

## Early rooting and flooding tolerance in cuttings from a *Populus deltoides* full-sib family under greenhouse conditions

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## Abstract

*Populus deltoides* is an important forest tree, with elite genotypes propagated mainly as unrooted dormant cuttings. Several areas where *P. deltoides* is planted periodically experience flooding episodes. The aims of this work were to analyze the early rooting capability and flooding tolerance of a *P. deltoides* full-sib family, and to identify growth, wood, and leaf traits correlating with flooding tolerance. We analyzed the early rooting capability of the parental genotypes and 30 clones from their  $F_1$  under greenhouse conditions. The rooting percentage of the cuttings ranged from 50 to 100%. There was a positive genetic correlation between shoot weight and root traits (number, biomass and total length). In a separate experiment, 2-month-old plants growing in pots from the same genotypes were subjected to two treatments: watered (control) and flooded for 35 days. Most genotypes showed an intermediate flooding tolerance with respect to the parental clones. Height, diameter, growth rate, biomass, plant leaf area, leaf number and leaf increase rate had a positive phenotypic correlation with flooding tolerance, while wood density did not. Height and diameter are traits recommended for selection because they correlate with flooding tolerance, are easy to measure, and have moderate to high narrow sense heritability.

**Key words:** eastern cottonwood - greenhouse cuttings - early rooting - narrow sense heritability - genetic correlation

## Introduction

*Populus deltoides* (eastern cottonwood) is widely cultivated in temperate regions worldwide because of its fast growth, either as a pure species or as selected parents of interspecific hybrids (Dickman and Kuzovkina 2014). Eastern cottonwood plantations are mainly established from unrooted stem dormant cuttings (Zhao et al. 2014). Rooting ability is crucial for *Populus* asexual propagation, and it is influenced by different factors such as pre-planting soaking, temperature, type of substrate, stock plant nutritional status, original position in the stock plant, and length and diameter of cuttings (Zalesny and Zalesny 2009, Zhao et al. 2014). The occurrence of clonal variation in terms of rooting capability in *P. deltoides* cuttings is extensively documented (e.g., Desrochers and Thomas 2003, Zalesny et al. 2005, Zalesny and Zalesny 2009).

The earliest stages of rooting are crucial for the successful establishment of plantations, when cuttings are developing their root system and leaf area (Zalesny and Zalesny 2009). The occurrence of a stress episode during the early phase of growth may compromise the success and future growth of *Populus* plantations. Drought altered the early rooting responses of hybrids poplars (Krabel et al. 2015), while an 18-days waterlogging episode during initial rooting caused different responses in cuttings of 9 *Populus* genotypes (Mc Carthy et al. 2018).

Flooding is likely to occur in areas where *P. deltoides* is planted, and the frequency and intensity of flooding episodes will increase due to climate change (Kreuswieser and Rennenberg 2014). There are clonal differences in flooding tolerance for eastern cottonwood and its hybrids (Gong et al. 2007, Guo et al. 2011, Luquez et al. 2012). A higher flooding tolerance among *P. deltoides* genotypes during early establishment will increase the success of plantations under climate change.

Traits like total leaf area, individual leaf area, leaf number and leaf number increment rate have shown correlation with growth along a wide variety of *Populus* species and hybrids (Rae et al. 2004, Monclus et al. 2005, Marron and Ceulemans 2006). These leaf traits may be affected by flooding, eventually causing a reduction in growth (Gong et al. 2007, Guo et al. 2011, Rodriguez et al. 2015). Wood density correlated with xylem cavitation resistance across a broad range of species (Hacke et al. 2001), indicating a relation with drought tolerance. But it is not known whether a similar relationship occurs between wood density and flooding tolerance in *Populus*.

The studies of quantitative genetics of flooding tolerance in poplar are scarce. Du et al. (2011) analyzed the 3 best F<sub>1</sub> individuals and the parents of 5 full-sib hybrid poplar families (a total of 20 genotypes). In some families, the F<sub>1</sub> individuals were more flood tolerant than the parents (Du et al. 2011). In a *Populus deltoides* F<sub>1</sub> full-sib family, most individuals of the offspring had a higher flooding tolerance than the parental genotypes (Rodriguez et al. 2020). Even when these results are limited to a few pedigrees, they indicate that it is possible to increase flooding tolerance through breeding in poplar. To this end, it is important to analyze more families and to identify traits that are relatively easy to measure and correlate with flooding tolerance. It is crucial the knowledge of the heritabilities and genetic correlations of the traits to be selected. In particular, the narrow sense heritability is a measure of the response to selection (Lynch and Walsh 1998). The genetic correlations are important because traits increasing stress tolerance might be correlated with detrimental traits. It would be desirable that the selection for increased flooding tolerance do not cause a reduction in tree growth or fitness in eastern cottonwood.

To our knowledge, this is the first quantitative genetic study of both early rooting and flooding tolerance on the same *Populus* family. We gathered data of growth, wood density and several root and leaf traits from two *P. deltoides* parental clones and 30 genotypes of their full-sib F<sub>1</sub> representing a range of individual growth. The aims of this work were: (1) to analyze the early rooting capability and the flooding tolerance under greenhouse conditions; (2) to determine which of the studied traits correlate with flooding tolerance and could be selected to increase tolerance to this stress in young *P. deltoides* plants obtained from cuttings; (3) to estimate genetic correlations and narrow sense heritabilities for both destructive labor intensive and non-destructive traits, to determine which ones could be adequate to select for productivity and for flooding tolerance in eastern cottonwood.

## Materials and Methods

### *Plant Material for both experiments*

The parental genotypes were obtained as the open pollinated progeny of selected female clones. The seeds were collected in the Mississippi Delta Area, introduced to Argentina between 1968 and 1979, and

subsequently selected for the poplar breeding program from the Instituto Nacional de Tecnología Agropecuaria (INTA). The female clone was registered as Nandi INTA (<https://inta.gob.ar/documentos/nandi-inta-populus-deltoides>, abbreviated Nandi) and the male clone as Carabelas INTA (<https://inta.gob.ar/variedades/carabelas-inta>, abbreviated CAR). The controlled cross was carried out in the year 2011 as part of the INTA breeding program. A full-sib F<sub>1</sub> progeny was obtained, from which a subset of genotypes representing a range of growth (i.e., including genotypes with good and poor growth) was selected for this work. The preliminary evaluation of growth of the F<sub>1</sub> progeny was done on plants growing on a stool bed. Both parental clones and 30 genotypes of the progeny were used for the rooting experiment, and the same genotypes minus one (31 in total) for the flooding experiment. This family was selected because the flooding tolerance of the parents was analyzed in a previous work, Nandi being more sensitive to this stress than CAR (Luquez et al. 2012).

#### *Rooting Experiment description*

One-year-old 20 cm dormant cuttings were collected from stool beds growing in the field at the INTA Delta Research Station during July 2017, and kept at 4°C until the beginning of the experiment. Before planting, the cuttings were soaked overnight in water and treated with fungicides to prevent diseases. The cuttings were planted in rectangular plastic trays (dimensions: 50 cm long, 18 cm wide and 14 cm deep) filled with vermiculite, which covered the lower half of the cutting. The planting took place on August 1<sup>st</sup>, 2017 and the trays were placed in a greenhouse in the city of La Plata (34° 59' 09" S; 57° 59' 42" W), with natural light (maximum irradiance 1500  $\mu\text{moles m}^{-2} \text{s}^{-1}$ ) and photoperiod. Five cuttings belonging to the same clone were arranged on each tray, and the trays were placed on benches on two different sites of the greenhouse: next to the walls and in the center, the latter receiving more time of maximum irradiance than the former. These two areas were treated as blocks, and the position of each tray within each block (= genotype) was completely randomized (see Fig. S1). Each tray was treated as a plot, and the measurements of all five cuttings pooled together. There were two trays of each genotype per block (4 trays and 20 cuttings per genotype in total). The trays were watered daily to keep the substrate moist and no fertilizer was added to the substrate, so the growth relied initially on the cutting's own reserves, until leaves developed and photosynthesis started (Fig. S1B).

The diameter of the cuttings was measured with a digital caliper as the average of two perpendicular measurements in the middle of each cutting. After 35 days, the rooting of the cuttings was evaluated. Rooting percentage (RP) was determined as the number of rooted cuttings over the total number of cuttings on each tray. The number of roots (RN), the average root length (ARL) and the total root length (TRL, the sum of the lengths of every individual root) were determined for each cutting. The shoot dry weight (SDW) and root dry weight (RDW) were determined after drying them at 65°C to constant weight. Some cuttings did not produce roots but developed callus tissue at the base from which roots could later develop (Zalesny and Zalesny 2009, Fig. S2A and Fig. S2B). In consequence, two rooting percentages were determined: actual rooting (cuttings with roots/total number of cuttings) and potential rooting (cuttings with roots or callus/total number of cuttings). The actual rooting percentage was used for further analyses, unless is stated otherwise. The abbreviations and units of all these traits measured in this experiment are summarized in Table 1.

### *Flooding Experiment description*

Cuttings of 20 cm long were planted in 5 L pots with garden soil (one cutting per pot), obtained and treated as described for the rooting experiment. The plantation date was 9<sup>th</sup> and 10<sup>th</sup> August, 2016. The plants were grown in a greenhouse under the natural irradiance and photoperiod of La Plata. 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 with 50 ml per pot of complete Hoagland solution. The flooding treatment was applied 60 days after planting, by placing the plants in 7 L pots sealed with a plastic bag, and filled with water up to 10 cm above soil level, as previously described (Luquez et al. 2012, Fig.S3). The flooding treatment lasted for 35 days. There were six repetitions per genotype and treatment (31 genotypes x 2 treatments x 6 repetitions = 372 plants in total) in a completely randomized layout. The experiment was surrounded with a border of plants that were not measured. An outline of the experiment is provided in Fig. S3.

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 fitted line. The basal diameter (D) was determined with a digital caliper. At the end of

the experiment, the total above ground dry weight (leaves plus stem, TDW) was determined after drying them to constant weight at 65°C. The basic wood density (BWD) was determined on a 10 cm subsample obtained from the basal part of the stem by fluid displacement as described in Achinelli et al. (2018). Before the beginning of 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 using the number of leaves above the mark, fitting a linear function in the same way as described for growth rate. The abscission rate (AR) was determined with the number of leaves below the mark, in the same way as LIR. The chlorophyll content of the tagged leaf was measured twice with a SPAD Minolta Chlorophyll Meter SPAD 502 (Osaka, Japan). A linear function was adjusted as described for growth rate, the leaf senescence rate (SEN) being the absolute value of the slope of the fitted line. At the end of the experiment, the total leaf area per plant (TLA) was determined with a LICOR LI3100 area meter (Lincoln, Nebraska, US). The individual leaf area (ILA) and specific leaf area (SLA) were determined on leaves expanded during the flooding episode. The leaf stomatal conductance ( $g_s$ ) was determined with a porometer Decagon SC1 on the abaxial side of the latest expanded leaf. The measurements were carried out on a cloudless day (December 12<sup>th</sup>, 2016) between 10.30 and 13.30 h, with an average irradiance of 1688  $\mu\text{moles m}^{-2} \text{s}^{-1}$ .

To quantify the flooding tolerance of the different genotypes, the Flooding Tolerance Index (FTI, Doffo et al. 2018) was determined using the total average dry weight for the watered or control ( $\text{TDW}_{\text{control}}$ ) and flooded or stressed ( $\text{TDW}_{\text{stressed}}$ ) treatments as follows:  $\text{FTI} = (\text{TDW}_{\text{stressed}} / \text{TDW}_{\text{control}}) \times 100$ .

### *Statistical analysis of the rooting experiment*

The 30  $F_1$  full-sib genotypes subset was analyzed together with both parental clones. Prior to analyses, the data were standardized to have zero mean and unit variance. The statistical analysis was carried on a plot mean basis (i.e., the average of all five cutting from a given genotype within each block). Plot means were considered appropriate for analyses because their use enabled improved normality of data for all traits (Jansson and Danell 1993). To test the significance of the genotype effects for all the rooting variables an analysis of covariance (ANCOVA) using a single-trait linear model that incorporated block and genotype as

fixed effects as well as the trait initial cutting diameter (D) as a covariate, was performed. The variable D was included in the linear model as a covariate, given that the cuttings had the same length, but their differences in diameter could have affected the amount of carbohydrates and other reserves that influence rooting. The Pearson correlation coefficient was used to determine phenotypic correlations using the mean values of each genotype and treatment. The ANCOVA and correlation (Pearson) analysis were carried out with R 3.5.0 (R Development Core Team 2017) using, respectively, the function `lm` from the base stats package and the function `correlation` from the *agricolae* package version 1.2-8 (de Mendiburu 2017).

The narrow sense heritability ( $h^2$ ) for each rooting trait and genetic correlations between all pairs of these traits were estimated using a multiple-trait mixed linear model with random block and genetic effects and **diameter** as a covariate. The rooting percentage was not included in the multiple-trait mixed model because the residuals were not normally distributed. All determinations were performed with the `breedR` R-package (Muñoz and Sanchez 2018) using the function `remlf90`, which is based on the programs `REMLF90` and `AIREMLF90` of `BLUPF90` library (Miszta 1999). The narrow sense heritability ( $h^2$ ) was calculated as:  $h^2 = \sigma_a^2 / \sigma_p^2$ , where  $\sigma_a^2$  is the additive genetic variance and  $\sigma_p^2$  is the phenotypic variance. The absence of spatial patterns in the residuals was also checked with `breedR` R-package (e.g., Cappa et al. 2019) for each rooting trait using a single-trait mixed linear model with random block and genotype effects and the trait D as covariate (not shown).

The genetic correlations were determined with two methods, first, using the `cov2cor` function from the base stats package, and second, calculating the Pearson correlation coefficient among the Best Linear Unbiased Prediction (BLUPs) of the genotype breeding values from the multiple-trait mixed linear model. Both predictions had a correlation above 0.99, in consequence the significances for the correlations of the second method are shown in the results.

A principal component analysis (PCA) was carried out to analyze the effects of the treatment and genotypes on the traits measured. The PCA was performed with the software `MVSP` (Kovach Computing Services, UK, <https://www.kovcomp.co.uk/mvsp/>), using the clonal phenotypic means for each treatment and genotype.



## Statistical analysis for the flooding assay

The 29  $F_1$  full-sib genotypes subset was analyzed together with both parental clones. Prior to analyses, the data were standardized to have zero mean and unit variance. An analysis of variance (ANOVA) using a single-trait linear model with fixed effects of treatment (i.e., control and flooding), genotype, and the interaction treatment  $\times$  genotype, was performed for all the flooding variables to test the significance of these effects. The ANOVA, phenotypic (Pearson) correlation and PCA analyses were carried out with the same statistical programs as the previous experiment. The ANOVA showed significant statistical differences among the two treatments and the PCA indicated that there were two distinct groups for control and flooded plants (see results below), so genetic correlations and heritability values were calculated separately for each treatment using a multiple-trait mixed linear model with a fixed trait mean and random genetic effects. To determine whether a growth, wood, morphological or physiological leaf traits correlated with the flooding tolerance index (FTI), these traits were correlated with FTI using the Pearson correlation coefficient. Bivariate plots of the FTI against all these studied traits were plotted to further elucidate these correlations.

## Results

### Rooting experiment

There were significant genotypic differences for all the traits measured (Table 1). Most traits did not differ among the parents, except for shoot dry weight (SDW), and they were higher on average in the  $F_1$  (Fig.1). The same can be appreciated in the PCA on a clonal basis, where root number (RN), rooting percentage (RP), root dry weight (RDW), total root length (TRL) and SDW were higher in most  $F_1$  genotypes than in both parental clones (Fig. S5). The actual rooting percentage (RP) was similar in both parental clones, 60% for CAR and 65% for Nandi, while in the  $F_1$  progeny ranged from 51 to 100% (Fig. 1 and Fig. S4). The potential rooting was 100% or very close for most genotypes (Fig. S4).

The narrow sense heritability ( $h^2$ , Table 1) was high for SDW, RN and average root length (ARL), but low to moderate for RDW and TRL.

RP had a high and statistically significant ( $p < 0.01$ ) phenotypic Pearson correlation with the rest of the traits, the relationship was negative for ARL and positive for the rest (Table 2, above the diagonal). ARL had a negative and statistically significant Pearson correlation with RN and no statistically significant correlation with other traits. SDW had a positive and statistically significant correlation with RDW, RN and TRL, and negative but no statistically significant correlation with ARL.

SDW had a positive and statistically significant genetic correlation with RN, RDW and TRL, and a negative one with ARL (Table 2, below the diagonal). RN had a strong negative correlation with ARL and a strong and positive correlation with TRL.

### *Flooding experiment*

Flooding had a statistically significant effect ( $p < 0.05$ ) on all variables measured except diameter (D), while genotype was statistically significant ( $p < 0.001$ ) for all variables except leaf increase rate (LIR), senescence rate (SEN) and stomatal conductance ( $g_s$ , Table 3). The interaction between genotype and treatment was statistically significant ( $p < 0.01$ ) for growth rate in height (GRH), total leaf area (TLA), individual leaf area (ILA), specific leaf area (SLA) and  $g_s$ , meaning that genotypic effects will be different according to treatment.

H, D, GRH and total dry weight (TDW) were reduced by flooding in Nandi as opposed to CAR, in which they did not change or were less reduced (Fig. 2). Basic wood Density (BWD) was increased by flooding in both the parental genotypes and the progeny (Fig. 2). TLA, ILA, LIR, leaf number (LN) and  $g_s$  (Fig.3) were less affected by flooding in CAR than in Nandi. The  $F_1$  progeny showed variability beyond the range of the parental genotypes for all traits (Fig. 2 and 3). The effects of flooding are clearly shown in the PCA (Fig. S6). The first component explains 39% of the variability, and mainly represents the effects of flooding, which increased SEN, basic wood density (BWD) and AR compared to control plants, while reducing the other variables. The second component reflects genotypic differences, because there were differences among clones in some traits.

The narrow sense heritability ( $h^2$ , Table 3) was higher for height (H) in both control and flooded treatments. The variables with the lowest heritability were SEN and the abscission rate (AR). The other traits

had moderate to low heritability, and sometimes the values were quite different between the control and the flooded treatment as for SLA.

According to the flooding tolerance index (FTI, Fig. 4), the parental clones had different responses to flooding, with clone Nandi being more flood-sensitive than clone CAR. FTI in the  $F_1$  showed intermediate values between the parents in most genotypes, with the parental clones almost at the extremes of the scale.

For the control treatment, the flooding tolerance index (FTI) had a negative and statistically significant phenotypic correlation only with ILA (Fig. 5). In flooded plants, FTI had a positive and statistically significant correlation with H, D, GRH, TDW, LN, LIR and TLA (Fig. 6). The SEN, SLA, BWD and *gs* traits did not show statistically significant correlations with FTI in any treatment (i.e., control and flooding).

The phenotypic Pearson correlations were higher and statistically significant ( $p < 0.05$ ) among the plant growth traits (H, D, GRH, TDW), and with some leaf traits known to be related to productivity (TLA, LN, LIR, Table 4). These correlations were significant in both control and flooded plants, but other traits had different correlations according to the treatment. For instance, BWD correlated with LN and LIR in the control treatment but not in the flooded treatment. Other variables like *gs* had a negative statistically significant correlation with H and LN in control plants but a positive statistically significant correlation in flooded plants. SLA correlated with TLA, ILA and TDW in control plants but not in flooded plants, and AR did not correlate with any other variable.

There was a statistically significant genetic correlation between growth traits (H, D, TDW) and LN for both control and flooded treatments (Table 5). TLA showed correlation with D, ILA and TDW for both control and flooded treatments. Other variables had different correlations according to the treatment, like *gs* with a negative correlation with H, LN and TDW for control plants, and a positive correlation for the same traits in flooded plants. BWD had a low but significant positive correlation with H in control plants but not in the flooded treatment. BWD had a negative correlation with AR, SEN and SLA in both control and flooded plants.

## Discussion

Poplar plantations are propagated from unrooted cuttings, and this is a crucial point for the deployment of new genotypes (Zhao et al. 2014). In early stages of establishment from dormant hardwood cuttings, *Populus* plantations are highly vulnerable to the occurrence of stresses, in consequence it would be desirable to select new genetic material with increased flooding tolerance at this stage. The analysis of heritability and genetic correlations are highly relevant for breeders to select for traits increasing the success of the establishment of eastern cottonwood plantations. In this work, we analyzed the rooting capability and flooding responses in cuttings of a full-sib  $F_1$  progeny of *P. deltoides*. We estimated the heritability and genetic correlations of relevant traits by means of multiple-trait mixed linear models. In addition, we identified several traits correlating with flooding tolerance.

#### *Early rooting of a full-sib family of P. deltoides.*

In this work, we aimed to determine clonal differences in early rooting capability between dormant rootless cuttings under environmental conditions that favor this process, except for the addition of rooting hormones (Desrochers and Thomas 2003, Zhao et al. 2014). The possibility of easily propagated elite clones is a key feature to realize genetic gains in poplar. *P. deltoides* rooting capability has a high genetic variation, with great differences at clone level, but strong environmental  $\times$  genotype ( $G \times E$ ) effects are present (Zalesny and Zalesny 2009). Poplar cuttings could develop lateral roots from latent root primordia, or basal roots from callus tissue originating at the base of the cutting as a wounding response (Zalesny and Zalesny 2009). In our material, most clones produced lateral roots and this was quantified, but some cuttings developed callus that may originate basal roots afterwards. The rooting percentage measured only with lateral roots (i.e., actual rooting) was high, but it reached 100% in most clones when cuttings with callus were included (potential rooting) (Fig.1 and S4). Overall, this family has a good rooting capability, since *P. deltoides* is more difficult to root from cuttings compared with other poplar species (Zalesny and Zalesny 2009). These percentages may be lower under field conditions, since the cuttings may suffer stresses that reduce rooting (Krabel et al. 2015, Mc Carthy et al. 2018).

We found a negative and statistically significant correlation between Average Root Length (ARL) and Root Number (RN) at both phenotypic and genetic levels (Table 2). This is different from previous results,

where a high and positive phenotypic and genetic correlation between ARL and RN was found for *P. deltoides* and some interspecific hybrids in a rooting field trial (Zalesny et al. 2005). The opposite result may be due to the differences in the genotypes analyzed and the experimental design. Zalesny et al. (2005) found positive genetic and phenotypic correlations between RDW, TRL and RN, as we did in our conditions (Table 2).

Our values for narrow-sense heritability ( $h^2$ ) for RN (Table 1) were inferior to the estimations of  $H^2$  for the same trait for a hybrid family of *P. deltoides* x *P. euramericana*, ranging from 0.80 to 0.85 (Zhang et al. 2009). This is due to the fact that  $h^2$  takes into account only the additive genetic variance, while  $H^2$  includes other genetic variance components as well (Lynch and Walsh 1998). We had higher  $h^2$  values for RDW and SDW than the broad-sense heritabilities ( $H^2$ ) estimated by Zalesny et al. (2005), likely because the heritability was estimated over different sites and years, increasing the environmental variance component.

Except for ARL, the rest of the traits had a positive genetic correlation with SDW (Table 2). This is an interesting result, because these traits (RN, TRL, RDW) are laborious to measure, especially under field conditions. The measurement of these traits by means of the shoot biomass is less time-consuming, and can be used as a surrogate of those traits for selection. Heilman et al. (1994) found that above-ground weight correlated positively with root weight in a set of 20 *P. deltoides*, 15 *P. trichocarpa* and 44 *P. deltoides* x *P. trichocarpa* hybrids growing in the field. In order to use shoot biomass as a surrogate for roots traits, it will be desirable to confirm whether this correlation holds for other *P. deltoides* pedigrees and under field conditions.

#### *Flooding tolerance correlated with some growth and leaf traits*

Previous results with poplar showed that some individuals in  $F_1$  offspring could have a higher flooding tolerance than the parental genotypes (Du et al. 2011, Rodríguez et al. 2020). The results for the Nandi x CAR family were completely different, the flooding tolerance of the progeny (measured as FTI) was intermediate among the parental genotypes (i.e., Nandi and CAR, Fig. 4). This is a strong indication of variability at clone level in *P. deltoides*, since different pedigrees had contrasting results for flooding tolerance. These results are in line with the occurrence of high genetic variability in the southern range of distribution of eastern cottonwood (Fahrenkrog et al. 2017), which is the region from where the female parents of clones Nandi and CAR were originally collected (Luquez et al. 2012).

The Flooding Tolerance Index (FTI) had a significant positive correlation with H, D, GRH, TDW, LN, LIR and TLA in flooded plants (Fig. 3). In another *P. deltoides* F<sub>1</sub> full-sib family (Rodríguez et al. 2020), H and D correlated with FTI in flooded plants, while there was no correlation of leaf traits in either control nor flooded plants. Du et al. (2011) found that height and diameter were the most reliable traits to select under flooding. It seems that H and D are more reliable for selection for early flooding tolerance than leaf traits, but it would be desirable to evaluate more families to confirm these results.

The use of H and D has other advantages, they had a strong and statistically significant positive phenotypic and genetic correlation with TDW in *P. deltoides*, and could be used as surrogates for plant biomass (Tables 4 and 5, Du et al. 2011, Rodríguez et al. 2020). Both measurements are non-destructive and could eventually be automated to screen a high number of genotypes in a breeding program. In our experiment, H and D had a similar or higher  $h^2$  (Table 3) than the reported for another *P. deltoides* pedigree (Rodríguez et al. 2020). Our results are similar to the broad sense heritability determined for H and D in a collection of 391 unrelated eastern cottonwood genotypes (Fahrenkrog et al. 2017).

Wood density correlates with xylem cavitation tolerance across a range of woody species (Hacke et al. 2001). We found that wood density increased in flooded plants, but it did not correlate with flooding tolerance (Fig.2 and 6). BWD had a moderate positive genetic correlation with H and a negative one with D in control but not in flooded plants (Table 5). In 10-year old poplar clones, wood density had a significant negative genetic correlation with both height and diameter (Pliura et al. 2007). These differences could be attributed to the different age of the trees, since wood density changes with age in poplar (Pliura et al. 2006).

Leaf traits have shown significant phenotypic correlations with growth in several *Populus* pedigrees (Rae et al. 2004, Monclus et al. 2005, Marron and Ceulemans 2006) and natural populations of different species like *P. nigra* (Guet et al. 2015), *P. balsamifera* (Soolanayakanahally et al. 2009), *P. trichocarpa* (Gornall and Guy 2007) and *P. tremuloides* (Kanaga et al. 2008), among others. In addition to the phenotypic correlations, we determined the genetic correlations between these traits that are more relevant for breeding. Some leaf traits had significant positive phenotypic and genetic correlations with H in both control and flooded treatment, like LN and LIR, but other like TLA were significant only for flooded plants (Table 4 and 5). The  $g_s$  had a positive correlation (genetic and phenotypic) with H in flooded plants but a negative one in control

plants. A possible explanation is that stomata close in flooded *Populus* plants, and the genotypes that keep a higher  $g_s$  are able to keep the photosynthetic activity and growth under stress conditions (Rodriguez et al. 2015, Du et al. 2011).

As a whole, the leaf traits have lower  $h^2$  than the growth traits (H, D and TDW). The comparison with published heritability values for leaf traits is not straightforward, because most works determined broad sense heritability, but our results are within the range of those published for other *Populus* species and hybrids (Marron and Ceulemans 2006, Kanaga et al. 2008).

A good rooting capability increases early growth of poplar plantations, and it is possible that clones with a superior rooting could also have an increased flooding tolerance. Willow genotypes with a vigorous early growth were better able to endure flooding than those with a lower growth rate, and consequently smaller size (Rodríguez et al. 2018). We compared the FTI scores of the flooding experiment with the rooting traits in the other experiment, but there was no statistically significant correlation with any of these variables (data not shown). The likely explanation for this lack of correlation is that the rooting of cuttings under waterlogged and non-waterlogged conditions is different, with genotypes that produced abundant roots under non-stress conditions rooted poorly under waterlogging (McCarthy et al. 2018). Eventually, the rooting of cuttings under waterlogging could be a predictor of the flooding tolerance of eastern cottonwood genotypes, but this hypothesis needs further confirmation.

Flood tolerance is highly dependent on the age of the plant, the length of the flooding period, the depth of the floodwater, and if the water is stagnant or running (Kozłowski 1997). In consequence, our data are relevant for field plantations of the same age, and with similar flooding conditions, and these results could not be extrapolated for older field plantations. But increasing flooding tolerance in young plants is still relevant for the establishment of poplar plantations. With more tolerant genotypes, a flooding episode at this early stage will cause less damage and, in consequence, a reduction of the cost to replace the lost plants in the field.

## Conclusions

We found genotypic variation in the early rooting capability of a full - sib  $F_1$  of eastern cottonwood and moderate to high narrow sense heritability values for the traits measured. The above ground biomass had a strong phenotypic and genetic correlation with the root traits of the cuttings. We could identify several traits that correlated with flooding tolerance in eastern cottonwood. Among these traits, H and D are more convenient for selection because they have a moderate to high heritability, are easy to measure, non-destructive and could be automated to screen a large number of trees. From the phenotypic and genetic correlations, we could determine that flooding tolerance does not imply a reduction of growth in this family. Additionally, we could identify some easy-to-measure traits that show a robust correlation with other more labor-intensive traits, like SDW with roots traits (RN, TRL, RDW) and H and D with above ground biomass (TDW). Further confirmation in field experimental trials should be a next step to conclusively correlate greenhouse results as a powerful tool of convenience to the breeder in poplar selection.

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**Table 1.** Trait full name, abbreviations, units, statistical significances of block, and genotype effects and the covariable diameter, and narrow-sense heritability ( $h^2$ ) estimation (and standard errors) for the different traits measured in the rooting experiment for the Nandi x CAR full-sib family.

Trait name	Abbreviation	Unit	Statistical significances <sup>1</sup>			$h^2$ (SE)
			Diameter	Block	Genotype	
Shoot Dry Weight	SDW	g	***	**	***	0.65 (0.10)
Root Number	RN	---	*	*	***	0.53 (0.10)
Root Dry Weight	RDW	mg	ns	*	***	0.22 (0.09)
Total Root Length	TRL	cm	ns	*	***	0.28 (0.10)
Average Root Length	ARL	cm	ns	ns	***	0.66 (0.09)
Rooting	RP	%	ns	ns	***	Not estimated

**NOTE:** <sup>1</sup> Statistical significance: ns: non-significant, \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ .

**Table 2.** Phenotypic Pearson correlation coefficients (upper part of the table, in italics, N = 32) and genetic correlations (lower part of the table) between the traits measured in the rooting experiment for the full-sib family Nandi x CAR. Statistically significant correlations in bold.

Trait	SDW	RN	RDW	TRL	ARL	RP
<b>SDW</b>		<i><b>0.62***</b></i>	<i><b>0.58***</b></i>	<i><b>0.60***</b></i>	-0.25	<i><b>0.66***</b></i>
<b>RN</b>	<i><b>0.75***</b></i>		<i><b>0.73***</b></i>	<i><b>0.88***</b></i>	<i><b>-0.56***</b></i>	<i><b>0.84***</b></i>
<b>RDW</b>	<i><b>0.41*</b></i>	0.32		<i><b>0.84***</b></i>	-0.12	<i><b>0.72***</b></i>
<b>TRL</b>	<i><b>0.60***</b></i>	<i><b>0.81***</b></i>	0.35		-0.20	<i><b>0.83***</b></i>
<b>ARL</b>	<i><b>-0.51**</b></i>	<i><b>-0.80***</b></i>	-0.08	<i><b>-0.50**</b></i>		<i><b>-0.47**</b></i>

**NOTE:** Statistical significances: \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ .

**Table 3.** Trait full name, abbreviations, units, statistical significances of genotype, treatment (i.e., control and flooding), and the interaction treatment × genotype effects; and narrow-sense heritability ( $h^2$ ) estimation (and standard errors) for the traits measured in the flooding experiment for the full-sib family Nandi x CAR.

Full name	Abbreviation	Units	Statistical significances <sup>1</sup>			$h^2$ Control	$h^2$ Flooded
			Genotype	Treatment	Interaction		
Final Height	H	cm	***	**	ns	0.58 (0.10)	0.80 (0.85)
Final Diameter	D	mm	***	ns	ns	0.36 (0.09)	0.52 (0.07)
Growth Rate in height	GRH	cm day <sup>-1</sup>	***	***	***	0.38 (0.10)	0.62 (0.12)
Total Dry Weight	TDW	g	***	*	ns	0.44 (0.10)	0.53 (0.09)
Basic Wood Density	BWD	g cm <sup>-3</sup>	***	***	ns	0.45 (0.10)	0.48 (0.11)
Total Leaf Area	TLA	cm <sup>2</sup>	***	***	**	0.28 (0.09)	0.24 (0.08)
Individual Leaf Area	ILA	cm <sup>2</sup>	***	***	***	0.45 (0.10)	0.33 (0.07)
Final Leaf Number	LN	---	***	**	ns	0.32 (0.09)	0.49 (0.10)
Leaf Increase Rate	LIR	Leaf day <sup>-1</sup>	ns	***	ns	0.29 (0.09)	0.40 (0.08)
Abscission Rate	AR	Leaf day <sup>-1</sup>	***	***	ns	0.19 (0.07)	0.27 (0.08)
Leaf Senescence Rate	SEN	SPAD units day <sup>-1</sup>	ns	***	ns	0.18 (0.08)	0.17 (0.05)
Specific Leaf Area	SLA	cm <sup>2</sup> g <sup>-1</sup>	***	***	**	0.43 (0.10)	0.15 (0.05)
Stomatal Conductance	gs	mmol m <sup>-2</sup> s <sup>-1</sup>	ns	***	***	0.24 (0.08)	0.20 (0.08)

**NOTE:** <sup>1</sup> Statistical significance: ns non - significant, \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .



**Table 4.** Phenotypic Pearson correlations coefficients for control (upper part of the table *-in italics-*) and flooded plants (lower part of the table) between different traits measured in the flooding experiment for the Nandi x CAR full-sib family. N = 31. Statistically significant correlations in bold. See Table 3 for traits full name and units.

	H	D	GRH	TLA	ILA	LN	LIR	AR	SEN	SLA	TDW	BWD	gs
H		<i>0.38*</i>	<i>0.61***</i>	0.21	-0.20	<i>0.51*</i>	<i>0.37*</i>	0.02	-0.24	-0.11	<b>0.56**</b>	0.16	<b>-0.43*</b>
D	<b>0.73***</b>		<i>0.41*</i>	<b>0.63***</b>	<b>0.39*</b>	<b>0.42*</b>	<b>0.41*</b>	0.27	-0.02	0.01	<b>0.74***</b>	-0.32	-0.26
GRH	<b>0.92***</b>	<b>0.68***</b>		0.31	0.19	0.19	<b>0.51**</b>	0.03	-0.14	0.34	0.24	-0.21	-0.06
TLA	<b>0.54***</b>	<b>0.66***</b>	<b>0.45*</b>		<b>0.77***</b>	<b>0.41*</b>	0.36	-0.08	-0.16	<b>0.45*</b>	<i>0.54**</i>	-0.27	-0.35
ILA	-0.06	0.04	-0.14	<b>0.62***</b>		-0.20	-0.08	-0.16	-0.06	<b>0.49***</b>	0.23	-0.12	-0.07
LN	<b>0.72***</b>	<b>0.77***</b>	<b>0.69***</b>	<b>0.68***</b>	-0.07		<b>0.63***</b>	-0.05	-0.16	-0.07	<b>0.49**</b>	<b>-0.32*</b>	<b>-0.46**</b>
LIR	<b>0.74***</b>	<b>0.74***</b>	<b>0.76***</b>	<b>0.46**</b>	-0.29	<b>0.78***</b>		0.34	-0.30	0.20	0.25	<b>-0.44*</b>	-0.16
AR	-0.23	-0.09	-0.17	-0.36	-0.27	-0.36	0.08		-0.07	0.07	-0.06	-0.11	0.01
SEN	0.09	0.12	0.09	0.27	0.35	-0.03	-0.09	-0.04		0.13	-0.20	-0.13	<b>0.40*</b>
SLA	0.14	-0.09	0.31	0.09	-0.08	0.14	0.10	-0.03	-0.02		<b>-0.42*</b>	-0.25	-0.06
TDW	<b>0.76***</b>	<b>0.83***</b>	<b>0.58***</b>	<b>0.79***</b>	0.29	<b>0.72***</b>	<b>0.62***</b>	-0.30	0.18	-0.31		0.01	<b>-0.37*</b>
BWD	0.15	-0.02	-0.01	-0.05	0.04	-0.05	-0.08	-0.19	-0.23	-0.32	0.15		-0.17
gs	<b>0.52**</b>	<b>0.45*</b>	<b>0.60***</b>	0.10	-0.29	<b>0.46**</b>	<b>0.47**</b>	-0.01	0.17	<b>0.36*</b>	0.19	-0.06	

**NOTE:** Statistical significances: \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ .

**Table 5.** Genetic correlations coefficients for control (upper part of the table *-in italics-*) and flooded plants (lower part of the table) between different traits measured in the flooding experiment for the Nandi x CAR full-sib family. Statistically significant correlations in bold. See Table 3 for traits full names and units.

	H	D	GRH	TLA	ILA	LN	LIR	AR	SEN	SLA	TDW	BWD	gs
H		<i>0.27</i>	<b>0.61***</b>	<i>0.10</i>	<i>-0.28</i>	<b>0.46**</b>	<i>0.31</i>	<i>-0.04</i>	<b>-0.43*</b>	<i>-0.14</i>	<b>0.51**</b>	<b>0.38*</b>	<b>-0.55***</b>
D	<b>0.74***</b>		<b>0.44*</b>	<b>0.70***</b>	<b>0.48**</b>	<b>0.36*</b>	<b>0.46*</b>	<i>0.34</i>	<i>0.09</i>	<i>0.02</i>	<b>0.73***</b>	<b>-0.36*</b>	<i>-0.26</i>
GRH	<b>0.94***</b>	<b>0.72***</b>		<b>0.39*</b>	<i>0.21</i>	<i>0.18</i>	<b>0.50*</b>	<i>-0.11</i>	<i>-0.14</i>	<b>0.42*</b>	<i>0.24</i>	<i>-0.24</i>	<i>-0.08</i>
TLA	<b>0.76***</b>	<b>0.65***</b>	<b>0.51**</b>		<b>0.78***</b>	<b>0.37*</b>	<b>0.47*</b>	<i>-0.03</i>	<i>0.01</i>	<i>0.34</i>	<b>0.53**</b>	<i>-0.33</i>	<b>-0.42*</b>
ILA	<i>-0.08</i>	<i>0.08</i>	<i>-0.18</i>	<b>0.56**</b>		<b>-0.27</b>	<i>-0.12</i>	<i>-0.23</i>	<i>-0.08</i>	<i>0.34</i>	<i>0.28</i>	<i>-0.22</i>	<i>-0.05</i>
LN	<b>0.73***</b>	<b>0.78***</b>	<b>0.75***</b>	<b>0.67***</b>	<i>-0.17</i>		<b>0.74***</b>	<i>0.18</i>	<b>-0.50**</b>	<i>-0.04</i>	<b>0.38*</b>	<i>-0.28</i>	<b>-0.61***</b>
LIR	<b>0.76***</b>	<b>0.86***</b>	<b>0.81***</b>	<b>0.50**</b>	<b>-0.41*</b>	<b>0.83***</b>		<b>0.44*</b>	<i>-0.31</i>	<i>0.27</i>	<i>0.21</i>	<b>-0.52**</b>	<i>-0.17</i>
AR	<b>-0.45*</b>	<i>-0.09</i>	<i>-0.34</i>	<b>-0.46**</b>	<b>-0.39*</b>	<b>-0.40*</b>	<i>0.01</i>		<i>-0.07</i>	<i>0.03</i>	<i>-0.05</i>	<i>-0.12</i>	<i>0.10</i>
SEN	<i>-0.06</i>	<i>0.11</i>	<i>-0.05</i>	<i>0.26</i>	<b>0.48**</b>	<i>-0.15</i>	<i>-0.11</i>	<i>0.27</i>		<b>0.33</b>	<b>-0.50**</b>	<i>-0.34</i>	<b>0.58***</b>
SLA	<i>0.21</i>	<i>-0.18</i>	<b>0.45**</b>	<i>-0.07</i>	<b>-0.46**</b>	<i>0.31</i>	<i>0.33</i>	<i>0.01</i>	<i>-0.09</i>		<b>-0.51**</b>	<b>-0.35*</b>	<i>-0.03</i>
TDW	<b>0.80***</b>	<b>0.84***</b>	<b>0.64***</b>	<b>0.81***</b>	<i>0.30</i>	<b>0.68***</b>	<b>0.62***</b>	<b>-0.47**</b>	<i>0.07</i>	<i>-0.30</i>		<i>0.11</i>	<b>-0.43*</b>
BWD	<i>0.20</i>	<i>-0.09</i>	<i>0.02</i>	<i>-0.07</i>	<i>0.14</i>	<i>-0.13</i>	<i>-0.21</i>	<b>-0.36*</b>	<b>-0.40*</b>	<b>-0.36*</b>	<i>0.17</i>		<i>-0.32</i>
gs	<b>0.68***</b>	<b>0.63***</b>	<b>0.73***</b>	<i>0.17</i>	<b>-0.40*</b>	<b>0.65***</b>	<b>0.63***</b>	<i>0.01</i>	<i>0.13</i>	<b>0.39*</b>	<b>0.37*</b>	<i>-0.02</i>	

NOTE: Statistical significance: \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ .

## Legends to the figures

**Fig. 1.** Phenotypic variability in the rooting experiment. CAR: male parental clone. NAN: female parental clone. F<sub>1</sub>: genotypes of the F<sub>1</sub> progeny.

**Fig. 2.** Phenotypic variability in the plant traits of the flooding experiment. CAR: male parental clone. NAN: female parental clone. F<sub>1</sub>: genotypes of the F<sub>1</sub> progeny. C: control treatment. F: flooded treatment.

**Fig. 3.** Phenotypic variability in the leaf traits of the flooding experiment. CAR: male parental clone. NAN: female parental clone. F<sub>1</sub>: genotypes of the F<sub>1</sub> progeny. C: control treatment. F: flooded treatment.

**Fig. 4.** Flooding Tolerance Index (FTI) of the parental clones and 29 genotypes of the F<sub>1</sub> from the full-sib family Nandi x CAR. FTI calculation was described in Material and Methods. Black: CAR (male parental clone). White: Nandi (female parental clone). Grey: genotypes of the F<sub>1</sub>.

**Fig. 5.** Bivariate plots and phenotypic Pearson correlation coefficients ( $r$ ) between FTI and the different traits measured for the control treatment, for the parental clones and 29 genotypes of the F<sub>1</sub> from the full-sib family Nandi x CAR.  $N = 31$ . \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ . Open symbols: non-significant correlation with FTI. Closed symbols: significant correlation with FTI. Full names of the traits are detailed in Table 3.

**Fig. 6.** Bivariate plots and phenotypic Pearson correlation coefficients ( $r$ ) between FTI and the different traits measured for the flooded treatment, for the parental clones and 29 genotypes of the F<sub>1</sub> from the full-sib family Nandi x CAR.  $N = 31$ . \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ . Open symbols: non-significant correlation with FTI. Closed symbols: significant correlation with FTI. Full names of the traits are detailed in Table 3.

# FIG. 1

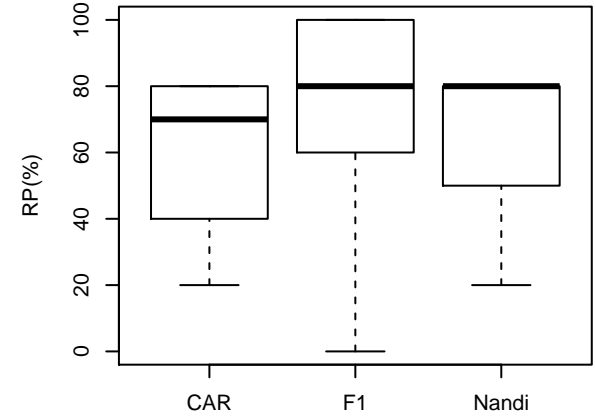
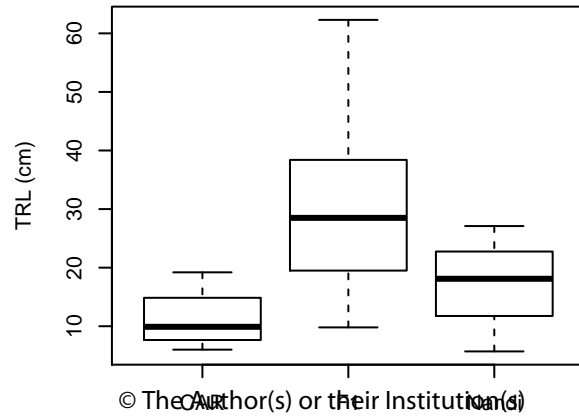
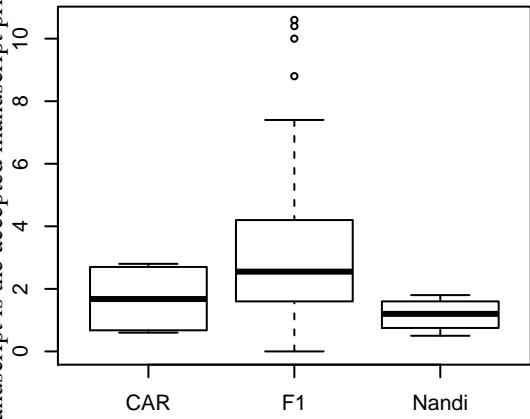
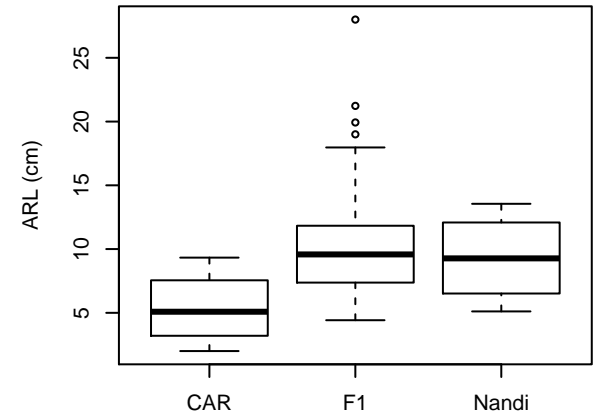
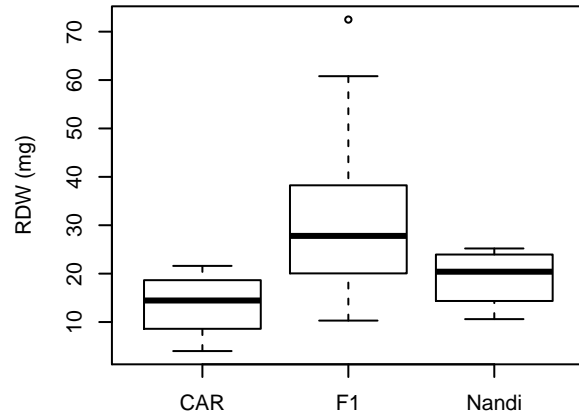
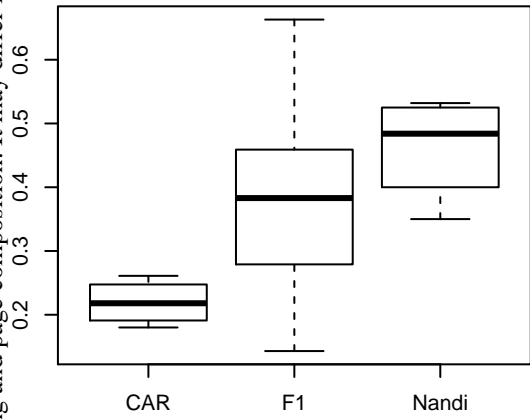


FIG. 2

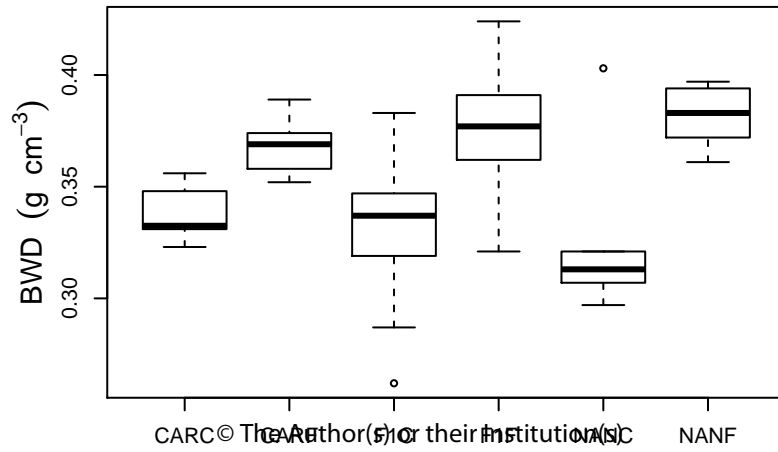
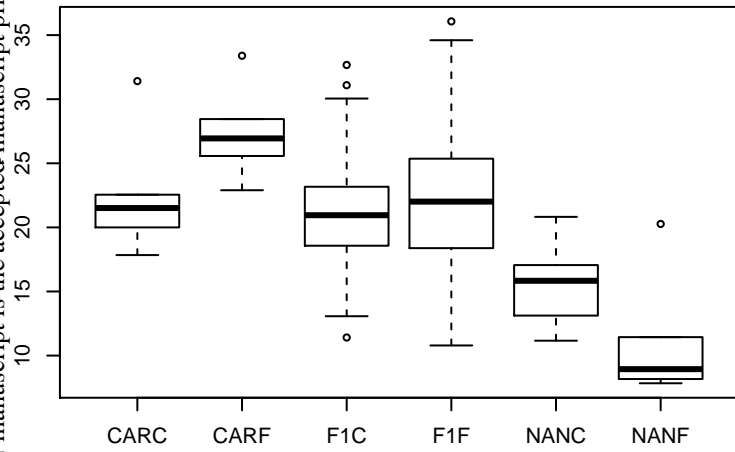
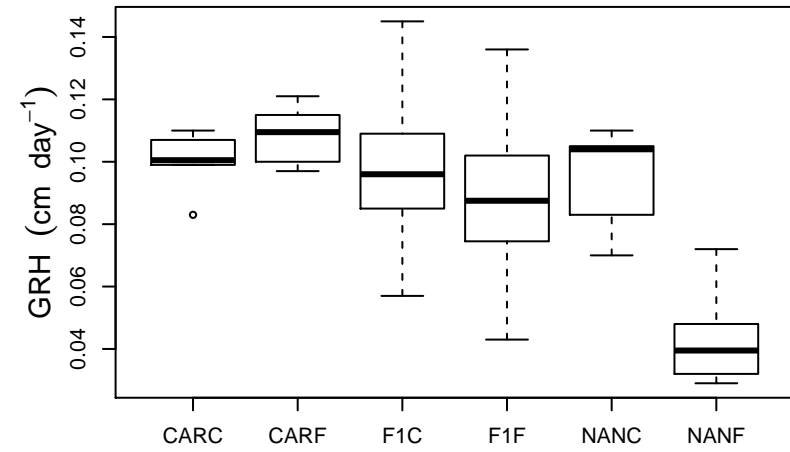
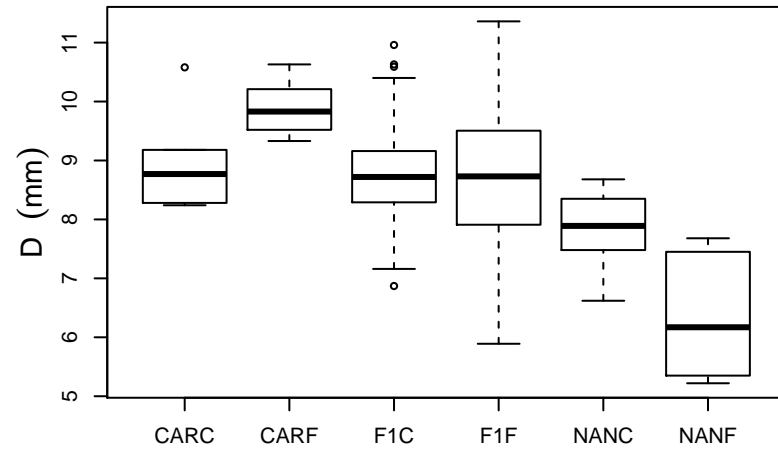
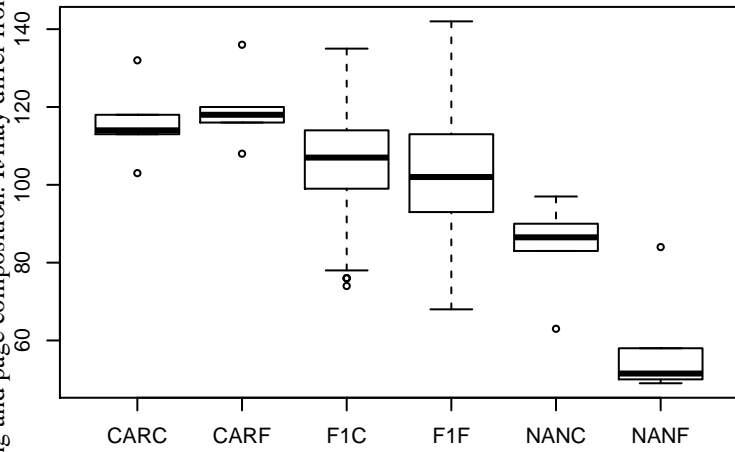
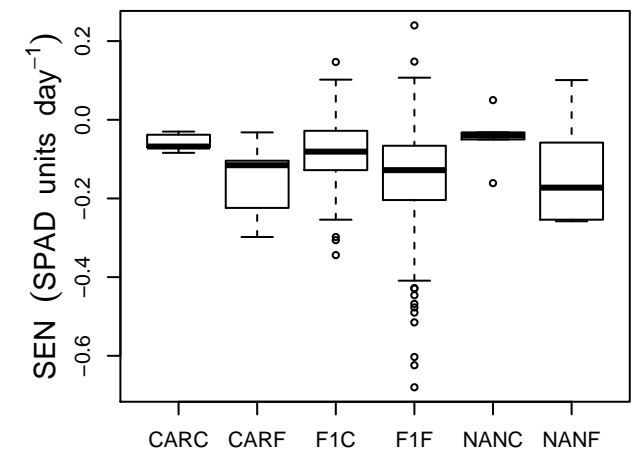
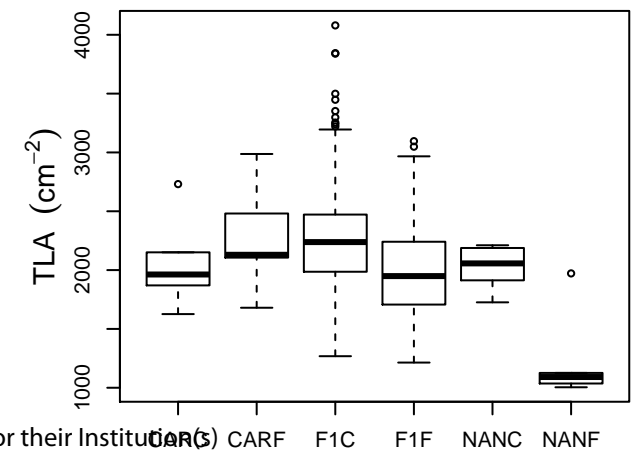
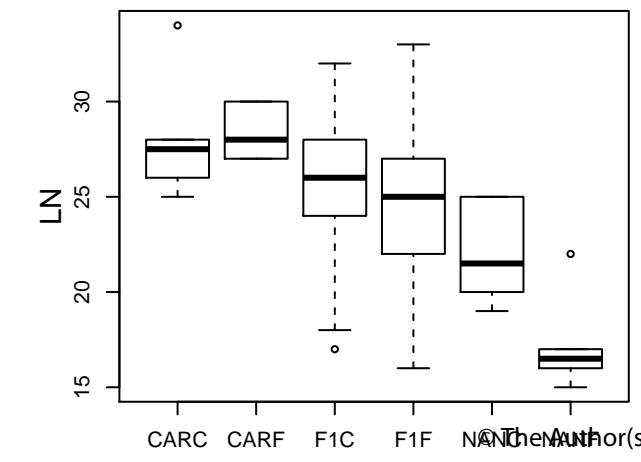
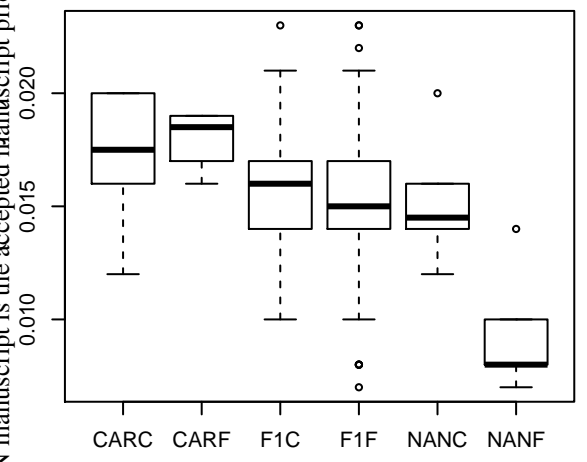
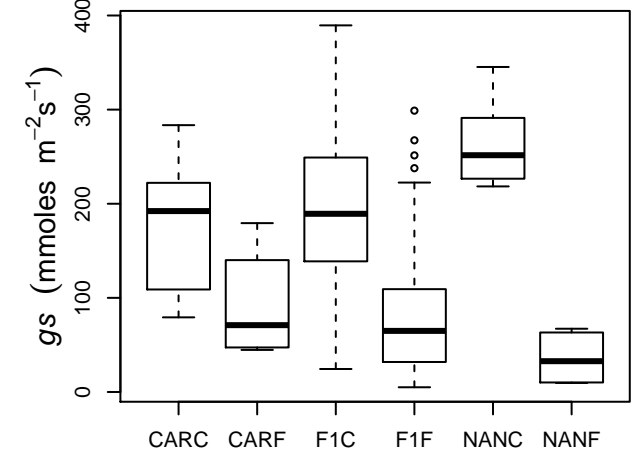
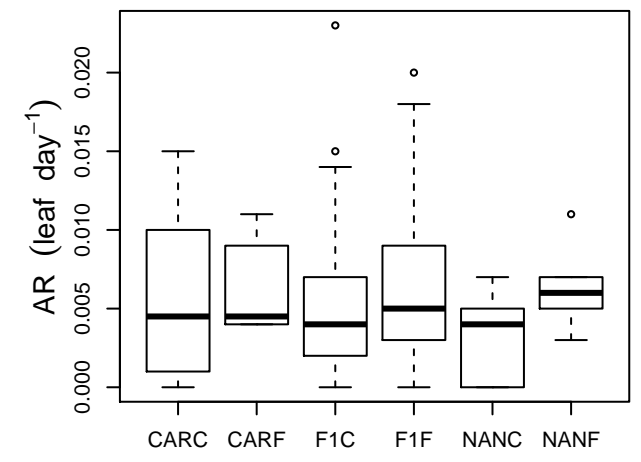
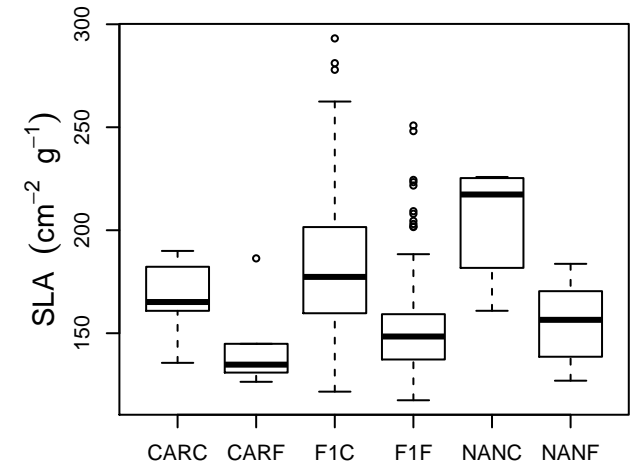
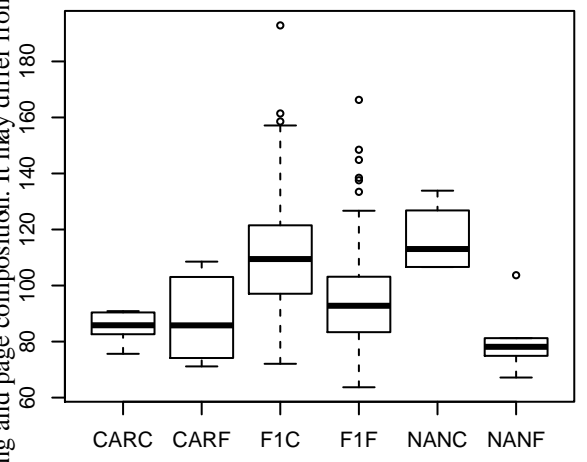


FIG 3



**FIG.4**

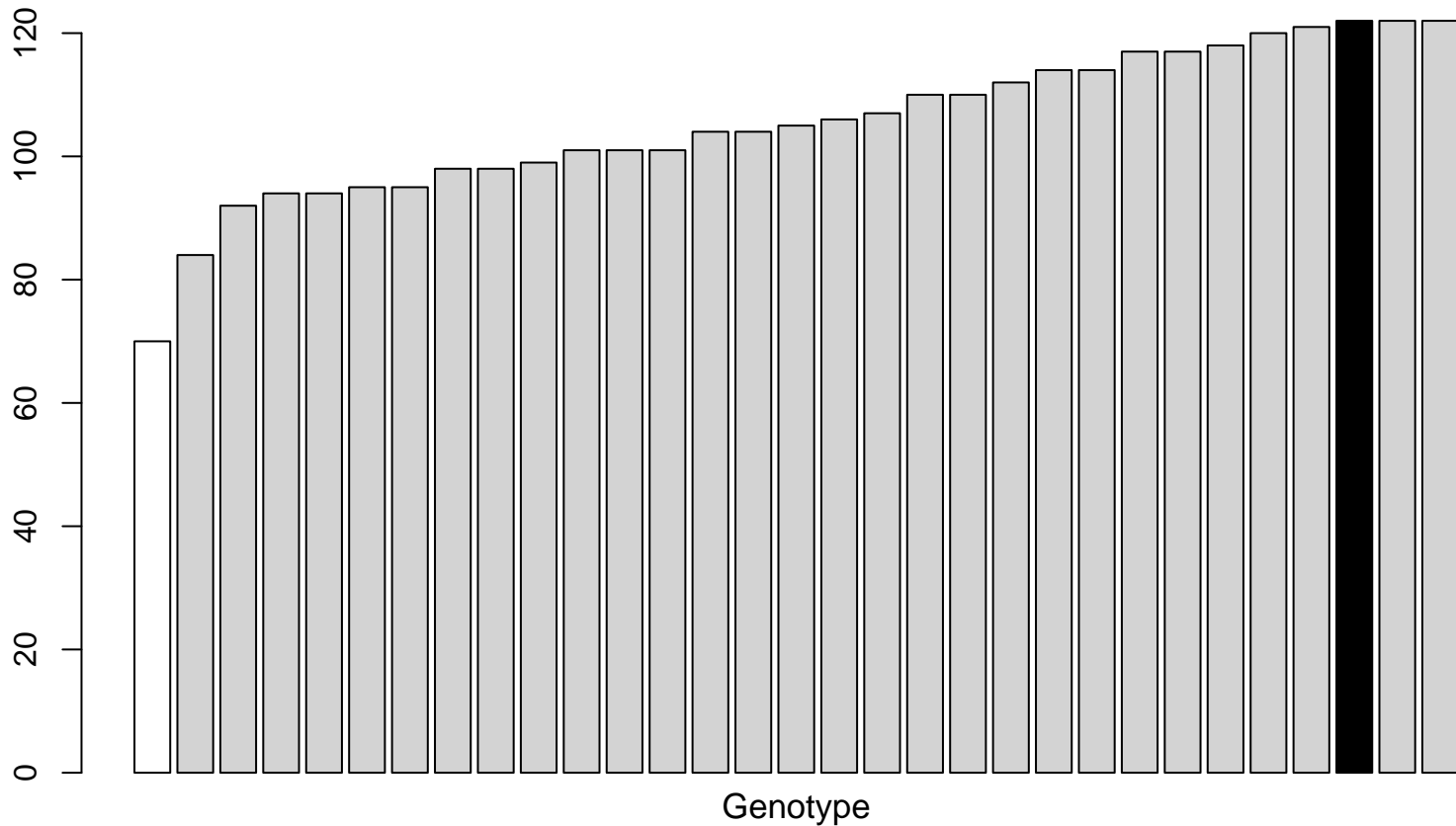


FIG.5

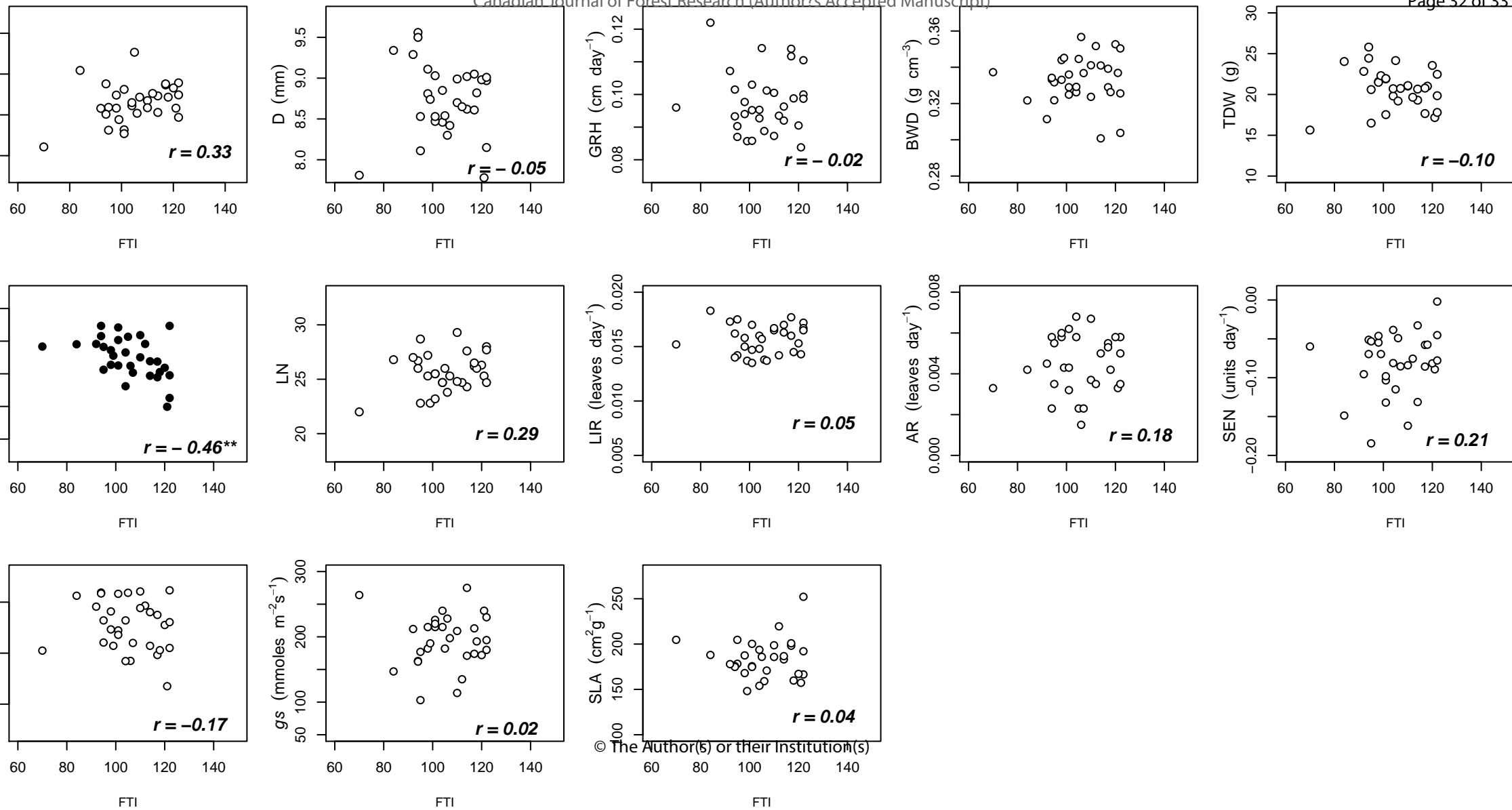




FIG.6

