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Bayesian Mapping Reveals Large-Effect Pleiotropic QTLs for Wood Density and Slenderness Index in 17-Year-Old Trees of *Eucalyptus cladocalyx*

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Abstract: *Eucalyptus cladocalyx* F. Muell is a tree species suitable for low-rainfall sites, even with annual average precipitation as low as 150 mm per year. Its wood is classified as highly durable and its permanence in soil is longer than 25 years, so it can be used for multiple applications. Given that about 41% of the world's land area is classified as drylands, added to the impact of climate change on the availability of water resources, it becomes necessary to use plant species that can tolerate environments with low water availability. In this study, a Bayesian analysis of genetic parameters showed that wood density (WD) was moderately heritable, with a posterior mean of $h^2 = 0.29$ and a Bayesian credibility region (90%) of 0.06–0.74, while the slenderness coefficient (SC) was highly heritable, with a posterior mean of $h^2 = 0.48$ and a Bayesian credibility region (90%) of 0.11–0.87. Through Bayesian regression analysis, we identified four and three significant associations for WD and SC, respectively. Another important finding of the bi-trait Bayesian analysis was the detection of three large-effect pleiotropic QTLs located on LG4 at 52 cM, on LG2 at 125 cM, and on LG6 at 81 cM. Bayesian bi-trait regression and the posterior probability of association indicated that three QTLs presented strong evidence of association with WD and SC. This provides convincing evidence that the loci qtlWD130/qtlSC130, qtlWD195/qtlSC195, and qtlWD196/qtlSC196 have a significant pleiotropic effect. The association mapping based on multivariate Bayesian regression was useful for the identification of genomic regions with pleiotropic effects. These loci can be used in molecular marker-assisted breeding to select trees with better wood density.

Keywords: Bayesian multivariate mapping; pleiotropy analysis; posterior probability of association

1. Introduction

The endemic species *Eucalyptus cladocalyx* F. Muell from southern Australia is naturally distributed in three geographical regions: Kangaroo Island, the Eyre Peninsula, and the Flinders Range [1]. An interesting feature is its natural evolution to tolerate environments where the annual rainfall is lower than 200 mm [2,3]. The trees of *E. cladocalyx* have tremendous potential for the production of wood with multiple applications, due to its straight and poorly branched stems [4]. Lundqvist et al. [5] compared the physical properties of the wood of three genotypes of eucalyptus tolerant to drought: *E. grandis* × *E. camaldulensis* hybrid, *E. gomphocephala* and *E. cladocalyx*, and concluded that *E. cladocalyx*

is one of the most suitable species to produce structural wood, given the high density of wood and rigidity of its microfibrils.

According to the Australian classification of natural durability of wood, *E. cladocalyx* is one of the most resistant to biodegradation [6], with a useful life of 25 years or more, in contact with the soil [7]. Natural durability has been described as the resistance of wood to biological decomposition by fungi, bacteria, insects, or marine organisms without copper/chromium/arsenate treatment to preserve it [8]. Therefore, considering the health problems and environmental pollution associated with the use of chemical preservatives, *E. cladocalyx* has been used to produce high natural durability wood with potential uses in the agricultural and industrial sectors [6,9].

The density of the wood is considered one of the most informative properties in terms of the physical and mechanical behavior of a tree, followed by the production of sawn wood [10]. It has been observed that a higher density of wood also implies greater natural durability [11]. Wood density is related to the structure of the cells of the vessels and the parenchyma [12], in which species resistant to cavitation have a greater density in the wood [13]. In this sense, density is positively correlated with tolerance to drought, so arid environments tend to be dominated by species with high wood density [13].

According to several authors, wood density is moderately to highly heritable in most forest species [10,14,15]. However, other studies have indicated that wood density can vary depending on local environmental conditions [13,16,17]. Bush et al. [6] determined that the wood density of *E. cladocalyx* varies considerably based on the different regions of origin of the species, presenting moderate heritability ($h^2 = 0.41$).

Association studies based on DNA markers provide a comprehensive approach to identify genetic loci associated with the phenotypic response of complex traits. Association studies have identified many quantitative trait loci (QTL) associated with wood density in *E. urophylla*, *E. grandis* [18], and *E. globulus* [19], along with microfibrils angle and total lignin content in *E. nitens* [19]. Wood density and other mechanical properties are highly variable among species and sites, and even between and within the trees themselves [20]. Due to the relevance of this type of trait for the wood industry, several studies have been developed to estimate genetic parameters and identify genes, or regions in the genome, related to the production of structural components of wood (for example, cellulose and lignin) and the spatial arrangement of the microfibrils [21–26]. In general, these studies have been explored with molecular markers and transcriptomic analyzes in the principal forest species. For example, Nakahama et al. [24] showed that genes related to the biosynthesis of lignocellulosic compounds (for example, cellulose synthase, invertase, l-cinnamate-4-hydroxylase and cinnamoyl-CoA reductase) and genes related to the modification of the cell wall (for example, expansins and xyloglucan endo-transglycosylase/hydrolase) are mostly expressed in *Eucalyptus* hybrids that have a high wood density.

On the other hand, a factor that is also relevant in the production of sawn wood is the tree growth stress, which is the product of the stresses of the same growth of the tree when forming a new layer of the trunk during the differentiation of the fibers [27]. One of the best indicators of this type of stress is the slenderness coefficient [28]. This variable reveals the capacity of a tree to sustain itself against these tensions and the tensions of the environment, e.g., wind [29]. Although the slenderness of a tree depends on environmental factors such as the availability of light, wind, and plant density [30–32], it has been concluded that this trait is also controlled by genetic components [33,34]. Moreover, the slenderness of a tree is also influenced by the density of its wood [35].

Due to the potential of *E. cladocalyx* for the production of high-quality wood at low-rainfall sites, and that wood density and the slenderness of trees are traits closely related to the quality of the wood, the following objectives were proposed: (i) to determine Bayesian genetic parameters of wood density, measured indirectly by means of Pilodyn, and the slenderness of *E. cladocalyx* trees grown under arid environmental conditions, (ii) to identify genomic regions associated with density of wood and

slenderness, using a key set of 130 microsatellite markers, and (iii) corroborate the pleiotropy of the loci associated with both traits, using a multivariate Bayesian regression approach.

2. Materials and Methods

2.1. DNA Extraction and Genotyping

The association analysis was focused on a sample of 245 *E. cladocalyx* trees, corresponding to 5 blocks × 49 half-sib families, from a provenance-progeny trial established in 2001 [2]. Forty-seven families come from five natural provenances: Flinders Chase (35°57′ S, 136°42′ E), Marble Range (34°30′ S, 135°30′ E), Mount Remarkable (32°43′ S, 138°06′ E), Cowell (33°38′ S, 136°40′ E) and Wirrabara (33°06′ S, 138°14′ E), distributed geographical in three regions of southern Australia (Eyre Peninsula, Flinders Ranges and Kangaroo Island), and two families from a local seed resource (31°40′ S, 71°14′ W). The trial was situated in a dryland area within the region of Coquimbo, Choapa Province (31°38′ S Latitude; 71°19′ W Longitude; and altitude of 297 m) in the south of the Atacama Desert, Chile. Total genomic DNA was isolated from juvenile leaves using the method CTAB according to da Silva [36] with some modifications. A total of 130 SSR polymorphic markers distributed across the genome of *E. cladocalyx* were used for genotyping the association population [37]. Polymerase chain reaction (PCR) was performed as follows: 0.3 uM of each primer, 40 ng of genomic DNA, 1 U of Taq DNA polymerase, 0.2 mM of each dNTP, 10 mM of Tris-HCl pH 8.3, 50 mM of KCl and 1.5 mM of $MgCl_2$. The amplification was run as follows: initial heat at 95 °C for 5 min followed by 40 cycles of 95 °C for 1 min, annealing temperature of each primer for 1 min, 72 °C for 1 min, and a final extension of 72 °C for 5 min. Finally, the PCR products were separated and visualized according to the methods described by Mora et al. [37].

2.2. Traits Assessment

Wood density (WD) of 17-year-old trees was indirectly estimated after removal of the bark based on Pilodyn penetration using a Pilodyn 6J Forest (PROCEQ, Zurich, Switzerland). Penetration depth (in millimeters) was measured two to three times per tree at breast height, until two identical measurements (at a precision of 2.0 mm) were obtained. The slenderness coefficient (SC) was calculated as follows: SC = height (m)/DBH (m) according to Benomar et al. [38], where DBH corresponds to the diameter at breast height. The measurements of height and DBH were taken from Arriagada et al. [2]. The Tukey-Kramer multiple comparison test was performed to determine significant differences between the means of each provenance for the traits evaluated.

2.3. Genetic Parameters

The subsequent marginal distributions for the parameters, narrow-sense heritability and variance components, were estimated using a Bayesian approach, via Gibbs sampling algorithm, in MTGSAM [39] using the following base model:

$$y = Xb + Zp + Wf + \varepsilon, \quad (1)$$

where y is the vector of observed values of WD and SC; b and p are vectors of fixed effect of block and provenance, respectively; f is the vector of random effects of family, and ε is the residual error. X, Z, and W are known incidence matrices. Posterior estimates for the narrow-sense heritability of traits, were calculated from posterior samples of variance components obtained by the model (1) detailed above, using the expression:

$$\hat{h}^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_\varepsilon^2}, \quad (2)$$

where $\hat{\sigma}_a^2$ and $\hat{\sigma}_\varepsilon^2$ are the additive genetic and residual variances. This Bayesian analysis was contrasted with the restricted maximum likelihood (REML) method in ASReml 3.4 [40]. Coefficients of additive genetic variation (*CVa*) were calculated from posterior samples of additive genetic variance as follows:

$$CVa = \frac{\sqrt{\hat{\sigma}_a^2}}{\overline{x}}, \tag{3}$$

where $\hat{\sigma}_a^2$ is the additive genetic variance and $\overline{x}$ is the overall mean. Additive genetic correlations between WD and SC was estimated by:

$$r_{xy} = \frac{\hat{\sigma}_{G_{xy}}}{\hat{\sigma}_{G_x}^2 \cdot \hat{\sigma}_{Gy}^2}, \tag{4}$$

where $\hat{\sigma}_{G_{xy}}$ correspond to posterior distribution samples of genotypic covariance between the traits, and $\hat{\sigma}_{G_x}^2$, $\hat{\sigma}_{G_y}^2$ correspond to posterior mean distribution samples of genotypic variance for WD and SC. Variance and covariance components were assumed to have an Inverted Wishart prior distribution. A uniform prior distribution (Flat) was considered for known environmental effects (blocks), whereas a normal distribution was used for the vector of traits of interest and additive genetic effects. The convergence and autocorrelation of the Gibbs chains were assessed using the tests available in the CODA library of the R program, Coventry, England [41].

2.4. Population Structure and Kinship Analysis

The software STRUCTURE, Stanford [CA], United States 2.3.2 [42] was used to cluster the individuals into a number (K) of genetically homogeneous subpopulations based on an admixture model with correlated allele frequencies between provenances. For each K value (previously K = 1–6), 10 runs were conducted separately each with 100,000 Monte Carlo Markov Chain (MCMC) replicates and a burn-in period of 10,000 iterations. The optimal K value was determined with the highest ΔK method [43]. The membership coefficient (Q) of each individual was used to form the population structure Q matrix. Only markers with allele frequencies of 5% or higher were included in data analyses. The software TASSEL, Ithaca [NY], United States 3.0 [44] was used to calculate the genetic relatedness among all pairs of individuals (kinship matrix).

2.5. Bayesian Association Mapping and Pleiotropy

The residuals from a general linear model for each trait were used as the adjusted phenotypes for the Bayesian association analysis (taking into account the effects of block and genetic structure). This approach is used to reduce the sources of unexplained variability, e.g., the effects of block and genetic structure, which implies a reduction in the error rate and increase the statistical power of association study. The probability that a locus is associated with a given trait was evaluated by the Bayes Factor (BF) and the Posterior Probability of Association (PPA) [45]. The BF is the ratio of marginal likelihoods between the probabilities of the model of association (M1) and a null model of no association (M0) [46]:

$$BF = \frac{P(\,Data|M_1)}{P(\,Data|M_0)}, \tag{5}$$

where the marginal likelihoods for $M1$ and $M0$ are defined by:

$$P(\,Data|M_l) = \int \left(\prod_{i=1}^{N} \sum_{k=0}^{2} P(\Phi|G_{ij} = k, \theta) p_{ijk} \right) P(\theta|M_l)\, d\theta, \tag{6}$$

in which θ denotes the regression parameters, G_i denotes the genotype of the ith individual at the jth marker, Φ denotes the phenotype of the ith individual in a study of N samples, and $p_{ijk} = P(G_{ij} = \mathrm{k})$

is the probability that the genotype at the jth marker of the ith individual is k [46]. The Laplace approximation was used to estimate the marginal likelihoods of M1 and M0 [46].

The PPA combines the evidence in the observed association data (BF) with the prior probability (π) that a marker is associated. The π and BF are used to compute the posterior odds (PO):

$$\mathrm{PO} = \mathrm{BF} \times \frac{\pi}{1-\pi}, \tag{7}$$

where π is the prior probability that a given marker is associated with a trait. BF was calculated using a Bayesian regression analysis in the SNPTEST software [46]. The PPA was calculated from this PO, as follows:

$$\mathrm{PPA} = \frac{\mathrm{PO}}{1+\mathrm{PO}}. \tag{8}$$

The possible pleiotropic effect of one loci associated with more of one trait was corroborated by a multivariate Bayesian regression in SNPTEST [46]. Additionally, the association mapping was performed in TASSEL 3.0 software [44], employing the unified mixed linear model (Q + kinship) [47].

3. Results

3.1. Genetic Parameters

The trees from Wirrabara had the highest average depth of penetration of pilodyn at 16.8 mm, in contrast to the individuals from Cowell (13.0 mm), indicating that the latter has a denser wood. In contrast, the mean slenderness coefficient varied between 83.4 and 109.3 for the trees from Wirrabara and Cowell, respectively. Regarding regions, the trees from the Flinders Mountain Range had the highest slenderness coefficient and the lowest wood density, while the individuals of the Kangaroo Island and the Eyre Peninsula presented the highest wood density and the lowest coefficient of slenderness (Table 1).

Table 1. Population means for wood density and slenderness coefficient in the populations of *E. cladocalyx*.

Provenance	Region	N_f	N_t	Wood Density	Slenderness
Wirrabara	Flinders Ranges	9	45	16.8 a	83.4 b
Mount Remarkable	Flinders Ranges	16	80	16.4 a	86.2 b
Illapel	Coquimbo	2	10	16.1 a	92.0 ab
Flinders chase	Kangaroo Island	8	40	13.8 b	101.3 a
Marble Range	Eyre Peninsula	4	20	13.6 b	87.3 ab
Cowell	Eyre Peninsula	10	50	13.0 b	109.3 a

N_f: total number of families evaluated; N_t: total number of trees evaluated. Mean values with the same letter in the same column indicate that the populations are not significantly different according to the Tukey-Kramer test ($p < 0.01$).

Based on subsequent marginal distributions, the density of the wood in *E. cladocalyx* showed a moderate heritability (mean of $\hat{h}_a^2 = 0.29$), with a credibility range (90%) of 0.06 to 0.74; while the heritability for the slenderness coefficient was high $\hat{h}_a^2 = 0.48$ (credibility region from 0.11 to 0.87). The posterior mean of the genetic correlation between wood density and the slenderness coefficient was negative with a value of 0.3 and a credibility range of −0.49 to −0.11. The estimates of heritability via REML were similar to those obtained by the Bayesian method, with values of 0.30 and 0.45, for wood density and slenderness coefficient, respectively. The genetic correlation between both traits was moderate and negative (−0.34). The estimations of variance components, the heritability values and their genetic correlation, based on the point estimates (mean, mode, and median) of the posterior marginal distributions (and from the REML method), are shown in Table 2.

Table 2. Variance components, heritability for wood density (WD) and slenderness coefficient (SC), and the genetic correlation between the traits in the population of *E. cladocalyx*.

Trait/Estimates	Bayesian Estimates						REML Estimates
	Mean	Median	Mode *	SD	90% Credible Sets		
					Lower	Upper	
Wood density							
$\hat{\sigma}_a^2$	1.7	1.3	0.6	1.3	0.3	4.5	1.7
$\hat{\sigma}_\varepsilon^2$	4.0	4.2	4.5	1.2	1.6	5.7	4.0
$\hat{h}_a^2$	0.29	0.23	0.12	0.21	0.06	0.74	0.30
CVa (%)	7.9	7.4	6.2	3.1	3.7	13.7	8.5
Slenderness coefficient							
$\hat{\sigma}_a^2$	164.0	155.0	86.1	88.1	37.7	317.0	152.6
$\hat{\sigma}_\varepsilon^2$	174.3	175.2	205.0	79.8	46.6	301.3	183.8
$\hat{h}_a^2$	0.48	0.47	0.34	0.24	0.11	0.87	0.45
CVa (%)	13.4	13.6	14.6	4.0	6.7	19.4	13.5
Genetic correlation	−0.30	−0.30	−0.30	0.12	−0.49	−0.11	−0.34

$\hat{\sigma}_a^2$: additive genetic variance, $\hat{\sigma}_\varepsilon^2$: residual variance, $\hat{h}_a^2$: heritability, *CVa*: additive coefficient of variation, SD: standard deviation. * Kernel density estimates of the mode from marginal posterior distributions.

3.2. Genetic Structure

The trees from the five Australian locations evaluated in this study were grouped into three genetically differentiated groups, which coincide with the three geographical regions from which each population originates. Additionally, individuals from Illapel (local population) were grouped mainly with individuals from the Flinders Mountain Range (Figure 1). Consistent with the above, the mean Fst value of a given cluster is not contained within the credibility regions of the other groups, which confirms the significant genetic differentiation (Table 3).

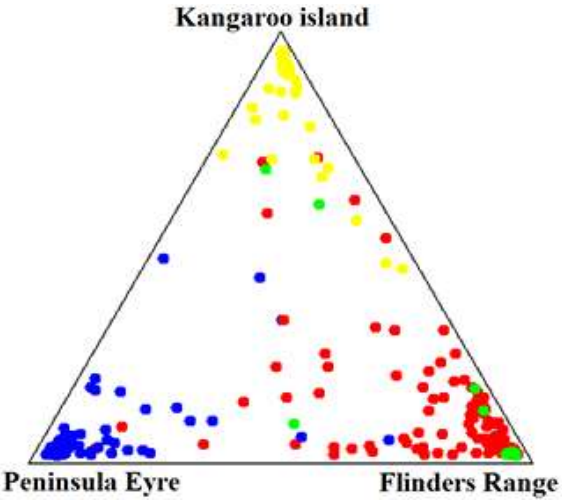

Figure 1. Bayesian clustering approach showing three groups genetically differentiated, according to the geographical origin of *E. cladocalyx* in Australia. The blue dots correspond to the populations from Marble Range and Cowell, the yellow dots correspond to the population from Flinders Chase, the red dots correspond to the populations from Wirrabara and Remarkable, and the green dots correspond to the local population taken from Illapel, Chile.

Table 3. Bayesian point estimation (mode, median, and mean) and 90% credible regions of *Fst* calculated in each group genetically differentiated of *E. cladocalyx*.

Cluster	Individuals	Mean	Median	Mode *	SD	90% Credible Sets	
						Lower	Upper
I	48	0.117	0.116	0.115	0.016	0.092	0.144
II	72	0.072	0.071	0.070	0.015	0.049	0.098
III	125	0.184	0.184	0.182	0.025	0.144	0.217

* Kernel density estimates of the mode from marginal posterior distributions.

3.3. Bayesian Association Mapping and Pleiotropy

A total of 13 putative associations, comprising 9 SSR markers, were identified for wood density and slenderness coefficient by the association mapping based on a unified mixed model (frequentist) (Table 4) [47]. Six loci were associated with WD, where the loci *qtl*WD130 located on chromosome 4 at 52.1 cM, explained the highest proportion of the phenotypic variance (17%). Similarly, seven markers were associated with SC, and the greater proportion of the phenotypic variance was explained by the loci *qtl*SR56 (21%). Four of these loci showed significant associations with WD and SC concomitantly. This suggests a common genetic control between the traits, which could result in possible pleiotropic effects for these loci. On the other hand, less loci were identified in the Bayesian association mapping (four and three loci associated with WD and SC, respectively), which presented a moderate (3 < BF <10) to extremely strong (100 < BF) evidence of association (model M1) (Figure 2 and Table 4), according to the scale presented by Andraszewicz et al. [48]. The posterior likelihood of association (PPA) indicated that loci with moderate evidence of association (3 < BF <10) have a PPA of less than 0.35, while loci with strong evidence of association (10 < BF < 30) showed a PPA between 0.35 and 0.61. Furthermore, loci with extremely strong evidence (100 > BF) have PPA values greater than 0.84 (Table 4).

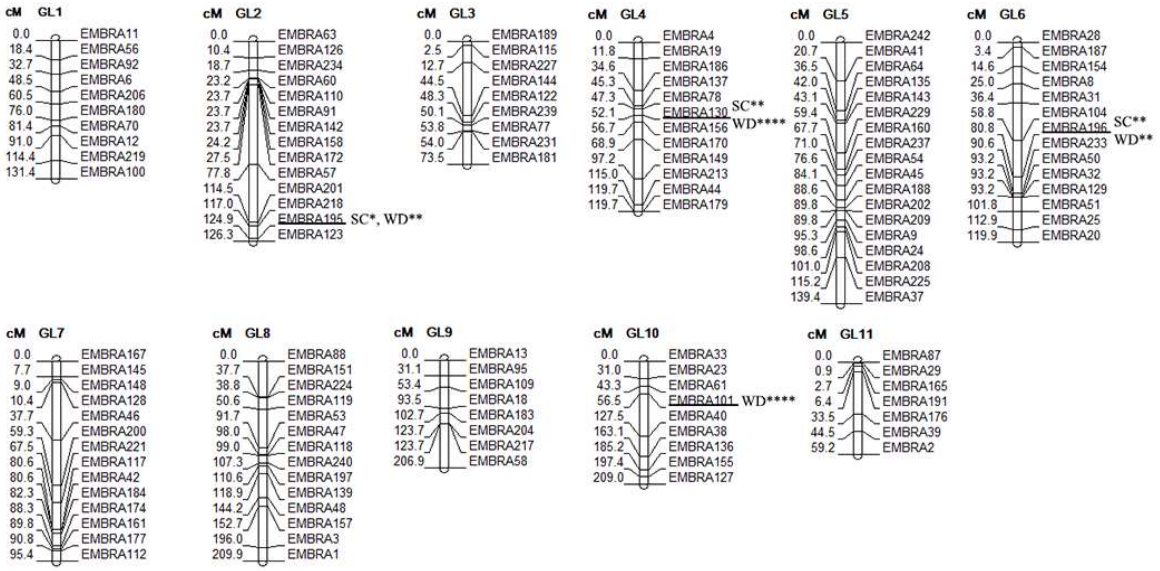

Figure 2. Loci associated with wood density (WD) and slenderness coefficient (SC) across the genome of *E. cladocalyx* (Bayesian multivariate mapping). To the left the distance in centiMorgan (cM) and to the right the name of the molecular markers (SSR, n = 130). LG: Linkage Group. * moderate evidence of association (3 < BF < 10); ** strong evidence of association (10 < BF < 30); ** very strong evidence of association (30 < BF < 100). **** extreme evidence of association (100 < BF). BF: Bayes Factor.

Table 4. Summary of loci associated with wood density (WD) and slenderness coefficient (SC) using a unified mixed linear model and Bayesian regression analysis, measured in 17-year-old trees of *E. cladocalyx* in arid conditions.

Traits	Locus	LG	Position (cM)	PV (%)	BF	PO	PPA
WD	*qtl*WD101 *	10	56.5	16.4	1,525,001	80,263	1.000
	*qtl*WD130 *	4	52.1	16.7	1,055	55.55	0.982
	*qtl*WD18	9	93.5	5.2	2.10	0.110	0.099
	*qtl*WD195 *	2	124.9	7.6	15.90	0.837	0.456
	*qtl*WD50	6	93.2	14.5	1.16	0.061	0.057
	*qtl*WD196 *	6	80.8	8.0	15.80	0.832	0.454
SC	*qtl*SC50	6	93.2	18.2	0.91	0.048	0.046
	*qtl*SC195 *	2	124.9	9.2	8.11	0.427	0.299
	*qtl*SC161	7	89.8	5.3	1.26	0.066	0.062
	*qtl*SC56	1	18.4	20.6	0.59	0.031	0.030
	*qtl*SC196 *	6	80.8	7.5	17.53	0.923	0.480
	*qtl*SC130 *	4	52.1	12.8	24.17	1.272	0.560
	*qtl*SC218	2	117	6.1	0.97	0.051	0.048
Pleiotropic loci	*qtl*WD130/*qtl*SC130 *	4	52.1	16.7/12.8	1911	100.58	0.990
	*qtl*WD195/*qtl*SC195 *	2	124.9	7.6/9.2	15.44	0.812	0.448
	*qtl*WD/*qtl*SC50	6	93.2	14.5/18.2	1.30	0.068	0.064
	*qtl*WD196/*qtl*SC196 *	6	80.8	8.0/7.5	24.01	1.264	0.558

PV: Proportion of phenotypic variance explained by each QTL. LG: Linkage group number. BF: Bayes Factor. PO: Posterior Odds. PPA: Posterior Probability of Association. * Loci identified through Bayesian regression analysis.

Three QTL associated with both traits were detected through a multivariate Bayesian analysis [46] on LG2 at 125 cM, on LG4 at 52 cM, and on LG6 at 81 cM. The bi-trait QTL analysis indicated that these loci presented a BF > 15, indicating that the evidence of association is between the categories of strong and extremely strong association. Furthermore, these loci have a PPA of 0.45 to 0.99, indicating a high probability of association (Table 4).

4. Discussion

4.1. Phenotypic Variability and Genetic Parameters

According to the results, wood density (WD) and the slenderness coefficient (SC) varied significantly among the provenances of *E. cladocalyx* evaluated in this study. The morphological variability of *E. cladocalyx* (such as stem straightness, flowering intensity, diameter and tree height) has been previously reported in other studies [49–51]. For example, Mora et al. [49] and Vargas-Reeve et al. [51] determined that trees from the Flinders Range have a diameter of the stem larger than trees from other natural regions of the species. In general, a larger diameter correlates negatively with SC in trees, in which it has been observed that trees with a greater diameter have a lower SC [28,52,53]. Consistently, the trees from Wirrabara and Mount Remarkable showed the lowest SC values, while the trees from Cowell were the slenderest. A high SC value indicates that trees are more susceptible to damage by mechanical pressures [29]. According to Navratil [54], trees with a SC greater than 100 have a high probability of suffering from wind damage. In this sense, the individuals of the Flinders Mountain Range would present a greater mechanical resistance than the trees from the Eyre Peninsula. Interestingly, the Flinders Chase, Cowell and Marble Range populations had the lowest penetration values of pilodyn (WD), which indicates that these trees have a higher wood density than those from the Flinders Range. It should be noted that Bush et al. [6] reported that trees from the Eyre peninsula have a lower wood density (based on the water displacement method, TAPPI [55]) than trees that come from the Flinders Mountain Range and Kangaroo Island. Moreover, these authors also demonstrated that there is a negative correlation between wood density and tree diameter in *E. cladocalyx*. The variability observed among the populations for WD and SC, had similarities with the

natural distribution of the same. Mora et al. [49] indicate that in genetically differentiated groups a reduction of genetic variability is observed. This presents evidence that the location of origin of an individual influenced the expression of their traits. Moreover, in WD the populations belonging to Eyre Peninsula and Kangaroo Island regions did not present significant differences between them (Table 1), indicating a possible genetic flow between these regions that is not shared with the Flinders Range region.

According to REML and Bayesian estimates, the heritability for WD and SC was moderate and high, respectively, indicating that the phenotypic variability observed between provenances for these traits is explained by an important additive genetic component. Bush et al. [6] determined that wood density in *E. cladocalyx* presents a narrow-sense heritability of 0.39, which could be considered moderate to high, while in *E. globulus*, Stackpole et al. [25] determined that wood density is a highly heritable trait, with a value of $h^2 = 0.51$. Our results are subtly lower than in previous studies in *E. cladocalyx* and in other *Eucalyptus* species. In general, the values of heritability, both broad and narrow, are comparatively lower in environments with low water availability [56–58], which would confirm the differences between our results and previous studies.

The SC is not a trait usually evaluated in the genetic studies of *Eucalyptus*. However, it has been determined that this trait has moderate to high heritability in other species. For example, Pastorino et al. [33] determined that the heritability of SC in cypress is 0.15, while Chaendaekattu and Mydin [34] reported a heritability for SC between 0.25 and 0.43 in trees of *Hevea brasiliensis*. It should be noted that the heritability for stem diameter and the height in trees of *E. cladocalyx*, variables related to SC, is low to moderate [49,59], however, the results of the present study suggest that SC is a more inheritable trait than height and diameter in *E. cladocalyx*.

The WD and SC presented a negative genetic correlation. Slender shafts tend to be denser [60], therefore, the degree of penetration of Pilodyn in the stem is expected to be lower in slender trees. A greater Pilodyn penetration implies that the tree is less dense, therefore, the correlation between the density and slenderness of a tree is expected to be negative.

4.2. Genetic Structure

The population of *E. cladocalyx* evaluated in this study is composed of three genetically differentiated groups, which is consistent with the findings of McDonald et al. [61], Mora et al. [37], and Arriagada et al. [2]. The individuals were grouped according to the three geographical regions from which the evaluated populations were taken (Flinders Range, Eyre Peninsula, and Kangaroo Island). In addition, between the determined genetic groups, there is a significant and moderate differentiation according to the values of Fst, which is consistent with previous studies in *E. cladocalyx* [61–63].

The effect of the genetic structure of the population on the analysis of association and detection of QTLs has been widely discussed [64,65]. Particularly, the presence of a significant genetic structure, and inadequate information on kinship among individuals can generate false marker-trait associations [66]. For all QTLs analyses, a full model considering the genetic structure effects was contrasted with a null model not including these effects. The genetic structure effect was significant for all association analyses; therefore, it was used for the detection of QTLs in this study [47]. In several plant species, the effect of the structure and the degree of kinship in the association studies has been evaluated [65,67].

4.3. Bayesian Association Mapping and Pleiotropy

Wood density is one of the most important indicators of sawn wood quality and pulp yield [24]. Several studies have shown that density has a moderate to high genetic control in different species of *Eucalyptus* [25,26], which suggests that this trait could be subject to genetic improvement through selection assisted by molecular markers. In this context, a significant number of studies have been conducted to identify markers associated with wood density in *Eucalyptus*. For example, Bundock et al. [68] detected QTLs associated with wood density in *E. globulus*, on chromosomes 6 and 11, which explained between 8% and 12% of the phenotypic variation, respectively. Thumma et al. [19]

identified QTLs that explained between 3.6% and 5.6% of the variation in wood density of *E. nitens*, using RFLP and SSR markers. These findings are concordant with our results. Some of the QTLs detected in this study explained about 16% of the phenotypic variation of wood density in *E. cladocalyx* (*qtl*WD130 and *qtl*WD101), which can be used as selection criteria for higher trees for this phenotype.

In the present study, QTL *qtl*WD195, located on chromosome 2 at a distance of 124.9 cM, explained 7% of the wood density variation of *E. cladocalyx*. Similarly, in the same chromosome, Gion et al. [18] reported two QTLs, at 123 cM and 124 cM, associated with wood density in hybrids of *E. urophylla* and *E. grandis*, explaining up to 10% of the phenotypic variation. Similarly, the QTL *qtl*WD101, identified on chromosome 10 at 56.5 cM, is consistent with a region indicated on the same chromosome by Freeman et al. [69] in *E. globulus*, which was also stable in several populations and sites. Furthermore, in this same chromosomal region, Hamilton et al. [70] identified QTLs associated with acoustic wave velocity, an indirect measure of wood density in *E. globulus*. According to our results and previous studies, chromosome 10 could be strongly involved in the genetic control of wood density in *E. cladocalyx* and other *Eucalyptus* species.

The SC has been widely used to measure the stability and resistance of the tree to the mechanical pressures of the environment. Moreover, like wood density, the slenderness coefficient is used to evaluate the potential of a tree for the production of paper and sawn wood [28,71]. Another important finding of the bi-trait Bayesian analysis was the existence of three large-effect pleiotropic QTLs located on LG4 at 52 cM, on LG2 at 125 cM, and on LG6 at 81 cM, indicating that both traits are genetically related.

The detection of QTL for the SC has been poorly addressed. However, the results of the present study can be contrasted with the mapping of previously reported QTLs for tree diameter and height variables, since both traits determine the slenderness of a tree. Freeman et al. [69] detected a QTL on chromosome 4 associated with the diameter at breast height (DBH) in *E. globulus*, whose position is close to *qtl*SC130, reported in the present study.

According to Stephens and Balding [45], the BF is comparable to the likelihood ratio, but compares two different models instead of two parameter values in a model. BF is often used as an indicator of the evidence of association of a locus to a trait, in which the moderate or strong categories are considered as definitive evidence against the null model (M0). According to this principle, PPA has been used as a probabilistic measure of posterior evidence, which combines the previous probability of association (π) and the evidence of association in the observed data (BF). Because π is small, the BF must be large to provide convincing evidence of an association.

We determined that a locus with a BF > 8 delivers a moderate evidence to determine that the hypothesis of association with the given trait is more likely than the null hypothesis of non-association. The unified mixed model indicated that 13 loci showed a significant association with traits. However, only six of these loci presented strong evidence of association (BF > 15 and PPA > 0.44) through the Bayesian regression analysis. Additionally, the Bayesian bi-trait regression (pleiotropy analysis) indicated that three of the four loci identified through the unified mixed model presented strong evidence of association with WD and SC. This provides convincing evidence that the loci *qtl*WD130/*qtl*SC130, *qtl*WD195/*qtl*SC195 and *qtl*WD196/*qtl*SC196, have a significant pleiotropic effect, being associated with both traits under study.

5. Conclusions

E. cladocalyx has a recognized potential to produce structural timber under arid environmental conditions. According to our results, wood density and the slenderness of the trees of *E. cladocalyx* presented a moderate and high genetic control, respectively. Several of the associations found for wood density were located in chromosomal regions previously reported for this trait in other *Eucalyptus* species, revealing collinearity of QTLs. In the context of marker-assisted selection, the QTLs associated with WD (4) and SC (2) can be used as selection criteria in breeding programs for the species under arid conditions. The QTLs identified in this study explained 48% and 20% of the observed genetic variance

in WD and SC, respectively. This may be an indication that the coverage of markers in insufficient. Thus, it would be appropriate to include a greater amount of markers in future studies.

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