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ORIGINAL PAPER

Genetic parameters of growth, straightness and wood chemistry traits in *Pinus pinaster*

Camille Lepoittevin · Jean-Pierre Rousseau · Audrey Guillemin · Christophe Gauvrit · François Besson · François Hubert · Denilson da Silva Perez · Luc Harvengt · Christophe Plomion

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Abstract
• ***Introduction*** Tree breeding is giving an increasing attention to wood properties in order to better fit the requirements of the saw, board, pulp and paper industries. In particular, it has been reported that lignin and cellulose content display moderate to high heritabilities making them prime candidates for genetic improvement of wood chemistry. Moreover, these traits have been shown to be negatively correlated at both phenotypic and genetic levels. However, they have generally been evaluated against a narrow genetic background, and little is known about their correlations with mandatory selection criteria such as growth and straightness.
• ***Materials and methods*** In this study, we first investigated the performance of near-infrared (NIR) spectroscopy combined with a non-destructive sampling method to assess chemical properties of wood in maritime pine. We afterwards estimated genetic parameters of growth, stem form and wood chemistry traits across a large genetic background in a progeny trial and clonally replicated progenies.
• ***Results*** Our results showed that removal of extractives prior to NIR spectra acquisition is highly recommended for achieving high accuracy in NIRS-PLSR prediction for wood chemistry traits in maritime pine.

We further observed moderate heritabilities (0.15–0.55) for the studied traits. Wood chemistry traits were genetically inter-correlated (e.g., negatively between lignin and cellulose), whereas correlations with growth were not significant, indicating that growth and chemical properties could be improved independently.

Keywords *Pinus pinaster* · Wood chemical properties · Growth · Heritability · Genetic correlations · Additivity · Dominance · Epistasis · Wood sampling

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C. Lepoittevin (✉) · F. Hubert · C. Plomion
INRA, UMR1202 BIOGECO,
33612 Cestas, France
e-mail: camille.lepoittevin@pierroton.inra.fr

C. Lepoittevin · F. Hubert · C. Plomion
UMR1202 BIOGECO, Université de Bordeaux,
33400 Talence, France

C. Lepoittevin · L. Harvengt
FCBA, Laboratoire de Biotechnologies,
77370 Nangis, France

J.-P. Rousseau · F. Besson
FCBA, Station Sud-Ouest,
33480 Moulis-en-Médoc, France

A. Guillemin · D. da Silva Perez
FCBA InTechFibres, Pôle Nouveaux Matériaux,
Domaine Universitaire,
38044 Grenoble, France

C. Gauvrit
INRA, UE0570 Domaine Expérimental de l’Hermitage,
33612 Cestas, France

1 Introduction

Lignin and cellulose are major components of wood, and the most abundant biopolymers on Earth. Lignin accumulates in the secondary cell wall of xylem cells, playing a key role in sap conduction, mechanical support and biotic and abiotic stress resistance (Plomion et al. 2001). Although lignin is used as a fuel for bio-power generation, it is undesirable in the conversion of wood into pulp since it causes discoloration and reduces paper brightness upon thermal or light exposure (Chiang et al. 1988). Its removal

is a major step in the papermaking process for "chemical pulps", which consumes large quantities of energy and chemicals. In addition, lignin acts as cement between fibres and is also responsible for the high energy consumption in thermo-mechanical pulp production due to energy required to separate fibres. Conversely, cellulose content is favourably correlated with pulp yield (Wallis et al. 1996; Kube and Raymond 2002). Breeders are, therefore, seeking ways to increase the cellulose to lignin ratio. Previous studies in various forest tree species showed that lignin and cellulose contents have moderate heritabilities and are strongly negatively correlated at both phenotypic and genetic levels (Baillères et al. 2002; Pot et al. 2002; Sykes et al. 2006; Da Silva Perez et al. 2007), highlighting the possibility of obtaining significant genetic gains for the cellulose to lignin content ratio by classical breeding methods. However, wood chemistry traits have generally been evaluated against a narrow genetic background such as diallel and factorial mating designs involving a low number of genotypes. Moreover, little is known about their correlations with mandatory selection criteria such as growth and straightness, or with other end-use properties such as wood density, modulus of elasticity or fibre-related traits (Pot et al. 2002; Da Silva Perez et al. 2007). Recent studies involving transgenic poplars and radiata pines suggest that the capacity for lignin reduction is limited: lignin contents reduced by less than 10% do not appreciably change tree growth characteristics (Pilate et al. 2002; Wagner et al. 2009; Voelker et al. 2010), whereas reductions in lignin content by about 20% can dramatically affect productivity (Leple et al. 2007; Wagner et al. 2009; Voelker et al. 2010). Considering the low natural variability of lignin content in conifer species (~3–4% in Costa e Silva et al. 1998; Pot et al. 2002; Hannrup et al. 2004), it can be hypothesized that breeding for low lignin content would not impact growth performance. Breeding for a slight decrease in lignin content, however, seems worthwhile given the volume of wood processed each year by the pulp industry (Peter et al. 2007).

In the last decade, wood phenotyping has been enhanced by the development of indirect tools such as the pilodyn penetrometer and the resistograph for measuring wood density (Wang et al. 1999; Bouffier et al. 2008), or near-infrared spectroscopy (NIRS) for predicting the wood chemical and physical properties of a large number of samples by fast optical measurements (reviewed by Tsuchikawa 2007). NIRS has been widely used in fields such as food, agriculture, pharmaceutical or chemical industries. However, its application in wood and paper science is recent (most of the results have been published in the 1990s) and appears as a cost- and time-efficient alternative to wet chemistry methods. On the other hand, due to the complexity of NIR spectra, all measurements are calibration dependent, which complicates wide-spread application (Da Silva Perez et al. 2008).

In this study, we first investigate the performance of NIRS combined with a non-destructive sampling method to assess wood chemical properties in maritime pine (*Pinus pinaster* Ait.), the principal conifer species used for large-scale reforestation in south-western Europe. We then estimate the genetic parameters of growth, stem form and wood chemistry traits using a progeny trial with clonally replicated individual offspring derived from control crosses, with the aim of assessing the relative importance of additive, dominance and epistatic effects for these traits in a material representing a broad genetic background. Finally, we estimate the phenotypic and genetic correlations between these traits and discuss the breeding opportunities for wood chemistry traits.

2 Materials and methods

2.1 Plant material

Two experimental trials, Hermitage (trial number 2-44-17) and Vaquey (trial number 33802), located in the Aquitaine region (south-western France) were examined (Table 1). The Hermitage trial was described in earlier publications where genetic parameters for a series of traits related to growth and density have been studied (Bouffier et al. 2008; Bouffier et al. 2009). Briefly, Hermitage is a 31-year-old progeny trial of plus-trees phenotypically selected in the Aquitaine forest for overall good growth and form. This material is commonly referred as the first generation breeding population (G_0). A total of 261 G_0 mother trees were crossed with a pollen mix collected from 28 unrelated G_0 trees, resulting in 8,667 individuals distributed in families of 12 to 36 half-sibs. This trial was installed on a humid sandy podzol moor site of low fertility (which represents 80% of the forested area in the Aquitaine region), using randomized complete block design with three, six or nine tree-row plots per family per block. Vaquey is a 13-year-old clonal trial of 189 trees from the second-generation breeding population (G_1) which were individually selected within full-sib progenies of 77 G_0 trees, based on their genetic value for growth and stem straightness. These 189 G_1 clones belonged to 78 full-sib families (some G_0 parents were used in several crosses) and were replicated three to five times, leading to a total number of 892 trees. This trial was established on a humic loamy clay soil using fully randomized single-tree plots. No field blocks were a priori defined. Material from this trial has been previously used for developing NIRS calibrations (Da Silva Perez et al. 2005), without any estimation of genetic parameters. These two trials will be referred as the

Table 1 Description of trials and sampling procedure

Trial		Hermitage (HST)	Vaquey (CT)
Plantation year		1975	1987
Blocks		4	0
Trees per plot		3, 6 or 9	1
Number of families or clones		261 families	189 clones/78 families
Number of trees		8,667	892
Seedlings		Bare roots	Containers
Plantation density		4×1.1 m	6×6 m
Soil		Humid sandy podzol moor	Loamy clay soil
Fertilisation		No	No
Growth (Ht, Gir, Diam)	Tree age (years)	8	8 (Ht), 13 (Diam)
	Number of families or clones	261	189 clones/78 families
	Number of trees	8,667	892
Form (Str)	Tree age (years)	8	–
	Number of families or clones	261	–
	Number of trees	8,667	–
Wood chemistry traits (Ext, Lign, Cell, Hemi, Mann, Gal, Xyl)	Tree age (years)	31	13
	Number of families or clones	105[a]	189 clones/78 families
	Number of trees	993	892
	Type of sample	Shavings from standing trees	Discs

[a] Families for which more than nine trees were still available in 2006, after two systematic thinning where half of the trees were removed in each family

Half-Sib Trial for Hermitage (HST), and the Clonal Trial for Vaquey (CT).

2.2 Data measurement

Table 1 describes the sampling procedure and the traits that have been measured on both trials. All the trees from the two trials were measured for growth traits, i.e. total height (Ht) and girth at 1.3 m (Gir) at 8 years for the HST, and Ht at 8 years and diameter at 1.3 m (Diam) at 13 years for the CT. Trees from the HST were also evaluated for stem deviation from verticality (Str) at 8 years (the deflection from to the vertical was measured at 1 m).

Chemical characterization of the samples was conducted separately for the two tests by NIRS. For the HST, the sampling was carried out at 31 years of age on 993 standing trees representing 105 different half-sib families, with seven to 12 trees per family. We collected shavings by drilling a hole of 2 cm in diameter and 5 cm in depth into the tree at breast height (1.3 m). The shavings were dried for 24 h at 60°C, ground in a SM-100 three-knife mill (Retsch, Haan, Germany) and sieved.

Near-infrared spectra acquisition was then carried out on the 40–60 mesh sawdust fraction using a MPA spectrometer with an integration sphere (Bruker Optics, Ettlingen, Germany). NIRS-partial least square (NIRS-PLSR) calibrations were developed using a subset of 98 samples measured for (1) extractives content (Ext) using an automatic SoxTec extractor (Foss, Hillrod, Denmark) and an acetone–water extraction sequence (Da Silva Perez et al. 2005), (2) lignin content (Lign) based on the Klason method (Schwanninger and Hinterstoisser 2002) and (3) polysaccharides: cellulose (Cell) and hemicellulose (Hemi) contents calculated from the wood monosugars contents liberated by acidic hydrolysis and analysed by HPLC (Puls et al. 1995). The glucose content was entirely attributed to cellulose (and therefore not studied), although it is known that a small fraction of this monosugar also enters in the composition of hemicelluloses. For this later component, the proportions of monosugars (mannose (Mann), galactose (Gal) and xylose (Xyl) content) can be variable and were included as wood chemistry traits. PLSR was performed according to Workman et al. (1996) and Martens and Naes (1989) using the OPUS Quant software (Bruker Optics).

NIRS-PLSR evaluation in the CT for Klason lignin, cellulose, hemicellulose, mannose, galactose and xylose contents at 13 years was carried out on extractive-free sawdust obtained from whole discs collected at breast height (1.3 m) as described in Da Silva Perez et al. (2005).

For both trials, the quality of NIRS-PLSR models was assessed using the rank (number of PLS eigen vectors used for the regression) and cross-validation results, i.e. coefficient of determination (R^2), and root mean square of error prediction (RMSECV) (Burns and Ciurczak 2008).

2.3 Statistical models for genetic parameter estimation

The individual-tree model was used to partition the phenotypic value of each tree in its genetic and environmental components.

The following model was used for the HST:

$$y = Xb + Z_1a + Z_2v + e \quad (1)$$

where y is a vector of observations on a trait, b is a vector of fixed block effects, a is a vector of random genetic effects of individual genotypes, v is a vector of random plot effects (block×half-sib family interactions), e is the vector of residuals, X, Z_1 and Z_1 are the incidence matrices linking observations to the effects. The random effects in model (1) were assumed to follow a normal distribution with means and variances defined by:

$$\begin{bmatrix} a \\ v \\ e \end{bmatrix} \sim N\left(\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} A\sigma_a^2 & 0 & 0 \\ 0 & I\sigma_v^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix} \right) \quad (2)$$

where 0 is a null matrix; A is the additive genetic relationship matrix (computed from a pedigree that takes into account all the relationships among individual genotypes); I is the identity matrix, σ_a^2 is the additive genetic variance, σ_v^2 the plot variance, and σ_e^2 is the residual variance. As the variances are assumed to be independent, the phenotypic variance σ_P^2 is defined as:

$$\widehat{\sigma_P^2} = \widehat{\sigma_a^2} + \widehat{\sigma_v^2} + \widehat{\sigma_e^2} \quad (3)$$

For the CT, two different models, named "full model" and "simplified model" were used. The full model is as follows:

$$y = Z_1a + Z_2f + Z_3c + e \quad (4)$$

where a and e are defined as above, f is a vector of random full-sib family effects, c is a vector of random effect of clones within full-sib families, Z_1, Z_2 and Z_3 are the incidence matrices linking the observations in y to the effects in a, f and c, respectively. The random effects in the model defined in Eq. 4 were assumed to follow normal distributions with means and variances defined by:

$$\begin{bmatrix} a \\ f \\ c \\ e \end{bmatrix} \sim N\left(\begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \end{bmatrix}, \begin{bmatrix} A\sigma_a^2 & 0 & 0 & 0 \\ 0 & I\sigma_f^2 & 0 & 0 \\ 0 & 0 & I\sigma_c^2 & 0 \\ 0 & 0 & 0 & I\sigma_e^2 \end{bmatrix} \right) \quad (5)$$

where 0, I, σ_a^2 and σ_e^2 are defined as above (observations on different ramets of a clone were treated as repeated measurements on a single genotype, therefore A is the matrix of relationships among individual genotypes as mentioned before); σ_f^2 is the non-additive genetic variance among full-sib families, and σ_c^2 is the non-additive variance among clones within full-sib families. This full model was used after excluding from the dataset the families with only one genotype, as they do not allow the estimation of dominance effects (Costa e Silva et al. 2009). Thus the full model was fitted for 36 families representing 143 clones and 705 phenotypes out of the 892 trees. The phenotypic variance σ_P^2, the total genetic variance σ_G^2, the dominance genetic variance σ_D^2 and the epistatic genetic variance σ_I^2 were defined as follows:

$$\widehat{\sigma_P^2} = \widehat{\sigma_a^2} + \widehat{\sigma_f^2} + \widehat{\sigma_c^2} + \widehat{\sigma_e^2} \quad (6)$$

$$\widehat{\sigma_G^2} = \widehat{\sigma_a^2} + \widehat{\sigma_f^2} + \widehat{\sigma_c^2} = \widehat{\sigma_a^2} + \widehat{\sigma_D^2} + \widehat{\sigma_I^2} \quad (7)$$

$$\widehat{\sigma_D^2} = 4 \times \widehat{\sigma_f^2} \quad (8)$$

$$\widehat{\sigma_I^2} = \widehat{\sigma_c^2} - 3 \times \widehat{\sigma_f^2} \quad (9)$$

For Eqs. 8 and 9, we assumed a large, random mating parental population with diploid inheritance and near linkage equilibrium at gene loci affecting the observed traits (Comstock et al. 1958; Foster and Shaw 1988). As $\widehat{\sigma_a^2}$ and $\widehat{\sigma_f^2}$ contain portions of epistasis with successively decreasing contributions of interactions involving larger groups of loci, the unbiased estimation of additive and dominance variances assumes that higher-order interloci interactions only represent a small portion of the total epistasis (Mullin and Park 1992; Wu 1996; Costa e Silva et al. 2004). Similarly, interactions involving groups of more than two or three loci are assumed in Eq. 9, since $\widehat{\sigma_I^2}$ contains only a fraction of the total epistasis with a major contribution of high-order interactions. In addition, non-genetic effects introduced by cloning ("C effects") (Foster and Shaw 1988; Costa e Silva et al. 2004) were assumed to be negligible or absent.

The simplified model entailed in dropping the family and clonal effects from the full model (4) described above, so that we could use the total dataset to estimate the variance components (i.e. 78 families, 189 genotypes and 892 phenotypes). In this model, the family structure is ignored although the kinship is accounted for. Dominance and epistatic genetic effects can no longer be estimated,

therefore only the total genetic variance $\widehat{\sigma_G^2}$ was estimated as described in Eq. 7.

The estimates of the fixed and random effects were obtained by solving Henderson's mixed model equations (Henderson 1975) using the average information REML algorithm (Gilmour et al. 1995) implemented in the ASReml v2.0 software (Gilmour et al. 2006). Wald tests as implemented in ASReml (Gilmour et al. 2006) and likelihood ratio tests were used to assess the statistical significance of the fixed and random effects, respectively.

Analyses were performed for each trait separately. Estimates of phenotypic, total genetic and additive genetic coefficients of variation ($\widehat{\mathrm{CV}_P}$, $\widehat{\mathrm{CV}_G}$ and $\widehat{\mathrm{CV}_A}$) as well as broad- and narrow-sense heritabilities ($\widehat{h^2_{\mathrm{bs}}}$ and $\widehat{h^2_{\mathrm{ns}}}$) were defined as follows:

$$\widehat{\mathrm{CV}_P} = \frac{\widehat{\sigma_P}}{\overline{X}};\ \widehat{\mathrm{CV}_G} = \frac{\widehat{\sigma_G}}{\overline{X}};\ \widehat{\mathrm{CV}_A} = \frac{\widehat{\sigma_a}}{\overline{X}}$$

$$\widehat{h^2_{\mathrm{bs}}} = \frac{\widehat{\sigma^2_G}}{\widehat{\sigma^2_P}};\ \widehat{h^2_{\mathrm{ns}}} = \frac{\widehat{\sigma^2_a}}{\widehat{\sigma^2_P}}$$

where $\overline{X}$ is the mean of the studied trait for the trial considered.

The estimated phenotypic ($\widehat{r_P}$), total genetic ($\widehat{r_G}$), and additive genetic ($\widehat{r_A}$) correlations between traits were obtained using bivariate extensions of the models previously described, for each pair of traits and each trial. They were evaluated as follows:

$$\widehat{r_P} = \frac{\widehat{\mathrm{Cov}_P}(x,y)}{\sqrt{\widehat{\sigma^2_{P_x}} + \widehat{\sigma^2_{P_y}}}} \qquad \widehat{r_G} = \frac{\widehat{\mathrm{Cov}_G}(x,y)}{\sqrt{\widehat{\sigma^2_{G_x}} + \widehat{\sigma^2_{G_y}}}} \qquad \widehat{r_A} = \frac{\widehat{\mathrm{Cov}_A}(x,y)}{\sqrt{\widehat{\sigma^2_{a_x}} + \widehat{\sigma^2_{a_y}}}}$$

where $\widehat{\mathrm{Cov}_P}(x,y)$, $\widehat{\mathrm{Cov}_G}(x,y)$ and $\widehat{\mathrm{Cov}_A}(x,y)$ are the phenotypic, genetic and additive genetic covariances between traits x and y, respectively. For the CT, the simplified model was used to estimate a total genetic correlations ($\widehat{r_G}$), including additive, dominance and epistatic effects. Approximate standard errors for estimated variances, heritabilities and correlation coefficients were calculated with ASReml using a standard Taylor series expansion (Sorensen and Gianola 2002; Gilmour et al. 2006).

Expected genetic gains were estimated independently for each trait by dividing the expected response (R) by the mean of the trait considered and expressed as a percentage. R was calculated for individual selection according to the following formula:

$$R = i \times \widehat{\sigma_P} \times \widehat{h^2_{\mathrm{ns}}}$$

with

- ***i*** selection intensity
- $\widehat{\sigma_P}$ estimated phenotypic standard deviation
- $\widehat{h^2_{\mathrm{ns}}}$ estimated narrow-sense heritability.

Expected genetic gains were only estimated for the HST, since our dataset did not allow accurate estimates of $\widehat{h^2_{\mathrm{ns}}}$ in the CT.

3 Results

3.1 Near-infrared spectroscopy calibrations

The performances of NIRS-PLSR calibrations for the prediction of chemical composition of maritime pine wood were assessed by cross validations and are shown in Table 2. Extractive, lignin, cellulose, mannose and galactose contents could be predicted by NIRS with a quite good accuracy in one or both trials, with low RMSECV and coefficients of determination (R^2) ranging from 0.70 to 0.96. The calibrations developed for the CT site generally performed better than those developed for the HST site, displaying higher R^2, lower rank and RMSECV. Calibrations showing a cross-validation R^2 below 0.7 (i.e. cellulose and mannose contents for the HST, and hemicellulose and xylose contents of both trials) were not used for genetic parameters estimation because of the low accuracy of their predictions.

3.2 Variance components

Estimates of variance components obtained from the analysis of the two trials are presented in Table 3. Growth and stem straightness showed moderate to high coefficients of phenotypic variation ($\widehat{\mathrm{CV}_P} = 15 - 25\%$ for growth and 54% for straightness), in agreement with previous results (Costa and Durel 1996; Pot et al. 2002). With the exception of Ext and Gal, $\widehat{\mathrm{CV}_P}$ for wood chemistry traits were low (<7%), consistent with most of the results reported in the literature (Costa e Silva et al. 1998; Pot et al. 2002; Hannrup et al. 2004). The fixed block effect and random plot effect in the HST were highly significant (p value of <0.1%) for all traits except for Lign, for which only the plot effect was significant. For both trials, $\widehat{\sigma^2_a}$ and $\widehat{\sigma^2_G}$ were highly significant with very low p values (<0.1%).

In the CT, $\widehat{\sigma^2_f}$ was accurately estimated and much lower than $\widehat{\sigma^2_a}$ for Ht, suggesting a low contribution of dominance effects on this trait. For Diam, both $\widehat{\sigma^2_f}$ and $\widehat{\sigma^2_c}$ were accurately estimated and again non-significant (p value of >5%) and much lower than $\widehat{\sigma^2_a}$, also indicating strong additive control. For the other traits, $\widehat{\sigma^2_f}$ and $\widehat{\sigma^2_c}$ were non-significant, with values similar to or greater than $\widehat{\sigma^2_a}$, and standard errors of the same magnitude as the estimates. In these cases, our dataset did not allow accurate estimates of non-additive variances, thus the simplified model was preferable to estimate heritabilities and correlations.

Table 2 Cross-validation performances of the NIRS-PLSR calibrations for the prediction of wood chemical composition in the Hermitage (HST) and Vaquey (CT) trials

Trait	Trial	Range (%)		Rank	Cross validation	
		Min	Max		R^2	RMSECV (%)
Ext	Hermitage	3.3	10.3	4	0.70	0.76
Lign	Hermitage	21.7	30.6	4	0.91	0.40
	Vaquey	25.8	32.7	3	0.96	0.40
Cell	Hermitage	36.8	47.4	3	0.53	1.51
	Vaquey	39.9	51.1	2	0.92	0.80
Hemi	Hermitage	20.1	28.2	8	0.34	1.22
	Vaquey	23.3	28.4	5	0.53	0.73
Mann	Hermitage	8.6	13.8	7	0.60	0.62
	Vaquey	14.5	20.0	1	0.87	0.55
Gal	Hermitage	1.4	6.1	10	0.82	0.49
	Vaquey	2.0	10.9	7	0.94	0.72
Xyl	Hermitage	3.7	8.2	8	0.30	0.86
	Vaquey	9.1	13.9	5	0.63	0.50

Data for Vaquey were taken from Da Silva Perez et al. (2005). Abbreviations as described in Section 2.2

Estimated broad-sense heritabilities in the CT were low to moderate, ranging from 0.17 to 0.23 for chemical-related traits and from 0.24 to 0.38 for growth. Narrow-sense heritabilities estimated in the HST ranged from 0.15 to 0.55, and were generally higher than broad-sense heritabilities estimated on the CT for the same traits using the full and simplified models (except Gir which was twice as low).

3.3 Genetic correlations

Estimated phenotypic and genetic correlations between traits are shown in Table 4 for the HST and Table 5 for the CT. In the HST, Ht and Gir were strongly correlated at the phenotypic and genetic levels ($\widehat{r_P} = 0.82$, $\widehat{r_A} = 0.86$), as expected, but weakly correlated with stem straightness at the genetic level ($\widehat{r_A} = 0.15$ and 0.24, respectively). In both trials, wood chemistry traits were significantly correlated, showing in most cases higher values for genetic than phenotypic correlations. The highest correlation observed was between Lign and Cell in the CT ($\widehat{r_G} = -0.98$ with a standard error of 0.01). We did not observe any significant genetic correlation between growth and chemical-related traits, although the phenotypic correlations were generally significant in the HST.

3.4 Expected genetic gains

Expected genetic gains were estimated for a selection of the 5% best units in the HST (Table 6). Although moderate to high genetic gains can be expected for most of the traits (8.51% to 39.98%), the expected genetic gain was very low for lignin content (1.51%).

4 Discussion

4.1 Rapid wood-quality assessment techniques

A prerequisite of a tree-breeding programme focusing on wood quality is the ability to measure whole-tree properties, which often implies the destructive sampling of discs to provide a bulk sample that represents the whole tree. Destructive sampling is extremely time-consuming and, owing to practical constraints, the number of trees that can be sampled is limited. An alternative to destructive sampling is to take an increment core or, as done in this study for the HST, to collect shavings by drilling a hole in the stem of a standing tree, and assume that the results are indicative of the whole-tree properties. However, this assumption can lead to erroneous results; indeed, we know that wood chemical composition shows significant radial variations, with mature wood (corewood as defined by Burdon et al. 2004) containing more cellulose and less lignin than juvenile wood (Zobel and Sprague 1998). The proportions of mature wood and juvenile wood in the powder obtained from increment cores or drill shavings are different from the true proportions of a stem section, and thus do not represent the whole tree. To illustrate this sampling bias, Augusto and Bert (2005) showed that using increment cores for determining nutrient contents generally leads to significant underestimations of whole-tree values in sapwood and overestimations in heartwood. Since the error associated with the drill sampling method increases with the drilling depth, restricting the sampling to the last rings should reduce this bias, but with the drawback of measuring only mature wood properties on adult trees. According to micro-density profiles of the HST (data from Bouffier et al.

Table 3 Variance components and genetic parameters estimated for Hermitage (HST) and Vaquey (CT) trials

Traits		Trials	Tree age (years)	Site mean	Variance estimates						Heritability estimates		CV estimates (%)		
					$\widehat{\sigma^2_v}$	$\widehat{\sigma^2_a}$	$\widehat{\sigma^2_f}$	$\widehat{\sigma^2_c}$	$\widehat{\sigma^2_G}$	$\widehat{\sigma^2_e}$	$\widehat{h^2_{bs}}$	$\widehat{h^2_{ns}}$	$\widehat{CV_P}$	$\widehat{CV_G}$	$\widehat{CV_A}$
Growth	Ht	Hermitage	8	5.80 m	2,640.00 (172.00)***	2,426.00 (541.00)***	–	–	–	3,344.00	–	0.29 (0.06)	15.8	–	8.5
	Ht	Vaquey (full model)	8	6.61 m	–	35.09 (15.44)***	5.41E−4 (1.2E−4)	3.88 (9.86)	38.97 (8.24)***	160.18	0.20 (0.03)	0.18 (0.07)	21.4	9.5	9.0
	Ht	Vaquey (simplified model)	8	6.52 m	–	–	–	–	52.58 (7.94)***	162.40	0.24 (0.03)	–	22.5	11.1	–
	Gir	Hermitage	8	28.18 cm	6.6 (0.63)***	7.79 (1.89)***	–	–	–	36.05	–	0.15 (0.04)	25.2	–	9.9
	Diam	Vaquey (full model)	13	20.38 cm	–	435.20 (85.60)***	2.86E−6 (1.7E−7)	2.63E−6 (1.60E−7)	435.20 (85.6)***	925.10	0.32 (0.05)	0.32 (0.05)	18.1	10.2	10.2
	Diam	Vaquey (simplified model)	13	20.07 cm	–	–	–	–	569.70 (90.5)***	913.00	0.38 (0.04)	–	19.2	11.9	–
Form	Str	Hermitage	8	9.11 cm	0.72 (0.19)***	7.03 (0.96)***	–	–	–	16.49	–	0.29 (0.04)	54.0	–	29.1
Chemical composition	Ext	Hermitage	31	6.7%	0.33 (0.07)***	0.58 (0.21)***	–	–	–	0.72	–	0.35 (0.12)	19.0	–	11.3
	Lign	Hermitage	31	26.8%	ns	0.16 (0.06)***	–	–	–	0.50	–	0.25 (0.09)	3.0	–	1.5
	Lign	Vaquey (full model)	13	30.2%	–	0.05 (0.15)***	0.08 (0.07)	0.08 (0.08)	0.21 (0.05)***	0.80	0.21 (0.04)	0.05 (0.15)	3.3	1.5	0.8
	Lign	Vaquey (simplified model)	13	30.2%	–	–	–	–	0.25 (0.05)***	0.84	0.23 (0.04)	–	3.5	1.7	–
	Cell	Vaquey (full model)	13	43.4%	–	0.18 (0.34)***	0.17 (0.16)	0.05 (0.19)	0.39 (0.11)***	1.84	0.17 (0.04)	0.08 (0.15)	3.5	1.4	1.0
	Cell	Vaquey (simplified model)	13	43.3%	–	–	–	–	0.42 (0.10)***	1.92	0.18 (0.04)	–	3.5	1.5	–
	Mann	Vaquey (full model)	13	17.1%	–	0.09 (0.11)***	0.05 (0.05)	0.04 (0.06)	0.17 (0.04)***	0.66	0.21 (0.04)	0.10 (0.13)	6.9	3.1	2.2
	Mann	Vaquey (simplified model)	13	17.1%	–	–	–	–	0.15 (0.04)***	0.68	0.19 (0.04)	–	7.0	3.0	–
	Gal	Hermitage	31	3.3%	0.15 (0.05)***	0.64 (0.18)***	–	–	–	0.37	–	0.55 (0.14)	33.1	–	24.6
	Gal	Vaquey (full model)	13	4.1%	–	0.02 (0.23)***	0.18 (0.13)*	0.10 (0.13)*	0.31 (0.08)***	1.27	0.20 (0.04)	0.01 (0.15)	30.6	13.6	3.6
	Gal	Vaquey (simplified model)	13	4.1%	–	–	–	–	0.28 (0.07)***	1.35	0.17 (0.04)	–	31.0	12.9	–

Standard errors are indicated in brackets. Effects that were not significant and not considered in the model are indicated by *ns*. The significance of random effects is indicated after each variance estimator:

* Significant at the 5% threshold

*** Significant at the 0.1% threshold

Table 4 Phenotypic and genetic correlations in the HST ($\hat{r_P}$ below and $\hat{r_A}$ above the diagonal, respectively)

	Ht	Gir	Str	Ext	Lign	Gal
Ht	–	0.86 (0.04)***	0.15 (0.12)	−0.46 (0.26)	−0.16 (0.25)	0.12 (0.23)
Gir	0.82 (0.004)***	–	0.24 (0.12)*	−0.10 (0.28)	−0.26 (0.26)	0.18 (0.25)
Str	0.27 (0.01)***	0.36 (0.01)***	–	−0.01 (0.21)	0.23 (0.19)	0.20 (0.17)
Ext	0.09 (0.04)*	0.14 (0.03)***	0.10 (0.03)***	–	−0.57 (0.20)**	−0.96 (0.13)***
Lign	−0.26 (0.03)***	−0.26 (0.03)***	−0.02 (0.03)	−0.32 (0.03)***	–	0.85 (0.12)***
Gal	−0.19 (0.04)***	−0.19 (0.03)***	0.04 (0.04)	0.03 (0.03)	0.57 (0.02)***	–

Standard errors are indicated in brackets after each coefficient. The significance of random effects is indicated after each variance estimator:

* Significant at the 5% threshold

** Significant at the 1% threshold

*** Significant at the 0.1% threshold

2008), 5 cm deep drillings corresponded to nine (for fast-growing trees) to 24 (for slow-growing trees) growth rings. Assuming a transition between juvenile and mature wood in maritime pine to occur around the 10th to the 12th growth ring (Zobel et al. 1972; Dumail et al. 1998), ~75% of the samples should mainly contain mature wood. Even if we generally avoided the sampling bias due to radial effects, the number of rings sampled greatly varied depending on the trees, thus a bias due to different annual climatic conditions is not possible to exclude. To our knowledge, the only published study using drill shavings to estimate conifer-wood chemical properties concluded that this sampling method gave errors that were too large for practical purpose (Jones et al. 2008). These authors used young trees (13-year-old loblolly pines), drilled holes from the cambium to the pith, and obtained poor NIRS-PLSR calibrations. Our sampling method was quite different in the HST (holes were drilled mainly in mature outerwood) and gave rather good calibrations, especially for lignin content (RMSECV of 0.40%).

NIRS analysis relies on developing a calibration that relates the NIR spectra of a large number of samples to their known chemical or physical properties. Once the calibration has been set, NIR spectra can be used to predict these properties with the advantages of minimal sample preparation, rapid acquisition times, non-contact and non-destructive spectral acquisition (So et al. 2004). In the present study, two different types of samples were used for NIR spectra acquisition: (1) raw sawdust for the HST samples with the idea to routinely apply this low-cost and time-saving method into breeding and (2) extractive-free sawdust for the CT samples. They led to different calibration performances, the latter giving higher NIRS-PLSR prediction accuracy for all the analysed traits. Such a result has previously been reported for eucalypts, with the improvement of calibration models for lignin content and sugar monomer composition after elimination of wood extractives (Baillères et al. 2002; Da Silva Perez et al. 2008). To explain such difference these authors hypothesized that polyphenolic compounds of wood extracts alter the lignin absorption bands located in the same spectral zones. Despite this result, NIRS calibration for lignin content based on non-extracted powder was of high quality in the present study, as it provided good predictions (RMSECV of 0.40%). However, this was not the case for cellulose and most of monosugar calibrations, which showed very low performance for the raw sawdust. Extractives are a complex group of cell wall and lumen

Table 5 Phenotypic and genetic correlations in the CT ($\hat{r_P}$ below and $\hat{r_G}$ above the diagonal, respectively)

	Diam	Lign	Cell	Mann	Gal
Diam	–	−0.007 (0.13)	−0.12 (0.14)	0.09 (0.14)	0.18 (0.14)
Lign	−0.04 (0.04)	–	−0.98 (0.01)***	−0.87 (0.05)***	0.64 (0.08)***
Cell	−0.02 (0.04)	−0.94 (0.004)***	–	0.89 (0.06)***	−0.79 (0.06)***
Mann	0.05 (0.04)	−0.66 (0.02)***	0.49 (0.03)***	–	−0.75 (0.09)***
Gal	0.05 (0.04)	0.82 (0.01)***	−0.86 (0.01)***	−0.61 (0.03)***	–

Standard errors are indicated in brackets after each coefficient. The significance of random effects is indicated after each variance estimator:

*** Significant at the 0.1% threshold

Table 6 Expected genetic gain for each trait considered independently for a selection of the 5% best individuals

Trait	Target	Expected genetic gain (%)
Ht	Increase	11.15
Gir	Increase	8.51
Str	Decrease	20.18
Ext	Decrease	12.15
Lign	Decrease	1.51
Gal	Decrease	27.50

Trait abbreviations are described in Section 2.2

chemicals mainly consisting of fats, fatty acids, fatty alcohols, phenols, terpenes, steroids, resin acids, waxes and many other minor organic compounds (Rowell 2005). Their interaction with cellulose and monosugar absorption bands is likely, although to our knowledge it has never been explicitly reported. The differences between calibrations based on raw or extractive-free sawdust could also be due to other factors such as the sampling age (CT was sampled at 13 years while the HST was sampled at 31 years). Sampling method (discs versus drill shavings) may also be involved, but this seems unlikely, as poor sugar calibrations have already been reported for young loblolly pine raw sawdust ground from discs, while lignin calibration performed well (Jones et al. 2008), or for young maritime pine raw sawdust ground from whole stems (data not shown). Actually, these technical issues for assessing cellulose content can be easily avoided provided that lignin content is accurately predicted, because lignin and cellulose contents are strongly negatively correlated at both phenotypic and genetic levels, as found in the CT ($\widehat{r_P} = -0.94$ and $\widehat{r_G} = -0.98$, with standard errors of 0.004 and 0.01, respectively). This cellulose–lignin balance has been largely studied in conifers (Chantre et al. 2002; Pot et al. 2002; Sewell et al. 2002; Pot et al. 2006; Da Silva Perez et al. 2007), and confirmed by transgenic studies in various plant species where a cross talk between both pathways has been suggested (Hu et al. 1999; Baucher et al. 2003; Park et al. 2004).

4.2 Genetic effects and heritabilities

Genetic variance among individuals is usually partitioned into additive and non-additive components, the latter being a global term including allelic interactions within loci (dominance) and interactions between genes at two or more loci (epistasis) (Falconer and Mackay 1996). Selection response for traits mainly controlled by non-additive effects is not predictable from parental performance and non-cumulative over generations. Although they have been intensively studied in the last decades, contradictory results have been published for growth and stem form, showing either low (Foster and Shaw 1988; Kusnandar et al. 1998; Isik et al. 2003; Baltunis et al. 2007; Da Silva Perez et al. 2007) or moderate (Cotterill et al. 1987; Pot et al. 2002; Hannrup et al. 2004) non-additive control. This heterogeneity could be due to the low sample sizes in the diallel and factorial designs employed. While these mating schemes are useful for the partitioning of the genetic variance into its sub-components, they generally involve a low number of parents (the number of parents ranged from six to 38 in the above-mentioned studies). However, large samples are generally needed to accurately estimate non-additive genetic variances (Foster and Shaw 1988; Costa e Silva et al. 2004). Recently, Costa e Silva et al. (2004) provided an elegant model to estimate dominance and epistatic effects using clonally replicated progenies of eucalypts: with 153 full-sib families originating from 79 parents, they estimated that dominance and epistatic effects accounted for between 0% and 4% and 0.4% of the phenotypic variance in stem diameter and for 0% and 5% of the phenotypic variance for pilodyn penetration (an indirect measure of wood density), respectively. In the present study, using the same model but a lower number of parents and families (47 and 36, respectively), we showed that non-additive effects for Ht as well as dominance effects for Diam were accurately estimated and not significantly different from 0. However, our experimental design did not allow to accurately estimate non-additive variances for wood chemistry traits, which again shows that large datasets are required to reduce the standard errors of these components to low values.

Interestingly, for the same traits, narrow-sense heritabilities (h^2_{ns}) estimated on the HST were generally higher than broad-sense heritabilities (h^2_{bs}) estimated on the CT, whereas the opposite was expected (or at least equality between both estimates). A first hypothesis is that the error variance in the CT is larger than in the HST, as it includes a component of the environmental variance due to the absence of blocks in the trial, thus h^2_{bs} may be underestimated. For example, when the block effect was omitted from model (1) in the HST, h^2_{ns} was underestimated by ~10% for form and wood chemistry traits and not significantly different from 0 for growth, because error and plot variances were greatly inflated. This illustrates the importance of controlling for environmental effects in large forestry trials, and also shows that growth traits are more sensitive to micro environmental conditions than wood chemistry traits. A spatial analysis of the CT would certainly improve heritability estimates (unfortunately, spatial data were not available for this trial). A second hypothesis is that changes with radial position could also be partly responsible for heritability variations between both trials, as wood chemistry traits were measured at different ages: 13 versus 31 years old for the CT and the HST,

respectively. This bias could even be amplified by our sampling method in the HST, as previously discussed. Costa and Durel (1996) and Danjon (1994) showed that heritability of growth-related traits increased with age in maritime pine, and wood density also seems to follow this trend (Bouffier et al. 2008; Gaspar et al. 2008). However, to our knowledge time-trend analysis of wood chemistry traits heritability has not yet been reported. Further analyses will be necessary to confirm or reject this hypothesis. A third hypothesis is that half-sib families in the HST included full-sib pairs of genotypes that were not accounted for in our pedigree matrix, leading to an over-estimation of h^2_{ns}. Recently, Gaspar et al. (2009) showed using molecular markers that the coancestry coefficient of maritime pine open-pollinated families was 0.130, instead of the 0.125 value usually assumed for half-sib families. This underestimation of offspring relationships did not dramatically change heritability estimates in the studied trial, but simulations showed that the presence of around 10% of full-sibs in half-sib families is enough to produce heritability over-estimation by ~10% (Gaspar et al. 2009). In our case, the progenies of the HST were not obtained by open pollination but by a controlled cross involving a pollen mix collected from 28 trees (i.e. a "polycross" trial), thus the family coancestry coefficient could be higher than that of open-pollinated families, resulting in significant bias in heritabilities. Only the genotyping of the half-sib families and their parents with highly polymorphic markers such as microsatellites, as in Gaspar et al. (2009), would allow quantification of this bias and allow more accurate variance estimates.

4.3 Perspectives for breeding applications

We observed low to moderate heritabilities for all the studied traits, and the expected genetic gains for mandatory traits of the maritime pine breeding programme (growth and stem form) were higher than that observed for lignin content. However, given the volume of wood processed each year by the pulp industry and its predicted increase, even slight modifications of wood chemistry trait performances would be of commercial value. Indeed, an increase in cellulose to lignin ratio would be advantageous in decreasing energy and chemical consumption (Rydholm 1965), as well as increasing pulp yield. A recent economic study on loblolly pine showed that a 4% decline in lignin content (from 29% to 25% after selection) could yield a 4% increase in mill profits, but the advantages of further decline were eroded substantially by the loss of mill biopower generation and the consequent need to purchase more power from the open market (Peter et al. 2007). Our study shows that a ~1.5% decrease in lignin content could be achieved in one maritime pine breeding generation with a 5% selection rate, in agreement with previous findings (Pot et al. 2002; Da Silva Perez et al. 2007). This breeding programme is in its third generation of selection, therefore significant genetic gain can be expected by classical breeding. However, genetic gains per unit of time are inevitably slow because of the long generation time and the fact that many traits can only be scored at rotation age. Further analyses of wood chemistry traits are thus needed to assess the extent of age–age correlations and genotype-by-environment interactions, to quantify better the trade-offs with other wood-quality-related traits or the relative importance of additive and non-additive effects in their genetic control. In this context, the sampling method and NIRS technique used in the present study may offer some advantages when compared with conventional destructive sampling and wet chemistry methods. This rapid assessment technique gives estimates with reasonable precision for lignin content, hence contributing to a reduction in measurement costs.

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