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

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Running head: Flooding tolerance and growth in *Populus deltoides*

Abstract

Climate change will increase the risk of flooding in several areas of the world where *Populus deltoides* (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 *Populus 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: 1) plants watered to field capacity (control); and 2) 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.

48 **Introduction**

49 *Populus deltoides* Marshall (eastern cottonwood) is a native species to eastern North
50 America, with a wide range of distribution from the Mexican Gulf coast in the south to the Great
51 Lakes in the north (Richardson et al. 2014). From the ecological viewpoint, *P. deltoides* is a
52 significant species in the floodplains of its native range (Rood et al. 2003). In addition to its
53 importance in natural ecosystems, *P. deltoides* is widely planted around the world, either as a pure
54 species or as an interspecific hybrid with other *Populus* species (Dickman and Kuzovkina 2014). In
55 some countries, *P. deltoides* is planted in areas that may experience episodes of flooding (Du et al.
56 2012, Luquez et al. 2012). The occurrence of flooding episodes will increase due to climate change
57 in several regions of the world (Kreuswieser and Rennenberg 2014), including the areas where the
58 eastern cottonwood is planted. Therefore, it is important to breed new clones with increased
59 flooding tolerance to face these adverse conditions.

60 The occurrence of genotypic variability for flooding tolerance in *P. deltoides* and its hybrids
61 with other species is well documented (Gong et al. 2007, Guo et al. 2011, Luquez et al. 2012).
62 Furthermore, there is extensive literature regarding the relationship between different physio-
63 morphological leaf traits and growth and productivity in poplars. For instance, total leaf area,
64 individual leaf area, leaf number, leaf number increment rate, carbon isotopic discrimination and
65 stomatal density have shown correlation with growth and productivity in different *Populus* species
66 and hybrids (Rae et al. 2004, Monclus et al. 2005, Marron and Ceulemans 2006, Al Afas et al. 2006,
67 Dillen et al. 2008). Traits such as leaf area, leaf number, specific leaf area and carbon isotopic
68 discrimination have shown variability in natural populations of *P. nigra* L. (Chamaillard et al. 2011,
69 Guet et al. 2015), *P. balsamifera* L. (Soolanayakanahally et al. 2009), *P. trichocarpa* Torrey & Gray
70 (Gornall and Guy 2007), *P. tremuloides* Michaux (Kanaga et al. 2008), *P. davidiana* Dode (Zhang et
71 al. 2004) and *P. deltoides* (Rowland 2001). Some of these leaf traits can be affected by flooding,
72 causing a negative impact on growth (Gong et al. 2007, Guo et al. 2011, Luquez et al. 2012,
73 Rodríguez et al. 2015). However, little is known about the relationship of these leaf traits with
74 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 Issaquenna 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 INTA's (Instituto Nacional de Tecnología Agropecuaria) poplar breeding program, resulting in an F1 of 190 full-sib individuals. From these F1, a subset of 30 genotypes

were 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 1st and the 2nd 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 6 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 November 9, 2015 and lasted for 35 days. An outline of the experimental design is provided in Supplementary Fig. 1.

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:

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

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 grounded to 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 400x and photographed with a digital camera (Olympus E-330). Ten fields for sample were counted with the software Image J (https://imagej.nih.gov/ij/, Schneider et al. 2012), and there were 3 replicates for the F1 and 4 replicates for each parental genotype. The field area was 0.0997 mm^2. The stomatal index (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 Image J.

The leaf stomatal conductance (gs) 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 µmoles m^{-2} s^{-1}. At least 4 to 5 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 (BLUPs) 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 Supplementary Table 2). The absence of spatial structure in the data was also checked using breedR.

The PCA (principal components analysis) was done with the software MVSP (Kovach Computing Services, UK, 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

182 measured several times, like height and stomatal conductance, only the last date was included in
183 the PCA. At this point, the differences between the treatments were maximized.

184

185

186 **Results**

187 In Principal Components Analysis (PCA, Fig. 1), the first component (PC1) represents the
188 variation related to the flooding treatment, separating control and flooded plants into two distinct
189 groups, as shown by the color code of the treatments. Due to the clear separation caused by
190 flooding, the correlations and heritability values were calculated separately for control and flooded
191 plants. The second principal component (PC2) represents the genotypic variation. PC1 and PC2
192 together explained 43% of the total variability. Most traits either decreased or were not affected by
193 flooding, except for SEN and AR, which increased with the stress treatment. As for gs, it was
194 reduced by flooding in both the parental genotypes and the progeny (Supplementary Fig. 2).

195 The PCA results were in accordance with those of ANOVA (Table 2). Most variables were
196 significantly reduced by the flooding treatment except for D, GRD, LIR and Δ. The variables
197 significantly affected by the genotype were final H, ILA, LN, gs and Δ. The mean values and
198 standard deviation of all traits for the parental genotypes and the F1 are shown in Supplementary
199 Table 1.

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

203 The phenotypic correlations (Table 3) differed for the control and flooded treatments on
204 several traits. H correlated positively with D ($r = 0.50$ $p < 0.01$ for control and $r = 0.67$ $p < 0.001$ for
205 flooded), GRH ($r = 0.65$ $p < 0.001$ for control and $r = 0.86$ $p < 0.001$ for flooded), LN ($r = 0.42$ $p <$
206 0.05 for control and $r = 0.60$ $p < 0.001$ for flooded), and LIR ($r = 0.40$ $p < 0.05$ for control and $r =$
207 0.37 $p < 0.05$ for flooded) in both control and flooded treatments, while it correlated negatively with
208 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). 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 ($r = 0.51$ $p < 0.01$). While in flooded plants, Δ had a positive correlation with gs ($r = 0.36$ $p < 0.05$), 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 flooding tolerance index (FTI) is depicted in Fig. 2. A very interesting result was that most F1 genotypes had a higher flooding tolerance than both parents. For the control treatment (Fig. 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, 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 plain 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 to obtain 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 and 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.

263

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

266 There is extensive literature on leaf traits variation and its correlation with growth and yield in
267 poplar crosses and natural populations. In this *P. deltoides* cross, we found genotypic variability on
268 several leaf traits, such as gs, ILA and LN. Similar results had been previously found for *P. deltoides*
269 (Rowland 2001), *P. nigra* (Chamaillard et al. 2011, Guet et al. 2015), *P. tremuloides* (Kanaga et al.
270 2008) and *P. balsamifera* (Soolanayakanahally at al. 2009). These traits were also significantly
271 reduced by flooding, as previously reported for *P. deltoides* and other species (Gong et al. 2007, Du
272 et al. 2008, Luquez et al. 2012). We did not find genotypic variability for stomatal density, probably
273 because the parental genotypes had similar leaf morphologies, in contrast to the segregation
274 reported for interspecific hybrid poplars with contrasting leaf traits (Al Afas et al. 2006, Dillen et al.
275 2008).

276 Δ represents a proxy for the photosynthesis to the stomatal conductance ratio (instantaneous
277 water use efficiency, Chamaillard et al. 2011), and it has shown genotypic variability among different
278 *Populus* species (Guet et al. 2015, Soolanayakanahally et al. 2009, Gornall and Guy 2007, Kanaga
279 et al. 2008, Zhang et al. 2004). We found a significant effect of the genotype but not of the treatment
280 on Δ, in spite of the reduction in gs in the flooded plants. In addition, we did not find a correlation in
281 either treatment between Δ and gs, as occurred with *P. nigra* (Guet et al. 2015). A possible
282 explanation for this result is that flooded leaves rely on remobilized carbon to compensate for the
283 photosynthetic reduction that occurs under flooding (Du et al. 2012, Rodriguez et al. 2015).
284 Previous results regarding responses to stress of Δ showed disparity. Δ did not change in response
285 to moderate drought in poplar (Monclus et al. 2009), but it was significantly affected in *P. davidiana*
286 under a limited water supply (Zhang et al. 2004).

287 We did not find phenotypic correlations between Δ and total growth for neither control nor
288 flooded plants, except for a moderate correlation with D in control plants. The results have been
289 variable for other *Populus* species, e.g., there was no correlation between Δ and growth in natural

290 populations of *P. nigra* (Chamaillard et al. 2011) while there was correlation in *P. balsamifera*
291 populations (Soolanayakanahally et al. 2009).
292
293 *Phenotyping and breeding for flooding tolerance*
294 Some of the most meaningful changes conveying adaptation/tolerance to flooding take place
295 in roots, not an easy-to-phenotype organ, least of all in a breeding program in which a high number
296 of genotypes are to be measured. Hence the need to identify non-destructive, easy-to-phenotype
297 traits (i.e., avoiding phenotyping of roots, if possible) that correlate with flooding tolerance, and
298 preferably without subjecting the plants to flooding. Leaf traits are obvious candidates, since they
299 are relatively easy to measure, show genotypic variability in *Populus* and are affected by flooding.
300 However, in the family analyzed, the morphological and physiological leaf traits did not show any
301 correlation with flooding tolerance (measured with FTI), with the exception of LIR, and only in the
302 non-flooded plants. Some growth traits showed correlation with flood tolerance. In particular, RSR,
303 H and D had a statistically significant positive correlation with FTI in flooded plants. These results
304 imply that a bigger size combined with a higher root biomass is a favorable combination of traits for
305 flooding tolerance in young plants obtained from cuttings. Similar results were obtained from
306 willows, in which young plants with a vigorous early growth were more able to cope successfully
307 with flooding (Rodríguez et al. 2018).
308 For breeding, it is important to know the heritability of the traits -in particular, the narrow
309 sense heritability-, which is a measure of the response to selection (Lynch and Walsh 1998). Our h^2
310 estimations rated from very low to moderate, and they differed in some traits for control and flooded
311 treatments. This is not surprising, since heritability values are highly influenced by factors such as
312 environmental conditions and plant age (Lynch and Walsh 1998). Most of the values published for
313 the traits measured in *Populus* are for broad sense heritability (H^2), therefore the comparisons are
314 not straightforward. For instance, Fahrenkrog et al. (2017b) reported H^2 values of 0.71 for height
315 and 0.51 for diameter for a collection of 391 unrelated genotypes of *P. deltoides* of a similar age to
316 the plants of our experiment, but in this case, the genotypic variance included other components

317 (dominance, epistasis) in addition to the additive genetic variance. For leaf traits, our results are
318 within the range of the H^2 values for other *Populus* species and hybrids (Marron and Ceulemans
319 2006, Kanaga et al. 2008, Monclus et al. 2009, Chamaillard et al. 2011).

320 Another important question for breeding is whether the traits under selection have genetic
321 correlations with potentially undesirable traits. For instance, Δ had a negative correlation with TDW
322 in control plants; this means that genotypes with higher water use efficiency will accumulate less
323 biomass. A negative genetic correlation between Δ and growth traits has also been found for
324 *Castanea sativa* (Lauteri et al 2004) and *Picea mariana* (Johnsen et al. 1999). On the other hand, H
325 and D show a positive genetic correlation between them and with total biomass under both flooded
326 and non-flooded conditions. Since H and D have a positive correlation with flooding tolerance, they
327 are obvious candidates for selection. The use of these traits to screen for flooding tolerance have
328 been already proposed for a set of hybrid poplar F1 populations (Du et al. 2008). A downside of this
329 is that H and D had a negative genetic correlation with RSR in control plants, implying that the
330 selection for an increased size in young plants will lead to a reduction of the root biomass. The
331 reduction in RSR per se should not necessarily be a drawback for flooding tolerance, but it could be
332 a disadvantage if the young plants face a drought episode, as it occurred with willows (Doffo et al.
333 2017). There are other examples in which genetic correlations place a constraint in adaptation
334 mechanism to stress. For instance, in *C. sativa* seedlings, a high Δ and limited growth appears as a
335 prerequisite for adaptation to dry environments (Lauteri et al. 2004).

336

337 **Conclusions**

338 As we hypothesized, there was transgressive segregation for flooding tolerance in an F1 full-
339 sib family of eastern cottonwood. We found genotypic variability in several leaf traits, including Δ,
340 that have never been assessed before for *Populus* under flooding stress. H, D and RSR correlated
341 with flooding tolerance, while most morphological and physiological leaf traits did not. In
342 consequence, growth traits will be more useful in screening for flooding tolerance than leaf traits. In
343 particular, height stands out, since it has a reasonable heritability, with the advantage of being non-

344 destructive and eventually being automated to screen a high number of genotypes in a breeding
345 program. A vigorous early growth is a trait to be selected for genotypes intended for areas with a
346 high risk of flooding.
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348
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358
359 **Authors' Contributions**
360 MER carried out the experiment, collected, analyzed and interpreted data, and revised the
361 manuscript. DL collected, analyzed and interpreted data, and revised the manuscript. SC
362 participated in the experiment design and revised the manuscript. VL designed the experiment,
363 analyzed and interpreted data and wrote the manuscript.
364
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524 Table 1. Traits, abbreviations and units of the measurements carried out on the parental genotypes
525 and F1 full-sib progeny for the A106 x ST67 family.
526

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	gs	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	----

527

528 Table 2. ANOVA results (with genotype and treatment as factors) and narrow sense heritability
529 values (h^2) for the different traits measured in the A106 x ST67 family. ns non - significant, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Between parentheses: standard error for heritability.
531

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)
gs	*	***	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)

Table 3. Phenotypic correlations (Pearson correlation coefficient) between different traits measured in the parental genotypes and the F1, for the A106 x 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. In bold: statistically significant correlations. *: p < 0.05; **: p < 0.01, ***: p < 0.001.

Trait	H	D	GRH	GRD	ILA	LN	LIR	AR	SEN	gs	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
gs	*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**

538 Table 4. Genetic correlations (Spearman correlation coefficient) between the Best Linear Unbiased Predictions (BLUPs) of the breeding values
539 for different traits measured in the parental genotypes and the F1, for the A106 x ST67 family. N = 32. Correlations for control plants in the
540 lower part of the table (in italics). Correlations for flooded plants in the upper part of the table. In bold: statistically significant correlations. *: p <
541 0.05; **: p < 0.01, ***: p < 0.001.
542

Trait	H	D	GRH	GRD	ILA	LN	LIR	AR	SEN	gs	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
gs	*-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**

543

Legends to the figures

Fig. 1. Principal Components Analysis (PCA) of the parental clones and 30 full-sib genotypes of the F1 belonging to a *Populus 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. ST67: male.

Fig. 2. Flooding Tolerance Index (FTI) of the parental clones and 30 full-sib genotypes of the F1 belonging to a *Populus deltoides* intraspecific cross. FTI calculation was described in Material and Methods. A106: female. ST67: male.

Fig. 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 *Populus 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.

Fig. 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 *Populus 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.

FIG.1

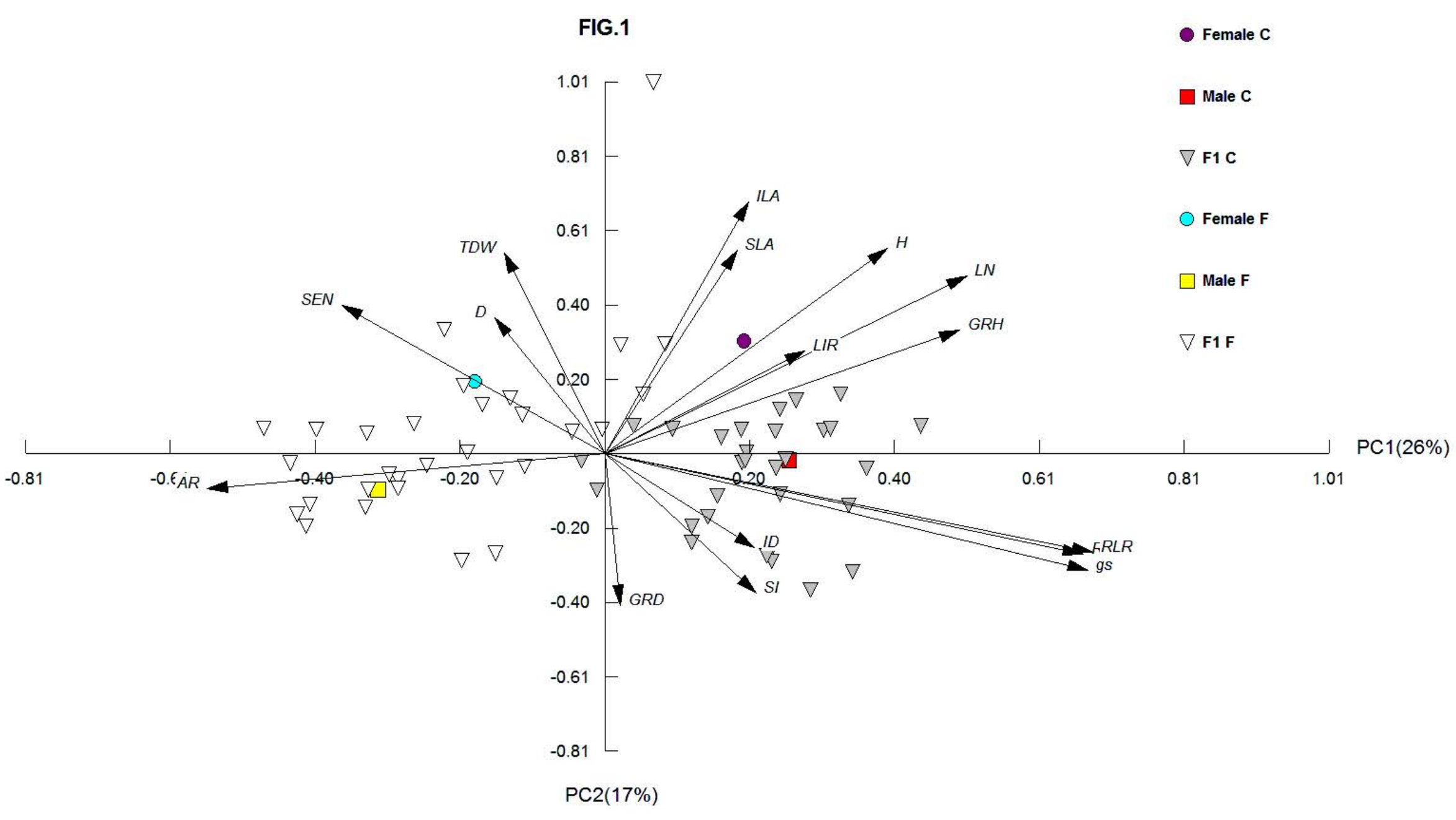

Female C
Male C
F1 C
Female F
Male F
F1 F
1.01
0.81
0.61
0.40
0.20
-0.20
-0.40
-0.61
-0.81
-0.81
-0.61
-0.40
-0.20
0.20
0.40
0.61
0.81
1.01
PC1(26%)
PC2(17%)
ILA
SLA
H
LN
GRH
LIR
TDW
SEN
D
AR
ID
SI
GRD
RLR
gs

FIG.2

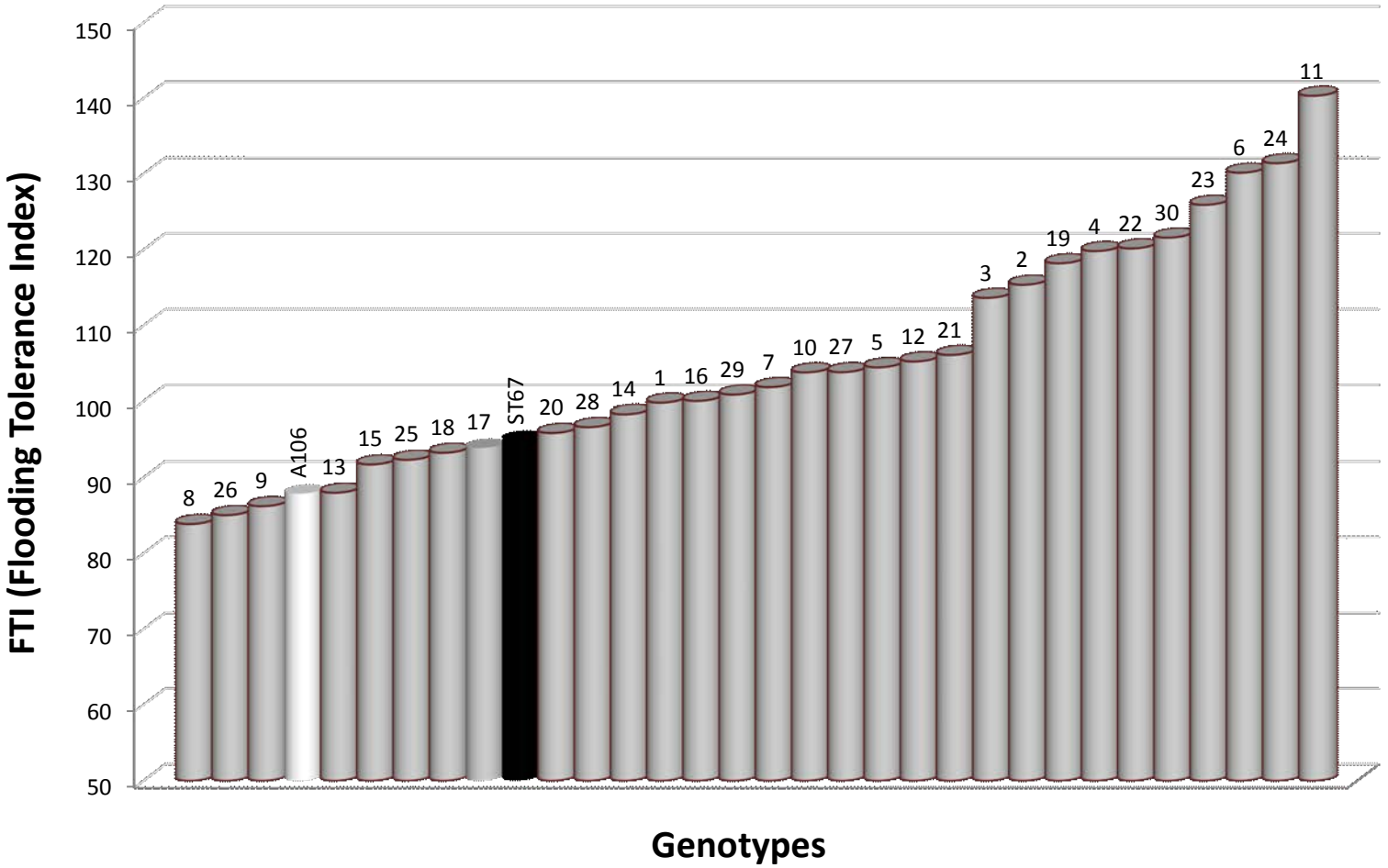

FTI (Flooding Tolerance Index)
150
140
130
120
110
100
90
80
70
60
50
8
26
9
A106
13
15
25
18
17
ST67
20
28
14
1
16
29
7
10
27
5
12
21
3
2
19
4
22
30
23
6
24
11
Genotypes

FIG.3

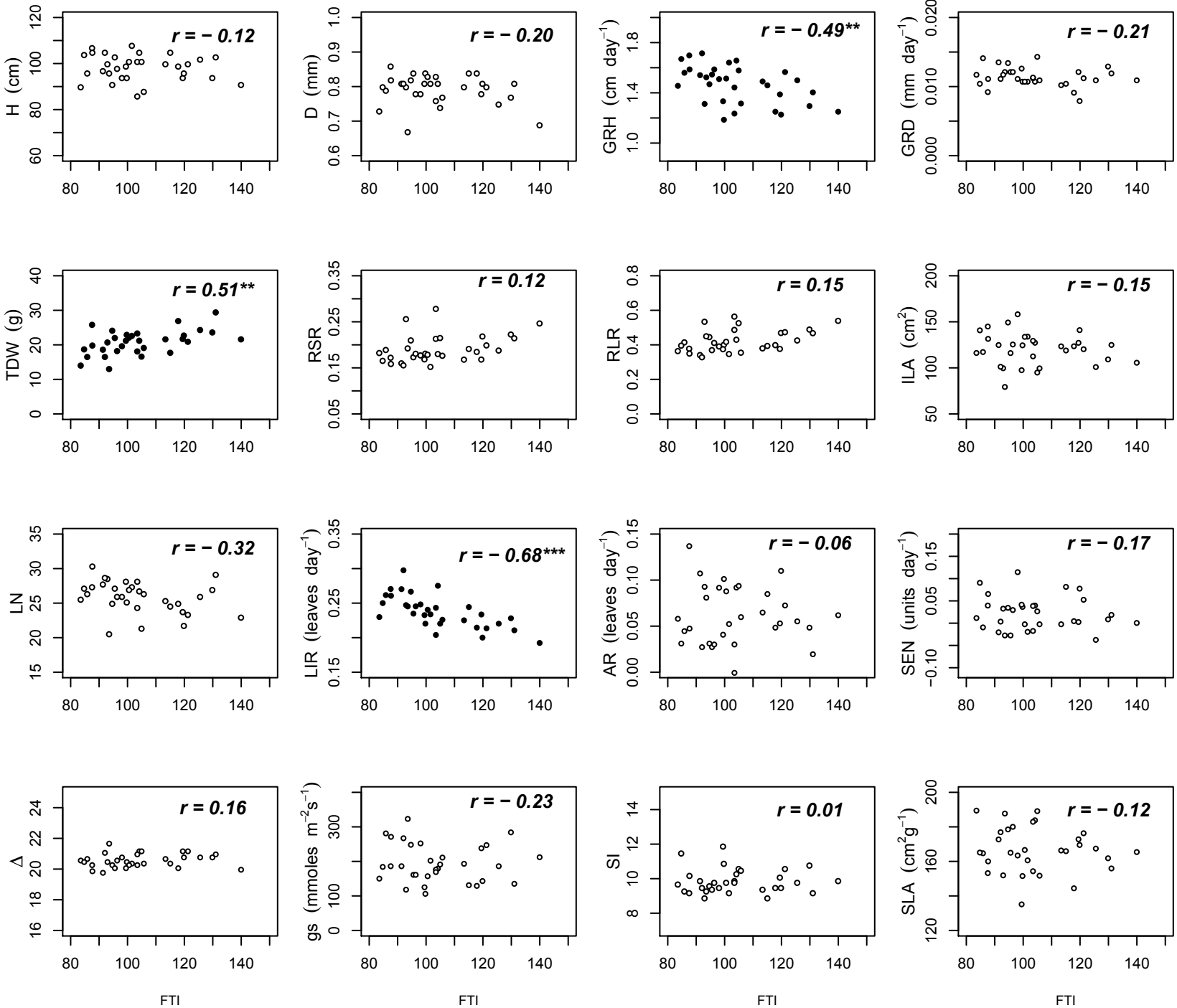
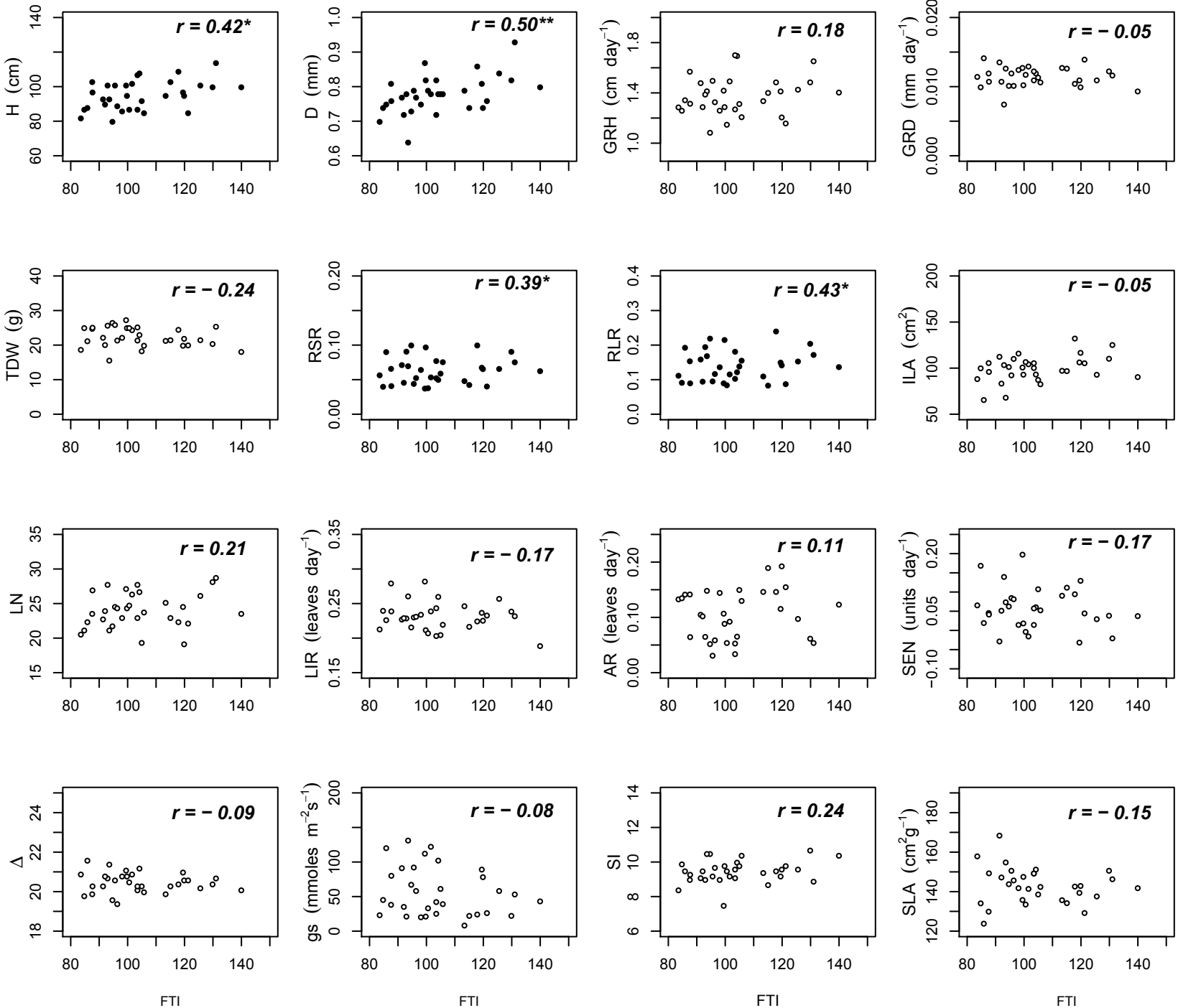

H (cm)
r = − 0.12
D (mm)
r = − 0.20
GRH (cm day−1)
r = − 0.49**
GRD (mm day−1)
r = − 0.21
TDW (g)
r = 0.51**
RSR
r = 0.12
RLR
r = 0.15
ILA (cm2)
r = − 0.15
LN
r = − 0.32
LIR (leaves day−1)
r = − 0.68***
AR (leaves day−1)
r = − 0.06
SEN (units day−1)
r = − 0.17
Δ
r = 0.16
gs (mmoles m−2s−1)
r = − 0.23
SI
r = 0.01
SLA (cm2g−1)
r = − 0.12
FTI
FTI
FTI
FTI

FIG.4


H (cm)
r = 0.42*
D (mm)
r = 0.50**
GRH (cm day^{-1})
r = 0.18
GRD (mm day^{-1})
r = − 0.05
TDW (g)
r = − 0.24
RSR
r = 0.39*
RLR
r = 0.43*
ILA (cm^2)
r = − 0.05
LN
r = 0.21
LIR (leaves day^{-1})
r = − 0.17
AR (leaves day^{-1})
r = 0.11
SEN (units day^{-1})
r = − 0.17
Δ
r = − 0.09
gs (mmoles m^{-2}s^{-1})
r = − 0.08
SI
r = 0.24
SLA (cm^{2}g^{-1})
r = − 0.15
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Supplementary Fig.1. An outline of the experimental design in this paper.

Experimental Design: completely randomized

Plants growing in pots

35 days

Final destructive sampling

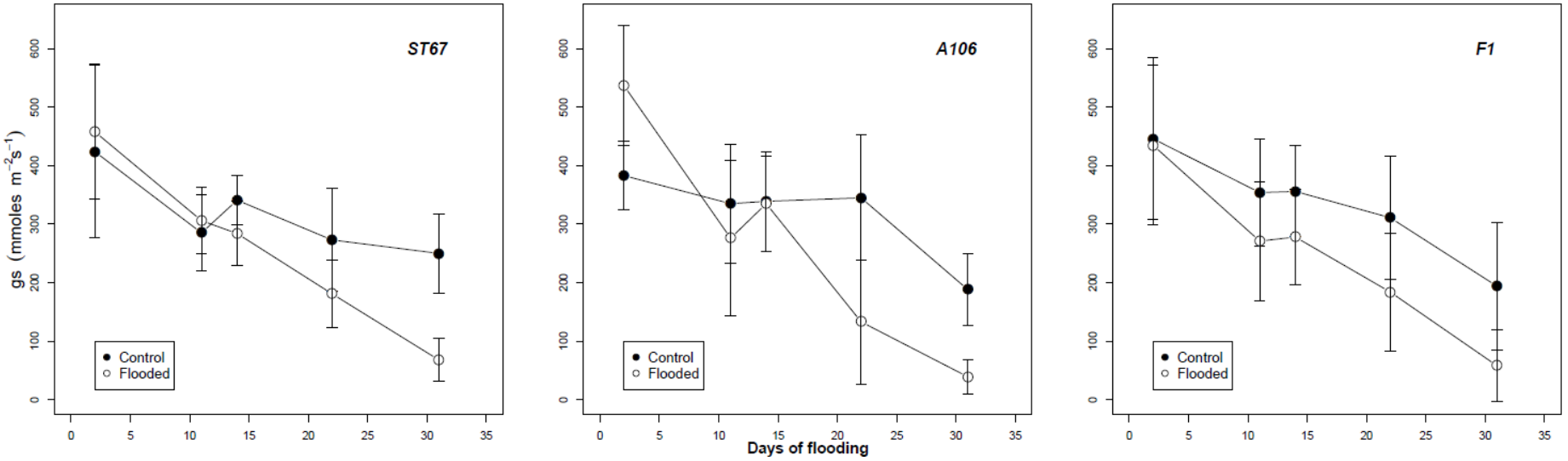
32 Genotypes: 2 parental clones + 30 genotypes of the F1

2 treatments: control (well drained) and flooded 10 cm above soil level (placed inside another pot with a plastic bag)

6 repetitions for each genotype and treatment: 384 plants

Supplementary Table 1. Average and one standard deviation (between parenthesis) of the traits measured for the parental clones and 30 full-sib genotypes of the F1 for the A106 X ST67 family. A106:female clone. ST67: male clone. C: control (watered to field capacity). F: flooded 10 cm above soil level. Complete name of the traits in Table 1 of the main text. * Data corresponding to the last date of measurement.

Trait	A106 C	A106 F	ST67 C	ST67 F	F1 C	F1 F
H*	105 (8)	103 (26)	99 (13)	101 (12)	98 (10)	95 (15)
D*	8.36 (0.88)	7.44 (1.41)	8.10 (1.03)	8.74 (0.91)	7.93 (7.81)	7.79 (1.10)
GRH	0.023 (0.004)	0.022 (0.002)	0.024 (0.003)	0.019 (0.001)	0.021 (0.004)	0.020 (0.003)
GRD	0.012 (0.003)	0.009 (0.006)	0.010 (0.002)	0.014 (0.002)	0.012 (0.003)	0.011 (0.003)
ILA	146 (21)	106 (12)	151 (30)	102 (21)	119 (23)	100 (22)
LN*	27 (4)	24 (6)	25 (1)	22 (1)	26 (3)	24 (4)
LIR	0.245 (0.045)	0.217 (0.029)	0.234 (0.023)	0.283 (0.036)	0.239 (0.035)	0.230 (0.034)
AR	0.086 (0.107)	0.190 (0.134)	0.041 (0.024)	0.108 (0.096)	0.061 (0.056)	0.103 (0.068)
SEN	0.047 (0.070)	0.047 (0.041)	0.036 (0.122)	0.064 (0.048)	0.022 (0.085)	0.059 (0.11)
gs*	188 (62)	39(29)	250 (68)	68 (37)	194 (110)	59 (62)
SLA	166.3 (25.8)	134.5 (27.8)	135.5 (4.8)	136.1 (1.1)	167.9 (24.8)	144.3 (18.1)
TDW	24.9 (6.5)	26.0 (8.8)	26.6 (4.8)	24.3 (5.4)	22.4 (5)	20.7 (5.6)
RSR	0.17 (0.04)	0.04 (0.02)	0.19 (0.06)	0.04 (0.01)	0.19 (0.06)	0.07 (0.03)
RLR	0.38 (0.11)	0.16 (0.08)	0.45 (0.18)	0.22 (0.05)	0.43 (0.12)	0.14 (0.07)
Δ	20.1 (0.5)	19.9 (0.5)	20.3 (0.9)	19.6 (0.8)	20.6 (0.8)	20.5 (0.7)
SI	9.01 (0.51)	9.18 (0.18)	10.47 (0.47)	9.64 (0.23)	9.44 (0.10)	9.94(1.13)



Supplementary Fig. 2. Stomatal conductance (gs) measured in the abaxial surface of the latest expanded leaf. For every genotype and treatment, 4-5 plants were measured in each date.

Table 2. Code for the determination of heritabilitity and breeding values with breedR, with R 3.5.1.

```
library(breedR)

wl<-read.table("data.txt",header=TRUE)
names(wl)

wl$D<-as.numeric(wl$D)


#EXAMPLE DIAMETER

# INDIVIDUAL TREE MIXED MODEL with LMM - REML
model1.1<- remlf90(fixed = D ~ 1,
                   genetic = list(model = c('add_animal'),
                                  pedigree = data[,1:3],
                                  id = 'clon'),
                   data = wl)

summary(model1.1)

model1.1$fixed
model1.1$ranef


#spatial analysis

coordinates(model1.1) <-data[, c('row', 'col')]
plot(model1.1, 'resid')


breedR.setOption(col.seq = c('yellow', 'red'))
plot(model1.1, 'phenotype')


variogram(model1.1)
```