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Research paper

Variability in flooding tolerance, growth and leaf traits in a *Populus deltoides* intraspecific progeny

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Climate change will increase the risk of flooding in several areas of the world where *Populus deltoides* Marshall (eastern cottonwood) is planted, so it would be desirable for this species to select for flooding tolerance. The aims of this work were to explore the variability in growth, leaf traits and flooding tolerance in an F1 full-sib intraspecific progeny of *P. deltoides*, to analyze the correlations of leaf and growth traits with flooding tolerance and to assess their suitability for use in breeding programs. Two-month-old parental clones and their progeny of 30 full-sib F1 genotypes were grown in pots and subjected to two treatments: (i) plants watered to field capacity (control) and (ii) plants flooded up to 10 cm above soil level for 35 days. Growth (height, diameter and biomass partition) and leaf traits (leaf size and number, specific leaf area, leaf senescence, abscission, stomatal conductance, carbon isotope discrimination, stomatal index) were measured. Flooding tolerance for each genotype was estimated as the ratio of the biomass of stressed plants to the biomass of control plants. Results showed segregation in terms of flooding tolerance in the F1 progeny. A significant genotype effect was found for leaf size and number, carbon isotopic discrimination and stomatal conductance, but it did not correlate with flooding tolerance. Height, diameter and root-to-shoot ratio had a positive phenotypic correlation with flooding tolerance, and there was a positive genetic correlation of height and diameter with biomass on both treatments. The narrow sense heritability values for the traits analyzed ranged from 0 to 0.56. We conclude that growth traits are more adequate than leaf traits for selection to increase flooding tolerance. A vigorous initial growth would increase flooding tolerance in young poplar plants.

Keywords: carbon isotopic discrimination, eastern cottonwood, F1, genetic correlation, heritability, leaf size.

Introduction

Populus deltoides Marshall (eastern cottonwood) is a native species to the eastern North America, with a wide range of distribution from the Mexican Gulf coast in the south to the Great Lakes in the north (Richardson et al. 2014). From the ecological viewpoint, *P. deltoides* is a significant species in the floodplains of its native range (Rood et al. 2003). In addition to its importance in natural ecosystems, *P. deltoides* is widely planted around the world, either as a pure species or as an interspecific hybrid with other *Populus* species (Dickman and Kuzovkina 2014). In some countries, *P. deltoides* is planted in

areas that may experience episodes of flooding (Du et al. 2012, Luquez et al. 2012). The occurrence of flooding episodes will increase due to climate change in several regions of the world (Kreuswieser and Rennenberg 2014), including the areas where the eastern cottonwood is planted. Therefore, it is important to breed new clones with increased flooding tolerance to face these adverse conditions.

The occurrence of genotypic variability for flooding tolerance in *P. deltoides* and its hybrids with other species is well documented (Gong et al. 2007, Guo et al. 2011, Luquez et al. 2012). Furthermore, there is an extensive literature regarding

the relationship between different physio-morphological leaf traits, and growth and productivity in poplars. For instance, total leaf area, individual leaf area, leaf number, leaf number increment rate, carbon isotopic discrimination and stomatal density have shown correlation with growth and productivity in different *Populus* species and hybrids (Rae et al. 2004, Monclus et al. 2005, Marron and Ceulemans 2006, Al Afas et al. 2006, Dillen et al. 2008). Traits such as leaf area, leaf number, specific leaf area and carbon isotopic discrimination have shown variability in natural populations of *Populus nigra* L. (Chamaillard et al. 2011, Guet et al. 2015), *Populus balsamifera* L. (Soolanayakanahally et al. 2009), *Populus trichocarpa* Torrey & Gray (Gornall and Guy 2007), *Populus tremuloides* Michaux (Kanaga et al. 2008), *Populus davidiana* Dode (Zhang et al. 2004) and *P. deltoides* (Rowland 2001). Some of these leaf traits can be affected by flooding, causing a negative impact on growth (Gong et al. 2007, Guo et al. 2011, Luquez et al. 2012, Rodríguez et al. 2015). However, little is known about the relationship of these leaf traits to flooding tolerance, and if they may be useful for breeding more flood-tolerant genotypes.

Since the genus is dioecious and wind-pollinated, there is a high degree of gene flow within natural *Populus* populations (Slavov and Zhelev 2010). In consequence, it is not surprising that the eastern cottonwood shows a high genetic diversity and a low level of population differentiation at the nucleotide level (Fahrenkrog et al. 2017a). Taking these facts into account, we hypothesize that the materials included in breeding programs still preserve an important amount of the genetic variability occurring in natural populations. When subjected to controlled crosses, we may expect the segregation of different traits at F1 and/or F2 level, including flooding tolerance.

We analyzed the parental genotypes and 30 full-sib genotypes of an F1 eastern cottonwood intraspecific progeny. The aims of this work were to explore the extent of the variability in growth, leaf traits and flooding tolerance in an F1 of an intraspecific cross of *P. deltoides*, to analyze the correlations of growth and leaf traits with flooding tolerance and to assess their suitability for use in breeding programs to increase the tolerance to this stress.

Materials and methods

Plant material, growth conditions and stress treatment

The parental clones were two *P. deltoides* individuals: the female clone named Australiano 106-60 (abbreviated A106) and the male clone named Mississippi Slim, locally known as Stoneville 67 (abbreviated ST67). The parental genotypes of the cross were open pollinated progeny of two selected female clones. The female parent of A106 was collected near College Station, Texas, while ST67 was selected from seeds of a female tree from Issaquena County, Mississippi (Luquez et al. 2012). This family was selected for the study due to the response to flooding

of the parental genotypes assessed in a previous work; both clones having an intermediate flooding tolerance compared with the other genotypes analyzed (Luquez et al. 2012). The cross was carried out in the year 2006, as part of the Instituto Nacional de Tecnología Agropecuaria's (INTA) poplar breeding program, resulting in an F1 of 190 full-sib individuals. From these F1, a subset of 30 genotypes was selected, representing a range of growth from outstanding individuals to very poor performers. In this paper, these 30 full-sib genotypes were analyzed together with the parental clones.

One-year-old cuttings of 25-cm long were planted in 5-l pots with a 1:1 mixture of soil and sand (one cutting per pot). Before planting, the cuttings were soaked overnight in water and treated with fungicides to avoid diseases. The planting date was between the first and the second of September 2015. The plants were grown under natural irradiance and photoperiod in a greenhouse in La Plata (34° 59' 09''S; 57° 59' 42'' W, elevation: 26 m above sea level). The pots were watered daily, keeping the substrate at field capacity. Before the beginning of the treatments, plants were pruned leaving only one shoot per cutting and fertilized twice with 50 ml per pot of complete Hoagland solution (Legget and Frere 1971). The experiment was a completely randomized design, with six repetitions for each genotype and treatment ($N = 384$ plants). The trial was surrounded with a border of plants that were not used for measurements. The control (non-flooded) plants were watered daily, and the flooded plants were placed inside a 10-l pot sealed with a plastic bag and filled with water up to 10 cm above soil level. The stress treatment started on 9 November 2015 and lasted for 35 days. An outline of the experimental design is provided in Figure S1 available as Supplementary Data at *Tree Physiology* Online.

Plant growth measurements

All measured variables with their abbreviations and units are listed in Table 1. Plant height (H) was measured every week with a graduated stick. For each plant, the height values were plotted vs time, and a linear function was adjusted. The growth rate in height (GRH) was determined as the slope of the straight line. The basal diameter (D) was determined with a digital caliper in the basal part of the shoot at the beginning and at the end of the experiment. The growth rate in diameter (GRD) was determined as described for GRH. At the end of the experiment, the total dry weight (TDW) of leaves, stem and roots was determined after drying them to constant weight in an oven at 65 °C. Root-to-shoot ratio (RSR) and root-to-leaf ratio (RLR) were calculated with those data.

The Flooding Tolerance Index (FTI, Fichot et al. 2009) was determined using the above-ground dry weight (AGDW) as follows:

$$FTI = (AGDW_{stressed} / AGDW_{control}) \times 100$$

Table 1. Traits, abbreviations and units of the measurements carried out on the parental genotypes and F1 full-sib progeny for the A106 × ST67 family.

Trait	Abbreviation	Units
Final height	H	cm
Final diameter	D	mm
Growth rate in height	GRH	cm day ⁻¹
Growth rate in diameter	GRD	mm day ⁻¹
Individual leaf area	ILA	cm ²
Final leaf number	LN	–
Leaf number increase rate	LIR	Leaves day ⁻¹
Abscission rate	AR	Leaves day ⁻¹
Leaf senescence rate	SEN	SPAD units day ⁻¹
Stomatal conductance	g_s	mmol m ⁻² s ⁻¹
Specific leaf area	SLA	cm ² g ⁻¹
Total dry weight	TDW	g
Root-to-shoot ratio	RSR	–
Root-to-leaves ratio	RLR	–
Carbon isotopic discrimination	Δ	‰
Stomatal index	SI	–

The values of FTI calculated with TDW (including roots) had a strong correlation with the estimation carried out with AGDW ($r = 0.98$, $P < 0.0001$, $N = 32$). Consequently, we kept only the FTI determined with AGDW on this work.

Physio-morphological leaf traits

Before starting the treatment, the latest expanded leaf was tagged with a colored wire. The leaves above and below the mark were counted, and the total leaf number (LN) was determined as the sum of both. The leaf increase rate (LIR) was determined in the same way as the growth rate, using the number of leaves above the mark. The abscission rate (AR) was determined by the number of leaves below the mark, as in LIR.

The chlorophyll content of the tagged leaf was measured twice with a Minolta Chlorophyll Meter SPAD 502 (Osaka, Japan), and a linear function was adjusted for the growth rate as described above, the leaf senescence rate (SEN) being the value of the slope multiplied by -1 . The latest leaf expanded during flooding was sampled for carbon isotopic discrimination (Δ) and stomatal index (SI). This sampling was carried out at the end of the experiment. To determine Δ , the leaf was dried at 35 °C until constant weight and ground to a powder with a mortar and a pestle. The determination of the carbon isotopic composition of the leaf (δC_{13leaf}) was carried out at the CATNAS laboratory—Centro de Aplicaciones de Tecnología Nuclear en Agricultura Sostenible (Facultad de Agronomía, Universidad de la República, Montevideo, Uruguay). The carbon isotopic composition of the air (δC_{13air}) was assumed to be -8‰ . Δ was calculated according to Farquhar et al. (1989):

$$\Delta = (\delta C_{13air} - \delta C_{13leaf}) / (1 + (\delta C_{13leaf} / 1000)) (\text{‰})$$

For stomata and cell counting, an imprint of the abaxial side of the leaf was made with transparent nail varnish and transparent tape. The imprints were mounted on slides, observed under the microscope at 400× and photographed with a digital camera (Olympus E-330). Ten fields for sample were counted with the software ImageJ (<https://imagej.nih.gov/ij/>, Schneider et al. 2012), and there were three replicates for the F1 and four replicates for each parental genotype. The field area was 0.0997 mm². The SI was determined according to Masle et al. (2005).

The leaf below the one used for SI was selected to determine individual leaf area (ILA) and specific leaf area (SLA). The leaves were scanned, and the area was determined with the software ImageJ.

The leaf stomatal conductance (g_s) was determined with a Decagon SC1 porometer on the abaxial side of the latest expanded leaf. The measurements were carried out between 10:30 and 13:30 h on cloudless days, with an average irradiance of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. At least four to five plants of each genotype and treatment were determined on each measurement date.

Statistical analysis

The ANOVA and correlation analysis were carried out with R 3.5.0 (R Development Core Team 2017), using the package agricolae version 1.2-8 (de Mendiburu 2017). The aov function was used for ANOVA, with clone, treatment and their interaction as factors. The Pearson and Spearman coefficients were used to calculate phenotypic and genetic correlations. The genetic correlations among traits were determined by relating the best linear unbiased predictions of the breeding values of each genotype (Luquez et al. 2008). The narrow sense heritability (h^2) and breeding values were estimated with the REML method using the breedR package (Muñoz and Sanchez 2018, script for R in Table S2 available as Supplementary Data at *Tree Physiology* Online). The absence of spatial structure in the data was also checked using breedR.

The principal components analysis (PCA) was done with the software MVSP (Kovach Computing Services, Pentraeth, Isle of Anglesey, United Kingdom, <https://www.kovcomp.co.uk/mvsp/>). The data were standardized and centered, using the clonal means of each treatment for the analysis. For the variables that were measured several times, like height and stomatal conductance, only the last date was included in the PCA. At this point, the differences between the treatments were maximized.

Results

In PCA (Figure 1), the first component (PC1) represents the variation related to the flooding treatment, separating control and flooded plants into two distinct groups, as shown by the color code of the treatments. Due to the clear separation

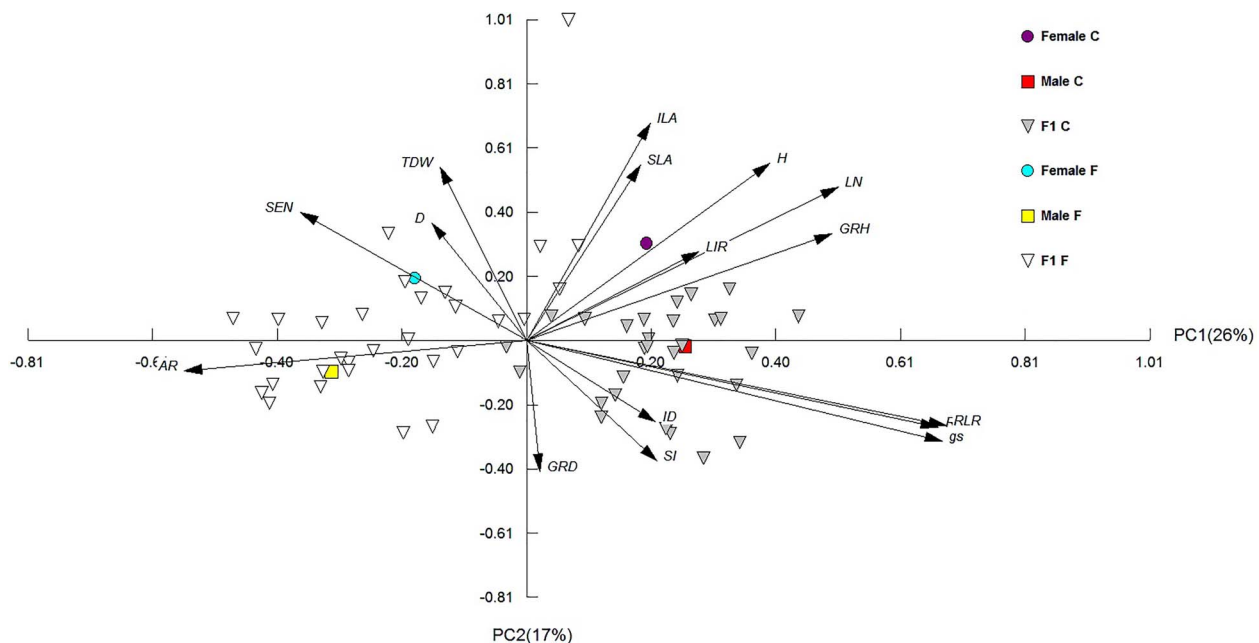


Figure 1. Principal components analysis of the parental clones and 30 full-sib genotypes of the F1 belonging to a *P. deltoides* intraspecific cross. The complete variable names and units are listed in Table 1. The analysis was carried out using the average values for each genotype and treatment. A106: female and ST67: male.

caused by flooding, the correlations and heritability values were calculated separately for control and flooded plants. The second principal component (PC2) represents the genotypic variation. PC1 and PC2 together explained 43% of the total variability. Most traits either decreased or were not affected by flooding, except for SEN and AR, which increased with the stress treatment. As for g_s , it was reduced by flooding in both the parental genotypes and the progeny (Figure S2 available as Supplementary Data at *Tree Physiology* Online).

The PCA results were in accordance with those of ANOVA (Table 2). Most variables were significantly reduced by the flooding treatment except for D, GRD, LIR and Δ . The variables significantly affected by the genotype were final H, ILA, LN, g_s and Δ . The mean values and standard deviation of all traits for the parental genotypes and the F1 are shown in Table S1 available as Supplementary Data at *Tree Physiology* Online.

The narrow sense heritability values (h^2) ranged from low to moderate for most traits (Table 2), and in some cases, they differed in control and flooded treatments. GRD and SEN showed h^2 values close to zero.

The phenotypic correlations (Table 3) differed for the control and flooded treatments on several traits. H correlated positively with D ($r = 0.50$, $P < 0.01$ for control and $r = 0.67$, $P < 0.001$ for flooded), GRH ($r = 0.65$, $P < 0.001$ for control and $r = 0.86$, $P < 0.001$ for flooded), LN ($r = 0.42$, $P < 0.05$ for control and $r = 0.60$, $P < 0.001$ for flooded) and LIR ($r = 0.40$, $P < 0.05$ for control and $r = 0.37$, $P < 0.05$ for flooded) in both control and flooded treatments, while it correlated negatively with RSR only in control plants ($r = 0.40$, $P < 0.05$). RSR and RLR

showed a strong and significant correlation between them on both treatments ($r = 0.98$, $P < 0.001$ for control and $r = 0.94$, $P < 0.001$ for flooded). D correlated with LN ($r = 0.54$, $P < 0.01$ for control and $r = 0.61$, $P < 0.001$ for flooded) and TDW ($r = 0.52$, $P < 0.01$ for control and $r = 0.49$, $P < 0.05$ for flooded) in both control and flooded plants. For the rest of the variables, there were significant correlations for only one treatment.

The genetic correlations among traits are depicted in Table 4. H showed a significant and positive genetic correlation on both treatments with D ($r = 0.51$, $P < 0.01$ for control and $r = 0.62$, $P < 0.001$ for flooded), LN ($r = 0.52$, $P < 0.01$ for control and $r = 0.56$, $P < 0.001$ for flooded), LIR ($r = 0.39$, $P < 0.05$ for control and $r = 0.39$, $P < 0.05$ for flooded) and TDW ($r = 0.52$, $P < 0.01$ for control and $r = 0.71$, $P < 0.001$ for flooded). D had a positive correlation on both treatments with LN ($r = 0.36$, $P < 0.05$ for control and $r = 0.65$, $P < 0.001$ for flooded) and TDW ($r = 0.75$, $P < 0.001$ for control and $r = 0.71$, $P < 0.001$ for flooded). D had a negative correlation with RLR ($r = -0.36$, $P < 0.05$) and RSR ($r = -0.36$, $P < 0.05$) in the control treatment, and a positive correlation for the same traits in flooded plants ($r = 0.40$, $P < 0.01$ for RSR and $r = 0.45$, $P < 0.01$ for RLR). GRH correlated positively on both treatments with GRD ($r = 0.41$, $P < 0.05$ for control and $r = 0.61$, $P < 0.001$ for flooded) and LIR ($r = 0.69$, $P < 0.001$ for control and $r = 0.39$, $P < 0.05$ for flooded). RSR and RLR had a strong correlation between them in both treatments, similar to the phenotypic correlations ($r = 0.91$, $P < 0.001$ for control and $r = 0.98$, $P < 0.001$ for flooded).

Table 2. ANOVA results (with genotype and treatment as factors) and narrow sense heritability values (h^2) for the different traits measured in the A106 × ST67 family. ns non-significant, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Standard error for heritability is given in parentheses.

Trait	Genotype	Treatment	Interaction	h^2 control	h^2 flooded
H	*	*	ns	0.30 (0.11)	0.30 (0.12)
D	ns	ns	ns	0.18 (0.11)	0.15 (0.11)
GRH	ns	***	*	0.34 (0.11)	0.28 (0.12)
GRD	ns	ns	ns	0.09 (0.10)	0.03 (0.09)
ILA	**	***	ns	0.56 (0.11)	0.43 (0.12)
LN	*	**	ns	0.45 (0.12)	0.38 (0.12)
LIR	ns	ns	ns	0.48 (0.11)	0.24 (0.12)
AR	ns	***	ns	0.26 (0.13)	0.32 (0.13)
SEN	ns	**	ns	0.00 (0.01)	0.00 (0.01)
g_s	*	***	ns	0.11 (0.12)	0.29 (0.12)
SLA	ns	***	ns	0.14 (0.12)	0.31 (0.13)
TDW	ns	*	ns	0.21 (0.13)	0.31 (0.13)
RSR	ns	***	ns	0.11 (0.12)	0.37 (0.12)
RLR	ns	***	*	0.12 (0.12)	0.42 (0.12)
Δ	*	ns	*	0.14 (0.15)	0.49 (0.13)
SI	ns	**	*	0.27 (0.18)	0.44 (0.16)

The other correlations were only significant for one of the treatments (control or flooded). For instance, in the control treatment, Δ had a negative correlation with LN ($r = -0.40$, $P < 0.05$) and TDW ($r = -0.48$, $P < 0.01$) but a positive one with SLA ($r = 0.51$, $P < 0.01$). Meanwhile in flooded plants, Δ had a positive correlation with g_s ($r = 0.36$, $P < 0.05$), RSR ($r = 0.37$, $P < 0.05$) and RLR ($r = 0.36$, $P < 0.05$), and a negative one with SEN ($r = -0.38$, $P < 0.05$).

The FTI is depicted in Figure 2. A very interesting result was that most F1 genotypes had a higher flooding tolerance than both parents. For the control treatment (Figure 3), FTI had a significant negative correlation with GRH ($r = -0.49$, $P < 0.01$) and LIR ($r = -0.68$, $P < 0.001$), and a positive one with TDW ($r = 0.51$, $P < 0.01$). In flooded plants (Fig. 4), FTI had a positive correlation with H ($r = 0.42$, $P < 0.05$), D ($r = 0.50$, $P < 0.01$), RSR ($r = 0.39$, $P < 0.05$) and RLR ($r = 0.43$, $P < 0.05$).

Discussion

Variability in flooding tolerance in the F1 progeny of the eastern cottonwood

There is extensive literature on hybrid vigor and transgressive segregation for different traits in F1 and F2 crosses of *Populus* (Slavov and Zhelev 2010). We show a considerable transgressive segregation for flooding tolerance at the intraspecific level in *P. deltoides*. To quantify flooding tolerance, we used an index that measures the ability to limit growth losses under stress (Fichot et al. 2009). Both parental genotypes and some of the individuals of the F1 population experienced a reduction in biomass under flooding (FTI below 100), but most F1 genotypes had a higher flooding tolerance than the parental clones. Several individuals of the F1 population had a higher above

ground biomass accumulation in flooded plants than in the non-flooded treatment; consequently, their FTI value was above 100. This increase in FTI is not a straightforward consequence of the reduction in the root-to-shoot ratio caused by flooding (Rodríguez et al. 2015), because there is an increase in the total biomass of the flooded plants on those genotypes (data not shown). The most interesting result is the possibility of obtaining clones with a higher flooding tolerance than that of the parental genotypes included in breeding programs. These results are consistent with the data indicating a high genetic variability within natural populations in the southern range of the eastern cottonwood distribution (Fahrenkrog et al. 2017a, 2017b), from where the parental genotypes of the male and female clones were collected.

One important challenge to face is that flood tolerance changes with the age of the plants (Glenz et al. 2006); hence, caution is needed when extrapolating results to older plants. For practical reasons, most of the evaluations for flooding tolerance are carried out in small plants growing in pots. In the case of a plantation from cuttings, the usual practice in *P. deltoides*, the establishment phase is the point of highest vulnerability regarding the survival of the plant. In consequence, the evaluation of flooding tolerance at this early stage is meaningful for the development of poplar plantations, even when the results may vary for older plants.

Flooding and genotypic effects on leaf traits and its correlation with growth in the eastern cottonwood

There is extensive literature on leaf traits variation and its correlation with growth and yield in poplar crosses and natural populations. In this *P. deltoides* cross, we found genotypic variability on several leaf traits, such as g_s , ILA and LN. Similar results had been previously found for *P. deltoides*

Table 3. Phenotypic correlations (Pearson correlation coefficient) between different traits measured in the parental genotypes and the F₁, for the A106 × ST67 family. *N* = 32. Correlations for control plants in the lower part of the table (in italics). Correlations for flooded plants in the upper part of the table. Statistically significant correlations are indicated by bold type. **P* < 0.05; ***P* < 0.01, ****P* < 0.001.

Trait	H	D	GRH	GRD	ILA	LN	LIR	AR	SEN	<i>g</i> _s	SLA	TDW	RSR	RLR	Δ	SI
H	1	0.67 **	0.86 **	-0.08	0.20	0.60 **	0.37 *	-0.17	0.07	-0.36	0.13	0.32	0.05	0.09	0.14	-0.20
D	0.50 **	1	0.49 **	-0.09	0.13	0.61 **	0.19	-0.28	0.20	-0.46 **	-0.02	0.49 *	-0.01	0.03	-0.09	-0.24
GRH	0.65 **	0.14	1	0.12	0.06	0.56 **	0.46 **	-0.27	0.02	-0.17	0.04	0.18	-0.03	0.01	0.18	-0.17
GRD	-0.23	-0.30	0.14	1	-0.53 *	-0.06	0.27	0.21	-0.18	0.32	-0.52	-0.18	-0.32	-0.27	0.37 *	-0.22
ILA	0.06	0.52 **	0.15	-0.34	1	0.31	-0.02	-0.19	-0.26	-0.34	0.97 **	0.31	0.40	0.33	0.01	-0.18
LN	0.42 *	0.54 **	0.27	0.04	0.16	1	0.37 *	-0.65 **	0.37	-0.12	0.27	0.46 *	-0.01	-0.02	0.06	-0.17
LIR	0.40 *	0.33	0.69 **	0.22	0.14	0.48 **	1	0.01	-0.23	0.13	-0.04	0.24	-0.43 *	-0.33	0.17	-0.25
AR	0.07	0.04	0.01	-0.26	0.13	-0.32	0.05	1	-0.23	0.09	-0.20	-0.48 *	-0.16	-0.14	0.13	-0.06
SEN	-0.08	-0.22	-0.12	-0.15	-0.38 *	0.10	0.02	-0.14	1	-0.13	-0.26	0.26	-0.03	0.01	-0.15	-0.15
<i>g</i> _s	0.15	-0.16	0.27	0.34	-0.14	-0.06	0.21	-0.05	-0.06	1	-0.25	-0.49 *	-0.14	-0.07	0.17	0.50 **
SLA	-0.18	-0.50 **	0.35	0.28	-0.03	-0.50 **	0.12	0.06	-0.02	0.15	1	0.19	0.40 *	0.32	0.10	-0.12
TDW	0.23	0.52 **	-0.26	-0.34	0.35	0.29	-0.27	-0.07	-0.18	-0.19	-0.58 **	1	-0.14	-0.18	-0.16	-0.37 *
RSR	-0.40 *	-0.01	-0.48 **	0.17	-0.03	-0.08	-0.07	0.04	-0.20	-0.10	-0.24	0.32	1	0.94 **	-0.10	0.20
RLR	-0.32	0.00	-0.44 *	0.15	-0.06	-0.09	-0.07	0.05	-0.16	-0.06	-0.26	0.38	0.98 **	1	-0.09	0.31
Δ	-0.06	-0.44 *	0.14	0.10	-0.25	-0.49 **	-0.08	0.05	-0.12	0.10	0.56 **	-0.28	-0.04	-0.02	1	-0.26
SI	-0.14	-0.06	-0.11	0.18	-0.13	0.03	-0.13	-0.12	0.29	0.03	-0.16	-0.05	-0.19	-0.15	-0.05	1

Table 4. Genetic correlations (spearman correlation coefficient) between the best linear unbiased predictions of the breeding values for different traits measured in the parental genotypes and the F₁, for the A106 × ST67 family. *N* = 32. Correlations for control plants in the lower part of the table (in italics). Correlations for flooded plants in the upper part of the table. Statistically significant correlations are indicated by bold type. **P* < 0.05; ***P* < 0.01, ****P* < 0.001.

Trait	H	D	GRH	GRD	ILA	LN	LIR	AR	SEN	<i>g</i> _s	SLA	TDW	RSR	RLR	Δ	SI
H	1	0.62 **	0.16	-0.01	0.27	0.56 **	0.39 *	0.15	-0.02	-0.02	0.11	0.71 **	0.26	0.37 *	0.07	-0.10
D	0.51 **	1	0.07	0.14	0.33	0.65 **	0.35	0.32	-0.22	-0.11	-0.33	0.71 **	0.40 *	0.45 **	-0.03	0.27
GRH	0.16	-0.09	1	0.61 **	-0.08	0.11	0.39 *	0.16	-0.05	0.33	0.33	-0.22	0.16	0.21	0.25	0.04
GRD	0.13	0.10	0.41 *	1	-0.07	0.15	0.19	0.21	-0.04	0.37 *	0.19	-0.36 *	0.26	0.27	0.29	0.11
ILA	0.17	0.49 **	0.15	-0.10	1	0.21	0.20	0.08	-0.07	-0.19	-0.19	0.49 **	0.09	0.10	-0.12	-0.03
LN	0.52 **	0.36 *	0.08	0.25	0.04	1	0.49 **	0.65 **	-0.32	-0.08	0.02	0.49 **	0.22	0.24	0.13	0.00
LIR	0.39 *	0.29	0.69 **	0.23	0.24	0.47 **	1	0.10	0.07	0.21	-0.26	0.20	-0.05	0.04	0.10	0.12
AR	0.07	-0.03	0.10	-0.15	-0.07	0.35 *	0.04	1	-0.20	0.15	0.22	0.12	0.04	-0.01	0.01	-0.09
SEN	0.07	0.20	0.08	-0.24	0.43 *	0.03	0.16	0.03	1	-0.01	0.14	-0.20	-0.05	-0.01	-0.38 *	0.09
<i>g</i> _s	-0.05	-0.29	0.41 *	0.42 *	-0.05	-0.08	0.34	0.12	0.01	1	0.06	-0.30	-0.02	-0.01	0.36 *	-0.09
SLA	-0.09	-0.32	0.47 **	0.33	0.11	-0.47 **	0.14	-0.07	-0.03	0.18	1	-0.20	0.34	0.33	0.29	-0.28
TDW	0.52 **	0.75 **	-0.22	-0.12	0.41 *	0.67 **	0.24	0.08	0.17	-0.49 **	-0.54 **	1	0.24	0.30	-0.05	0.05
RSR	-0.48 **	-0.41 *	-0.43 *	-0.27	-0.38 *	-0.28	-0.52 **	0.02	0.02	-0.22	-0.08	-0.17	1	0.98 **	0.37 *	-0.04
RLR	-0.26	-0.36 *	-0.46 **	-0.10	-0.30	-0.25	-0.48 **	0.00	0.02	-0.22	-0.07	-0.09	0.91 **	1	0.36 *	0.02
Δ	0.01	-0.42 *	0.09	0.19	-0.14	-0.40 *	-0.14	-0.10	0.12	-0.05	0.51 **	-0.48 **	0.19	0.28	1	-0.19
SI	-0.14	-0.32	-0.16	-0.35 *	-0.48 **	-0.17	-0.24	0.04	-0.08	0.18	-0.13	-0.33	0.30	0.43 *	0.17	1

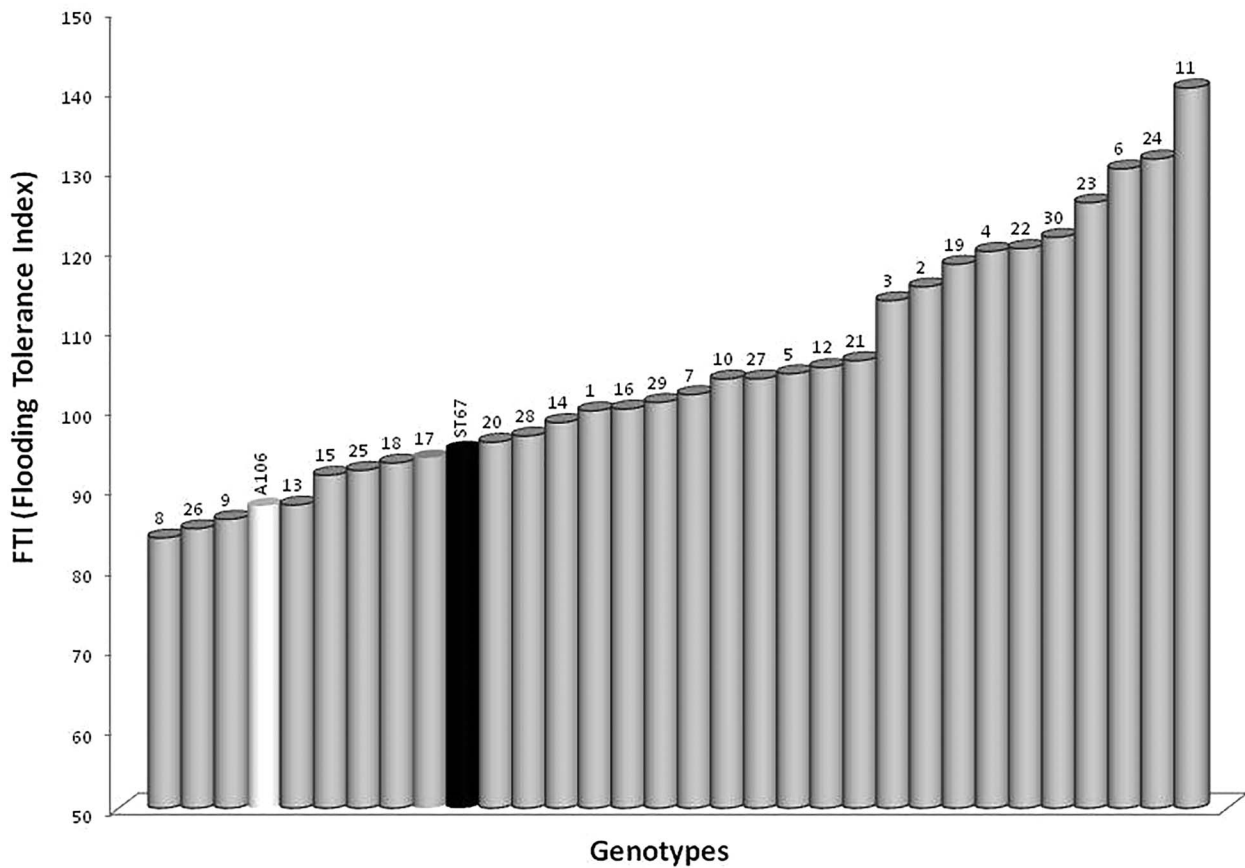


Figure 2. The FTI of the parental clones and 30 full-sib genotypes of the F1 belonging to a *P. deltoides* intraspecific cross. The FTI calculation was described in the Material and methods. A106: female and ST67: male.

(Rowland 2001), *P. nigra* (Chamaillard et al. 2011, Guet et al. 2015), *P. tremuloides* (Kanaga et al. 2008) and *P. balsamifera* (Soolanayakanahally et al. 2009). These traits were also significantly reduced by flooding, as previously reported for *P. deltoides* and other species (Gong et al. 2007, Du et al. 2008, Luquez et al. 2012). We did not find genotypic variability for stomatal density, probably because the parental genotypes had similar leaf morphologies, in contrast to the segregation reported for interspecific hybrid poplars with contrasting leaf traits (Al Afas et al. 2006, Dillen et al. 2008).

Δ represents a proxy for the photosynthesis to stomatal conductance ratio (instantaneous water-use efficiency, Chamaillard et al. 2011), and it has shown genotypic variability among different *Populus* species (Zhang et al. 2004, Gornall and Guy 2007, Kanaga et al. 2008, Soolanayakanahally et al. 2009, Guet et al. 2015). We found a significant effect of the genotype but not of the treatment on Δ , in spite of the reduction in g_s in the flooded plants. In addition, we did not find a correlation in either treatment between Δ and g_s , as occurred with *P. nigra* (Guet et al. 2015). A possible explanation for this result is that flooded leaves rely on remobilized carbon to compensate for the photosynthetic reduction that occurs under flooding (Du et al. 2012, Rodríguez et al. 2015). Previous results regarding

responses to stress of Δ showed disparity. Δ did not change in response to moderate drought in poplar (Monclus et al. 2009), but it was significantly affected in *P. davidiana* under a limited water supply (Zhang et al. 2004).

We did not find phenotypic correlations between Δ and total growth for either control nor flooded plants, except for a moderate correlation with D in control plants. The results have been variable for other *Populus* species; e.g., there was no correlation between Δ and growth in natural populations of *P. nigra* (Chamaillard et al. 2011), while there was correlation in *P. balsamifera* populations (Soolanayakanahally et al. 2009).

Phenotyping and breeding for flooding tolerance

Some of the most meaningful changes conveying adaptation/tolerance to flooding take place in roots, not an easy organ to phenotype, least of all in a breeding program in which a high number of genotypes are to be measured, hence, the need to identify non-destructive, easy-to-phenotype traits (i.e., avoiding phenotyping of roots, if possible) that correlate with flooding tolerance and preferably without subjecting the plants to flooding. Leaf traits are obvious candidates, since they are relatively easy to measure, show genotypic variability in *Populus* and are affected by flooding. However, in the family analyzed,

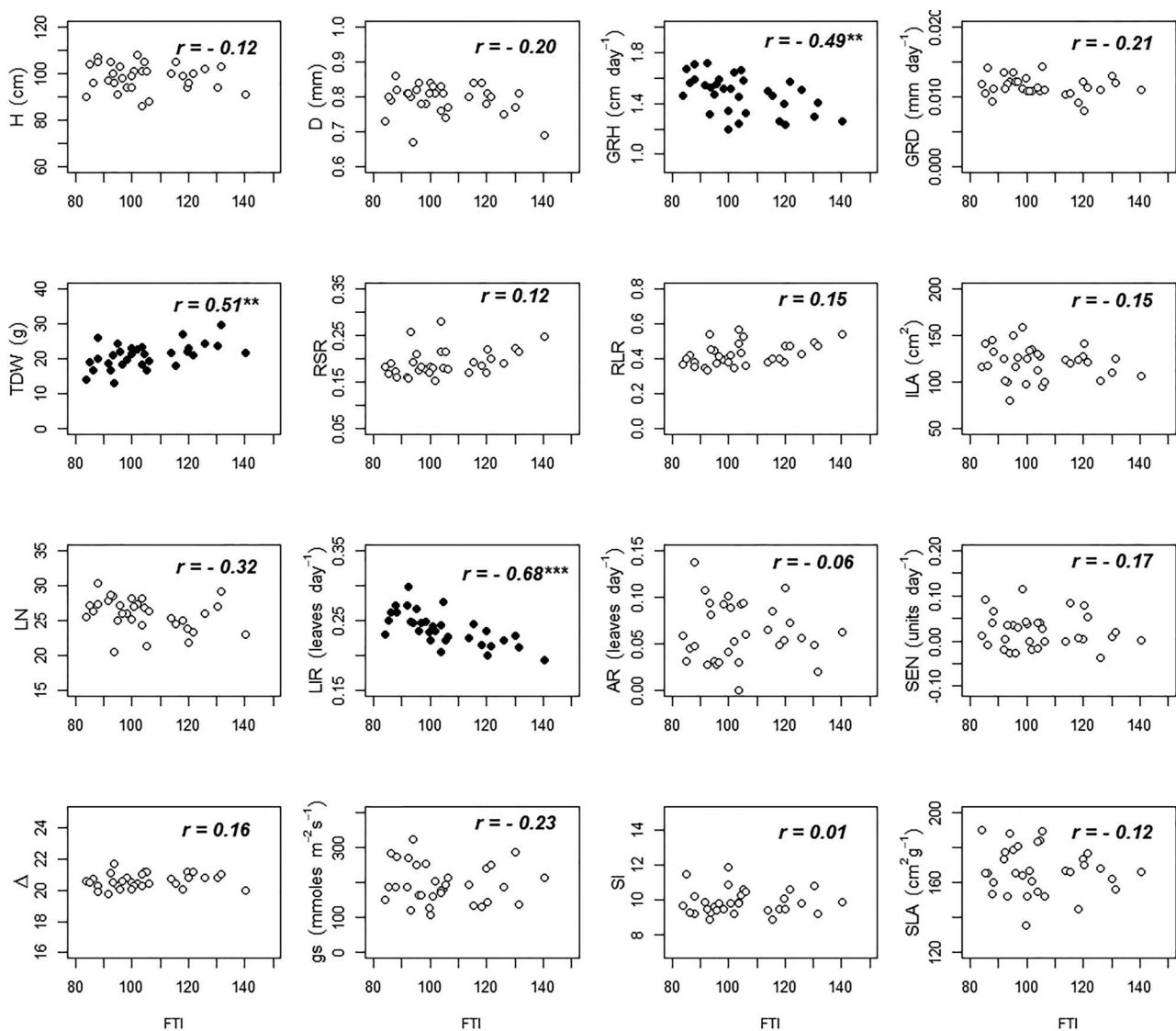


Figure 3. Pearson correlation coefficient between FTI and the different traits measured for the control treatment, for the parental clones and 30 full-sib genotypes of the F1 of a *P. deltoides* intraspecific cross. $N = 32$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Open symbols: non-significant correlation with FTI. Closed symbols: significant correlation with FTI.

the morphological and physiological leaf traits did not show any correlation with flooding tolerance (measured with FTI), with the exception of LIR, and only in the non-flooded plants. Some growth traits showed correlation with flood tolerance. In particular, RSR, H and D had a statistically significant positive correlation with FTI in flooded plants. These results imply that a bigger size combined with a higher root biomass is a favorable combination of traits for flooding tolerance in young plants obtained from cuttings. Similar results were obtained from willows, in which young plants with a vigorous early growth were more able to cope successfully with flooding (Rodríguez et al. 2015).

For breeding, it is important to know the heritability of the traits, in particular, the narrow sense heritability, which is a measure of the response to selection (Lynch and Walsh 1998). Our h^2 estimations rated from very low to moderate, and they

differed in some traits for control and flooded treatments. This is not surprising, since heritability values are highly influenced by factors such as environmental conditions and plant age (Lynch and Walsh 1998). Most of the values published for the traits measured in *Populus* are for broad sense heritability (H^2); therefore, the comparisons are not straightforward. For instance, Fahrenkrog et al. (2017b) reported H^2 values of 0.71 for height and 0.51 for diameter for a collection of 391 unrelated genotypes of *P. deltoides* of a similar age to the plants of our experiment, but in this case, the genotypic variance included other components (dominance, epistasis) in addition to the additive genetic variance. For leaf traits, our results are within the range of the H^2 values for other *Populus* species and hybrids (Marron and Ceulemans 2006, Kanaga et al. 2008, Monclus et al. 2009, Chamaillard et al. 2011).

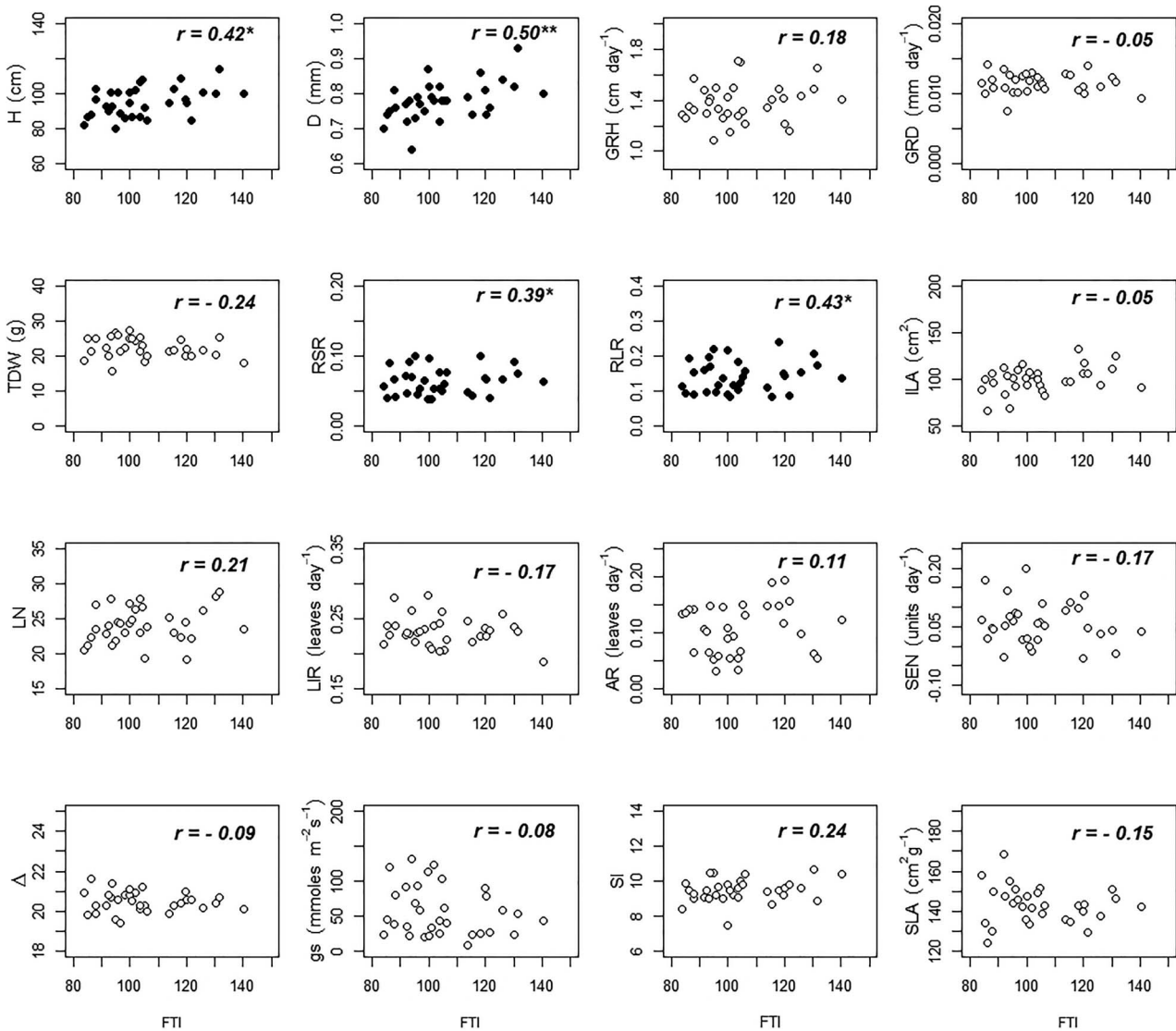


Figure 4. Pearson correlation coefficient between FTI and the different traits measured for the flooded treatment, for the parental clones and 30 full-sib genotypes of the F1 of a *P. deltoides* intraspecific cross. $N = 32$. * $P < 0.05$; ** $P < 0.01$, *** $P < 0.001$. Open symbols: non-significant correlation with FTI. Closed symbols: statistically significant correlations with FTI.

Another important question for breeding is whether the traits under selection have genetic correlations with potentially undesirable traits. For instance, Δ had a negative correlation with TDW in control plants; this means that genotypes with higher water-use efficiency will accumulate less biomass. A negative genetic correlation between Δ and growth traits has also been found for *Castanea sativa* (Lauteri et al. 2004) and *Picea mariana* (Johnsen et al. 1999). On the other hand, H and D show a positive genetic correlation between them and with total biomass under both flooded and non-flooded conditions. Since H and D have a positive correlation with flooding tolerance, they are obvious candidates for selection. The use of these traits to screen for flooding tolerance has been already proposed for a set of hybrid poplar F1 populations (Du et al. 2008). A downside of this is that H and D had a negative

genetic correlation with RSR in control plants, implying that the selection for an increased size in young plants will lead to a reduction of the root biomass. The reduction in RSR per se should not necessarily be a drawback for flooding tolerance, but it could be a disadvantage if the young plants face a drought episode, as occurred with willows (Doffo et al. 2017). There are other examples in which genetic correlations place a constraint in adaptation mechanism to stress. For instance, in *C. sativa* seedlings, a high Δ and limited growth appears as a prerequisite for adaptation to dry environments (Lauteri et al. 2004).

Conclusions

As we hypothesized, there was transgressive segregation for flooding tolerance in an F1 full-sib family of eastern cottonwood.

We found genotypic variability in several leaf traits, including Δ , that have never been assessed before for *Populus* under flooding stress. H, D and RSR correlated with flooding tolerance, while most morphological and physiological leaf traits did not. In consequence, growth traits will be more useful in screening for flooding tolerance than leaf traits. In particular, height stands out, since it has a reasonable heritability, with the advantage of being non-destructive and eventually being automated to screen a high number of genotypes in a breeding program. A vigorous early growth is a trait to be selected for genotypes intended for areas with a high risk of flooding.

Supplementary Data

Supplementary Data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

Authors' contributions

M.E.R. carried out the experiment, collected, analyzed and interpreted data, and revised the manuscript. D.L. collected, analyzed and interpreted data, and revised the manuscript. S.C. participated in the experiment design and revised the manuscript. V.M.C.L. designed the experiment, analyzed and interpreted data, and wrote the manuscript.

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