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Genetic variation in productivity, leaf traits and carbon isotope discrimination in hybrid poplars cultivated on contrasting sites

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Abstract –

• We examined the relationships between productivity, leaf traits and carbon isotope discrimination in bulk leaf matter (Δ_1) and in phloem sap (Δ_s) from more than 5-year-old trees belonging to *Populus deltoides* × *P. nigra* and *Populus trichocarpa* × *P. deltoides*; trees were grown in alluvial and non alluvial sites in a commercial poplar plantation.
• On both sites, a large genetic variability was evidenced for all variables. The genotypic ranking remained stable between years for all variables, while it differed between sites. Δ_1 scaled positively with Δ_s and neither Δ_1 nor Δ_s were correlated with productivity. A significant genotype by site interaction was evident for all variables. The non alluvial site resulted in lower productivity, and in thicker/denser leaves with lower nitrogen and carbon contents. Noteworthy, the genotypic ranking for Δ_1 measured at the alluvial site was similar to that previously established in a glasshouse.
• As observed in previous studies from younger trees, there is a potential to select genotypes, combining high productivity and high water-use efficiency, for growth in moderately drought-prone areas.

hybrid poplars plantation / soil characteristics / genetic variability / carbon isotope discrimination (Δ) / leaf traits

Résumé – Variation génétique de la productivité, de caractères foliaires et de la discrimination isotopique vis-à-vis du carbone chez des hybrides de peuplier cultivés sur des sites contrastés.

• La productivité, des caractères foliaires et la discrimination isotopique vis-à-vis du carbone de la matière organique des feuilles (Δ_1) et de la sève élaborée (Δ_s) ont été étudiés chez *Populus deltoides* × *P. nigra* et *Populus trichocarpa* × *P. deltoides* à partir d'arbres âgés de plus de cinq ans, cultivés en peupleraie sur des sites alluviaux et non alluviaux.
• Des différences génotypiques ont été observées pour toutes les variables. Le classement des génotypes était conservé entre deux années, alors qu'il variait entre sites une année donnée. Δ_1 était corrélé positivement avec Δ_s et aucun lien n'a été détecté entre Δ_1 ou Δ_s et productivité. Une interaction significative entre génotype et site a été observée pour toutes les variables. Les arbres du site non alluvial se caractérisaient par une plus faible productivité et des feuilles plus épaisses/denses avec des teneurs en azote et en carbone plus faibles. De façon intéressante, le classement des génotypes pour Δ_1 était maintenu entre les expériences réalisées sur site alluvial et celles précédemment menées en serre.
• Toutes expériences confondues, il semble possible de sélectionner des génotypes productifs et efficaces afin d'étendre les plantations à des terrains enclins à des sécheresses modérées.

peupleraie / caractéristiques du sol / variabilité génétique / discrimination isotopique vis-à-vis du carbone (Δ) / caractères foliaires

1. INTRODUCTION

Poplars are among the fastest growing trees under temperate latitudes. Their high productivity is associated with a tight dependency upon water availability (Barigah et al., 1994; Ceulemans et al., 1988; Tschaplinski and Blake, 1989; Tschaplinski et al., 1994; Zsuffa et al., 1996). Poplar cultivation tends to extend from flood plains and bottomlands towards

uplands where soil water availability is subjected to seasonal changes. To sustain this trend, it might be useful to select cultivars with improved water-use efficiency over the growth cycle. Intrinsic water-use efficiency (W_i), i.e., the ratio between net CO₂ assimilation and stomatal conductance, is an important component of this efficiency which may be indirectly estimated at leaf level, via the carbon isotope discrimination (Δ) (Farquhar and Richards, 1984; Farquhar et al., 1989; Monclus et al., 2006; Ponton et al., 2001). A large genetic variability of Δ has already been reported among poplar genotypes (Marron et al., 2005; Monclus et al., 2005 and 2006; Zhang et al., 2004).

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† In memoriam (September 2006).

The selection of genotypes combining a satisfactory productivity and high water-use efficiency would be a considerable advantage for growth in moderately drought-constrained areas, as stated by Braatne et al. (1992) for poplar and by Condon et al. (2002) for cereals.

The links between productivity and Δ differ according to poplar species: a positive relationship was found for *Populus davidiana* (Dode) Schneider (Zhang et al., 2004), whereas none was detected for *Populus trichocarpa* \times *P. deltoides* (TD hybrids) and *Populus deltoides* \times *P. nigra* (DN hybrids) (Marron et al., 2005; Monclus et al., 2005; 2006; Rae et al., 2004). The lack of correlation observed for these two hybrids suggests the possibility to select genotypes displaying simultaneously a high productivity and water-use efficiency.

A detailed study with several commercial poplar genotypes (DN hybrids) grown as young cuttings in glasshouse (3-month-old rooted cuttings; Marron et al., 2005) or in a nursery (1 to 4-year-old rooted cuttings coppiced each year; Monclus et al., 2005; 2006) indicated consistent genotypic differences in W_i , and a lack of correlation between W_i and productivity. The stability of these observations remains to be established under a wider range of environmental conditions and with older trees, as some juvenility effects might interfere with the observed ranking. Therefore, we have extended the comparative approach to trees at least 5 years old grown in a commercial poplar plantation for wood production, and managed with conventional forestry practices. Tested clones were genotypes of DN hybrids and TD hybrids. Two sites were chosen in the plantation according to soil water availability and fertility. Genetic variability was studied on alluvial and non alluvial sites on the basis of 12 and 11 genotypes, respectively. The site effect was quantified by comparing 9 genotypes common to both sites. Comparisons with previous experiments carried out in glasshouse and in nursery were done using 9 DN genotypes. The productivity of each tree was computed as the ratio between the circumference measured at 1.30 m height and the age of the tree; leaf traits encompassed specific leaf area and carbon and nitrogen contents; carbon isotope discrimination was used as a surrogate for intrinsic water-use efficiency.

2. MATERIAL AND METHODS

2.1. Study site

The experiment was carried out in the commercial poplar plantation of *Le Moulin de Bariteau* (Vienne, France, 47° 7' N, 0° 10' E; 36 m a.s.l.) which extends over 200 ha and includes about 34 000 hybrid poplar trees. The climate is oceanic, characterized by mild temperatures (11.1 °C annual mean) and a constant distribution of precipitation (650 mm per year for the last three decades) (Météo France, <http://www.meteofrance.com>). The poplar plantation occupies both sides of the *Chavenay* river, upstream of its junction with the *Négron* river and the adjacent slopes.

Soil fertility and soil water availability were analysed during 1994, for areas located on the river banks and for areas located on adjacent slopes, by the *Association Forêt Cellulose* (AFOCEL) in the *Laboratoire de la chambre d'agriculture de la Sarthe* (LARCA 72, Le Mans, France). Data related to soil characteristics are available

at <http://www.peupliersdefrance.org/indexbariteau.htm>. Soil fertility was rated according to its cationic exchange capacity (CEC; meq. 100g⁻¹) and its saturation rate (S/CEC; %); ratio between soil base cationic content (S; meq. 100 g⁻¹) and CEC. Maximal available soil water (PAW_M; mm) was assessed from soil texture (Jamagne et al., 1977). Based on LARCA data, two sites, named alluvial and non alluvial, were selected for the present study. The alluvial site displayed a CEC above 40 meq. 100 g⁻¹, a S/CEC of 100%, a PAW_M above 250 mm and the level of the water table near the soil surface year round; this zone was restricted to the banks of the *Chavenay*. The non alluvial site displayed a CEC below 23 meq. 100 g⁻¹, a S/CEC of about 90% and a PAW_M ranging from 100 to 200 mm; this site was on the adjacent slopes.

2.2. Plant material

Fourteen poplar genotypes including 10 *Populus deltoides* \times *P. nigra* (DN hybrids) and 4 *Populus trichocarpa* \times *P. deltoides* (TD hybrids) were selected; among them, 9 grew on both sites (Tab. I). Overall, 357 trees were studied; their age ranged from 6 to 14-year-old and their height varied from 14 to 28 m. All trees were grown at 7 \times 7 m spacing.

2.3. Plant measurements

From July 18 to 21, 2005, one mature leaf was collected from the lowest branch of each tree and six calibrated discs (2 cm²) were cut from each lamina. Discs were dried at 60 °C during two days, weighted and specific leaf area (SLA, cm² g⁻¹) was computed. During the same period, phloem sap was collected from each tree with the method described by Scartazza et al. (2004) and modified by Gessler et al. (2004). On July 19, from 11:00 a.m. to 5:00 p.m., a thin strip of bark (2 cm long and 1 cm wide) still attached to the trunk was removed and plunged into tubes filled with 1 mL pure water for 4 h. This mixture was membrane filtered (0.45 μ m pore size, Millipore), put into tin capsules and oven-dried at 60 °C over one day. From July 10 to July 13, 2006, the sampling was repeated from mature leaves of trees grown in the alluvial site only. During November 2006, the productivity of each tree was estimated as the mean annual radial increment (RI, mm y⁻¹; i.e., the ratio between the circumference measured at 1.30 m height and the age of the tree), assuming a circular section of the trunk.

Carbon isotope composition ($\delta^{13}\text{C}$) and carbon and nitrogen contents (C_M , N_M ; mg g_{DW}⁻¹) of bulk leaf material were assessed from 1 mg homogenous dry powder obtained from the oven-dried calibrated discs. $\delta^{13}\text{C}$ of phloem sap was analysed from the dried phloem extracts. These analyses were conducted by the Scottish Crop Research Institute (Dundee, Scotland, UK) from a CF-IRMS (Europa Scientific ANCA-NT 20-20 Stable Isotope Analyser with ANCA-NT Solid/Liquid Preparation Module, Europa Scientific Ltd., Crewe, UK). $\delta^{13}\text{C}$ was calculated relative to Pee Dee Belemnite limestone standard as described in Craig (1957):

$$\delta^{13}\text{C} = \frac{(R_{\text{sample}} - R_{\text{standard}})}{(R_{\text{standard}})} \times 1000 (\text{‰}),$$

where R_{sample} and R_{standard} are the ¹³C/¹²C ratio in plant tissue and the standard, respectively. Carbon isotope discrimination (Δ_1 and Δ_s

Table I. Plant material description including hybrid crosses, genotypes, abbreviations, origins and the number of trees used for each genotype and for each type of site. The age (years) of trees are indicated into brackets.

Hybrid	Genotype	Abbreviation	Origin	Alluvial site	Non alluvial site
				Number of tree (Age)	Number of tree (Age)
<i>P. deltoides</i> × <i>P. nigra</i>	A4A	A4	Italy	10 (6)	
	Brenta	Bt	Italy		6 (5) + 10 (6)
	Dorskamp	Ds	The Netherlands	10 (6)	10 (5)
	I214	I2	Italy	10 (7)	30 (7)
	I45/51	I4	Italy	10 (9)	9 (10) + 10 (14)
	Koster	Ks	The Netherlands	9 (6)	
	Lambro	Lb	Italy		9 (5) + 10 (6)
	Luisa-Avanzo	La	Italy	9 (7)	
	Mella	Ml	Italy	9 (6)	4 (5)
<i>P. trichocarpa</i> × <i>P. deltoides</i>	Soligo	Sg	Italy	9 (6)	3 (5)
	Beaupré	Bp	Belgium	10 (9) + 10 (8)	20 (12)
	Boelare	Bl	Belgium	10 (9)	30 (7) + 10 (13)
	Hunnegen	Hn	Belgium	10 (7)	30 (7)
	Raspalje	Rs	Belgium	10 (8)	10 (5) + 10 (6) + 10 (8) + 10 (7)

for bulk leaf matter and phloem sap respectively, ‰) was calculated according to Farquhar and Richards (1984), as:

$$\Delta = \frac{(\delta_{\text{air}} - \delta_{\text{plant}})}{(1 + (\delta_{\text{plant}}/1000))} (\text{‰}),$$

where δ_{air} and δ_{plant} are $\delta^{13}\text{C}$ of the atmospheric CO_2 (assumed to be close to -8‰) and the sample, respectively.

2.4. Statistical analyses

Results were analysed with the SPSS statistical software package (SPSS, Chicago, IL, USA) and with R software (version 2.0.1, A Language and Environment, Copyright 2004). For each variable, the normality of the distribution was tested with a Shapiro–Wilk test. Levels of significance were * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and ns = non significant. Genotypic means are expressed with their standard error.

Genetic variability was evaluated for each site by a two-way ANOVA using the following model: $Y_{ijk} = \mu + H_i + H_i(G_j) + \varepsilon_{ijk}$; where Y_{ijk} refers to individual values, μ is the general mean, H_i is the effect of hybrid cross i , $H_i(G_j)$ is the effect of genotype j in hybrid cross i and ε_{ijk} is the error. For each site, relationships between variables were computed by linear correlation (Pearson's coefficients), and illustrated by a principal component analysis (PCA) using genotypic means. The variables were standardized and orthogonal factors (= F1 and F2 axis) were successively built as linear combinations of these variables to maximize the fraction variability explained by these factors. Variables were first represented on the plane defined by the two main factors of the PCA; their coordinates were their linear correlation coefficients (Pearson's coefficient) with these factors. Variables projected as supplementary variables in the main plane F1 × F2 were obtained by computing linear correlations between these variables and scores F1 and F2 obtained from the initial PCA.

Station effect was evaluated by a three-way ANOVA using the following model: $Y'_{ijkl} = \mu + H_i + H_i(G_j) + S_k + H_i \times S_k + H_i(G_j) \times S_k + \varepsilon_{ijkl}$;

where Y'_{ijkl} refers to individual values, μ is the general mean, H_i is the effect of hybrid cross i , $H_i(G_j)$ is the effect of genotype j in hybrid cross i , S_k is the site effect, $H_i \times S_k$ is the hybrid cross by site interaction, $H_i(G_j) \times S_k$ is the genotype in hybrid cross by site interaction and ε_{ijkl} is the error. This analysis was performed with the 9 genotypes common to both sites; noteworthy, each genotype displayed the same age on alluvial and non alluvial sites (Tab. I).

Crossing comparisons with previous studies realized in our research unit were analysed by rank correlation (Spearman's coefficient), from DN hybrids. As a whole, 9 genotypes were common with previous studies; among them 7 belonged to the alluvial site (Dorskamp, I214, I45/51, Koster, Luisa-Avanzo, Mella and Soligo) and 7 belonged to the non alluvial site (Brenta, Dorskamp, I214, I45/51, Lambro, Mella and Soligo).

3. RESULTS

3.1. Genetic variability in the alluvial site

During 2005 and 2006, significant differences were recorded between DN hybrids and TD hybrids for all variables, except for Δ_s measured in 2005 and for Δ_l measured in 2006 (Fig. 1). Overall, DN hybrids displayed higher values of RI, N_M and C_M than TD hybrids, while they displayed the lowest values of SLA and Δ_l , at least during 2005. A significant genotype effect within hybrid cross was detected for all variables in 2005 and 2006. With all genotypes grouped, the genotype ranking (Spearman coefficients, r_s) remained stable from 2005 to 2006 for N_M ($r_s = 0.59^*$), SLA ($r_s = 0.92^{***}$) and Δ_l ($r_s = 0.84^{**}$) whereas it varied significantly for C_M ($r_s = 0.46^{\text{ns}}$). In 2005, genetic variability was of the same magnitude for Δ_l and for Δ_s with values ranging from 20.1‰ to 22.3‰ and genotypic ranking remained comparable between Δ_l and Δ_s ($r_s = 0.68^*$). Values measured for SLA and

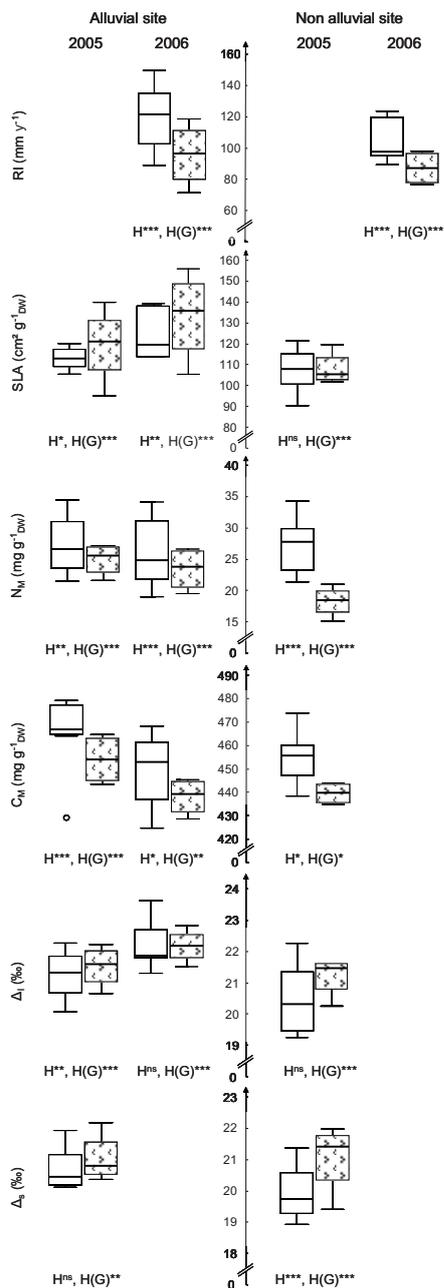


Figure 1. Genetic variability of productivity, leaf traits and variables related to water-use efficiency in DN hybrids (white boxes) and TD hybrids (hyphenated boxes) from two sites, during 2005 and 2006. Sites were alluvial (8 DN hybrids and 4 TD hybrids) and non alluvial (7 DN hybrids and 4 TD hybrids). Box plots are shown for mean annual radial increment (RI, mm y^{-1}), specific leaf area (SLA, $\text{cm}^2 \text{g}^{-1} \text{DW}$), leaf nitrogen and carbon contents (N_M and C_M , $\text{mg g}^{-1} \text{DW}$), and leaf and phloem sap carbon isotope discrimination (Δ_1 and Δ_s , respectively, ‰). Each box represents the quartile below (Q1) and above (Q3) the median value. Vertical bars show minimum and maximum values except for genotypes away from 1.5 times from the top of the inter-quartile (Q3–Q1) range. Values outside this range are represented by open circles. Levels of significance are indicated for the hybrid cross effect (H) and for the genotype in hybrid cross one [H(G)] (ns = non significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

Δ_1 were significantly higher in 2006 than in 2005; these differences could be explained by a higher cumulated rainfall (computed between January and August of each year) in 2006 than in 2005 (350 mm vs. 224 mm) (*Chambre d'agriculture d'Indre et Loire*, <http://www.indre-et-loire.chambagri.fr>). Genetic variability was of the same magnitude during the two years for Δ_1 (2.3%), while it was higher in 2006 than in 2005 for SLA.

With all genotypes grouped, the main factorial plane of the PCA ($F1 \times F2$, Figs. 2a and 2b) explained 68.8% of the genetic variability, with 39.9% for $F1$ alone, while the $F3$ axis did not differentiate the genotypes (data not shown). Two independent groups of variables were identified from the $F1 \times F2$ plane (Fig. 2a). The first one included Δ_1 and Δ_s and the second one RI and N_M . In each group, the variables were positively correlated (Tab. II). There was no clear grouping among genotypes in the $F1 \times F2$ plane (Fig. 2b). Genotypes Koster and Dorskamp displayed high RI whereas Hunnegen displayed the lowest one; Raspalje had high values of Δ whereas Mella and Soligo displayed the lowest values. Interestingly, we were unable to detect any correlation between RI and Δ (Tab. II). The projection on the main plane ($F1 \times F2$) of the variables measured in 2006, as supplementary variables, showed a similar distribution to 2005 except for C_M (Fig. 2a).

3.2. Genetic variability in the non alluvial site – comparison with the alluvial site

During 2005, significant differences were recorded between DN hybrids and TD hybrids for RI, N_M , C_M and Δ_s (Fig. 1). Overall, DN hybrids displayed higher values of RI, N_M and C_M than TD hybrids, while they displayed the lowest values of Δ_s . A significant genotype in hybrid cross effect was detected for all variables (Fig. 1). With all genotypes grouped, genotypic ranking remained comparable between Δ_1 and Δ_s ($r_s = 0.86^{**}$). The main plane of the general PCA ($F1 \times F2$), performed at genotypic level (Figs. 2c and 2d), explained 90.8% of the genetic variability, with 63% for $F1$ alone, while the $F3$ axis did not differentiate the genotypes (data not shown). Three groups of variables were defined from the $F1 \times F2$ plane (Fig. 2c); the first group included Δ_1 and Δ_s , the second one N_M and C_M and the third one included SLA and RI. The $F1$ axis of the PCA opposed Δ (Δ_1 and Δ_s) to N_M and C_M ; within each group, variables were positively correlated while they were negatively correlated between groups (Tab. II). The $F2$ axis was defined by SLA and RI, but these variables did not correlate (Tab. II). Genotypes I214, I45/51 and Boelare displayed a high RI, a large SLA and high values of Δ_1 and Δ_s while Raspalje, Dorskamp, Mella and Brenta displayed low values of RI, SLA, Δ_1 and Δ_s ; Soligo and Lambro displayed low values of Δ_1 and Δ_s while Beaupré and Hunnegen were characterized by high values of Δ_1 and Δ_s (Fig. 2d). Interestingly, we were unable to detect any correlation between RI and Δ (Tab. II).

A significant site effect was detected for all variables except Δ_s and Δ_1 (Appendix A1). Values recorded for RI, SLA, N_M and C_M were significantly lower in the non alluvial site.

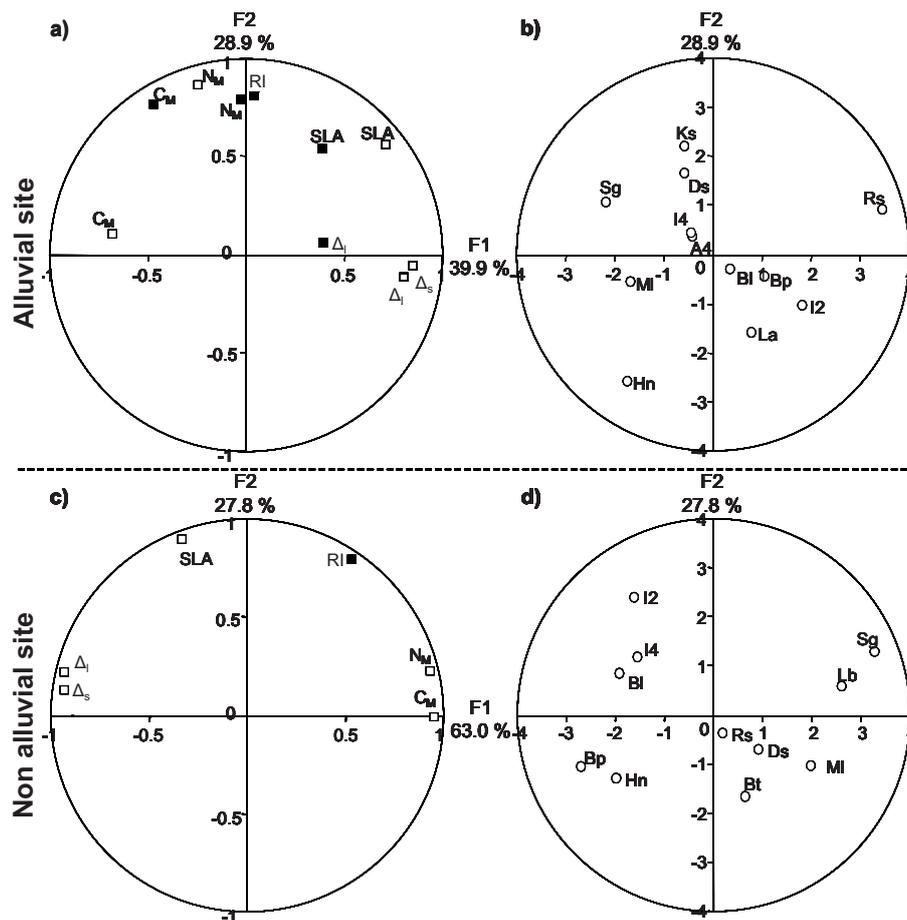


Figure 2. Factorial analysis (Principal Components Analysis) of genetic variability measured in the alluvial (a and b) and non alluvial sites (c and d). For the alluvial site, the distribution of 10 variables (a) and the projection of the 12 hybrid poplars genotypes (b) are shown in the main factorial plane F1 × F2 of PCA. F1 and F2 are linear combinations of the 5 variables measured during 2005 (white squares) plus mean annual radial increment (RI) measured in 2006 (black square). The others variables recorded during 2006 in the alluvial site (black squares) were projected as supplementary variables on the main F1 × F2 plane. For the non alluvial site, the distribution of 6 variables (c) and the projection of the 11 hybrid poplar genotypes (d) are shown in the main factorial plane F1 × F2 of a PCA. F1 and F2 are linear combinations of the 5 variables measured during 2005 (white squares) plus mean annual radial increment (RI) measured in 2006 (black square). See Table 1 for genotype abbreviations.

A significant hybrid cross by site interaction was detected for N_M and SLA only (Appendix A1). TD hybrids displayed greater decrease in N_M and SLA than DN hybrids. A significant genotype in hybrid cross by site interaction was observed for all variables (Appendix A1). With all genotypes grouped, genotypic ranking varied significantly for RI, SLA, Δ_1 , Δ_s and C_M , while it remained stable for N_M ($r_s = 0.68^*$).

3.3. Comparison of field grown trees with rooted cuttings grown under either nursery (Monclus et al., 2005 and 2006) or glasshouse (Marron et al., 2005) conditions

Although growth conditions, plant size and age of the plants largely differed among the experiments, the genetic variability detected during experiments was of similar magnitude for all

leaf traits, except SLA. SLA displayed a greater variability and higher values in the glasshouse ($190 < SLA \text{ (cm}^2 \text{ g}_{DW}^{-1}) < 235$; Marron et al., 2005) than in the nursery ($104.4 < SLA \text{ (cm}^2 \text{ g}_{DW}^{-1}) < 126.3$) or in the poplar plantation ($90.3 < SLA_{2005} \text{ (cm}^2 \text{ g}_{DW}^{-1}) < 121.3$). In all experiments, the productivity index did not correlate with Δ measured from bulk leaf dry matter. Only one significant and positive correlation was detected between values of Δ_1 measured in 2005 or 2006 in the alluvial site and values previously measured in glasshouse from younger cuttings (Fig. 3).

4. DISCUSSION

Compared to TD hybrids, DN hybrids displayed the highest productivity (estimated via RI), the highest water-use efficiency (estimated via Δ) and thicker/denser leaves (estimated

Table II. Linear correlations (Pearson's coefficients) between mean annual radial increment (RI, mm y⁻¹), specific leaf area (SLA, cm² g⁻¹), leaf carbon and nitrogen contents (C_M, N_M; mg g_{DW}⁻¹) and, leaf and phloem sap carbon isotope discrimination (Δ_l and Δ_s respectively, ‰). Correlations were computed at genotype level, from 12 poplar genotypes cultivated in alluvial site (above the diagonal line) and from 11 poplar genotypes cultivated in non alluvial site (below the line) in 2005. Significant correlations are indicated in bold (ns = non significant; * P ≤ 0.05; ** P ≤ 0.01; *** P ≤ 0.001; nd = not determined).

		SLA		C _M		N _M		Δ _l		Δ _s	RI
		2005	2006	2005	2006	2005	2006	2005	2006	2005	
SLA	2005		0.78**	-0.46 ^{ns}	0.12 ^{ns}	0.31 ^{ns}	0.50 ^{ns}	0.41 ^{ns}	0.20 ^{ns}	0.48 ^{ns}	0.33 ^{ns}
	2006	nd		-0.16 ^{ns}	0.45 ^{ns}	0.35 ^{ns}	0.67*	0.31 ^{ns}	0.19 ^{ns}	0.17 ^{ns}	0.35 ^{ns}
C _M	2005	-0.34 ^{ns}	nd		0.45 ^{ns}	0.31 ^{ns}	0.02 ^{ns}	-0.26 ^{ns}	0.17 ^{ns}	-0.39 ^{ns}	0.02 ^{ns}
	2006	nd	nd	nd		0.65*	0.47 ^{ns}	-0.42 ^{ns}	-0.06 ^{ns}	-0.49 ^{ns}	0.70*
N _M	2005	-0.09 ^{ns}	nd	0.94***	nd		0.66*	-0.30 ^{ns}	0.03 ^{ns}	-0.15 ^{ns}	0.48 ^{ns}
	2006	nd	nd	nd	nd	nd		-0.15 ^{ns}	-0.09 ^{ns}	-0.16 ^{ns}	0.62*
Δ _l	2005	0.45 ^{ns}	nd	-0.86**	nd	-0.77**	nd		0.77**	0.69*	0.01 ^{ns}
	2006	nd	nd	nd	nd	nd	nd	nd		0.43 ^{ns}	0.07 ^{ns}
Δ _s	2005	0.41 ^{ns}	nd	-0.81**	nd	-0.78**	nd	0.91***	nd		0.07 ^{ns}
	RI	0.46 ^{ns}	nd	0.49 ^{ns}	nd	0.62*	nd	-0.30 ^{ns}	nd	-0.41 ^{ns}	

via SLA) with higher N_M and C_M contents at both sites. The site effect was similar for all variables between hybrid cross except for SLA and N_M; TD hybrids displayed the greatest decline in SLA and N_M when planted on the non alluvial site compared with DN hybrids. Nevertheless, all these results must be taken cautiously because of the unbalanced number of genotypes within each hybrid cross.

4.1. Genetic variability of productivity, leaf traits and variables related to water-use efficiency – year and site effects

Significant genotype variability was observed within each hybrid cross for all traits in alluvial and non alluvial sites. Such genetic variability had already been observed from younger plant material in previous studies in hybrid poplars growing in a glasshouse or in the field (Al Afas et al., 2007; Ceulemans and Deraedt, 1999; Marron et al., 2005; Monclus et al., 2005; 2006; Voltas et al., 2006).

All genotypes grouped, genotypic rankings were comparable between 2005 and 2006 for most of the variables measured from the alluvial site showing stability overtime in genetic variability. Such stability over 2 years had already been observed in nursery from 3-year-old rooted cuttings of 29 DN hybrid genotypes (Monclus et al., 2006). Δ_l and SLA increased between 2005 and 2006; these variations can be attributed to inter-annual climatic changes and more particularly, to higher cumulated rainfall measured in 2006 than in 2005 (350 mm vs. 224 mm). Such environmental effects had similarly been observed by Monclus et al. (2006).

In 2005, a significant interaction between genotype in hybrid cross and site was detected for all variables. Taking into account that each genotype displayed the same age between alluvial and non alluvial sites, these results suggest that the effect of soil fertility and soil water availability on productivity,

on variables related to WUE and on leaf traits strongly depends on the genotype. As a whole, RI was clearly larger in the alluvial site than in the non alluvial one; this can be easily explained by higher soil fertility and soil water availability in the alluvial site. Differences between sites were corroborated by differences in some leaf traits such as SLA and N_M. The lowest values of SLA and N_M measured in the non alluvial site can be ascribed to lower soil water availability and fertility (Abrams et al., 1990; Cunningham et al., 1999; Li et al., 2005; Marron et al., 2002; Monclus et al., 2006; Niinemets et al., 1998; Poorter and de Jong, 1999). N_M decreased between alluvial and non alluvial sites while genotypic ranking remained stable suggesting that N_M variations mainly depended on the environment.

It is noteworthy that the genotype ranking was maintained for Δ_l between experiments conducted in a glasshouse and in the alluvial station only. In both cases, plants grown on fertilized and well-watered soils and the collected leaves were exposed to low irradiance. Therefore, the genotype ranking for Δ_l was conserved regardless of the age of the plants whereas it strongly varied for plants of similar age when grown under contrasting environmental conditions. Further experiments are needed to build a consistent data basis for age and site related effects on genotype rankings for Δ.

4.2. Relationships between productivity, leaf traits and variables related to WUE

Although Δ_l was estimated from leaves located on the lowest branches and thus from shade leaves, a strong and positive relationship was observed between Δ_l and Δ_s during 2005, for both sites. While Δ_l is an integrative indicator of intrinsic water-use efficiency at a leaf life time-scale (Farquhar and Richards, 1984), Δ_s integrates the whole canopy but may reflect short-term fluctuations of C_i/C_a (Cernusak et al., 2003;

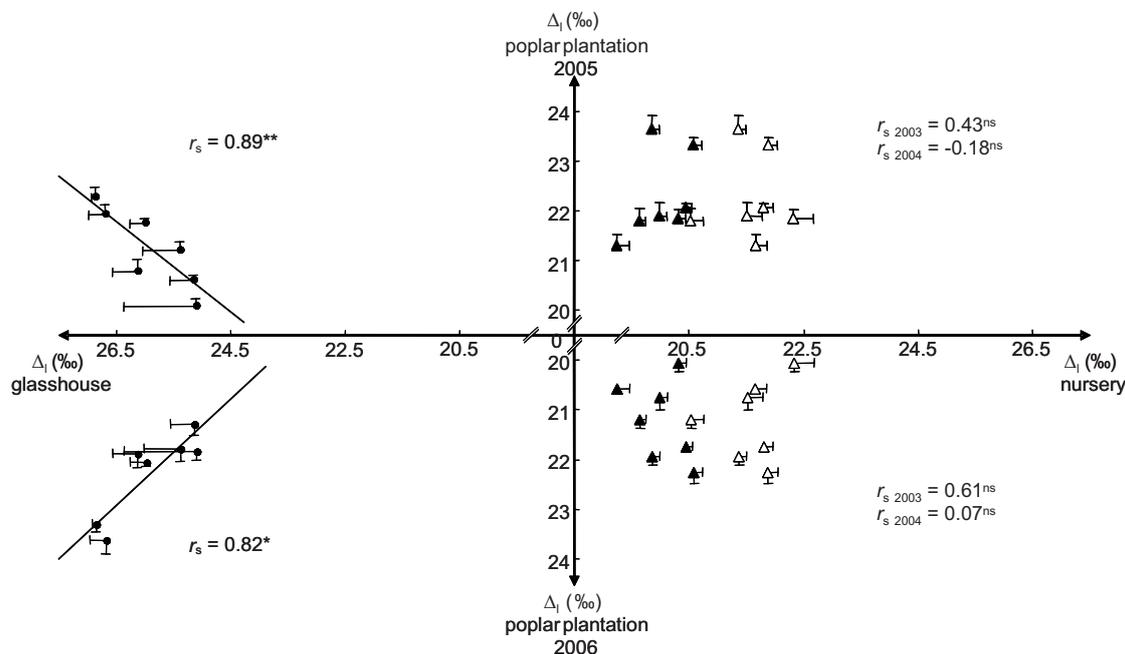


Figure 3. Relationship between leaf carbon isotope discrimination (Δ_1 , ‰) measured in the alluvial site in 2005 and 2006, and leaf carbon isotope discrimination measured for the same genotypes in glasshouse in 2001 (black circles) (Marron et al., 2005) and in nursery in 2003 (black triangles) (Monclus et al., 2005) and 2004 (White triangles) (Monclus et al., 2006). Glasshouse and nursery measurements had been recorded from plants grown under well-watered condition. Genotypic means \pm standard error are indicated ($n = 7$). Rank correlations (Spearman coefficients, r_s) are indicated with level of significance (ns = non significant; * $P \leq 0.05$; ** $P \leq 0.01$).

Gessler et al., 2001; Keitel et al., 2003; Pate and Arthur, 1998). This similarity is remarkable with respect to the potential causes for differences in measurements at such different time and spatial scales. Nevertheless, as previously shown for mature leaves, Δ_1 could be an accurate predictor of the carbon isotope signature of the whole canopy (Cernusak et al., 2003; Gessler et al., 2001; Pate and Arthur, 1998).

No relationship has been observed between productivity and variables related to water-use efficiency (Δ), as previously described for *Populus fremontii* (Leffler and Evans, 1999), for TD hybrids (Rae et al., 2004) and for DN hybrids (Marron et al., 2005; Monclus et al., 2005; 2006; Voltas et al., 2006). The lack of correlation between productivity and water-use efficiency observed earlier on young individuals was thus confirmed at larger ages and sizes, and under very different growth conditions. From a practical point of view, these results support the idea that there is a potential for improving intrinsic water-use efficiency in poplar without necessarily reducing overall productivity. In 2005, RI scaled positively with N_M in the non alluvial site only; this result suggests that N_M would be a limiting factor for productivity in the non alluvial site. A negative correlation was observed between Δ and N_M in the non alluvial site suggesting that genetic variability of photosynthetic capacity could also contribute to WUE variability (Monclus et al., 2005; 2006). As already shown in the nursery studies, Δ did not correlate with SLA, suggesting that the variability of Δ cannot be explained by genotype variations in internal conductance to CO_2 transfer into the leaves, which may be greater in thicker leaves.

5. CONCLUSIONS

Experiments were conducted with DN and TD hybrids older than five years and grown in alluvial and non alluvial sites in a commercial poplar plantation. A large genetic variability in productivity, leaf traits and variables related to WUE was observed from mature trees, similar to that previously observed from young cuttings cultivated in glasshouse or in nursery. The genotypic ranking for WUE was maintained between young cuttings grown in glasshouse and trees grown in the alluvial site, while it was broadly disrupted for trees of similar age when grown on contrasting sites. No correlation was detected between productivity and Δ , as previously observed in a glasshouse or in a nursery, supporting the conclusion that there is a potential for improving WUE in poplar without necessarily reducing overall productivity. Interestingly for European poplar growers, we were able to detect one genotype, Soligo, combining a satisfactory productivity and a large WUE whatever the site. Moreover, we were also able to put forward one genotype, Dorskamp, combining a satisfactory productivity and low WUE in the alluvial site only; this latter genotype could be used as a medium for phytoremediation of polluted soils.

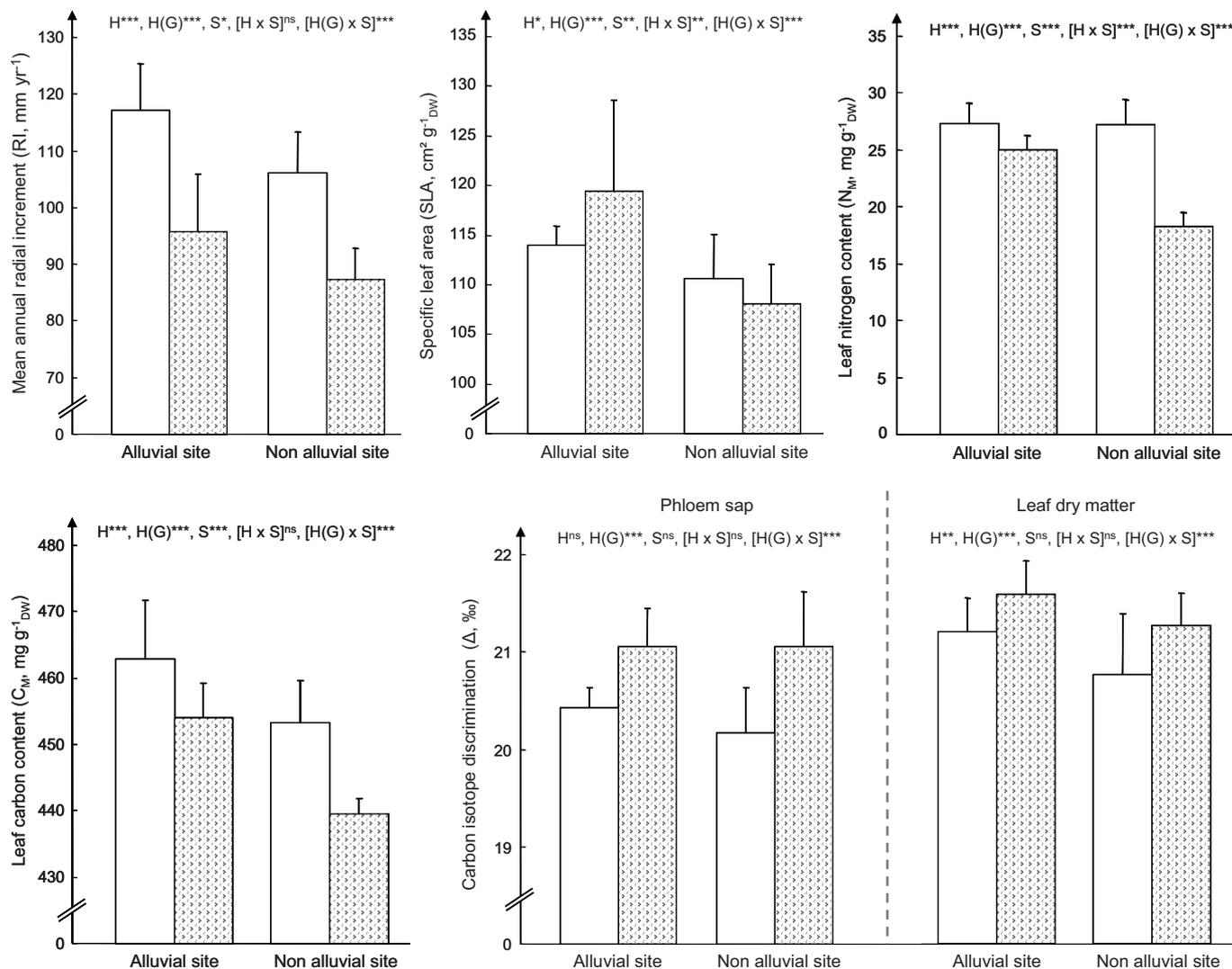
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APPENDIX



Appendix A1. Comparison between alluvial and non alluvial sites for mean annual radial increment (RI, mm yr⁻¹) specific leaf area (SLA, cm² g⁻¹ DW), leaf nitrogen and carbon contents (N_M and C_M, mg g⁻¹ DW), and leaf dry matter and phloem sap carbon isotope discrimination (Δ, ‰). Comparison was performed from 9 hybrid poplars (5 DN hybrids (white bars) and 4 TD hybrids (hyphenated bars)) common to the two sites. Means ± standard errors were presented for each site. Levels of significance are indicated for the hybrid cross effect (H), for the genotype in hybrid cross effect [H(G)], for the site effect (S), for the hybrid cross by site interaction (H × S) effect and for the genotype in hybrid cross by site interaction [H(G) × S] effect (ns = non significant; * *P* ≤ 0.05; ** *P* ≤ 0.01; *** *P* ≤ 0.001).