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Heterosis Estimation for Growth Traits and Wood Properties of *Eucalyptus urophylla* under Two Sites in Southern China[1]

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Abstract

Heterosis is a quicker, cheaper and easier method for increasing plantation production, and heterosis studies can provide the basis for exploitation of valuable hybrid combinations in breeding programs. Growth traits, wood properties, and bark percentage were assessed for 6 × 6 full diallel progenies of *E. urophylla* in southern China measured at age 4 years. Analysis of variance showed that there were significant differences on all studied traits except dynamic modulus of elasticity among combinations. The mean diameter at breast height, wood basic density, and dynamic modulus of elasticity were 9.40 to 12.20 cm, 0.45 to 0.47 g cm^{-3}, and 5.04 to 5.72 GPa, respectively. The coefficients of variation ranged from 18.18% to 50.43% for growth traits, 9.07% to 55.24% for wood properties and 22.26% to 23.93% for bark percentage. The heterosis estimation on diameter at breast height, wood basic density, and dynamic modulus of elasticity were -24.07% to 18.74%, -14.23% to 9.17% and -25.53% to 16.28%. U22×U56 generally had higher heterosis of growth traits and wood properties through three sites. Same combinations had different original and reciprocal heterosis estimation at two sites even on same traits. The combinations of same parents always had similar original and reciprocal heterosis estimation.

Key words: Full Diallel Progenies, Bark Percentage, Eucalyptus Urophylla, Breeding Strategy

1 INTRODUCTION

Wood is one of the earth's most important renewable building materials which can be used for different purposes as the properties of wood are comparable with those of other structural materials (Nazmul et al. 2012). Hence, with timber supply deficit, the availability of land, and aiming to have a eucalypt resource of sufficient size to support veneer production and paper industry (Arnold et al. 2013; McKenney, 1998), eucalypt plantations have been developed and now covered an estimated area of more than 4.0 million hectares in China (Chen and Chen, 2013), principally in Guangdong, Guangxi, Hainan Island, Yunnan, Sichuan and Fujian provinces while more than 1.4 million hectares in Guangxi (Liley, 2014). Unfortunately, the mean annual increment in volume of eucalypt plantations in China is lower than that in the tropical and subtropical regions of the other parts of the world (Yang, 2003; Xu, 2003; Toit *et al.* 2010), suggesting that great effort for improving productivity of eucalypt plantations should be put in practice in China.

Eucalyptus urophylla S. T. Blake, plays an important role in forestry industry in humid and sub-humid tropical region, naturally distributed on the mountain slopes and valleys of the large island of Timor and the nearby Indonesian islands of Wetar, Alor, Pantar, Lomblen, Adonara and Flores, at an altitudinal range from 90 and 3000 m, which is the widest of any eucalypt species (Wright and Osorio, 1996; Kien *et al.* 2009). Intensive breeding has been reported to raise mean annual increment of clonal plantation, provenances and families of *E. urophylla* (Wei and

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Borralho, 1997; Leite *et al.* 2002; Kien *et al.* 2009; Quang *et al.* 2009; Wu *et al.* 2013). Further, genetic improvement of wood properties affecting the quality of pulpwood and peeled veneer products is of general interest to tree breeders worldwide (Blackburn et al. 2012). Therefore, breeding including heterosis estimation also should be progressed from improving mostly growth and form traits to combining growth with wood properties.

In this paper, we compare the variance of difference combination, estimate the coefficients of variation of different traits, and examine heterosis value of difference crosses in 6×6 full diallel *E. urophylla* trials at aged 4 years. This information will be used to develop appropriate breeding strategies for this species in southern China.

2 MATERIALS AND METHODS

2.1 Trial Description

Three 6×6 full diallel E. urophylla trials were established in early 2006 on contrasting sites in central Guangdong province in China. This province has a subtropical humid monsoon climate. There is a summer rainfall maximum and winters are generally dry and cool. The mean rainfall of two sites is near 1750 mm per year. All the soil is classified as Lateritic red earth. The site details for three sites are presented in Table 1. One site, Gonghe town (22°34′N, 112°51′E, 30m asl.) in Heshan City, is located near urban area of Jiangmen city. The second site, Lantang town (23°25′N, 114°50′E, 100m asl.), is near Zijing county of Heyuan city.

The 6×6 full-diallel mating design was made during August to October in 2004. Thirty-four full diallel combinations and open pollination progenies of six parents were collected in April 2005. The field design was a randomized complete block with 6 replications and 5-tree line plots planted with a spacing of 3.0m×2.0m. The planting pits (50cm×50cm×40cm) were prepared and compound fertilizer was applied in the first two years with individual tree applications.

TABLE 1 SITE DETAILS FOR 6 ×6 FULL DIALLEL *E. UROPHYLLA* TRIALS ESTABLISHED IN 2006 IN GUANGDONG

Site	PH Value	Organic Content ($g.kg^{-1}$)	Total N ($g.kg^{-1}$)	Total P ($g.kg^{-1}$)	Total K ($g.kg^{-1}$)	Available N ($mg.kg^{-1}$)	Available P ($mg.kg^{-1}$)	Available K ($mg.kg^{-1}$)	Available B ($\mu g.g^{-1}$)	Mean annual temperature (℃)	Mean min. temp. coldest month (℃)	Mean max. temp. hottest month (℃)
Gong-he	4.10	12.79	0.42	0.18	5.13	34.25	0.53	21.63	0.26	22.3	16.7	28.3
Lan-tang	4.64	8.39	0.32	0.17	1.07	22.91	5.98	15.28	0.17	20	16~17	26~27

2.2 Data Collection

Growth traits measurements, bark and 5 mm diameter increment cores were collected at aged 4 years old. Diameter at breast height over bark (DBH in cm) and height (HGT in m) were measured for all trees. Individual tree volume over bark (VOL in m3) was calculated using the following formula as per Lu *et al.* (2009):

$$VOL = HGT \times DBH^2 / 30\ 000 \quad (1)$$

Bark percentage (Bark Perc) for each individual tree was defined as the ratio of the area of bark at breast height (1.3m) to total cross-sectional area at the same height (Wu *et al.* 2011). All progenies were assessed for Pilodyn penetration (PP) and stress wave velocity (SWV) using Pilodyn and Fakopp microsecond timer tools respectively. PP was measured using a 6-J Forest Pilodyn fitted with a 2.5 mm steel pin, by removing a small section of bark (approximately 40mm×20mm) at 1.3m. Wood basic density (BD) was determined using the water displacement method, with weight of water displaced by immersion of core and oven dry weight (Kien *et al.* 2008; Wu *et al.* 2011). SWV was calculated by dividing the test span (1500mm) by the mean measurement stress wave transmission time (Wang *et al.* 2000), in km s^{-1}. The SWV combined with green wood density were used to estimate dynamic modulus of elasticity (MOE) (Gpa) (Wang *et al.* 2000).

Statistical Analysis

Statistic analyses were conducted using GLM procedures in SAS statistical software to detect difference for growth traits and wood properties, as well as bark percentage. The line model on one trait (Wu *et al.* 2015), y_{ijk} is:

$$y_{ijk} = \mu + E_i + B_{j(i)} + C_k + \varepsilon_{ijk} \quad (2)$$

Where Y_{ijk} is the phenotypic value of the individual of the k^{th} combination in j^{th} block within i^{th} site, μ is the overall mean, E_i is the fixed effect of i^{th} site, $B_{j(i)}$ is the fixed effect of j^{th} block within i^{th} site, C_k is the fixed effect of k^{th} combination, and ε_{ijk} is the random error (or residual).

F_1 hybrid heterosis was calculated as follow (Bahman *et al.* 1975):

$$H = \frac{F_i - \overline{OP}}{\overline{OP}} \times 100\% \quad (3)$$

Where F_i is the average value of studied traits for the hybrid progenies, $\overline{OP}$ is average value of studied traits for the open pollination progenies of better parent.

3 RESULTS AND DISCUSSION

3.1 Variance Analysis and Coefficient Variation

The analysis of variance of studied traits among sites, replicates and combinations is presented in Table 2. The results showed that there were significant differences on growth traits, BD, bark percentage, PP and MOE among sites, implying that these traits were prone to be affected by environment. The joint analysis of all families showed significant combinations effects for all traits except MOE, indicating the less difference of MOE among combinations. Meanwhile significant differences on DBHOB, BD, SWV and MOE at 0.01 level among replicates were also found.

TABLE 2 MEAN VALUES, RANGES AND VARIANCE ANALYSIS FOR THE STUDIED TRAITS AT THREE SITES

Trait	Site	Mean	Min.	Max.	SD	SE	CV (%)	Variance Analysis		
								Site	Replication	Combination
HGT(m)	Gong-he	17.04	6.8	24.2	3.10	0.11	18.18	<0.0001	0.0541	<0.0001
	Feng-an	13.32	5.4	19.8	2.72	0.16	20.43			
DBH (cm)	Gong-he	12.20	5.00	18.80	2.52	0.09	20.63			
	Feng-an	9.40	4.4	14.3	1.83	0.10	19.50	<0.0001	0.0012	<0.0001
VOL (m^3)	Gong-he	0.09	0.01	0.24	0.05	0.00	49.09	<0.0001	0.5630	<0.0001
	Feng-an	0.04	0.01	0.11	0.02	0.00	50.43			
BD ($g\ cm^{-3}$)	Gong-he	0.45	0.13	0.78	0.05	0.00	11.24			
	Feng-an	0.47	0.33	0.65	0.04	0.00	9.07	<0.0001	0.0163	<0.0001
BK (cm)	Gong-he	0.53	0.20	1.25	0.15	0.01	28.02	0.2196	0.1922	<0.0001
	Feng-an	0.50	0.20	1.06	0.13	0.01	26.52			
Bark Perc (%)	Gong-he	16.65	6.28	32.90	0.04	0.00	23.93			
	Feng-an	20.48	10.25	36.38	0.05	0.00	22.26	<0.0001	0.7037	0.0002
PP	Gong-he	12.09	6.00	19.00	1.71	0.06	14.18	0.0024	0.0018	<0.0001
	Feng-an	11.8	7.40	17.40	1.74	0.10	14.72			
SWV ($km\ s^{-1}$)	Gong-he	3.36	2.65	4.82	0.29	0.01	8.69	0.1921	<0.0001	<0.0001
	Feng-an	3.85	2.56	4.95	0.36	0.02	9.24			
MOE (GPa)	Gong-he	5.04	1.49	11.14	1.11	0.05	22.08	<0.0001	0.9724	0.5195
	Feng-an	5.72	2.56	11.47	3.16	0.19	55.24			

HGT = height; DBH= diameter at breast height over bark; VOL = volume; BD = wood basic density; BK = bark thickness; Bark Perc = Bark percentage; PP = Pilodyn pin penetration; SWV = stress wave velocity; MOE = dynamic modulus of elasticity; SD = standard deviation; SE = standard error; CV = genotypic coefficient of variation

The mean HGT, DBH and Vol at 4 years were 13.32 to 17.04 m, 9.40 to 12.20 cm and 0.04 to 0.09 m^3 respectively (Table 2). Gong-he site had the best growth yield while Feng-an plantation was lower than others. The DBH increments per year were ranged from 2.35 to 3.05 cm. These results were somewhat bigger than published material on E. urophylla (Bao et al. 2002), E. globulus (Stackpole et al. 2010) and other species in Pakistan (Mahmood et al. 2003), India (Varghese et al. 2008), Vietnam (Kien et al. 2010) and Australia (Kube et al. 2001) whereas lower than intra-species hybrids within Eucalyptus nitens Maiden and E. globulus and inter-specific E. nitens × globulus at age 4 years (Volker *et al*. 2008)

The mean BD values ranged from 0.45 to 0.47 g cm-3 at 4 years (Table 2), agreeing with previous studies by Kube et al. (2001) and Bao et al. (2002). Nevertheless, the present wood density values were somewhat lower than those reported for E. urophylla (Wei and Borralho, 1997; Kien et al. 2008; Wu et al. 2013), probably due to the smaller growth age of this trial. Wood basic density based on increment cores was assessed 0.51 g cm-3 at four E. urophylla progeny trials in south east China (Wei and Borralho, 1997) and averaged 0.51 g cm-3 for E. urophylla across two trials in northern Vietnam at the age of eight and nine years (Kien et al. 2008).

It is well known that MOE can be used to estimate growth stresses, which are involving in effecting end splitting of logs, without the need for harvesting trees and (Raymond *et al.* 2004). Our results showed that the MOE values ranged from 5.04 to 5.72 GPa (Table 2), indicating that studied material had higher end splitting of logs. Findings of the value of MOE were somewhat smaller than previous studies, in which *Eucalyptus cloeziana* F. Muell showed the highest value (14.2 to 15.7 GPa), followed by *Eucalyptus pilularis* Smith (12.2 to 13.5 GPa) and *Eucalyptus dunnii* Maiden (10.7 to 12.6 GPa) (Warren *et al.* 2009).

The coefficient of variation of all traits at three sites is given in Table 2. Coefficients of variation ranged from 18.18% to 50.43% for growth traits, 9.07% to 55.24% for wood properties and 22.26% to 23.93% for bark percentage. These were higher than the variability observed in previous studies by Wei and Borralho (1997), in which coefficients of variation for wood properties ranged from 7.9% to 13.6% for *E. urophylla* in southeast China, and Kien *et al.* (2008), in which coefficients of variation ranged from 5.3% to 5.9% for wood basic density and 4.8% to 5.2% for Pilodyn pin penetration, implying the relative high potential for plus tree selection in these trials. However, the coefficient of variation for MOE at Feng-an was considerable larger than that at other sites. The possible explanation could be in part due to the relatively larger coefficient of variation for green wood density. The coefficient of variation for VOL (49.09%-50.43%) was larger than that for other traits, whereas the coefficient of variation of SWV (8.69%-9.24%) was relatively small, indicating that the scope for selection among families is limited.

3.2 Estimation of Heterosis among Crosses

Heterosis values were estimated as the percentage deviation of the F1 performance from the midparent, better parent and the best parent (Paramathma et al. 1997). Estimation of heterosis on growth traits in E. urophylla are given in Table 3. On the part of original heterosis, the estimation on HGT, DBH, VOL and Bark Percentage were -23.29% to 18.80%, -19.18% to 16.49%, -36.93% to 43.44% and -14.97% to 11.96%, respectively. The original heterosis estimation of HGT had similar range with DBH. The range of Bark Percentage was higher than that of HGT and DBH. VOL had the highest range than others probably due to the product between HGT and DBH, agreeing with previous studies by Hong (2009). Bao et al. (2002) reported that the heterosis estimation of growth traits ranged from 5.2% to 60.6% in E. urophylla at 9 years. Volker et al. (2008) revealed that the heterosis for intra-specific hybrid and inter-specific hybrids were ranged from 0% to 11.3% and from -24.0% to 14.3%. On the other hand, the reciprocal heterosis estimation on HGT, DBH, VOL and Bark Percentage were -22.12% to 18.50%, -24.07% to 18.74%, -52.13% to 50.13% and -23.72% to 17.21%, respectively. Just like the original heterosis, the reciprocal heterosis estimation of HGT had similar range with DBH and VOL had the highest range than others. U2×U21, U2×U22, U21×U56, U21×U64, U21×DU1, U22×U56 and U22×DU1 had better original and reciprocal heterosis estimation than others at Gong-he. Further, U22×U56 and U22×DU1 had better original and reciprocal heterosis estimation than others at Feng-an. It is interesting to find that the same combinations had different original and reciprocal heterosis estimation at different sites even on same traits. For example, the original heterosis on VOL of U2×U21 at Gong-he and Feng-an were 39.18% and -27.06% respectively. However, U22×U56 generally had higher heterosis of growth traits at all sites.

TABLE 3 ESTIMATION OF HETEROSIS ON GROWTH TRAITS AT THREE SITES

Site	Cross	Original Heterosis %				Reciprocal Heterosis %			
		HGT	DBH	VOL	Bark Perc	HGT	DBH	VOL	Bark Perc
Gomg-he	U2×U21	7.86	15.78	39.18	-14.71	-	-	-	-
	U2×U22	9.43	5.08	15.67	-17.22	12.76	9.92	29.03	-7.50
	U2×U56	-8.43	-19.18	-36.00	-4.42	-13.38	-13.17	-25.51	-0.21
	U2×U64	-17.31	-14.05	-31.21	9.23	8.30	18.74	50.13	-8.50
	U2×DU1	-	-	-	-	18.50	9.34	29.17	-7.88
	U21×U22	-11.19	-12.68	-32.94	-6.43	-6.70	-4.27	-17.43	-23.72
	U21×U56	9.24	14.47	43.44	-8.72	11.62	18.24	47.39	-10.75
	U21×U64	8.71	13.20	39.50	9.99	-	-	-	-
	U21×DU1	4.54	16.49	34.18	-12.36	5.67	11.48	23.88	-18.93
	U22×U56	13.78	7.68	24.78	-14.97	12.55	3.14	12.12	-12.19
	U22×U64	-	-	-	-	7.15	14.03	41.81	-6.04
	U22×DU1	18.80	5.45	23.94	11.96	5.82	4.42	7.44	-10.45
	U56×U64	-14.36	-3.02	-9.30	0.10	5.84	17.96	50.55	-1.77
	U56×DU1	6.12	-0.83	0.42	-5.18	4.94	0.48	2.00	-1.55
	U64×DU1	-	-	-	-	5.26	-1.06	6.76	1.12
Feng-an	U2×U21	-6.92	-11.10	-27.06	2.54	-	-	-	-
	U2×U22	-2.26	-1.50	-3.73	-4.48	-	-	-	-
	U2×U56	-23.29	-16.69	-36.93	7.47	-8.77	-2.79	-7.64	10.75
	U2×U64	-	-	-	-	-6.41	2.53	-3.01	9.85
	U2×DU1	-	-	-	-	-2.41	-2.04	-6.17	-6.19
	U21×U22	-	-	-	-	-22.12	-24.07	-52.13	17.21
	U21×U56	-5.81	-8.34	-21.37	-0.60	-8.97	-10.10	-23.42	5.64
	U21×DU1	-	-	-	-	-16.21	-11.84	-34.47	-4.66
	U22×U56	-	-	-	-	4.85	7.95	16.99	5.40
	U22×U64	-	-	-	-	-7.05	2.09	-6.31	1.72
	U22×DU1	-	-	-	-	5.80	3.36	9.18	-5.25
	U56×DU1	-11.58	-5.88	-21.37	6.57	-6.26	0.72	-5.17	4.81

HGT = height; DBH= diameter at breast height over bark; VOL = volume; Bark Perc = Bark percentage.

Estimation of heterosis on wood properties in E. urophylla is given in Table 4. On the part of original heterosis, the estimation on BD, PP, SWV and MOE were -11.97% to 7.69%, -10.25% to 13.25%, -5.12% to 5.50% and -18.19% to 16.28%, respectively. Bao et al. (2002) reported that the heterosis estimation of extraction ranged from -9.4% to 23.2% in E. urophylla at aged 9 years. On the other hand, the reciprocal heterosis estimation on BD, PP, SWV and MOE were -14.23% to 9.17%, -20.40% to 15.31%, -14.53% to 8.09% and -25.53% ~ 8.49%, respectively. In general, the range of heterosis on wood properties was smaller than that on growth traits. U2×U21, U21×U64, U22×U56, U56×DU1 and U64×DU1 had better original and reciprocal heterosis estimation than others at Gong-he. Further, U2×U64, U21×U22, U21×U56, U22×U64 and U56×DU1 had better original and reciprocal heterosis estimation than others at Feng-an. Just like growth traits, the same combinations had different original and reciprocal heterosis estimation at different sites even on same traits. For example, the original heterosis on BD of U2×U21 at Gong-he and Feng-an were -1.71% and 1.84% respectively. However, U22×U56 generally had higher heterosis of wood properties at all sites. Generally speaking, the combinations by same parents always had similar original and reciprocal heterosis estimation.

TABLE 4 ESTIMATION OF HETEROSIS ON WOOD PROPERTIES AT THREE SITES

Site	Cross	Original Heterosis %				Reciprocal Heterosis %			
		BD	PP	SWV	MOE	BD	PP	SWV	MOE
Gomg-he	U2×U21	-1.71	4.12	2.09	5.87	-	-	-	-
	U2×U22	0.62	-4.69	2.26	7.91	1.57	-5.08	0.11	-4.08
	U2×U56	-1.74	0.98	-3.30	-9.75	1.26	0.07	-1.26	-0.25
	U2×U64	2.30	-0.86	-0.83	2.36	-14.23	11.10	-6.09	-25.53
	U2×DU1	-	-	-	-	-3.90	-2.13	-1.97	-6.09
	U21×U22	-7.61	13.25	-3.66	-8.34	-3.54	6.42	-0.01	1.11
	U21×U56	-11.97	3.41	3.55	-3.09	-2.50	2.29	0.55	1.53
	U21×U64	-2.84	5.41	1.30	3.68	-	-	-	-
	U21×DU1	-8.08	11.28	1.12	-1.29	-9.99	9.29	-2.49	-12.34
	U22×U56	7.69	0.12	5.50	16.28	2.93	3.41	1.14	8.49
	U22×U64	-	-	-	-	-13.12	15.31	-6.01	-25.41
	U22×DU1	-2.04	0.69	2.28	1.27	-6.50	-2.26	2.13	4.85
	U56×U64	-1.50	12.91	-5.07	-18.19	-10.08	6.89	-4.19	-17.48
	U56×DU1	2.21	7.06	-0.67	5.65	1.23	-1.57	2.06	7.85
	U64×DU1	-	-	-	-	-2.23	5.54	1.00	3.36
Feng-an	U2×U21	1.84	-7.04	-5.12	4.25	-	-	-	-
	U2×U22	4.71	-4.24	-5.07	7.47	-	-	-	-
	U2×U56	-2.10	-10.25	6.66	-0.57	-3.16	7.77	4.04	7.41
	U2×U64	-	-	-	-	-5.63	0.02	8.09	1.40
	U2×DU1	-	-	-	-	-0.61	-7.18	-9.60	5.21
	U21×U22	-	-	-	-	9.17	9.49	-14.53	3.32
	U21×U56	-5.28	4.46	0.14	1.84	-5.60	6.39	3.90	3.15
	U21×DU1	-	-	-	-	2.27	-20.40	-4.52	4.08
	U22×U56	-	-	-	-	-1.21	17.94	-7.25	3.80
	U22×U64	-	-	-	-	-5.32	12.50	4.81	1.03
	U22×DU1	-	-	-	-	-0.18	20.45	-13.26	6.34
	U56×DU1	-7.31	8.67	2.31	8.58	-7.31	8.67	2.31	8.58

BD = wood basic density; PP = Pilodyn pin penetration; SWV = stress wave velocity; MOE = dynamic modulus of elasticity.

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