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Integrated drought responses of black poplar: how important is phenotypic plasticity?

Marie Garavillon-Tournayre^a, Aurélie Gousset-Dupont^a, Florian Gautier^b, Pierrick Benoit^a, Pierre Conchon^a, Romain Souchal^a, David Lopez^a, Gilles Petel^a, Jean-Stéphane Venisse^a, Catherine Bastien^b, Philippe Label^{a,†} and Boris Fumanal^{a,*} 

^aUniversité Clermont Auvergne, INRA, PIAF, F-63000 Clermont-Ferrand, France

^bINRA Centre Val de Loire, UAGPF, F-45075 Orléans, France

Correspondence

*Corresponding author,
e-mail: boris.fumanal@uca.fr

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Climate change is expected to increase drought frequency and intensity which will threaten plant growth and survival. In such fluctuating environments, perennial plants respond with hydraulic and biomass adjustments, resulting in either tolerant or avoidant strategies. Plants' response to stress relies on their phenotypic plasticity. The goal of this study was to explore physiology of young *Populus nigra* in the context of a time-limited and progressive water deficit in regard to their growth and stress response strategies. Fourteen French 1-year-old black poplar genotypes, geographically contrasted, were subjected to withholding water during 8 days until severe water stress. Water fluxes (i.e. leaf water potentials and stomatal conductance) were analyzed together with growth (i.e. radial and longitudinal branch growth, leaf senescence and leaf production). Phenotypic plasticity was calculated for each trait and response strategies to drought were deciphered for each genotype. Black poplar genotypes permanently were dealing with a continuum of adjusted water fluxes and growth between two extreme strategies, tolerance and avoidance. Branch growth, leaf number and leaf hydraulic potential traits had contrasted plasticities, allowing genotype characterization. The most tolerant genotype to water deficit, which maintained growth, had the lowest global phenotypic plasticity. Conversely, the most sensitive and avoidant genotype ceased growth until the season's end, had the highest plasticity level. All the remaining black poplar genotypes were close to avoidance with average levels of traits plasticity. These results underpinned the role of plasticity in black poplar response to drought and calls for its wider use into research on plants' responses to stress.

Abbreviations – Ψ_{pd} , predawn leaf water potential; Ψ_{md} , midday leaf water potential; θ_{max} , maximum annual average temperature; θ_{min} , minimum annual average temperature; D , main branch diameter gain; g_s , stomatal conductance; H , tree height before experiment; L , new branch length gain; Light, mean number of sunny days per years; N , number of new leaves per branch; p_{-} , plasticity of; PCA, principal component analysis; SLA, specific leaf area; Rain, mean number of rainy days per years; RDPI, Relative Distance Plasticity Index; T , total leaf number variation.

[†]These authors contributed equally to this work.

Introduction

Ongoing global climate change is expected to increase both the intensity and the frequency of future extreme climatic events (Beniston et al. 2007, Burkett et al. 2014). In temperate countries, drought episodes will be part of the more intense climatic changes (van Mantgem et al. 2009, Anderegg et al. 2013, Cisneros et al. 2014), and have already been held responsible for some observed forest die-offs (Bréda et al. 2006).

Trees physiological responses to water deficit result in hydraulic and carbon cycle adjustments, which have already been well described (Parker 1956, Bréda et al. 2006). Stomatal closure initiates the phenotypic responses to edaphic water deficit (Tardieu and Simonneau 1998, Marron et al. 2002, Bogeat-Triboulot et al. 2007). Stomatal aperture regulation acts as a safety barrier that reduces water loss by leaf transpiration (Stalfelt 1955, Barrs 1971, Brodribb and Holbrook 2003, Araújo et al. 2011). The evaporative demand of the plant persists despite water constraints, leading to a progressive tension increase in the water column (Tyree and Sperry 1988, Sellin 1999, Sack and Holbrook 2006). If water deficit is prolonged, cavitation events can occur in the vascular system (Milburn 1966, Nardini et al. 2001, Brodribb and Holbrook 2003, Cochard et al. 2007). Nevertheless, xylem functionality could be recovered after rewatering (McCully 1999, Brodribb and Cochard 2009). These hydraulic variations are evenly supported by morphological and physiological changes at the whole plant scale, such as a decrease of cellular elongation and surface of new leaves growing during drought (Battaglia et al. 1998, Pedrol et al. 2000, Marron et al. 2002, Iogna et al. 2013, Bizet et al. 2014). Even with non-critical levels of dehydration, carbon assimilation is strongly reduced (Thomas and Eamus 1999, Lambers et al. 2008, Hamanishi et al. 2012). As a consequence, biomass production falls dramatically (Bray 1997, Caruso et al. 2002, Monclus et al. 2006, Bonhomme et al. 2008), and ultimately ceases. In the final stages of severe drought response, combined hydraulic failure and carbon depletion can result in plant death (McDowell et al. 2008, Sala et al. 2010, Sevanto et al. 2014). Plants response strategies depicted in different species are complex, owing to strong regulation of physiological traits that enables plants to tolerate water stress, and avoid instant death.

Poplar is widely used as a tree model (Bradshaw et al. 2000, Taylor 2002) to study physiological responses to water deficit in plants. *Populus nigra* grows in a wide range of geographical climates (Cagelli and Lefèvre 1995), most of which are in riparian stands and wetlands (Rameau et al. 1989, Isebrands and Richardson

2014). Black poplar is a pioneer species characterized by a strong colonization power during juvenile status when growth rate supported by high water consumption is the highest. This meso-hygrophilous species, used in breeding programs, is known to be particularly vulnerable to water deficit despite its rusticity (Bréda et al. 2006). Significant natural variations in phenotypic responses have been shown among European genotypes subjected to moderate drought suggesting their adaption to local climatic events (Viger et al. 2016). These results were supported by an integrated study revealing that genetic and morphological characteristics of 13 European *P. nigra* populations should depend on adaptive differentiation and historic local events (DeWoody et al. 2015). In addition, French genotypes exhibiting the greatest genetic admixture (DeWoody et al. 2015) showed heterogeneous drought responses (Viger et al. 2016). Regarding to these results, conclusions should be tried on larger set of French black poplar genotypes responding to drought to explore their phenotypic and genetic particularities, and estimate the degree of the response variability. Two water deficit response strategies have been identified in poplar species and hybrids regarding to water fluxes and biomass production parameters: tolerance and avoidance (Marron et al. 2003, Monclus et al. 2006, Giovannelli et al. 2007). The tolerant strategy is defined by the capacity of a perennial plant to sustain growth and biomass production by maintained stomatal aperture or a loosely regulated one (Passioura 2002), while limiting dehydration by leaf osmotic adjustments (Gebre et al. 1994, Marron et al. 2002, Hanin et al. 2011, Barchet et al. 2013, Martorell et al. 2014). The avoidant strategy is based on limiting water loss through quick stomatal closure and/or leaf abscission (Couso and Fernandez 2012). Similar strategies have been identified in other poplar species, with a broad range of variations in physiological trait regulation (Brignolas et al. 2000); notably, stomatal sensitivity (Hamanishi et al. 2012), leaf abscission, leaf production (Marron et al. 2002) and general plant growth (Giovannelli et al. 2007). However, there is a continuum of responses depicting these two strategies according to individuals in a population, or even in the same individual according the environment. To cope with environmental changes, perennial plants have three options: local adaptation (Kawecki and Ebert 2004), new colonization (Sax et al. 2007) or phenotypic plasticity. Phenotypic plasticity is defined as the capacity of a genotype to produce different phenotypes in response to environmental conditions (Bradshaw 1965, Sultan 2000, Nicotra et al. 2010). A part of the total phenotypic plasticity can be evaluated using many indices (Valladares et al. 2006) focused on plant traits. The level of plasticity depends on species (Iogna et al. 2013, McKown et al.

2014), genotypes (Bizet et al. 2014), the considered plant trait (Couso and Fernandez 2012) and growth conditions.

Levels of phenotypic plasticity can become adaptive and inheritable especially when expressed by plants under recurrent environmental modifications. Adaptive plasticity can benefit survival, growth and reproduction (Van Kleunen and Fischer 2005, Chevin and Lande 2009, Ghalambor et al. 2015), as suggested for adaptive receptor sensitivity to environmental cues (Nicotra et al. 2010). Energy consumption required for the modification of a stable trait must be proportional to the intensity of environmental stress (Valladares and Niinemets 2008) to avoid ineffective extreme phenotypic plasticity (DeWitt et al. 1998, Steinger et al. 2003). The environmental predictability, i.e. the match between environmental cues and the level of plant phenotypic plasticity, appears crucial for the efficiency of plant phenotypic adjustments (Moran 1992, Reed et al. 2010). In an environment which will undergo drastic changes, as expected in the future, plant plasticity should play a major role in plant interactions, selection pressure, competition for resources and plant persistence (Parmesan and Hanley 2015).

The aim of this study was to characterize the main early physiological responses of young black poplar during a time-limited progressive drought. The followed goals were (1) to evaluate the extent of phenotypic plasticity involvement in drought responses, and (2) to decipher relationships between phenotypic plasticity and response strategies to water deficit. Fourteen black poplar genotypes, from contrasted geographical origins in France, were subjected to water withholding until they reached a severe water deficit, as measured by predawn leaf water potential. Physiological and growth traits were monitored during the water treatment. Genotype strategies integrating trait plasticities were studied using a multivariate analysis.

Materials and methods

Plant material and experimental design

Fourteen European *P. nigra* genotypes were sampled by INRA UR 0588 AGPF (Orléans, France) in natural riparian populations (Villar and Forestier 2009) from different French river basins characterized by different climatic conditions (Table 1). They were kept in annually coppiced mother-stocks at the ONF nursery of Guéméné-Penfao (47.62°N, 1.84°E). Eight cuttings per genotype (1 cm collar diameters, 30 cm height) were planted in 10 l pots in spring 2012. Pot substrate was composed of one-third black peat and two-thirds local Limagne topsoil, added with 20 g of Osmocot

Pro fertilizer (17:11:10 N, P, K, 2 MgO). Pots were set up in the common garden at Clermont Auvergne University, Clermont-Ferrand (45.76°N, 3.12°E) using a complete randomized block design. Trees were spaced 1 m apart and surrounded by additional randomized clones from the different genotypes tested in order to minimize edge effects. Pots were watered automatically twice a day (0.5 l in the morning and afternoon) until the onset of water deficit. The experiment ran from June 11, 2013 to June 18, 2013 on 107 one-year-old black poplars (401 ± 10 leaves, 16 ± 1 mm of stem diameter). Trees height before experiment start was not different between clones within genotypes ($P = 0.127$). However, two genotypes among the 14 had significantly different heights ($P = 0.002$) from 75 ± 6 cm for ERS-12 to 125 ± 7 cm for SPM-40. Four clones per genotype were subjected to water deficit (except two for 77–308 and ERS-12 genotypes) by withholding water until all genotypes exceeded at least a predawn leaf water potential of -2 MPa, corresponding to 8-day treatment and severe water deficit (Marron et al. 2003). Pots were weighed daily to check water deficit (mean water loss of $1.6\% \pm 0.1$ per day and per pot; Fig. S1, Supporting Information). Four control trees per genotype were well-watered (only three ERS-12 trees). The soil of droughted trees was covered with plastic bags sealed with silicone at the tree collar to prevent rehydration by rain and morning dew. At the end of the experiment, the trees were re-watered and monitored until the season's end (October 19, 2013).

Water movements

Predawn and midday leaf water potential (Ψ_{pd} and Ψ_{md} , expressed in MPa) were measured on rank-four leaves from the top with a Scholander pressure chamber (Model 600; PMS Instrument Company, Albany, OR) every 2 days. Ψ_{pd} was measured from 04 h 30 min to 05 h 15 min, i.e. before sunrise, (1) to control steady state of water status along the soil–plant–atmosphere continuum for control trees and (2) to reach at least $\Psi_{pd} = -2$ MPa for the droughted trees. Ψ_{md} was measured between 13 h 00 and 13 h 45, i.e. at peak transpiration.

Stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$) was measured daily between 13 h 00 and 13 h 45 on rank-four leaves using an SC-1 Leaf Porometer (Decagon Devices, Pullman, WA).

Plant growth and development

Poplar architecture features sylleptic and proleptic branching (Hallé et al. 1978, Ceulemans et al. 1990, Wu and Hinckley 2001). Tree organs were annotated

Table 1. Annual averages of climatic parameters between 1981 and 2010 defining the native environmental conditions of the 14 black poplar genotypes studied. ^aA minimum of 1 mm of rainfall and 120 W m⁻² of sunshine per day was taken into account for number of days counting. ^bThe hottest month was July for each sampling origin.

Genotype	Latitude	Longitude	Sampling origin	Average temperature (°C)		Cumulative rainfall			Total sunshine	
				Minimal	Maximum	Height (mm)	Number of days ^a	Height (mm), for the hottest month ^b	Duration (h)	Number of days ^a
STR-10	48.37	7.49	Strasbourg	6.6	15.3	665.0	114.9	72.7	1692.7	59.8
STR-16	48.37	7.49	Strasbourg	6.6	15.3	665.0	114.9	72.7	1692.7	59.8
ERS-05	48.25	7.43	Erstein	6.1	15.7	607.3	103.9	66.8	1799.0	62.2
ERS-10	48.25	7.43	Erstein	6.1	15.7	607.3	103.9	66.8	1799.0	62.2
ERS-12	48.25	7.43	Erstein	6.1	15.7	607.3	103.9	66.8	1799.0	62.2
RHN-35	48.16	7.41	Rhinau	6.1	15.7	607.3	103.9	66.8	1799.0	62.2
RHN-38	48.16	7.41	Rhinau	6.1	15.7	607.3	103.9	66.8	1799.0	62.2
SPM-12	47.51	1.48	Saint-Pryvé Saint-Mesmin	5.8	16.7	702.3	113.8	59.9	1743.6	60.3
SPM-28	47.51	1.48	Saint-Pryvé Saint-Mesmin	5.8	16.7	702.3	113.8	59.9	1743.6	60.3
SPM-40	47.51	1.48	Saint-Pryvé Saint-Mesmin	5.8	16.7	702.3	113.8	59.9	1743.6	60.3
ALL-29	46.24	3.19	Val d'Allier	6.0	16.7	779.5	116.7	71.6	1861.7	68.7
77-308	45.54	5.12	Meximieux	8.1	16.9	831.9	104.1	77.7	2001.9	81.1
72-501	45.35	5.36	Saint-Genix sur Guiers	6.3	16.2	934.3	106.4	86.6	2065.9	88.1
BDG	44.04	0.54	Garonne	8.4	18.5	712.2	107.1	51.3	1982.4	75.7

to take into account these two branching processes: the stem, the longest past year sylleptic shoot (named main branch) and one proleptic shoot developed on the main branch (named new branch). Measurements of radial growth (D, at 5 cm from the collar or terminal bud scar) and longitudinal growth (L) were performed during the water deficit period.

Total leaf number per tree were counted before and after the water deficit then divided by leaf number at the beginning of the experiment (T, %). Apical meristems were marked before the experiment in order to count the number of new leaves produced during the stress on the new branch (N). Three rank-four leaves per tree grown during the water deficit were sampled (except one stressed leaf for 77-308 and BDG, and none for stressed ERS-12 trees owing to lack of leaves) to evaluate the specific leaf area (SLA). Then for each fresh leaf, leaf area (A, mm²) was measured using IMAGEJ software (version 1.48; Schneider et al. 2012) and leaf dry mass (M, mg) was obtained after 4 days drying at 60°C. Mean SLA (mm⁻² mg⁻¹) per tree was calculated as SLA = A/M.

Statistical analysis

The effects of water deficit and genotype were analyzed over time on stomatal conductance, predawn and midday leaf water potentials of trees using mixed effects linear model and contrast analysis using post hoc Tukey's test.

Physiological and growth traits measured at the maximum water deficit (day 8) were analyzed using a multivariate principal component analysis (PCA) on standardized values. Shapiro-Wilk's test was used to

check Gaussian distribution for all variables. Climatic variables ($\theta_{\min}$, minimum annual average temperature; $\theta_{\max}$, maximum annual average temperature; Rain, mean number of rainy days per year; Light, mean number of sunny days per year) describing the original environment of each genotype and their height (H, trees height before experiment) were added to the PCA as supplementary variables. Eigenvalues were calculated for the first five dimensions. The contributions of each variable (C_{var}) and each individual (C_{ind}) to these dimensions were compared with theoretical equivalent contribution (C_{th}), defined as: $C_{\text{th}} = 1/\text{variable number}$. Variables and individuals with a significant contribution ($C_{\text{var}} > C_{\text{th}}$ or $C_{\text{ind}} > C_{\text{th}}$) to the dimensions and a quality of representation (range of explicative dispersion, $\cos^2$) in the plane above 0.60 were retained to help describe genotype behaviors during the water deficit. V-test results (Lebart et al. 1995) singled out genotypes belonging to the first two dimensions. These screening criteria allowed choosing the most informative traits, which were then compared among treatment and between genotypes using two-way ANOVA and post hoc Tukey's test.

The plasticity of the most informative physiological and growth trait (D, L, N, T, Ψ_{pd} and Ψ_{md}) was calculated using the Relative Distance Plasticity Index (RDPI; Valladares et al. 2006) varying from 0 to 1 (respectively low and high plasticity) for each variable and genotype as: $\text{RDPI} = \Sigma(|x_{ij} - x_{ij'}|/(x_{ij} + x_{ij'}))/n$, where x_{ij} defines the value of a variable in treatment i (well-watered or droughted) for trees j, $|x_{ij} - x_{ij'}|$ is the absolute difference obtained between all pairs of trees from two different treatments, and n is the total number of pairs. The

corresponding variables were named after their physiological counterpart and prefixed p_. The relationships of trait plasticity indexes were analyzed using a PCA. Kruskal and Wallis test has been performed on each RDPI trait to estimate the genotype effect (Table S3).

Statistical analyses were performed using R software version 3.2.1 with $\alpha = 0.05$. FACTOMINER (Le et al. 2008) and FACTOEXTRA R packages were used to perform a PCA analysis and visualized results.

Results

Evolution of physiological plant status during progressive water deficit

Stomatal conductance (g_s), predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potentials were significantly different ($P < 0.001$) between well-watered and droughted trees over time (Fig. 1). No significant genotype effect has been detected over time on Ψ_{pd} ($P = 0.150$) and Ψ_{md} ($P = 0.142$). The g_s value of trees under water deficit significantly fell throughout the time course compared with well-watered ones, from 171 ± 19 at day 2 to 32 ± 3 mmol H₂O m⁻² s⁻¹ at day 3, and then stabilized until the end of the experiment (Fig. 1A). A notable variability in g_s was also observed on well-watered trees during the experiment (from 146 ± 31 to 512 ± 49 mmol m⁻² s⁻¹). Predawn and midday leaf water potentials of well-watered poplars remained stable during the progressive water treatment, respectively $\Psi_{pd} = 0$ MPa and $\Psi_{md} = -1$ MPa (Fig. 1B, C). The expected decrease of Ψ_{pd} and Ψ_{md} were significant respectively after four ($P < 0.001$) and eight ($P < 0.001$) days of water deficit, reaching -2.8 ± 0.1 MPa for Ψ_{pd} and -2.6 ± 0.2 MPa for Ψ_{md} at day 8.

Whole trait relationships

The global response of trees to severe water deficit (day 8) was studied by PCA of the eight measured physiological and growth traits along with five supplementary quantitative variables. On the main plane representing 62% of global variance (Fig. 2A), individuals projection revealed the effect of water deficit on individual clustering, and split individuals into two groups along the first PCA dimension (correlation = 0.83, $P < 0.001$). The underlying structure of variance was summarized by few traits (Fig. 2B): total leaf number variation (T) and predawn leaf water potential (Ψ_{pd}). They were respectively positively and negatively correlated with the first dimension. Midday leaf water potential (Ψ_{md}), radial (D) and longitudinal (L) growths and number of new leaves per new branch (N) contribute to the variance of the first axis in a lesser extent. SLA and stomatal conductance (g_s) did

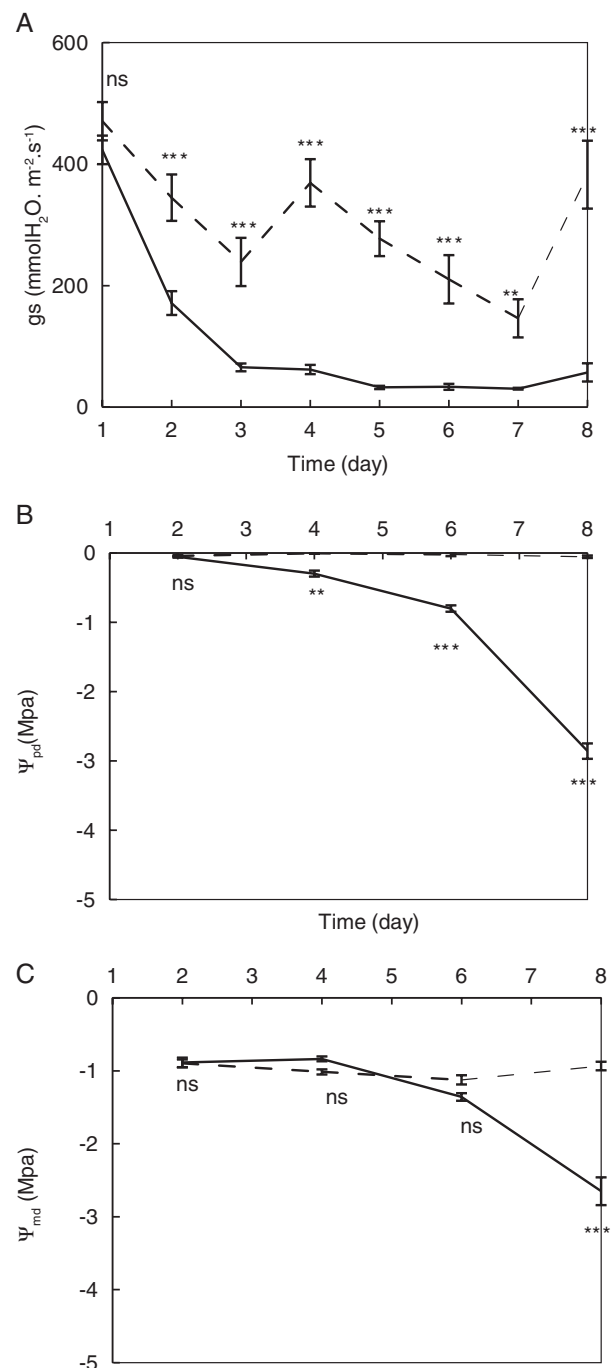


Fig. 1. Mean ($\pm$ se) stomatal conductance (A) and predawn (B) and midday (C) leaf water potential of black poplars in well-watered (dotted line) and water deficit (solid line) conditions. Results of mixed effects linear model and post hoc Tukey's test are indicated (* $0.01 < P < 0.05$; ** $0.01 < P < 0.001$; *** $P < 0.001$; ns, non-significant).

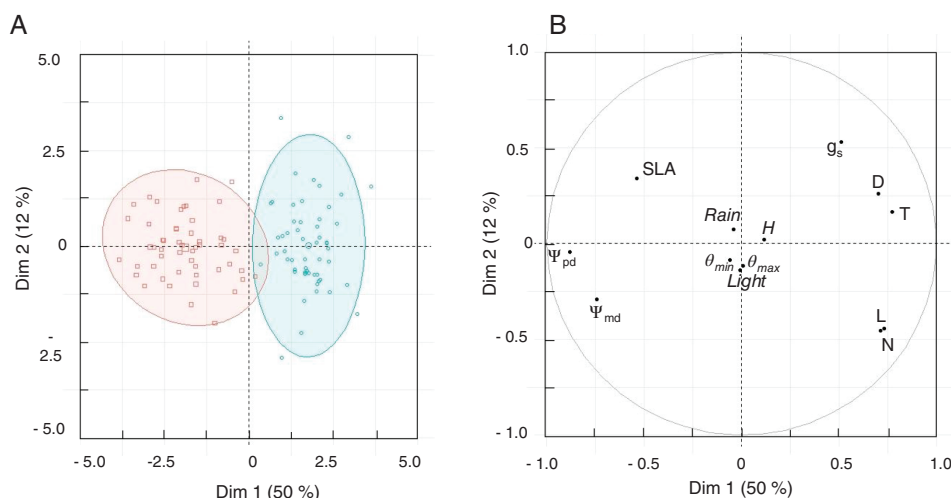


Fig. 2. PCA on physiological and growth traits of 14 black poplar tree genotypes at the maximal water deficit (day 8). Individual trees projected on the first two PCA dimensions, representing 62% of global variance. (A) Droughted (red dots) and well-watered (blue dots) trees with their 95% confidence ellipses. (B) Eight physiological and growth variables (with correlation circles) projected on the first two PCA dimensions, including five climatic and tree traits supplementary variables (italic). g_s = stomatal conductance; Ψ_{pd} = predawn leaf water potential; Ψ_{md} = midday leaf water potential; D = main branch diameter gain; L = new branch length gain; T = total leaf number variation; N = number of new leaves per branch; SLA = specific leaf area; θ_{min} = minimum annual average temperature; θ_{max} = maximum annual average temperature; Rain = mean number of rainy days per years; Light = mean number of sunny days per years; H = tree height before experiment.

Table 2. Test of genotype and treatment effect on physiological and growth traits of 14 black poplar genotypes at the maximal water deficit (day 8) using two-way ANOVA. *Significant difference for $P < 0.05$.

Description of physiological traits	Two-way ANOVA P -values significance		
	Genotype	Treatment	Genotype $\times$ Treatment
<i>Water movement traits</i>			
Stomatal conductance (g_s , $\text{mmol m}^{-2} \text{s}^{-1}$)	0.175	<0.001*	0.458
Predawn leaf water potential (Ψ_{pd} , MPa)	0.096	<0.001*	0.369
Midday leaf water potential (Ψ_{md} , MPa)	0.602	<0.001*	0.726
<i>Growth and development traits</i>			
Branch			
Main branch diameter gain (D, mm)	0.030*	<0.001*	0.294
New branch length gain (L, cm)	0.009*	<0.001*	0.039*
Leaf			
Total leaf number variation (T, %)	0.135	<0.001*	0.018*
Number of new leaves per branch (N)	0.092	<0.001*	0.556
SLA ($\text{mm}^2 \text{mg}^{-1}$)	0.331	<0.001*	0.069

not bring significant contribution to the variance on the main plane. Projecting genotypes onto the PCA main plane revealed weak correlation with the second dimension (correlation=0.11, $P > 0.05$). Climatic parameters describing the original environment of genotypes and the height of trees, analyzed as supplementary variables, did not yield any significant contribution to global variance on the main PCA plane.

Water movements

Trees lost on average $7.7 \pm 0.3\%$ of water content ($P < 0.001$) when water deficit was maximal at day 8

(Fig. S1). There were no significant differences between genotypes ($P > 0.05$).

Water deficit induced a steady decrease of Ψ_{pd} in droughted trees until day 8 ($P < 0.001$; Table 2) reaching different rates according to genotype: from -1.9 ± 0.2 MPa for SPM-12 to -4.1 ± 0.3 MPa for ERS-12 (Fig. 3). At day 8, Ψ_{md} of well-watered trees ranged from -0.7 ± 0.1 MPa to -1.4 ± 0.2 MPa between genotypes. Among droughted trees, Ψ_{md} fell ($P < 0.001$; Table 2) from -1.8 ± 0.2 MPa for SPM-12 to -3.7 ± 0.3 MPa for ERS-12 genotype (Fig. 3), without any significant differences among genotypes ($P > 0.05$).

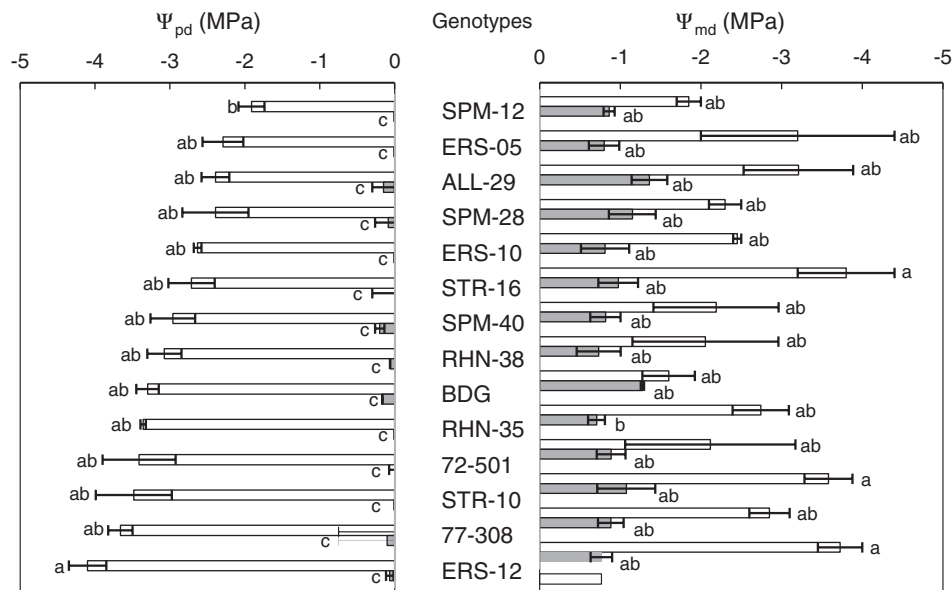


Fig. 3. Mean ($\pm$ SE) predawn (Ψ_{pd}) and midday (Ψ_{md}) leaf water potential measured on 14 well-watered (gray bars) and droughted (white bars) black poplar genotypes at the maximal water deficit (day 8). Results of two-way ANOVA and post hoc HSD Tukey's test are indicated by letters. Same letters indicate no differences between groups.

Plant growth and development

Total leaf number variation (T) increased by $53 \pm 16\%$ for control trees on average (from 414 ± 14 to 516 ± 20 leaves; Fig. 4A). It peaked on average at $35\% \pm 5$ among droughted trees (from 388 ± 14 to 229 ± 20 leaves; $P < 0.001$; Table 2) reaching 90% for genotypes 77–308 and ERS-12 (Fig. 4A) because of leaf fall following drought stress. Genotype and treatment effects interacted significantly for this trait ($P = 0.018$; Table 2).

Globally, number of new leaves (N) was significantly reduced by water deficit ($P < 0.001$; Table 2), although this trend was not always significant at the genotype level. However, N of two droughted genotypes, BDG and STR-16 ($P < 0.01$; Fig. 4B), decreased by twofold compared with control trees. Similarly, a significant increase of SLA in water deficit treatment was observed ($P < 0.001$; Table 2; Fig. S2) but no difference was noted between genotypes.

The main effect of water deficit was clearly detected on longitudinal (L) and radial (D) growth ($P < 0.001$; Table 2). However, only genotype $\times$ treatment interaction was significantly detected for L ($P = 0.039$; Table 2). When examined at the genotype level, decrease in L under water deficit was significant for BDG, RHN-35, SPM-28 and SPM-40 ($P < 0.05$; Table S1). The main effect of water deficit on D decrease was the most marked in STR-10 and RHN-35 genotypes ($P < 0.05$; Table S1).

Phenotypic plasticity

Plasticity of physiological and growth traits was derived as RDPI for each genotype, and analyzed using PCA (Fig. 5). Variance was mostly explained by the first three PCA dimensions (26, 22 and 21%, respectively). The first two dimensions represented 48% of global variance (Fig. 5A). p_L and p_Psi_{pd} contributed weakly to the variance of the first dimension (29 and 27%; Table S2). The variance represented on the second dimension was mainly because of p_T contributing to 47%, followed by p_Psi_{md} , contributing to 18%. The genotype factor explained 55, 54 and 30% of the variance of the first three dimensions respectively (Fig. 5C, D). ERS-05 was significantly and positively correlated to the first dimension ($P = 0.01$) although SPM40 ($P = 0.01$) and BDG ($P = 0.002$) were negatively correlated (Fig. 5C). ERS-12 and RHN-35 were positively correlated with the second dimension ($0.03 < P < 0.04$) and anticorrelated with SPM-12, SPM-40 and ALL-29 ($P = 0.02$). BDG was the only genotype significantly correlated with the third dimension ($P = 0.01$). p_Psi_{pd} , p_T and p_D were associated with the genotypes significantly well represented on the first three dimensions. The significant genotype effect was confirmed on these three traits ($P < 0.05$; Table S3).

PCA highlighted four traits for which plasticity was genotype-specific: p_Psi_{pd} , p_D , p_L and p_T . p_Psi_{pd} showed the highest RDPI (Table 3) among all the genotypes ($RDPI > 0.9$). p_D ranged between 0.18 ± 0.05 (72–501) and 0.57 ± 0.02 (77–308), p_L

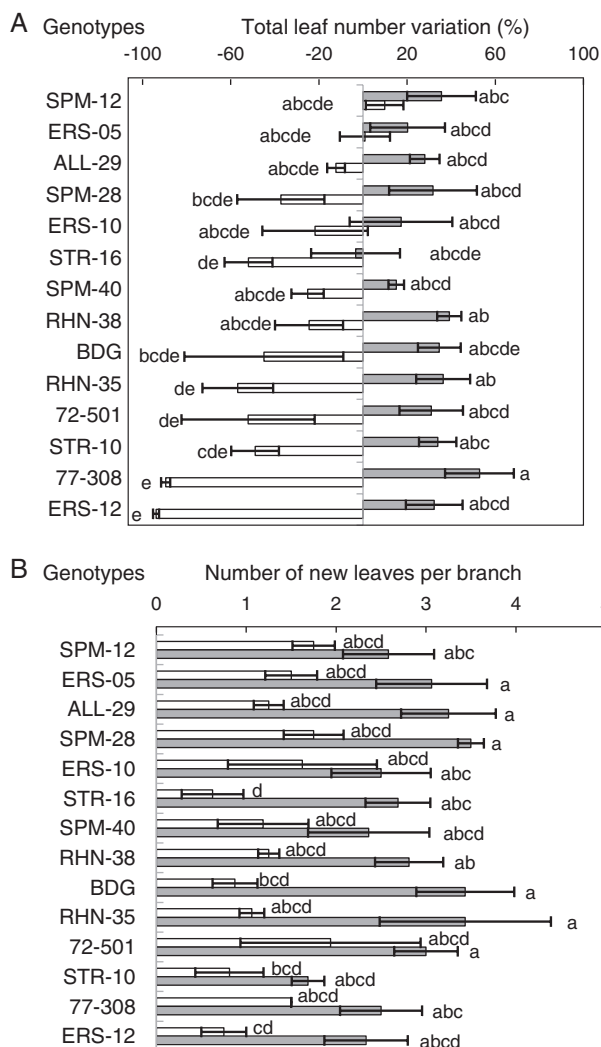


Fig. 4. Mean ($\pm$ se) total leaf number variation (A) and number of new leaves per branch (B) of 14 well-watered (gray bars) and droughted (white bars) black poplar genotypes at the maximal water deficit (day 8). Results of two-way ANOVA and post hoc HSD Tukey's test are indicated by letters. Same letters indicate no differences between groups.

from 0.27 ± 0.03 (ERS-05) and 0.67 ± 0.19 (SPM-28), and p_T from 0.13 ± 0.02 (SPM-12) to 0.91 ± 0.01 (ERS-12). $p_{\Psi_{md}}$ was lower for BDG (0.15 ± 0.08) than for the other genotypes (from 0.36 ± 0.02 for SPM-12 to 0.66 ± 0.01 for ERS-12). p_N ranged between 0.20 ± 0.04 (SPM-12) and 0.65 ± 0.13 (STR-16).

Discussion

Black poplars belong to riparian species with high evaporative demand during juvenile stage (Bazzaz 1979). Surprisingly, this pioneer species could also colonize dry soil habitats (Rameau et al. 1989). One way to explore its acclimatization capacity face to future climate

disturbances is to study its response trait plasticity. In this study, 14 contrasting native black poplar genotypes were exposed to progressive severe water deficit to explore the diversity of responses of this species. Our main goal was to evaluate the importance of plasticity in plant response strategy to water deficit.

Black poplars were severely stressed after an 8-day water deficit

Considering global variance of each physiological trait measured (Fig. 2), an 8-day water deficit was enough to induce a strong response for all the 14 black poplar genotypes. They were characterized by well-known changes in physiological traits, confirming their reached severe water deficit status as reported for many species. We observed a rapid decrease in stomatal conductance (Tardieu and Simonneau 1998, Marron et al. 2002, Marron et al. 2003, Aasamaa et al. 2004, Bogeat-Triboulot et al. 2007, Almeida-Rodriguez et al. 2010), a progressive increase of tension in the water column (Shackel et al. 1997, Fu et al. 2006, Almeida-Rodriguez et al. 2010, Coccozza et al. 2010, Barchetet al. 2013), leaf abscission (Marron et al. 2002, Viger et al. 2016), and a reduction of radial and longitudinal growth (Morabito et al. 2006, Giovannelli et al. 2007). A relation between the decrease of water fluxes (g_s , Ψ_{pd} , Ψ_{md} ; Fig. 2B) and growth traits (T, N) was observed in all the genotypes, as previously described by Lambs et al. (2006), Chamailard et al. (2011) and Viger et al. (2016) on other black poplar genotypes.

Black poplar genotypes exhibited a wide range of response to water deficit

At the genotype level, variability of response to water deficit was detectable. ERS-12, 77-308, and STR-10 regulated mostly water movements (Ψ_{pd} , Ψ_{md}), while STR-16 and BDG were mainly characterized by a decrease of growth (T, N). Little changes occurred in SPM-12, which sustained the highest Ψ_{pd} (-1.9 MPa), still producing new leaves and keeping existing leaves during water deficit. On the other end of the response gradient, ERS-12, 77-308, and STR-10 experienced Ψ_{pd} around -4 MPa and stopped growing. To ensure that all the genotypes were able to continue growth as normal following, we re-watered them and measured their growth restart before the end of the growing season (Table S1). At that point, one genotype (ERS-12) failed to recover its growth, possibly because of vascular system and/or photosynthesis pathway damages (Fichot et al. 2015, Jajic et al. 2015, Chen et al. 2016). The following year, we checked the growth of all the plants. All the genotypes, even those experiencing severe stress with extreme Ψ_{pd} were able

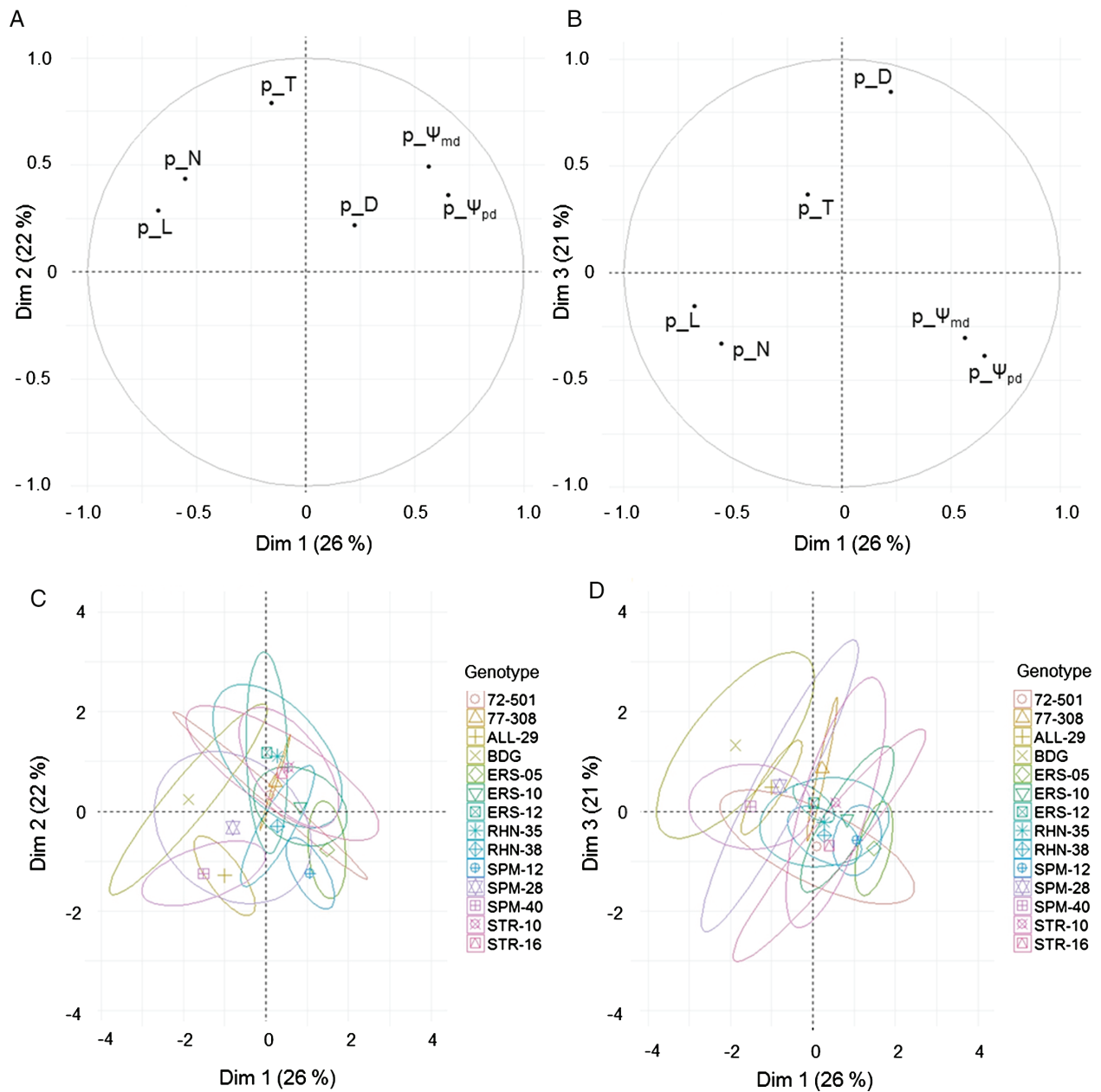


Fig. 5. PCA of phenotypic plasticity as a RDPI calculated for six traits on 14 black poplar genotypes at the maximal water deficit (day 8). Data structuring is detected on (A, C) the first plane (dimension 1 and 2) and (B, D) the second plane (dimensions 1 and 3). (A, B) Correlation circles of traits RDPI on the first three dimensions. (C, D) Trees are identified according to genotype factor on the two planes with 95% confidence ellipses drawn around each genotype barycenter. p_{-} = plasticity of; Ψ_{pd} = predawn leaf water potential; Ψ_{md} = midday leaf water potential; D = main branch diameter gain; L = new branch length gain; T = total leaf number variation; N = number of new leaves per branch.

to recover from this severe water deficit (including ERS-12 plants). Similar observations have been made on severely droughted poplar species (Brignolas et al. 2000, Marron et al. 2003, Almeida-Rodriguez et al. 2010, Coccozza et al. 2010, Barchet et al. 2013).

Genotypes modulated evapotranspiration similarly during water deficit: it is thus surprising that SPM-12

has been able to grow in such conditions. Black poplars mostly responded by leaf fall during water deficit except for two genotypes (SPM-12 and ERS-05). In black poplars (Regier et al. 2009, Coccozza et al. 2010) and several other poplar species (Pelah et al. 1997, Marron et al. 2002, Bogeat-Triboulot et al. 2007, Barchet et al. 2013, Cao et al. 2013, Hamanishi et al. 2015), authors have

Table 3. Mean ($\pm$ se) RDPI of 14 black poplar genotypes at the maximal water deficit (day 8) for six physiological traits.

Genotypes	$p_{\Psi_{pd}}$	p_D	p_L	p_T	$p_{\Psi_{md}}$	p_N
SPM-12	1.00 $\pm$ 0.01	0.27 $\pm$ 0.05	0.41 $\pm$ 0.04	0.13 $\pm$ 0.02	0.36 $\pm$ 0.02	0.20 $\pm$ 0.04
ERS-05	1.00 $\pm$ 0.01	0.30 $\pm$ 0.05	0.27 $\pm$ 0.03	0.14 $\pm$ 0.03	0.57 $\pm$ 0.09	0.33 $\pm$ 0.07
ALL-29	0.89 $\pm$ 0.01	0.38 $\pm$ 0.04	0.48 $\pm$ 0.08	0.19 $\pm$ 0.02	0.39 $\pm$ 0.07	0.43 $\pm$ 0.05
SPM-28	0.93 $\pm$ 0.01	0.42 $\pm$ 0.20	0.67 $\pm$ 0.19	0.38 $\pm$ 0.15	0.36 $\pm$ 0.03	0.34 $\pm$ 0.07
ERS-10	1.00 $\pm$ 0.01	0.44 $\pm$ 0.06	0.47 $\pm$ 0.07	0.30 $\pm$ 0.12	0.53 $\pm$ 0.01	0.39 $\pm$ 0.07
STR-16	1.00 $\pm$ 0.01	0.41 $\pm$ 0.15	0.41 $\pm$ 0.13	0.35 $\pm$ 0.06	0.60 $\pm$ 0.03	0.65 $\pm$ 0.13
SPM-40	0.89 $\pm$ 0.01	0.28 $\pm$ 0.01	0.60 $\pm$ 0.03	0.22 $\pm$ 0.04	0.39 $\pm$ 0.14	0.43 $\pm$ 0.12
RHN-38	0.96 $\pm$ 0.01	0.25 $\pm$ 0.01	0.45 $\pm$ 0.05	0.31 $\pm$ 0.08	0.52 $\pm$ 0.09	0.38 $\pm$ 0.04
BDG	0.91 $\pm$ 0.01	0.50 $\pm$ 0.13	0.57 $\pm$ 0.05	0.66 $\pm$ 0.14	0.15 $\pm$ 0.08	0.59 $\pm$ 0.09
RHN-35	1.00 $\pm$ 0.01	0.41 $\pm$ 0.02	0.55 $\pm$ 0.16	0.54 $\pm$ 0.10	0.59 $\pm$ 0.04	0.49 $\pm$ 0.04
72-501	1.00 $\pm$ 0.01	0.18 $\pm$ 0.05	0.47 $\pm$ 0.19	0.55 $\pm$ 0.16	0.48 $\pm$ 0.07	0.40 $\pm$ 0.09
STR-10	1.00 $\pm$ 0.01	0.52 $\pm$ 0.17	0.56 $\pm$ 0.04	0.46 $\pm$ 0.08	0.56 $\pm$ 0.02	0.43 $\pm$ 0.18
77-308	0.95 $\pm$ 0.01	0.57 $\pm$ 0.02	0.49 $\pm$ 0.01	0.87 $\pm$ 0.02	0.53 $\pm$ 0.02	0.23 $\pm$ 0.01
ERS-12	0.97 $\pm$ 0.01	0.45 $\pm$ 0.05	0.59 $\pm$ 0.24	0.91 $\pm$ 0.01	0.66 $\pm$ 0.01	0.50 $\pm$ 0.09

reported that species without leaf fall under drought may favor solute availability and sustain cellular homeostasis. The question remains unanswered for SPM-12, in which growth was maintained during water deficit.

SPM-12 strategy face to water deficit conditions was considered as 'tolerant' according to previous findings on poplar species (Marron et al. 2002, Monclus et al. 2006, Coccozza et al. 2010). By contrast, the strategy of ERS-12 was closer to the description of 'avoidant', as described for *P. simonii* $\times$ *P. balsamifera* (Almeida-Rodriguez et al. 2010). Based on this definition, the 12 other black poplar genotypes could also be regarded as 'avoidant'. Although poplar is known to be meso-hygrophilous to slightly hygrophilous in regularly flooded environments, it can sustain growth under occasional short drought periods, in accordance with some reports of French black poplar colonizing dry substrates (Rameau et al. 1989).

Phenotypic plasticity related to drought strategy was genotype-specific

Phenotypic plasticity (Fig. 5) was evaluated for traits related to water movements ($p_{\Psi_{pd}}$, $p_{\Psi_{md}}$) and growth (p_L , p_D , p_T , p_N). Multivariate analysis retained $p_{\Psi_{pd}}$, p_D , p_L and p_T as genotype-specific, characterizing the diversity of plasticity responses of black poplar genotypes. Growth traits show medium plasticity levels as previously observed for black poplar genotypes (Guet et al. 2015), *Populus* $\times$ *euramericana* (Bizet et al. 2014), *Pinus pinaster* (Alía et al. 1997, Lamy et al. 2014), *Pinus halepensis* (Baquedano et al. 2008) or *Quercus coccifera*. Discriminatory power of plasticities among genotypes was higher for the growth traits p_D , p_T , p_L than for $p_{\Psi_{pd}}$, owing to large differences in plasticity levels between genotypes (e.g. 0.13 < RDPI

<0.9 for p_T). The highest plastic trait to water deficit was $p_{\Psi_{pd}}$ with RDPI from 0.89 to 1.0 (Table 3).

Black poplar genotypes developed different plasticity patterns to withstand a short-term severe water deficit (Fig. 5): ERS-05 was characterized by a high $p_{\Psi_{pd}}$ and a low p_L/p_D contrary to BDG and SPM-40. Two genotypes (ERS-12 and RHN-35) were associated with high p_T in contrast with SPM-12, SPM-40 and ALL-29. In addition, the least plastic black poplar genotype (SPM-12, Table 3) was tolerant to severe water deficit, whereas the most plastic genotype (ERS-12) was avoidant. Earlier reports, of low plasticities of fitness-related traits (i.e. total biomass and tillering, Couso and Fernandez 2012), Ψ_{50} (Fichot et al. 2010), water fluxes, gas exchange and antioxidant traits (Baquedano et al. 2008) associated with drought tolerance, support this result. Differences in plasticity among genotypes could be partially explained by different selection pressure on traits occurring in their native environments (Valladares et al. 2007). Plants adapted to a predictable environment and under low selection pressure, offer low plasticity in response to non-predictable water deficit because they are unable to adjust rapidly their physiological traits (Henry and Aarssen 1997, Valladares et al. 2005, Ghalambor et al. 2007, Willis et al. 2008, Reed et al. 2010). A low plasticity can be efficient to sustain primary metabolism (Valladares et al. 2000). However, these plants take a risk: low plastic genotypes are usually close to their hydraulic safety margin and incur hydraulic failure (McDowell et al. 2008). Conversely, species responding to water deficit by high plasticity could allocate resources to non-essential phenotypic modifications, which could be detrimental to their fitness (Alpert and Simms 2002, Steinger et al. 2003). Parmesan and Hanley (2015) pointed out the complex impact of plasticity on plant performances under climate

change, which also depends on non-climatic constraints (i.e. interactions between species). Transplantation experiments suggested that their plasticity level depend on species (Willis et al. 2008, Early and Sax 2014, Hargreaves et al. 2014, Dalmaris et al. 2015) and was not necessarily conditioned by climatic factors (Early and Sax 2014, Bradley et al. 2015).

In our experiment, medium-to-high plasticity was concomitant to avoidance for 13 black poplar genotypes out of the 14 tested. Unexpectedly, the more plastic genotype was the more avoidant whereas the least plastic one was drought tolerant. Our results suggested that an avoidant strategy combined with a certain amount of response trait plasticity might reduce the plant performances for resources allocation and threatened poplar persistence in occasionally droughted environments. A better understanding of the molecular basis of the phenotypic plasticity could be approached by studying the relationships between phenotypic plasticity and transcriptional responses of poplars subjected to drought, as recently shown by some authors (i.e. Cohen et al. 2010, Hamanishi et al. 2010, Hamanishi et al. 2015, Viger et al. 2016).

Author contributions

B. F. designed the study; F. G., B. F., P. B., P. C. and R. S. performed the ecophysiological measurements; M. G. T. and B. F. performed the statistical analysis; P. L., A. G., C. B., J. S. V., D. L. and G. P. contributed to the interpretation of results; M. G. T., B. F., A. G. and P. L. wrote the manuscript, with the contribution of all authors.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Branch growth traits of 14 well-watered (WW) and droughted (WS) black poplar genotypes subjected to 8-day water deficit.

Table S2. Principal component analysis loadings of the six RDPI traits.

Table S3. Test of genotype effect on plasticity degree using RDPI estimated on physiological and growth traits of 14 black poplar genotypes at the maximal water deficit (day 8).

Fig. S1. Mean percentage evolution of weight loss of 14 black poplars growing in pots subjected to progressive 8-day water deficit.

Fig. S2. Mean ($\pm$ se) Specific Leaf Area of 14 well-watered (gray bars) and droughted (white bars) black poplar genotypes at the maximal water deficit (day 8).

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