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

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Key words :

Water use efficiency
Transpiration efficiency
Nocturnal transpiration
Water deficit
Poplar
Intraspecific diversity

Abbreviations :

A: net CO₂ assimilation rate,
A_{mean}: mean of net CO₂ assimilation rate measured *in situ*,
A_{sat}: net CO₂ assimilation rate measured under light-saturated conditions,
C_i: CO₂ internal concentration,
Cumult: cumulated water loss,
DMincr: total dry mass increment,
DTR: diurnal transpiration rate,
δ¹³C: carbon isotope composition,
FinalH: final stem height,
FinalD: final stem diameter,
g: stomatal conductance to water vapour,
g_m: mesophyll conductance for CO₂,
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,
V_{Cmax}: maximum CO₂ carboxylation rate,
WUE: water use efficiency,
W_i: leaf intrinsic water use efficiency
W_{isat}: leaf intrinsic water use efficiency measured under light-saturated conditions,
W_{imean}: mean leaf intrinsic water use efficiency measured *in situ*.

1 **Abstract**

2
3 Poplar plantations, widely used for the production of woody biomass, might be at high risk from the
4 climate change-induced increase in the frequency of drought periods. Therefore, selecting improved
5 genotypes, which are highly productive but with a high water use efficiency (WUE), is becoming a
6 major target. The use of automated weighing systems in controlled environments facilitates the
7 estimation of cumulated water loss and whole plant transpiration efficiency (TE). Differences in TE and
8 leaf level intrinsic WUE as well as the contribution of underlying ecophysiological traits were
9 determined in three contrasting *P. nigra* genotypes. Strong differences in TE among the selected
10 genotypes were congruent with differences in leaf level intrinsic WUE. Our data show that a high total
11 leaf area was overcompensated by a low per leaf area transpiration rate, leading to higher TE in highly
12 productive genotypes originating from cool locations. Nocturnal water loss was relatively low but
13 contributed to variations in TE among genotypes. In response to drought, leaf level WUE increased
14 but not TE, suggesting that carbon losses due to whole plant respiration could offset the drought-
15 induced increase in intrinsic WUE.

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19 **Highlights**

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

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29 1. Introduction

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

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

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

74 Commercial poplar genotypes have been selected primarily for high productivity or resistance to foliar
75 rust but not for high WUE (Monclus et al., 2006). The lack of correlation between productivity and W_i
76 across 29 *Populus x canadensis* hybrids suggested that it would be possible to select genotypes
77 which combine high productivity and high WUE (Monclus et al., 2005). Conversely, a negative
78 relationship between TE and productivity was found in the Asian species *P. davidiana* (Zhang et al.,
79 2004), questioning the independence between productivity and WUE. European black poplar (*P. nigra*,
80 L.) is a key pioneer tree species, essential for the dynamics of riparian habitats and for soil
81 stabilisation. Further, it has an economic value as a parent pool for genetic breeding of *P. x*
82 *canadensis* cultivars (Chamaillard et al., 2011; Sow et al., 2018). *P. nigra* has a wide natural
83 distribution with populations growing in different climatic conditions across Europe and showing
84 significant genetic differentiation as well as phenotypic variation in growth rate, plant architecture and
85 leaf size (DeWoody et al., 2015; Viger et al., 2016).

86 To improve our understanding of the determinants of TE and their responses to drought, we
87 determined TE in three contrasting *P. nigra* genotypes, which originate from different regions and
88 which strongly differ in terms of growth and leaf morphology (DeWoody et al., 2015; Wildhagen et al.,
89 2018). Here, we analysed underlying ecophysiological traits as well as leaf level estimators of WUE.
90 Our first aim was to investigate which traits explained differences in TE among genotypes under
91 optimal watering conditions. Here we test the following hypotheses i) The differences in TE among
92 genotypes are driven by transpiration rate rather than by biomass accumulation rate ii) The leaf level
93 WUE is a main driver of whole plant TE iii) Unproductive water losses may decouple whole plant TE
94 from leaf level WUE. A second aim was to determine if TE would be changed differently among
95 genotypes in response to drought, and which underlying traits would drive this acclimation.

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99 2. Material and methods

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101 2.1. Plant material and growth conditions

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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;

110 FERTIL S.A.S, Boulogne Billancourt, France) and $1 \text{ g l}^{-1} \text{ CaMg}(\text{CO}_3)_2$. Plants were grown in two
111 compartments of a glasshouse located at Champenoux, France ($48^\circ 45' 09.3'' \text{N}$, $6^\circ 20' 27.6'' \text{E}$), under
112 natural light conditions with daily maxima of irradiance ranging from 150 to $1000 \mu\text{mol m}^{-2} \text{s}^{-1}$
113 photosynthetically active radiation (PAR, Fig. 2). Environmental conditions in the greenhouse were
114 affected by weather conditions, but the temperature was maintained between 15 and 26°C (Fig. 2).
115 After planting, plants were watered 2–4 times a day –according to plant size and weather conditions–
116 to 85% of field capacity with an automated weighing and watering system (Buré et al., 2016). The
117 position of plants in the greenhouse was rotated at each weighing event.

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119 *2.2. Control of water deficit*

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

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

$$133 \quad \text{REW}_{\text{soil}} = \left(\frac{\text{SWC} - \text{SWC}_{\text{wiltingpoint}}}{\text{SWC}_{\text{fieldcapacity}} - \text{SWC}_{\text{wiltingpoint}}} \right) \times 100\%,$$

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

135

136 *2.3. Growth, gas exchange, transpiration rate and transpiration efficiency*

137 *Height and diameter*

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

141 *Total leaf area*

142 For each genotype, a relationship between leaf area and maximal leaf width was built from a sample
143 of approximately 80 leaves taken from the full range of leaf sizes. Regression coefficients were over
144 0.98 for each of the three genotypes. The width of all leaves of each plant was measured once a week
145 and converted to area using the established relationships. Individual leaf areas were summed to
146 calculate the total leaf area (LA) of each plant. Spline adjustment (interspline function, R) was used to
147 estimate LA for dates in between days of measurement.

148 *Dry biomass*

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

153

154 *Gas exchange and intrinsic water use efficiency*

155 Gas exchange was measured *in situ* in the greenhouse. Net CO₂ assimilation (A) and stomatal
156 conductance to water vapour (g) were measured using two inter-calibrated portable photosynthesis
157 systems LI-COR 6200 (LI-COR® Inc, Lincoln, NE, USA). Measurements were performed on the
158 youngest fully expanded mature leaves at the beginning of the experiment, corresponding to the 8th–
159 10th leaf down from the first apical leaf, between 11:00–12:00 twice a week over the five week-
160 experiment. Intrinsic water use efficiency at the leaf level (Wi) was calculated as the ratio of A/g. A, g
161 and Wi were averaged over the five last measurement days corresponding to the steady drought
162 period during the three last weeks (A_{mean} , g_{mean} and $W_{i\text{mean}}$, respectively) and these means were used
163 for the ANOVA (Tables 1 and 2).

164 We also estimated the photosynthetic capacity by measuring gas exchange under light-saturated
165 conditions, with calibrated Li-6400 XT portable gas analyzers (LI-COR® Inc, Lincoln, NE, USA) 4-6
166 days before the harvest. CO₂ concentration was 400 $\mu\text{mol mol}^{-1}$, light intensity (PAR) was 1500
167 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and block temperature was 25 °C. All measurements were performed in the corridor next
168 to the greenhouse compartments, on the same leaf used for *in situ* gas exchange. For each plant, the
169 same procedure was followed. We waited for stomatal conductance to reach a steady state (typically
170 after 20–30 min), then the A-C_i (C_i: CO₂ internal concentration) curve was performed by changing the
171 [CO₂] entering the leaf chamber with the following steps: 400, 300, 250, 200, 150, 100, 50, 400, 400,
172 500, 600, 700, 800, 1000, 1200 and 1500 $\mu\text{mol mol}^{-1}$, typically with 2–3 min between each step.
173 Maximum carboxylation rate ($V_{c\text{max}}$), maximum electron transport rate (J_{max}) and mesophyll
174 conductance (g_m) were estimated with the method by Ethier and Livingston (2004) that fits A-C_i curves
175 with a non-rectangular hyperbola version of Farquhar's biochemical model of leaf photosynthesis
176 (Farquhar et al., 1980). This is based on the hypothesis that g_m reduces the curvature of the Rubisco-
177 limited portion of an A-C_i response curve. The Rubisco kinetic traits and specificity for CO₂/O₂ were
178 characterized *in vitro* as described previously (Galmes et al., 2014). The values of the Rubisco
179 Michaelis-Menten constants for CO₂ (K_c), and O₂ (K_o) and the chloroplast CO₂ compensation point (Γ^*)
180 were obtained at 15, 25 and 35 °C and adjusted to the measured temperature using the Arrhenius
181 function (see details on Rubisco kinetic traits and specificity for CO₂/O₂ in the supplementary material
182 and methods).

183

184 *Transpiration rates*

185 Daily transpiration rate (TR) was calculated on a daily basis as the ratio between the water loss over
186 24 h and LA on that day, and then averaged over the whole experimental period. Days 17, 18, 26, 27,
187 28, 29 were used to calculate a mean diurnal transpiration rate (DTR) and a mean nocturnal
188 transpiration rate (NTR), using the ratio between water loss during the 05:00–22:00 period and the

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

193

194 *Transpiration efficiency*

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

199

200 *$\delta^{13}\text{C}$ determination*

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

210

211 *2.4. Statistical analyses*

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

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

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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 ($V_{C\text{max}}$, J_{max} , A_{sat} , g_{sat} , g_m , C_i , $W_{i\text{sat}}$) or *in situ* (A_{mean} , g_{mean} , $W_{i\text{mean}}$) 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 $\delta^{13}\text{C}$ (Table 1). The Italian genotype had a higher TE and $\delta^{13}\text{C}$ than the French, which had a much higher TE and $\delta^{13}\text{C}$ than the Spanish. There were no significant differences among genotypes in instantaneous WUE ($W_{i\text{sat}}$ and $W_{i\text{mean}}$), 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

270 this reduction in growth rate occurred later for the Spanish and the Italian genotypes (at day 11 and
271 15, respectively; Supp Fig 1). Stem diameter growth was also reduced but it seemed less sensitive
272 than stem height growth in the French and Italian genotypes (-30 % for diameter growth rate versus -
273 40% for height growth rate) and more sensitive for the Spanish genotype (-40% versus -30%) (Supp
274 Fig 1). For all genotypes, the decrease of stem diameter growth became significant from day 15.
275 Although only few significant genotype x environment interactions were detected in the ANOVA, post-
276 hoc Tukey's HSD tests suggested some species-specific drought responses. The total dry mass
277 tended to be less reduced under drought in the Italian genotype (-20%), compared to that of the
278 Spanish and French (-34 and -38%) (Table 2). Growth allocation was also differentially affected by
279 drought among genotypes. In particular, the Italian genotype maintained allocation to roots during
280 drought so that its root dry mass was not affected and its RootF increased (Table 2). The leaf fraction
281 of the French genotype was reduced but not its RootF, whereas allocation was not changed in the
282 Spanish genotype (Table 2). Drought reduced LA in all three genotypes, but the effect was most
283 pronounced in the French genotype (Table 1, Supp Fig 2). Drought also reduced the total leaf number
284 of the Spanish and French genotypes (Supp Fig 2). In the Italian genotype, drought reduced LA but
285 not the number of leaves, indicating that leaf growth rate was more sensitive than leaf production rate
286 by the meristem (Supp Fig 2).

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

297 298 3.3 Correlations

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

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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 ($\delta^{13}\text{C}$), and by instantaneous measurements (W_{isat} , W_{imean}) although differences were not significant. Guet et al. (2015) tested genotypes from geographically close populations and also found higher WUE ($\delta^{13}\text{C}$) 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 $\delta^{13}\text{C}$, 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

351 night than the other tested genotypes, and showed the highest TE. In our *P. nigra* experiment, Φ_w
352 ranged from 1.9% to 4.2%, which is a similar range to estimations for tropical tree species (1.2% to
353 5.2%; Cernusak et al., 2009) or for another plantation tree species such as *Eucalyptus grandis* (5%,
354 Benyon, 1999). Higher Φ_w (9 to 30%) were found for other poplar species (Cirelli et al., 2016; Rohula
355 et al., 2014), indicating that nocturnal transpiration was relatively low in *P. nigra*, and that a gain in TE
356 due to reduced Φ_w would be small. However, it may be that introgressing *P. nigra* into other *Populus*
357 species could reduce nocturnal water losses and increase TE. Differences in Φ_w were more closely
358 related to differences in NTR than in DTR, suggesting that stomatal regulation during the night was
359 partly independent from daytime regulation. Maintaining a significant nocturnal transpiration might
360 enhance nutrient acquisition (Kupper et al., 2012), prevent a build-up of CO₂ within the leaves (Marks
361 and Lechowicz, 2007), or facilitate a fast increase of net photosynthesis during early morning (Dawson
362 et al., 2007).

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364 **Genotypic differences in TE are related to origin and biomass allocation**

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

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

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

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397 ***Drought increased intrinsic WUE but not TE***

398 Globally, the three genotypes responded similarly to drought and we found only two significant
 399 genotype \times drought interactions in the statistical model ($\delta^{13}\text{C}$ and NTR). As expected, biomass of all
 400 compartments decreased, or tended to decrease, under drought. Growth was clearly more sensitive
 401 than assimilation rate. Consequently, growth limitation was independent from carbon supply, as
 402 already found in other poplar species (Bogeat-Triboulot et al., 2007; Cohen et al., 2010). However,
 403 growth allocation showed some genotype specific patterns. The French genotype decreased mainly
 404 the leaf fraction, as found in a previous study (Durand et al., 2019). The Italian genotype, which
 405 showed the least reduction in biomass under drought, decreased mainly the stem fraction. These
 406 responses differ from those recorded in the same genotype in previous experiments (Durand et al.,
 407 2019; Viger et al., 2016), suggesting a strong plasticity of biomass allocation.

408 The drought-induced increase in leaf level WUE ($W_{i\text{mean}}$, $\delta^{13}\text{C}$) was most likely due to a decrease in
 409 stomatal conductance, mainly observable in the *in situ* measurements, whereas neither photosynthetic
 410 capacity nor assimilation rates were significantly changed. Increased $\delta^{13}\text{C}$ and thus increased intrinsic
 411 WUE under drought due to stomatal closure is a classical response in plants showing luxurious water
 412 consumption in well watered conditions, as shown for different poplar species (Monclus et al., 2006;
 413 Viger et al., 2016). In addition, changes in intrinsic WUE seemed genotype dependent: genotype \times
 414 drought interaction was significant for $\delta^{13}\text{C}$ and almost significant for $W_{i\text{mean}}$ ($p_{\text{int}} = 0.060$). The French
 415 genotype showed a significant and greater increase in leaf level WUE, linked to a relatively stronger
 416 reduction in g_{mean} than in the two other genotypes. These results suggest differences in stomatal
 417 regulation among these genotypes, as already found in a shorter drought experiment (Durand et al.,
 418 2019).

419 Surprisingly, TE was not increased under drought in any genotype, indicating that there were other
 420 factors apart from leaf level processes, which negated the effects of improved intrinsic WUE on TE.
 421 One such process could be the nocturnal water loss, which decouples leaf level from whole plant
 422 water use efficiency (Cernusak et al., 2007). However the observed reduction in NTR and Φ_w under
 423 drought should have had a positive effect on TE and did therefore not offset the increased leaf intrinsic
 424 WUE. Another factor decoupling whole plant from leaf level WUE could be carbon losses other than
 425 day respiration by leaves (Cernusak et al., 2007). An increased whole plant respiration under drought
 426 would decrease TE and therefore would offset the increase of leaf intrinsic WUE. Published effects of
 427 water deficit on respiration are not consistent, from inhibition to stimulation (Brito et al., 2018; Flexas et
 428 al., 2005) and here leaf respiration after 30 minutes in darkness was not affected by drought (P-value
 429 = 0.41; data not shown). However whole plant carbon losses might depend also on leaf fraction. The
 430 observed decrease in leaf fraction under drought, which was stronger in the French genotype, implies

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

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436 **Conclusions**

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

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448 **Conflict of interest statement**

449 The authors have no conflicts of interest to declare.

450

451 **Author contributions**

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

457

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491 [Metrologie/chap2-ns-J2M-2016/Art02-ns-J2M-2016](https://www6.inra.fr/cahier_des_techniques/Les-Cahiers-parus/Les-N-Speciaux/Mesure-et-Metrologie/chap2-ns-J2M-2016/Art02-ns-J2M-2016).

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631 **Figure legends:**

632

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

637

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

641

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

645

646 **Figure 4:** Correlations between traits in control plants (upper part) and in drought-subjected plants
647 (lower parts) (n=18 for each subplot). Only significant correlations were displayed (P-value<0.05).

648 A_{mean} : mean of net CO₂ assimilation rate measured *in situ*, A_{sat} : net CO₂ assimilation rate measured
649 under light-saturated conditions, C_i : CO₂ internal concentration, CumulT: cumulated water loss,
650 DMincr: total dry mass increment, DTR: diurnal transpiration rate, $\delta^{13}\text{C}$: carbon isotope composition,
651 FinalH: final stem height, FinalD: final stem diameter, g_m : mesophyll conductance for CO₂, g_{mean} :
652 stomatal conductance to water vapour measured *in situ*, g_{sat} : stomatal conductance to water vapour
653 measured under light-saturated conditions, J_{max} : maximum photosynthetic electron flux, LA: total leaf
654 area, LeafF: leaf fraction, Φ_w : proportion of unproductive water loss to productive water loss, NTR:
655 nocturnal transpiration rate, RootF: root fraction, TE: whole plant transpiration efficiency, TotalDM:
656 total dry mass, TR: daily transpiration rate, $V_{c\text{max}}$: maximum CO₂ carboxylation rate, $W_{i\text{sat}}$: leaf intrinsic
657 water use efficiency measured under light-saturated conditions, $W_{i\text{mean}}$: mean leaf intrinsic water use
658 efficiency measured *in situ*.

659

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

666
667
668 **Table1: Results of Two-way ANOVA of different traits.** Significance and adjusted correlation
669 coefficient of the model, significance of the factors (genotype and drought) and of the interaction.
670 Marginal mean \pm s.e. are given for the three genotypes and for the treatments. Different letters denote
671 significant differences between groups according to Tukey post-hoc tests.

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

683
684 **Table 2: Complement of Table 1.** Mean \pm s.e. of different traits within each genotype x treatment
685 group (n=4 - 6). Different letters denote significant difference between groups according to Tukey post-
686 hoc tests. Acronyms are identical to those in Table 1.

691 **Supplementary material**

692
693 **Supplementary material and methods:** Rubisco kinetic traits and specificity for CO_2/O_2
694 characterisation

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

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

697 **Supplementary Figure 1:** Growth rate in height and in stem diameter of the three genotypes over the
698 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 CO₂ assimilation rate, stomatal conductance and intrinsic water use
702 efficiency over the 5-week experiment.

703

Table 1

| | | model | | Factors | | | French | | | Italian | | | Spanish | | | Control | | Drought | |
|--------------------|--------------------------------------|-------|-------|---------|---------|-------|----------------------|---|----------------------|---------|----------------------|---|----------------------|---|----------------------|---------|--|---------|--|
| | | model | R2 | Genot. | Drought | G x D | | | | | | | | | | | | | |
| 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 | | | |
| δ 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 | m ² | *** | 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 | | | |
| V _{Cmax} | 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 | | | |

Comment citer ce document :

Bogeat-Triboulot, M.-B., Buré, C., Gerardin, T., Chuste, P.-A., Le Thiec, D., Hummel, I., Durand, M., Wildhagen, H., Douthe, C., Molins, A., Galmés, J., Smith, H., Flexas, J., Polle, A., Taylor, G., Brendel, O. (2019). Additive effects of high growth rate and low transpiration rate drive differences in whole plant transpiration efficiency among black poplar genotypes.

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 |
| δ 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 | m ² | 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 |
| V _{Cmax} | 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 |

Comment citer ce document :

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Figure 1

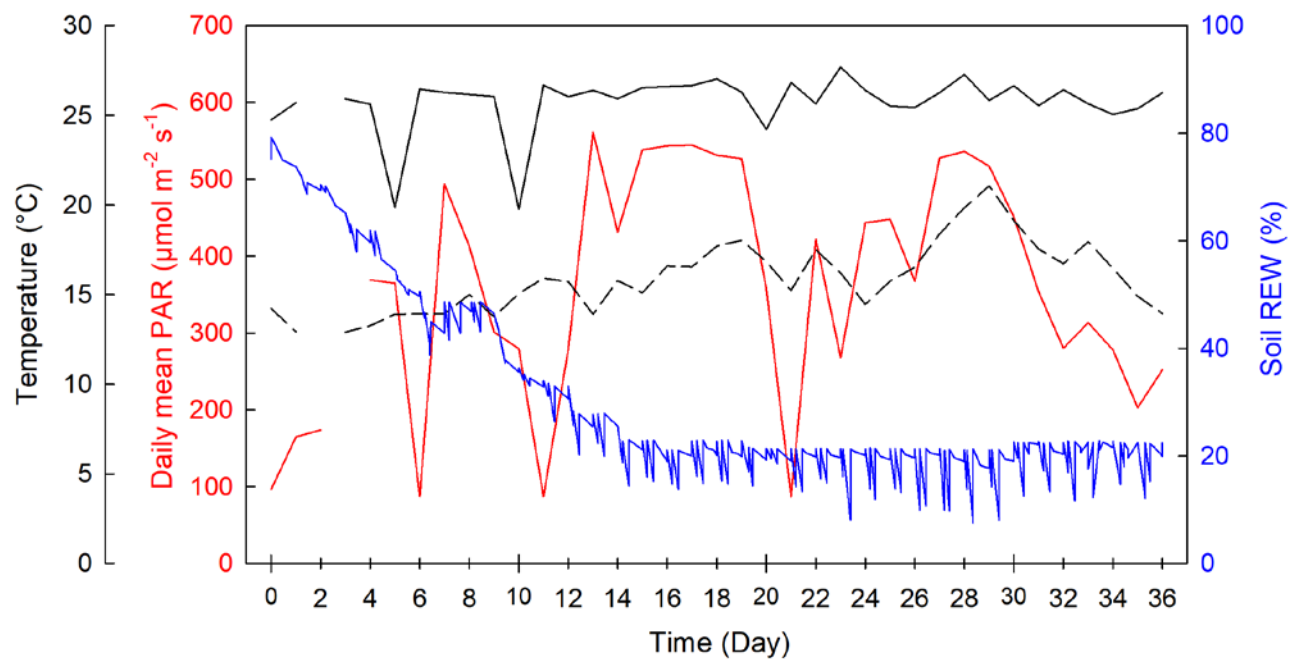


Figure 2

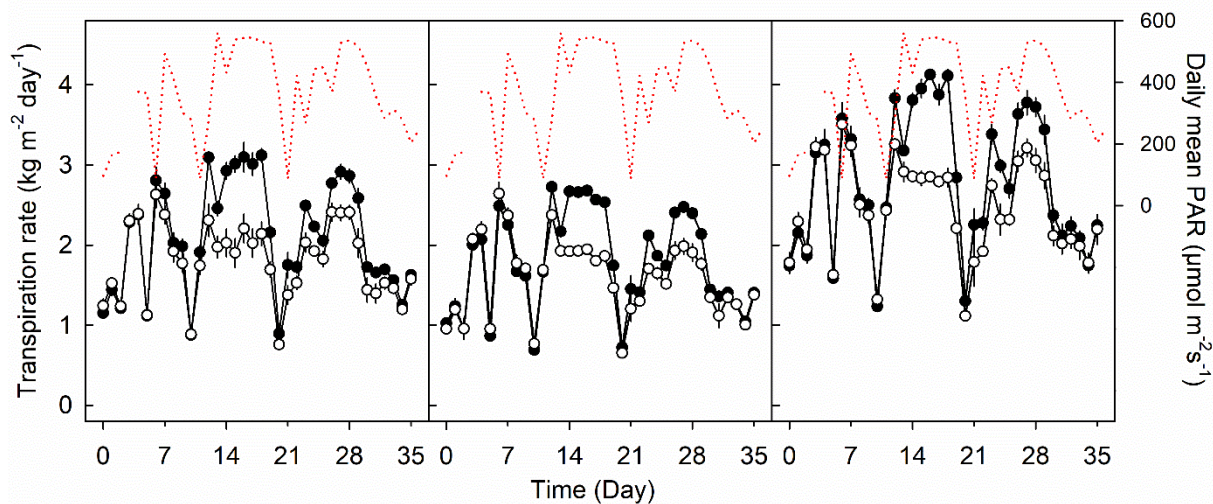


Figure 3

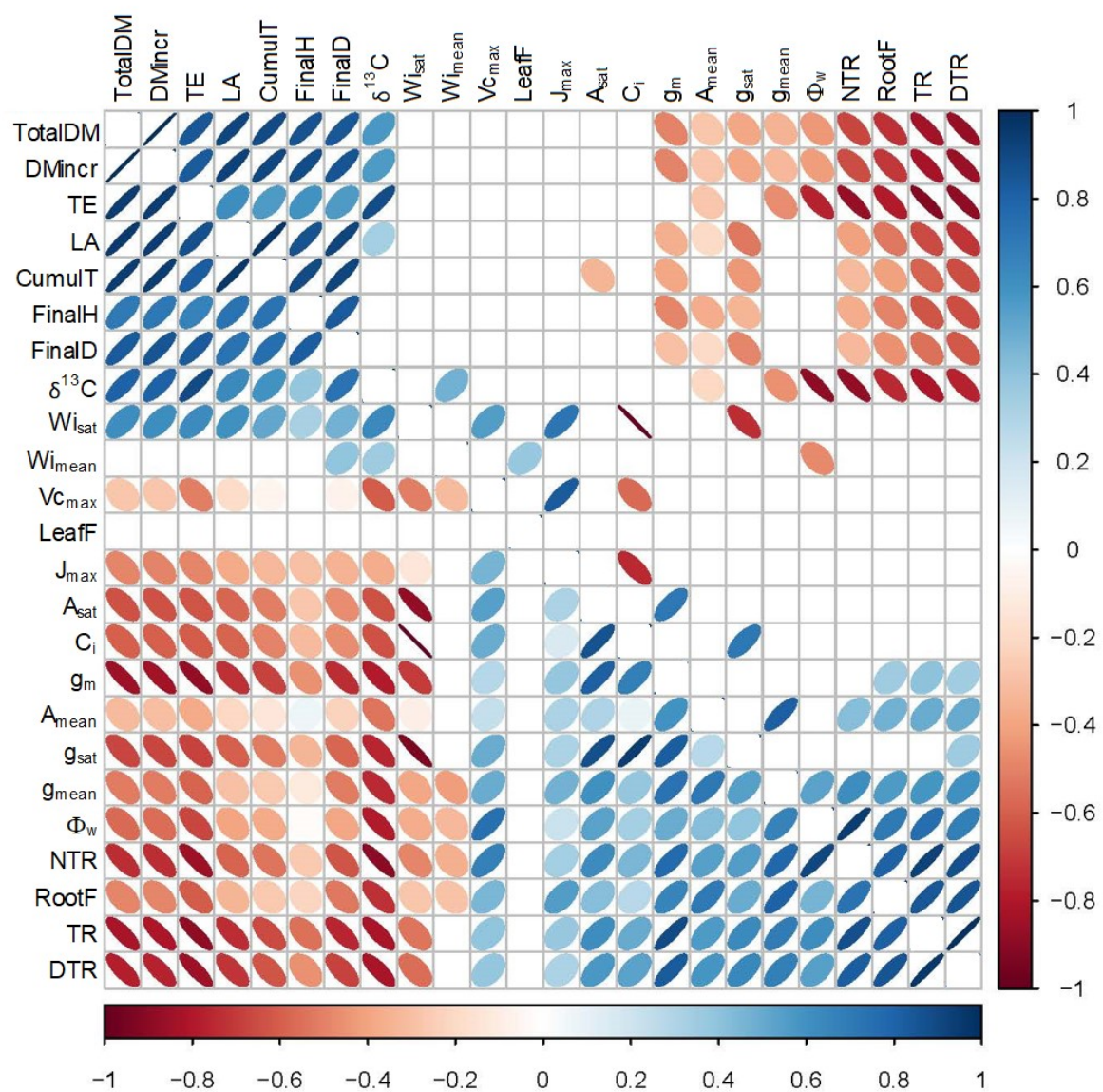


Figure 4

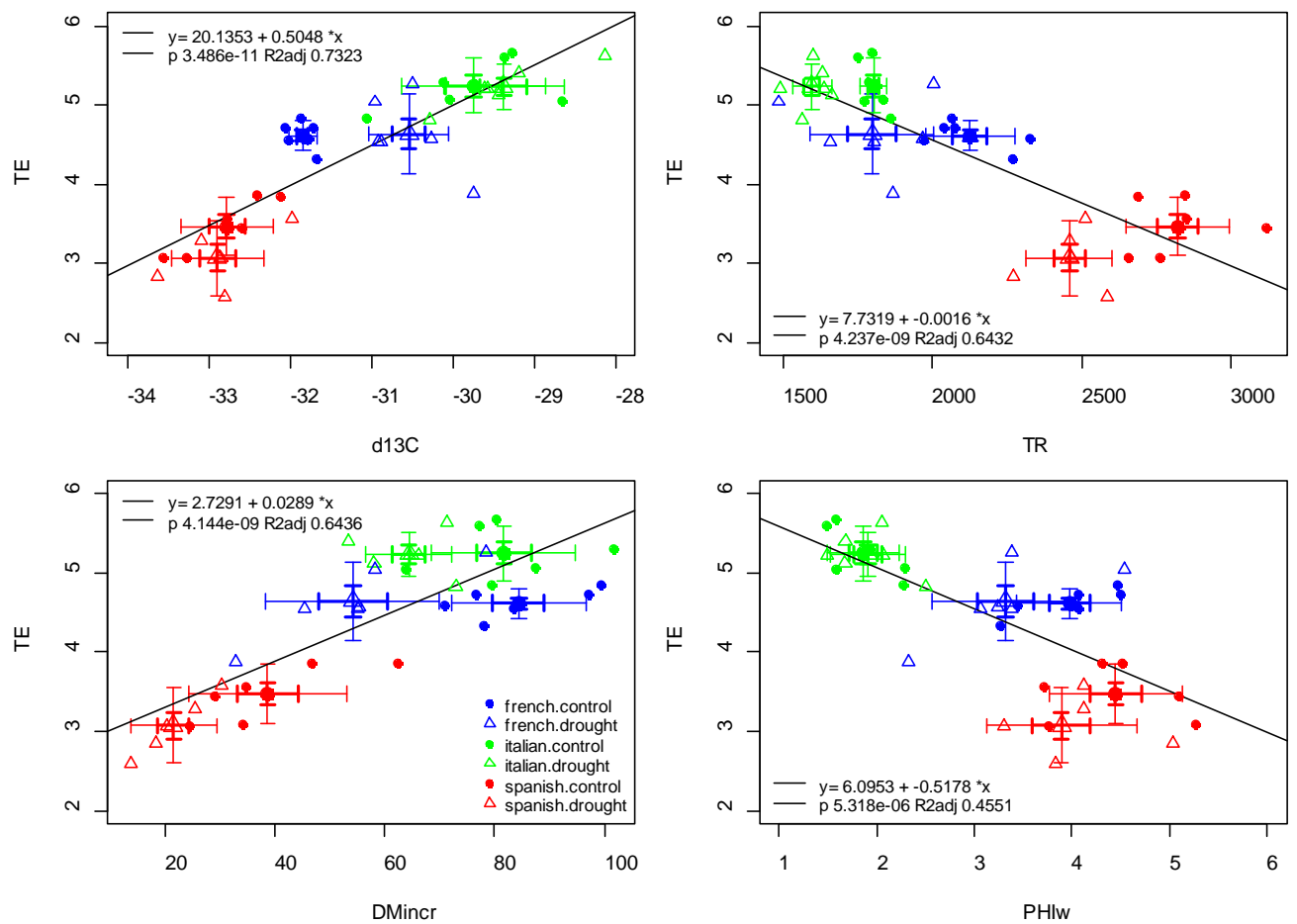


Figure 5

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 <i>et al.</i> 2016 | x | |
| 414_R | CAAATCCTCCAGACGTAGAGC | Chen <i>et al.</i> 1998 | | x |
| 991_R | CGGTACCAGCGTGAATATGAT | Chen <i>et al.</i> 1998 | | x |
| 1494_R | GATTGGGCCGAGTTTAATTAC | Chen <i>et al.</i> 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 94°C for 2 min, 55°C for 30 s, 72°C for 4 min, followed by 30 cycles of 94°C for 30 s 56°C for 45 s and 72°C for 1 min, and a final elongation at 72°C 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 BigDye™ 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 Genbank 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 *rbcL* 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 CO₂/O₂ 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 MgCl₂, 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 °C. 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 MgCl₂, 10 mM DTT, 1 mM KH₂PO₄, 0.5 mM EDTA, 1 mM benzamidine, 1 mM aminocaproic acid and 10 mM NaHCO₃. 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 NaH¹⁴CO₃ to adjust the specific radioactivity to 3.7 × 10¹⁰ Bq mol⁻¹. The remaining extract volume was frozen immediately in liquid nitrogen to measure the Rubisco active site concentration.

Rates of Rubisco ¹⁴CO₂-fixation using the activated protein extract were measured at 15, 25 and 35°C, each at two concentrations of O₂ (0 and 21% v/v). In all the cases, nine different concentrations of ¹⁴CO₂ were used (0 to 93 μM, each with a specific radioactivity of 3.7 × 10¹⁰ 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 MgCl₂, 0.4 mM RuBP and about 100 W-A units of carbonic anhydrase), and equilibrated either with nitrogen (N₂) or a mixture of O₂ and N₂ (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 ¹⁴C was determined by liquid scintillation counting, following removal of acid-labile ¹⁴C by evaporation. The Michaelis-Menten constant for CO₂ (*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}*^o) was extrapolated from the corresponding *V_{max}* value after allowance was made for the Rubisco active site concentration, as determined by [¹⁴C]CPBP binding (Yokota & Calvin, 1985).

The Rubisco specificity for CO₂/O₂ (*S_{co}*) 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 MgCl₂, 0.15

mg mL⁻¹ carbonic anhydrase, 2 mM NaH¹⁴CO₃ (18.5 kBq mol⁻¹), 20 μL activated Rubisco from purified extracts and 2.5 μM RuBP. The basic buffer was pre-equilibrated with CO₂-free air at the temperature of measurement. RuBP oxygenation was calculated from the oxygen consumption and carboxylation from the amount of ¹⁴C 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 CO₂ (K_c), the Michaelis-Menten constant for CO₂ measured under 21% O₂ conditions (K_c^{air}), the maximum rate of carboxylation (k_{cat}^c), the specificity for CO₂/O₂ ($S_{c/o}$) and the CO₂ 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 CO₂ (K_c) and O₂ (K_o), the Michaelis-Menten constant for CO₂ measured under 21% O₂ conditions (K_c^{air}), the maximum rate of carboxylation (k_{cat}^c), the specificity for CO₂/O₂ ($S_{c/o}$) and the chloroplast CO₂ compensation point (Γ^*).

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

| Parameter | C | | ΔH_a (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:

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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 |
| | | | | | | | | | |

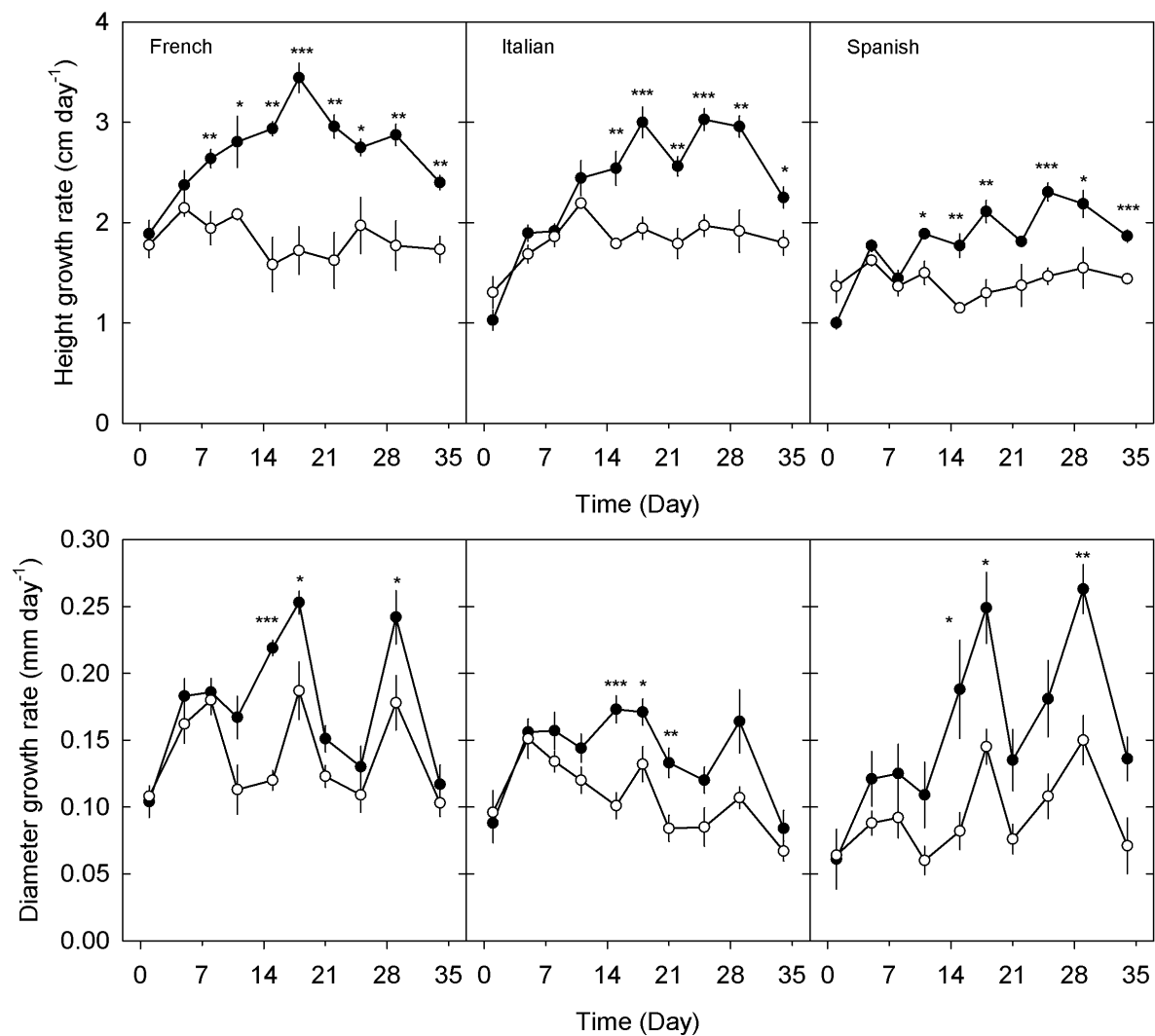
DeWoody, J., Trewin, H., Taylor, G., 2015. Genetic and morphological differentiation in *Populus nigra* L.: isolation by colonization or isolation by adaptation? *Mol. Ecol.* 24, 2641-2655.

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 \pm s.e., n=4 – 6.

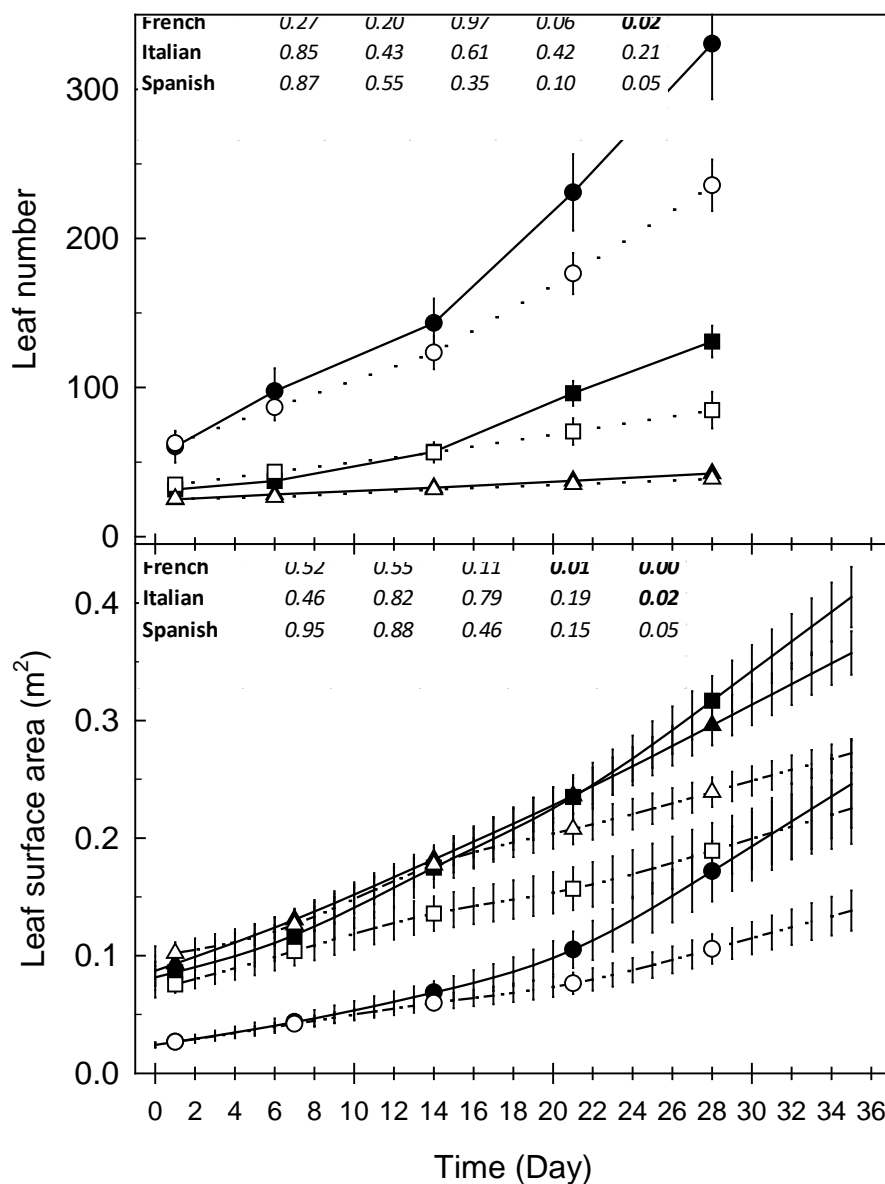
| | French | Italian | Spanish |
|---------|------------------|------------------|------------------|
| control | -0.23 \pm 0.01 | -0.08 \pm 0.02 | -0.22 \pm 0.04 |
| drought | -0.29 \pm 0.01 | -0.26 \pm 0.02 | -0.20 \pm 0.04 |

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



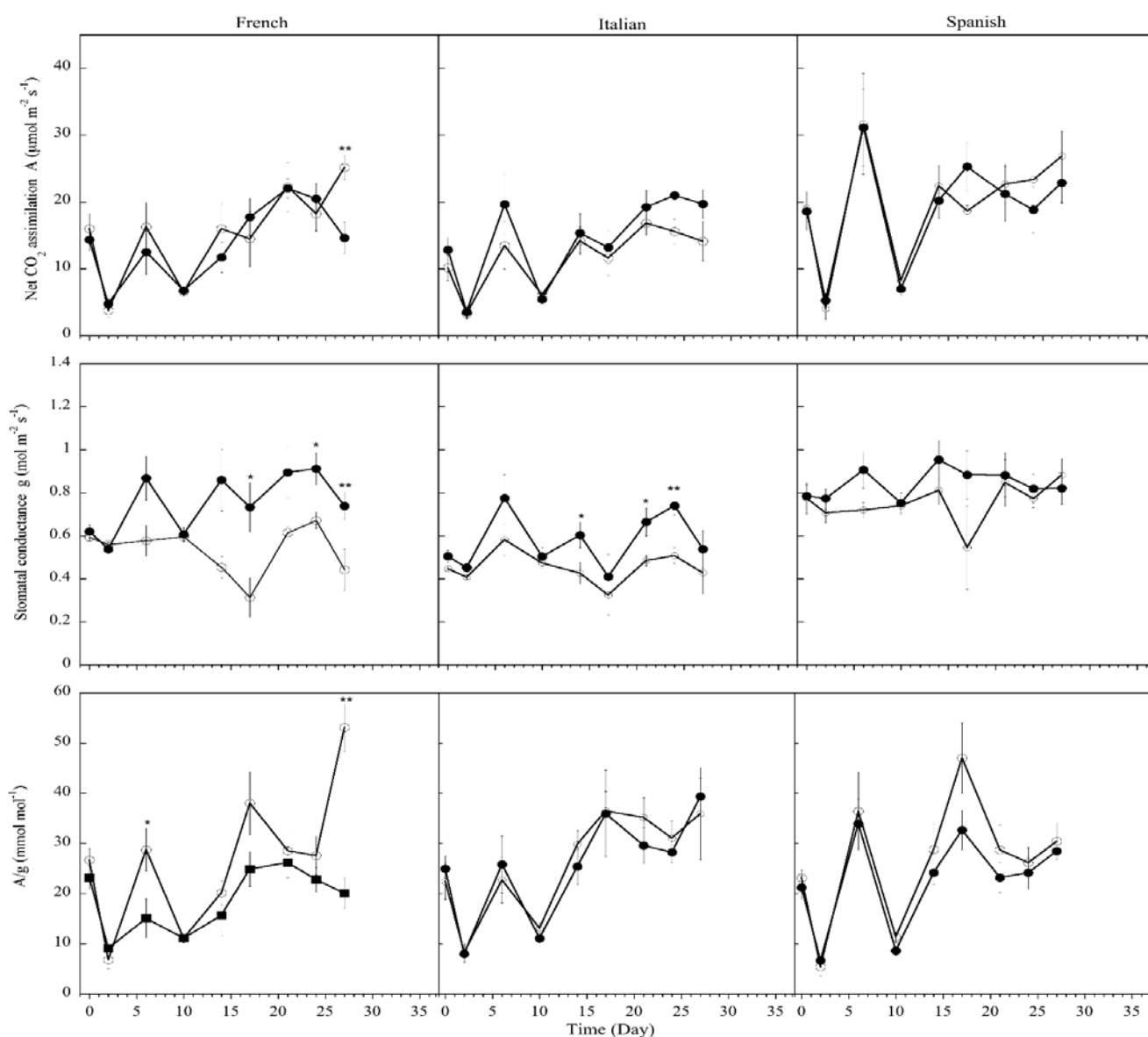
Supplementary Figure 1 :

Growth rate in height and in stem diameter of the three genotypes over the 5-week experiment. Mean \pm 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 \pm 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 CO₂ 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).