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Productivity, water-use efficiency and tolerance to moderate water deficit correlate in 33 poplar genotypes from a *Populus deltoides* × *Populus trichocarpa* F₁ progeny

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Summary Genotypic variability for productivity, water-use efficiency and leaf traits in 33 genotypes selected from an F₁ progeny of *Populus deltoides* Bartr. ex Marsh × *Populus trichocarpa* L. was explored under optimal and moderate water-deficit conditions. Saplings of the 33 genotypes were grown in a two-plot open field at INRA Orléans (France) and coppiced every year. A moderate water deficit was induced during two successive years on one plot by withholding irrigation, while the second one remained irrigated (control). Stem biomass and leaf structure (e.g., specific leaf area and leaf area) were measured in 2004 and 2005 and functional leaf traits (e.g., carbon isotope discrimination, Δ) were measured only in 2004. Tolerance to water deficit was estimated at genotype level as the ability to limit losses in biomass production in water deficit versus control trees. Stem biomass, leaf structure and Δ displayed a significant genotypic variability whatever the irrigation regime. For all traits, genotype ranks remained stable across years for similar irrigation conditions. Carbon isotope discrimination scaled negatively with productivity and leaf nitrogen content in controls. The most productive genotypes were the least tolerant to moderate water deficit. No relationship was evidenced between Δ and the level of tolerance to water deficit. The relationships between traits evidenced in this collection of *P. deltoides* × *P. trichocarpa* F₁ genotypes contrast with the ones that were previously detected in a collection of *P. deltoides* × *Populus nigra* L. cultivars tested in the same field trial.

Keywords: broad-sense heritability, carbon isotope discrimination and leaf traits, drought tolerance, genotypic variability, hybrid poplars, open field.

Introduction

Poplars are among the superior hardwood trees used for wood production under temperate latitudes and are also widely used for pulp production, plywood confection or bioenergy (Zsuffa et al. 1996). A large genetic variability in terms of rapid juvenile growth and photosynthetic capacity is found within the *Populus* genus, particularly among their interspecific hybrids (Monclus et al. 2006). Many breeding programmes have thus been developed with an ultimate goal being the selection of the most productive genotypes. Because productivity of cottonwood species and hybrid cottonwoods depends upon water availability (e.g., Braatne et al. 1992, Kim et al. 2008), global warming and expected water limitation are likely to have a significant impact on poplar cultivation. These fast environmental changes require adaptation of breeding strategies towards a more sustainable poplar wood production by combining growth potential and tolerance to both biotic and abiotic constraints. Water-use efficiency (WUE), the ratio of biomass produced to the amount of water used and tolerance to water deficit are critical links between poplar wood production and water management (Monclus et al. 2006).

A recent effort has been made to decipher the genetic basis and the relationships between WUE and productivity

[†] In memoriam (September 2006).

in *Populus deltoides* Bartr. ex Marsh \times *Populus nigra* L. genotypes (D \times N hybrids) under contrasting water regimes (Marron et al. 2005, Monclus et al. 2005, 2006). High levels of genotypic variability for productivity and for WUE were found under ample water supply or moderate water deficit. In addition, Monclus et al. (2006) were unable to detect any unfavourable correlation between productivity, WUE and tolerance to moderate water deficit estimated from the loss of height and diameter growth (Passioura 2002). Altogether, these findings suggest that it should be possible to select genotypes combining large levels of productivity, WUE and drought tolerance (Monclus et al. 2006).

The successful application of indirect selection strategies requires morphophysiological traits that are cheap and easy to record on a large number of genetic units. Their selection efficiency also depends on their genetic correlation with the target objective and higher heritability values under the testing conditions (Tuberosa et al. 2002). Stem circumference, tree height and maximal individual leaf area (LA_{MAX}) have been found to scale frequently positively with biomass production within the *Populus* genus and are therefore reliable indexes of enhanced productivity (Ceulemans 1990, Pellis et al. 2004, Rae et al. 2004, Zhang et al. 2004, Marron et al. 2005, 2006, Marron and Ceulemans 2006, Monclus et al. 2005, 2006). In contrast, other leaf traits such as specific leaf area (SLA) or leaf nitrogen content per dry mass (N_M) failed to be efficient predictors because of their tight dependency upon environmental conditions (Poorter and Van der Werf 1998, Shipley 2002, Marron et al. 2005, Monclus et al. 2005). Evidence of significant genetic variability for WUE has been successfully observed in different poplar species directly from the assessment of leaf gas exchange (ratio of net CO_2 assimilation rate versus stomatal conductance, often called intrinsic WUE, W_i) and indirectly from leaf carbon isotope discrimination (Δ) (Rae et al. 2004, Zhang et al. 2004, Marron et al. 2005, Monclus et al. 2005, 2006, Bonhomme et al. 2008). Carbon isotope discrimination correlated negatively with stomatal conductance and W_i in D \times N hybrids, but did not correlate with productivity. This suggests that Δ is as expected a valuable indirect predictor for WUE (Farquhar et al. 1989, Monclus et al. 2006) to include in poplar breeding programmes, and that selecting for a higher WUE may not come necessarily at the expense of a lower productivity. Finally, the best predictor identified in D \times N hybrids for tolerance to moderate water deficit was the ratio between the leaf area under optimal water supply versus moderate water deficit (Monclus et al. 2006).

The links between productivity and Δ may differ according to the genetic background. A positive relationship was found for *Populus davidiana* (Dode) Schneider (Zhang et al. 2004), whereas no link was evidenced with D \times N (Marron et al. 2005, Monclus et al. 2005, 2006) and in an F_2 progeny of *Populus trichocarpa* L. \times *P. deltoides* (Rae et al. 2004). The lack of relationship between productivity and Δ suggests that WUE is mainly driven by stomatal conductance

to water vapour, whereas a positive relationship indicates that WUE is mainly controlled by photosynthetic capacity (Farquhar et al. 1989). In this study, we explored the analysis of relationships between productivity, WUE and tolerance to water deficit in an F_1 progeny of *P. deltoides* \times *P. trichocarpa* (D \times T hybrids). The objectives of this study were to

- (i) quantify the genetic variability in productivity and WUE under optimal conditions and a moderate water deficit and to detect genetic variation on tolerance to moderate water deficit;
- (ii) investigate the phenotypic relationships between productivity, WUE and tolerance to water deficit;
- (iii) examine inter-annual stability in the relationships between traits;
- (iv) evaluate the relevance of several leaf traits as simple predictors of productivity, WUE and tolerance to water deficit.

The experiment consisted of a random set of 33 F_1 D \times T genotypes grown in the same field trial as the one previously described for D \times N hybrids (Monclus et al. 2005, 2006). Water deficit was induced during two successive summers (2004 and 2005) by withholding irrigation from one plot while the second one was used as an irrigated control.

Materials and methods

Plant material and growth conditions

A subset of 33 cloned genotypes was selected among a set of 342 F_1 genotypes derived from a cross between *P. deltoides* '73028-62' as female and *P. trichocarpa* '101-74' as male. This subset is representative of the genetic variability for productivity observed in the whole progeny as recorded in an earlier test (unpublished data). Saplings of the 33 clones and their two parents were grown together with the 29 D \times N hybrids described by Monclus et al. (2006) in a common two-plot open field experiment established at INRA Orléans (47°46' N and 1°52' E; 110 m a.s.l.) during January 2001. The complete list of D \times N hybrid genotypes can be found in Monclus et al. (2006). The two plots were established 15 m apart from each other on a homogeneous soil with 25-cm-long woody-stem cuttings of the 64 genotypes. Each plot was divided into five randomized complete blocks (one single tree of each genotype per block). Spacing between trees was 1.2 m between rows and 0.5 m within rows. A double row of commercial varieties bordered each plot to minimize edge effects.

Plants were coppiced at the end of every year. Bud flush occurred synchronously for all genotypes in each plot (during April) along the 2 years of study. A systematic pruning was made during May every year, leaving only a single 40-cm-high shoot per stool. During 2001–2003, the two plots

were irrigated every second day (20 mm of water) with overhead sprinklers. During 2004 and 2005, they were irrigated until June 15 and 25, respectively. From this date to the end of the growing season, water deficit was induced by irrigation cessation on one of the plots, whereas the second was kept irrigated and used as a control.

Environmental conditions and soil–water potential

During the 2004 and 2005 growing seasons, daily irradiance ($\text{J m}^{-2} \text{d}^{-1}$), air temperature (minimum, mean and maximum, $^{\circ}\text{C d}^{-1}$), potential evapotranspiration (mm d^{-1}) and rainfall (mm d^{-1}) were recorded at a closely located weather station (XARIA, Degreane Horizon, Cuers, France; see Appendix Table A1). Cumulative rainfall from June 15 to September 30 reached 220 and 150 mm in 2004 and 2005, respectively. Soil–water availability was monitored by measuring predawn leaf water potential (Ψ_{pd} , MPa) with a Scholander-type pressure chamber on a mature leaf once a week throughout the growing season (Améglio et al. 1999, Brignolas et al. 2000). Dynamics of soil–water deficit are described in detail in Monclus et al. (2006). During both years, Ψ_{pd} declined by 0.3 MPa as compared to controls, from early September onwards.

Structural traits

On 7 January 2005 and 10 November 2005, the final stem height (Ht, cm), final circumference (Circ, mm) and fresh biomass (Biom_F , g f wt) were recorded. The tolerance to water deficit was defined as the ability to limit losses in Biom_F production (Passioura 2002) and was thus quantified as $(\text{Biom}_F - \text{water-deficit}) / (\text{Biom}_F - \text{well-watered}) \times 100$. Leaf structure traits were assessed on 10 September 2004 and 5 September 2005. One fully expanded and illuminated mature leaf (foliar index 17, Monclus et al. 2005) was collected on each plant. The leaf was then photocopied to yield the LA_{MAX} (cm^2). The leaf area was estimated from scanned photocopies with an image analyser (UTHSCSA *Image Tool* program developed at the University of Texas Health Science Center at San Antonio, Tx and available from the Internet by anonymous FTP from <http://ddsdx.uthscsa.edu/dig/itdesc.html>). Six calibrated discs of lamina (2 cm^2) were sampled from the leaf, dried and weighed to compute SLA ($\text{cm}^2 \text{g}^{-1}$).

Functional traits

The six calibrated discs of leaf lamina were ground to a fine powder for the analysis of leaf carbon isotope composition ($\delta^{13}\text{C}$), carbon (C_M) and nitrogen (N_M) content. All analyses were performed at the technical platform of functional ecology at the INRA-Nancy. One-milligram subsamples of ground material were enclosed in tin capsules and combusted. The CO_2 produced by combustion was purified and its $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio was analysed by a continuous flux isotope ratio mass spectrometer (IRMS Delta S, Finnigan

MAT, Bremen, Germany) with a precision over measurements of $\pm 0.14\text{‰}$. The discrimination between atmospheric CO_2 (δ_{air} assumed to be close to -8‰) and plant material (δ_{plant}) was calculated as $\Delta = (\delta_{\text{air}} - \delta_{\text{plant}}) / (1 + (\delta_{\text{plant}}/1000))$ according to Farquhar and Richards (1984). C_M and N_M were expressed on a dry mass basis ($\text{mg g}^{-1} \text{DW}$).

Statistical analyses

Data were evaluated by linear correlation (Pearson's coefficients), rank correlation (Spearman's coefficient) and analysis of variance (ANOVA) using the SPSS statistical software package (SPSS, Chicago, IL). For all traits and treatments, individual values of F₁ genotypes were adjusted to the block effects when significant and genotype mean values were calculated from adjusted values. For each variable, the normality of the distribution of residuals was tested using the Shapiro–Wilk test. All statistical tests were considered significant at $P \leq 0.05$. Genotypic mean values are expressed with their standard error (SE) or with their confidence interval ($\alpha = 0.05$).

For each variable and treatment (control versus water deficit), restricted maximum likelihood estimates of genotypic, block and residual variance components (σ^2_G , σ^2_B and σ^2_e , respectively) were computed, and broad-sense heritability was calculated at genotype level as $h^2_{\text{genot}} = \sigma^2_G / (\sigma^2_G + \sigma^2_e/n)$, where n is the average number of replicates per genotype. For each variable and treatment, the coefficient of genotypic variation (CV) was computed as $\text{CV} = (s \times 100)/\mu$, where s is the standard deviation and μ is the grand mean.

Results

Genotypic variability and correlations among traits under well-watered conditions

A significant genotypic effect was detected for all variables among the 33 F₁ genotypes during 2004 and 2005 (Figures 1 and 2). Genotype ranking remained stable between years for all traits measured in 2004 and 2005 (Table 1). Estimates of h^2_{genot} ranged from 0.25 for Biom_F and Circ to 0.44 for Ht; the CV ranged from 1.1% to 25.9% for C_M and Biom_F , respectively (Table 1). Genotypic performances for Δ varied along a 1.8‰ range and displayed a moderate heritability ($h^2_{\text{genot}} = 0.39$; Figure 2C; Table 1). Nitrogen content (N_M) ranged from 20 to 27 mg g^{-1} ($h^2_{\text{genot}} = 0.20$; CV = 6.7%; Figure 2E; Table 1). Carbon content (C_M) displayed significant genotypic differences, but showed the lowest values for h^2_{genot} and CV (Figure 2D; Table 1). During 2004 and 2005, Biom_F , Circ and LA_{MAX} were positively correlated (Table 2). During 2004, N_M scaled positively with Biom_F , Circ and SLA (Table 2), whereas Δ scaled negatively with Biom_F , Circ and N_M and positively with SLA (Table 2; Figure A1).

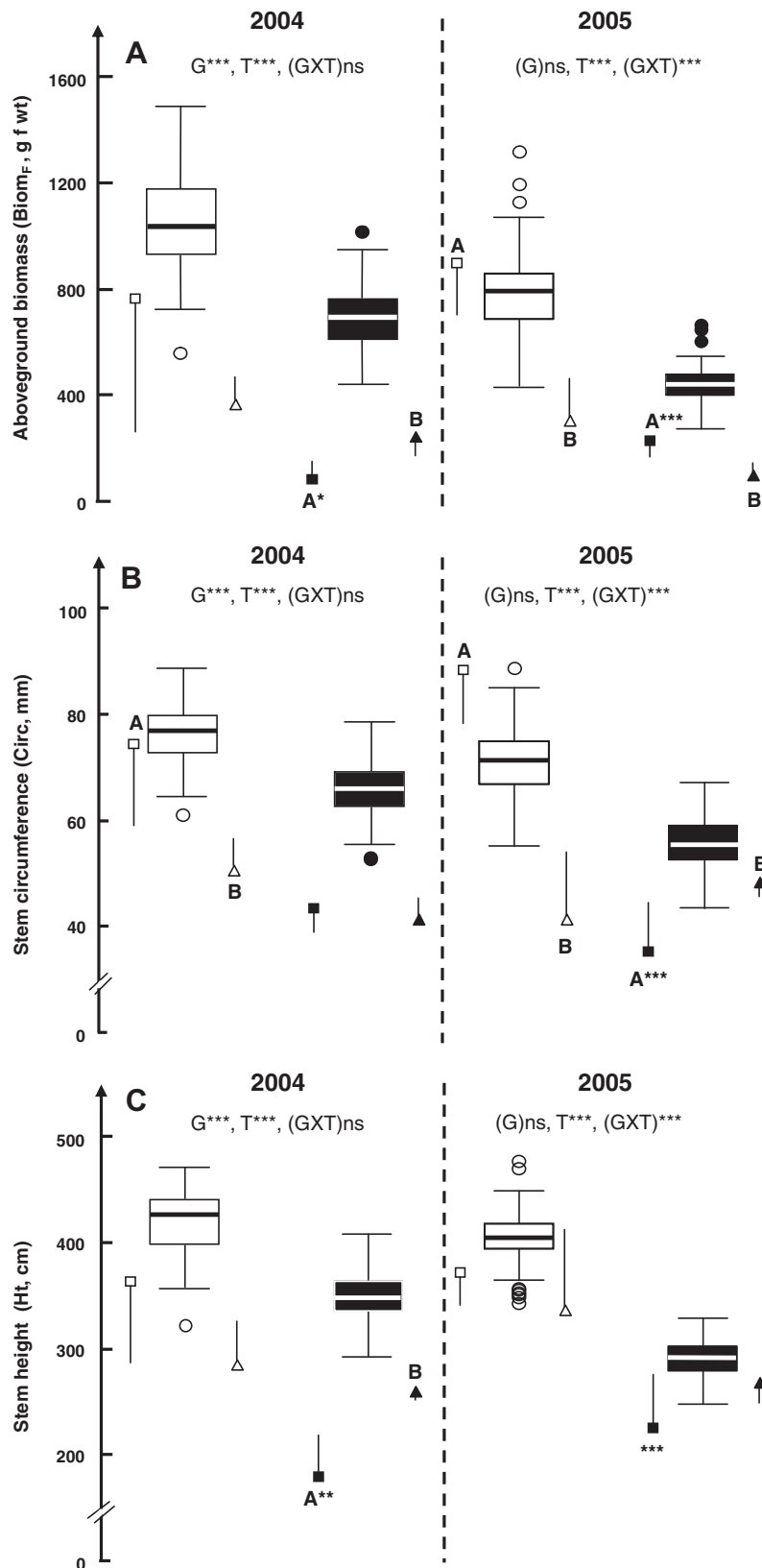


Figure 1. Genotypic variability of traits related to productivity among 33 F₁ *P. deltoides* × *P. trichocarpa* grown in the field under contrasted water supply during 2004 and 2005. Box plots of (A) aboveground biomass (Biom_F, g f wt), (B) stem circumference (Circ, mm) and (C) stem height (Ht, cm). White boxes, controls; black boxes, water deficit. Each box represents the quartile below (Q₁) and above (Q₃) the median value. Vertical bars represent minimum and maximum values except when the latter are away 1.5 times from the top of the interquartile (Q₃–Q₁) range. Values beyond this range are represented as circles. For each year, Genotype (G), Treatment (T) and ‘Genotype × Treatment’ interaction (G × T) effects were tested by a two-way ANOVA. Levels of significance: **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001 and ns for nonsignificant. The two parents *P. trichocarpa* and *P. deltoides* are identified by triangles and squares, respectively; mean values are presented with their confidence intervals (α = 0.05), significant differences between the two parents are indicated by different letters (A and B) and water-deficit-induced differences are indicated by asterisks.

For all traits, values recorded from the female parent *P. deltoides* were within the range of variations recorded from the 33 genotypes (Figures 1 and 2A, C and E). In con-

trast, values recorded from the male parent *P. trichocarpa* were below the range of variations for Biom_F, Ht, Circ, LA_{MAX} and Δ (Figures 1 and 2).

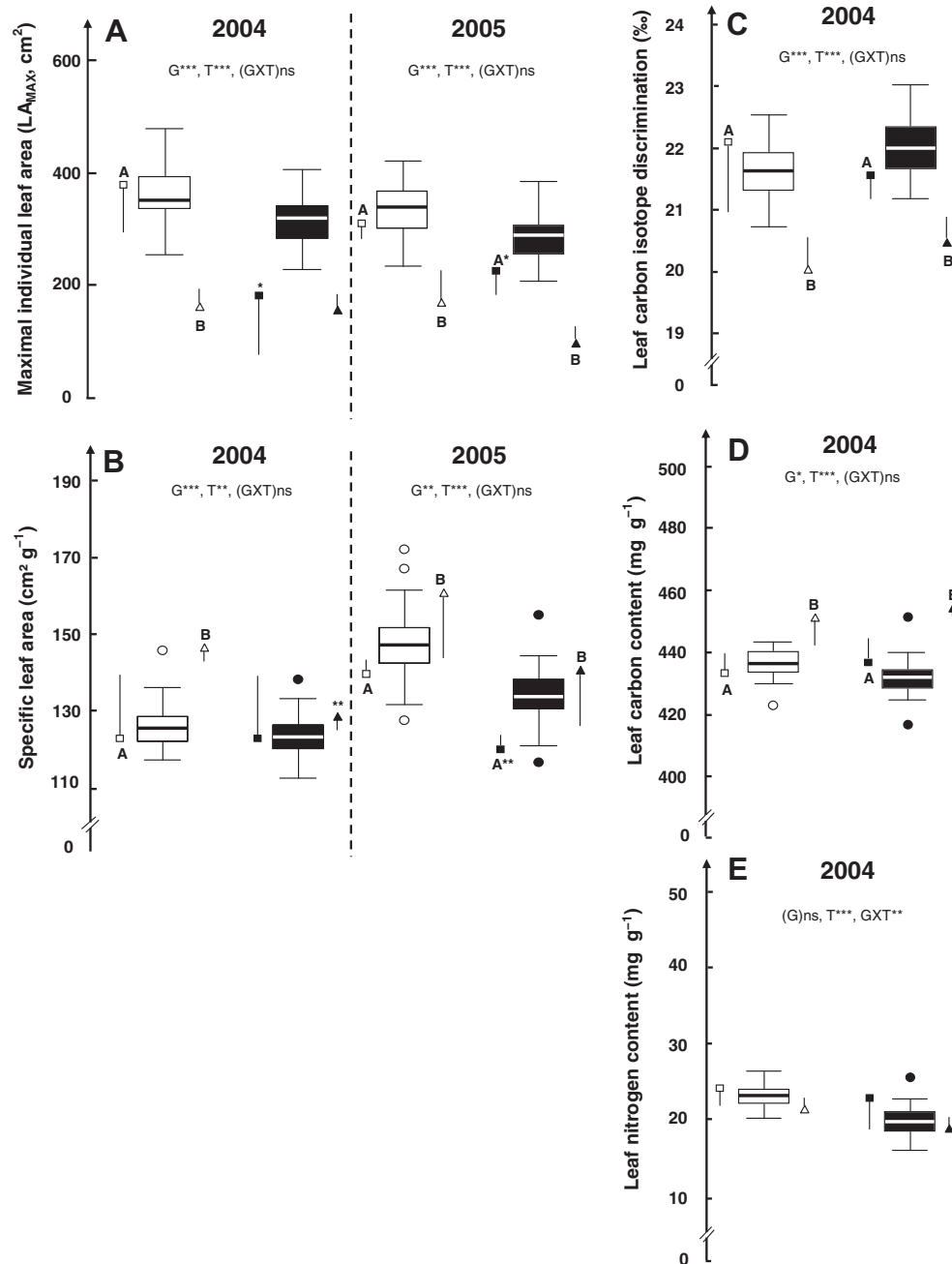


Figure 2. Genotypic variability of leaf traits of 33 F₁ *P. deltoides* × *P. trichocarpa* grown in the field under contrasted water supplies during 2004 and 2005. Box plots of (A) maximal individual leaf area (LA_{MAX} , cm²), (B) specific leaf area (SLA, cm² g⁻¹), (C) leaf carbon isotope discrimination (Δ , ‰), (D) leaf carbon (C_M , mg g⁻¹) and (E) nitrogen content (N_M , mg g⁻¹). Symbols as in Figure 1.

Impact of moderate water deficits on genotypic variability and correlations among traits

Irrigation was withheld from mid-June to the end of the growing season during 2004 and 2005. Cumulative rainfall was significantly lower in 2005 than in 2004, especially in August (26 mm versus 122 mm). However, a similar and significant decrease in Ψ_{pd} of about -0.3 MPa was observed during September 2004 (Monclus et al. 2006) and September 2005 in the plot subjected to a moderate

water deficit with respect to control. Thus, the water deficit in our study might be characterized as being moderate, long-lasting, with a peak in September.

During 2004, the water deficit induced significant reductions in $Biom_F$, H_t and $Circ$ in most genotypes (Figure 1). Even though no 'G × T' interaction was evidenced, the reduction in $Biom_F$ ranged from 8% to 54% depending on the genotype (Figures 1 and 3A). The genotype ranking and the range of genotypic variability were not significantly

Table 1. General mean values (\pm SE), broad-sense heritability (h^2_{genot}), genotypic variation coefficients (CV, %) and Spearman's coefficients for 33 F_1 *P. deltoides* \times *P. trichocarpa* grown in the field under contrasted water supply (control, WW versus water deficit, WD) during 2004 and 2005. Aboveground biomass (Biom_F, g f wt), stem circumference (Circ, mm), stem height (Ht, cm), leaf carbon isotope discrimination (Δ , ‰), maximal individual leaf area (LA_{max}, cm²), specific leaf area (SLA, cm² g⁻¹) and leaf carbon (C, mg g⁻¹) and nitrogen content (N, mg g⁻¹). Δ , C_M and N_M not available for 2005 (nd). Levels of significance: * $P \leq 0.05$, ** $P \leq 0.01$ and *** $P \leq 0.001$.

Variables	Year	General mean (\pm SE)		h^2_{genot}		CV (%)		Spearman's coefficients		
		WW	WD	WW	WD	WW	WD	WW versus WD	2004 versus 2005	
									WW	WD
Biom _F	2004	1043.5 (26.4)	698.5 (17.1)	0.25	0.31	19.6	20.3	0.69***	0.54**	0.64***
	2005	797.1 (25.2)	426.2 (13.2)	0.25	ns	25.9	21.3	0.35*		
Circ	2004	75.9 (0.8)	65.5 (0.7)	0.25	0.29	8.4	9.5	0.65***	0.62***	0.58***
	2005	70.9 (0.9)	56.3 (0.7)	0.27	ns	11.3	9.4	0.26 ^{ns}		
Ht	2004	415.2 (3.6)	350.4 (3.1)	0.44	0.43	8.1	8.1	0.70***	0.61***	0.55**
	2005	402.8 (4.3)	290.7 (2.9)	0.21	0.13	8.3	7	0.27 ^{ns}		
Δ	2004	21.64 (0.05)	22.02 (0.06)	0.39	0.36	2	2.2	0.70***	nd	nd
	2005	nd	nd	nd	nd	nd	nd	nd		
LA _{MAX}	2004	395.3 (6.2)	349.1 (5.4)	0.25	0.29	12.2	12.8	0.61***	0.71***	0.55**
	2005	367.7 (5.0)	321.1 (5.6)	0.35	0.15	12.3	13	0.49**		
SLA	2004	126.1 (0.7)	123.5 (0.7)	0.25	0.29	3.9	4.4	0.55**	0.59***	0.31ns
	2005	147.4 (1.4)	134.8 (0.9)	0.35	0.15	6.1	4.9	0.52**		
C _M	2004	436.6 (0.8)	432.4 (0.7)	ns	0.44	1.1	1.4	0.48**	nd	nd
	2005	nd	nd	nd	nd	nd	nd	nd		
N _M	2004	22.7 (0.2)	19.4 (0.2)	0.20	0.36	6.7	10	0.35*	nd	nd
	2005	nd	nd	nd	nd	nd	nd	nd		

Table 2. Linear correlations (Pearson's coefficients) between traits recorded during 2004 and 2005 in 33 F_1 *P. deltoides* \times *P. trichocarpa* full-sib offspring grown in the field under water deficit (upper triangle) and for controls (lower triangle). See text and Figures 1 and 2 for abbreviations. C_M, N_M and Δ were only recorded during 2004. Level of significance: * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ and ns for nonsignificant. Correlations with $0.05 < P < 0.1$ are indexed 't'.

		Biom _F	Ht	Circ	LA _{MAX}	SLA	C _M	N _M	Δ
Biom _F	2004		0.91***	0.97***	0.66***				
	2005		0.88***	0.97***	0.52**				nd
Ht	2004	0.65***		0.90***	0.55**				
	2005	0.82***		0.88***	0.49**				nd
Circ	2004	0.64***	0.38*		0.68***				
	2005	0.98***	0.84***		0.56***				nd
LA _{MAX}	2004	0.53**		0.67***					
	2005	0.47**	0.42*	0.54**					nd
SLA	2004						-0.47**		0.34 ^t
	2005								nd
C _M	2004			0.47**					
	2005	nd	nd	nd	nd	nd			nd
N _M	2004	0.39*		0.35*	0.32 ^t	0.38*			-0.43*
	2005	nd	nd	nd	nd	nd	nd		nd
Δ	2004	-0.43*	-0.33 ^t	-0.38*		0.54**		-0.38*	
	2005	nd	nd	nd	nd	nd	nd	nd	

modified by water deficit for Biom_F, Circ and Ht (Table 1; Figure 1). A negative relationship was evidenced between Biom_F of controls and the extent of decrease in biomass induced by water deficit (Figure 3B). All leaf traits were affected by water deficit, but no 'G \times T' interaction was

detected, except for N_M. Maximal individual leaf area, SLA, N_M and C_M all decreased in response to drought, whereas Δ increased. Water deficit did not modify either the genotype ranking for LA_{MAX}, SLA, N_M, C_M and Δ or the amplitude of genotypic variability and h^2_{genot} and

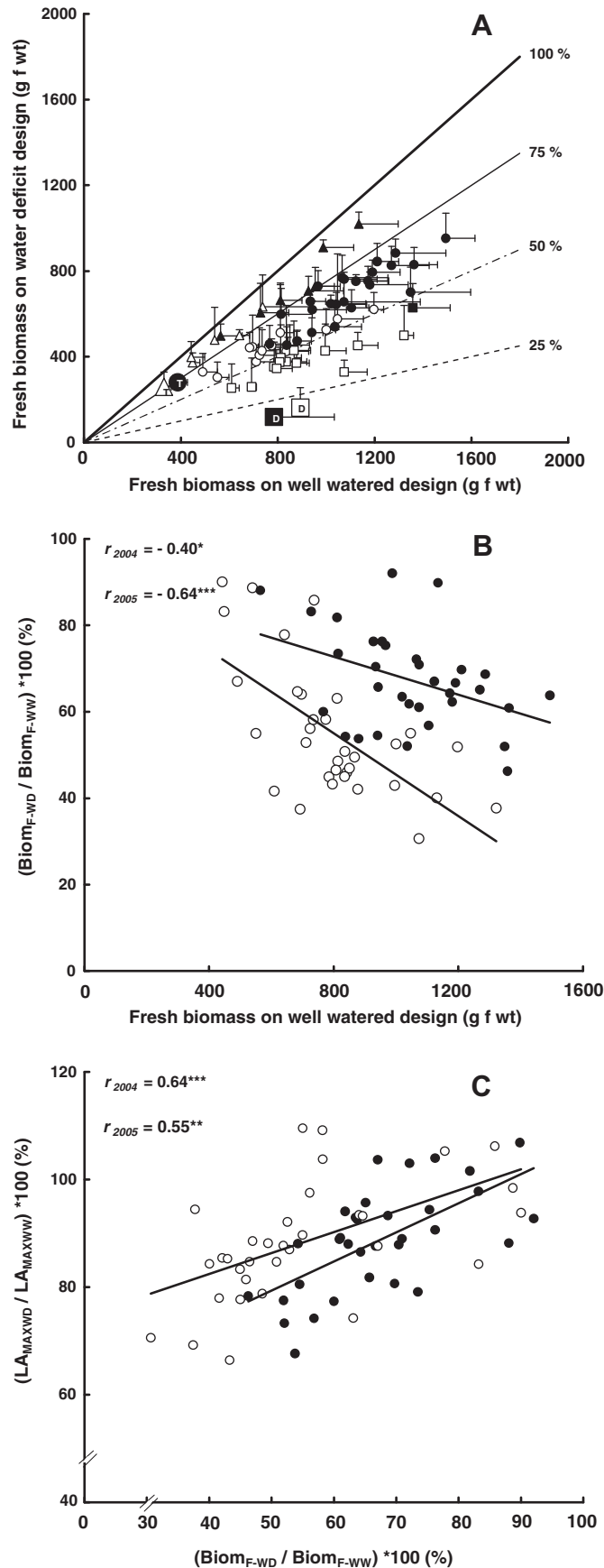


Figure 3. Variability of water-deficit induced reductions in biomass and relationship with productivity or leaf traits in 33 F₁ *P. deltoides* × *P. trichocarpa*. (A) Relationship between Biom_F (g f wt) measured on control and water-deficit plots on 7 January 2005 (closed symbols) and 10 November 2005 (open symbols). Mean values are presented with their SEs. Triangles, circles and squares identify genotypes for which the decrease in mean biomass in response to water deficit ranged from 0% to 25%, 25% to 50% and 50% to 75%, respectively. The two parents *P. trichocarpa* and *P. deltoides* are identified by capital letters (T and D, respectively). (B) Linear correlations (Pearson's coefficient) computed in 2004 (closed symbols; r_{2004}) and 2005 (open symbols; r_{2005}) between the ratio of aboveground biomass measured under water deficit versus control ($\text{Biom}_{F-WD} / \text{Biom}_{F-WW}$) and Biom_{F-WW}. (C) Linear correlations (Pearson's coefficient) computed in 2004 (closed symbols; r_{2004}) and 2005 (open symbols; r_{2005}) between the ratio of Biom_{F-WD} versus Biom_{F-WW} and leaf area measured under water deficit (LA_{MAXWD}) versus control (LA_{MAXWW}).

CV (Table 1; Figure 2). Carbon isotope discrimination was negatively correlated with N_M and scaled positively with SLA as in control conditions, but no correlation was evidenced between Δ and $Biom_F$, $Circ$ and LA_{MAX} (Table 2). A linear and positive relationship was detected between the relative water-deficit-induced decline of $Biom_F$ and that of LA_{MAX} (Figure 3C).

On 15 June 2005, that is 10 days before the onset of the water deficit, Ht of the stools was smaller for trees subjected to water deficit during 2004 than for controls (data not shown). This was assigned to a remnant effect of the 2004 water deficit rather than to a later budflush on the stressed plot. A more severe decrease was observed for $Biom_F$, Ht and $Circ$ in 2005 than in 2004 in response to water deficit (Figures 1 and 3A; Table 1). Contrary to 2004, a significant 'G \times T' interaction was detected for $Biom_F$, Ht and $Circ$, which could be attributed to a cumulative impact of 2004 and 2005 water deficits and a higher rainfall deficit in August 2005 (Figure 1; Table 1). Decreased h^2_{genot} and CV were observed for $Biom_F$, Ht and $Circ$ (Figure 1; Table 1). The relative reduction in $Biom_F$ induced by water deficit scaled negatively with $Biom_F$ of controls, similar to that during 2004 (Figure 3B). During 2005, water deficit also induced a larger decrease in LA_{MAX} and SLA than during 2004, but did not modify either genotype ranking or genotypic variability (Table 1; Figure 2A and B). Only in 2005, h^2_{genot} of LA_{MAX} and SLA decreased in response to water deficit (Table 1). A linear and positive relationship was detected between the relative, water-deficit-induced, decline of $Biom_F$ and that of LA_{MAX} reduction, as was the case during 2004 (Figure 3C).

Compared to the 33 F_1 genotypes, *P. deltoides* displayed the greatest sensitivity to water deficit and the greatest LA_{MAX} reduction, whereas *P. trichocarpa* displayed a slight sensitivity and no reduction in LA_{MAX} (Figure 1A). In response to water deficit, the two parents displayed decreased SLA and N_M , but stable values of Δ , like the 33 F_1 genotypes.

Discussion

Genotypic variability in productivity and Δ under well-watered conditions

Several traits related to productivity, leaf structure and leaf function were tested among 33 genotypes belonging to an F_1 D \times T hybrid progeny. The female parent *P. deltoides* differed from the male parent *P. trichocarpa* by a greater productivity, a lower WUE (as inferred from Δ) and leaves with a higher density/thickness.

A large genotypic variability was detected during 2004 and 2005 among the 33 F_1 genotypes for all traits. Similar levels of variability have already been reported for either related or non-related poplar clones (Bradshaw and Stettler 1995, Wu et al. 1997, Ceulemans and Deraedt 1999, Rae et al. 2004, Marron et al. 2005, Monclus et al. 2005, 2006, Bonhomme et al. 2008). Although the values

recorded during 2005 differed markedly from those of 2004 for most of the variables, genotypic variability and genotype ranking remained comparable and stable from year to year (Niinemets et al. 1998, Barbour et al. 2002, Monclus et al. 2005, 2006).

The area of the largest leaf (LA_{MAX}) was strongly and positively correlated with productivity indicators ($Biom_F$, Ht and $Circ$) across the two successive years of investigation. This correlation confirms that the genotypic variability for productivity can be appreciated from LA_{MAX} in all poplar species and crosses tested so far (Ridge et al. 1986, Barigah et al. 1994, Souch and Stephens 1998, Pellis et al. 2004, Rae et al. 2004, Zhang et al. 2004, Marron et al. 2005, Monclus et al. 2005, 2006). A positive relationship was also evidenced between leaf nitrogen content (N_M) and $Biom_F$, in agreement with many studies that have proposed that the genetic variability for productivity is driven by a combination of total leaf area per stool and photosynthetic capacity (Barigah et al. 1994).

A negative relationship was detected between $Biom_F$ and Δ ; i.e., the most productive genotypes seemed to be simultaneously the most efficient for water use. Similar results have already been shown in other tree species such as *Castanea sativa* Mill., *Pinus pinaster* Aiton or *Larix occidentalis* Nutt. (Guehl et al. 1994, Cassasoli et al. 2004, Zhang et al. 2004). This relationship confirms that there is a potential for improving WUE in poplar without reducing overall productivity, and suggests a control of Δ by photosynthetic capacities rather than stomatal conductance (Farquhar et al. 1989). However, it must be noted that this pattern contrasts with the one previously found for an F_2 progeny of D \times T hybrids and for a large panel of D \times N hybrids (Rae et al. 2004, Marron et al. 2005, Monclus et al. 2006). The relationship between productivity and WUE thus seems to be a function of the genetic background and probably of the degree of kinship.

Impact of water deficits on productivity and WUE

In 2004 and 2005, withholding irrigation induced comparable homogeneous, moderate and long-lasting soil–water deficits, as evidenced from a relative small decline in Ψ_{pd} (–0.3 MPa), which was recorded in the first week of September 2004 (Monclus et al. 2006) and 2005. Although all genotypes (parents and F_1) were subjected to the same soil–water deficit, they differed widely in their sensitivity to water deficit, as inferred from the loss of biomass production. During both years, the two parents differed strongly in this respect: the *P. deltoides* parent displayed an 80% reduction of biomass, whereas no variation was evidenced for *P. trichocarpa*. During 2004, the impact of water deficit on $Biom_F$ varied between 8% and 54% reduction depending on the genotype. During 2005, it ranged from 12% to 70% and a significant 'G \times T' interaction was detected, which modified the genotype ranking for Ht and $Circ$ compared to controls. The larger impact of

water deficit during 2005 was due, at least in part, to a cumulative effect of the 2004 deficit on regrowth dynamics, as shown by the lower values of Ht during spring 2005 on the trees that were subjected to water deficit in 2004.

Water deficit induced decreases in LA_{MAX}, N_M, C_M and SLA in all genotypes. Many other studies have found a similar impact of decreased soil–water potential and increased vapour pressure deficit on these leaf traits (Abrams et al. 1990, Niinemets et al. 1998, Marron et al. 2002, 2003, Wright et al. 2004, Monclus et al. 2006). Unexpectedly, the 2004 water deficit induced a small increase of Δ (decrease of WUE), suggesting that the net CO₂ assimilation rate was relatively more affected than stomatal conductance (Damesin et al. 1997, Korol et al. 1999, Barbour et al. 2002, Monclus et al. 2006).

Variables related to productivity (Biom_F, Ht and Circ) were tightly correlated to LA_{MAX} under water deficit as well as in controls. This result, combined with the finding that LA_{MAX} displays moderate broad-sense heritability, suggests that LA_{MAX} can be used in the breeding programmes as an index of enhanced productivity, irrespective of environmental conditions used for genotypic evaluation. Under water deficit, the correlation observed between Biom_F and Δ in controls disappeared. This was due to the fact that Δ is still partly controlled by the isotopic signature of the carbon assimilated before the onset of the water deficit, whereas growth was immediately affected by water deficit. As under well-watered conditions, Δ scaled negatively with N_M and positively with SLA, suggesting that the genotypic variability in Δ was still driven by the net CO₂ assimilation rate (Farquhar et al. 1989).

During 2004 and 2005, negative relationships were evidenced between tolerance to water deficit and productivity recorded on controls, i.e., the most productive genotypes displayed a greater susceptibility to water deficit. As already evidenced from different poplar species (Monclus et al. 2006), the magnitude of leaf area reduction in response to water deficit seemed to be a good indicator of the magnitude of aboveground biomass reduction. Although the relationships were evidenced between productivity and tolerance to water deficit or Δ , no direct correlation was detected between tolerance to water deficit and Δ , as already reported in D × N hybrids (Monclus et al. 2006). However, productive genotypes were sensitive to water deficit and most of them displayed a low Δ , highlighting that a high tolerance to water deficit was not associated with a high WUE.

Conclusion

This study demonstrated the occurrence of a significant genotypic variability for productivity, Δ (used as a proxy of WUE) and drought tolerance within a *Populus* D × T F₁ family represented by 33 genotypes and their two parents. We showed that productive genotypes display high

WUE and low tolerance to soil–water deficit, but no direct relationship is evidenced between tolerance and WUE similar to what was previously observed in the D × N hybrids growing in the same experimental field design (Monclus et al. 2006). We also confirmed that the LA_{MAX} and the magnitude of leaf area reduction are tightly associated with productivity and drought tolerance, respectively. The moderate broad-sense heritability estimates recorded for LA_{MAX} and Δ suggest that these two traits could be used as indexes of productivity, WUE and tolerance to water deficit in future poplar breeding programmes, but these results still require a validation from older trees. Nevertheless, all these findings suggest that this F₁ mapping progeny displays sufficient variability for productivity, WUE and drought tolerance to dissect the complex inheritance of these traits. In this context, additional studies on the complete F₁ progeny are currently in progress to precise genomic determinants of productivity, WUE and tolerance to water deficit via a QTL (Quantitative Trait Loci) approach.

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Appendix

Table A1. Environmental growth conditions (temperatures, evapotranspiration and daily irradiance) of mature leaves collected on 10 September 2004 and 5 September 2005 from the same field design. No significant difference (ANOVA) has been recorded between 2004 and 2005.

	22 August to 10 September 2004	17 August to 5 September 2005	<i>P</i>
Daily maximum temperature (°C d ⁻¹)	24.8	26.3	ns
Daily minimum temperature (°C d ⁻¹)	12.2	12.4	ns
Daily mean temperature (°C d ⁻¹)	18.5	19.3	ns
Evapotranspiration (mm d ⁻¹)	3.8	4.1	ns
Daily irradiance (J cm ⁻² d ⁻¹)	1715	1739	ns

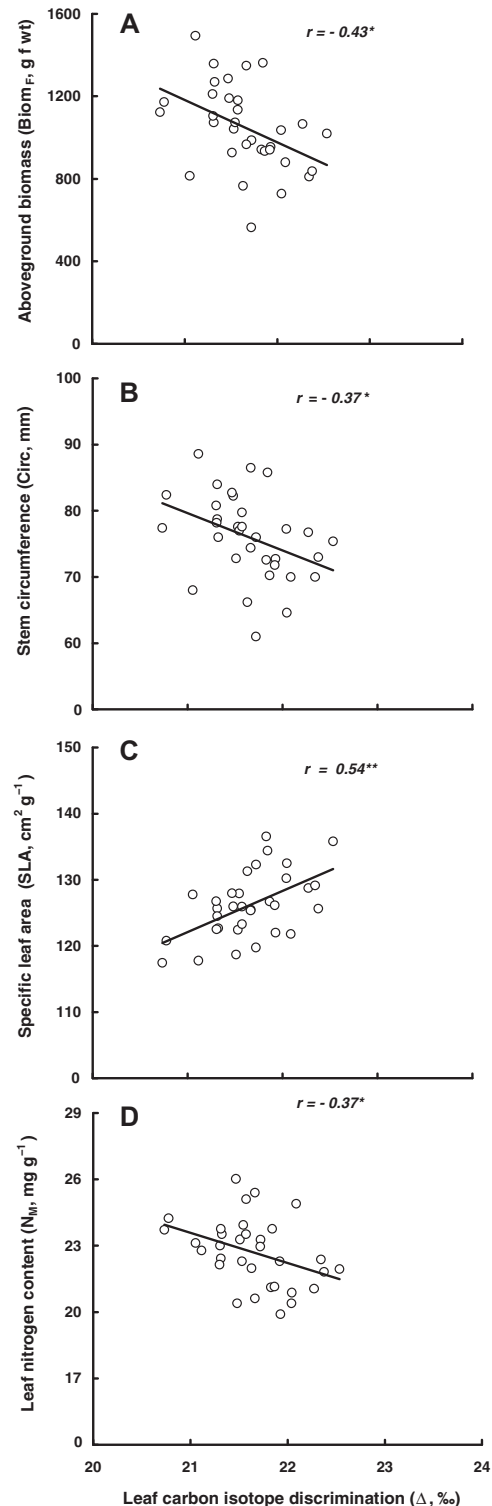


Figure A1. Relationships between leaf carbon isotope discrimination (Δ , ‰) and (A) aboveground biomass ($Biom_F$, g f wt), (B) stem circumference (Circ, mm), (C) specific leaf area ($cm^2 g^{-1}$) and (D) leaf nitrogen content (N_M , $mg g^{-1}$). Relationships were established from the F₁ genotypic mean values ($n = 33$) computed from the data collected in 2004 under well-watered conditions. Pearson's coefficient (r) and their level of significance ($*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$) were indicated for each relationship.