GENETIC PARAMETERS AND GENOTYPE BY ENVIRONMENT INTERACTION OF *EUCALYPTUS GRANDIS* POPULATIONS USED IN INTRA-SPECIFIC HYBRID PRODUCTION IN SOUTH AFRICA

Gert J van den Berg[1,3], Steven D Verryn[2,3], Paxie W Chirwa[3] and Francois van Deventer[1]

[1]Mondi Forests (Pty) Ltd, P.O. Box 12, Hilton, 3245, South Africa.

[2]Creation Breeding Innovations cc, 75 Kafue Street, Lynnwood Glen, Pretoria, 0081, South Africa

[3]Faculty of Natural and Agricultural Sciences, University of Pretoria, 2 Lynnwood Road, Hatfield, Pretoria, 0002, South Africa

Corresponding author : gert.vandenberg@mondigroup.co.za

Abstract

In South Africa, *Eucalyptus grandis* is an important species due to its fast growth and general sustainability of its timber for a range of products. However, *E. grandis* is susceptible to fungal diseases like *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 *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 inter-specific 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 (GxE) 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 aims of this study were firstly to determine the magnitude of GxE of *E. grandis* across the three sites; secondly to estimate the genetic parameters for growth of the *E. grandis* parents selected for intra-specific crosses; and lastly to identify the best parents to use for intra- and inter-specific crosses in future hybrid breeding programmes.

Results of our study indicated that GxE 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 intra-specific 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 GCA values from our study are also good general combiners in inter-specific 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

Introduction

In South Africa, approximately 520 000 ha are planted to the *Eucalyptus* genus (DAFF 2010). *Eucalyptus grandis* Hill ex Maiden is one of the main species planted either as a pure species crop, or as an inter-specific 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 like *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 like Zululand in South Africa (Retief and Stanger 2009). Lately, *E. grandis* is also succumbing to infestation by the gall wasp *Leptocybe invasa*. However, *Eucalyptus urophylla* 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 inter-specific 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 inter-specific hybrid populations.

Estimating GxE 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 GxE is lacking.

The aims of this study were therefore to (1) estimate the genetic parameters for growth of the *E. grandis* parents selected for intra-specific crosses; (2) to determine the magnitude of GxE of an *E. grandis* full-sib population and (3) to identify the best *E. grandis* parents to use for intra- and inter-specific crosses in future hybrid breeding programmes.

Materials and methods

Breeding material

The mating design consisted of a partial diallel with 46 *E. grandis* parents (**Appendix 1**). The parents were selected from 37 unrelated families (second generation) in a series of four progeny trials (**Appendix 2**). 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 4 and 15 times.

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 1**. Each trial was planted in a randomised complete block design (RCB) and replicated five times across each site. Each family was planted in a 6 tree line plot and at various spacing as indicated in **Table 1**. Trial measurements were done when the trees were 7 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).

Statistical analysis

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 GxE was also eliminated (Burdon 1977; Eisen and Saxon 1983; Hill 1984). The data for this study was standardized 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.

Single site analysis of the diallel

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

$$y_{ijkl} = \mu + R_i + \mathrm{f}_j + \mathrm{m}_k + sca_{jk} + e_{ijkl}$$

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;

f_j or m_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 BLUP 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).

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

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

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

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

Heritabilities were estimated as:

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

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

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

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

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:

$$y_{ijklm} = \mu + S_i + R_{j(i)} + f_k + m_l + sca_{kl} + S{*}f_{ik} + S{*}m_{il} + S{*}sca_{ikl} + e_{ijklm}$$

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;

f_k or m_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;

$S*f_{ik}$ or $S*m_{il}$ = the random GCA by Site Interaction;

$S*sca_{jkl}$ = 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):

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

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 ($\mathrm{r_{Bgca}}$ and $\mathrm{r_{Bsca}}$) correlations were estimated as follow:

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

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

Table 1: Site and trial information of *E. grandis* full-sib progeny trials.

	A	B	C
District	Zululand	KwaZulu-Natal 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 x 3m	3m x 2m	3m x 3m
Number of families	116	81	66

Table 2: 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	Height	Volume	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 trees 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 trees 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 trees 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

Table 3: 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	328.8±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	90.2±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	64.3±34.8	1632.74±56.7

Type B correlation measures GxE 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.

Results

Single site analysis

Mean DBH, height, volume per tree and survival for each site are presented in **Table 2**. 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.

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**). Hence, $\hat{}_a^2$ and h_i^2 followed the same trend as indicated in **Table 4**. 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.

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, $\hat{}^2_{\mathrm{d}}$ (0.14, 0.10 and 0.13) were also the lowest at this site **Table 4**. 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, $\hat{}^2_{\mathrm{d}}$ (106.37 and 79.3 for DBH) were higher than $\hat{}^2_{\mathrm{a}}$ (96.5 and 57.9 for DBH), and d^2 (0.26 and 0.26 for DBH) were therefore also higher than h^2_{i} (0.24 and 0.19 for DBH). However, at trial C $\hat{}^2_{\mathrm{a}}$ (75.6 for DBH) was higher than $\hat{}^2_{\mathrm{d}}$ (42.9 for DBH) for all three growth traits. Total genetic variation was highest at site A (H^2_{i} = 0.38) and lowest at site C (H^2_{i} = 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).

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 5**. 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 $\hat{}^2_{\mathrm{d}}$ (0, 258.3, 0 and 0), and h^2_{i} (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 6**). 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.

Table 4: Genetic parameter for diameter at breast height (DBH), height and tree volume for three *E. grandis* full-sib progeny trials established 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	57.9±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
$\hat{\sigma}_e^2$	328.8±8.9	256.15±7.9	262.49±9.1	224.34±4.5	88.05±2.7	90.2±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

Table 5: 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

Table 6: 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

Overall, the Zululand site pair (A&C) had the highest GCA (116.91) and SCA (64.57) estimates for volume and was under more total genetic control (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 1**).

This result was also noticeable when GxE for GCA values was estimated, and the r_{Bgca} was equal to 1 for the Zululand site pair A&C (**Table 6**). This is an indication that there is no GxE between these two sites with regards to the growth performance of progeny from particular parents. However, GxE 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. GxE 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 1**). Further GxE studies therefore needs to be conducted for the KwaZulu-Natal Midlands region. 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 5

Table 7: 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

parents for the two Zululand sites combined is given in **Table 7**. 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 7**).

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 GxE detected in this region. Although our study indicated that GxE 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 GxE, is the severity of diseases at the different sites. For instance, Van Heerden and Wingfield (1999) indicated a significant GxE 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 GxE effect ($r_B = 0.64$) for *E. grandis* clones across three environments in Colombia. One exception was the study done by Miranda et al. (2015). They have reported significant differences in GxE effect for an open pollination *E. grandis* seedling population across four sites in Brazil. Despite this divergence in the literature, it's 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 intra-specific 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 4 different sites in Brazil, and h_{i}^2= 0.09 across all 4 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. Nevertheless, the information from this trial series is useful to construct a production population with parents with high GCA values.

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 either commercial production or 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 done 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.

It must be kept in mind though that *E. grandis* is susceptible to fungal diseases like *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* x *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 inter-specific hybrid combinations with *E. urophylla* needs to be conducted in order to develop the best inter-specific hybrid breeding strategy.

Conclusions

The study was set out to investigate the magnitude of GxE of *E. grandis* across three sites, as well as to estimate the genetic parameters for growth of *E. grandis* parents selected for intra-specific crosses, which will also be used for inter-specific 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 intra-specific 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 GxE 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 GxE 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 GxE 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 GxE 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 inter-specific 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 inter-specific hybrid combinations with *E. urophylla* needs to be conducted.

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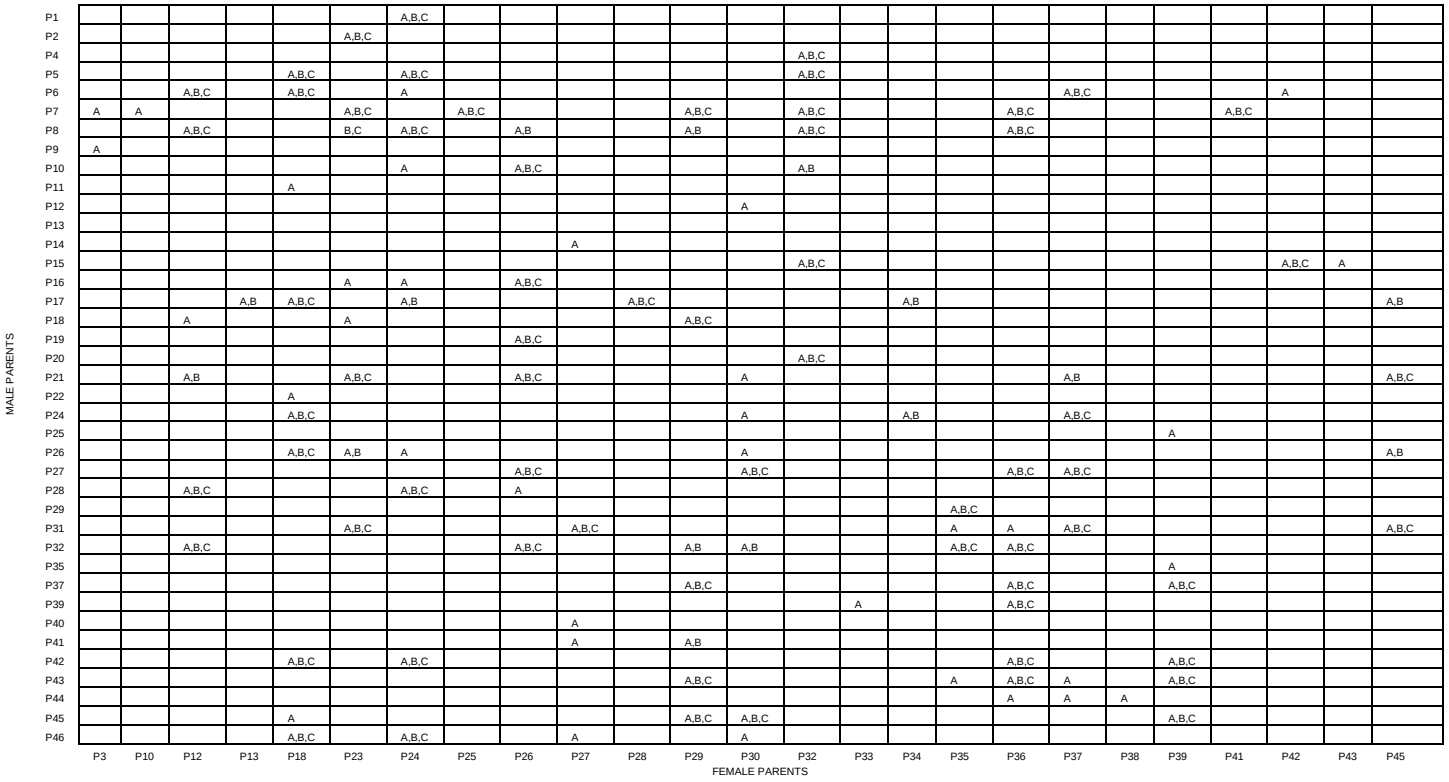
Appendix 1: Diagram indicating crosses in the mating design of the partial diallel. The sites at where the full-sib families were established are designated by A, B or C.



Appendix 2: 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