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

Jean-Marc Bouvet[1,2,3], Chrissy Garel Makouanzi Ekomono[4,5], Oliver Brendel [6], Jean-Paul Laclau[7,8], Jean-Pierre Bouillet[2,7,8], Daniel Epron[6,9]

[1]Cirad, UMR AGAP, Amélioration Génétique et Adaptation des Plantes Tropicales et Méditerranéennes, Montpellier, France

[2]Cirad, dispositif de Recherche et d'Enseignement en Partenariat "Forêt et biodiversité à Madagascar", Antananarivo 101, Madagascar

[3] Agap, Univ Montpellier, CIRAD, INRA, Montpellier Sup-Agro, Montpellier, France

[4]ENSAF, Ecole Nationale Supérieure d'Agronomie et de Foresterie, Université Marien NGOUABI, République du Congo

[5] Centre de Recherche sur la Durabilité et la Productivité des Plantation Industrielles, Pointe-Noire République du Congo

[6] Université de Lorraine, AgroParisTech, INRA, UMR SILVA Nancy, France

[7] CIRAD, UMR Eco&Sols, F-34398 Montpellier, France

[8] Eco&Sols, Univ Montpellier, CIRAD, INRA, IRD, Montpellier, SupAgro, Montpellier, France

[9] Laboratory of Forest Hydrology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

Corresponding author: Jean Marc Bouvet[1,2,3]

Email: jean-marc.bouvet@cirad.fr, Phone: +261 320233513

Highlights

- Selecting *Eucalyptus* ideotypes is a major challenge for plantations in dry zones
- Genomic selection improves selection accuracy of breeding and clonal populations
- Water use efficiency (WUE), volume, and wood traits are under a strong genetic control
- Correlations between volume, WUE, lignin and cellulose are low in *Eucalyptus*
- Index selection can lead to suitable genetic gains to enhance WUE and wood properties

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, $\delta^{13}C$, as a proxy transformed into intrinsic WUE, Wi), as well as lignin (KL) and holocellulose (HCEL) contents. The variance components were mainly additive for $\delta^{13}C$(Wi), 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 Wi (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 -0.265 and 0.265]) as were the correlations due to environmental effects (between -0.267 and 0.348), 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 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 Wi, 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 Wi, 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 Wi, 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.

Key words: eucalypt hybrid, growth, water use efficiency, wood chemistry, genomic selection, heritability, genetic correlation, index selection

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 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 (de Oliveira 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 (Roussel et al., 2009a, b; Rasheed et al., 2013). Similar results were observed for *Eucalyptus* trees (Osorio et al., 1994, 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 δ^{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 δ^{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 (Stape et al., 2010, Christina et al., 2016). 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 δ^{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 Republic of the 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 3,000 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 (Aikake, 1974), especially using autoregressive functions to take into account spatial effects and competition. Finally, the following model was implemented:

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

Where $\mathbf{y}$ 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, $\mathbf{col} \sim N(\mathbf{0}, \sigma^2_{col}\mathbf{Id})$ was the vector of random spatial environmental effects due to the field design column, with σ^2_{col} being the variance related to the spatial effects, $\mathbf{Id}$ was the identity matrix, $\mathbf{r{:}b} \sim N(\mathbf{0}, \sigma^2_{r:b}\mathbf{Id})$ was the vector of random spatial environmental effects due to field design row by block interaction, with $\sigma^2_{r:b}$ being the variance related to the spatial effects. The genetic effects were defined by: $\mathbf{a_f} \sim N(\mathbf{0}, \sigma^2_{af}\mathbf{A^H_{fG}})$, $\mathbf{a_m} \sim N(\mathbf{0}, \sigma^2_{am}\mathbf{A^H_{mG}})$ and $\mathbf{d} \sim N(\mathbf{0}, \sigma^2_{d}\mathbf{D^H_{G}})$ with $\mathbf{A^H_{fG}}[c,c]$ and $\mathbf{A^H_{mG}}[c,c]$ the molecular-based female and male additive relationship matrices, $\mathbf{D^H_{G}}[c,c]$ the dominance relationship matrix.

$\boldsymbol{X}$, $\boldsymbol{Z_{col}}$, $\boldsymbol{Z_{r:b}}$, $\boldsymbol{Z_p}$, 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 $\mathbf{A^H_{fG}}[c,c]$ $\mathbf{A^H_{mG}}[c,c]$ 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 $h^2= \sigma^2_a/\sigma^2_p$ (eq. 1) and $H^2= \sigma^2_g/\sigma^2_p$ (eq. 2). The dominance variance ratios representing the part of dominance variance in total variance was defined by $d^2= \sigma^2_d/\sigma^2_p$ (eq. 3). These ratios were calculated with σ^2_a, σ^2_g and σ^2_p defined as the additive ($\sigma^2_a= \sigma^2_{am}+\sigma^2_{af}$) (eq. 4), the total genetic ($\sigma^2_g= \sigma^2_{am}+\sigma^2_{af}+ \sigma^2_d$) (eq. 5) and the phenotypic variances ($\sigma^2_p= \sigma^2_{am}+\sigma^2_{af}+ \sigma^2_d+\sigma^2_e/2$) (eq. 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 $\rho_a= (cov_{a(1,2)})/(\sigma_{a1})(\sigma_{a2})$ (eq. 7)
the dominance correlation $\rho_d= (cov_{d(1,2)})/(\sigma_{d1})(\sigma_{d2})$ (eq. 8)
the total genetic correlation $\rho_g= (cov_{g(1,2)})/(\sigma_{g1})(\sigma_{g2})$ (eq. 9)
the residual (environmental) correlation $\rho_e= (cov_{e(1,2)})/(\sigma_{e1})(\sigma_{e2})$ (eq. 10)

where $cov_{(1,2)}$ 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 for the four traits of a tree "I" defined by the vector "$\mathbf{u_i}$" or total genetic BLUP for the four traits "**gi**" 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 $\mathbf{H_{ui}} = \mathbf{u}_i\ \mathbf{a_u}$. (eq. 11) based on additive values and $\mathbf{H_{gi}} = \mathbf{g}_i\ \mathbf{a_g}$ (eq. 12) based on total genetic values where $\mathbf{a_u}$ or $\mathbf{a_g}$ are the 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 (Cotteril 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 (Pesek and Baker 1969). The economic coefficients were calculated by $\mathbf{a_u} = \mathbf{G_u}^{-1}\ \mathbf{P}\ \mathbf{G_u}\ \mathbf{d}$ (eq 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 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 equations 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, W_i, KL, HCEL, respectively, which provided a major influence of volume production on the selection index. The second set tested was 40%, 40%, 10%, 10% for V55, W_i, KL, HCEL, respectively, which emphasized volume production and water use efficiency. The third set tested was 30%, 30%, 30%, 10% for V55, Wi, KL, HCEL, respectively, which emphasized volume production, with good water use efficiency for charcoal production. The fourth set tested was 30%, 30%, 10%, 30% for V55, W_i, KL, HCEL, respectively, which emphasized volume production, with good water use efficiency for pulp production. The fifth set tested was 25%, 25%, 25%, 25% for V55, W_i, KL, HCEL, 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}.

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):

$$\Delta G_u = \frac{\frac{1}{n}\sum_1^n (BLUP_{a\_sel})}{\bar{y}} \quad \text{(eq. 14)}$$

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

where BLUP was the best linear unbiased predictor estimated using the model 1, $BLUP_{a/g\_sel}$ being the $BLUP_{a/g}$ of the selected trees using the index or independent culling level method, n was the number of selected trees and μ 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 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 (Figure 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 (h^2=0.290) and the highest for $\delta^{13}C$ (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 ($\rho_a < 0.300$) between V55 and wood chemical traits and low negative additive genetic correlations between V55 and $\delta^{13}C$ ($\rho_a \approx$ -0.265 (or -0.260) for W_i). Similarly, correlations between wood chemical traits and $\delta^{13}C$ (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 $\delta^{13}C$ (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 = 0.348). 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=1.45 (µmol mol^{-1})$^{-1}$, a_3=1.48 %$^{-1}$ and a_4=1.34 %$^{-1}$, while they were a_1=10 m^{-3}, a_2=-1.12 (µmol mol-1)$^{-1}$, a_3=-0.21 %$^{-1}$ and a_4=0.73 %$^{-1}$ 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=9.90 (µmol mol-1)$^{-1}$, a_3= 9.80 %$^{-1}$ and a_4=1.98 %$^{-1}$ for the equal emphasis method, while they were a_1=10 m^{-3}, a_2=30.42 (µmol mol-1)$^{-1}$, a_3= 32.67 %$^{-1}$ and a_4=7.25 %$^{-1}$ 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 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* (Rambolorimanana 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 (Cotteril 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 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 (μmol 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.

Acknowledgements

We are particularly grateful to the field team of the Centre de Recherche sur la Durabilité des Plantations Forestières (CRDPI) in the Congo for the collection of wood cores and the measurement of the growth traits. We are also grateful for the laboratory assistance of the UMR AGAP for the NIRS measurements, especially Sophie Nourrissier-Mountou and Gilles Chaix for the extraction of cellulose and lignin from δ13C samples, and Pierre Epron for his technical support in the laboratory. Isotope analyses were carried out at the Technical Platform for Functional Ecology (PTEF OC 081) of UMR SILVA.

Funding

This work was partly supported by the WUEtree project (ANR-13-ADAP-0012) and by funding from the different partners associated with the project: Cirad, Université de Lorraine and CRDPI. UMR

SILVA is supported by a grant overseen by the French National Research Agency (ANR) as part of the Investissements d'Avenir programme (ANR-11-LABX-0002-01, Lab of Excellence ARBRE).

References

Akaike, H., 1974. A new look at the statistical model identification. Trans Autom Control 19: 716–723.

Aubry C.A., Adams, W.T., Fahey, T.D., 1998. Determination of relative economic weights for multitrait selection in coastal Douglas-fir Can. J. For. Res. 28: 1164–1170

Albaugh, J.M., Dye, P. J., King, J.S., 2013. Eucalyptus and Water Use in South Africa, Int. J. For. Res., vol. 2013, Article ID 852540, 11 pages. https://doi.org/10.1155/2013/852540.

Bartholomé, J., Mabiala, A., Savelli, B., Bert, D., Brendel, O., Plomion, C., Gion, J.M., 2015. Genetic architecture of carbon isotope composition and growth in Eucalyptus across multiple environments. New Phytol. 206, 1437–1449. https://doi.org/10.1111/nph.13301

Battie Laclau, P., Delgado-Rojas, J.S, Christina, M., Nouvellon, Y., Bouillet, J.-P., De Cassia Piccolo, M., Moreira M.Z., De Moraes Gonçalves, J.L., Roupsard, O., Laclau, J.-P., 2016. Potassium fertilization increases water-use efficiency for stem biomass production without affecting intrinsic water-use efficiency in *Eucalyptus grandis* plantations. For. Ecol. Manage., 364 : 77-89.http://dx.doi.org/10.1016/j.foreco.2016.01.004

Benner, R., Fogel, M. L., Sprague, E.K., Hodson, R. E., 1987. Depletion of 13C in lignin and its implications for stable carbon isotope studies. Nature, 329, 708–710.

Berlin, M., Gunnar, J., Lönnstedt, L., Danell, Ö., Ericsson, T., 2012. Development of economic forest tree breeding objectives: review of existing methodology and discussion of its application in Swedish conditions. Scan. J. For. Res., 27: 681_691 http://dx.doi.org/10.1080/02827581.2012.672586

Bogeat-Triboulot, M.B., Buré, C., Gerardin, T., Chuste, P.A., Le Thiec, D., Hummel, I., Brendel, O. (2019). Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes. Environ. Exp. Bot., 166(May). https://doi.org/10.1016/j.envexpbot.2019.05.021

Booth, T.H., 2013. Eucalypt plantations and climate change. For. Ecol. Manage. 301, 28–34. DOI: 10.1016/j.foreco.2012.04.004

Bouvet, J.M., Vigneron, P., Gouma, R., Saya, A.R., 2003. Trends in variances and heritabilities with age for growth traits in eucalyptus spacing experiments. Silvae Genet., 52, 121-133.

Bouvet J.-M., Vigneron P., Villar E., Saya, A.R., 2009a. Determining the optimal age for selection by modelling the age-related trends in genetic parameters in *Eucalyptus* hybrid populations. Silvae Genet., 58 (3) : p. 102-112.
https://doi.org/10.1515/sg-2009-0014

Bouvet, J.M., Saya, A.R., Vigneron, P., 2009b. Trends in additive, dominance and environmental effects with age for growth traits in *Eucalyptus* hybrid populations. Euphytica 165, 35-54. http://dx.doi.org/10.1007/s10681-008-9746-x

Bouvet, J.M., Makouanzi, G., Cros, D., Vigneron, P., 2016. Modeling additive and non-additive effects in a hybrid population using genome-wide genotyping: Prediction accuracy implications. Heredity 116, 146-157. https://doi.org/10.1038/hdy.2015.78 https://doi.org/10.5061/dryad.g73t2

Bowling, D.R., Pataki, D.E., Randerson, J.T., 2008. Carbon isotopes in terrestrial ecosystem pools and CO2 fluxes. New Phytol. 178, 24–40.

Brendel, O., Le Thiec, D., Scotti-Saintagne, C., Bodenes, C., Kremer, A., Guehl, J.-M., 2008. Quantitative trait loci controlling water use efficiency and related traits in *Quercus robur* L. Tree Genet. Genomes 4, 263–278 https://doi.org/10.1007/s11295-007-0107-z

Brendel., O., 2014. Is the coefficient of variation a valid measure for variability of stable isotope abundances in biological materials? Rapid Commun. Mass Spectrom., 4, 370–376 https://doi.org/10.1002/rcm.6791

de Oliveira Castro, C.A., Resende, R.T., Bhering, L.L., Cruz, C.D., 2016. Brief history of Eucalyptus breeding in Brazil under perspective of biometric advances. Ciência Rural, 46, n.9, set, 2016.

Chaix G., Nourissier S., Ramananantoandro T., Makouanzi G., Filho M.T., 2015. Near infrared spectroscopy for eucalyptus wood chemical compounds. In : International Symposium on Wood Science and Technology. Tokyo: JWRS, 1 p. IAWPS 2015, 2015-03-15/2015-03-17, Tokyo (Japon).

Chen, J., Chang, S.X., Anyia, A.O., 2011. Gene discovery in cereals through quantitative trait loci and expression analysis in water-use efficiency measured by carbon isotope discrimination. Plant Cell Environ., 34(12):2009-23. doi: 10.1111/j.1365-3040.2011.02397.x

Christina, M., Nouvellon, Y., Laclau, J.-P., Stape, J. L., Bouillet, J.-P., Lambais G.R., le Maire, G., 2016. Importance of deep water uptake in tropical eucalypt Forest. Funct. Ecol., doi: 10.1111/1365-2435.12727

Condon, A., Richards, R., Rebetzke, G., Farquhar, G., 2004. Breeding for high water-use efficiency. J. Exp. Bot. 55, 2447-2460.

Condon, A.G., Farquhar, G.D., Richards, R.A., 1990. Genotypic variation in carbon isotope discrimination and transpiration efficiency in wheat. Leaf gas exchange and whole plant studies. Aust. J. Plant Physiol., 17, 9–22.

Correia, I., Almeida, M.H., Aguiar, A., Alia, R., David, T.S., Santos Pareira, J., 2008. Variations in growth, survival and carbon isotope composition (13C) among Pinus pinaster populations of different geographic origins. Tree Physiol., 28, 1545–1552

Cotterill, P.P., Jackson, N., 1985. On index selection I. Methods of determining economic weight. Silvae Genetica 34,6-63.

Cotterill, P.P., Dean, C.A., 1990. Successful tree breeding with index selection. CSIRO. Division of Forestry and Forest Products 1990, Victoria Australia pp.xiii + 80 pp. ref.108

Cumbie, W.P., Eckert, A., Wegrzyn, J., Whetten, R., Neale, D., Goldfarb, B., 2011. Association genetics of carbon isotope discrimination, height and foliar nitrogen in a natural population of *Pinus taeda* L. Heredity, 107, 105-114.

Denis, M., Favreau, B., Ueno, S., Camus-Kulandaivelu, L., Chaix, G., Gion, J.M., Nourrisier-Mountou, S., Polidori J., Bouvet, J.M., 2013. Genetic variation of wood chemical traits and association with underlying genes in *Eucalyptus urophylla*. Tree Genet. Genomes, 9, 927-942.

Epron, D., Laclau, J.-P., Almeida, J.C.R., Gonçalves, J.L.M., Ponton, S., Sette Jr, C.R., Delgado-Rojas, J.S., Bouillet, J.-P., Nouvellon, Y., 2012. Do changes in carbon allocation account for the growth response to potassium and sodium applications in tropical *Eucalyptus* plantations? Tree Physiol., 32, 667-679.

FAO, 2010. Global Forest Resources Assessment 2010 Main report. FAO Forestry paper 163

Fardusi, M.J., Ferrio, J.P., Comas, C., Voltas, J., Resco de Dios, V., Serrano, L., 2016. Intra-specific association between carbon isotope composition and productivity in woody plants: A meta-analysis. Plant Sci., 251, 110-118.

Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the Relationship between Carbon Isotope Discrimination and the Intercellular Carbon Dioxide Concentration in Leaves. Aust. J. Plant Physiol., 9, 121-37

Farquhar, G., Richards, R.A., 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust. J. Plant Physiol., 11, 539–552.

Fernandes, T.J.G., Del Campo, A.D., Herrera, R., Molina, A.J., 2016. Simultaneous assessment, through sap flow and stable isotopes, of water use efficiency (WUE) in thinned pines shows improvement in growth, tree-climate sensitivity and WUE, but not in WUEi. For. Ecol. Manage., 361, 298–308.

Gallais, A., 1990. Théorie de la Sélection en Amélioration des Plantes. Masson: Paris, France, 588 p.

Gilmour, A.R., Gogel, B., Cullis, B.R., Thompson, R., 2006. ASReml, User Guide. Release 2.0. VSN International Ltd: Hemel Hempstead, UK.

Greaves, B.L., Borralho, N.M. G., Raymond, C. A., 1997. Breeding objective for plantation Eucalypts grown for production of kraft pulp. For. Sci., 43, 465–472, https://doi.org/10.1093/forestscience/43.4.465

Hazel, L. N. 1943. The genetic basis for constructing selection indexes. Genetics 28:476.

Hazel, L.N., Dickerson, G. E., Freeman, A. E., 1994. The Selection Index-Then, Now, and for the Future. J Dairy Sci 77:3236-3251

Hsiao, T.C., Acevedo, E., 1974. Plant responses to water deficits, water use efficiency, and drought resistance. Agr. Meteorol., 14, 59–84.

Hein, P.R.G., Bouvet, J.M., Mandrou, E., Vigneron, P., Clair B., Chaix, G., 2012. Age trends of microfibril angle inheritance and their genetic and environmental correlations with growth, density and chemical properties in *Eucalyptus urophylla* S.T. Blake wood. Ann. For. Sci., 69, 681-691.

Kanpanon, N., Kasemsap, P., Thaler, P., Kositsup, B., Rattanawong, R., Epron, D., 2017. Large variation in carbon isotope composition of bulk leaf material of rubber trees from a germplasm collection: an opportunity for improving water use efficiency in breeding programs. J. Rubber Res., 20, 58–70.

Konate, N.M., Dreyer, E., Epron, D., 2016. Differences in carbon isotope discrimination and whole-plant transpiration efficiency among nine Australian and Sahelian Acacia species. Ann. For. Sci., 73:995–1003.

Le Roux, D., Stock, W.D., Bond, W.J., Maphanga, D., 1996. Dry mass allocation, water use efficiency and d13C in clones of Eucalyptus grandis, *E. grandis x camaldulensis* and *E. grandis x nitens* grown under two irrigation regimes. Tree Physiol., 16, 497-502.

Li, C., Berninger, F., Koskela, J., Sonninen, E., 2000. Drought responses of Eucalyptus microtheca provenances depend on seasonality of rainfall in their place of origin Funct. Plant Biol., 27, 231-238.

Li, C., 2002. Carbon isotope composition, water-use efficiency and biomass productivity of Eucalyptus microtheca populations under different water supplies Physiol. Plantarum, 2000 - Wiley Online Library

Li, C., Wang, K., 2003. Differences in drought responses of three contrasting Eucalyptus microtheca F. Muell. Populations. For. Ecol. Manage., 179, 377-385.

Makouanzi, G., Chaix, G., Nourissier, S., Vigneron, P., 2017. Genetic variability of growth and wood chemical properties in a clonal population of *Eucalyptus urophylla × Eucalyptus grandis* in the Congo. South. Forest., 80, 151–158.

Mandrou, E., Hein, P.R.G., Villar, E., Vigneron, P., Plomion, C., Gion, J.-M., 2012. A candidate gene for lignin composition in Eucalyptus: cinnamoyl-CoA reductase (CCR). Tree Genet. Genomes, 8, 353–364.

Mareschal, L., Nzila, J.D.D., Turpault, M.P., Thongo M'Bou, A., Mazoumbou, J.C., Bouillet, J.P., Ranger, J., Laclau, J.P., 2011. Mineralogical and physico-chemical properties of Ferralic Arenosols derived from unconsolidated Plio-Pleistocenic deposits in the coastal plains of Congo. Geoderma, 162, 159–170.

Marguerit, E., Bouffier, L., Chancerel, E., Costa, P., Lagane, F., Guehl, J.-M., Brendel, O., 2014. The genetics of water-use efficiency and its relation to growth in maritime pine. J. Exp. Bot., 65, 4757–4768. https://doi.org/10.1093/jxb/eru226

Monclus, R., Dreyer, E., Delmotte, F.M., Villar ,M., Delay, D., Boudouresque, E., Petit, J.-M., Marron, N., Bréchet, C., Brignolas, F., 2005. Productivity, leaf traits and carbon isotope discrimination in 29 *Populus deltoides x P. nigra* clones. New Phytol., 167, 53-62

Nunes, F.N., Barros, N.F., de Novais, R.F., da Silva, I.R., Stape, J.L., 2016. Carbon isotope discrimination and differential drought tolerance in eucalypt clones. Sci. For., Piracicaba 44, 895-903

Oehlert, G. W., 1992. A Note on the Delta Method , The American Statistician, vol. 46, no 1, p. 27-29

Olbrich, B. W., Le Roux, D., Poulter, A. G., William, JB, William, D S, 1993. Variation in water use efficiency and 13C levels in *Eucalyptus grandis* clones. J. Hydrol., 150, 615-633

Osorio, J., Pereira, J.S., 1994. Genotypic differences in water use efficiency and C-13 discrimination in *Eucalyptus globulus*. Tree Physiol., 14, 871–882

Osório, J., Osório M.L., Chaves, M.M., Pereira, J.S., 1998. Effects of water deficits on 13C discrimination and transpiration efficiency of *Eucalyptus globulus* clones. Funct. Plant Biol., 25, 645-653.

Pešek, J., Baker, R.J., 1969. Desired improvement in relation to selection indices. *Can. J. Plant Sci.* 49: 803-804 http:// dx.doi.org/10.4141/cjps69-137.

Poke, F.S., Potts, B.M., Vaillancourt, R.E., Raymond, C.A., 2006. Genetic parameters for lignin, extractives and decay in *Eucalyptus globulus*. Ann. For. Sci., 63, 813–821

Plomion, C., Bartholomé, J., Bouffier, L., Brendel, O., Cochard, H., De Miguel, M., Porté, A., 2016. Understanding the genetic bases of adaptation to soil water deficit in trees through the examination of water use efficiency and cavitation resistance: maritime pine as a case study. J. Plant Hydraul., 3, 008. https://doi.org/10.20870/jph.2016.e008

Rambolarimanana, H., Ramamonjisoa, L., Verhaegen, D., Leong Pock Tsy, J.M., Jacquin, L., Cao-Hamadou, T.V., Makouanzi, C.G., Bouvet, J.-M., 2018. Performance of multi-trait genomic selection for *Eucalyptus robusta* breeding program. Tree Genet. Genomes, 14 , 13 p.

Ramirez-Valiente, J. A., Lorenzo, Z., Soto, A., Valladares, F., Gil, L., Aranda, I., 2009. Elucidating the role of genetic drift and natural selection in cork oak differentiation regarding drought tolerance. Mol. Ecol., 18(18), 3803–3815.

Rasheed, F., Dreyer, E., Richard, B., Brignolas, F., Montpied, P., Le Thiec, D., 2013. Genotype differences in 13C discrimination between atmosphere and leaf matter match differences in transpiration efficiency at leaf and whole-plant level in hybrid *Populus deltoides × nigra*. Plant. Cell. Envir., 36, 87-102. - doi: 10.1111/j. 1365-3040.2012.02556.x

R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing: Vienna, Austria.

Roussel, M., Le Thiec, D., Montpied, P., Ningre, N., Guehl, J.-M., Brendel, O. 2009a. Diversity of water use efficiency among *Quercus robur* genotypes: contribution of related leaf traits. Ann. For. Sci., 66, 408.

Roussel, M., Dreyer, E., Montpied, P., Le-Provost, G., Guehl, J.-M., Brendel, O., 2009b. The diversity of (13)C isotope discrimination in a Quercus robur full-sib family is associated with differences in intrinsic water use efficiency, transpiration efficiency, and stomatal conductance. J. Exp. Bot., 60, 2419–2431.

Shelbourne, C. J. A., Low C., B., 1980. Multi-trait selection and genetic gains of *Pinus radiata* progenies at five sites New Zealand Journal of Forestry Science Vol. 10 No. 2 (1980): 307-24

Smith, H.F. 1936. A discriminant function of plant selection. Ann. Eugenics, 7: 240-250.

Schröder, T., Pereira, L. D., dos Santos Ananias Hofiço, N., 2013. Comparing methods for total Volume estimation in *Eucalyptus grandis* W. Hill ex Maiden Floresta e ambiente, 20(4), 480-486

Stape, J.L., Binkley, D., et al. 2010. The Brazil Eucalyptus Potential Productivity Project: Influence of water, nutrients and stand uniformity on wood production. For. Ecol. Manage., 259, 1684-1694.

Stackpole, D.J., Vaillancourt, R.E., Alves, A., Potts, B.M., 2011. Genetic Variation in the Chemical Components of Eucalyptus globulus Wood - Genes Genomes Genet., 1,151-159.

Verlinden, M.S., Fichot, R., Broeckx, L. S., Vanholme, B., Boerjan, W., Ceulemans, R., 2015. Carbon isotope compositions ($\delta^{13}C$) of leaf, wood and holocellulose differ among genotype of poplar and between previous land uses in a short-rotation biomass plantation. Plant. Cell. Environ., 38, 144–156.

Wartelle, R., Dreyfus, J., 2010. Synthèse bibliographique sur la production de biomasse d'origine arborée. Chambre d'Agriculture de Picardie; Programme CASDAR Agroforesterie, p 31.

Wei X., Borralho N.M.G., 1999. Objectives and selection criteria for pulp production of *Eucalyptus urophylla* plantations in South East China. Forest Genetics 6(3); 181-190

White, T. L., Adams, W.T., Neale D. B., 2007. Forest Genetics CABI Pub., 682 pages

Xu, Z., Prasolovab, N., Lundkvist, K., Beadled, C., Leamanb, T., 2003. Genetic variation in branchlet carbon and nitrogen isotope composition and nutrient concentration of 11-year-old hoop pine families in relation to tree growth in subtropical Australia. For. Ecol. Manage., 186, 359–371.

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.

					Variance components								Variance ratios						
Trait	Mean	Min	Max	CV %	σ^2_{af}	SE	σ^2_{am}	SE	σ^2_d	SE	σ^2_r	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).

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)

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		Equal emphasis method	Observed relative gain ΔGu (%)				Desired gain method	Observed relative gain ΔG_u (%)				R: Spearman correlation[b]
	s(%)	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, 0.081, 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 coefficient were: m^{-3}, $(\mu mol\ mol^{-1})^{-1}$, $\%^{-1}$ and $\%^{-1}$, for V55, HCEL, KL and W_i respectively

[b] Spearman correlation for the index ranking.

[c] actual economic weights; [d] transformed economic weights

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		Equal emphasis method	Observed relative gain ΔG_g (%)				Desired gain method	Observed relative gain ΔG_g (%)				R: Spearman correlation[b]
	s (%)	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 coefficient were: m^{-3}, $(\mu mol\ mol^{-1})^{-1}$, $\%^{-1}$ and $\%^{-1}$, for V55, HCEL, KL and W_i respectively

[b] Spearman correlation for the index ranking.

[c] actual economic weights; [d] transformed economic weights

Table 5: Economic coefficients, expected relative additive (ΔG_u) and total genetic gain (ΔG_g) following selection on indices using economic weights determined by the desired gain method. The expected gains were calculated for an intensity of selection of 10% and 1%. 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		Equal emphasis	Observed relative gain ΔG_u (%)				Desired gain method	Observed relative gain ΔG_u (%)			
	s (%)	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[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	1.2, 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		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 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 coefficient were: m^{-3}, $(\mu mol\ mol^{-1})^{-1}$, $\%^{-1}$ and $\%^{-1}$, for V55, HCEL, KL and W_i respectively

[c] actual economic weights; [d] transformed economic weights

Figure 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 ($\delta^{13}C$ in ‰), c) the lignin content (KL in %) and d) the holo-cellulose content (HCEL in %).