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Abstract

Classical quantitative genetics and quantitative trait dissection analysis (QTL) approaches were used in order to investigate the genetic determinism of wood cellulose carbon isotope composition ($\delta^{13}C$, a time integrated estimate of water use efficiency) and of diameter growth and their relationship on adult trees (15 years) of a forest tree species (maritime pine). We used a half diallel experimental set-up to (1) estimate heritabilities for $\delta^{13}C$ and ring width and (2) to decompose the phenotypic $\delta^{13}C$ / growth correlation into its genetic and environmental components. We found considerable variation for $\delta^{13}C$ (range of over 3‰) and for ring width (range of over 5 mm) and significant heritabilities (narrow sense 0.17 / 0.19 for $\delta^{13}C$ and ring width, respectively, 100% additivity). The significant phenotypic correlation between $\delta^{13}C$ and ring width was not determined by the genetic component, but was attributable to environmental components. Using a genetic linkage map of a full-sib family, four significant and four suggestive QTLs were detected for $\delta^{13}C$, the first for $\delta^{13}C$ in a forest tree species, as far as known to the authors. Two significant and four suggestive QTLs were found for ring width. No co-location of QTLs was found between $\delta^{13}C$ and growth.

Keyword Index :

stable isotope, ^{13}C, growth, water use efficiency, heritability, quantitative trait, Pinus pinaster, tree rings

Introduction

In given environmental conditions, trees with high water-use efficiency at the leaf level (intrinsic WUE, defined as the ratio of net CO_2 assimilation rate *A* to stomatal conductance for water vapour *g*) can maintain higher growth rates under water limited conditions than trees with lower WUE (Sun *et al.* 1996; Nguyen-Queyrens *et al.* 1998). Measurements of plant carbon isotope composition ($\delta^{13}C$) provide time-integrated estimates of WUE (Farquhar, O'Leary & Berry 1982; Farquhar & Richards 1984) that can be applied to adult trees (Zhang & Marshall 1994; Zhang & Marshall 1995; Guehl *et al.* 1995; Sun *et al.* 1996). Assessments of differences in WUE among- and within-tree species are facilitated by the crown- and time-integrative nature of tree ring $\delta^{13}C$.

Forest tree species are known to be among the most polymorphic species of the flora (Hamrick, Godt & Sherman-Broyles 1992). Genotypic differences in leaf $\delta^{13}C$ of conifer species were found among provenances in common garden studies (Zhang, Marshall & Jaquish 1993; Zhang *et al.* 1994; Zhang *et al.* 1995; Guehl *et al*. 1995; Nguyen-Queyrens *et al.* 1998). It has been suggested that provenance differences of $\delta^{13}C$ might be determined by differences in stomatal sensitivity to changes in vapour pressure deficit (Zhang *et al.* 1995) and/or differences in plant hydraulic characteristics (Guehl *et al.* 1995). However, differences of $\delta^{13}C$ among genetic families within provenances of *Picea mariana* (Mill.) were found to be mainly determined by differences in photosynthetic capacity (Johnsen & Major 1995; Major & Johnsen 1996). Similar indications were obtained for maritime pine (*Pinus pinaster* Ait.) by Guehl *et al.* (1995).

Tree growth is an important goal for forest tree breeding programs. To avoid inadvertent negative selection for growth when selecting for high WUE, it is important to know if $\delta^{13}C$ and growth are genetically linked. Positive but weak phenotypic relationships between $\delta^{13}C$ and height or diameter growth (Flanagan & Johnsen 1995; Johnsen *et al.* 1999; Nguyen-Queyrens *et al.* 1998) have been found among trees within different forest tree species. Genetic parameters calculated for physiological or morphological traits can disentangle phenotypic relationships into genotypic and environmental components. Johnsen *et al.* (1999) found strong genetic correlations between $\delta^{13}C$ and tree height or tree diameter. They concluded that *A* was determining $\delta^{13}C$ and growth performance and thus constituted probably the link between the two traits. However, since $\delta^{13}C$ as an indicator of WUE could be either controlled by *A* and / or by *g*, there is not necessarily a strong relationship between *A* and $\delta^{13}C$. This suggests that the existence of a genetic correlation between $\delta^{13}C$ and growth is depending on the factor by which WUE is controlled.

Adaptive traits, like $\delta^{13}C$, are characterised by high phenotypic variation among and within populations of forest tree species (Meinzer *et al.* 1992; Zhang *et al.* 1993; Zhang *et al*. 1995; Flanagan *et al.* 1995; Nguyen-Queyrens *et al.* 1998). Moreover, high heritabilities for $\delta^{13}C$ have been found for non-woody (Matus, Slinkard & Van Kessel 1995; Asay, Johnson & Palazzo 1998) and woody species (Johnsen *et al.* 1999). The development of genetic mapping (Tanksley 1993) has made it possible to localize genetic factors controlling quantitative traits (QTLs, Quantitative Trait Loci). High heritability of a trait is a favourable factor for quantitative trait dissection analysis.

In crop plant breeding, improvement of WUE has been an important aim and therefore the first QTLs for $\delta^{13}C$ were detected in tomato (Martin *et al.* 1989). Mansur *et al.* (1993) found in a preliminary investigation of $\delta^{13}C$ on soybean one large genomic region that could be responsible for as much as 53% of the observed variation. In a study of three weeks old barley plants (Pakniyat *et al.* 1997), twelve AFLP markers were detected for $\delta^{13}C$, two of these markers alone accounted for 53.2% of the variation. QTLs for water use efficiency, as measured by the ratio of dry weight to water used, were found in soybean (Mian *et al.* 1996; Mian, Ashley & Boerma 1998).

Detection of QTL on woody species, however, is still in development, due to long generation time and therefore the lack of controlled crosses. Genetic maps have often to be constructed from F1 full-sib progenies. Carlson *et al.* (1991) were the first to show that RAPD primers could be screened for informative markers segregating in a 1:1 ratio in diploid tissue of full-sib progenies. Grattapaglia and Sederoff (1994) extended this idea in constructing parental maps of an interspecific eucalyptus hybrid family in a mapping strategy named “two-way pseudo-testcross”. It was further used in conifers (Kubisiak *et al.* 1996, Arcade *et al.* 2000) with RAPDs and AFLPs. For maritime pine the genome coverage required for linkage map construction and QTL analysis was achieved by using RAPD markers (Plomion *et al*.1995a; Plomion, O'Malley & Durel 1995b) and AFLP analysis (Costa *et al.* 2000).

Our objectives were: 1) To estimate the variability and heritability of $\delta^{13}C$ and ring width in a forest tree species (*Pinus pinaster* Ait.) using a half-diallel experimental design. 2) To investigate the phenotypic correlation between $\delta^{13}C$ and growth. 3) To separate the phenotypic correlation between $\delta^{13}C$ and growth into a genetic and an environmental component. 4) To dissect $\delta^{13}C$ and ring width into mendelian inherited components (quantitative trait dissection analysis) using a *Pinus pinaster* Ait. full-sib family. 5) To compare QTLs for $\delta^{13}C$ with QTLs for growth.

Material and Methods

Half Diallel

A twelve by twelve half-diallel of maritime pine (*Pinus pinaster* Ait.) was used to estimate the variability, heritability and genetic correlations among the studied traits. Parental trees were crossed in 1980 and seeds from the controlled crosses sown in a nursery in spring 1982 and planted in autumn 1982. The 12 parents were trees phenotypically selected for stem growth and straightness in the local provenance of the Landes de Gascogne. The half-diallel was located in Cestas (Gironde, France, 944°N44' 0°W44') on a semi-humid podzolic soil. Spacing was 4 m between rows and 1.1 m between individual trees, i.e. 2272 trees/ha. No selfed crosses were analysed, therefore the half-diallel consisted of 66 families (12 female and 11 male parents) of 15 to 15 individuals each. Three families were not available, therefore only 63 families were analysed. A parentage test was performed using 3 microsatellites (Gerber *et al.* 2000) confirming the authenticity of the progenies used in the half diallel. The experimental design consisted of 74 incomplete randomised blocks (the large number of blocs is due to the fact that the presented half-diallel is part of a much larger complete diallel). For the present study, 564 trees were cut in march 1997 (trees were fifteen years old). Disks were sampled, dried in a greenhouse and analysed for carbon isotopic composition ($\delta^{13}C$) and ring width as described below.

Full-sib family

A three-generation outbred pedigree comprising 202 fifteen-year-old trees was used to study the genetic architecture of the studied traits, i.e. the number, genome location and effect of Quantitative Trait Loci. The four grand parents were trees phenotypically selected for stem growth and straightness in the local provenance of the Landes de Gascogne and grafted in clonal archives. These grand parents were tested in a polycross progeny test and classified according to their breeding value as "Vigor +" (for vigorous trees) and "Vigor -" (for less vigorous trees). Each of the parental trees is the result of the cross of one "Vigor +" and one "Vigor -" grandparent. The two parental trees were crossed in 1980 and seeds from the controlled cross-sown in spring 1982. They produced progeny seedlings that were planted in autumn 1982. The family was located in Malente (Gironde, France, 44° N 3230' 0° W 47') on a semi-humid podzolic soil. Spacing was 4 m between rows and 1.1 m between individual trees, i.e. 2272 trees / ha. The trees were felled in March 1997 and stem discs were cut, dried in a greenhouse and analysed for $\delta^{13}C$ and ring width. From all the analysed families, 16 trees with no visible growth in the last four years before harvest were removed from the analysis.

Ring width measurements

Wood subsamples were taken from four positions on the circumference of the stem discs for the last four years of growth (1993 to 1996). As the trees were cut in march 1997, this includes any growth during winter 96/97 utilising reserve material from the summer 1996 growth period. Two different methods were used for ring width measurements. For the half diallel experiment, ring width was measured using the indirect X ray-method first described by Polge (1966). For the full-sib family, the ring widths were measured at the four sampling points on the circumference using a

semi-automatic system consisting of a digitising tablet linked to a computer (precision 0.1 mm standard deviation). The width was averaged for each ring. To make the ring width data comparable to the $\delta^{13}C$ measurements, for each tree the mean growth was calculated for the years 1993 to 1996 (mean ring width: MRW), using an arithmetic mean.

Isotope measurements

The $\delta^{13}C$ was measured of a bloc of four rings, which represents a ring-width weighted mean of the $\delta^{13}C$ of each ring. The sampled blocks of wood were cut by hand into small pieces, pre-ground in a centrifugal mill (Tecator, Cyclotech 1093 Sample Mill, Höganäs, Sweden) and milled to a fine powder in a ball mill (Retsch, MM2000, Haan, Germany). Cellulose was extracted after an acidic acid /nitric acid procedure described in Brendel, Iannetta & Stewart (2000). In brief, the method uses a concentrated nitric acid / 80% acetic acid on-to-ten dilution (0.2 cm^3 in 2 cm^3) to digest lignin, proteins and hemicelluloses in 50 mg of powdered wood sample. The digested molecule fragments are then washed out using ethanol, remainders of acid are removed during a water wash. The samples are dried chemically with a pure ethanol / acetone progression and physically in a vacuum centrifugal evaporator (speed vac) at 100 hPa for 2 h. The original protocol (Brendel *et al.* 2000) was modified to include two extraction cycles, a 0.5 molar NaOH wash replacing the water wash to remove acids more thoroughly and prolonging the ethanol washes to 5 min at 60 °C. For $\delta^{13}C$ analysis, 1 mg cellulose subsamples were combusted and analysed for ^{13}C composition using a continuous flow isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen, Germany). Carbon isotope composition was calculated relative to the Pee Dee Belemnite standard as (Craig 1957):

$$\delta^{13}C = \frac{R_{sa} - R_{sd}}{R_{sd}} \times 1000[‰] \qquad (1),$$

where R_{sa} and R_{sd} are the $^{13}C/^{12}C$ ratios of the sample and the standard, respectively. The discrimination between the $\delta^{13}C$ of atmospheric CO_2 ($\delta_{air} \approx$ -8‰) and the $\delta^{13}C$ of plant material (δ_{plant}) was calculated as (Farquhar *et al.* 1984) :

$$\Delta = \frac{\delta_{air} - \delta_{plant}}{1 + \delta_{plant} / 1000} \qquad (2).$$

Intrinsic WUE was estimated from discrimination using a modified equation from (Farquhar *et al.* 1982) :

$$WUE = \frac{A}{g} = \frac{c_a}{1.6}\left(\frac{b - \Delta}{b - a}\right)\left[\frac{\text{mol}\,CO_2}{\text{mol}\,H_2O}\right] \qquad (3),$$

where c_a is the atmospheric CO_2 concentration (estimated as $360 \cdot 10^{-6}$ mol mol^{-1}), b is the net fractionation caused by carboxylation (27‰) and Δ is the discrimination between the $\delta^{13}C$ of atmospheric CO_2 and the $\delta^{13}C$ of cellulose (Equation 2).

Estimation of genetic parameters

The normality of the distribution of the traits for both experimental set-ups was tested using Smirnov-Kolmogorov test. While $\delta^{13}C$ was normally distributed, a small distortion from the normality was observed for ring width (p-value = 0.01). However, this distortion was considered to be too small to necessitate an adjustment.

Analyses of variance for block and family effects in the half diallel were carried out with the OPEP software (Baradat 1989; Baradat & Labbé 1995) according to the following model derived from the “Henderson III” model (Searle 1971):

$$Y_{ijk} = \mu + B_i + F_j + \varepsilon_{ijk} \qquad (4)$$

where Y_{ijk} is the value of the trait for the individual k belonging to the family j, located in the block i, B_i is the fixed effect of the ith block, F_j is the random effect of the jth family and ε_{ijk} is the random residual comprising : individual deviation from family mean and family x block interactions. When block and family effects were significant, data were adjusted to the block effect, prior to the decomposition of family effect. The half diallel analysis was carried out with OPEP using the model presented below, it is derived from the simplification of the random diallel model described by Garretsen & Keuls (1977) (Baradat & Desprez-Lousteau 1997) which is adapted to non orthogonal trials with reciprocal crosses :

$$Y_{ijk} = \mu + a_i + a_j + s_{ij} + \varepsilon_{ijk} \qquad (5),$$

where Y_{ijk} is the value of the trait for the individual k corresponding to the cross between the male i and the female j, a_i (a_j) is the general combining ability (GCA) of the i^{th} (j^{th}) parent, s_{ij} is the specific combining ability (SCA) of the cross between the ith and the jth parent and ε_{ijk} is the residual term. The additive and dominance variances are: $\sigma^2_A = 4\sigma^2_a$ and $\sigma^2_D = 4\sigma^2_s$, whereas the phenotypic variance is : $\sigma^2_P = \sigma^2(Y_{ijk}) = 2\sigma^2_a + \sigma^2_s + \sigma^2_\varepsilon$ .The narrow and broad sense heritabilities were calculated as $h^2_{ns} = \sigma^2_A / \sigma^2_P$ and $h^2_{bs} = (\sigma^2_A + \sigma^2_D) / \sigma^2_P$, respectively. The percentage of additivity is calculated as the additive variance divided by the sum of additive plus dominance variances: $\sigma^2_A / (\sigma^2_A + \sigma^2_D)$. Genetic and environmental correlations were computed with OPEP using a multi-trait analysis of variance and covariance: (1) "estimated" genetic and environmental correlations were calculated according to the additive and dominance effects assessed in the random model (Equation 5; parental level), and (2) “predicted” correlations were assessed from the individual breeding values of each tree (individual level; figures 2 a-c). However, as the estimated and the predicted correlations gave similar results, only the correlation coefficients based on the prevalent "estimated" results were used in the discussion. Standard errors of estimates of heritabilities were computed using the robust Jackknife method (Lebart *et al.* 1979).

QTL detection

Two genetic maps corresponding to the female and male parents of the full-sib family were established using AFLP markers, genotyped on a subset of 90 F1. The whole mapping population was further genotyped with evenly spaced markers to increase the statistical power of QTL detection (Chagné *et al.* submitted). In order to reduce the intra-trial environmental background noise, the data were adjusted for the block effect. We used the two-way pseudo-test cross mapping strategy to construct the linkage map (Grattapaglia & Sederoff 1994). Twelve linkage groups were found for the female map, equalling the number of chromosomes for *Pinus pinaster*. For the male map 15 linkage groups were detected, however the combination of the male and the female maps into a consensus map using ¾/¼-segregating markers yields 12 linkage groups for each parent. For QTL analysis however, only the ½/½-segregating markers could be used.

For QTL analysis, MultiQTL software (A. Korol, http//www.multiqtl.com) was used. In a first step, QTLs were detected by interval mapping using a LOD threshold of 1.5 and a one-QTL-model (one QTL per linkage group). In a second step, these QTLs were taken as co-factors (composite interval mapping, CIM; introduced by Jansen & Stam 1994 and Zeng 1994), allowing individual QTL to be detected independently to the background noise. In a third step, a two QTL model (Korol *et al.* 1998) using CIM was applied, first testing if two QTLs are significant and then testing if two QTLs are more significant than one QTL. Standard deviations for the positions of the QTLs were calculated using a bootstrap method.

As there are difficulties involved when using asymptotic approximations of LOD statistics(fixed LOD level) for QTL detection (Doerge &Churchill 1996), a permutation approach was used to determine appropriate significance thresholds. Two theoretical critical thresholds were considered, the first corresponding to a per linkage group type I error of 5% allowing the detection of “suggestive” QTL and the second corresponding to a genome wise type I error of 5% allowing the detection of “significant” QTL. Theoretical critical threshold corresponding to a genome wise type I error of 5% were calculated for each chromosome taking into account the number of markers in each chromosome. If α_m is the critical threshold at the marker level corresponding to a 5% genome wise type I error, the α_c (critical threshold at the chromosome level) for a chromosome comprising n markers would be: $\alpha_c = 1 - (1 - \alpha_m)^n$. These theoretical thresholds were compared to the thresholds associated with the LOD obtained by CIM at the chromosome level after 1000 permutations of the data. The proportion of phenotypic variance explained by each QTL was estimated using the coefficient of determination (R^2, estimated by CIM, 1000 permutations), which is based on the partial correlation of a putative QTL with the trait adjusted for cofactors in the multi-locus model.

Results

Trait distributions

Means, ranges and variabilities for $\delta^{13}C$ were very similar between the half diallel and the full-sib experimental designs (Table 1, Figure 1). For MRW (mean ring width) growth was higher in the half diallel by 0.66 mm and also the range of observed values was larger (Table 1). However, the coefficient of variation was slightly higher for the full-sib experimental design than for the half diallel. This was also true when the coefficients of variation for MRW were calculated using the block effect adjusted data (data not shown).

Half diallel

Analysis of variance including family and block effect (Equation 4) indicated variation among families for both $\delta^{13}C$ and MRW, which justified the decomposition of the family effect according to Equation 5. Taking into account the significant block effect for $\delta^{13}C$, data were adjusted prior to the genetic decomposition. The narrow sense heritabilities were highly significant ($p < 0.005$) for $\delta^{13}C$ and for MRW and close in their values (Table 2). No dominance effects were detected for these two traits, thus narrow sense and broad sense heritabilities are equal and additivities are 100%.

The phenotypic correlation between mean ring width and $\delta^{13}C$ associated faster growth with less negative $\delta^{13}C$ values (higher WUE) and was significant with a coefficient of correlation of $r = 0.45$ (Table 3 and Figure 2a). The correlation was not significant (Table 3) for the genetic component (additive effect), whereas the environmental component was highly significant with a strong correlation coefficient ($r = 0.52$).

Full-sib family

A significant positive phenotypic correlation between MRW and $\delta^{13}C$ of the full-sib family ($r = 0.39$; $p < 0.005$; Figure 3) was observed. For $\delta^{13}C$, eight QTLs were found on seven linkage groups (chromosomes) and for MRW six QTLs on four linkage groups (Table 4). Using the one-QTL model, six QTLs were detected for $\delta^{13}C$ and two for MRW. With the two-QTL model, one pair of QTLs was found for $\delta^{13}C$ and two pairs for MRW. For $\delta^{13}C$, two of the QTLs detected with the one-QTL model and the QTL-pair detected with the two-QTL model and for MRW one QTL-pair are "significant QTLs" at a probability corresponding to a 5% genome wise type I error. All other detected QTLs are "suggestive QTLs" at a probability corresponding to a 5% chromosome type I error. For $\delta^{13}C$, QTLs were detected on the male and the female maps, however not on the same chromosomes. A multi-locus model, including the male and female maps, explained 51.4% of the phenotypic variation of $\delta^{13}C$, the major QTL at chromosome 6 alone explaining 12.4%. For MRW, no QTLs were found on the female map and a multi-locus model for the male map explained 42.9% of the observed phenotypic variation. No co-localisation for a QTL of $\delta^{13}C$ and of MRW was found.

Discussion

Trait distributions

Standard deviations and ranges were similar for half diallel as well as for the full-sib (Table 1). For $\delta^{13}C$ the coefficients of variation were nearly the same, whereas the coefficient of variation for mean ring width was higher for the full-sib than for the half diallel (Table 1). Variability of traits might be expected to be higher in a half diallel with twelve parental trees, than in one full-sib family. However, it has to be taken into account that parental trees for both experiments were selected from the same provenance (Landes, Gascogne) and therefore might be genetically close. Further, with a polygenic complex trait such as $\delta^{13}C$, due to transgression, even parents with only a small difference in a measured trait can produce offspring with extreme values (Prioul *et al.* 1997). For $\delta^{13}C$, the average of family variation of the half diallel (0.55‰ standard deviation within a range of 0.0‰ to 1.0‰ standard deviations) was similar to the variation found for the 186 trees of the full-sib family (0.63‰ standard deviation). Similarly, for MRW, the average of standard deviations within half-diallel families was 0.94 mm, close to the 0.99 mm standard deviation found for the full-sib family. Using Equations 2 and 3, the measured $\delta^{13}C$ values transformed into a range of WUE of 67 µmol CO_2 / mol H_2O to 100 µmol / mol for the half-diallel and a range of 65 µmol / mol to 95 µmol / mol for the full-sib. This represents for the half diallel and the full-sib a variation from one to one-and-a half times the WUE.

Heritabilities and quantitative trait dissection analysis

The heritabilities for mean ring width and $\delta^{13}C$ were found to be significant, similar between the two traits and of rather moderate value. Therefore selective crossings can improve growth and WUE. The heritabilities for ring width are comparable to values found in the literature for maritime pine or other conifers. Danjon (1994) found for maritime pine trees from the same provenance as used in the present study narrow sense heritabilities for diameter growth ranging from 0 to 0.45 for different experimental set-ups (40 to 100 half or full-sib families). Blada (1999) found for a *Pinus cembra* L. 10x10 full-diallel narrow sense heritabilities for diameter from 0.23 to 0.32 and broad sense heritabilities from 0.50 to 0.59.

For $\delta^{13}C$ there are not yet any publications known to the authors that estimated heritability for maritime pine, and there are only a few publications of estimates of heritability for $\delta^{13}C$ for other species. Narrow sense heritability estimates by Johnsen *et al*. 1999 for *Picea mariana* are lower for diameter growth (0.14) than for $\delta^{13}C$ (0.54). For non-woody species, heritabilities for $\delta^{13}C$ can be high (broad sense heritabilities for *Lens culinaris* Medikus 0.73, Matus *et al*. 1995 and for *Agropyron desertorum* (Fischer ex Link) Schultes 0.90, Asay *et al.* 1998), however it was shown that water stress could reduce the heritability of $\delta^{13}C$ (Ehdaie & Waines 1994, Johnson *et al.* 1990). An explanation for the moderate heritabilities found in the present study could therefore be the integrative properties of $\delta^{13}C$ measured on cellulose of several rings, together with the possibility of frequent water stress. The present study was located in the south-west of France, where summer drought is

common (Nguyen-Queyrens *et al.* 1998). The half diallel was created from the descendants of trees selected for growth vigour. This might have restricted the genetic base compared to natural populations and hence lowered the detectable heritability of growth.

Existing QTLs for maritime pine were localized for traits related to growth (Plomion, Durel & O'Malley 1996, Gerber, Lascoux & Kremer 1997). We were able to provide here the first example of QTL observations for $\delta^{13}C$ in a forest tree species. The four significant QTLs found for $\delta^{13}C$ explained nearly one-third of the phenotypic variation observed for this trait. Several experiments (Prioul *et al.* 1997) have shown that even for complex traits, such as growth or carbon isotope discrimination, the expected number of major loci is quite small, a small number of genetic factors is predominantly determining a quantitative trait. No co-localisations of QTLs for $\delta^{13}C$ and QTLs for MRW were observed, suggesting no common genetic control for these two traits. However, underestimation of number of QTL is inherent to the methodology of QTL detection.

Relationships between $\delta^{13}C$ and MRW

The phenotypic correlations between $\delta^{13}C$ and growth (ring width) found for the half diallel and full-sib experimental designs are significant with moderate coefficients of correlation (Figure 2a, Figure 3, Table 3) and the estimated regressions are similar in slope and intercept. Among trees in the same environmental conditions, this suggests that an increased growth relates to a higher WUE. Depending on a plants' physiology, a difference in WUE could be predominantly determined by stomatal conductance and/or by assimilation rate. The Farquhar model of carbon isotope discrimination (Farquhar, Ehleringer & Hubick 1989) predicts that an increasing photosynthetic capacity will decrease Δ. Positive as well as negative correlations have been found between photosynthesis and growth (Johnsen *et al.* 1995), however when assuming a positive correlation between photosynthetic capacity and growth, a positive correlation between $\delta^{13}C$ and growth could suggest a predominantly assimilation rate based control of $\delta^{13}C$. This is in agreement with results for black spruce: differences among families were found to be mainly determined by differences in photosynthesis (Johnsen *et al.* 1995), whereby differences in photosynthesis were rather the result of non-stomatal limitations than of stomatal limitation (Major *et al.* 1996). If $\delta^{13}C$ would be controlled by stomatal conductance, the Farquhar model predicts a negative correlation between $\delta^{13}C$ and growth. Therefore, the positive correlation between $\delta^{13}C$ and growth suggests that the variation of WUE among the measured trees is rather controlled by assimilation than by stomatal conductance. This was the case for the half diallel as well as for the full-sib family.

The calculated genetic and environmental correlations between $\delta^{13}C$ and MRW indicate that the phenotypic correlation is mainly based on environmental influence. This result is in agreement with the lack of colocalisations between QTLs for $\delta^{13}C$ and for MRW. The bloc effect was included in the estimation of the genetic parameters, hence the environmental correlation is probably due to micro-environmental influences on each individual tree. As the model also accounts for any

type of genetic effect, including intra-family genetic variation, the observed large environmental variation therefore suggests for the two measured traits a high sensitivity to micro-environmental conditions. This also suggests for growth and water use efficiency a high non-genetic placticity to adjust to environmental conditions. The strong environmental correlation that was found for the two traits is therefore probably due to a substantial environmental influence of less negative $\delta^{13}C$ with increased growth and *vice versa*.

The non-significant genetic correlation in the presented study is in opposition to the strong genetic correlation between $\delta^{13}C$ and tree growth (height and diameter) found for black spruce (Johnsen *et al.* 1999). Several factors might have contributed to this discrepancy. 1) Johnsen *et al.* (1999) suggested assimilation rate as common control for $\delta^{13}C$ and growth. However, correlations between assimilation rate and growth found in the literature range from negative over non significant to positive relationships (Johnsen *et al.* 1995). Therefore, even if $\delta^{13}C$ is determined by assimilation rate, if growth is not determined by assimilation rate, then there might be no correlation between $\delta^{13}C$ and growth. 2) Furthermore, genetic control was rather moderate for both traits, which might have lowered the significance of a genetic correlation. 3) It has also to be taken into account that in the present study carbon isotope discrimination measured on cellulose of main stem wood was compared to the average diameter growth of four years, whereas Johnsen *et al.* (1999) compared $\delta^{13}C$ of needle material with height or trunk diameter. These complex traits might include the action of a number of slightly different genes and a common genetic control might exist for the combination of needle $\delta^{13}C$ to height or diameter and not for the combination of trunk cellulose to mean annual growth of the same growth period.

The lack of a genetic correlation between δ13C and MRW found in the half-diallel is in agreement with the lack of co-localizations between QTLs for δ13C and for MRW found in the full-sib experimental design. Albeit the lack of a genetic correlation, both traits were found to be heritable and significant QTLs were detected. This opens new perspectives for the investigation of the genetic determinism of water use efficiency and the identification of groups of genes involved in drought responses.

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7

Tables

Table 1 Means, standard deviations (SD), coefficients of variation (SD / mean), ranges (maximum – minimum) and probability (p-value in %; * significant at 5% level; ns not significant) of block and family effects (Equation 4) of the two experimental designs for $\delta^{13}C$ (in [‰]) and mean ring width (MRW; in [mm])

		N	Mean	SD	Coef. of Var.	Range	Block effect	Family effect
$\delta^{13}C$	Half diallel	564	-26.21	0.61	0.02	3.64	2.8*	2.3*
	Full-sib	186	-26.48	0.63	0.02	3.23	0.0*	---
MRW	Half diallel	564	2.88	1.10	0.38	6.77	ns	0.2*
	Full-sib	186	2.11	0.99	0.47	5.15	0.9*	---

Table 2 Half diallel: Heritabilities for $\delta^{13}C$ and mean ring width (MRW) with standard deviations and 95% confidence interval in parentheses. As there were no dominance effects detected and therefore additivity is 100%, the narrow sense (ns) and broad sense (bs) heritabilities are equal.

	$h^2_{ns} = h^2_{bs}$	%additivity
$\delta^{13}C$ [‰]	0.17 ± 0.06 (0.06 – 0.29)*	100%
MRW [mm]	0.19 ± 0.06 (0.06 – 0.31)*	100%

* significant at the 5% level

Table 3 Half diallel: Correlations between mean ring width and $\delta^{13}C$; r is the correlation coefficient on the family level, estimated by OPEP software with standard deviation and 95% confidence interval in parentheses; r' is the correlation coefficient estimated by linear regression analysis using the calculated individual tree breeding values which are shown in Figure 2.

	r	r'
Phenotypic correlation	0.45 ± 0.057 (0.33 – 0.56)*	0.46*
Genetic correlation	0.27 ± 0.21 (0.07 – 0.15) NS	0.02 NS
Environmental correlation	0.52 ± 0.16 (0.20 – 0.83)*	0.79*

* significant at the 5% level; NS : not significant

1Table 4 Full sib: Results of the composite interval mapping analysis for $\delta^{13}C$ and
2mean ring width (MRW) using MultiQTL software. The p-value associated with the
3LODs were calculated using 1000 permutations of the data, standard deviation of
4position (SD) was calculated using the bootstrap method (1000 permutations); in
5case of a significant QTL-pair for a chromosome, Lod and p-value are given for the
6tests of (I) two QTLs *versus* no QTLs and (II) two QTLs *versus* one QTL (difference
7of Lod for two and for one QTL).

	map	Chr[a]	N[b]	Position ± SD[c]	Lod	P-value[d]	Dir.[e]	R^2	R^2_{total}
$\delta^{13}C$	male	3a	149	4.3±13.2	1.78	0.021*	+	0.047	0.268
	male	6	164	102.7±21.6	4.40	0.001***	+	0.124	
	male	8	85	0.0±15.7	1.85	0.021*	-	0.050	
	male	9	183	104.6±28.5	1.90	0.033*	-	0.047	
	female	2	84	209.1±56.4	2.30	0.019*	+	0.065	0.246
	female	5	164	99.9±22.0	1.98	0.0033***	-	0.062	
	female	12	153	1: 0.0±36.7 2: 135.4±27.1	I : 4.24 II: 1.88	I : 0.002*** II: 0.036*	- +	0.119	
MRW	male	2b	180 153	1: 47.2±15.9 2: 51.8±11.1	I : 2.49 II: 1.57	I : 0.048* II: 0.027*	- +	0.181	0.429
	male	5	145	56.8±33.4	2.41	0.022*	-	0.065	
	male	6	174 168	1: 7.5±31.0 2: 133.7±22.0	I : 4.24 II: 2.68	I : 0.001*** II: 0.002*	- +	0.124	
	male	11	165	11.6±30.9	1.73	0.040*	+	0.059	

8[a] chromosome ID
9[b] number of full-sibs with available data for QTL detection
10[c] Lod score peak position (from the top) of the chromosome (cM)
11[d] Probability for the null hypothesis of no QTL at the chromosome level
12 *** : probability corresponding to a 5% genome wise type I error (significant QTL)
13 * : probability corresponding to a 5% chromosome type I error (suggestive QTL)
14[e] direction of influence of presence of the allele for each QTL

Figure legends

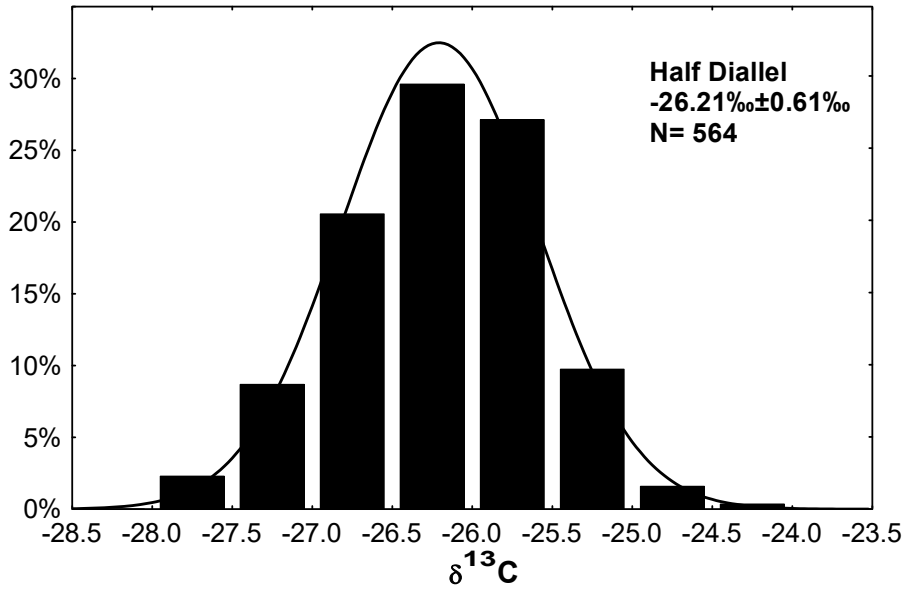
Figure 1 Distribution of cellulose $\delta^{13}C$ (raw data; not corrected for block effect) for a) the half diallel experiment and b) the full-sib experiment; parameters for the normal distributions as in Table 1

Figure 2 Linear correlations between mean ring width (MRW) and $\delta^{13}C$ for the half diallel experiment using data corrected with the individual tree breeding values (OPEP software): a) phenotypic, b) genetic (additive effects) and c) environmental correlations; all data are adjusted for block effect, data for genetic and environmental correlations are centred and standardized by the mean.

Figure 3 Phenotypic linear correlation between mean ring width (MRW) and $\delta^{13}C$ for the full-sib experiment; data adjusted for block effect

Figure 1 a b

a)



b)

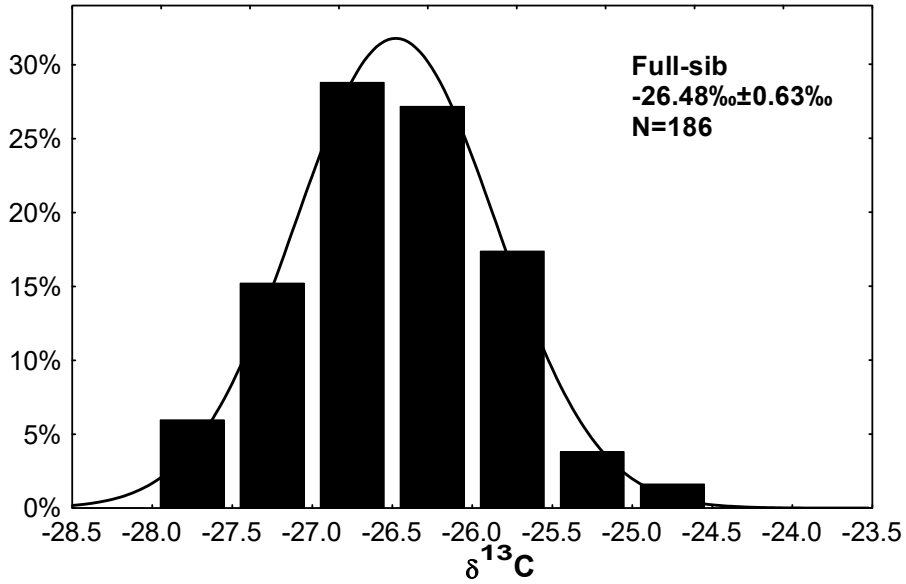
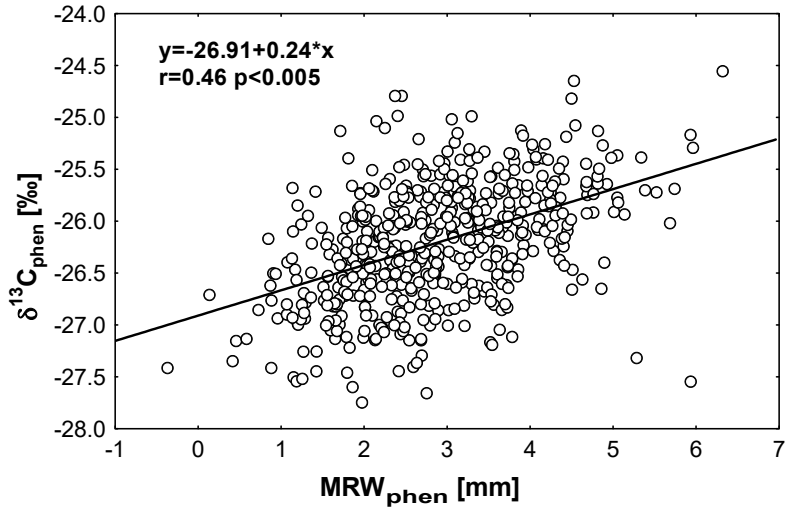
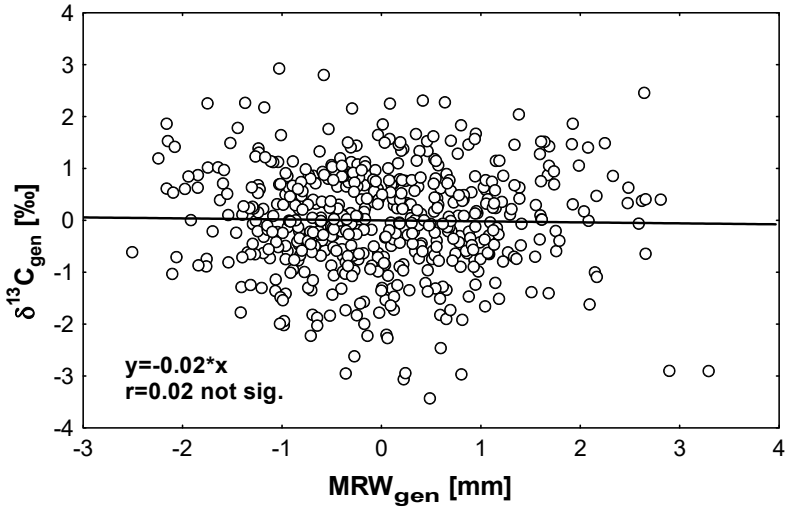


Figure 2 a b c

a)phenotypic correlation



b)genetic correlation



c)environmental correlation

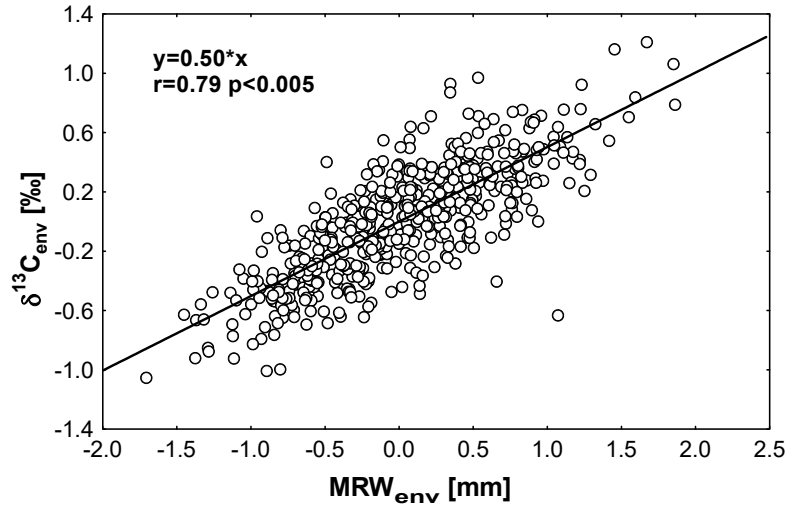
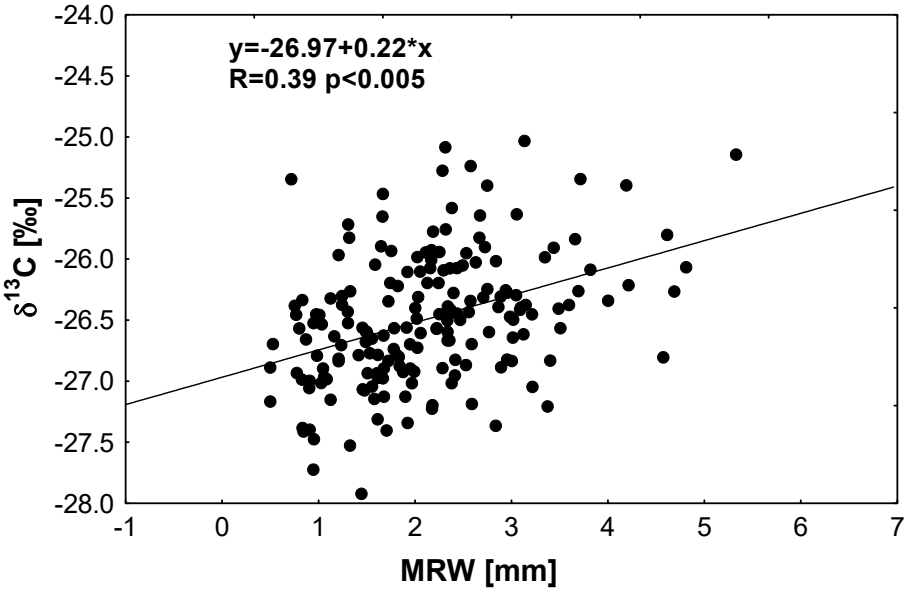


Figure 3


y=-26.97+0.22*x
R=0.39 p<0.005
δ13C [‰]
MRW [mm]
-24.0
-24.5
-25.0
-25.5
-26.0
-26.5
-27.0
-27.5
-28.0
-1
0
1
2
3
4
5
6
7