Stable marker-trait associations for growth across different ages in *Eucalyptus cladocalyx* through the use of microsatellitesAssociações genéticas do crescimento de *Eucalyptus cladocalyx* usando marcadores microssatélites, evidenciam estabilidade a diferentes idadesCarlos Maldonado¹, Rodrigo Ivan Contreras-Soto², Ismael Fernando Schegoscheski Gerhardt³, Antônio Teixeira do Amaral Júnior⁴ e Freddy Mora⁵

Resumo

A estabilidade de loci de características quantitativas em diferentes idades é de particular interesse nos programas de melhoramento de árvores. O objetivo deste trabalho foi identificar associações genéticas para o crescimento, em 47 famílias de meios-irmãos de *Eucalyptus cladocalyx*, medido em diferentes idades, usando marcadores microssatélites. A altura total da árvore (H) foi medida aos 3, 9, 10 e 13 anos (H03, H09, H10 e H13, respectivamente), enquanto o diâmetro à altura do peito (DBH) foi medido aos 9, 10 e 13 anos (DBH09, DBH10 e DBH13, respectivamente). Baseado nas análises de modelos mistos (REML/BLUP), os caracteres de crescimento apresentaram herdabilidade moderada variando de $h^2 = 0,15$ a $h^2 = 0,29$ para H, e de $h^2 = 0,16$ a $h^2 = 0,22$ para o DBH. O marcador EMBRA208 foi estatisticamente consistente em todas as idades avaliadas para altura, e os marcadores EMBRA191, EMBRA208 e EMBRA32 para o DBH, sugerindo uma alta estabilidade das associações nas diferentes idades avaliadas. Esses resultados têm implicações práticas importantes para a seleção assistida por marcadores.

Palavras-chave: marcadores EMBRA, condições áridas, ampliações de espécies cruzadas.

Abstract

Stability of quantitative trait loci (QTL) across different ages is of particular interest in tree breeding programs. This study aimed to identify stable marker-trait associations for growth in 47 half-sib families of *Eucalyptus cladocalyx* across different ages using microsatellite markers. Total tree height (H) was measured at ages 3, 9, 10 and 13 years (H03, H09, H10 and H13, respectively), while diameter at breast height (DBH) was measured at ages 9, 10 and 13 years (DBH09, DBH10 and DBH13, respectively). Based on a mixed modeling approach (REML/BLUP), growth traits were found to be moderately heritable ranging from $h^2 = 0.15$ to $h^2 = 0.29$ for H, and from $h^2 = 0.16$ to $h^2 = 0.22$ for DBH. The marker EMBRA208 was statistically consistent at all ages studied for tree height, and the markers EMBRA191, EMBRA208 and EMBRA32 for DBH, indicating high stability across different ages of *E. cladocalyx*. These results have important practical implications for marker-assisted selection.

Keywords: EMBRA markers, arid conditions, cross-species amplification, mixed models.

INTRODUCTION

The genus *Eucalyptus* consists of approximately 700 species and subspecies, and represents the most planted trees worldwide. In the forest industry, this tree genus is one of the most important and studied in the world (MORA; ARRIAGADA, 2016; SCHMIT et al., 2015). Some species present high adaptation to various ecosystems from tropical regions to arid areas with prolonged dry periods. In particular, *Eucalyptus cladocalyx* represents a species that grows naturally in the semi-arid zone of South Australia, with a discontinuous distribution across three regions: Flinders Ranges,

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Eyre Peninsula and Kangaroo Island. This species has been studied for multiple applications such as honey production, timber resources and environmental (BUSH et al., 2011; BALLESTA et al., 2015). In this context, and considering its ability to grow in dry land conditions, breeding programs have been undertaken in Australia and Chile focusing to improve key economic traits such as timber and poles as well as wood density (BALLESTA et al., 2015; BUSH et al., 2011; MORA et al., 2016a; VARGAS-REEVE et al., 2013).

In tree breeding programs there has been a considerable interest in the use of molecular information as marker-assisted selection (MAS), which involves selection of specific traits based on the use of molecular marker instead of the traditional phenotypic selection (FREEMAN et al., 2013). A main DNA marker used in MAS is called microsatellite or simple sequence repeats (SSR), as they are highly polymorphic (multi alleles), co-dominants, highly reliable and transferable across species (BRONDANI et al., 2006; COLLARD; MACKILL, 2008). In the genus *Eucalyptus* the availability of a robust genetic map with highly transferable SSR markers has been proposed as an effective tool for the advance of genomic undertakings including quantitative trait loci (QTL) validation across pedigrees, co-localization of QTL and candidate genes for guiding association mapping experiments and eventually improves MAS (BRONDANI et al., 2006). In addition, the importance of SSR for the *Eucalyptus* genome analysis has been highlighted and applied in different species such as *E. grandis* × *E. urophylla* (BRONDANI et al., 2006), *E. camaldulensis* (SILVA et al., 2009) and *E. globulus* (FREEMAN et al., 2013).

Among the proposed techniques to improve MAS are marker-trait associations, which may identify QTL used as direct tool for early selection (MORA et al., 2016a). However, the potential use of MAS may be questioned considering that QTL effects may vary during the tree growth stages and/or across environments. Consequently, the QTL stability in different genetic backgrounds considering time and space is essential for assisted selection. Therefore, for the effective use of QTL in crop/tree breeding, it requires to be stable across time and space (BUNDOCK et al., 2008). Several studies have described QTL stability, particularly with respect to time. For example, Verhaegen et al. (1997) investigated the stability of growth and wood density over three different ages in a F1 population of *E. grandis* × *E. urophylla*; moreover Bundock et al. (2008) examined *E. globulus* for 6 years and found QTL associated to height and diameter at breast height (DBH). Verhaegen et al. (1997) further indicates that repeat measures on the same individuals for different ages improve the statistical power for QTL detection.

In the last few years a big number of studies have considered the use of mixed linear modeling (MLM) approach for QTL analysis in *Eucalyptus* (CAPPA et al., 2013; FREEMAN et al., 2009; SCHMIT et al., 2015). This model combines both fixed and random effects to adjust association tests with molecular markers (MORA et al., 2016b). Population structure and kinship among individuals are the major confounding effects when marker trait association is implemented; in fact, they are included into the MLM to reduce spurious associations or to minimize false positives rates (YU et al., 2006). An additional advantage of MLM is their use to fit the complexity of phenotypic longitudinal or repeated measures data sets into the tree breeding programs (SCHMIT et al., 2015). The implementation of molecular marker-assisted selection in genetic improvement of forest tree species, presents challenges related to the long generation times and poor juvenile/mature trait correlations (FREEMAN et al., 2013). The traits of interest in tree breeding programs (e.g. tree height or diameter at breast height) can be expressed more than once in each individual to generate longitudinal data or repeated measures over time (SCHMIT et al., 2015), allowing the identification of QTL at different ages. However, not all loci present stability over time, which can be regarded as suitable for specific years, limiting selection process. The use of repeated measures over time could provide a more realistic search for QTL throughout the genome, allowing the identification of putative stable QTL. Therefore, information on QTL stability is essential for efficient MAS, being particularly beneficial in early selection programs of forest species (BUNDOCK et al., 2007; FREEMAN et al., 2013). Thus, this study aimed to identify stable marker-trait associations for growth (height and diameter at breast height) in 47 half-sib families of *Eucalyptus cladocalyx* across different ages, using microsatellite markers from *Eucalyptus* spp.

MATERIALS AND METHODS

Plant material and phenotypic data

The longitudinal stability analysis of QTL was carried out using a progeny trial of *E. cladocalyx*, which was hand-planted in 2001 in the Choapa Province, Chilean administrative region of Coquimbo (31° 40' S Latitude; 71° 14' W Longitude; 297 m altitude, mean annual rainfall of around 200 mm, and 15°C mean annual temperature). The trial was arranged according to a randomized complete block design with 47 half-sib families, 30 blocks and single-tree plots. Further information about the progeny trial can be obtained from Ballesta et al. (2015). Total tree height (H) was measured at ages 3, 9, 10 and 13 years (H03, H09, H10 and H13, respectively), while diameter at breast height (DBH) was measured at ages 9, 10 and 13 years (DBH09, DBH10 and DBH13, respectively). DBH at age 3 was not included because the trees were not high enough to measure them. Variance components and heritability were estimated using the following mixed model:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{g} + \boldsymbol{\varepsilon}$$

where $\mathbf{y}$ is the vector of observed responses for each growth trait in a determined year of measurement; $\mathbf{X}$ and $\mathbf{Z}$ are known incidence matrices relating $\mathbf{y}$ to $\boldsymbol{\beta}$ and $\mathbf{g}$, respectively; $\boldsymbol{\beta}$ is the vector with the block effect; $\mathbf{g}$ is the family effect vector; $\boldsymbol{\varepsilon}$ is the residual or within-plot error. In the statistical analysis per year: Variance ($\mathbf{g}$) = $\mathbf{G} = \mathbf{I}\sigma_f^2$ and Variance ($\boldsymbol{\varepsilon}$) = $\mathbf{R} = \mathbf{I}\sigma_e^2$, where $\mathbf{I}$ is an identity matrix with dimension equal to number of records; σ_f^2 and σ_e^2 are the family and residual variances, respectively. Variance components were estimated by using the Restricted Maximum Likelihood (REML) method computed in the SAS software by the MIXED procedure (SAS INSTITUTE INC., 2007).

The Bayesian Information Criterion (BIC) was used to confirm the significance of the random family effect on all of the phenotypic traits. The narrow-sense heritability (h^2) of growth traits, was estimated by the following expression:

$$h^2 = \frac{4\sigma_f^2}{\sigma_f^2 + \sigma_e^2}$$

The longitudinal analysis was performed using the following statistical model:

$$\mathbf{y}_i = \mathbf{X}\boldsymbol{\beta}_i + \mathbf{Z}_1\mathbf{g}_i + \mathbf{Z}_2\mathbf{w}_i + \boldsymbol{\varepsilon}_i$$

where i represents the years of measurement, $\mathbf{y}_i$ is the vector of observed responses for each growth trait measured in the i -th year; $\mathbf{X}$, $\mathbf{Z}_1$ and $\mathbf{Z}_2$ are known incidence matrices relating $\mathbf{y}_i$ to $\boldsymbol{\beta}_i$, $\mathbf{g}_i$ and $\mathbf{w}_i$, respectively; $\boldsymbol{\beta}_i$ is the vector with the block effect and years of measurement; $\mathbf{g}_i$ is the family effect vector; $\boldsymbol{\varepsilon}_i$ is the residual or within-plot error. The following variance-covariance structures (estimated using REML) were tested using the REPEATED statement of MIXED procedure (SAS INSTITUTE INC. 2007; SCHMIT et al., 2015): Variance Components (VC), Compound Symmetry (CS), Unstructured (UN), Banded Main Diagonal (UN(1)) and First-Order Autoregressive (AR(1)) (SAS INSTITUTE INC. 2007).

DNA extraction and genotyping

Leaf tissues were collected from 13-year-old individual trees and stored at -20°C until further use. Total genomic DNA was extracted using the method CTAB for plant tissues described by Silva (2010) with minor modifications. Integrity and concentration of the DNAs was visualized on 0.8% agarose gels and compared with standard markers, ProMega-Marker 50–8000 kb Lambda Ladder (Promega, Madison, WI, USA). Ten SSR markers distributed on five linkage groups of *Eucalyptus* (LG5, LG6, LG9, LG10 and LG11, BRONDANI et al., 2006) were selected for the marker-trait association analysis. The molecular markers were selected because they were found to be associated with growth traits in other species of *Eucalyptus* (BRONDANI et al., 1998, 2002, 2006, FREEMAN et al., 2009, 2013, KULLAN et al., 2012).

The PCR amplifications were performed in a 20 µL reaction volume containing 2 µL of template DNA (diluted in 1:100), 1X Green GoTaq reaction PCR Buffer (Promega, Madison, WI, USA), 2.5 mM MgCl₂, 0.4 mM dNTPs, 100 nM of each primer and 0.3 U Taq DNA polymerase (Promega, Madison, WI, USA). Specific annealing temperatures were carried out as described by Brondani et al. (2006) (Table 1). The PCR products were separated on 10% (w/v) denaturing polyacrylamide gel

in a run with 1X TBE at 80v for 18h, and stained with Red gel nucleic acid stain (Biotium, CA, USA). The sizes of products were estimated with a low range DNA ladder (Thermo Scientific, Lithuania, EU) as a molecular weight reference.

Table 1. Microsatellite primers used for the marker-trait association analysis of growth in *Eucalyptus cladocalyx* at different ages.

Tabela 1. Marcadores microssatélites utilizados na análise de associação genética do crescimento em *Eucalyptus cladocalyx* medido a diferentes idades.

SSR	Primer	Sequence	Annealing temperature (°C)	Expected size (bp)	Linkage group
EMBRA33	Forward Reverse	CAATTTGCATGTCCAGTTTG GCAGAAGTTGATTGAAAGCA	58	122	10
EMBRA61	Forward Reverse	GGCATAACGAGTTGTTCT AGAGTATAATCAGCGCCT	58	186	10
EMBRA37	Forward Reverse	CACCTCTCCAACTACACAA CTCCTCTCTCTCACCATTTC	56	124	5
EMBRA208	Forward Reverse	AAGCTATTGGTGTCCCGAA TATGTATGCGATGTTGGTGT	58	116	5
EMBRA204	Forward Reverse	CTCGTGTGGTTATGTGAAC GCTTGTCTACTATGCACATGA	56	147	9
EMBRA18	Forward Reverse	CAGCTAGGATGTTAGACTTGG GCACACCTAGAATTTTCAAACCTA	56	87	9
EMBRA32	Forward Reverse	ATCAGCCTCCACGTTCTA GGAGAAGGAAGGTGTATCAA	56	65	6
EMBRA104	Forward Reverse	CACCGATCTCCACTATGC TTTTTTCAAGGCAAAAGTG	60	99	6
EMBRA39	Forward Reverse	GCATTCGTACTCATTTTCAA GCATCGAGAGTGGATTAGTT	54	146	11
EMBRA191	Forward Reverse	GATCCAGCTGTGTACATC AGGTGTGTCTCTCTGTTG	56	207	11

Population structure

The Hardy-Weinberg equilibrium (HWE) analysis was performed at each locus using the Markov chain Monte Carlo (MCMC) method implemented in GENETPOP 4.3 (ROUSSET, 2008). The population structure was inferred using the Bayesian model implemented in the software STRUCTURE 2.3.4 (PRITCHARD et al., 2000). The no-admixture model was employed to analyze the data set with prior population information. Six runs were performed to setting number of clusters (K), hypothesizing that the optimum K would vary between 1 and 6. The K values were estimated with 20 independent runs of MCMC sampling for each possible K value, with a burn-in of 5000 and 50000 iterations of Gibbs chains. The number of K groups was estimated using the method described by Evanno et al. (2005), with the mean likelihood L(K) over 20 runs for each K. After determining the most likely K value, a new analysis was performed with a burn-in period of 100000, 1 million MCMC iterations and step of 100.

Identification of stable marker-trait associations

Marker-trait associations were performed for each combination of traits under study on the selected markers using a Mixed Linear Model (MLM) that includes the genetic marker, kinship matrix and population structure (YU et al., 2006). This analysis was carried using TASSEL software Version 5.0 (BRADBURY et al., 2007). This mixed model includes both fixed and random effects. Incorporating random effects gives MLM the ability to aggregate information about relationships between individuals. The statistical model can be described as follows:

$$\mathbf{y}_b = \mathbf{S}\mathbf{a} + \mathbf{Q}\mathbf{v} + \mathbf{Z}\mathbf{\mu} + \mathbf{\varepsilon}$$

where $\mathbf{y}_b$ is a vof the predicted genotypic values of the individuals obtained from the phenotypic analyses (using the BLUP/REML procedure), $\mathbf{a}$ is a vector of molecular markers (fixed), $\mathbf{v}$ is a vector of the population structure effect (fixed), $\mathbf{\mu}$ is a vector of polygene background effects (random) and $\mathbf{\varepsilon}$ a vector of residual effects. $\mathbf{S}$, $\mathbf{Q}$ and $\mathbf{Z}$ are incidence matrices relating $\mathbf{y}$ to $\mathbf{a}$, $\mathbf{v}$ and $\mathbf{\mu}$, respectively. A kinship matrix was included in the above mixed model, which was estimated using the software TASSEL to define the degree of genetic covariance between each pair of individual trees.

RESULTS

Significant additive genetic variation (or family effects) was observed. According to the Bayesian information criterion (BIC) there was very strong evidence against the reduced models where the additive effects are assumed to be zero (i.e. $\sigma_a^2 = 0$) for height and diameter ($\Delta \text{BIC} > 10$, Table 2). By choosing the fitted candidate model corresponding to the minimum value of BIC, one is attempting to select the candidate model corresponding to the highest Bayesian posterior probability (NEATH; CAVANAUGH, 2012). The banded main diagonal structure (UN(1)) was selected as the best variance-covariance structure according to all information criteria used (Table 3). Based on REML analyses, growth traits were found to be moderately heritable ranging from $h^2 = 0.15$ to $h^2 = 0.29$ for tree height at ages 9 and 3 years, respectively, and for DBH ranging from $h^2 = 0.16$ to $h^2 = 0.22$ at age 10 and 13, respectively (Table 2). Correlation coefficients among best linear unbiased prediction values (at family level) are shown in Table 4. All correlation coefficients were statistically different from zero at the 0.01 probability level.

Table 2. Variance components and heritability for growth in half-sib families of *Eucalyptus cladocalyx* grown under arid conditions.

Tabela 2. Componentes de variância e herdabilidade do crescimento em famílias de meios-irmãos de *Eucalyptus cladocalyx* avaliadas sob condições áridas.

	DBH9	DBH10	DBH13	H03	H9	H10	H13
-2 RLL	4844	5012	4673	13194	13741	13159	12403
Delta BIC	12.4	12.2	19.3	36	11.1	10.1	29.5
$\hat{\sigma}_f^2$	0.217	0.206	0.280	231	634	698	1313
$\hat{\sigma}_e^2$	4.919	4.948	4.899	3003	16361	17902	17541
h^2	0.169	0.160	0.216	0.286	0.149	0.150	0.279

DBH9, DBH10 and DBH13 are diameter at breast height at age 9, 10 and 13 respectively; H03, H9, H10 and H13 are total tree height at age 3, 9, 10 and 13 respectively; BIC: Bayesian information criterion. RLL: restricted maximum log likelihood; $\hat{\sigma}_f^2$: family variance; $\hat{\sigma}_e^2$: residual variance; h^2 : heritability (*stricto sensu*).

Table 3. Variance-covariance structures used for longitudinal data analyses in *Eucalyptus cladocalyx*.

Tabela 3. Estruturas de variâncias e covariâncias usadas na análise longitudinal em *Eucalyptus cladocalyx*.

IC	Total Tree Height					Diameter at Breast height				
	VC	CS	UN	UN(1)	AR(1)	VC	CS	UN	UN(1)	AR(1)
AIC	266.6	268.6	IL	204.1	242.8	147.9	149.9	110.1	104.1	141.7
AICC	266.7	268.7	IL	204.7	242.9	148.0	150.1	111.5	104.5	141.9
BIC	268.5	272.4	IL	213.6	246.6	149.8	153.7	121.5	109.8	145.5

IC: Information criteria; AIC: Akaike's information criterion; AICC: Akaike's information criterion corrected; BIC: Bayesian information criterion. VC: variance components; CS: compound symmetry; UN: Unstructured; UN(1): banded main diagonal; AR(1): first-order autoregressive; IL: Infinite likelihood (LITTELL et al., 2002).

Table 4. Correlation coefficients among best linear unbiased prediction values (family level).

Tabela 4. Coeficientes de correlação entre os valores da melhor predição linear não viesada (nível familiar).

Traits	H03	H9	H10	H13	DBH9	DBH10	DBH13
H03	1						
H9	0.57**	1					
H10	0.56**	0.92**	1				
H13	0.58**	0.7**	0.72**	1			
DBH9	0.68**	0.84**	0.81**	0.57**	1		
DBH10	0.66**	0.78**	0.77**	0.5**	0.95**	1	
DBH13	0.72**	0.67**	0.7**	0.7**	0.8**	0.81**	1

** Significant at the 0.01 probability level.

In the present study, population structure of half-sib families from different regions was investigated using a Bayesian clustering approach. According to the structure analysis, the most probable number of groups genetically differentiated is three ($K=3$; Figure 1). The marker-trait associations (performed using a mixed model with genetic structure and kinship matrix) detected for growth traits are summarized in Table 5, which accounted for 10.8 to 42.6% of the phenotypic variation. Of the ten primers considered in this study, four were associated with both growth traits (with a significance level of 5%), across the different ages evaluated. The analysis of repeated measurements

over time or age showed that the markers EMBRA208, EMBRA191 and EMBRA32 were statistically significant across time for DBH, and the marker EMBRA208 for total tree height.

Figure 1. Probabilities of membership (genetic structure) for half-sib families of *Eucalyptus cladocalyx* assessed using SSR markers.

Figura 1. Probabilidades de formação de grupos geneticamente diferenciados (estrutura populacional) em famílias de meios-irmãos de *Eucalyptus cladocalyx* avaliadas usando marcadores SSR.

Table 5. Summary of marker-trait associations ($p < 0.05$) detected for tree height (H03, H9, H10 and H13 at age 3, 9, 10 and 13, respectively), and diameter at breast height (DBH9, DBH10 and DBH13 at age 9, 10 and 13, respectively) in *Eucalyptus cladocalyx*.

Tabela 5. Resumo das associações genéticas ($p < 0,05$) encontradas para altura das árvores (H03, H9, H10 e H13 aos 3, 9, 10 e 13 anos, respectivamente) e diâmetro à altura do peito (DBH9, DBH10 e DBH13 aos 9, 10 e 13 anos, respectivamente) em *Eucalyptus cladocalyx*.

Trait	SSR marker	F	p	PV(%)
H03	EMBRA208	7.465	0.010	22.9
H9	EMBRA208	5.715	0.023	20.3
H10	EMBRA208	6.021	0.020	25.3
H13	EMBRA191	9.140	0.005	28.8
H13	EMBRA208	6.949	0.013	24.9
HL	EMBRA208	7.423	0.010	26.2
DBH9	EMBRA208	4.666	0.038	13.6
DBH9	EMBRA32	8.401	0.007	30.5
DBH9	EMBRA191	5.259	0.029	13.4
DBH10	EMBRA32	7.881	0.010	42.6
DBH10	EMBRA191	6.457	0.019	22.0
DBH13	EMBRA208	4.283	0.046	13.9
DBH13	EMBRA32	8.622	0.006	33.2
DBH13	EMBRA191	4.603	0.040	11.3
DAPL	EMBRA208	5.704	0.023	16.5
DAPL	EMBRA32	8.217	0.007	27.5
DAPL	EMBRA191	5.691	0.023	14.2

HL and DAPL = longitudinal analysis by mixed model with repeated measure in SAS; PV(%): phenotypic variation explained by markers.

DISCUSSION

Several studies of forest trees have addressed the stability of QTL, particularly for growth in *Eucalyptus* (BUNDOCK et al., 2008; THUMMA et al., 2010; VERHAEGEN et al., 1997). For instance, Verhaegen et al. (1997) reported putative QTL for height and diameter at breast height at 18, 26 and 38 months in an *E. grandis* x *E. urophylla* F1 cross. Of this, fifteen percent were stable across years. Similarly, Bundock et al. (2008) detected QTL with slight evidence for the first measurements of height growth at year 1 and diameter at year 2 in *E. globulus*, and showed clear evidence for later expression of this QTL at year 6. These reports are similar with our result for DBH and height in *E. cladocalyx*. In the present study, the marker EMBRA208 was statistically consistent at all ages studied for tree height, and the markers EMBRA191, EMBRA208 and EMBRA32 for DBH, indicating a putative stable QTL for height and DBH of *E. cladocalyx*. Moreover, the analysis of repeated measurements over age determined that the EMBRA208 marker had a stable association with height. On the other hand, EMBRA32, EMBRA191 and EMBRA208 also showed stability across age for DBH. This result indicates that these markers not only present a significant association in each age, but they also proved to be significant in the analysis of repeated measurements (Table 5). Previously, molecular markers have been reported for height and DBH in the *Populus* and *Corymbia* genera (DILLEN et al., 2008; RAE et al., 2008). More recently, Vargas-Reeve et al. (2013) reported high genetic correlation between height and diameter at breast height in *E. cladocalyx*. On the other hand, common loci influencing the variation in multiple traits, often including traits which are not strongly phenotypically correlated, is consistent with other QTL (GION et al., 2011; POT et al., 2006; THUMMA et al., 2010; UKRAINETZ et al., 2008) and association (e.g. THUMMA et al., 2009) studies in tree species.

In the present study, some markers explained a high proportion of the phenotypic variation. For instance, the microsatellite markers EMBRA208, EMBRA191 and EMBRA32 explained up to 13.6%, 22% and 42.6%, respectively, of the phenotypic variation for DBH. Moreover, in the longitudinal analysis, these markers explained 17%, 14% and 28% (EMBRA208, EMBRA191 and EMBRA32, respectively) of the phenotypic variation for DBH. For total tree height, the marker EMBRA208 explained up to 25% of the phenotypic variation, and explained 26% when this was considered as repeated measurement over time. These values are higher than previous results reported for DBH and height in *Eucalyptus* species. Thumma et al. (2010), for instance, reported a phenotypic variation ranged from 3.1 to 5.6% and 2.7 to 6.4% for DBH and height, respectively. In fact, the estimated percentage of phenotypic variation explained for growth traits have usually been found to be moderate (<15%). The relatively small population size of our study would explain the high overestimated values of the phenotypic variation explained for DBH and total tree height. However, the genomic position of the QTL reported here were broadly consistent with the genomic regions reported in past studies (i.e. GION et al., 2011; FREEMAN et al., 2009, 2013, THUMMA et al., 2010). In *E. globulus* with 2 and 7 years-old trees, Freeman et al. (2009) and Freeman et al. (2009; 2013) detected genomic regions located near to the SSR marker EMBRA37 and DArT markers associated to DBH, respectively. Both of these markers are located at the LG5 near to the EMBRA208. Similarly, QTL for DBH has been reported at the same genomic regions in *E. nitens* (THUMMA et al., 2010) and *E. grandis* (GION et al., 2011). In addition, QTL stability for this trait with these markers has been previously described by Verhaegen et al. (1997) in *E. grandis* and then by Thumma et al. (2010) in *E. nitens*. Furthermore, Thumma et al. (2010) reported a cluster of QTL for 3 year-old and 4 year-old trees for height and DBH, respectively at LG5, which possibly reflects the high correlation among these traits.

CONCLUSION

Based on mixed modeling analyses, growth traits were found to be moderately heritable. The marker EMBRA208 was statistically consistent in all ages studied for tree height, and the markers EMBRA191, EMBRA208 and EMBRA32 for DBH, indicating a high stability across different ages of *E. cladocalyx*. These results have important and practical implications for marker-assisted selection in *E. cladocalyx*.

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