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This paper is published in Environmental and Experimental Botany :

Article title: Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes
Article reference: EEB3784
Journal title: Environmental and Experimental Botany
Corresponding author: Dr Oliver Brendel
First author: Dr. Bogeat-Triboulot
First published version available online: 19-JUN-2019
DOI information: 10.1016/j.envexpbot.2019.05.021

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Key words :
Water use efficiency
Transpiration efficiency
Nocturnal transpiration
Water deficit
Poplar
Intraspecific diversity

Abbreviations :

A: net CO_2 assimilation rate,
A_{mean}: mean of net CO_2 assimilation rate measured *in situ*,
A_{sat}: net CO_2 assimilation rate measured under light-saturated conditions,
C_i: CO_2 internal concentration,
CumulT: cumulated water loss,
DMincr: total dry mass increment,
DTR: diurnal transpiration rate,
$\delta^{13}C$: carbon isotope composition,
FinalH: final stem height,
FinalD: final stem diameter,
g: stomatal conductance to water vapour,
g_m: mesophyll conductance for CO_2,
g_{mean}: stomatal conductance to water vapour measured *in situ*,
g_{sat}: stomatal conductance to water vapour measured under light-saturated conditions,
J_{max}: maximum photosynthetic electron flux,
LA: total leaf area,
LeafDM: leaf dry mass,
LeafF: leaf fraction,
Φ_w: proportion of unproductive water loss to productive water loss,
NTR: nocturnal transpiration rate,
RootF: root fraction,
StemF: stem fraction,
TE: whole plant transpiration efficiency,
TotalDM: total dry mass,
TR: daily transpiration rate,
Vc_{max}: maximum CO_2 carboxylation rate,
WUE: water use efficiency,
Wi: leaf intrinsic water use efficiency
Wi_{sat}: leaf intrinsic water use efficiency measured under light-saturated conditions,
Wi_{mean}: mean leaf intrinsic water use efficiency measured *in situ*.

Abstract

Poplar plantations, widely used for the production of woody biomass, might be at high risk from the climate change-induced increase in the frequency of drought periods. Therefore, selecting improved genotypes, which are highly productive but with a high water use efficiency (WUE), is becoming a major target. The use of automated weighing systems in controlled environments facilitates the estimation of cumulated water loss and whole plant transpiration efficiency (TE). Differences in TE and leaf level intrinsic WUE as well as the contribution of underlying ecophysiological traits were determined in three contrasting *P. nigra* genotypes. Strong differences in TE among the selected genotypes were congruent with differences in leaf level intrinsic WUE. Our data show that a high total leaf area was overcompensated by a low per leaf area transpiration rate, leading to higher TE in highly productive genotypes originating from cool locations. Nocturnal water loss was relatively low but contributed to variations in TE among genotypes. In response to drought, leaf level WUE increased but not TE, suggesting that carbon losses due to whole plant respiration could offset the drought-induced increase in intrinsic WUE.

Highlights

- The Spanish genotype was less water use efficient than northern genotypes
- Low WUE was linked to a high transpiration rate and a large root system
- A trade-off appeared between total leaf surface and per surface transpiration rate
- Intrinsic WUE was increased by drought but not transpiration efficiency

1. Introduction

To limit the increasing global temperature, there is an urgent need to reduce greenhouse gas emissions coming from fossil fuels. Biofuels which come from dedicated crops and tree plantations can contribute to meet this target (Sannigrahi et al., 2010) and poplar plantations are widely used for the production of woody biomass (Navarro et al., 2018). On the other hand, future climate change is projected to reduce the productivity of plantation forestry in the coming decades through changes in net primary production (Hanson and Weltzin, 2000). Moreover Domec et al. (2015) suggested that intensively managed plantations are more drought-sensitive than natural forests. Considering this, as well as the high vulnerability of poplars to drought-induced cavitation (Fichot et al., 2015), poplar plantations might be at high risk from the climate change-induced increase in the frequency of droughts. To meet the worldwide increasing demand of wood biomass in the context of climate change, selecting improved tree genotypes, which are highly productive but with a high water use efficiency (WUE), is becoming a major target.

At the whole plant level, WUE is called transpiration efficiency (TE) and is defined as the ratio between the biomass accumulated and the water transpired over a defined period of time. At the leaf level, WUE is reflected by intrinsic WUE (Wi), the ratio between net CO_2 assimilation rate (A) and stomatal conductance of water vapour (g). For any one plant, the relationship between A and g is curvilinear, approaching asymptotically a maximum A when stomata are fully open. Under optimal watering conditions, stomata are often more open than required to achieve a maximum A under the given atmospheric conditions resulting in “luxurious” water consumption. During an increasing soil water deficit, this results in stomatal closure affecting A less than proportionally, thereby increasing Wi (see for example Suppl Fig 2 of Marguerit et al., 2014). In the case of large-scale screening of poplar genotypes for WUE (Kruse et al., 2012; Viger et al., 2013), an indirect estimation of Wi is often used by measuring the carbon stable isotope composition ($\delta^{13}C$) of organic material such as leaf, wood or extracted cellulose (Bussotti et al., 2015; Farquhar et al., 1982). However, even if $\delta^{13}C$ is measured on wood or extracted cellulose, it still represents a spatio-temporal assimilation-weighted integration of leaf level processes during daytime (A and g). Therefore, $\delta^{13}C$ does not include processes in other plant parts and those occurring during the night, which can contribute to variations in biomass accumulation and water loss, and thus TE. These processes relate to respiration of the whole plant during day and night (except leaves during the daytime as this is included in net CO_2 assimilation), water losses from plant organs other than leaves and also water losses from leaves during the night (Cernusak et al., 2007). Thus, choosing water efficient genotypes for tree plantations on the base of the whole plant transpiration efficiency could be more judicious than on the more widely used leaf level estimates ($\delta^{13}C$, Wi). However, the estimation of TE in adult trees in the field is challenging, because of the difficulties of estimating both the biomass increase, especially that of the root system, and the water use of a whole tree over long time periods. TE of a single tree can be estimated by an allometric estimation of aboveground biomass increase and direct sap flow measurements (Navarro et al., 2018). However, the root biomass increase is ignored and such measurements are not feasible on a large number of individuals. Biomass increments of potted plants can be more easily assessed and the use

of automated weighing systems facilitates the estimation of cumulated water use in controlled environments. Such systems are either based on multiple balances (Cirelli et al., 2012) or robotic systems (Buré et al., 2016; Granier et al., 2006) and allow many plants to be weighed at a high frequency, thus both controlling soil humidity and quantifying water loss. This in turn allows an accurate estimation of TE and underlying traits as well as comparisons with $\delta^{13}C$ or Wi.

Commercial poplar genotypes have been selected primarily for high productivity or resistance to foliar rust but not for high WUE (Monclus et al., 2006). The lack of correlation between productivity and Wi across 29 *Populus* × *canadensis* hybrids suggested that it would be possible to select genotypes which combine high productivity and high WUE (Monclus et al., 2005). Conversely, a negative relationship between TE and productivity was found in the Asian species *P. davidiana* (Zhang et al., 2004), questioning the independence between productivity and WUE. European black poplar (*P. nigra*, L.) is a key pioneer tree species, essential for the dynamics of riparian habitats and for soil stabilisation. Further, it has an economic value as a parent pool for genetic breeding of *P.* × *canadensis* cultivars (Chamaillard et al., 2011; Sow et al., 2018). *P. nigra* has a wide natural distribution with populations growing in different climatic conditions across Europe and showing significant genetic differentiation as well as phenotypic variation in growth rate, plant architecture and leaf size (DeWoody et al., 2015; Viger et al., 2016).

To improve our understanding of the determinants of TE and their responses to drought, we determined TE in three contrasting *P. nigra* genotypes, which originate from different regions and which strongly differ in terms of growth and leaf morphology (DeWoody et al., 2015; Wildhagen et al., 2018). Here, we analysed underlying ecophysiological traits as well as leaf level estimators of WUE. Our first aim was to investigate which traits explained differences in TE among genotypes under optimal watering conditions. Here we test the following hypotheses i) The differences in TE among genotypes are driven by transpiration rate rather than by biomass accumulation rate ii) The leaf level WUE is a main driver of whole plant TE iii) Unproductive water losses may decouple whole plant TE from leaf level WUE. A second aim was to determine if TE would be changed differently among genotypes in response to drought, and which underlying traits would drive this acclimation.

2. Material and methods

2.1. Plant material and growth conditions

Three genotypes of *Populus nigra* L., originating from individual trees of natural populations in France (Drôme 6; FR-6), Italy (La Zelata; IT1) and Spain (Ebro 2; SP-2) (DeWoody et al., 2015) and showing different leaf morphology were studied in controlled conditions (Fig. 1). Mean temperatures and precipitations of the three locations are provided in Supp Table 1. Growth, gas exchange and TE were measured on a subset of plants grown as part of the experiment described by Wildhagen et al. (2018), with six replicates per genotype x treatment. Briefly, woody cuttings were obtained from clonal propagation and were planted in 10 l plastic pots filled with a 1:1 (v/v) mixture of peat and sand, amended with a slow release fertiliser (4 g l^{-1} of Nutricote T100, 13:13:13 NPK and micronutrients;

FERTIL S.A.S, Boulogne Billancourt, France) and 1 g l^{-1} $CaMg(CO_3)_2$. Plants were grown in two compartments of a glasshouse located at Champenoux, France (48°45'09.3"N, 6°20'27.6"E), under natural light conditions with daily maxima of irradiance ranging from 150 to 1000 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR, Fig. 2). Environmental conditions in the greenhouse were affected by weather conditions, but the temperature was maintained between 15 and 26°C (Fig. 2). After planting, plants were watered 2–4 times a day –according to plant size and weather conditions– to 85% of field capacity with an automated weighing and watering system (Buré et al., 2016). The position of plants in the greenhouse was rotated at each weighing event.

2.2. Control of water deficit

After six weeks of growth, plants of each genotype were randomly assigned to either a control or a drought treatment for five weeks (day 0 was 21 May 2013). Control plants were watered to 85% soil relative extractable water content (REW_{soil}) by the automated system for the whole experiment. REW_{soil} of control plants oscillated between 65 and 85% (data not shown). For drought-treated plants, REW_{soil} was progressively decreased to reach 20% in two weeks and then maintained at this target level for the following three weeks (Fig. 2).

The control of the available soil water content (SWC) was based on a calibration between volumetric SWC measured by Time Domain Reflectometry (Trime Pico-32, IMKO) and pot weight. Target weights were defined individually for each pot, and were updated every day during the first two weeks to control the SWC decrease (Fig. 2) and were corrected for plant biomass increment using allometric relationships once a week. Each plant was thus submitted to the same stress level, irrespective of plant size and water consumption. Available water was expressed as soil relative extractable water content (REW_{soil}), which is defined as:

$$REW_{soil} = \left(\frac{SWC - SWC_{wiltingpoint}}{SWC_{fieldcapacity} - SWC_{wiltingpoint}} \right) \times 100\%,$$

with SWC at wilting point = 3%; SWC at field capacity = 32%.

2.3. Growth, gas exchange, transpiration rate and transpiration efficiency

Height and diameter

Plant height was measured from the soil surface to the shoot apex twice per week. The stem base was photographed with a ruler attached to the stem for scale calibration, twice per week. Stem diameter was measured from picture analysis with ImageJ (Schneider et al, 2012).

Total leaf area

For each genotype, a relationship between leaf area and maximal leaf width was built from a sample of approximately 80 leaves taken from the full range of leaf sizes. Regression coefficients were over 0.98 for each of the three genotypes. The width of all leaves of each plant was measured once a week and converted to area using the established relationships. Individual leaf areas were summed to calculate the total leaf area (LA) of each plant. Spline adjustment (interspline function, R) was used to estimate LA for dates in between days of measurement.

Dry biomass

At the end of the experiment, all plants were harvested. For each plant, the cutting, stem, roots and leaves were separated, dried at 70 °C for 48 h and weighed. Growth allocation was estimated through the calculation of root, leaf and stem biomass fractions (root biomass, leaf biomass and stem biomass over total biomass, respectively).

Gas exchange and intrinsic water use efficiency

Gas exchange was measured *in situ* in the greenhouse. Net CO_2 assimilation (A) and stomatal conductance to water vapour (g) were measured using two inter-calibrated portable photosynthesis systems LI-COR 6200 (LI-COR® Inc, Lincoln, NE, USA). Measurements were performed on the youngest fully expanded mature leaves at the beginning of the experiment, corresponding to the 8th–10th leaf down from the first apical leaf, between 11:00–12:00 twice a week over the five week-experiment. Intrinsic water use efficiency at the leaf level (Wi) was calculated as the ratio of A/g. A, g and Wi were averaged over the five last measurement days corresponding to the steady drought period during the three last weeks (A_{mean}, g_{mean} and Wi_{mean}, respectively) and these means were used for the ANOVA (Tables 1 and 2).

We also estimated the photosynthetic capacity by measuring gas exchange under light-saturated conditions, with calibrated Li-6400 XT portable gas analyzers (LI-COR® Inc, Lincoln, NE, USA) 4-6 days before the harvest. CO_2 concentration was 400 µmol mol^{-1}, light intensity (PAR) was 1500 µmol m^{-2} s^{-1} and block temperature was 25 ºC. All measurements were performed in the corridor next to the greenhouse compartments, on the same leaf used for *in situ* gas exchange. For each plant, the same procedure was followed. We waited for stomatal conductance to reach a steady state (typically after 20–30 min), then the A-C_i (C_i: CO_2 internal concentration) curve was performed by changing the [CO_2] entering the leaf chamber with the following steps: 400, 300, 250, 200, 150, 100, 50, 400, 400, 500, 600, 700, 800, 1000, 1200 and 1500 µmol mol^{-1}, typically with 2–3 min between each step. Maximum carboxylation rate (Vc_{max}), maximum electron transport rate (J_{max}) and mesophyll conductance (g_m) were estimated with the method by Ethier and Livingston (2004) that fits A-C_i curves with a non-rectangular hyperbola version of Farquhar's biochemical model of leaf photosynthesis (Farquhar et al., 1980). This is based on the hypothesis that g_m reduces the curvature of the Rubisco-limited portion of an A-C_i response curve. The Rubisco kinetic traits and specificity for CO_2/O_2 were characterized *in vitro* as described previously (Galmes et al., 2014). The values of the Rubisco Michaelis-Menten constants for CO_2 (K_c), and O_2 (K_o) and the chloroplast CO_2 compensation point (Γ^*) were obtained at 15, 25 and 35 °C and adjusted to the measured temperature using the Arrhenius function (see details on Rubisco kinetic traits and specificity for CO_2/O_2 in the supplementary material and methods).

Transpiration rates

Daily transpiration rate (TR) was calculated on a daily basis as the ratio between the water loss over 24 h and LA on that day, and then averaged over the whole experimental period. Days 17, 18, 26, 27, 28, 29 were used to calculate a mean diurnal transpiration rate (DTR) and a mean nocturnal transpiration rate (NTR), using the ratio between water loss during the 05:00–22:00 period and the

following 22:00–05:00 period, respectively, and LA. The 22:00–05:00 was chosen as a period of full darkness (astronomic sunset to sunrise). The proportion of unproductive water loss to productive water loss Φ_w (Farquhar et al., 1989) was estimated as Φ_w =NTR*9/(DTR*15) as the unproductive time (civil sunset to sunrise) was approximately 9 h during the experiment.

Transpiration efficiency

Transpiration efficiency (TE) was calculated as the ratio between the biomass gain (final total dry biomass – mean initial total dry biomass) and the cumulative water loss over the experiment period. For each genotype, the mean initial total dry biomass was estimated on a separate set of four plants harvested at day 0 (4.2 g, 6.2 g and 4.0 g for the French, Italian and Spanish genotypes, respectively)

$\delta^{13}C$ determination

The first leaf that had completely developed during the drought stress (mature at the harvest time) was harvested for carbon isotope analysis; dried for 48 h in an oven at 70 °C and ground into a fine powder. Subsamples of 1 mg ± 0.1 mg were weighed into tin capsules. The carbon isotopic composition was measured with a coupled isotope ratio mass spectrometer (Thermo-Finnigan; Delta S, Bremen, Germany). $\delta^{13}C$ was calculated according to the international standard (Vienna Pee Dee Belemnite, VPDB) using the following equation: $\delta^{13}C = (Rs – Rstd)/Rstd \times 1000$, where Rs and Rstd are the isotopic ratios $^{13}C/^{12}C$ of the sample and the standard, respectively. The precision of spectrometric analysis (standard deviation of $\delta^{13}C$) was assessed with a calibrated, internal laboratory reference material with a matrix close to the measured samples (oak leaves, n = 16, SD = 0.05 ‰).

2.4. Statistical analyses

All statistical analyses were performed with R (R Core Team, 2018). All data-sets were tested for outliers using the generalized ESD test (Extreme Studentized Deviate, Rosner and Bernard, 1983). Only outliers for which evidence for analytical errors were found were actually removed from the analyses.

A two-way ANOVA model with interaction was run for traits in Tables 1 and 2, using genotype and treatment as factors and type III sum of squares (Anova function of the car library). As a large number of variables were tested, the model significance was adjusted using False Discovery Rate (p-adjust function with the "fdr" option). Significant differences among factor levels were computed using Tukey's Highest Significant Difference test (HSD.test function of the agricolae package). Normality of the residuals was tested using Shapiro-Wilk test (shapiro.test function). Variables that showed a Shapiro-Wilk test with $p<0.05$ were transformed using the boxCox function (car package). Then the above described ANOVA was run again for all transformed variables and the significance levels were compared with those of untransformed variables. Only one result changed, the interaction for Wi_{mean} became significant (0.036 for transformed versus 0.060 for untransformed), therefore we presented the results of untransformed variables. The correlation analysis was conducted with the cor function using the Pearson method and the matrix was ordered according to the first principal component axis.

3. Results

3.1. Genotype differences

We tested the influence of genotype and drought on poplar traits by two-way ANOVA. We did not find significant genotype x drought interactions for 26 out of 28 variables tested (Table 1). Therefore, differences between genotypes are presented based on overall means. After 11 weeks of growth, the development of the three genotypes differed significantly. The Spanish genotype was much smaller in height, stem diameter and biomass than the other two genotypes (Table 1). This difference in height was the result of a smaller growth rate of the Spanish genotype (2.5 cm day^{-1}) compared to those of the French and Italian genotypes (3–3.5 cm day^{-1}) (Fig supp 1). The differences in stem diameter growth rates between genotypes were smaller than those of height growth rates (Fig supp 1). In addition, the Spanish genotype had many branches (more than the French whereas the Italian had none, data not shown) and many leaves, but it showed the smallest total leaf area (LA) due to much smaller leaves (Table 1, Fig 1, Fig supp 2). The relative allocation of growth to the roots was another important difference between genotypes: the root fraction (RootF) of the Spanish genotype was higher than that of the French genotype, which was higher than that of the Italian genotype (Table 1).

These differences in growth were accompanied by differences in ecophysiological traits. The Italian genotype had the lowest daily transpiration rate (TR, 1.70 kg m^{-2} day^{-1}), diurnal transpiration rate (DTR, 126 g m^{-2} h^{-1}) and nocturnal transpiration rate (NTR, 3.9 g m^{-2} h^{-1}) and also the lowest proportion of unproductive water loss to productive water loss (Φ_w, 1.9 %) (Fig. 3, Table 1). The Spanish genotype showed a very high TR (2.66 kg m^{-2} day^{-1}) and DTR (193 g m^{-2} h^{-1}), in accordance with a significantly higher stomatal conductance (g_{mean}, 0.88 mol m^{-2} s^{-1}), and a very high NTR (13.5 g m^{-2} h^{-1}) and Φ_w (4.2 %) (Fig. 3, Table 1). However, the Spanish genotype had a very small LA, resulting in a significantly lower cumulative water loss over the experiment (CumulT) than those of the two other genotypes (Table 1).

Traits related to gas exchange measured in optimal conditions (Vc_{max}, J_{max}, A_{sat}, g_{sat}, g_m, C_i, Wi_{sat}) or *in situ* (A_{mean}, g_{mean}, Wi_{mean}) were similar in the French and the Italian genotypes (Table 1). The Spanish genotype showed higher g_m, g_{sat}, g_{mean} and A_{mean} (and a tendency for higher A_{sat}) compared to the two other genotypes (Table 1).

The three genotypes differed significantly in whole plant transpiration efficiency (TE), which was corroborated by the integrated leaf level intrinsic WUE as estimated by $\delta^{13}C$ (Table 1). The Italian genotype had a higher TE and $\delta^{13}C$ than the French, which had a much higher TE and $\delta^{13}C$ than the Spanish. There were no significant differences among genotypes in instantaneous WUE (Wi_{sat} and Wi_{mean}), but the trait values showed a similar gradient as for TE across genotypes, confirming that the Spanish genotype had the lowest WUE.

3.2 Drought effect

The drought stress was applied for five weeks by reducing soil REW to 20%. Stress level was moderate so that drought-exposed trees still grew but at a reduced rate (Table 1, Table 2, Fig Supp 1). Drought significantly reduced the growth rate in height of the French genotype as early as day 8, while

this reduction in growth rate occurred later for the Spanish and the Italian genotypes (at day 11 and 15, respectively; Supp Fig 1). Stem diameter growth was also reduced but it seemed less sensitive than stem height growth in the French and Italian genotypes (-30 % for diameter growth rate versus -40% for height growth rate) and more sensitive for the Spanish genotype (-40% versus -30%) (Supp Fig 1). For all genotypes, the decrease of stem diameter growth became significant from day 15. Although only few significant genotype x environment interactions were detected in the ANOVA, post-hoc Tukey's HSD tests suggested some species-specific drought responses. The total dry mass tended to be less reduced under drought in the Italian genotype (-20%), compared to that of the Spanish and French (-34 and -38%) (Table 2). Growth allocation was also differentially affected by drought among genotypes. In particular, the Italian genotype maintained allocation to roots during drought so that its root dry mass was not affected and its RootF increased (Table 2). The leaf fraction of the French genotype was reduced but not its RootF, whereas allocation was not changed in the Spanish genotype (Table 2). Drought reduced LA in all three genotypes, but the effect was most pronounced in the French genotype (Table 1, Supp Fig 2). Drought also reduced the total leaf number of the Spanish and French genotypes (Supp Fig 2). In the Italian genotype, drought reduced LA but not the number of leaves, indicating that leaf growth rate was more sensitive than leaf production rate by the meristem (Supp Fig 2).

The moderate drought level applied here did not significantly affect the following leaf traits: photosynthetic capacity (Vc_{max}, J_{max}), mesophyll conductance to CO_2 (g_m) and net CO_2 assimilation rate (A_{sat} and A_{mean}). By contrast, stomatal conductance decreased under drought as compared to well-watered conditions (g_{sat} and g_{mean}) (Table 1). TR, DTR, NTR and Φ_w (p=0.054) were also strongly decreased. Consequently, the cumulative water loss (CumulT) was lowered under drought. The estimates of intrinsic water use efficiency (Wi_{sat}, Wi_{mean}, $\delta^{13}C$) indicated a significant increase of WUE at the leaf level by 35%. By contrast, TE did not respond to drought as the biomass accumulation (DMincr) and CumulT were similarly affected within each genotype (Table 1, Table 2). However, DMincr and CumulT were more reduced by drought in the French and the Spanish genotypes (approximately -40%) compared to the Italian genotype (-22%).

3.3 Correlations

A correlation analysis based on individual data highlighted, in both well-watered and drought conditions, that TE strongly correlated with $\delta^{13}C$ (R = 0.88 and 0.90 for control and drought, respectively, Fig. 4, Fig. 5A), but the relationship was weaker with Wi_{sat} (R = 0.26 and 0.62) and Wi_{mean} (R = 0.42 and 0.22) (Fig. 4). TE correlated more strongly with DMincr (R = 0.83 and 0.94) than with CumulT (R = 0.56 and 0.83) (Fig. 4). TE was also positively correlated with dry mass accumulation rate (DMinc/36 days) and was related negatively with the transpiration rates (daily, diurnal and nocturnal), and with g_{mean} to a lesser extent (Fig 4, 5B and 5C). Traits related to photosynthetic capacity and assimilation rate were weakly related to TE and DMincr under control conditions, but they were slightly negatively related under drought. It is also noticeable that transpiration rates (TR, DTR and NTR) were highly negatively correlated to traits related to LA and overall biomass accumulation, and positively correlated to the relative investment in roots (RootF) and to g_{mean} (Fig 4). Φ_w and NTR were positively correlated to TR and negatively to TE (Fig 4 and 5D).

4. Discussion

Transpiration efficiency differs strongly among genotypes

Transpiration efficiency (TE) is a long term whole plant measure of WUE, estimated as the ratio between biomass accumulation and water loss over time. TE variations can originate from different processes, daytime leaf processes such carbon assimilation rate and stomatal conductance but also from unproductive water losses such as nocturnal transpiration, and from carbon losses such as respiration of non-photosynthetic organs. In this study, we measured TE and traits related to TE in three contrasting *P. nigra* genotypes. We found a strong genotype effect for TE, where the Italian genotype showed the highest value (5.2 g kg^{-1}) and the Spanish genotype the lowest value (3.3 g kg^{-1}). This TE range was similar to that found in the same French and Italian genotypes in an earlier study (4.9 and 5.4 g kg^{-1}, respectively; Durand et al., 2019) and in other *P. nigra* genotypes grown under high vapour pressure deficit (3.1 to 5.9 g kg^{-1}) (Rasheed et al., 2015).

Differences in transpiration rate and in the proportion of unproductive water loss explain the genotypic differences in TE

The genotypic differences in TE were corroborated by the integrated measure of leaf level intrinsic WUE ($\delta^{13}C$), and by instantaneous measurements (Wi_{sat}, Wi_{mean}) although differences were not significant. Guet et al. (2015) tested genotypes from geographically close populations and also found higher WUE ($\delta^{13}C$) for Italian genotypes compared to French genotypes grown in a plantation with fertile soil and wet conditions. By contrast, Viger et al. (2016) compared *P nigra* genotypes coming from geographically close French, Italian and Spanish populations in a greenhouse experiment and did not find similar differences of carbon isotope discrimination among these populations.

The strong correlation between TE and $\delta^{13}C$, indicates that a significant part of the differences in TE among plants were driven by leaf level processes. Similarly strong correlations were found for P. *nigra* by Rasheed et al. (2015) and for *P. deltoides x nigra* crosses by Guo et al. (2011) and Rasheed et al. (2013). The weak difference in photosynthetic traits that we observed did not explain the genotypic differences in TE whereas the higher stomatal conductance of the Spanish genotype could clearly explain its low TE. Also all three transpiration rates, TR (day scale), DTR (diurnal) and NTR (nocturnal), correlated strongly and negatively with TE, indicating that the water efficient genotypes were transpiring less per leaf area, during the day as well as during the night. The French and Spanish genotypes with high NTR showed more negative values of predawn leaf water potential under control conditions (Supp Table 2), suggesting that the equilibration of the water potential between plant and soil was less complete for these genotypes than for the Italian one, which could be due to high NTR. The nocturnal transpiration represents an unproductive water loss (Farquhar et al., 1989) and therefore, in theory, impacts TE independently from leaf level WUE (Cernusak et al., 2007). Interestingly, the proportion of unproductive water loss to productive water loss (Φ_w) also correlated strongly and negatively with TE. Indeed the Italian genotype transpired proportionally less during the

night than the other tested genotypes, and showed the highest TE. In our *P. nigra* experiment, Φ_w ranged from 1.9% to 4.2%, which is a similar range to estimations for tropical tree species (1.2% to 5.2%; Cernusak et al., 2009) or for another plantation tree species such as *Eucalyptus grandis* (5%, Benyon, 1999). Higher Φ_w (9 to 30%) were found for other poplar species (Cirelli et al., 2016; Rohula et al., 2014), indicating that nocturnal transpiration was relatively low in *P. nigra*, and that a gain in TE due to reduced Φ_w would be small. However, it may be that introgressing *P. nigra* into other *Populus* species could reduce nocturnal water losses and increase TE. Differences in Φ_w were more closely related to differences in NTR than in DTR, suggesting that stomatal regulation during the night was partly independent from daytime regulation. Maintaining a significant nocturnal transpiration might enhance nutrient acquisition (Kupper et al., 2012), prevent a build-up of CO_2 within the leaves (Marks and Lechowicz, 2007), or facilitate a fast increase of net photosynthesis during early morning (Dawson et al., 2007).

Genotypic differences in TE are related to origin and biomass allocation

The three studied genotypes were chosen as representative of contrasting populations in terms of individual leaf size and of location in Europe: the Italian genotype had the largest leaves and the Spanish genotype the smallest leaves and a smaller LA than the two other genotypes. The observed strong correlation between TE and DMincr was mainly due to the smaller plant size and the lower TE of the Spanish genotype. The observed difference in plant size is in accordance with Viger (2011), who showed higher growth and larger leaves for *P. nigra* from central Europe with relatively wet climate (such as the Italian genotype) compared to trees from regions with hot and dry Mediterranean summers (such as the Spanish genotype). Also in hybrid poplars, individual leaf area was a good predictor of growth rate and productivity (Marron et al., 2007). Our data suggest that the genotypes with a high growth rate, a high individual leaf area and a high total LA showed a much lower per leaf area transpiration rate, reducing total water loss, and resulting in a higher TE.

The variation of TE, TR and growth rate among the genotypes appears coherent with the climatic gradient across their region of provenance. A high transpiration rate is expected to lead to a strong leaf cooling effect, which could be advantageous for plants growing in hot climates and having access to water. The constitutively higher investment into roots as compared to leaves by the Spanish genotype (higher RootF) is consistent with a higher TR and thus supports the hypothesis of a higher water flow requirement due to a hotter and drier climate. Overall, the differences in biomass allocation among the genotypes resulted in the observed strong negative correlation between TE and RootF. Similarly, it was shown for different provenances of *Castanea sativa,* that ecotypes from regions with low precipitation and higher mean temperature had lower intrinsic WUE (Lauteri et al., 1997) and a deeper rooting pattern (personal comm. M. Lauteri). Other *C. sativa* populations from drought prone sites also showed lower intrinsic WUE, and lower growth and total biomass (Lauteri et al., 2004; Pliura and Eriksson, 2002). Also a maritime pine ecotype originating from a dry and hot location in Morocco (Tamjoute) had a lower growth rate and a lower intrinsic WUE than two ecotypes from wet and cooler locations in France (Landes, Porto-Vecchio) (Guehl et al., 1995). Overall, the leaf cooling effect of transpiration and the high carbon allocation to the root system might be an adaptive strategy resulting

in lower TE for ecotypes from hot environments where deep water is available. Using neutral markers and phenotypic measurements, DeWoody et al. (2015) showed that isolation by distance played a major role in the differentiation among the western European *P. nigra* populations. In addition, they showed that adaptive differentiation also occurred for small-leaf populations from the Mediterranean area, supporting the idea that the Spanish specificities could result from local adaptation.

Drought increased intrinsic WUE but not TE

Globally, the three genotypes responded similarly to drought and we found only two significant genotype × drought interactions in the statistical model ($\delta^{13}C$ and NTR). As expected, biomass of all compartments decreased, or tended to decrease, under drought. Growth was clearly more sensitive than assimilation rate. Consequently, growth limitation was independent from carbon supply, as already found in other poplar species (Bogeat-Triboulot et al., 2007; Cohen et al., 2010). However, growth allocation showed some genotype specific patterns. The French genotype decreased mainly the leaf fraction, as found in a previous study (Durand et al., 2019). The Italian genotype, which showed the least reduction in biomass under drought, decreased mainly the stem fraction. These responses differ from those recorded in the same genotype in previous experiments (Durand et al., 2019; Viger et al., 2016), suggesting a strong plasticity of biomass allocation.

The drought-induced increase in leaf level WUE (Wi_{mean}, $\delta^{13}C$) was most likely due to a decrease in stomatal conductance, mainly observable in the *in situ* measurements, whereas neither photosynthetic capacity nor assimilation rates were significantly changed. Increased $\delta^{13}C$ and thus increased intrinsic WUE under drought due to stomatal closure is a classical response in plants showing luxurious water consumption in well watered conditions, as shown for different poplar species (Monclus et al., 2006; Viger et al., 2016). In addition, changes in intrinsic WUE seemed genotype dependent: genotype × drought interaction was significant for $\delta^{13}C$ and almost significant for Wi_{mean} (p_{int} = 0.060). The French genotype showed a significant and greater increase in leaf level WUE, linked to a relatively stronger reduction in g_{mean} than in the two other genotypes. These results suggest differences in stomatal regulation among these genotypes, as already found in a shorter drought experiment (Durand et al., 2019).

Surprisingly, TE was not increased under drought in any genotype, indicating that there were other factors apart from leaf level processes, which negated the effects of improved intrinsic WUE on TE. One such process could be the nocturnal water loss, which decouples leaf level from whole plant water use efficiency (Cernusak et al., 2007). However the observed reduction in NTR and Φ_w under drought should have had a positive effect on TE and did therefore not offset the increased leaf intrinsic WUE. Another factor decoupling whole plant from leaf level WUE could be carbon losses other than day respiration by leaves (Cernusak et al., 2007). An increased whole plant respiration under drought would decrease TE and therefore would offset the increase of leaf intrinsic WUE. Published effects of water deficit on respiration are not consistent, from inhibition to stimulation (Brito et al., 2018; Flexas et al., 2005) and here leaf respiration after 30 minutes in darkness was not affected by drought (P-value = 0.41; data not shown). However whole plant carbon losses might depend also on leaf fraction. The observed decrease in leaf fraction under drought, which was stronger in the French genotype, implies

an increase in the fraction of only respiring organs, which should in turn increase the whole plant respiration and contribute to offset the drought-induced increase of intrinsic WUE. This hypothesis is also congruent with the highest TE of the Italian genotype, which had the highest LA and should therefore have lower carbon losses by whole plant respiration relative to its size.

Conclusions

Strong differences in TE among the selected genotypes were congruent with differences in WUE at the leaf level. Our data suggest that a high total leaf area is offset by a low per leaf area transpiration rate, leading to higher TE in highly productive genotypes from cool locations. Nocturnal water loss contributes to variations in TE but are relatively low in *P. nigra*, reducing the possibility to improve TE in this species by selecting genotypes with low Φ_w. However, Φ_w has been shown to be much higher for other poplar species, and introgression of black poplar might provide a gain in nocturnal water losses. Our data also suggest that carbon losses due to whole plant respiration might contribute to the TE differences among genotypes and could offset the drought-induced increase in intrinsic WUE. Future studies should include measurements of respiratory carbon losses of different plant organs.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Author contributions

MBBT, OB, HW, CD, DLT, IH, HKS, JF, AP, and GT conceived the original research plans. MBBT, CB, CD, JF, HW, PAC, TG, DLT, OB, HKS, AM and JG performed the greenhouse experiment and the analytical measurements. MBBT, HW, HKS, CD, IH, DLT, MD, AM, JG and OB analysed the data; MBBT and OB wrote the article with contributions of all authors. All authors approved the final version of the manuscript.

Funding

This research received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under the grant agreement n°FP7-311929 (WATBIO), and UMR Silva was supported by the French National Research Agency through the Laboratory of Excellence ARBRE (ANR-12-LABXARBRE-01).

Acknowledgments :

We thank Alexi Marchal, Josselin Groux, Carole Antoine, Nathalie Aubry and Billy Valdes-Fragoso for their help with leaf surface area and growth measurements and Christian Hossann who performed the isotopic measurements at the Plateforme Technique d'Ecologie Fonctionnelle (PTEF) (OC 081, INRA Nancy, France). We acknowledge the providers of the original P. nigra genotypes 'France 6J-29' (INRA, Paris, France represented by G. Pilate) and 'Spain RIN2-new' (CITA,Zaragosa, Spain,

represented by JV Lacasa Azlor) and C. Bastien (INRA, Orleans, France) for providing the stock cuttings.

References

Benyon, R.G., 1999. Nighttime water use in an irrigated *Eucalyptus grandis* plantation. Tree Physiol. 19, 853-859.

Bogeat-Triboulot, M.B., Brosche, M., Renaut, J., Jouve, L., Le Thiec, D., Fayyaz, P., Vinocur, B., Witters, E., Laukens, K., Teichmann, T., Altman, A., Hausman, J.F., Polle, A., Kangasjarvi, J., Dreyer, E., 2007. Gradual soil water depletion results in reversible changes of gene expression, protein profiles, ecophysiology, and growth performance in *Populus euphratica*, a poplar growing in arid regions. Plant Physiol. 143, 876-892.

Brito, C., Dinis, L.-T., Ferreira, H., Moutinho-Pereira, J., Correia, C., 2018. The role of nighttime water balance on *Olea europaea* plants subjected to contrasting water regimes. J. Plant Physiol 226, 56-63.

Buré, C., Bénard, A., Bogeat-Triboulot, M.A., Brendel, O., Gross, P., Hummel, I., Le Thiec, D., Radnai, F., 2016. Un automate d'irrigation contrôle la sécheresse et quantifie la transpiration chez de jeunes arbres. Le cahier des techniques de l'INRA https://www6.inra.fr/cahier_des_techniques/Les-Cahiers-parus/Les-N-Speciaux/Mesure-et-Metrologie/chap2-ns-J2M-2016/Art02-ns-J2M-2016.

Bussotti, F., Pollastrini, M., Holland, V., Brueggemann, W., 2015. Functional traits and adaptive capacity of European forests to climate change. Environ. Exp. Bot. 111, 91-113.

Cernusak, L.A., Winter, K., Aranda, J., Turner, B.L., Marshall, J.D., 2007. Transpiration efficiency of a tropical pioneer tree (*Ficus insipida*) in relation to soil fertility. J. Exp. Bot 58, 3549-3566.

Cernusak, L.A., Winter, K., Turner, B.L., 2009. Physiological and isotopic (delta 13C and delta 18O) responses of three tropical tree species to water and nutrient availability. Plant, Cell and Environment 32, 1441-1455.

Chamaillard, S., Fichot, R., Vincent-Barbaroux, C., Bastien, C., Depierreux, C., Dreyer, E., Villar, M., Brignolas, F., 2011. Variations in bulk leaf carbon isotope discrimination, growth and related leaf traits among three *Populus nigra* L. populations. Tree Physiol. 31, 1076-1087.

Cirelli, D., Equiza, M.A., Lieffers, V.J., Tyree, M.T., 2016. Populus species from diverse habitats maintain high night-time conductance under drought. Tree Physiol. 36, 229-242.

Cirelli, D., Lieffers, V.J., Tyree, M.T., 2012. Measuring whole-plant transpiration gravimetrically: a scalable automated system built from components. Trees - Struct. Funct. 26, 1669-1676.

Cohen, D., Bogeat-Triboulot, M.B., Tisserant, E., Balzergue, S., Martin-Magniette, M.L., Lelandais, G., Ningre, N., Renou, J.P., Tamby, J.P., Le Thiec, D., Hummel, I., 2010. Comparative transcriptomics of drought responses in *Populus*: a meta-analysis of genome-wide expression profiling in mature leaves and root apices across two genotypes. BMC Genomics 11, 630.

Dawson, T.E., Burgess, S.S.O., Tu, K.P., Oliveira, R.S., Santiago, L.S., Fisher, J.B., Simonin, K.A., Ambrose, A.R., 2007. Nighttime transpiration in woody plants from contrasting ecosystems. Tree Physiol. 27, Ecol Soc Amer-575.

DeWoody, J., Trewin, H., Taylor, G., 2015. Genetic and morphological differentiation in *Populus nigra* L.: isolation by colonization or isolation by adaptation? Mol. Ecol. 24, 2641-2655.

Domec, J.C., King, J.S., Ward, E., Oishi, A.C., Palmroth, S., Radecki, A., Bell, D.M., Miao, G.F., Gavazzi, M., Johnson, D.M., McNulty, S.G., Sun, G., Noormets, A., 2015. Conversion of natural forests to managed forest plantations decreases tree resistance to prolonged droughts. For. Ecol. Manage. 355, 58-71.

519 Durand, M., Brendel, O., Buré, C., Le Thiec, D., 2019. Altered stomatal dynamics induced by changes
520 in irradiance and vapour-pressure deficit under drought: impacts on the whole plant transpiration
521 efficiency of poplar genotypes. New Phytol.
522 Ethier, G.J., Livingston, N.J., 2004. On the need to incorporate sensitivity to CO2 transfer conductance
523 into the Farquhar-von Caemmerer-Berry leaf photosynthesis model. Plant, Cell and Environment
524 27, 137-153.
525 Farquhar, G.D., Caemmerer, S.V., Berry, J.A., 1980. A biochemical model of photosynthesis CO2
526 fixation in leaves of C3 species. Planta 149, 78-90.
527 Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and
528 photosynthesis. Annual Review of Plant Physiology and Molecular Biology 40, 503-537.
529 Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the Relationship between Carbon Isotope
530 Discrimination and the Intercellular CO2-concentration in Leaves. Australian Journal of Plant
531 Physiology 9, 121-137.
532 Fichot, R., Brignolas, F., Cochard, H., Ceulemans, R., 2015. Vulnerability to drought-induced cavitation
533 in poplars: synthesis and future opportunities. Plant Cell Environ. 38, 1233-1251.
534 Flexas, J., Galmes, J., Ribas-Carbo, M., Medrano, H., 2005. The Effects of Water Stress on Plant
535 Respiration., in: H., L., M., R.-C. (Eds.), Advances in Photosynthesis and Respiration. Springer,
536 Dordrecht, pp 85-94.
537 Galmes, J., Kapralov, M.V., Andralojc, P.J., Conesa, M.A., Keys, A.J., Parry, M.A.J., Flexas, J., 2014.
538 Expanding knowledge of the Rubisco kinetics variability in plant species: environmental and
539 evolutionary trends. Plant Cell Environ. 37, 1989-2001.
540 Granier, C., Aguirrezabal, L., Chenu, K., Cookson, S.J., Dauzat, M., Hamard, P., Thioux, J.J., Rolland, G.,
541 Bouchier-Combaud, S., Lebaudy, A., Muller, B., Simonneau, T., Tardieu, F., 2006. PHENOPSIS, an
542 automated platform for reproducible phenotyping of plant responses to soil water deficit in
543 *Arabidopsis thaliana* permitted the identification of an accession with low sensitivity to soil water
544 deficit. New Phytol. 169, 623-635.
545 Guehl, J.-M., Nguyen-Queyrens, A., Loustau, D., Ferhi, A., 1995. Genetic and environmental
546 determinants of water-use efficiency and carbon isotope discrimination in forest trees, in:
547 Sandermann, H., Bonnet-Masimbert, M. (Eds.), Eurosilva: contribution to forest tree physiology.
548 Results from Eurosilva projects, presented at Dourdan, France, 7-10 November 1994. Editions
549 Colloques de l'INRA, Paris, pp. 297-321.
550 Guet, J., Fichot, R., Ledee, C., Laurans, F., Cochard, H., Delzon, S., Bastien, C., Brignolas, F., 2015. Stem
551 xylem resistance to cavitation is related to xylem structure but not to growth and water-use
552 efficiency at the within-population level in *Populus nigra* L. J. Exp. Bot 66, 4643-4652.
553 Guo, P., HaiTao, X., Xing, H., Weilun, Y., 2011. Discrimination of water use efficiency (WUE) among
554 three Populus deltoids clones. Journal of Beijing Forestry University 33, 19-24.
555 Hanson, P.J., Weltzin, J.F., 2000. Drought disturbance from climate change: response of United States
556 forests. Sci. Total Environ. 262, 205-220.
557 Kruse, J., Hopmans, P., Rennenberg, H., Adams, M., 2012. Modern tools to tackle traditional
558 concerns: Evaluation of site productivity and *Pinus radiata* management via δ13C- and δ18O-
559 analysis of tree-rings. For. Ecol. Manage. 285, 227-238.
560 Kupper, P., Rohula, G., Saksing, L., Sellin, A., Lõhmus, K., Ostonen, I., Helmisaari, H.S., Sõber, A., 2012.
561 Does soil nutrient availability influence night-time water flux of aspen saplings? Environ. Exp. Bot.
562 82, 37-42.
563 Lauteri, M., Pliura, A., Monteverdi, M.C., Brugnoli, E., Villani, F., Eriksson, G., 2004. Genetic variation
564 in carbon isotope discrimination in six European populations of *Castanea sativa* Mill. originating
565 from contrasting localities. Journal of Evolutionary Biology 17, 1286-1296.
566 Lauteri, M., Scartazza, A., Guido, M.C., Brugnoli, E., 1997. Genetic variation in photosynthetic
567 capacity, carbon isotope discrimination and mesophyll conductance in provenances of *Castanea*
568 *sativa* adapted to different environments. Functional Ecology 11, 675-683.

Marguerit, E., Bouffier, L., Chancerel, E., Costa, P., Lagane, F., Guehl, J.-M., Plomion, C., Brendel, O., 2014. The genetics of water-use efficiency and its relation to growth in maritime pine. J. Exp. Bot 65, 4757-4768.

Marks, C.O., Lechowicz, M.J., 2007. The ecological and functional correlates of nocturnal transpiration. Tree Physiol. 27, 577-584.

Marron, N., Dillen, S.Y., Ceulemans, R., 2007. Evaluation of leaf traits for indirect selection of high yielding poplar hybrids. Environ. Exp. Bot. 61, 103-116.

Monclus, R., Dreyer, E., Delmotte, F.M., Villar, M., Delay, D., Boudouresque, E., Petit, J.M., Marron, N., Brechet, C., Brignolas, F., 2005. Productivity, leaf traits and carbon isotope discrimination in 29 *Populus deltoides x P-nigra* clones. New Phytol. 167, 53-62.

Monclus, R., Dreyer, E., Villar, M., Delmotte, F.M., Delay, D., Petit, J.M., Barbaroux, C., Le Thiec, D., Brechet, C., Brignolas, F., 2006. Impact of drought on productivity and water use efficiency in 29 genotypes of *Populus deltoides x Populus nigra*. New Phytol. 169, 765-777.

Navarro, A., Portillo-Estrada, M., Arriga, N., Vanbeveren, S.P.P., Ceulemans, R., 2018. Genotypic variation in transpiration of coppiced poplar during the third rotation of a short-rotation bio-energy culture. GCB Bioenergy 10, 592-607.

Pliura, A., Eriksson, G., 2002. Genetic variation in juvenile height and biomass of open-pollinated families of six *Castanea sativa* Mill. Populations in a 2 × 2 factorial temperature x watering experiment. Silvae Genetica 51, 152-160.

R Core Team, 2018. A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rasheed, F., Dreyer, E., Richard, B., Brignolas, F., Brendel, O., Le Thiec, D., 2015. Vapour pressure deficit during growth has little impact on genotypic differences of transpiration efficiency at leaf and whole-plant level: an example from *Populus nigra* L. Plant Cell Environ. 38, 670-684.

Rasheed, F., Dreyer, E., Richard, B., Brignolas, F., Montpied, P., Le Thiec, D., 2013. Genotype differences in C-13 discrimination between atmosphere and leaf matter match differences in transpiration efficiency at leaf and whole-plant levels in hybrid *Populus deltoides x nigra*. Plant Cell Environ. 36, 87-102.

Rohula, G., Kupper, P., Raeim, O., Sellin, A., Sober, A., 2014. Patterns of night-time water use are interrelated with leaf nitrogen concentration in shoots of 16 deciduous woody species. Environ. Exp. Bot. 99, 180-188.

Rosner, Bernard, 1983. Percentage Points for a Generalized ESD Many-Outlier Procedure. Technometrics 25, 165-172.

Sannigrahi, P., Ragauskas, A.J., Tuskan, G.A., 2010. Poplar as a feedstock for biofuels: A review of compositional characteristics. Biofuels Bioprod. Biorefining 4, 209-226.

Sow, M.D., Segura, V., Chamaillard, S., Jorge, V., Delaunay, A., Lafon-Placette, C., Fichot, R., Faivre-Rampant, P., Villar, M., Brignolas, F., Maury, S., 2018. Narrow-sense heritability and P-ST estimates of DNA methylation in three *Populus nigra* L. populations under contrasting water availability. Tree Genet. Genomes 14.

Viger, M., 2011. Physiology, genetics and genomics of drought adaptation in *Populus*, School of Biological Sciences. University of Southampton, p. 235.

Viger, M., Rodriguez-Acosta, M., Rae, A.M., Morison, J.I.L., Taylor, G., 2013. Toward improved drought tolerance in bioenergy crops: QTL for carbon isotope composition and stomatal conductance in *Populus*. Food Energy Secur. 2, 220-236.

Viger, M., Smith, H.K., Cohen, D., Dewoody, J., Trewin, H., Steenackers, M., Bastien, C., Taylor, G., 2016. Adaptive mechanisms and genomic plasticity for drought tolerance identified in European black poplar (*Populus nigra* L.). Tree Physiol. 36, 909-928.

Wildhagen, H., Paul, S., Allwright, M., Smith, H.K., Malinowska, M., Schnabel, S.K., Paulo, M.J., Cattonaro, F., Vendramin, V., Scalabrin, S., Janz, D., Douthe, C., Brendel, O., Bure, C., Cohen, D., Hummel, I., Le Thiec, D., van Eeuwijk, F., Keurentjes, J.J.B., Flexas, J., Morgante, M., Robson, P., Bogeat-Triboulot, M.B., Taylor, G., Polle, A., 2018. Genes and gene clusters related to genotype

and drought-induced variation in saccharification potential, lignin content and wood anatomical traits in *Populus nigra*. Tree Physiol. 38, 320-339.
Zhang, X.L., Zang, R.G., Li, C.Y., 2004. Population differences in physiological and morphological adaptations of *Populus davidiana* seedlings in response to progressive drought stress. Plant Sci. 166, 791-797.

Figure legends:

Figure 1: A) general view of the plants in the greenhouse on day seven. B, C and D) Pictures of typical plants of the Italian, French and Spanish genotypes, respectively, on day 21. Mean individual leaf area was calculated as the ratio between total leaf area and leaf number on day 28 (mean ± s.e., n=6).

Figure 2: A. Minimum and maximum temperature in the greenhouse (dotted black and plain black lines, respectively), mean PAR radiation over 8:00 to 19:00 (red line) and mean soil relative extractable water in the drought-subjected plants (blue line) over the 5 week-experiment.

Figure 3: Daily transpiration rate of the three genotypes under well-watered conditions (black circle) and under drought (white circle) over the 5 week-experiment. Mean PAR radiation over 8:00 to 19:00 (red dotted line). Mean ± s.e, n=6.

Figure 4: Correlations between traits in control plants (upper part) and in drought-subjected plants (lower parts) (n=18 for each subplot). Only significant correlations were displayed (P-value<0.05). A_{mean}: mean of net CO_2 assimilation rate measured *in situ*, A_{sat}: net CO_2 assimilation rate measured under light-saturated conditions, C_i: CO_2 internal concentration, CumulT: cumulated water loss, DMincr: total dry mass increment, DTR: diurnal transpiration rate, $\delta^{13}C$: carbon isotope composition, FinalH: final stem height, FinalD: final stem diameter, g_m: mesophyll conductance for CO_2, g_{mean}: stomatal conductance to water vapour measured *in situ*, g_{sat}: stomatal conductance to water vapour measured under light-saturated conditions, J_{max}: maximum photosynthetic electron flux, LA: total leaf area, LeafF: leaf fraction, Φ_w: proportion of unproductive water loss to productive water loss, NTR: nocturnal transpiration rate, RootF: root fraction, TE: whole plant transpiration efficiency, TotalDM: total dry mass, TR: daily transpiration rate, Vc_{max}: maximum CO_2 carboxylation rate, Wi_{sat}: leaf intrinsic water use efficiency measured under light-saturated conditions, Wi_{mean}: mean leaf intrinsic water use efficiency measured *in situ*.

Figure 5: Correlation between whole plant transpiration efficiency (TE) and A) carbon isotope composition ($\delta^{13}C$), B) daily transpiration rate (TR), C) biomass increment (DMincr) and D) proportion of unproductive water loss to productive water loss (Φ_w). Each point corresponds to a plant. The blue, green and red symbols denote the French, the Italian and the Spanish genotypes, respectively. Closed and open symbols denotes control and drought treatments, respectively. Internal whiskers represent s.e., external whiskers represent 95% confidence interval.

Table1: **Results of Two-way ANOVA of different traits.** Significance and adjusted correlation coefficient of the model, significance of the factors (genotype and drought) and of the interaction. Marginal mean ± s.e. are given for the three genotypes and for the treatments. Different letters denote significant differences between groups according to Tukey post-hoc tests.

A_{mean}: mean of net CO_2 assimilation rate measured *in situ*, A_{sat}: net CO_2 assimilation rate measured under light-saturated conditions, C_i: CO_2 internal concentration, CumulT: cumulated water loss, DMincr: total dry mass increment, DTR: diurnal transpiration rate, $\delta^{13}C$: carbon isotope composition, FinalH: final stem height, FinalD: final stem diameter, g_m: mesophyll conductance for CO_2, g_{mean}: stomatal conductance to water vapour measured *in situ*, g_{sat}: stomatal conductance to water vapour measured under light-saturated conditions, J_{max}: maximum photosynthetic electron flux, LA: total leaf area, LeafF: leaf fraction, Φ_w: proportion of unproductive water loss to productive water loss, NTR: nocturnal transpiration rate, RootF: root fraction, TE: whole plant transpiration efficiency, TotalDM: total dry mass, TR: daily transpiration rate, Vc_{max}: maximum CO_2 carboxylation rate, Wi_{sat}: leaf intrinsic water use efficiency measured under light-saturated conditions, Wi_{mean}: mean leaf intrinsic water use efficiency measured *in situ*.

Table 2: Complement of Table 1. Mean ± s.e. of different traits within each genotype x treatment group (n=4 - 6). Different letters denote significant difference between groups according to Tukey post-hoc tests. Acronyms are identical to those in Table 1.

Supplementary material

Supplementary material and methods: Rubisco kinetic traits and specificity for CO_2/O_2 characterisation

Supplementary Table 1: Climatic data at the locations of the three populations

Supplementary Table 2: Predawn leaf water potential (MPa) of the three poplar genotypes

Supplementary Figure 1: Growth rate in height and in stem diameter of the three genotypes over the 5-week experiment.

699 **Supplementary Figure 2:** Leaf number and total leaf surface area of the three genotypes over the 5-
700 week experiment.
701 **Supplementary Figure 3:** Net CO_2 assimilation rate, stomatal conductance and intrinsic water use
702 efficiency over the 5-week experiment.
703

Table 1

		model		Factors													
		model	R2	Genot.	Drought	G x D	French			Italian			Spanish			Control			Drought		
TE	g kg^{-1}	***	0.85	***	n.s.	n.s.	**4.6** ± 0.1	b		**5.2** ± 0.1	a		**3.3** ± 0.1	c		**4.4** ± 0.2	a		**4.4** ± 0.2	a	
$\delta^{13}C$	‰	***	0.85	***	*	*	**-31.2** ± 0.2	b		**-29.6** ± 0.2	a		**-32.8** ± 0.1	c		**-31.5** ± 0.3	b		**-30.9** ± 0.4	a	
Wi_{sat}	µmol mol^{-1}	*	0.24	n.s.	**	n.s.	**66.3** ± 5.9	a		**75.8** ± 7.8	a		**56.0** ± 6.7	a		**55.8** ± 5.0	b		**76.3** ± 5.3	a	
Wi_{mean}	µmol mol^{-1}	n.s.	0.15	n.s.	**	n.s.	**32.2** ± 4.4	a		**33.2** ± 2.4	a		**28.7** ± 2.2	a		**26.7** ± 1.5	b		**36.0** ± 3.0	a	
CumulT	kg	***	0.61	***	***	n.s.	**12.9** ± 1.3	a		**12.2** ± 0.7	a		**8.5** ± 1.0	b		**13.5** ± 0.8	a		**8.9** ± 0.6	b	
TR	kg m^{-2} day^{-1}	***	0.91	***	***	n.s.	**1.96** ± 0.07	b		**1.70** ± 0.03	c		**2.66** ± 0.07	a		**2.25** ± 0.11	a		**1.92** ± 0.09	b	
DTR	g m^{-2} h^{-1}	***	0.87	***	***	n.s.	**148** ± 7	b		**126** ± 5	c		**193** ± 8	a		**175** ± 8	a		**136** ± 7	b	
NTR	g m^{-2} h^{-1}	***	0.88	***	***	**	**8.9** ± 0.7	b		**3.9** ± 0.2	c		**13.4** ± 1.0	a		**10.4** ± 1.2	a		**7.0** ± 0.8	b	
Φ_w	%	***	0.75	***	n.s.	n.s.	**3.6** ± 0.2	a		**1.9** ± 0.1	b		**4.2** ± 0.2	a		**3.4** ± 0.3	a		**3.0** ± 0.2	a	
FinalH	m	***	0.75	***	***	n.s.	**1.46** ± 0.06	a		**1.24** ± 0.04	b		**1.03** ± 0.03	c		**1.36** ± 0.06	a		**1.14** ± 0.04	b	
FinalD	mm	***	0.72	***	***	n.s.	**11.4** ± 0.3	a		**10.4** ± 0.3	b		**8.4** ± 0.6	c		**11.0** ± 0.4	a		**9.2** ± 0.4	b	
TotalDM	g	***	0.77	***	***	n.s.	**73.5** ± 6.0	a		**79.2** ± 4.1	a		**34.9** ± 4.2	b		**73.1** ± 6.0	a		**53.0** ± 5.2	b	
DMincr	g	***	0.77	***	***	n.s.	**69.3** ± 5.9	a		**73.0** ± 3.9	a		**30.8** ± 4.2	b		**68.2** ± 5.8	a		**48.1** ± 5.1	b	
LA	m²	***	0.63	***	***	n.s.	**0.32** ± 0.03	a		**0.31** ± 0.02	a		**0.20** ± 0.03	b		**0.34** ± 0.02	a		**0.22** ± 0.02	b	
LeafF	g g^{-1}	***	0.67	***	**	n.s.	**0.347** ± 0.009	c		**0.422** ± 0.006	a		**0.393** ± 0.006	b		**0.398** ± 0.007	a		**0.376** ± 0.011	b	
RootF	g g^{-1}	***	0.68	***	*	n.s.	**0.143** ± 0.004	b		**0.129** ± 0.004	c		**0.175** ± 0.004	a		**0.144** ± 0.006	a		**0.152** ± 0.005	a	
Vc_{max}	µmol m^{-2} s^{-1}	n.s.	-0.06	n.s.	n.s.	n.s.	**170** ± 11	a		**154** ± 12	a		**171** ± 11	a		**166** ± 10	a		**164** ± 9	a	
J_{max}	µmol m^{-2} s^{-1}	n.s.	0.04	n.s.	n.s.	n.s.	**171** ± 8	a		**176** ± 5	a		**184** ± 9	a		**170** ± 6	a		**183** ± 5	a	
g_m	mol m^{-2} s^{-1}	***	0.46	***	n.s.	n.s.	**0.42** ± 0.06	b		**0.37** ± 0.07	b		**0.90** ± 0.11	a		**0.49** ± 0.07	a		**0.58** ± 0.10	a	
A_{sat}	µmol m^{-2} s^{-1}	n.s.	0.08	n.s.	n.s.	n.s.	**21.1** ± 0.6	a		**19.5** ± 1.6	a		**24.3** ± 1.8	a		**21.7** ± 1.1	a		**21.6** ± 1.4	a	
g_{sat}	mol m^{-2} s^{-1}	*	0.27	*	*	n.s.	**0.35** ± 0.03	b		**0.31** ± 0.05	b		**0.49** ± 0.06	a		**0.44** ± 0.04	a		**0.33** ± 0.04	b	
C_i	µmol mol^{-1}	*	0.23	n.s.	**	n.s.	**272** ± 9	a		**259** ± 12	a		**287** ± 11	a		**288** ± 8	a		**256** ± 8	b	
A_{mean}	µmol m^{-2} s^{-1}	*	0.21	**	n.s.	n.s.	**18.2** ± 1.4	b		**16.1** ± 0.9	b		**24.4** ± 2.5	a		**20.1** ± 1.7	a		**19.1** ± 1.5	a	
g_{mean}	mol m^{-2} s^{-1}	***	0.47	***	**	n.s.	**0.68** ± 0.06	b		**0.53** ± 0.03	b		**0.88** ± 0.07	a		**0.79** ± 0.05	a		**0.60** ± 0.05	b	

Table 2 :

		French control	French drought	Italian control	Italian drought	Spanish control	Spanish drought
TE	g kg^{-1}	**4.61 ±** 0.07 b	**4.64 ±** 0.20 ab	**5.25 ±** 0.13 a	**5.23 ±** 0.11 a	**3.47 ±** 0.14 c	**3.07 ±** 0.17 c
$\delta^{13}C$	‰	**-31.84 ±** 0.07 c	**-30.54 ±** 0.19 b	**-29.74 ±** 0.34 ab	**-29.37 ±** 0.29 a	**-32.78 ±** 0.22 cd	**-32.89 ±** 0.22 d
Wi_{sat}	µmol mol^{-1}	**54.6 ±** 7.5 b	**77.9 ±** 6.4 ab	**59.8 ±** 7.9 ab	**91.7 ±** 9.1 a	**53.6 ±** 11.2 b	**58.9 ±** 7.1 ab
Wi_{mean}	µmol mol^{-1}	**21.9 ±** 1.4 b	**42.4 ±** 6.6 a	**31.6 ±** 2.8 ab	**34.9 ±** 4.1 ab	**26.5 ±** 1.7 ab	**30.8 ±** 4.1 ab
CumulT	kg	**16.4 ±** 1.0 a	**9.4 ±** 1.1 c	**13.8 ±** 0.9 ab	**10.6 ±** 0.6 bc	**10.3 ±** 1.3 bc	**6.3 ±** 0.6 c
TR	kg m^{-2} day^{-1}	**2.13 ±** 0.06 c	**1.80 ±** 0.08 d	**1.80 ±** 0.02 d	**1.60 ±** 0.03 d	**2.82 ±** 0.07 a	**2.46 ±** 0.05 b
DTR	g m^{-2} h^{-1}	**166.0 ±** 6.0 b	**129.5 ±** 7.2 cd	**141.5 ±** 2.2 c	**109.7 ±** 4.5 d	**216.7 ±** 5.8 a	**168.5 ±** 4.7 b
NTR	g m^{-2} h^{-1}	**10.9 ±** 0.3 b	**6.9 ±** 0.5 c	**4.4 ±** 0.3 cd	**3.4 ±** 0.2 d	**16.0 ±** 1.1 a	**10.8 ±** 0.9 b
Φ_w	%	**4.0 ±** 0.2 ab	**3.3 ±** 0.3 b	**1.9 ±** 0.1 c	**1.9 ±** 0.1 c	**4.5 ±** 0.3 a	**3.9 ±** 0.3 ab
FinalH	m	**1.63 ±** 0.04 a	**1.29 ±** 0.07 bc	**1.35 ±** 0.05 b	**1.13 ±** 0.03 cd	**1.09 ±** 0.03 cd	**0.97 ±** 0.05 d
FinalD	mm	**12.2 ±** 0.2 a	**10.6 ±** 0.3 ab	**11.1 ±** 0.4 ab	**9.7 ±** 0.3 b	**9.6 ±** 0.7 b	**7.1 ±** 0.3 c
TotalDM	g	**88.6 ±** 4.9 a	**58.3 ±** 6.3 bc	**88.1 ±** 5.5 a	**70.4 ±** 3.4 ab	**42.5 ±** 5.7 cd	**25.7 ±** 3.0 d
DMincr	g	**84.4 ±** 4.7 a	**54.2 ±** 6.2 bc	**81.7 ±** 5.1 a	**64.3 ±** 3.1 ab	**38.6 ±** 5.6 cd	**21.5 ±** 2.8 d
LA	m²	**0.41 ±** 0.03 a	**0.23 ±** 0.03 cd	**0.36 ±** 0.02 ab	**0.27 ±** 0.01 bc	**0.25 ±** 0.04 cd	**0.14 ±** 0.02 d
LeafF	g g^{-1}	**0.369 ±** 0.008 b	**0.325 ±** 0.012 c	**0.426 ±** 0.010 a	**0.417 ±** 0.007 a	**0.397 ±** 0.007 ab	**0.388 ±** 0.011 ab
RootF	g g^{-1}	**0.140 ±** 0.006 bc	**0.146 ±** 0.007 b	**0.117 ±** 0.002 c	**0.141 ±** 0.004 b	**0.174 ±** 0.006 a	**0.176 ±** 0.005 a
Vc_{max}	µmol m^{-2} s^{-1}	**164 ±** 22 a	**176 ±** 9 a	**171 ±** 17 a	**137 ±** 16 a	**164 ±** 17 a	**179 ±** 16 a
J_{max}	µmol m^{-2} s^{-1}	**163 ±** 13 a	**179 ±** 8 a	**173 ±** 8 a	**179 ±** 8 a	**175 ±** 12 a	**195 ±** 12 a
g_m	mol m^{-2} s^{-1}	**0.36 ±** 0.08 b	**0.48 ±** 0.08 b	**0.46 ±** 0.11 b	**0.26 ±** 0.06 b	**0.74 ±** 0.17 ab	**1.07 ±** 0.08 a
A_{sat}	µmol m^{-2} s^{-1}	**20.7 ±** 1.0 ab	**21.5 ±** 0.8 ab	**22.0 ±** 1.0 ab	**17.1 ±** 2.8 b	**22.6 ±** 2.8 ab	**26.2 ±** 1.8 a
g_{sat}	mol m^{-2} s^{-1}	**0.41 ±** 0.04 ab	**0.29 ±** 0.03 ab	**0.41 ±** 0.05 ab	**0.21 ±** 0.05 b	**0.49 ±** 0.09 a	**0.50 ±** 0.07 a
C_i	µmol mol^{-1}	**291 ±** 12 a	**253 ±** 10 a	**282 ±** 13 a	**235 ±** 13 a	**291 ±** 18 a	**282 ±** 11 a
A_{mean}	µmol m^{-2} s^{-1}	**17.3 ±** 1.1 ab	**19.2 ±** 2.7 ab	**17.7 ±** 0.6 ab	**14.5 ±** 1.5 b	**25.2 ±** 4.6 a	**23.6 ±** 2.4 ab
g_{mean}	mol m^{-2} s^{-1}	**0.83 ±** 0.05 ab	**0.54 ±** 0.05 bc	**0.61 ±** 0.02 bc	**0.45 ±** 0.04 c	**0.92 ±** 0.11 a	**0.83 ±** 0.10 ab

Figure 1

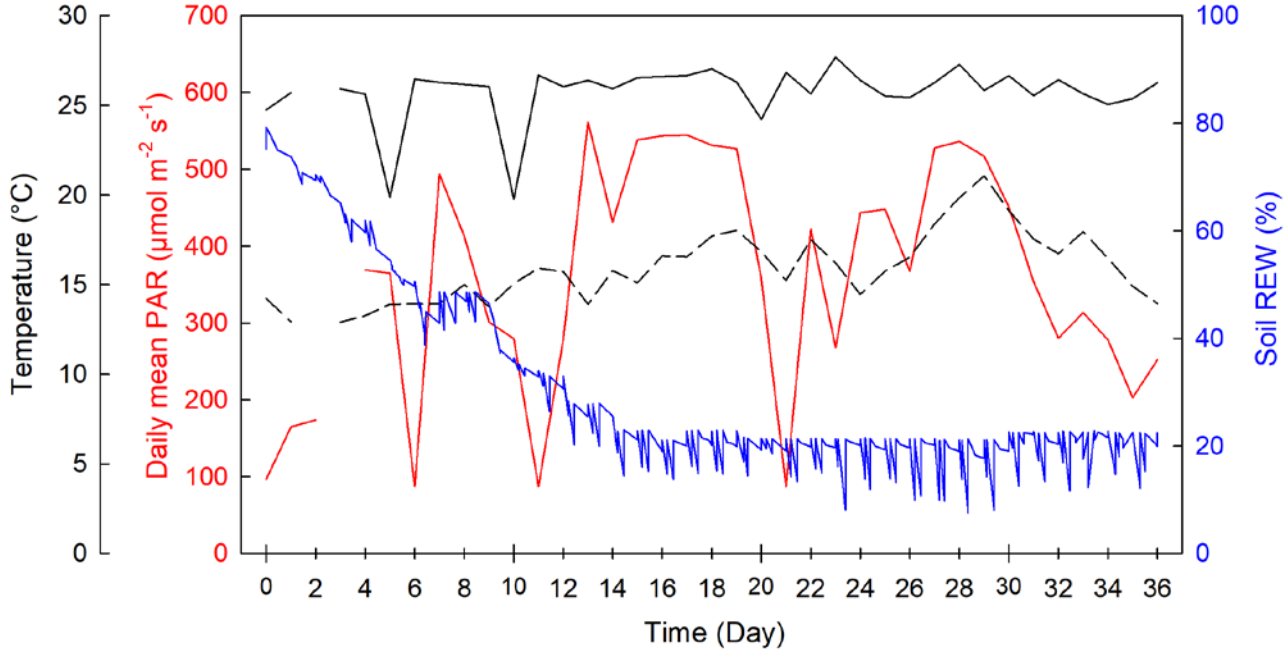


Figure 2

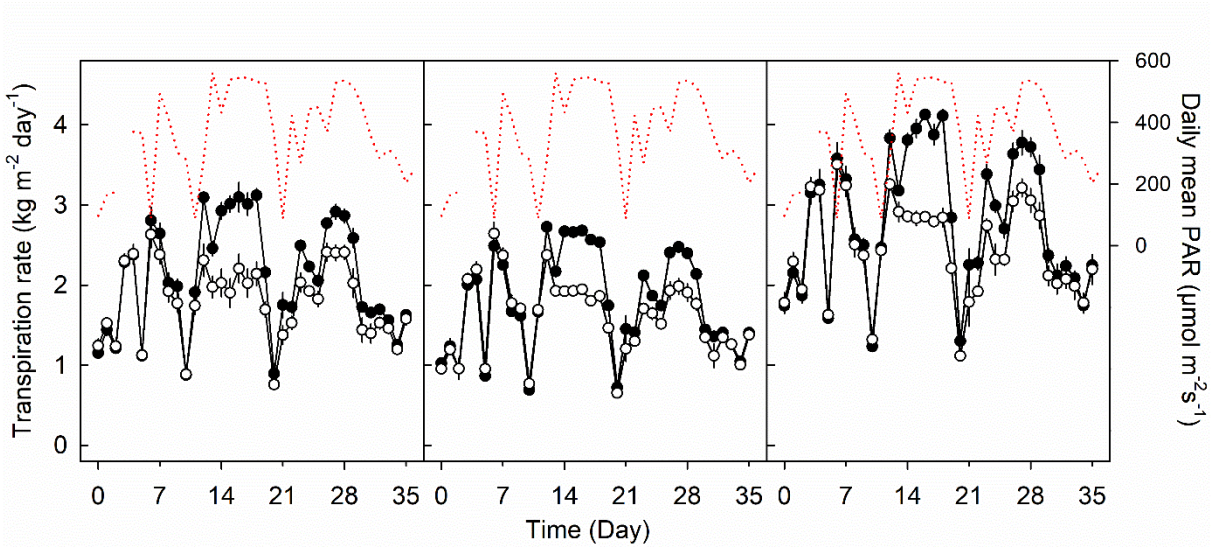


Figure 3

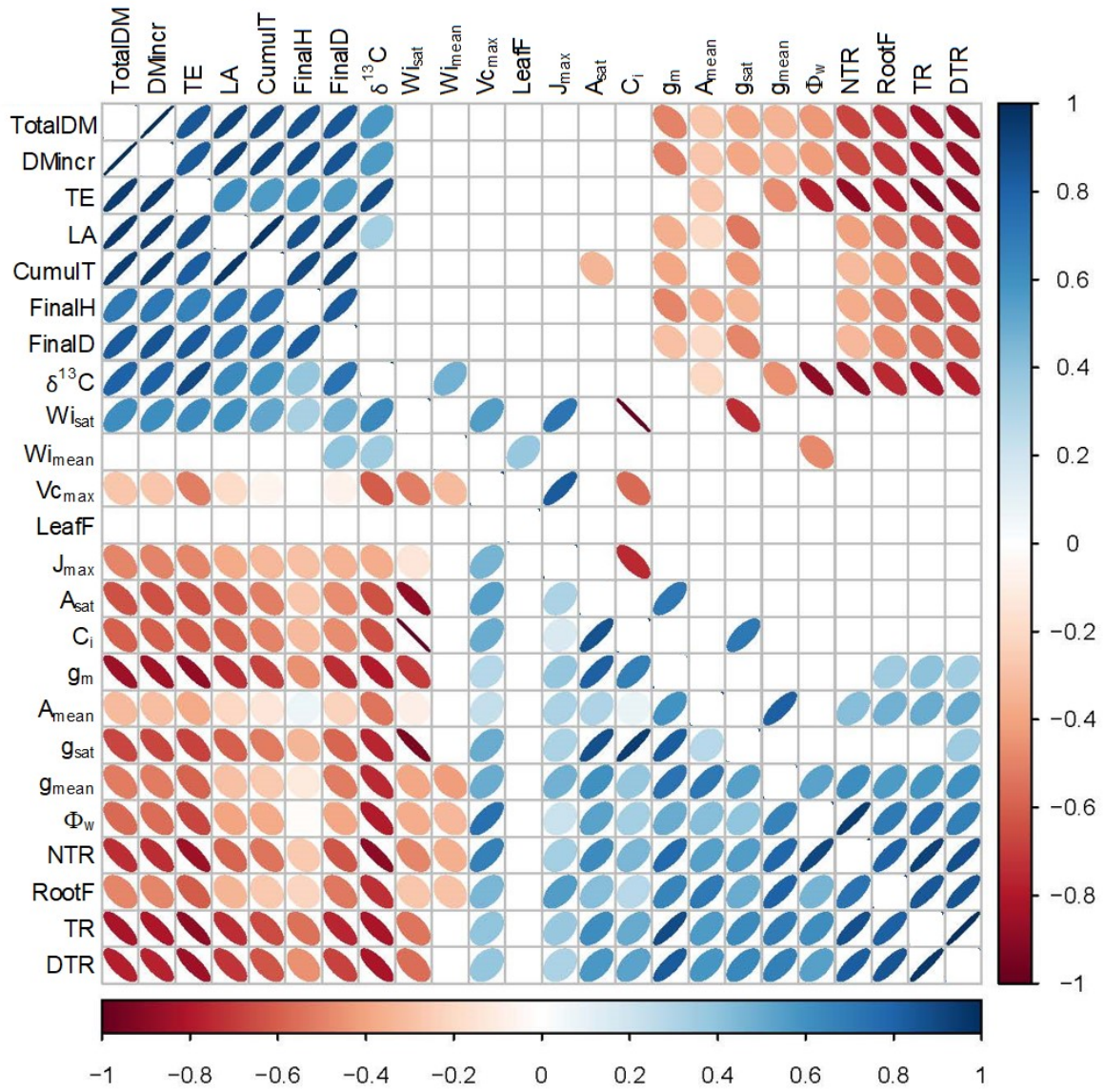


Figure 4

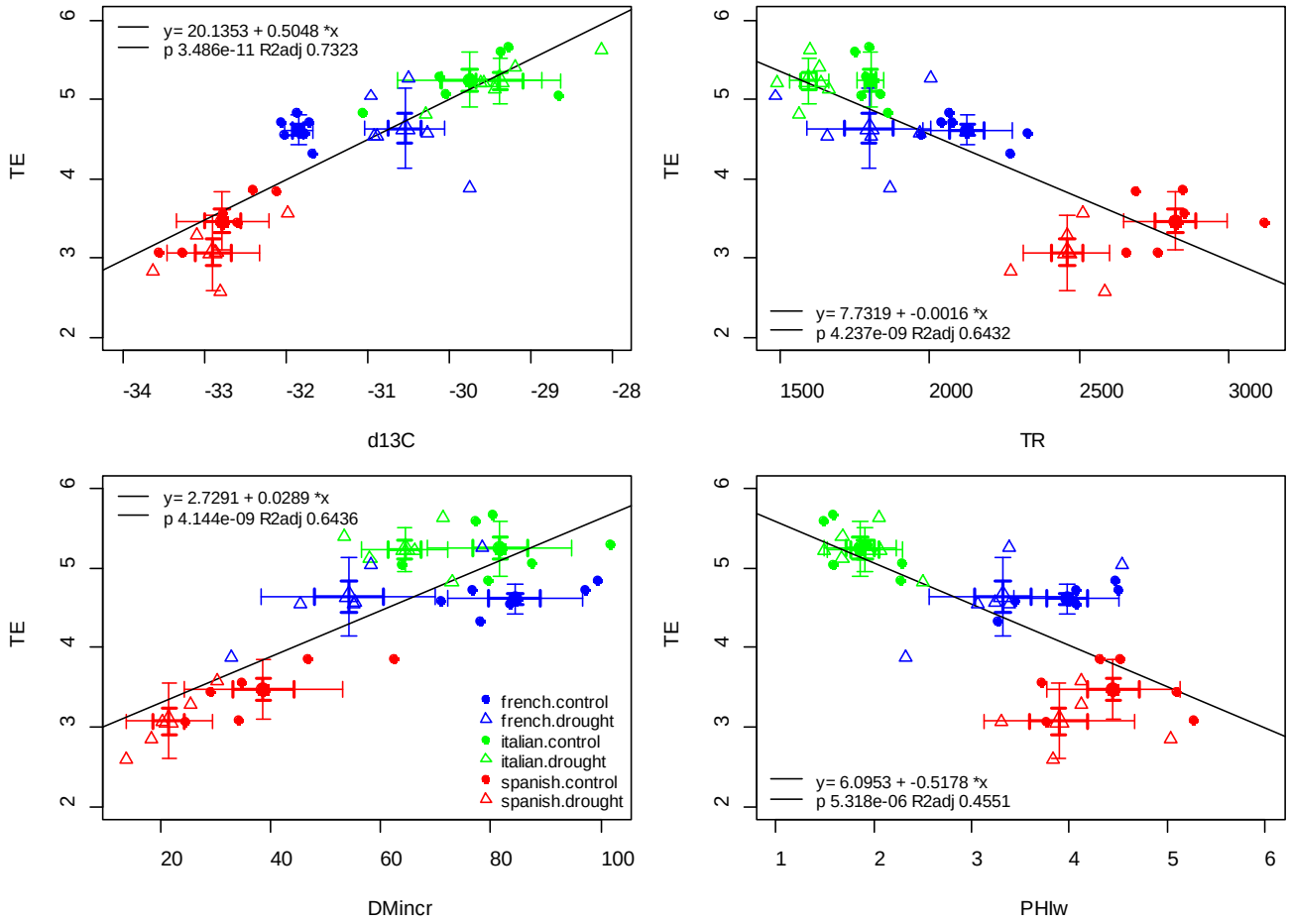


Figure 5

Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes

Bogeat-Triboulot MB et al, 2019

https://doi.org/10.1016/j.envexpbot.2019.05.021

Supplementary data

Supplementary Material and Methods: Rubisco characterisation

1. Sequence of the Rubisco large subunit gene (rbcL)

Total genomic DNA was isolated from leaf sample of the three genotypes independently and purified using the DNeasy™ Plant Minikit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The primers used for amplification and sequencing are listed in Table 1.

Primer	Sequence	Reference	Amplification	Sequencing
esp 2_F	ATGAGTTGTAGGGAGGGAC	Hermida *et al*. 2016	x	
414_R	CAAATCCTCCAGACGTAGAGC	Chen *et al.* 1998		x
991_R	CGGTACCAGCGTGAATATGAT	Chen *et al.* 1998		x
1494_R	GATTGGGCCGAGTTTAATTAC	Chen *et al.* 1998	x	x

Table 1: List of the primers used for amplification and sequencing.

PCR reactions were performed in 50 µl using EmeraldAmp GT PCR Master Mix (Takara, Shiga, Japan). PCR program for amplifications comprised initial cycle at 94ºC for 2 min, 55ºC for 30 s, 72ºC for 4 min, followed by 30 cycles of 94ºC for 30 s 56ºC for 45 s and 72ºC for 1 min, and a final elongation at 72ºC for 5 min. Amplifications were carried out on a 96-well SensoQuest labcycler (Progen Scientific Ltd., South Yorkshire, UK). The PCR products were separated on 2% agarose gels and purified using Illustra GFX PCR DNA and Gel band Purification kit (GE Healthcare Life Science, Buckinghamshire, England). The amplified PCR products were cloned using the TOPO TA cloning kit (Invitrogen, USA) following manufacturer's instructions, and sequenced with an ABI 3100 Genetic analyzer using the ABI BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, California). Sequence chromatograms were checked and manually corrected,

and the contigs were assembled and aligned using MEGA 5.0 (Tamura *et al.*, 2011). The sequences were submitted to Genebank with the accession number: Populus_nigra_Drome_6.sqn Populus_nigra_Drome_6 MK757467.

At the genomic level, a few DNA mutations were found in *rbc*L of the three genotypes but all of them were synonymous and provided an identical amino acid sequence. Therefore, the functional characterisation of Rubisco catalytic traits was performed only in the French genotype.

2. Rubisco kinetics and specificity for CO_2/O_2 characterization

Fresh leaf tissue of the French genotype was sampled in full sunlight, immediately frozen in liquid nitrogen and 0.4-0.5 g was ground in a mortar with 2 ml of ice-cold extraction buffer containing 100 mM Bicine (pH 8.2), 6% (w/v) PEG 4000, 2 mM $MgCl_2$, 0.1 mM EDTA, 1 mM benzamidine, 1 mM aminocaproic acid, 50 mM 2-mercaptoethanol, 10 mM DTT, 2 μM pepstain A, 10 μM E64, 10 μM chymostatin, 2 mM PMSF and 2.5% (w/v) PVPP. The homogenate was clarified by centrifugation at 13000 × g during 4 min at 4 ºC. Then, 1 mL of the supernatant was applied to a Sephadex PD-10 column (GE Healthcare, UK) pre-equilibrated with desalt buffer, containing 100 mM Bicine (pH 8.2), 20 mM $MgCl_2$, 10 mM DTT, 1 mM KH_2PO_4, 0.5 mM EDTA, 1 mM benzamidine, 1 mM aminocaproic acid and 10 mM $NaHCO_3$. The protein peak (in 1 ml) was supplemented with protease inhibitors (4 μM pepstain A, 20 μM E64 and 20 μM chymostatin) and 250 μL of this mixture were supplemented with sufficient carrier-free $NaH^{14}CO_3$ to adjust the specific radioactivity to 3.7 x 10^{10} Bq mol^{-1}.The remaining extract volume was frozen immediately in liquid nitrogen to measure the Rubisco active site concentration.

Rates of Rubisco $^{14}CO_2$-fixation using the activated protein extract were measured at 15, 25 and 35°C, each at two concentrations of O_2 (0 and 21% v/v). In all the cases, nine different concentrations of $^{14}CO_2$ were used (0 to 93 μM, each with a specific radioactivity of 3.7 × 10^{10} Bq mol^{-1}), as described previously (Galmés *et al*., 2014). Measurements were performed in 7 ml septum capped scintillation vials, containing reaction buffer (yielding final concentrations of 110 mM Bicine-NaOH pH 8.0, 22 mM $MgCl_2$, 0.4 mM RuBP and about 100 W-A units of carbonic anhydrase), and equilibrated either with nitrogen (N_2) or a mixture of O_2 and N_2 (21:79). Assays (1.0 ml total volume) were started by the prompt addition of 10 μL of activated leaf extract, and quenched after 1 min by the addition of 0.2 ml of 10 M formic acid. Acid-stable ^{14}C was determined by liquid scintillation counting, following removal of acid-labile ^{14}C by evaporation. The Michaelis-Menten constant for CO_2 (K_c) was determined from the fitted data as described by Bird *et al*. (1982). Replicate measurements (n = 4-5) were made using independent protein preparations from different individuals. For each sample, the maximum rate of carboxylation (K_{cat}^c) was extrapolated from the corresponding V_{max} value after allowance was made for the Rubisco active site concentration, as determined by [^{14}C]CPBP binding (Yokota & Canvin, 1985).

The Rubisco specificity for CO_2/O_2 ($S_{c/o}$) was also measured at 15, 25 and 35°C (n = 7-8) using purified leaf extracts obtained as in Galmés *et al.* (2006) and the oxygen electrode method described by Parry, Keys & Gutteridge (1989), using a DW1 oxygen electrode (Hansatech, Kings Lynn., UK). Reaction mixtures contained (final concentrations) 100 mM Bicine-NaOH (pH 8.2), 10 mM $MgCl_2$, 0.15

mg mL^{-1} carbonic anhydrase, 2 mM $NaH^{14}CO_3$ (18.5 kBq mol^{-1}), 20 µL activated Rubisco from purified extracts and 2.5 µM RuBP. The basic buffer was pre-equilibrated with CO_2-free air at the temperature of measurement. RuBP oxygenation was calculated from the oxygen consumption and carboxylation from the amount of ^{14}C incorporated into PGA when all the RuBP had been consumed.

The Rubisco kinetics at three different temperature, with the Michaelis-Menten constant (K_M) for CO_2 (K_c), the Michaelis-Menten constant for CO_2 measured under 21% O_2 conditions (K_c^{air}), the maximum rate of carboxylation (K_{cat}^c), the specificity for CO_2/O_2 ($S_{c/o}$) and the CO_2 compensation point in absence of dark respiration (Γ^*) are presented in Table 2.

Temperature	**15°C**		**25°C**		**35°C**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
K_c (µM)	4.96	0.59	9.38	0.60	15.46	0.61
K_c^{air} (µM)	6.64	0.45	13.60	0.76	24.64	1.85
k_{cat}^c (s^{-1})	1.35	0.13	2.17	0.06	3.69	0.18
k_{cat}^c /K_c(μM^{-1} s^{-1})	0.30	0.04	0.23	0.02	0.23	0.02
$S_{c/o}$(mol mol^{-1})	122.10	1.86	81.24	2.86	68.08	2.15
K_c (µmol mol^{-1} air)	110	13.04	275.75	17.60	590.25	23.18
K_c^{air} (µmol mol^{-1} air)	147.80	9.77	400	22.36	941.25	70.79
K_o (µM)	414.4	92.1	615.7	60.5	301.3	17.5
Γ^{**} (µmol mol^{-1})	30.76	0.48	50.52	1.92	64.97	2.09

Table 2: Rubisco kinetic parameters measured at three different temperature, with the Michaelis-Menten constant for CO_2 (K_c) and O_2 (K_o), the Michaelis-Menten constant for CO_2 measured under 21% O_2 conditions (K_c^{air}), the maximum rate of carboxylation (k_{cat}^c), the specificity for CO_2/O_2 ($S_{c/o}$) and the chloroplast CO_2 compensation point (Γ^*).

The parameters of the Arrhenius function (C, the scaling factor and ΔHa, the activation energy), which describe the temperature dependence, were calculated for each Rubisco kinetic parameter (Table 3).

Parameter	**C**		**ΔHa (KJ mol^{-1})**	
	Mean	**SE**	**Mean**	**SE**
K_c (µM)	18.45	0.80	40.25	2.06
K_c^{air} (µM)	21.97	1.56	48.09	3.82
k_{cat}^c (s^{-1})	18.54	1.70	44.14	4.33
$S_{c/o}$(mol mol^{-1})	-4.67	0.76	-22.64	1.85
K_c (µmol mol^{-1} air)	29.45	0.96	59.10	2.47
K_c^{air} (µmol mol ^{1} air)	33.11	1.94	53.95	9.58
Γ^* (µmol mol^{-1})	13.77	0.48	24.60	1.63

Table 3: Parameters of the Arrhenius function for each Rubisco kinetic parameter

3. References:

*Bird I, Cornelius M, Keys A. (1982) Affinity of RuBP Carboxylases for Carbon Dioxide and Inhibition of the Enzymes by Oxygen. Jounal of Experimental Botany **33**, 1004–1013.*

Chen ZD, Wang XQ, Sun HY, Han Y, Zhang ZX, Zou YP, Lu AM. (1998) Systematic position of the Rhoipteleaceae: Evidence from nucleotide sequences of rbcL gene. Acta Phytotaxonomica Sinica 36, 1-7.

Galmés J, Medrano H, Flexas J (2006) Acclimation of Rubisco specificity factor to drought in tobacco: discrepancies between in vitro and in vivo estimations. Journal of Experimental Botany 57, 3659–67.

Galmés J, Kapralov MV, Andralojc PJ, Conesa MÀ, Keys AJ, Parry MAJ, Flexas J. (2014) Expanding knowledge of the Rubisco kinetics variability in plant species: environmental and evolutionary trends. Plant, Cell and Environment doi: 10.1111/pce.12335.

Galmés, J, Hermida-Carrera, C, Laanisto, L, Niinemets, Ü (2016). A compendium of temperature responses of Rubisco kinetic traits: variability among and within photosynthetic groups and impacts on photosynthesis modeling. Journal of Experimental Botany, 67(17), 5067-5091.

Parry MAJ, Keys AJ, Gutteridge S. (1989) Variation in the specificity factor of C_3 higher plant Rubisco determined by the total consumption of ribulose-P_2. Journal of Experimental Botany 40, 317–320.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution, 28(10), 2731-2739.

Yokota A, Canvin D T (1985) Ribulose bisphosphate carboxylase/oxygenase content determined with [^{14}C] carboxypentitol bisphosphate in plants and algae. Plant Physiology, 77(3), 735-739.

Supplementary Table 1:

Climatic data at the location of the three populations from which the genotypes come from (from Dewoody et al, 2015).

Country	Population	Latitude	Longitude	Average annual temperature (°C)	Maximum temperature of warmest month (°C)	Minimum temperature of coolest month (°C)	Average annual precipitation (mm)	Precipitation of wettest month (mm)	Precipitation of driest month (mm)
France	Drôme 6	44.75	4.92	12.4	28.1	0.0	840	95	41
Italy	La Zelata	45.26	8.98	13.0	29.0	-1.0	982	122	55
Spain	Ebro 2	41.58	-1.00	13.7	29.5	1.3	365	53	17

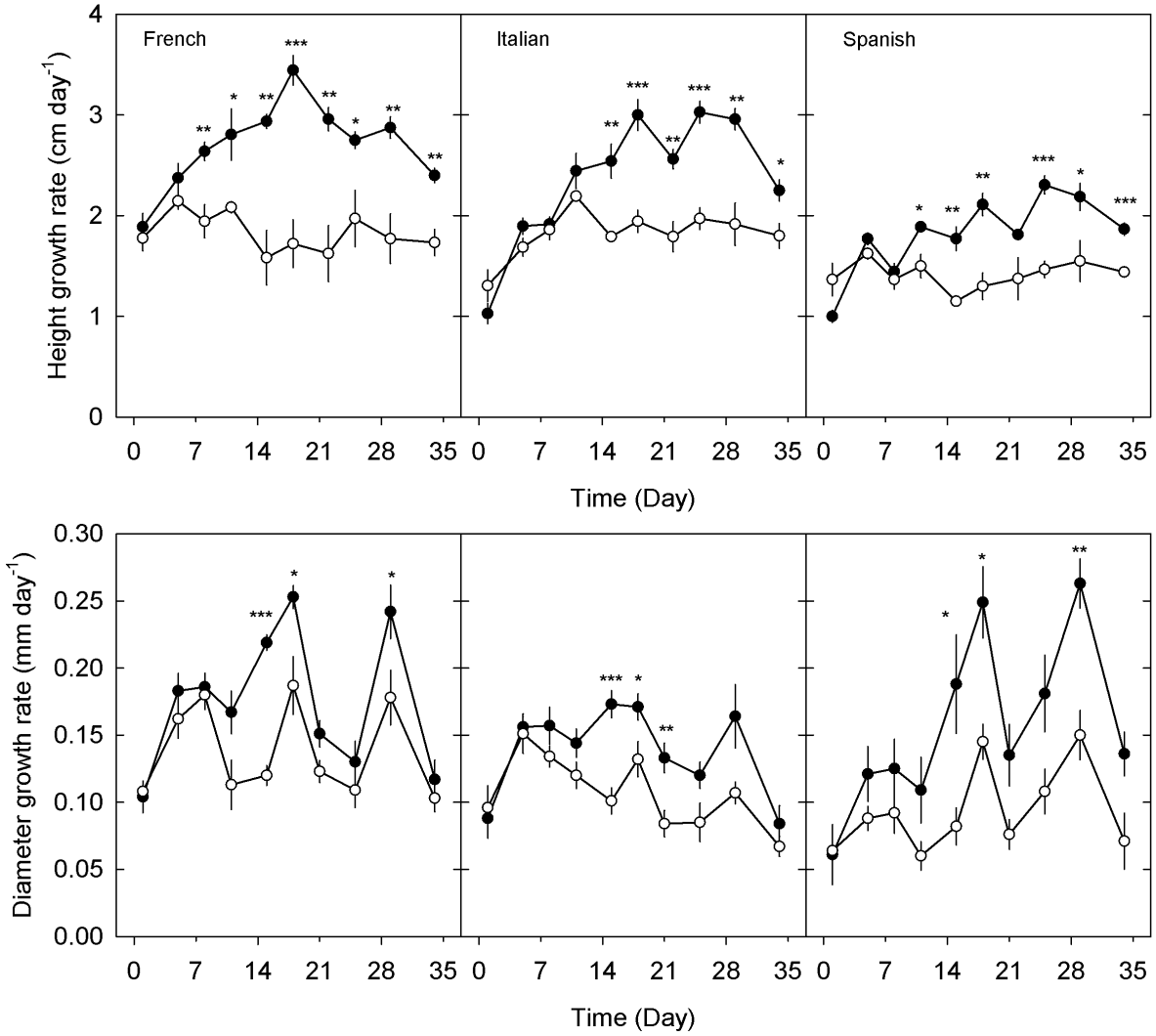
DeWoody, J., Trewin, H., Taylor, G., 2015. Genetic and morphological differentiation in Populus nigra L.: isolation by colonization or isolation by adaptation? Mol. Ecol. 24, 2641-2655.

Supplementary Table 2:

Predawn leaf water potential (MPa) of the three poplar genotypes measured on day 14 with a Scholander chamber in another batch of plants of the same experiment (Wildhagen *et al*, 2018). Mean ± s.e., n=4 – 6.

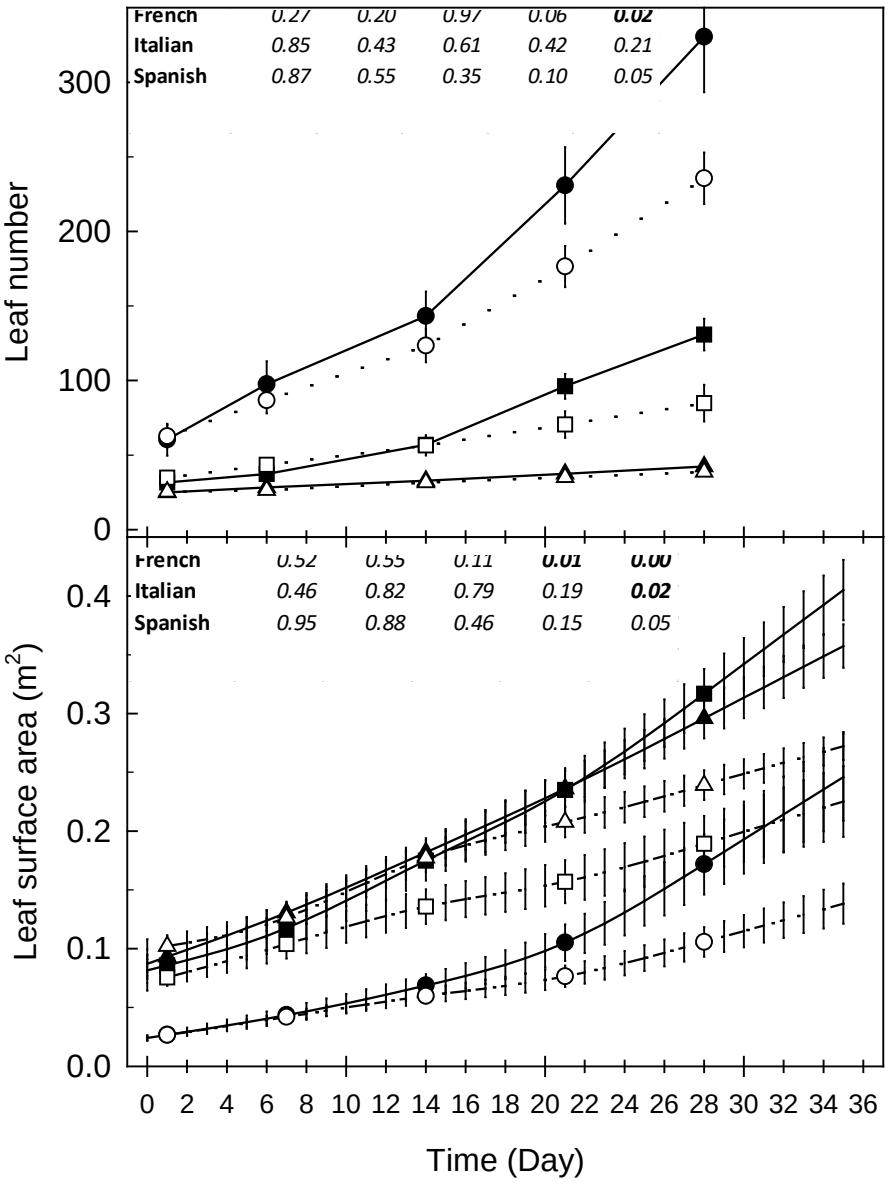
	French	Italian	Spanish
control	-0.23 ± 0.01	-0.08 ± 0.02	-0.22 ± 0.04
drought	-0.29 ± 0.01	-0.26 ± 0.02	-0.20 ± 0.04

Wildhagen, H, Paul, S, Allwright, M, Smith, HK, Malinowska, M, Schnabel, SK, Paulo, MJ, Cattonaro, F, Vendramin, V, Scalabrin, S, et al. 2018 Genes and gene clusters related to genotype and drought-induced variation in saccharification potential, lignin content and wood anatomical traits in Populus nigra. Tree Physiol. ***38****, 320-339. DOI : 10.1093/treephys/tpx054*



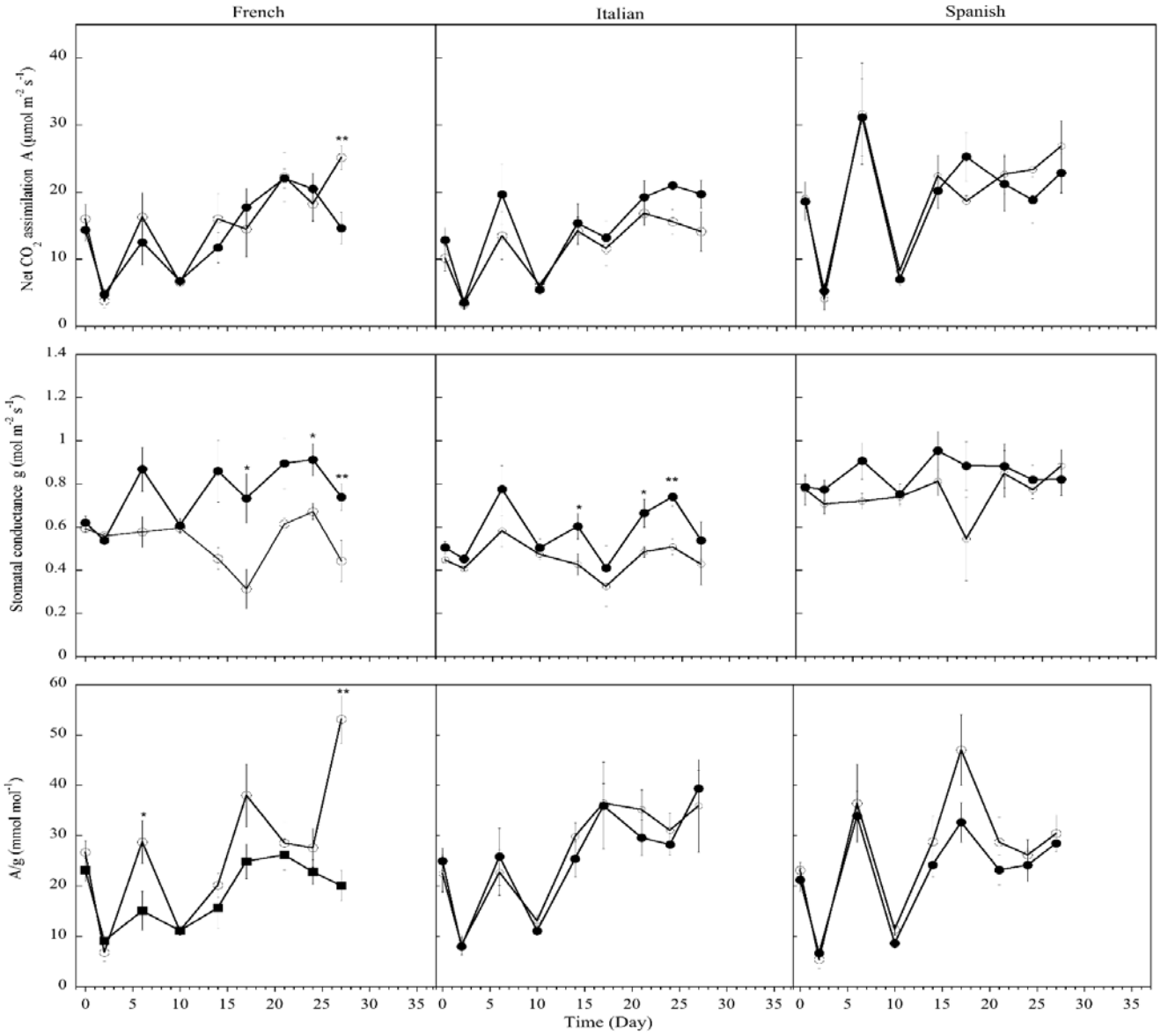
Supplementary Figure 1 :

Growth rate in height and in stem diameter of the three genotypes over the 5-week experiment. Mean ± s.e. (n=6). Closed and open symbols denote control and drought treatments, respectively. Symbols ***, **, * denote significance level (<0.001, 0.01 and 0.05, respectively).



Supplementary Figure 2:

Leaf number and total leaf area of the three genotypes over the 5-week experiment. Mean ± s.e. (n=6). Closed and open symbols denotes control and drought treatments, respectively. Squares, triangles and circles denote the French, the Italian and the Spanish genotypes, respectively. Closed and open symbols denote control and drought treatments, respectively. Total leaf area was measured at five time points (symbols) and estimated at the other dates from the adjustment of an interspline function (R). P-values of t-test between control and drought treatment within each genotype are given.



Supplementary Figure 3

Net CO_2 assimilation rate, stomatal conductance and intrinsic water use efficiency over the 5-week experiment. Closed and open symbols denote control and drought treatments, respectively. Symbols ***, **, * denote significance level (>0.001, 0.01 and 0.05, respectively).