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Genetic Control of Wood Basic Density and Bark Thickness and Their Relationships with Growth Traits of *Eucalyptus urophylla* in South East China

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Abstract

Wood basic density based on increment cores, Pilodyn penetration, bark thickness and relative bark thickness (measured as a ratio between bark thickness and diameter) were assessed at 4 *Eucalyptus urophylla* progeny trials in south east China. The results showed that both basic density and Pilodyn penetration are under strong genetic control, with heritabilities of 0.71 and 0.64, respectively. Heritabilities for bark thickness and relative bark thickness were also very high (h^2 = 0.45 and 0.40 respectively). Wood density had weak unfavourable genetic correlations with diameter, height and volume growth. Genetic correlations between relative bark thickness and growth were generally negative. Genotype by site interaction was unimportant for wood basic density, bark thickness, and relative bark thickness. The implications of these genetic parameters on the breeding strategies for this species are discussed.

Key words: heritability, genetic correlation, genotype by environment interaction, REML, *Eucalyptus urophylla*.

FDC: 165.5; 561; 176.1.

Introduction

In recent years, breeding objectives in tree improvement have moved from volume per hectare alone, to include also wood properties and their impact on industrial end products (Borralho *et al.*, 1993; Zobel and Jett, 1995; Dieters *et al.*, 1996; Greaves *et al.*, 1996). Of the range of wood properties, basic density is one of the most important, affecting every aspect of the quality and quantity of pulp (Higgins, 1984; Harding and Li, 1989; Zobel and Jett, 1995). Relative ease of measurement and generally high heritability also makes it the most studied wood characteristic in eucalypts (Rudman, 1970; Harding and Li, 1989; Borralho, 1992; Chafe, 1994; Li *et al.*, 1995; Raymond, 1995; Greaves *et al.*, 1996). In general, density in eucalypts has been reported to be under strong genetic control (Zobel and Jett, 1995), with individual heritabilities ranging between 0.4 and 0.84 (Rudman *et al.*, 1969; Otebeye and Kellison, 1980; Wang *et al.*, 1984; Malan, 1988; Dean *et al.*, 1990; Borralho *et al.*, 1992; Raymond, 1995; Zobel and Jett, 1995; Greaves *et al.*, 1996). Genetic correlations between basic density and growth rate have been weak but often unfavourable (Malan, 1988, 1991; Borralho *et al.*, 1992; Greaves *et al.*, 1996).

Measurement of wood density is expensive and time consuming and often needs to destroy sample trees, and that has restricted the number and accuracy of the studies published (Raymond, 1995). Recently, Pilodyn penetration, an indirect method for determining wood basic density, has proven effective in assessing large number of trees in eucalypts (Moura *et al.*, 1987; Dean *et al.*, 1990; Greaves *et al.*, 1996). Despite being less accurate than direct measurement for disks, Greaves *et al.*, (1996) demonstrated that Pilodyn assessment can yield the same amount of gain as direct selection due to its cheaper cost and higher selection intensity.

As *Eucalyptus urophylla* plantations in southern China and elsewhere are being established mainly for pulping (Ikemori *et al.*, 1986; Li *et al.*, 1995), growth and wood properties

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affecting the pulping process need to be addressed in order to carry out efficient breeding strategies. There have been some reports on the genetics of growth for the species (MORI *et al.*, 1990; KURINOBU *et al.*, 1996; NIRSATMANTO *et al.*, 1996), but the genetics of wood basic density remains poorly understood. Several studies have looked at the phenotypic variation for wood basic density and fibre length in China (GOULD, 1987; HARDING and LI, 1989; LI, 1992; CHAI *et al.*, 1993). They found large variation between provenances and individual trees and suggest a great potential for improvement at the provenance level; however, genetic parameters are not yet available.

The purpose of this paper is to determine the genetic control of wood basic density and bark thickness in *E. urophylla*, test the effectiveness of Pilodyn as an indirect criterion for wood basic density and look at the genetic relationship between growth traits and wood and bark traits. This information will be used to develop appropriate selection strategies for *E. urophylla* breeding programs in south east China.

Material and Method

Progeny trials

The 4 progeny trials used in this study were established at Dongmen State Forest Farm located in Guangxi Zhuang Autonomous Region. The trials, locally identified as 46D, 46W, 54 and 77, include 100, 50, 90 and 200 open-pollinated families associated with 4, 4, 8 and 12 provenances respectively. Field design was a randomised complete block with single tree plots in a spacing of 3 m x 2 m in all trials. Selective thinning based on visible assessment of growth and tree form was done at all trials at age 1 to 3 years. A more detailed description of soil and climate condition in the trials can be found in (WEI and BORRALHO, 1997).

Measurement

Pilodyn penetration (PP) were measured using a 6J Forest Pilodyn, by removing a small section of bark at 1.3 m above the ground and taking 2 Pilodyn shots at each tree. Bark thickness (BK) was measured right after PP by the same device. Relative bark thickness (BKR) was expressed as a ratio between BK and DBH. Since DBH being used to calculate BKR at trials 46D and 54 were taken at previous years, BKR at these 2 trials were overestimated. All surviving trees in 46D, 46W and 54, and in 3125 trees out of 4263 surviving trees in trial 77 were measured. In order to determine the reliability and genetic correlation of PP with wood basic density (DEN), increment cores were also taken on 459 trees from 9 randomly selected blocks in trial 54.

DEN was based on the gravimetric method (HARDING, 1988). A 5 mm increment core was taken at 1.3 m from every sampled tree and immediately stored in a plastic tube with 2 ends sealed, and later taken to a freezer. Three weights in grams were taken for every sample: weight in water (w_1) and weight in air (w_2) and oven dry weight (w_3). DEN (kg/m^3) was then calculated as:

$$DEN = \frac{w_3}{w_2 - w_1} * 1000 \qquad (1)$$

DBH was measured at 1.3 m by a measuring tape and HT by poles for each tree. Volume of a single tree (m^3/stem) was calculated by the following formula used in (ZHOU and LIANG, 1991):

$$VOL = 0.00002618(DBH)^2(HT) \qquad (2)$$

Statistical analysis

The model used for the single site analysis was (in matrix notation):

$$y = Xb + Ws + Za + e \qquad (3)$$

where y is a vector of the observation of PP (given as the average of the two measurements per tree), BK, BKR, DEN, DBH and HT at 2 ages (before and after the thinning), b is a vector of the fixed effects of block, s is the vector of random provenance effect, and a is a vector of the random effect of individual tree, and X, W, and Z are known incidence matrices. The model used across trials 46D, 46W and 54 was:

$$y = Xb + W_1 s + W_2 fs + Za + e \qquad (4)$$

where fs is a vector of the random effect of family by site interaction, and W_1 and W_2 are known incidence matrices. Records on each individual site were standardised by multiplying the ratio between phenotypic standard deviation of pooled data and the phenotypic standard deviation of each site (VISSCHER *et al.*, 1991).

Variance components were estimated using REML VCE v3.2 by (GROENEVELD, 1996). Significant test of provenance differences was conducted by the procedure PROC GLM of SAS® program. Open pollinated families were assumed to have a coefficient of relationship of ¼ (HOUSE and BELL, 1994).

Within provenance heritability at a single site was estimated as:

$$h^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_e^2} \qquad (5)$$

and across sites as:

$$h^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_{fs}^2 + \hat{\sigma}_e^2} \qquad (6)$$

Weighted heritability across 4 trial was estimated following CUNNINGHAM *et al.* (1977) and WOOLASTON *et al.* (1990):

$$h^2 = \sum_{i=1}^{n} z_i h_i^2 \qquad (7)$$

where weighting factor z_i is the inverse of the variance σ_i^2 of the heritability for the ith trial:

$$z_i = \frac{1/\sigma_i^2}{\sum_{j=1}^{n} 1/\sigma_j^2} \qquad (8)$$

Genetic correlation between trait i and j was as:

$$r_{ij} = \frac{\hat{\sigma}_{a_{ij}}^2}{\hat{\sigma}_{a_i}\hat{\sigma}_{a_j}} \qquad (9)$$

Results and Discussion

Performance of PP, DEN and BK

Mean PP, BK, BKR and DEN for each site are given at *table 1*. Trial mean and coefficient of variation for PP were relatively stable across the 4 trials, with PP ranging between 11.2 mm and 12.6 mm. Trial 77 had a slightly higher PP (12.6

mm) probably due to its younger age, when wood is usually less dense. On the other hand, BK differs considerably amongst the various sites, being highest (10.3 mm and 9.3 mm) on the 2 oldest trials (46D and 46W) and lowest (6.9 mm) on the youngest trial (77). This is simply related to tree size: the larger the tree, the thicker the bark. BKR might be similar across 4 trials. The higher proportions in trial 46D (7.4%) and 54 (6.4%) were apparently due to the different ages when DBH were measured earlier than that of BK. Nevertheless, the coefficient of variation for PP, BK and BKR were similar across all sites. DEN, which was measured directly from cores, ranged between 320 kg/m^3 and 619 kg/m^3 with an average of 513 kg/m^3. Ikemori *et al.* (1986) reported that the most suitable range of basic density for pulpwood in eucalypts was 480 kg/m^3 to 570 kg/m^3. There are considerably higher density values than those found in this *E. urophylla* study; this highlights the potential for improvement in wood density of *E. urophylla*, particularly when managed under a short-rotation regime.

Table 1. – Statistics of pilodyn penetration (PP in mm), bark thickness (BK in mm), relative bark thickness (BKR in %) and wood density (DEN in kg/m^3) in 4 *E. urophylla* progney trials in south east China.

Traits	Sites	Age	No trees measured	Mean ± s.d.	CV (%)	Individual Range
PP	46D	7	934	12.0 ± 1.63	13.6	7.6 ~ 18.5
	46W	7	647	11.2 ± 1.46	13.0	6.9 ~ 19.1
	54	6	2069	12.0 ± 1.54	12.8	7.6 ~ 20.1
	77	4	3125	12.6 ± 1.55	12.3	7.8 ~ 19.9
BK	46D	7	934	10.3 ± 3.30	32.0	3.2 ~ 32.0
	46W	7	647	9.3 ± 3.58	38.5	2.2 ~ 29.7
	54	6	2069	8.7 ± 3.08	35.4	1.0 ~ 28.0
	77	4	3125	6.9 ± 2.52	36.5	1.0 ~ 20.8
BKR	46D	7	934	7.4 ± 2.1	29.1	3.2~16.6
	46W	7	647	5.7 ± 1.2	34.5	1.7~20.5
	54	6	2069	6.4 ± 1.9	29.9	0.1~24.2
	77	4	3125	6.0 ± 1.8	30.1	0.1~16.9
DEN	54	6	459	513 ± 40	7.9	320.8 ~ 619.1

Provenance differences

Different traits had different response to seedlot origins. PP did not show significant differences between provenances in trial 46D and 46W but did so in trial 54 and 77. However, this significant difference disappeared when the 4 poorly represented (79, 12960) or non-native provenances (13243, var) in trial 54 were excluded in the test. DEN shared the same pattern as PP in trial 54, significant differences if including all provenances but no differences if excluding those 4 provenances. Two extreme values were from the Upper Ulanu River provenance with the highest of 540 kg/m^3 and the Aracruz seed orchard in Brazil the lowest of 494 kg/m^3 (*Table 2*). This might suggest an ineffective selection from native provenance for wood basic density or a negative indirect selection due to negative correlations with growth (*Figure 1*). The variation found in this study was somewhat smaller than those in Chai *et al.* (1993) and Ngulube (1989). Chai *et al.* (1993) reported a range between 471 kg/m^3 and 558 kg/m^3 on 5 provenances and Ngulube (1989) observed the range between 438 kg/m^3 and 564 kg/m^3 on 8 provenances. Both of them were sampled from a few old trees.

In contrast, BK and BKR showed significant differences between provenances at all trials. It was also true in trial 54 even when the 4 provenances from Brazil, Congo, Timor Island in Indonesia and the poorly represented Alor Island, were excluded. It is interesting to note that the faster growing provenances do not necessary have thicker bark or a higher proportion of bark. For instance, the seedlot from Brazil had the best performance in DBH and HT but the lowest BKR (4.4%) and a low BK (7.1 mm) in trial 54. This is also true for the seedlot from Mt. Lewotobi in trial 77.

Table 2. – Generalised Least Square Means (in kg/m^3) of basic density, bark thickness and relative bark thickness for provenances of *E. urophylla* progeny trial 54 in south east China.

Seedlot	Locations	Basic Density	Bark Thickness	Relative Bark Thickness
79	Upper Ulanu River, Alor, IND	533	10.4	9.0
12960	34 km N of Soe, Timor, IND	487	6.5	6.0
13011	Mt. Lewotobi, Flores Island, IND	503	8.8	5.9
13243	Republic of the Congo	511	6.3	5.1
14531	Mt. Egon, Flores Island, IND	513	8.7	6.4
14532	Mt. Lewotobi, Flores Island, IND	508	7.8	5.6
var	Seed Orchard, Aracruz, BRZ	493	7.1	4.4
Dongmen	Seed Production Area, PRC	514	8.3	5.8

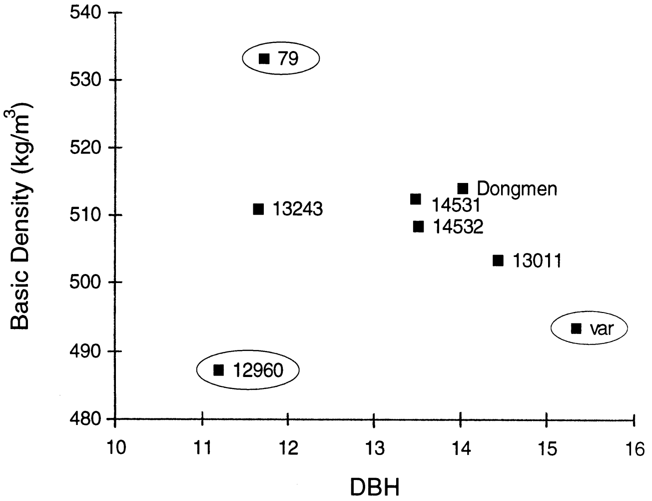

Figure 1. – Relationship between generalised least square means of DBH and wood basic density for 8 provenances at *E. urophylla* progeny trial in China. The location of provenances is listed at *table 1*.

Estimates of heritability in PP, DEN, BK and BKR

Variance components and estimates of heritability for PP, DEN, BK and BKR across all trials are listed in *table 3*. Wood density was highly heritable, either when based on cores (DEN), or indirect Pilodyn assessments (PP). The across-site weighted mean heritability for PP was h^2 = 0.66, whereas for DEN in trial 54 it was h^2 = 0.71. The high heritability estimates for PP and DEN were consistent with results found in other eucalypts (Malan, 1988, 1991, 1993; Dean *et al.*, 1990; Borralho *et al.*, 1992; Raymond, 1995; Greaves *et al.*, 1996). Raymond (1995) summarised 16 studies in eucalypts and found heritability varied from 0.05 to 0.84 with an average of 0.54. Nevertheless, the lower accuracy of PP compared with DEN was also reported in *E. nitens* (Greaves *et al.*, 1996).

Estimates of heritability for bark properties were also very high, with weighted means of h^2 = 0.50 for BK and h^2 = 0.47 for BKR. Although their heritability estimates varied from site to site, BK (h^2 = 0.34 to 0.62) and BKR (h^2 = 0.30 to 0.55) were consistently shown to be under strong genetic control.

The proportion of variance due to provenance effect was very small for PP (s^2 = 0.00 to 0.03) in all trials and DEN in trial 54 (s^2 = 0.00 to 0.01) (*Table 3*). This further indicated that selection of provenances for wood basic density would not be efficient. However, there were higher provenance effects for BK and BKR. Given the performance of growth (Wei and Borralho, 1997), it can be concluded that selection of

Table 3. – Estimates of additive genetic variance (σ_a^2), residual variance (σ_e^2), phenotypic variance (σ_p^2), and within provenance heritabilities and their standard error (h^2 ± s.e.) for PP, BK and DEN in 3 *E. urophylla* progeny trials in south east China. Weighted mean of heritability across 4 trials were also included.

Traits	Trial	σ_a^2	σ_s^2	σ_e^2	σ_p^2	h^2 ± s.e.	s^2 ± s.e.
PP	46D	2.553	0.001	0.193	2.747	0.93 ± 0.14	0.00 ± 0.01
	46W	1.420	0.031	0.640	2.092	0.69 ± 0.05	0.01 ± 0.01
	54	1.581	0.082	0.730	2.393	0.68 ± 0.12	0.03 ± 0.05
	77	1.211	0.065	1.143	2.419	0.51 ± 0.07	0.03 ± 0.02
	weighted mean					0.66 ± 0.04	0.01 ± 0.01
DEN	54	1083	13	428	1524	0.71 ± 0.20	0.01 ± 0.02
BK	46D	7.885	1.626	4.926	14.437	0.62 ± 0.08	0.11 ± 0.05
	46W	5.378	1.601	6.797	13.777	0.52 ± 0.08	0.12 ± 0.09
	54	3.006	0.949	5.763	9.718	0.34 ± 0.07	0.10 ± 0.06
	77	3.470	0.036	2.794	6.300	0.55 ± 0.07	0.10 ± 0.05
	weighted mean					0.50 ± 0.04	0.11 ± 0.03
BKR	46D	1.410	0.690	1.342	3.442	0.51 ± 0.07	0.20 ± 0.08
	46W	0.913	0.711	2.178	3.803	0.30 ± 0.12	0.19 ± 0.14
	54	1.259	1.197	1.949	4.405	0.39 ± 0.08	0.27 ± 0.14
	77	1.762	0.023	1.445	3.230	0.55 ± 0.07	0.01 ± 0.08
	weighted mean					0.47 ± 0.04	0.14 ± 0.05

Table 4. – Estimates of additive genetic variance (σ_a^2), residual variance (σ_e^2), family by environment interaction variance (σ_{fs}^2), phenotypic variance (σ_p^2), and within provenance heritability across 3 *E. urophylla* progeny trial in south east China. Estimates at the second line were based on the same dataset as the first line but the interaction item was not included in analysis.

Traits	σ_a^2	σ_s^2	σ_{fs}^2	σ_e^2	σ_p^2	-log*L*	h^2 ± s.e.	s^2 ± s.e.
PP	0.713	0.007	0.008	0.250	0.978	2187.7	0.74 ± 0.11	0.01 ± 0.02
	0.740	0.009		0.240	0.988	2187.9	0.76 ± 0.09	0.01 ± 0.02
BK	0.357	0.204	0.000	0.543	1.104	2205.6	0.40 ± 0.07	0.18 ± 0.06
	0.359	0.202		0.545	1.107	2205.6	0.40 ± 0.06	0.18 ± 0.09
BKR	0.339	0.223	0.001	0.509	1.071	2098.9	0.40 ± 0.08	0.21 ± 0.08
	0.338	0.225		0.509	1.071	2098.9	0.40 ± 0.06	0.21 ± 0.10

provenances from Alor Island or Flores Island would only be effective for bark thickness but not for growth and wood density.

Site by genotype interaction was not statistically significant for PP, BK and BKR (*Table 4*). Estimates of variance and heritability using the pooled data across the 3 trials (46D, 46W and 54) were almost identical to those when the interaction term was ignored. This was expected, given the similar environmental conditions across sites and the generally more stable genotype performance across environments found in wood properties (ZOBEL and JETT, 1995).

Correlations between PP, BK, DEN and growth traits

Correlations between wood, bark properties and growth traits across the 3 sites and in site 77 are presented in *table 5*. Unfavourable correlations were consistently observed between growth and density. Genetic correlations between PP and DBH, HT or VOL were generally positive (= 0.07 to 0.33, hence unfavourable). Genetic correlations between PP and DBH (r_g = 0.13 to 0.33) were apparently higher than corresponding correlations between PP and HT (r_g = 0.07 to 0.15), a result also observed in previous studies (Zobel and van Buijtenen, 1989; Yanchuk and Kiss, 1993).

Table 5. – Genetic (± standard error, above diagonal) and phenotypic correlations (below diagonal) correlations between PP, BK, BKR and DBH, HT and VOL across 3 trials (46D, 46W and 54) and at a single trial 77 of *E. urophylla* progeny in south east China.

	Site	DBH	HT	VOL	PP	BK	BKR
DBH	pooled		0.83±0.02	0.99±0.00	0.33±0.03	0.51±0.02	–0.15±0.02
	77		0.74±0.03	0.98±0.00	0.13±0.04	0.57±0.03	0.26±0.04
HT	pooled	0.80		0.88±0.02	0.15±0.04	0.25±0.03	–0.36±0.03
	77	0.75		0.85±0.02	0.07±0.04	0.07±0.06	–0.22±0.05
VOL	pooled	0.97	0.80		0.30±0.03	0.46±0.03	–0.21±0.02
	77	0.96	0.82		0.14±0.04	0.46±0.04	0.13±0.05
PP	pooled	0.21	0.07	0.20		0.14±0.03	–0.07±0.02
	77	0.11	0.00	0.09		–0.20±0.03	–0.28±0.03
BK	pooled	0.52	0.28	0.47	0.11		0.76±0.03
	77	0.53	0.26	0.47	–0.04		0.93±0.03
BKR	pooled	–0.04	–0.22	–0.09	–0.04	0.79	
	77	0.08	–0.10	0.02	–0.10	0.87	

As expected, BK showed a positive relationship with DBH and HT (r_g = 0.13 to 0.55), with bigger trees tending to have thicker bark. However, when expressed as the ratio of BK to DBH (BKR), it generally showed negative correlations with DBH, HT or VOL (r_g = –0.15 to –0.36) except with DBH and VOL (r_g = 0.13 and 0.26) at trial 77. This provides a possibility to improve bark proportion simultaneously with growth traits. Phenotypic correlations were generally weaker than genetic correlations for all trait combinations both at and across the 3 trials and at trial 77.

The correlations between DEN and other traits in trial 54 are listed at *table 6*. An important result is the near perfect genetic correlation between DEN and PP. This suggested that PP is a very effective indirect indicator for density and confirms previous studies in other eucalypt species (Dean *et al.*, 1990; Greaves *et al.*, 1996). Another result from *table 6* is the moderately high negative genetic correlation between DEN and both DBH and VOL. This is consistent with the positive correlation between PP and growth found across 4 trials. All correlations shared the same pattern as cross-site analysis.

Table 6. – Genetic (± standard error, above diagonal) and phenotypic correlations (below diagonal) between PP, BK, DEN and growth traits (DBH, HT and VOL) at E54.

	DBH5	HT5	VOL	PP	DEN	BK	BKR
DBH5		0.92±0.01	1.00±0.00	0.35±0.03	–0.36±0.04	0.37±0.04	–0.42±0.03
HT5	0.86		0.92±0.01	0.04±0.03	–0.04±0.04	0.40±0.05	–0.34±0.04
VOL	0.98	0.87		0.32±0.03	–0.34±0.04	0.37±0.05	–0.42±0.03
PP	0.25	0.09	0.25		–1.00±0.01	0.02±0.04	–0.27±0.04
DEN	–0.23	–0.11	–0.22	–0.80		–0.05±0.06	0.24±0.05
BK	0.47	0.25	0.44	0.14	–0.03		0.68±0.04
BKR	–0.28	–0.41	–0.29	–0.03	0.14	0.69	

Implications for tree improvement in E. urophylla

The implications of this study are on one hand that it should be relatively easy to select superior individuals for each trait individually, as growth (Kurinobu *et al.*, 1996; Nirsatmanto *et al.*, 1996; Wei and Borralho, 1997), bark thickness, and wood basic density have a high heritability. Our data seems to proved some evidence of realised gains in growth, with the better performance of the Dongmen and Aracuz seedlots, both selected on the basis of previous phenotypic selection. But, on other hand, improvement based on growth traits alone could prejudice wood density (*Table 5* and *6*). This is consistent with the observed very low wood density in the Aracruz seedlot which was previously selected for growth (*Table 2*). Care should be taken regarding selection for growth and density among provenances or landraces. For the selection of superior individuals, an index assigning appropriate weights to wood basic density and volume should be defined, with the exact coefficients dependent on the specific breeding objectives (Borralho *et al.*, 1993; Greaves *et al.*, 1996).

Conclusion

Wood basic density was under strong genetic control in *E. urophylla*, either based on direct measurement of increment cores (h^2 = 0.71) or indirect measurement of Pilodyn penetration (h^2 = 0.66), with the 2 traits being highly correlated. This confirms that Pilodyn penetration is a very useful predictor of wood basic density in this species. Bark thickness and relative bark thickness also have high heritabilities (0.45 and 0.40, respectively). Relative bark thickness was generally negatively correlated with growth traits. While considerable success in breeding should be expected from selection for wood basic density, bark thickness or volume individually, combined selection should appropriately consider the effect of provenance and the appropriate relative weighting of the different traits, given that all are likely to be relevant to improve an aggregate breeding objective of pulp production.

Another promising result from this study was the small genotype by environment interaction observed for Pilodyn penetration and bark thickness, confirming earlier result for growth traits (Wei and Borralho, 1997). Although a testing program covering a wide range of environments is needed, the results suggest that a single breeding population for *E. urophylla* in Guangxi region seems to be an appropriate strategy for this species.

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Cryopreservation: an Alternative Method for the Conservation of Endangered Populations of Two Iberian Pines (*Pinus nigra* Arnold and *Pinus sylvestris* L.)

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Summary

Seed conservation in traditional seedbanks is a possibility for preserving genetic resources, but the rapid decrease in the viability of germplasm of forest trees and the problems associated with collection and multiplication, recommend the use of other storage methods. We have studied the effect of cryopreservation on the viability of *Pinus nigra* and *Pinus sylvestris* seeds. The results showed that germination is not significantly different in any of the blocks included in our experimental design (cryopreservation x desiccation). All this suggests that cryopreservation can be an economical and practical method in the conservation of seeds of *Pinus* species.

Key words: Cryopreservation, diversity loss, *Pinus nigra, Pinus sylvestris,* seeds.

FDC: 232.315.2; 232.318; 174.7 *Pinus nigra;* 174.7 *Pinus sylvestris;* (460).

Introduction

The most conspicuous cause of the loss of biodiversity is habitat destruction induced by man's activities (Wilson, 1985; McNeely et al., 1995). Forested areas in temperate regions have suffered a severe reduction in size as a result of habitat fragmentation. Thus, isolated and threatened populations can be highly affected by both natural disturbances, such as wildfires, or man-induced changes, such as acid rain (Eney and Petzold, 1987), or urban development. Extreme events can lead to local extinctions and a consequent loss of genetic variability. The smaller the area and the more severe the disturbance, the higher the rate of local loss of populations of tree species. In this sense several Iberian populations of *Pinus sylvestris* on the southern boundary of its distribution, and of *Pinus nigra* subsp. *salzmannii* are clearly threatened due