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Selecting for water use efficiency, wood chemical traits and biomass with genomic selection in a *Eucalyptus* breeding program

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ABSTRACT

The selection of ideotypes combining high biomass production, high water use efficiency (WUE) and adequate wood properties is a major challenge in improving forest trees for industrial plantations. This issue was addressed in a field experiment evaluating 1130 clones of *Eucalyptus urophylla * Eucalyptus grandis*. Genomic selection using 3303 SNPs and the GBLUP model was carried out at age 55 months to select genitors and clones for stem volume (V55), WUE (using stable carbon isotope composition in stemwood, δ^{13}C as a proxy, transformed into intrinsic WUE, W_i), as well as lignin (KL) and holocellulose (HCEL) contents. The variance components were mainly additive for δ^{13}C(W_i), KL and HCEL, while they were highly non-additive for V55 (the ratio of dominance to additive variance was 130%). The narrow sense heritability was higher for W_i ($h^2 = 0.704$) than for volume and wood traits (< 0.5), showing that this trait was under strong genetic control. Additive and total genetic correlations among traits were low (between -0.260 and 0.260) as were the correlations due to environmental effects (between -0.267 and 0.344), which suggests a rather independence among traits. The equal emphasis and the desired gain index selection methods were used to assess economic weights and to estimate the expected additive and total genetic gains. Different breeding objectives were defined with percentages weighting the economic coefficients or the desired gain for the equal emphasis or desired gain method, respectively. When the breeding objective gave emphasis to volume production using the percentages of 70%, 10%, 10%, and 10% for V55, HCEL, KL and W_i, respectively, positive gains were observed in both methods, but the correlation between index rankings were 0.686 and 0.635 for breeding and clonal selection, respectively. With a more balanced breeding objective using percentages such as 25%, 25%, 25%, 25% for V55, HCEL, KL and W_i, respectively, both index methods gave close positive gains and similar rankings, the correlation between index rankings ranging from 0.941 to 0.982 for parent selection and from 0.883 to 0.903 for clone selection. With more contrasted percentages between V55 and W_i, such as (80%, 10%, 10%, 0%) or (90%, 10%, 10%, -10%), the gain increased for V55 and was close to the maximum and decreased markedly for Wi. This study explores the magnitude of economic coefficients for index selection and shows that positive genetic gains can be achieved by associating biomass, a proxy of WUE and wood chemical traits. It provides encouraging results for selecting *Eucalyptus* varieties adapted to dry zones while maintaining good performances regarding other economic traits.

1. Introduction

Forest plantations are viewed as one means of meeting the growing demand for biomass in temperate (Wartelle and Dreyfus, 2010) as well as in sub-tropical and tropical regions (FAO, 2010). Tree breeding programs have been carried out with many tree species to produce

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varieties presenting high volume and lignin concentration for fuelwood production, or high volume and cellulose concentrations for the pulp and paper market. Such programs are conducted for *Eucalyptus* commercial plantations in many parts of the Mediterranean and tropical zones with intensive silviculture (Castro et al., 2016). However, the duration of drought periods is expected to increase because of the climate change in many regions and *Eucalyptus* plantations are expected to extend to drier areas. Both concerns raise a major threat to the sustainability of intensively managed plantations, for example in Brazil (Booth, 2013) and in South Africa (Albaugh et al., 2013). Selection criteria enhancing the efficiency of water use for biomass production are therefore required to develop genotypes better adapted to water-limited areas making it possible to use less water for the same biomass production. These critical questions related to selection for wood products and water resource use efficiency have promoted research in ecophysiology and genetics, with some efforts to combine them.

Research in ecophysiology has improved our understanding of tree adaptation by addressing complex traits that play a critical role in adaptation. This is the case of water use efficiency (WUE), which can be defined as the ratio between harvested biomass and the amount of water used to produce this biomass (Hsiao and Acevedo, 1974). At the leaf level, WUE is defined as the ratio between net CO_2 assimilation rate to stomatal conductance for water vapor and is called intrinsic WUE (W_i). Discrimination against $^{13}CO_2$ during photosynthesis is used as a time-integrated surrogate for W_i (Farquhar and Richards, 1984; Condon et al., 1990). Therefore, differences in W_i among plants are thought to translate into differences in stable carbon isotope composition ($\delta^{13}C$) of plant organic material. $\delta^{13}C$ has proven to be robust in predicting differences in WUE in both leaves and whole plants, among plant species or genotypes (Condon et al., 2004), including tree species (Rasheed et al., 2013; Roussel et al., 2009b, 2009a). Similar results were observed for *Eucalyptus* trees (Osorio and Pereira, 1994; Osório et al., 1998; Li et al., 2000). Owing its ease of measurement by isotopic mass ratio spectrometry, $\delta^{13}C$ allows large-scale screening of phenotypes under a range of environments and is therefore a tool for disentangling genotypic and environmental effects using different experimental approaches and models (Chen et al., 2011).

Although our understanding of the genetic bases of $\delta^{13}C$ has improved in *Araucaria* (Xu et al., 2003), *Quercus* (Brendel et al., 2008), *Eucalyptus* (Bartholomé et al., 2015), and *Pinus* (Cumbie et al., 2011; Marguerit et al., 2014; Plomion et al., 2016), the factors influencing the variations of this proxy of WUE in breeding populations remain poorly understood. Research is still needed to gain insight into the genetic and environmental effects in phenotype variation and plasticity. The relationships between WUE, growth and wood traits have been little documented, and the results are still partial and sometimes inconsistent. Depending on experiments, biomass production and WUE can be positively correlated (Le Roux et al., 1996), negatively correlated (Monclus et al., 2005) or not correlated (Cumbie et al., 2011). It is difficult to determine whether these divergent results are due to sampling or to species or environmental effects. A meta-analysis showed a positive global intra-specific correlation between $\delta^{13}C$ and height (Gr = 0.28, P < 0.0001), a stronger correlation for biomass than for height (Gr = 0.68, P < 0.0001), and a non-significant correlation for diameter (Gr = 0.04, P < 0.64) (Fardusi et al., 2016). However, the authors did not study the influence of genetic and environmental effects on these correlations. Better knowledge of genetic and environmental correlations is a key issue in guiding tree breeding programs. This is especially critical when WUE and growth traits are negatively correlated genetically and when increasing genetic gain in WUE leads to a decrease in productivity. Similarly, increased genetic gain in WUE should not be at the expense of wood quality, which will depend on the end use of the wood (high lignin content for fuelwood or high cellulose content for pulpwood). Few data are available for understanding how genetic and environmental conditions are shaping the relationships between WUE, biomass production and wood properties.

Several questions must be addressed, especially for *Eucalyptus* species planted in marginal zones where water availability may become a critical issue (Christina et al., 2016; Stape and Binkley, 2010). What is the contribution of additive and non-additive gene effects in the expression of WUE, biomass production and wood property traits? What is the magnitude of the genetic and environmental correlations between WUE and other traits? Can we carry out an effective index selection combining suitable performances for biomass production, WUE and wood property traits? Using a field experiment with clones from *Eucalyptus* hybrid families planted in humid tropical conditions on a soil with a low water holding capacity, the objectives of our study were: (i) to gain insights into the genetic and environmental components controlling $\delta^{13}C$, stem volume and wood chemical traits, (ii) to assess the genetic and environmental correlations between those traits, (iii) to analyze the effect of multi-trait selection on the genetic gain for the traits of interest, and (iv) to draw the consequences in terms of selection of ideotypes for different breeding objectives.

2. Material and methods

2.1. Field experimental data

The study was conducted using a *Eucalyptus* progeny trial located east of Pointe-Noire (11°59′21″E, 4°45′51″S) in the People's Republic of Congo. Rainfall averaged 1200 mm/year. The soils were deep Ferralic Arenosols characterized by low water retention, a very low level of organic matter and poor cationic exchange capacity (Mareschal et al., 2011). The plant material resulted from controlled pollination crosses of thirteen *Eucalyptus urophylla* S.T. Blake females and nine *Eucalyptus grandis* Hill ex Maid. males according to a factorial mating design. Parents of each species came from different provenances in order to maximize the variability. No clear genetic structure was detected using molecular information (data not shown). The membership of a group was not considered in genetic analyses. These crosses generated 69 full-sib families and 1415 progenies. Each of the 1415 progenies was replicated three times using cuttings and a clonally replicated progeny test was planted at a stocking density of 833 trees ha^{-1}. The field experiment was a complete block design with three replications. Twenty-five trees replicated in three blocks represented each full-sib family. The total number of trees used in this study was reduced to 3596, representing 1130 clones, because of natural mortality.

2.2. Measured traits

Total tree height (HT) and circumference at breast height (C) were measured 55 months after planting and used to calculate a proxy of the total tree volume (V55) using the cylinder formula with a stem form factor of 0.3 (Schröder et al., 2013). To avoid difficulties in statistical mixed model convergence and variance estimation, the volume data were normalized using a logarithmic transformation.

Wood samples were collected at age 55 months from 1690 trees for near-infrared spectroscopy (NIRS) analyses. The wood chips were collected by drilling each tree to the heart of the stem at a height of 1.3 m. The wood chips were dried and ground (particle size 4 mm) and NIRS models were used to estimate Klason lignin (KL) and holo-cellulose content (HCEL). We used existing NIRS models of multiple *Eucalyptus* species that included samples from this study (Denis et al., 2013). The model was built using about 3000 wood samples collected from six *Eucalyptus* species and hybrid plantations of ages ranging from 5 to 30 years located in Congo, Senegal and Brazil. One hundred samples were selected using the Mahalanobis distance calculated from spectral information to measure wood chemistry traits through wet chemistry (Chaix et al., 2015), then spectral and wet chemistry data were used to calibrate NIRS predictions (Denis et al., 2013).

Stable carbon isotope composition ($\delta^{13}C$) of wood was measured on the same samples as those used for NIRS after grinding them to a fine

powder (< 0.1 mm). One mg of the powder was enclosed in a tin capsule and analyzed with an elemental analyzer (Carlo Erba, NA 1500-NC, Milan, Italy) coupled to an isotope-ratio mass spectrometer (Finnigan, Delta S, Bremen, Germany). $\delta^{13}C$ was expressed according to the international standard (Vienna Pee Dee Belemnite, VPDB). The accuracy of the analyses (standard deviation below 0.2‰) was assessed by repeated measurements of several laboratory standards. Due to the large number of sampled trees (1690), $\delta^{13}C$ was measured on wood rather than on cellulose. Cellulose has the advantage of being more homogeneous chemically and is produced with fewer biochemical steps from transported phloem sugars, therefore providing a less noisy integration of the isotopic leaf signal than wood. The difference between wood and cellulose $\delta^{13}C$ might be mainly impacted by variations in the content of lignin, which is isotopically lighter (Bowling et al., 2008). Therefore, on a subsample of 100 trees, cellulose and lignin were extracted using the methods of Chaix et al. (2015) and isotopic analyses were done as described above. The linear regression between wood and cellulose $\delta^{13}C$ was highly significant (p < 0.001) and strong (adjusted R^2 was 0.97). The $\delta^{13}C$ of extracted lignin was on average 2.9‰ more negative that the values for cellulose, which is close to the offset observed by Benner et al. (1987) of 4‰ to 7‰ for other forest tree species. However, the difference between $\delta^{13}C$ in wood and $\delta^{13}C$ in cellulose was steady and not related to the amount of lignin in the wood. The genotype ranking was similar using $\delta^{13}C$ measured either on cellulose or on bulk wood. These results showed that wood $\delta^{13}C$ was a robust estimator of cellulose $\delta^{13}C$ in our dataset.

Because the CV of $\delta^{13}C$ cannot be calculated, as $\delta^{13}C$ is not an absolute value but a deviation from a standard (Brendel, 2014), we converted the $\delta^{13}C$ of each tree into intrinsic WUE (W_i) using the Farquhar equation (Farquhar and Richards, 1984): $W_i = Ca (1 − (\delta^{13}C_{air} − \delta^{13}C − 4.4)/22.6)/1.6$ with $Ca = 400$ ppm and $\delta^{13}C_{air} = -8‰$. It must be stressed that W_i is a linear transformation of $\delta^{13}C$ for statistical purposes and not a robust estimate of intrinsic WUE which would have required use of a more complex set of equations for which we did not have all the parameters required.

2.3. Statistical model

We used a linear mixed model combining genetic and environmental effects to analyze the data. Different models were tested using AIC criteria (Akaike, 1974), especially using autoregressive functions to take into account spatial effects and competition. Finally, the following model was implemented:

$$y = X\beta + Z_{col}col + Z_{r:b}r: b + Z_c a_f + Z_c a_m + Z_c d + \varepsilon \quad \text{[model (1)]}$$

where **y** was the vector of the phenotypic variable (V55, $\delta^{13}C$, W_i, KL and HCEL measured at 55 months), $\boldsymbol{\beta}$ was the vector of fixed effects due to the general mean and blocks, $col \sim N(0, \sigma_{col}^2 \mathbf{Id})$ was the vector of random spatial environmental effects due to the field design column, with σ_{col}^2 being the variance related to the spatial effects, **Id** was the identity matrix, $r:b \sim N(0, \sigma_{r:b}^2 \mathbf{Id})$ was the vector of random spatial environmental effects due to field design row by block interaction, with $\sigma_{r:b}^2$ being the variance related to the spatial effects, $\varepsilon \sim N(0, \sigma_e^2 \mathbf{Id})$ was the vector of random spatial environmental effects due to microenvironmental effect impacting the tree with σ_e^2 being the environmental variance. The genetic effects were defined by: $a_f \sim N(0, \sigma_{af}^2 \mathbf{A_{fG}^H})$, $\mathbf{a_m} \sim N(0, \sigma_{am}^2 \mathbf{A_{mG}^H})$ and $\mathbf{d} \sim N(0, \sigma_d^2 \mathbf{D_G^H})$ with $\mathbf{A_{fG}^H}[c,c]$ and $\mathbf{A_{mG}^H}[c,c]$ the molecular-based female and male additive relationship matrices, $\mathbf{D_G^H}[c,c]$ the dominance relationship matrix calculated with the Hadamard product between male and female additive matrices. The number of clones is c = 1130.

$\boldsymbol{X}, \boldsymbol{Z_{col}}, \boldsymbol{Z_{r:b}}, \boldsymbol{Z_p}$, and $\boldsymbol{Z_c}$ were the incidence matrices connecting the fixed and random effects to the data. The female and male additive molecular marker-based coancestry matrices $\mathbf{A_{fG}^H}[c,c]$ $\mathbf{A_{mG}^H}[c,c]$ were derived from the haplotypes of each progeny according to Van Raden's estimator using 3303 single nucleotide polymorphism markers, see

details in Bouvet et al. (2016). The variance component estimation based on the REML method and the BLUP calculations were done using the ASReml version 3 package (Gilmour et al., 2006) implemented in R software (R Development Core Team, 2011).

Narrow- and broad-sense heritabilities were defined by

$$h^2 = \sigma_a^2/\sigma_p^2 \tag{1}$$

and

$$H^2 = \sigma_g^2/\sigma_p^2 \tag{2}$$

The dominance variance ratios representing the part of dominance variance in total variance was defined by

$$d^2 = \sigma_d^2/\sigma_p^2 \tag{3}$$

These ratios were calculated with σ_a^2, σ_g^2 and σ_p^2 defined as the additive

$$(\sigma_a^2 = \sigma_{am}^2 + \sigma_{af}^2) \tag{4}$$

the total genetic

$$(\sigma_g^2 = \sigma_{am}^2 + \sigma_{af}^2 + \sigma_d^2) \tag{5}$$

and the phenotypic variances

$$(\sigma_p^2 = \sigma_{am}^2 + \sigma_{af}^2 + \sigma_d^2 + \sigma_e^2/2) \tag{6}$$

The correlation estimates were obtained using model (1) in the multivariate formulation (Rambolarimanana et al., 2018). The correlations related to genetic and environmental effects between traits 1 and 2 were calculated with the following formulas:

the additive genetic correlation $\rho_a = (\text{cov}_{a(1,2)})/(\sigma_{a1})(\sigma_{a2}) \tag{7}$

the dominance genetic correlation $\rho_d = (\text{cov}_{d(1,2)})/(\sigma_{d1})(\sigma_{d2}) \tag{8}$

the total genetic correlation $\rho_g = (\text{cov}_{g(1,2)})/(\sigma_{g1})(\sigma_{g2}) \tag{9}$

the residual (environmental) correlation $\rho_e = (\text{cov}_{e(1,2)})/(\sigma_{e1})(\sigma_{e2}) \tag{10}$

where $\text{cov}_{(1,2)}$ is the covariance between traits 1 and 2 and σ_1, σ_2 are the standard deviations of traits 1 and 2, respectively. Approximate standard errors for linear functions of variance components were calculated using the pin.R function. This function, proposed by Ian White (2013) (http://www.homepages.ed.ac.uk/iwhite/asreml/), applies the delta method for the estimation of approximate standard errors (Oehlert, 1992).

The best linear unbiased predictors (BLUP) related to the additive (BLUP$_u$) and total (BLUP$_g$) genetic effects were computed by solving the mixed model equations. The BLUP$_u$ was calculated by the addition of male and female random coefficients estimated with the ASReml package. The BLUP$_g$ was calculated with the addition of male, female and male by female interaction random coefficients estimated with the ASReml package.

2.4. Index selection and genetic gain

We investigated the multiple trait selection combining four traits: V55, HCEL, KL and W_i. We considered the breeding context of massal selection starting from a breeding population composed of the 1130 hybrid clones. Two genetic gains were calculated: (i) the additive genetic gain based on breeding values, resulting from selection and crossings of the selected parents in successive generations, and (ii) the total genetic gain based on total genetic values for dissemination of clones as varieties.

The approach was derived from the index selection method (Smith 1936; Hazel 1943). We considered the breeding objective (Hazel 1943), also defined as the aggregate breeding value "H" (Hazel et al., 1994) and developed in tree breeding (Cotterill and Dean, 1990; Berlin et al., 2012). It was calculated using the additive BLUP$_u$ for the four traits of a

Table 1
Mean and variance components for additive female (σ^2_{af}), additive male (σ^2_{am}), dominance (σ^2_d) and residual (σ^2_r) effects and variance ratios for the traits measured at age 55 months: the individual tree volume (V55), the stable carbon isotope composition ($\delta^{13}C$), the intrinsic water use efficiency (W_i), the klason lignin content (KL) and the holo-cellulose content (HCEL). Standard errors of the estimations (SE) and coefficients of phenotypic variation (CV) are indicated.

Trait	Mean	Min	Max	CV %	Variance components								Variance ratios						
					σ^2_{af}	SE	σ^2_{am}	SE	σ^2_d	SE	σ^2_e	SE	h^2	SE	d^2	SE	H^2	SE	d^2/h^2
V55 (m^3)[a]	3.95	−4.00	5.89	28.83	0.302	0.139	0.236	0.138	0.742	0.283	0.882	0.048	0.306	0.096	0.422	0.123	0.727	0.046	1.379
$\delta^{13}C$ ($^0/_{00}$)	−29.37	−31.14	−27.24	nd[b]	0.081	0.022	0.096	0.023	0.000	0.000	0.121	0.006	0.704	0.027	0.000	0.000	0.704	0.027	0.000
W_i (µmol mol^{-1})	62.31	42.70	85.84	9.32	9.891	2.709	11.768	2.810	0.000	0.000	14.851	0.747	0.704	0.027	0.000	0.000	0.704	0.027	0.000
KL (%)	27.73	20.98	34.74	6.32	0.817	0.215	0.383	0.196	0.000	0.000	1.397	0.068	0.460	0.045	0.000	0.000	0.460	0.045	0.000
HCEL (%)	67.19	58.93	76.32	3.64	1.079	0.319	0.444	0.299	0.000	0.000	2.369	0.115	0.290	0.045	0.000	0.000	0.290	0.045	0.000

h^2: narrow sense heritability, H^2: broad sense heritability and d^2: proportion of dominance variance to additive variance.

[a] The average of V55 without logarithmic transformation was 0.079 m^3 and its coefficient of variation was 74%.

[b] The coefficient of variation for $\delta^{13}C$ cannot be calculated (see Brendel, 2014).

tree "i" defined by the row vector "u_i" or total genetic BLUP$_g$ for the four traits "g_i" estimated with model (1) and economic weights a_1, a_2, a_3 and a_4 for V55, W_i, KL, HCEL, respectively. For an individual i, **H** was defined as

$$H_{ui} = \mathbf{a_u}'\mathbf{u_i} \tag{11}$$

based on additive values and

$$H_{gi} = \mathbf{a_g}'\mathbf{g_i} \tag{12}$$

based on total genetic values where $\mathbf{a_u}$ or $\mathbf{a_g}$ are the row vectors of economic coefficients for parents and clone selection, respectively.

As we did not have any appropriate bio-economic data or economic function linking traits to economic values, we used the following two methods to define economic weights (Cotterill and Jackson, 1985). The first was based on equal emphasis (Shelbourne and Low 1980). The economic weight for the trait "t" was defined by $a_t = s_t/\sigma_{at}$, where s_t is a coefficient related to the relative importance of the trait "t" and σ_{at} is the additive standard deviation of the trait "t". The economic weights for different economic breeding objectives were calculated using a set "s" (s_1, s_2, s_3, s_4) of percentages giving the relative importance of the trait in the index (the different values "s_t" are given below).

The second method was based on the desired gain (Pešek and Baker, 1969). The economic coefficients were calculated by

$$\mathbf{a_u} = \mathbf{G_u}^{-1}\mathbf{P}\,\mathbf{G_u}\mathbf{d} \tag{13}$$

where $\mathbf{G_u}$ is the additive variance-covariance matrix among traits, **P** is the phenotypic variance-covariance matrix among traits and **d** the column vector of desired gain. The set of relative importance "s" was used to estimate the desired gain of the trait "t" $d_t = s_t *\Delta G_{umax,t}$, as the proportion of the maximal gain for each corresponding trait in the index. $\Delta G_{umax,t}$ was defined using Eqs. (14) and (15) (see below).

For both approaches, we used a set of percentages "s" defining the relative importance of each trait in the breeding objective. This first set was 70%, 10%, 10%, 10% for V55, HCEL, KL, W_i respectively, which provided a major influence of volume production on the selection index. The second set tested was 40%, 10%, 10%, 40% for V55, HCEL, KL, W_i respectively, which emphasized volume production and water use efficiency. The third set tested was 30%, 10%, 10%, 30%, 30% for V55, HCEL, KL, W_i respectively, which emphasized volume production, with good water use efficiency for charcoal production. The fourth set tested was 30%, 30%, 10%, 30% for V55, HCEL, KL, W_i respectively, which emphasized volume production, with good water use efficiency for pulp production. The fifth set tested was 25%, 25%, 25%, 25% for V55, HCEL, KL, W_i respectively, with a balanced weight among the traits.

To facilitate comparison among methods, economic weights were presented as actual values as well as transformed so that V55 has a relative weight of 10 m^{-3}.

Similar procedure was conducted for determining the economic

weights in the case of clonal selection using the total genetic standard deviation σ_g and the total genetic variance and covariance matrix $\mathbf{G_g}$.

To estimate the relative genetic gain in each trait induced by these different breeding objectives as the consequence of selection on index, the following formulas were used (White et al., 2007):

$$\Delta G_u = \frac{\frac{1}{n}\sum_1^n (BLUP_{u\_sel})}{\bar{y}} \tag{14}$$

$$\Delta G_g = \frac{\frac{1}{n}\sum_1^n (BLUP_{g\_sel})}{\bar{y}} \tag{15}$$

$BLUP_{u/g\_sel}$ being the BLUP (estimated with the model (1)) of the selected trees using the index method, n was the number of selected trees and $\bar{y}$ was the overall mean. The additive relative genetic gain ΔG_u was calculated using the $BLUP_u$. The total relative genetic gain ΔG_g was calculated using $BLUP_g$. The gains were calculated using two selection intensities (the proportion of selected individuals to the total number of trees in the population) of 10% for parent selection and 1% for clone selection.

3. Results

Phenotypic variabilities were highly variable depending on the traits (Table 1). The log-transformed volume stood out with a coefficient of variation (CV) of 18.9%, whereas the wood property traits showed CVs around 5%. The $\delta^{13}C$ values converted to intrinsic WUE (W_i), showed a CV of 9%. The distribution of V55 showed a marked skewness and the distribution of the three other traits was close to normal (Fig. 1).

3.1. Variance components and variance ratios

Variance components were well estimated by the linear mixed model, without convergence problems (Table 1). The female and male variance (σ^2_{af} and σ^2_{am}, respectively) showed close estimates for V55 and $\delta^{13}C$ (and W_i), whereas σ^2_{af} was much higher than σ^2_{am} for HCEL and KL. This result suggested a higher variability of the *E. urophylla* parent set than the *E. grandis* parent set for these latter traits. The variance ratios showed clear differences between V55 and the other traits. The V55 variable was characterized by a large part of the dominance variance as shown by $d^2/h^2 = 1.379$. On the other hand, we noted the absence of dominance variance for $\delta^{13}C$ (and W_i), KL and HCEL with d^2/h^2 equal to zero.

As expected, the narrow sense heritability (h^2) was smaller than the broad sense heritability (H^2), except when the dominance variance was null. This was the case for $\delta^{13}C$ (and W_i), KL and HCEL with similar values of h^2 and H^2. Differences in heritability estimates were high among traits. Narrow sense heritability was the smallest for HCEL
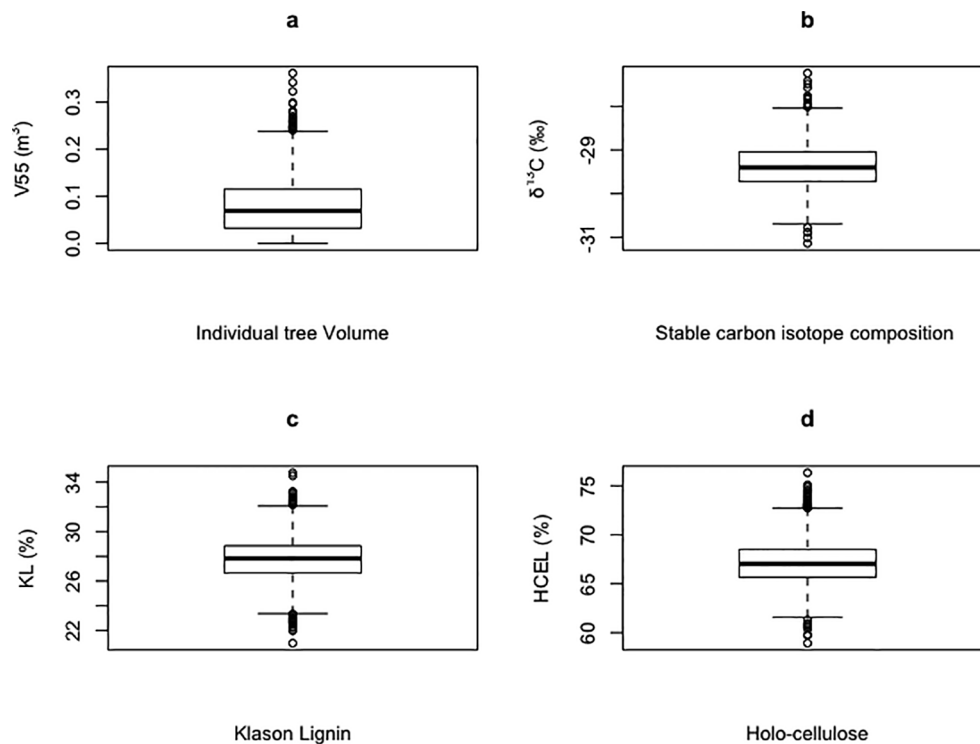
Fig. 1. Box plots showing the distribution of the studied traits: (a) the individual tree volume at age 55 months (V55 in m^3) represented before log transformation, (b) the stable isotope composition (δ^{13}C in ‰), (c) the lignin content (KL in %) and (d) the holo-cellulose content (HCEL in %).

(h^2 = 0.290) and the highest for δ^{13}C (h^2 = 0.704). This trend changed markedly for broad sense heritability where V55 showed the highest estimate as a result of the marked dominance variance (H^2 = 0.727).

3.2. Correlations

Globally, our results stressed the low to moderate genetic and environmental correlations between traits (Table 2). We noted small positive additive genetic correlations (ρ_a < 0.300) between V55 and wood chemical traits and low negative additive genetic correlations

Table 2
Genetic, environmental and phenotypic correlations between the different traits (the volume (V55), the intrinsic water use efficiency (W$_i$), the klason lignin content (KL) and the holo-cellulose content (HCEL). Standard errors of the estimations (SE) were estimated using model (1).

Traits		V55		HCEL		KL	
		estimate	SE	estimate	SE	estimate	SE
HCEL	ρ_a	0.118	0.126				
	ρ_d	0.000	0.000				
	ρ_g	0.118	0.126				
	ρ_e	−0.267	0.031				
	ρ_p	−0.125	0.039				
KL	ρ_a	0.238	0.092	−0.257	0.112		
	ρ_d	0.000	0.000	0.000	0.000		
	ρ_g	0.261	0.146	−0.257	0.112		
	ρ_e	0.344	0.030	0.023	0.031		
	ρ_p	0.298	0.036	−0.079	0.035		
W$_i$	ρ_a	−0.260	0.088	−0.101	0.100	−0.192	0.085
	ρ_d	0.000	0.000	0.000	0.000	0.000	0.000
	ρ_g	−0.260	0.088	−0.101	0.100	−0.192	0.08
	ρ_e	0.195	0.033	−0.045	0.032	0.104	0.032
	ρ_p	−0.034	0.039	−0.065	0.036	−0.038	0.036

ρ_a, ρ_d ρ_g ρ_e ρ_p are the additive, dominance, total genetic, residual (environmental) and phenotypic genetic correlations (Eq. (7)) to (Eq. (10)).

between V55 and δ^{13}C (or W$_i$) (ρ_a = −0.260). Similarly, correlations between wood chemical traits and δ^{13}C (or W$_i$) were not strong and negative estimates were observed (ρ_a = -0.101 and ρ_a = -0.192 for HCEL and KL, respectively). The correlations due to the dominance effect were null for all the combinations of δ^{13}C (or W$_i$), KL or HCEL because the estimates of the dominance variance were null. As a result, the total genetic correlations were equal to the additive genetic correlations. Most of the environmental correlations were small (ρ_e < 0.200 in absolute value), except between V55 and KL (ρ_e = 0.344). Similar patterns were noticed for phenotypic correlations.

3.3. Index economic coefficients and genetic gains

The method used greatly influenced the estimation of economic coefficients (Table 3). The equal emphasis method provided coefficients whose values were proportional to the percentage set "s" expressing the relative importance of each trait in the breeding objective. For example, with a breeding objective favoring gain in volume, s = (70%;10%;10%;10%), the transformed economic weights with the equal emphasis method were a_1 = 10 m^{-3}, a_2 = 0.85%$^{-1}$, a_3 = 0.96%$^{-1}$ and a_4 = 0.23 (µmol mol^{-1})$^{-1}$, while they were a_1 = 10 m^{-3}, a_2 = −1.12^{-1}, a_3 = −0.21%$^{-1}$ and a_4 = 0.73 (µmol mol^{-1})$^{-1}$ with the desired gain method for V55, HCEL, KL and Wi, respectively. The difference between the two methods was greater for an equal percentage of each trait in the breeding objective, s = (25%;25%;25%;25%), with a_1 = 10 m^{-3}, a_2 = 5.94%$^{-1}$, a_3 = 6.69%$^{-1}$ and a_4 = 1.58%$^{-1}$ (µmol mol^{-1})$^{-1}$ for the equal emphasis method, while they were a_1 = 10 m^{-3}, a_2 = 30.42%$^{-1}$, a_3 = 32.67%$^{-1}$ and a_4 = 7.25 (µmol mol^{-1})$^{-1}$ with the desired gain method for V55, HCEL, KL and Wi, respectively. Regarding the additive genetic gain ΔG_u, both methods provided similar estimates, except for s = (70%;10%;10%;10%). In that case, ΔG_u for W$_i$ was 0.2% with the equal emphasis method and 7.1% with the desired gain method (Table 3). In consequence, the Spearman correlation was the lowest with s = (70%;10%;10%;10%) (r = 0.686), while it was close to one

Table 3
Expected relative additive genetic gain (ΔG_u) following selection on indices using economic weights determined either by the equal emphasis method or by the desired gain method. The expected gains were calculated for an intensity of selection of 10%. Economic weights were estimated using different sets of percentages "s" representing the relative importance of each trait in the breeding objective. Single trait selection indicates the maximum relative gain for each trait.

Selection method (parents)	s(%)[e]	Equal emphasis method	Observed relative gain ΔG_u (%)				Desired gain method	Observed relative gain ΔG_u (%)				R: Spearman correlation[b]
		Index economic coefficients[a]	V55	HCEL	KL	W_i	Index economic coefficients	V55	HCEL	KL	W_i	
Multitrait	70;10;10;10	0.95, 0.08, 0.09, 0.02[c] 10.00, 0.85, 0.96, 0.23[d]	12.0	0.5	1.4	0.2	1.86, −0.01, 0.14, 0.19 10.00, −1.12, −0.21, 0.73	7.2	−0.1	0.42	7.1	0.686
	40;10;10;40	0.54, 0.08, 0.09, 0.08 10.00, 1.49, 1.67, 1.58	5.1	0.1	0.4	8.1	1.52, 0.36, 0.57, 0.35 10.00, 2.37, 3.75, 2.35	3.0	0.0	0.8	8.7	0.982
	30;30;10;30	0.41, 0.24, 0.09, 0.06 10.00, 5.94, 2.23, 1.58	5.3	0.8	0.2	6.5	0.71, 1.02, 0.86, 0.29 10.00, 14.34, 12.17, 4.09	3.3	0.6	1.5	6.4	0.903
	30;10;30;30	0.41, 0.08, 0.27, 0.06 10.00, 1.98, 6.69, 1.58	5.3	−0.1	2.6	5.7	0.79, 0.72, 1.13, 0.31 10.00, 9.12, 14.21, 3.84	3.2	0.2	2.1	6.5	0.966
	25;25;25;25	0.34, 0.20, 0.23, 0.05 10.00, 5.94, 6.69, 1.58	6.2	0.6	2.3	4.2	0.37, 1.13, 1.21, 0.27 10.00, 30.42, 32.67, 7.25	3.2	0.6	2.2	5.0	0.941
Single trait	$\Delta G_{u\ max}$		13.0	1.7	4.4	10.3		13.0	1.7	4.4	10.3	

[a] Units for the index coefficients were: m^{-3}, $\%^{-1}$, $\%^{-1}$ and $(\mu mol\ mol^{-1})^{-1}$ for V55, HCEL, KL and W_i respectively.
[b] Spearman correlation for the index ranking.
[c] Actual economic weights.
[d] Transformed economic weights.
[e] The order of the percentages is the same as the order of the traits in the following columns.

Table 4
Expected relative total genetic gain (ΔG_g) following selection on indices using economic weights determined by either the equal emphasis method or the desired gain method. The expected gains were calculated for an intensity of selection of 1%. Economic weights were estimated using different sets of percentages "s" representing the relative importance of each trait in the breeding objective. Single trait selection indicates the maximum relative gain for each trait.

Selection method (clones)	s (%)[e]	Equal emphasis method	Observed relative gain ΔG_g (%)				Desired gain method	Observed relative gain ΔG_g (%)				R: Spearman correlation[b]
		Index economic coefficients[a]	V55	HCEL	KL	W_i	Index economic coefficients	V55	HCEL	KL	W_i	
Multitrait	70;10;10;10	6.18, 0.89, 0.91, 0.81[c] 10.00, 1.44, 1.47, 1.31[d]	20.4	0.4	2.2	4.8	0.72, 0.48, 0.66, 0.20 10.00, 6.65, 9.21, 2.75	6.6	−0.18	4.4	13.1	0.635
	40;10;10;40	1.77, 0.41, 0.46, 1.76 10.00, 2.32, 2.60, 9.94	11.2	0.0	3.0	13.6	0.58, 0.95, 1.21, 0.45 10.00, 16.17, 20.73, 7.79	6.3	−0.15	4.6	13.6	0.885
	30;30;10;30	1.76, 1.70, 0.59, 1.72 10.00, 9.66, 3.35, 9.77	12.1	0.3	2.2	12.9	0.17, 1.91, 1.56, 0.40 10.00, 108.99, 89.43, 22.96	6.5	0.03	4.3	13.1	0.849
	30;10;30;30	1.76, 0.57, 1.73, 1.72 10.00, 3.24, 9.83, 9.77	7.1	−0.3	4.7	13.4	0.29, 1.36, 1.96, 0.42 10.00, 46.30, 66.67, 14.35	5.2	−0.2	4.9	13.2	0.883
	25;25;25;25	1.77, 1.78, 1.73, 1.72 10.00, 10.06, 9.77, 9.72	7.5	−0.1	4.4	13.3	0.05, 1.97, 2.04, 0.39 10.00, 339.09, 349.25, 66.76	5.5	−0.1	4.6	13.1	0.903
Single trait	ΔG_{gmax}		23.2	3.0	6.9	15.0		23.2	3.0	6.9	15.0	

[a] Units for the index coefficients were: m^{-3}, $\%^{-1}$, $\%^{-1}$ and $(\mu mol\ mol^{-1})^{-1}$ for V55, HCEL, KL and W_i respectively.
[b] Spearman correlation for the index ranking.
[c] Actual economic weights.
[d] Transformed economic weights.
[e] The order of the percentages is the same as the order of the traits in the following columns.

with the other sets "s".

The same approach was used for clonal selection considering a selection intensity of 1% and including non-additive effects (Table 4). As expected, the total genetic gains ΔG_g were higher than additive genetic gains, especially for V55, KL and Wi. There was a marked difference between methods with s = (70%;10%;10%;10%) for ΔG_g estimates, leading to the smallest correlation between indices (r = 0.635). With the other sets "s", both methods led to close estimates of ΔG_g, r varying between 0.883 and 0.903. We noticed that ΔG_g for V55 was far from the maximum gain (23.2%), which was particularly marked for the desired gain method with ΔG_g smaller than 7% for V55.

4. Discussion

The approach developed in this study was based on a large sample of 1130 eucalyptus clones belonging to 69 full-sib families that were connected through the crossing of 13 females and 9 males. Each clone was represented by three ramets, allowing the estimation of genetic and environmental variations. Measurements were conducted at 55 months of age, which is a relevant stage for the prediction of adult performance in the context of fast-growing *Eucalyptus* species (Bouvet et al., 2009a). Our genetic and statistical models used relationship matrices estimated from a dense genome-wide genotyping with SNPs. This genomic information leads to better estimates of the variance components than classical pedigree approaches (Bouvet et al., 2016). Variance components and correlations of important traits made it possible to estimate gains with different strategies of index selection and breeding goals.

4.1. Part of additive, dominance and environmental effects in trait variation

Volume, Klason lignin and holocellulose presented the means and coefficients of variation usually observed in experiments in similar conditions with the same *Eucalyptus* hybrid (Denis et al., 2013). Very few experiments to our knowledge have measured the variability of $\delta^{13}C$ based on a large *Eucalyptus* clonal population. In our study, $\delta^{13}C$ varied between -31 and -27‰; the range was quasi-similar to the one found in a nearby *Eucalyptus* stands based on a single full-sib family in the Congo (Bartholomé et al., 2015) and close to the range reported in *Eucalyptus grandis* trees in a Brazilian experiment (between -26 and -28‰, Epron et al., 2012). A similar range was reported for 49 wild genotypes of *Hevea brasiliensis* in Northern Thailand (Kanpanon et al., 2017), in a full-sib family with *Quercus robur* (Brendel et al., 2008) and in progeny trial of *Pinus pinaster* (Marguerit et al., 2014). This similarity can be explained by the variation of the intrinsic WUE, and hence of $\delta^{13}C$, which is physiologically constrained by the gradient of CO_2 concentrations between the atmosphere and the intercellular air space (Farquhar et al., 1982).

The additive and dominance variance components in this study, as well as their relative magnitude, were consistent with previous genetic studies carried out on *Eucalyptus*. They showed a marked dominance variance for volume and stressed a preponderance of the additive variance for physical and chemical wood traits (Bouvet et al., 2009b). The narrow sense heritability was higher for wood chemical traits than for volume, which was consistent with other studies estimating this parameter for lignin (Poke et al., 2006; Stackpole et al., 2011; Hein et al., 2012; Mandrou et al., 2012; Makouanzi et al., 2017) and holocellulose (Rambolarimanana et al., 2018). The pattern was different for the broad sense heritability (H^2), with higher values for V55 than for wood traits. This might result from the preponderance of dominance variance in complex traits such as V55 and from the inability of the model to take into account inter-tree competition. This effect may have been captured by the male-by-female interaction inflating the dominance variance. A previous study showed that the dominance variance for volume increases with tree age and competition in Congolese *Eucalyptus* plantations (Bouvet et al., 2003).

Our results stressed the high heritability of $\delta^{13}C$ (Wi) and were

consistent with previous findings, although the plant tissue and the heritability magnitude differed: in *Pinus* sp. with needles (Cumbie et al., 2011; Correia et al., 2008) and wood (Marguerit et al., 2014), in *Populus* sp. with leaves and wood (Monclus et al., 2005; Verlinden et al., 2015), in *Araucaria cunninghamii* with branchlets (Xu et al., 2003), and in *Quercus suber* with leaves (Ramirez-Valiente et al., 2009). For *Eucalyptus*, studies addressing the genetic determinism of $\delta^{13}C$ are scarce: two showed a high genetic variability among tree populations (e.g. Li and Wang, 2003), or among provenances of *E. microtheca* at the nursery stage (Li, 2002). Li and Wang (2003) and Li (2002) were based on $\delta^{13}C$ measurements in leaves. However, most of the studies were carried out on a very small number of genotypes (Olbrich et al., 1993; Le Roux et al., 1996; Nunes et al., 2016). Other studies analyzed the genetic architecture through quantitative trait loci of carbon isotope composition in stem wood (Bartholomé et al., 2015) but did not give any estimation of the variances. Very few studies have addressed the variance components of wood $\delta^{13}C$ using a large population in field conditions. Marguerit et al. (2014) using about 1900 individuals of *Pinus pinaster* distributed at three sites is among the rare studies in the literature. As far as we know, our study is among the first for *Eucalyptus* using more than 1000 clones in field conditions.

4.2. Correlation among traits

The additive correlations between V55 and wood chemical properties (KL, HCEL) were weak but positive (Table 2). Similar results were recently reported for *Eucalyptus robusta* (Rambolarimanana et al., 2018), but previous studies showed that wood chemical traits and volume are generally poorly correlated in *Eucalyptus* (Hein et al., 2012; Denis et al., 2013). More generally, results on other species show small to moderate correlations between $\delta^{13}C$ and growth traits in, for example, *Araucaria cunninghamii* (Xu et al., 2003), *Pinus* sp. (Cumbie et al., 2011; Marguerit et al., 2014) and *Populus* sp. (Verlinden et al., 2015).

Studies addressing the correlation between wood $\delta^{13}C$ (Wi) and growth traits are scarce, and differences between hardwood and softwood species are still poorly documented. The origin of correlation, pleiotropy or linkage disequilibrium (statistical association) remains unknown. With our data, the additive correlation between Wi ($\delta^{13}C$) and V55 was negative (-0.260) and the environmental correlation was positive (0.195). The sign inversion between additive and environmental correlations suggested a correlation due to linkage disequilibrium (Gallais, 1990), i.e. random gene association resulting from a sample effect. However, the estimates were small with high standard error and further studies are needed to draw relevant conclusions.

4.3. Implication for index selection

One of the objectives of our study was to address the selection of *Eucalyptus* ideotypes for different breeding objectives in a context of decreasing water availability. The novelty was the combination of traits related to biomass, wood chemical properties and water use efficiency, the latter trait having not yet been included, to our knowledge, in the multi-trait selection of *Eucalyptus*. Because we did not have any economic information, such as the net profit or net worth of a tree, to estimate economic weights, as done in previous studies for *Eucalyptus* (Greaves et al., 1997; Wei and Borralho, 1999) and softwood species (Aubry et al., 1998), we used two methods based on trait variation and co-variation (Cotterill and Jackson, 1985). Each method produced different sets of economic weights and the consequences were analyzed comparing relative genetic gains. In the following section we mainly focus the discussion on V55 and W_i due to the importance of both traits in sustainable plantations.

Except in the case of s = (70%;10%;10%;10%), for both index methods, the genetic gain in V55 was lower than the genetic gain in W_i and much lower than the maximum gain $\Delta G_{u\ max}$ or $\Delta G_{g\ max}$ (Tables 3

Table 5

Economic coefficients, expected relative additive (ΔG_u) and total genetic gain (ΔG_g) following selection on indices using economic weights determined by equal emphasis and the desired gain method. The expected gains were calculated for an intensity of selection of 10% (parent selection) and 1% (clone selection). Economic weights were estimated using two sets of percentages "s" representing contrasted trait importance. Single trait selection indicates the maximum relative gain for each trait.

Selection method (parents)	Equal emphasis	Observed relative gain ΔG_u (%)				Desired gain method	Observed relative gain ΔG_u (%)			
s (%)[e]	Index economic coefficients[a]	V55	HCEL	KL	W_i	Index economic coefficients	V55	HCEL	KL	W_i
Multitrait	80;10;10;0	1.09, 0.08, 0.09, 0.00[c] 10.00, 0.74, 0.84, 0.00[d]	12.7	0.5	1.6	−3.8	1.97, −0.13, 0.03, 0.14 10.00, −0.69, 0.02, 0.69	9	−0.1	0	5.8
	90;10;10;-10	1.20, 0.08, 0.09, −0.02 10.00, 0.66, 0.74,-0.17	12.5	0.5	1.6	−5.6	2.01, −0.26, −0.14, 0.08 10.00, −1.25, −0.67, 0.38	11	−0.1	−0.4	3.6
Single trait	ΔG_{umax}		13	1.7	4.4	10.3		13	1.7	4.4	10.3

Selection method (clones)	Equal emphasis	Observed relative gain ΔG_g (%)				Desired gain method	Observed relative gain ΔG_g (%)			
s (%)	economic coefficients	V55	HCEL	KL	W_i	Index economic coefficients	V55	HCEL	KL	W_i
Multitrait	80;10;10;0	0.70, 0.08, 0.09, 0.00 10.00, 0.66, 0.74, −0.17	22.3	0.6	1.8	−3.5	0.76, 0.33, 0.48, 0.11 10.00, 4.21, 6.27, 1.45	9.5	−0.1	4	13.2
	90;10;10;−10	0.79, 0.08, 0.09, −0.02 10.00, 1.02, 1.15, −0.27	21.9	0.2	1.9	−7.9	0.81, 0.17, 0.30, 0.02 10.00, 2.05, 3.65, 0.30	17.8	0.2	5	2.1
Single trait	ΔG_{gmax}		23.2	3	6.9	15		23.2	3	6.9	15

[a] Units for the index coefficients were: m^{-3}, $\%^{-1}$, $\%^{-1}$ and $(\mu mol\ mol^{-1})^{-1}$ for V55, HCEL, KL and W_i respectively.

[c] Actual economic weights.

[d] Transformed economic weights.

[e] The order of the percentages is the same as the order of the traits in the following columns.

and 4). In the case of genetic additive gain (Table 3), the economic weights for V55 were higher than for W_i; this can be easily noticed with the transformed coefficients equal to 10 m^{-3} for V55 when it varies from 0.23 to 7.25 $(\mu mol\ mol^{-1})^{-1}$ for Wi, whatever the index method. With this higher economic weight, we may have been expected to achieve a greater additive genetic gain in V55 compared to the other traits and especially W_i, but this was not the case (Table 3). This pattern might result from both the same selection intensity on W_i and V55, and from the negative genetic and phenotypic correlations between W_i and V55 (Table 2).

By imposing more contrasted percentages between V55 and W_i in the vector s, for example s = (80%;10%;10%;0%) or s = (90%;10%;10%;-10%), we obtained much higher coefficients for V55 than for W_i with both index methods (Table 5). As a result, the additive and total genetic gains were much higher for V55 than for W_i (Table 5). Moreover, negative additive genetic gain on W_i were achieved with the equal emphasis method when the gains remained positive with the desired gain method (Table 5).

These new sets "s" highlighted the impact of the different methods on the genetic gains resulting from selection. Our results showed that the desired gain method, even with contrasted coefficients, yielded contrasted but positive gains among traits. This can be attributed to the method that used all the genetic information i.e. the genetic and phenotypic co-variation among traits, to estimate the index coefficients. In addition, this method is generally more intelligible for breeders than the equal emphasis method or methods based on economic function, because breeders are used to dealing with the concept of genetic gain. With methods using economic function, the difficulty is to define the net worth of a tree given that it may change according to the user. For example, the net profit for the *Eucalyptus* grower can be quite different from that of the pulp companies and sawmill companies (Berlin et al., 2012). However, the desired gain method provides coefficients with errors correlated with the accuracy of the variance co-variance matrix that may influence the quality of ranking. The equal emphasis method is simpler, but does not use the all the genetic information (only variances and not co-variances). Whatever the method, the reliability of economic coefficient estimates depends on the quality of the estimation of genetic parameters.

5. Conclusion

Eucalyptus is an important genus for forest plantations in marginal zones unsuitable for agriculture where many commercial companies have made great efforts to optimize biomass production. The assessment of WUE is crucial for expanding *Eucalyptus* plantations to dry zones or coping with the consequences of climate change. Although recent research has stressed the need to improve the measurement of $\delta^{13}C$ as a proxy of whole plant WUE based on aboveground biomass (Battie Laclau et al., 2016; Fernandes et al., 2016; Konate et al., 2016, Bogeat-Triboulot et al., 2019), from a technical point of view the measurement of $\delta^{13}C$ in wood can be done in large-scale breeding populations. However, it is not yet routinely implemented in *Eucalyptus* improvement programs. Our study provides new data on the heritability of wood $\delta^{13}C$, its relationship with other major traits and the issues raised by trade-offs in index selection. We implemented two index selection methods to explore the set of economic weights for contrasting breeding objectives. We showed that index coefficients can be determined to achieve positive gains for each trait. Our findings are encouraging and show that inclusion of wood $\delta^{13}C$ in the selection process may lead to *Eucalyptus* varieties adapted to marginal zones still presenting good performance for biomass and wood chemical traits. Although the present study was based on a substantial number of clones, further studies involving other *Eucalyptus* genetic backgrounds are needed to better assess the inclusion of WUE in breeding objectives.

Authors statement

J-M.B. and C.G.M.E conceived and planned the experiments and C.G.M.E carried out the experiments and field measurements.

J-M.B planned and carried out the genetic data analyses. D.E, O.B.

and C.G.M.E contributed to sample collection and preparation and D13C and Wood chemical analyses.

J-M.B., J-P. L., J-P. B., O.B., C.G.M.E and D.E. contributed to the interpretation of the results. J-M.B. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foreco.2020.118092.

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