		clone(fam)	261.51	30.22	8.65	<0.001
		error	1402.99	13.91	100.82	<0.001
	All	strategy	2.24	23.57	0.10	0.462
		female	4.17	16.08	0.26	0.397
		male	26.55	23.50	1.13	0.129
		full-sib hybrid families	80.81	31.90	2.53	0.006
		clone(fam)	253.78	23.11	10.98	<0.001
		error	1242.83	9.58	129.69	<0.001

The *E. grandis* (female) and *E. urophylla* (male) effects were not significant for volume, regardless of the breeding strategy followed (**Table 6.4**). This resulted in additive genetic variation ($\hat{\sigma}^2_A$=61.44) only explaining 9.6% of the total genetic variance ($\hat{\sigma}^2_G$=638.46) when all the data was combined (**Table 6.5**). A similar trend was detected for the CHBS and AHBS clonal populations where additive genetic variance explained 29.2% (117.8/402.96) and 11.8% (95.8/810.91) of the total genetic variance respectively. In all the analysis, *E. urophylla*

parents contributed more to the additive genetic variation than the *E. grandis* parents. The h^2_m was 0.07 for the CHBS clonal population, 0.08 for the AHBS clonal population and 0.05 for the combined clonal populations, whereas h^2_f was 0.06, 0.00 and 0.01 respectively.

With regards to non-additive genetic effects, the clone within family effect was highly significant (*p<0.001*) for the CHBS, AHBS and combined clonal populations (**Table 6.4**). The full-sib hybrid family effect was significant (*p<0.05*) for the AHBS and combined clonal population, but not for the CHBS clonal population. Non-additive genetic variation ($\hat{\sigma}^2_{NA}$=577.02) explained 90.3% of the total genetic variation ($\hat{\sigma}^2_G$=638.46) of the combined clonal population (**Table 6.5**). Similar values were obtained for the CHBS clonal population (71%) and the AHBS population (88%). Fifty six percent of the non-additive variation ($\hat{\sigma}^2_{NA}$=577.02) could be explained by the dominance variance ($\hat{\sigma}^2_D$= 323.24) for the combined clonal population. The rest of the non-additive genetic variation was explained by the variation among clones within a full-sib hybrid family ($\hat{\sigma}^2_{c(fm)}$=253.78).

A discrepancy was noted between the CHBS and AHBS clonal populations where dominance variance explained 21% and 63% of the non-additive genetic variation respectively. The main cause of the relatively low dominance variation present in the CHBS clonal population could be due to the fact that in many cases, only one or two individuals were selected from a specific cross to test as clones. Hence, the data from the CHBS clonal population might be inadequate to partition the non-additive genetic variance into dominance and clone within family variation.

In order to try and shed some light onto the nature of non-additive and additive genetic effects, the random clonal within family effect was dropped from the linear model. It is apparent from **Table 6.4** that when the clone within family effect was dropped from the model, the full-sib hybrid family effect changed from insignificant (*p<0.327*) to highly significant (*p<0.001*) for the CHBS clonal population. This resulted in an increase of d^2 from 0.03 to 0.23 for the CHBS clonal population (**Table 6.5**). An increase in d^2 was also detected for the AHBS clonal population (from 0.20 to 0.27) and for the combined clonal population (from 0.16 to 0.28). The $\hat{\sigma}^2_A$ however, stayed low for the CHBS, ABHS and combined clonal populations and only explained 11.3% (84.17/741.69) of the total genetic variation of the

combined population (**Table 6.5**). This is an indication that the effects which the model previously allocated to remaining genetic effects among clones within a family are now mostly being absorbed by the inferred dominance genetic component of variation. This result points to a potential strong confounding effect between dominance and other epistatic terms present in the clone effect.

Nevertheless, results from both the CHBS and AHBS hybrid breeding strategies indicated that a breeding strategy to capture non-additive genetic effects will be the most appropriate strategy to follow. The same phenomenon was noted by van den Berg et al. (2016) for the GU seedling population from where the clonal material of the CHBS population was selected from.

Table 6.5 Genetic parameters at four years for volume of two *E. grandis* × *E. urophylla* clonal populations derived from a conventional and an accelerated breeding strategy.

	Conventional		**Accelerated**		**All**	
	with clone	without clone	with clone	without clone	with clone	without clone
Female (*E. grandis*)						
$\hat{\sigma}_f^2$	28.49±27.51	29.89±27.06	0±0	0±0	4.17±16.21	11.45±18.97
$\hat{\sigma}_{Af}^2$	113.96±110.05	119.56±108.26	0±0	0±0	16.68±64.75	45.80±75.89
h_f^2	0.06±0.07	0.05±0.04	0±0	0±0	0.01±0.04	0.02±0.04
Male (*E. urophylla*)						
$\hat{\sigma}_m^2$	30.41±23.75	33.76±23.49	47.90±54.31	68.19±57.89	26.55±23.72	30.56±22.92
$\hat{\sigma}_{Am}^2$	121.64±94.99	135.04±93.97	191.60±217.22	272.76±231.52	106.20±94.91	122.54±91.74
h_m^2	0.07±0.06	0.06±0.04	0.08±0.10	0.11±0.11	0.05±0.04	0.05±0.04
Full-sib hybrid family						
$\hat{\sigma}_{fm}^2$	15.08±31.49	132.25±33.80	113.40±54.24	163.00±50.68	80.81±31.64	164.38±34.59
$\hat{\sigma}_D^2$	60.32±125.98	529.00±135.17	453.60±216.96	652.00±202.70	323.24±126.55	657.52±138.33
d^2	0.03±0.08	0.23±0.08	0.20±0.09	0.27±0.11	0.16±0.06	0.28±0.07
Clone(fam)						
$\hat{\sigma}_c^2$	224.84±33.71		261.51±29.75		253.78±22.81	
c^2	0.12±0.08		0.12±0.07		0.12±0.05	
$\hat{\sigma}_A^2$	117.80	127.30	95.80	136.38	61.44	84.17
$\hat{\sigma}_{NA}^2$	285.16	529.00	715.11	652.00	577.02	657.52
$\hat{\sigma}_G^2$	402.96	656.30	810.91	788.38	638.46	741.69
H_i^2	0.22		0.37	0.33	0.31	0.31
$\hat{\sigma}_e^2$	1464.49±15.01	1614.63±16.72	1402.99±11.76	1613.05±14.97	1432.39±9.58	1615.99±11.44
$\hat{\sigma}_{total}^2$	1867.45	2270.93	2213.90	2401.43	2070.85	2357.68

6.4.2 Realised genetic gains of two hybrid breeding strategies

Best linear unbiased prediction estimates for each full-sib hybrid family and clone within a full-sib hybrid family were directly interpreted as percentage gain due to the way the data was standardised. The realised gain for each clone was calculated by adding the BLUP estimates of the full-sib hybrid family and the clone within that family. In order to quantify the potential gains per unit time for the two strategies, the average gain of the top 5% of each population was calculated (**Table 6.6**). This amounted to a total of eight clones (selected from six families) for the CHBS clonal population with an average gain of 31.3% (2.7% family gain + 28.6% clone gain) over the population mean and 19.4% gain over the commercial GU clonal control. The top 5% of the AHBS population equated to 11 clones (selected from seven

families) with an average gain of 41.6% (14.0% family gain + 27.6% clone gain) over the population mean and 28.1% gain over the commercial GU clonal control. The difference in gains of the top 5% of two clonal populations was more profound when the time it took to test the material was considered in the calculation. The percentage gain per year was calculated at 3.1% and 5.5% over the population mean for the top 5% of the CHBS and AHBS clonal populations respectively (**Figure 6.2**).

Table 6.6 Genetic gains of the top 5% of clones from the conventional and accelerated clonal populations.

	Conventional					**Accelerated**				
	% gain for fam (n=6)	% gain for clone (n=8)	% total gain	Length of cycle (years)	% gain per year	% gain for fam (n=7)	% gain for clone (n=11)	% total gain	Length of cycle (years)	% gain per year
% gain over population mean	2.7	28.6	31.3	10	3.1	14.0	27.6	41.6	7.5	5.5
% gain over GU clone control	1.7	17.7	19.4	10	1.9	9.5	18.6	28.1	7.5	3.7
% gain over *E. grandis* seedling control	3.8	40.1	43.9	10	4.4	17.3	34.0	51.3	7.5	6.8
% gain over GC clone control	5.0	52.7	57.7	10	5.8	22.2	43.7	65.9	7.5	8.8
% gain over *E. grandis* clone1 control	5.0	52.9	57.9	10	5.8	21.8	43.1	64.9	7.5	8.7
% gain over E. grandis clone2 control	5.3	55.8	61.1	10	6.1	22.9	45.2	68.1	7.5	9.1

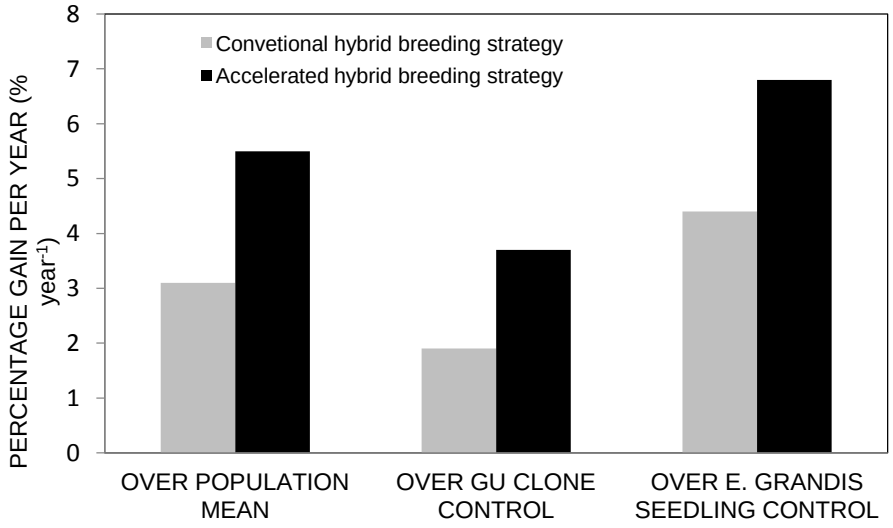

Figure 6.2 Realised gains of the conventional and accelerated hybrid breeding strategies.

6.5 DISCUSSION

It was evident in our study that the testing of GU seedlings over a shorter time period had a positive impact on volume gains per unit time. The percentage gain (over the GU commercial clonal control) per year was higher for the AHBS (3.7%) than for the CHBS (1.9%) (**Table 6.6**). It was surprising that the absolute gain of the AHBS was larger than for the CHBS before the time factor was taken into account. One may expect that the clonal population derived from the hybrid progeny trial to outperform the clonal population derived from the HSSB, especially taking into account that the selection intensity for the CHBS was higher (2%) than that of the AHBS (5.5%). A possible explanation could be the higher family gains of the top 5% of clones of the AHBS (14% gain over population mean) when compared to that of the CHBS (2.7% gain over population mean) (**Table 6.6**). However, it must be kept in mind that the selections for the AHBS were done visually and no family data was used as in the case of the CHBS. In addition, extra costs associated with the CHBS, such as the establishment, maintenance and measurements of GU seedling progeny trials, will also have a negative impact on the cost effectiveness of this strategy.

Another important issue to consider when an improvement strategy is applied to plant or animal species is the proportion of additive variance to the total genetic variance (Lynch and Walsh 1998). The results from our study indicated that additive genetic variation explained minority (between 10% and 30%) of the genetic variation present in GU clonal populations, regardless of the hybrid breeding strategy followed. Although there is little information available on genetic parameters of GU clonal populations, some authors reported the same phenomenon for GU hybrid seedling populations (Rezende and de Resende 2000, Vigneron et al. 2000, Bouvet et al. 2009, Retief and Stanger 2009, Van den Berg et al. 2016). For instance, Retief and Stanger (2009) reported that dominance genetic effects accounted for nearly 60% of the total genetic variance in a GU hybrid factorial study in Zululand, and Bouvet et al. (2009) reported an average $\hat{\sigma}_D^2/\hat{\sigma}_A^2$ ratio of 1.2 for a relatively large GU hybrid seedling population (684 families). In addition, some results on eucalypt pure species populations also indicated a positive $\hat{\sigma}_D^2/\hat{\sigma}_A^2$ ratio (Van Wyk 1990, Vaillancourt et al. 1995, Hodge et al. 1996, Hardner and Tibbits 1998, Volker et al. 2008).

One explanation of the relatively high non-additive variance could be due to the nature of dominance variance. The dominance effect between alleles and their frequency will determine the magnitude of the dominance variance (Gallais 1991, Lynch and Walsh 1998). Dominance variance exceeds additive variance in the case of overdominance and/or in the case of total dominance when frequencies of alleles are different when a model of one locus and two alleles are used (Lynch and Walsh 1998). Although the case of overdominance must be considered with caution (Birchle et al. 2006), it may explain some of the relatively high dominance variance present in perennial plants such as *Eucalyptus* (Bouvet et al. 2009).

It must also be borne in mind that the selection process of first selecting elite pure species parents to use for hybrid crossing, followed by the selection of ortets from the hybrid population to test as clones, may influence the additive and non-additive variance estimates of the clonal populations. This effect was evident in our study when a decrease in h_{i}^2 from 0.12 to 0.06 was noted when the GU hybrid seedling population (Van den Berg et al. 2016) from where the ortets were selected, was compared to the CHBS clonal population. Nevertheless, our results indicate that following the current selection processes, a breeding strategy to capture non-additive genetic effects in GU hybrids will be the most appropriate strategy to follow.

Three strategies that could potentially exploit non-additive variance are reciprocal recurrent selection (RRS) (Comstock et al. 1949), reciprocal recurrent selection with forward selection (RRS-SF, Nikles 1992) and the development and crossing of inbred lines. The implications of these strategies for tree improvement have been discussed by various authors (Vigneron 1991, Nikles 1992, Dungey et al. 2000, Shelbourne 2000, Hettasch et al. 2005). Kerr et al. (2004) did a simulation study comparing RRS, RRS-SF, recurrent selection for general combining ability (RS-GCA, Jenkins 1940) and the hybrid swarm strategy over five cycles of breeding. Results from this study suggest that the RRS-SF strategy yielded the highest genetic gains per year in cases where non-additive variance is higher than additive variance and when the pure-hybrid correlations are negative or close to zero.

However, majority of theses studies were based on seedling populations, and did not consider the effects of clonal testing. Currently, GU hybrids are commercially deployed as

clones and not as seedlings. Hence, a combined strategy to provide a superior GU hybrid seedling source, and to test ramets of selected ortets cost effectively needs to be applied. Based on the results of our study and other authors, a strategy combining RRS-SF and AHBS will most likely result in the most cost effective genetic gains.

6.6 CONCLUSIONS

Our study showed that the testing of GU seedlings over a shorter time period had a positive impact on volume gains per unit time when the clonal populations of the conventional and accelerated hybrid breeding strategies were compared. Furthermore, the additional cost savings associated with the AHBS will also help to justify the continuation of this strategy.

Overall, both the AHBS and CHBS clonal populations provided similar genetic information. Results indicated that non-additive variance explained the majority (88% and 71%) of the genetic variation in the AHBS and CHBS GU hybrid clonal sub-populations respectively. It is recommended that a hybrid breeding strategy to capture the non-additive genetic effects should be adopted and combined with a strategy that minimises the time spend on testing GU hybrid material as seedlings.

Although the study has offered an evaluative perspective on *Eucalyptus* hybrid breeding, information on the economic impact of the two hybrid strategies was limited. A future experiment designed to quantify the economic impact associated with cost savings will help to shed light on this discourse.

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CHAPTER 7 SYNTHESIS AND CONCLUSION

7.1 INTRODUCTION

As the global human population increases, the demand for wood is increasing. Plantation forestry is one way to address the wood scarcity problem and will help to address the demand for timber from natural forests (Fenning and Gershenzon 2002). The clonal deployment of eucalypt hybrid material showed the biggest gains in plantation forestry worldwide and can go a long way to fulfil present and future global wood needs (Griffin et al. 2000). However, in order to have a sustainable supply of wood in a demanding and competitive market, it is of utmost importance that the forestry industry has specific breeding and deployment strategies in place to optimise gains in productivity (Rezende et al. 2014).

In this context, the study was set out to firstly review the conventional eucalypt hybrid breeding strategy for the hybrid breeding cycle of *E. grandis* × *E. urophylla* in Zululand. Information on genetic parameters of *E. grandis* and *E. urophylla* pure species populations, as well as *E. grandis* × *E. urophylla* hybrid seedling and clonal populations needed quantification. The pure-hybrid and ortet-ramet correlation values are essential to develop a suitable hybrid breeding strategy and were explored in this study.

Secondly, this study has sought to know whether accelerating the hybrid breeding cycle by reducing the testing time of hybrid seedlings can result in increased genetic gains per unit time. The literature on this subject and specifically in the context of *E. grandis* × *E. urophylla* hybrid clonal populations in Zululand is inconclusive on several vital questions within the hybrid breeding discourse. The study sought to answer some of these questions.

7.2 SUMMARY OF RESEARCH FINDINGS

The main empirical findings are chapter specific and were summarised within the following chapters: Chapter 3: Genetic parameters of intraspecific hybrids of *Eucalyptus grandis*, Chapter 4: Estimates of genetic parameters and genetic gains for growth traits of two *Eucalyptus urophylla* populations in Zululand, South Africa, Chapter 5: Genetic parameters of interspecific hybrids of *Eucalyptus grandis* and *E. urophylla* seedlings and cuttings, and

Chapter 6: Realised genetic gains and estimated genetic parameters of two *Eucalyptus grandis* × *E. urophylla* hybrid breeding strategies. This section will synthesize the empirical findings to answer the study's questions.

The conclusion in Chapter 3 is that G×E did not have a significant impact on the performance of *E. grandis* in Zululand, and that a single population will be sufficient for the breeding of this species in Zululand. The additive genetic variation in the *E. grandis* population was sufficient to identify and select parents with high GCA values that could produce progeny with 28.4% genetic gains through intraspecific crosses. This chapter also highlighted that additional genetic gains could potentially be achieved through vegetative propagation due to the relatively large portion of non-additive genetic variation present.

Chapter 4 described *E. urophylla* populations grown in Zululand. As with the *E. grandis* population, the conclusion is that a single *E. urophylla* breeding population should be sufficient for Zululand due to the moderate levels of G×E. The relatively large provenance and family variation detected for the two *E. urophylla* populations provides an adequate source to select for genetic gains and to maintain genetic diversity for hybrid and pure species breeding. Although results from the first two empirical chapters showed the potential to select for additive gene effects in the *E. grandis* and *E. urophylla* pure species populations grown in Zululand, the question on the performance of the parents as pure species parents versus hybrid parents, still needed to be explored and was reported on in the third empirical chapter.

Results from Chapter 5 in this study indicated that non-additive genetic variance plays a significant role in determining the growth performance of *E. grandis* × *E. urophylla* hybrid seedlings and clones and explained up to 71% of the total genetic variation. Due to the pre-eminence of non-additive variance, the pure-hybrid combining ability correlations were weak, especially for clonal populations. It would therefore seem that GCA is not a good predictor of GHA for growth performance in the observed populations. Results from this chapter also indicated a weak ortet-ramet correlation for *E. grandis* × *E. urophylla* hybrids. This suggests that the conventional strategy of first screening *E. grandis* × *E. urophylla* seedlings in progeny trials should be revisited. A strategy to accelerate the time spent on testing *E. grandis* × *E. urophylla* as seedlings needed investigation and was explored in Chapter 6.

Chapter 6 showed that accelerating the conventional hybrid breeding strategy by shortening the testing time of *E. grandis* × *E. urophylla* seedlings resulted in more realised

genetic gains per unit time. Furthermore, the additional cost savings associated with the AHBS will also help to justify the continuation of this strategy. As far as genetic parameters go, results indicated that non-additive variance explained the majority of the genetic variation regardless of the strategy followed.

7.3 OVERALL LIMITATIONS OF THE RESEARCH

The study was conducted in Zululand on existing *E. grandis, E. urophylla* and *E. grandis* × *E. urophylla* populations obtained from the conventional hybrid breeding strategy. As a direct consequence of this methodology, the study encountered a number of limitations, which need to be considered.

Firstly, all the *E. grandis* and *E. urophylla* parents used for interspecific crossing were selected on their mature age phenotypes for growth and tree form. Hence, the assumption that crosses are made from parents selected randomly from the population of interest has been violated. This selection process might explain the lack of additive variance present in the observed *E. grandis* × *E. urophylla* hybrid populations.

Secondly, in view of the selected nature of *E. grandis* × *E. urophylla* ortets and the limited numbers of individuals per family, results on the *E. grandis* × *E. urophylla* clonal populations must be interpreted cautiously. Due to this limitation, the family effect of clonal populations of the conventional hybrid breeding strategy was insignificant, and the genetic ortet-ramet correlation could, therefore, not be estimated.

7.4 RECOMMENDATIONS AND FUTURE RESEARCH

The scale of this debate is extensive and in order to develop a suitable and cost effective eucalypt hybrid breeding strategy, there is a need for more studies to allow further assessments of this subject. Exploring the following as future research strategies can facilitate the attainment of this goal:

- A quantitative genetics study incorporating hybrid breeding material derived from interspecific crosses between randomly selected *E. grandis* and *E. urophylla* parents.

- An investigation of the genetic parameters and ortet-ramet genetic correlations of *E. grandis* × *E. urophylla* clonal populations that consist of a wider range (unbiased) full-sib hybrid families and more selections from each hybrid family.
- To expand this research to other important eucalypt hybrid species and economically important traits such as pulp yield and basic wood density.

In addition to quantitative genetic studies, developing a genomic selection model for *E. grandis* × *E. urophylla* populations could assist tree breeders to further reduce the testing time of the hybrid material by omitting the testing phase of the hybrid seedlings. High genetic gains from genomic selections have been achieved in cattle breeding by eliminating expensive progeny testing and consequently reduced the breeding cycle (Schefers and Weigel 2012). Since genomic selection has revolutionized the cattle industry, there has been an increasing interest in it among tree breeders (Grattapaglia and Resende 2011, Goddard et al. 2011, Zapata-Valenzuela et al. 2013). However, genomic selection studies are in the developmental phase and majority of studies are proof of concept (Isik, 2014). In addition, genomic selection models have mainly been developed for breeding strategies that do not exploit non-additive genetic variation through clonal deployment (Grattapaglia, 2014). In my study, it was evident that majority of the genetic variation was explained by non-additive genetic variation for the *E. grandis* × *E. urophylla* populations. Research into developing genomic selection models to predict total genotypic values for tree hybrids species such as *E. grandis* × *E. urophylla* that are deployed as clonal varieties, is needed (Grattapaglia, 2014).

In spite of the limitations of this study, it has attained its two primary objectives namely: to review the conventional hybrid breeding strategy and to investigate an accelerated hybrid breeding strategy. From the results of the study, the overall recommendation is to adopt a hybrid breeding strategy that captures non-additive genetic effects in combination with the described accelerated strategy.

7.5 RESEARCH CONTRIBUTION AND IMPLICATIONS

The research contributes to knowledge on a number of aspects related to *Eucalyptus* hybrid breeding. One of the important issues this study addresses is the prolonged testing time associated *Eucalyptus* hybrid breeding. The benefits of hybrid breeding are often diluted or

lost due to the prolonged time taken to breed such material for commercial use. This study indicates that the potential impact of hybrid breeding can be increased by 1.8% per year for volume through fast-tracking the conventional hybrid breeding process in *E. grandis* × *E. urophylla* breeding and deployment. The economic benefits of such gains in the forestry industry have been described by various authors. For instance, Wei and Borralho (1999) reported that an improvement of 1 m^3 ha^{-1} in volume will reduce the production costs of 1 ton pulp by $0.38 under a standard pulp regime in China. In our accelerated scenario, this means that the genotypes that were selected for commercial deployment after 7.5 years of testing could reduce the pulp production costs by approximately $18 per ton of pulp with a volume increase of 47 m^3 ha^{-1} when compared to the commercial control. In the case of the conventional hybrid strategy this equates to a potential reduction in pulp production costs of $12.3 after 10 years of testing. Hence, implementing the accelerated strategy will reduce the pulp production costs two fold ($2.4 versus $1.2 per year of testing) when compared to the conventional hybrid breeding strategy. However, these costs were calculated for a pulp regime in China in 1999, and a cost benefit analysis study for the scenario in my study needs to be conducted.

The study also revealed the importance of capturing non-additive genetic variation in *E. grandis* × *E. urophylla* hybrid populations in achieving increased genetic gains. The hybrid breeding strategies described in this study have not taken the high proportion of non-additive genetic variation into consideration when it was designed. Adopting a strategy such as the reciprocal recurrent selection with forward selection (RRS-SF, Nikles 1992) could further increase the genetic gains by 0.015 (units of the F1 standard deviation, Kerr et al. 2004).

Adopting the recommended eucalypt hybrid breeding strategy as indicated in this study will help forestry companies to maximise genetic gains per unit time instead of per cycle of breeding as described by White et al. (2007). An increase in productivity over a shorted time period will enable the forestry industry to keep up with the increasing demand for timber globally. Additional benefits of the suggested strategy are quicker returns on money invested in tree breeding, as well as to manage risk better by reacting quicker to the upward trend of pest and disease introductions. Overall, the study has offered an evaluative perspective on *Eucalyptus* hybrid breeding, and will enable companies to construct more efficient *Eucalyptus* hybrid breeding strategies.

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