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ORIGINAL ARTICLE

Narrow-sense heritability and P_{ST} estimates of DNA methylation in three *Populus nigra* L. populations under contrasting water availability

Mamadou Dia Sow[1] · Vincent Segura[2] · Sylvain Chamaillard[1] · Véronique Jorge[2] · Alain Delaunay[1] · Clément Lafon-Placette[1,3] · Régis Fichot[1] · Patricia Faivre-Rampant[4] · Marc Villar[2] · Franck Brignolas[1] · Stéphane Maury[1]

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Abstract

In a context of climate change and forest decline, a better understanding of the sources of tree flexibility involved in phenotypic plasticity and adaptation is needed. These last years, the role of epigenetics in the response to environmental variations has been established in several model plants at the genotype level but little is known at the level of natural populations grown in pedoclimatic sites. Here, we focused on three French natural populations of black poplar, a key pioneer tree from watersheds, planted in common garden and subjected to controlled variations of water availability. We estimated common genetic parameters such as narrow-sense heritability (h^2), phenotypic differentiation index (P_{ST}), and the overall genetic differentiation index (F_{ST}) from genome-wide SNPs to evaluate the extent of epigenetic variations. Indeed, global DNA methylation levels from individuals exposed to drought or irrigated in a common garden were used. We found that the three populations were not distinguished by their levels of DNA methylation. However, a moderate drought was associated to a significant decrease in DNA methylation in the populations. Narrow-sense heritability and P_{ST} estimates of DNA methylation were similar to those found for biomass productivity. Heritability and P_{ST} were higher when trees were subjected to drought than in control condition. Negative genetic correlations between global DNA methylation and height or biomass were detected in drought condition only. Altogether, our data highlight that global DNA methylation acts as a genetic marker of natural population differentiation under drought stress in a pedoclimatic context.

Keywords DNA methylation · h^2 · P_{ST} · F_{ST} · Poplar · Water availability

Introduction

Trees are sessile organisms with a long lifespan that are constantly exposed to environmental changes over decades. In order to survive, they need to develop mechanisms enabling them to respond and to survive to recurring stress (Bruce et al. 2007). Over the last decades, forest tree declines have been reported around the world in relation to heat stress and drought (Allen et al. 2010; IPCC 2014). The ability of trees to survive through their phenotypic plasticity or potential of adaptation is a fundamental question. However, the sources of flexibility in trees and the underlying molecular mechanisms have not been

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✉ Stéphane Maury
stephane.maury@univ-orleans.fr

[1] LBLGC, INRA, Université d'Orléans, EA 1207 USC 1328, 45067 Orleans, France

[2] BioForA, INRA, ONF, 45075 Orleans, France

[3] Present address: Department of Botany, Charles University, CZ-12800 Prague, Czech Republic

[4] INRA, US1279 EPGV, CEA-IG/CNG, 91057 Evry, France

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fully established (van Kleunen and Fischer 2005; Bradshaw 2006; Nicotra et al. 2010; Bräutigam et al. 2013; Plomion et al. 2016). In this context, it has been shown that the genetic variability (sequence variations) is not the only source of phenotypic variation and heritability and this phenomenon was called missing heritability (Maher 2008). Among possible mechanisms, epigenetics with the modifications of chromatin marks such as DNA methylation and histone marks altering gene expression seems of first importance (Nicotra et al. 2010; Mirouze and Paszkowski 2011; Feil and Fraga 2012; Niederhuth et al. 2016). The influence of environmental factors on epigenetic marks, and on the resulting changes in gene expression and phenotype, has attracted considerable attention these last years particularly in model plants such as Arabidopsis, maize, poplar but mainly at the individual (genotype) level (Bräutigam et al. 2013; Plomion et al. 2016; Richards et al. 2017). Epigenetic mechanisms could constitute an additional layer of heritable phenotypic variation and evolutionary potential for natural populations since these modifications can be reversibly induced by environmental stimuli and eventually inherited by future generations (Law and Jacobsen 2010; Richards et al. 2017). However, the ecological significance in terms of acclimation and adaptation still needs evidence particularly using field studies and population epigenomics (Richards et al. 2017). In addition, the interacting complexity between genetic and epigenetic variations and the evolutionary role of these variations in natural populations have only been recently investigated in few studies in plants (Shen et al. 2014; Dubin et al. 2015; Robertson et al. 2017). Finally, all these recent studies are mainly on annual plants (Dubin et al. 2015; Kawakatsu et al. 2016; Verhoeven et al. 2016; Richards et al. 2017; Sork 2017) and there is a need of data for natural tree populations originated from different pedoclimatic sites.

Poplar (*Populus* spp.) is a model tree with sequenced genomes (http://www.phytozome.net/poplar.php), genetic diversity, fast juvenile growth, and large water requirements (Tuskan et al. 2006; Jansson and Douglas 2007) relevant for dissecting the ecophysiological and molecular determinants of tolerance to water deficit in trees (Marron et al. 2003; Monclus et al. 2006; Fichot et al. 2015; Hamanishi et al. 2012; Bizet et al. 2015; Toillon et al. 2016). Accordingly, poplar has been particularly used to investigate the role of epigenetics in the response of trees to environmental variations (Bräutigam et al. 2013; Plomion et al. 2016). DNA methylation was shown to vary across hybrids, in correlation to their biomass productivity and in response to water deficit (Gourcilleau et al. 2010; Raj et al. 2011). In addition, characteristics of gene-body DNA methylation and relationships with tissue-specific or stress gene expression were established (Vining et al. 2012; Lafon-Placette et al. 2013; Bräutigam et al. 2013; Liang et al. 2014; Lafon-Placette et al. 2018). Recently, the epigenetic component of site-dependent growth performances (Guarino et al. 2015; Schönberger et al. 2016) or the developmental phenotypic plasticity at the shoot apical meristem level in response to environmental bud break conditions (Conde et al. 2017) and water availability (Lafon-Placette et al. 2018) was reported.

In this study, we focused on natural populations of European black poplar (*Populus nigra* L.), a key pioneer tree species widely used not only for its economic interest as a parent pool for genetic breeding of *Populus* × *canadensis* poplars but also for its ecological value in the dynamics of riparian habitats and soil stabilization. However, *P. nigra* is one of the most threatened tree species in Europe as a result of global climate changes, fragmentation and loss of its native habitat and lack of genetic diversity (de Rigo et al. 2016). We assessed the extent of genetic variation of global DNA methylation from three natural populations of *P. nigra* acclimated in a common garden and evaluated under irrigated and drought regimes. The ecophysiological characterization of these populations has already been reported (Chamaillard et al. 2011). Global DNA methylation, a widely used epigenetic indicator in plants (Lambé et al. 1997; Trap-Gentil et al. 2011; Teyssier et al. 2014; Alonso et al. 2015; Alonso et al. 2016; Plomion et al. 2016), was assessed in the shoot apical meristem of cloned individuals of the three populations under irrigated and non-irrigated regimes as previously reported (Gourcilleau et al. 2010; Lafon-Placette et al. 2013; Condé et al. 2017; Lafon-Placette et al. 2018). We made use of recently available SNP data (Faivre-Rampant et al. 2016) in order to reconstruct genomic relationships between genotypes within and between populations and estimated the narrow-sense heritability (h^2) and phenotypic differentiation index (P_{ST}) for global DNA methylation. Finally, we estimated genetic correlations between global DNA methylation and biomass productivity traits.

Materials and methods

Plant material and experimental design

The three *P. nigra* populations (NOH, RAM, and SPM) originate from France were investigated in this study. The NOH population originates from Southern France along the Nohèdes river (42° 37′ 24″ N, 2° 16′ 36″ E, 1000 m), the RAM population from South-Eastern France along the Drôme river (44° 45′ 08″ N, 4° 54′ 01″ E, 187 m), and the SPM population from Central France along the Loire river (47° 52′ 19″ N, 1° 49′ 24″ E, 91 m) (Chamaillard et al. 2011). Clonal copies of genotypes from the three populations were grown under two watering regimes as reported in Chamaillard et al. (2011). Cuttings were sampled on 30 sexually mature trees without any prior phenotypic selection in

each population and were then planted in two plots in a common garden in spring 2006 (INRA Centre Val-de-Loire research site Loiret, France). The two plots were split into five randomized complete blocks with one cutting of each genotype per block (i.e., 30 genotypes × 3 populations in each block). In 2007, the two plots were equally watered with overhead sprinklers during the growing season in order to promote growth. In 2008, irrigation was withheld in one of the two plots from June 18, 2008 to the end of the growing season (drought), while the second one remained regularly irrigated. Predawn leaf water potential (Ψ_{pd}) was monitored each week from June 18 to August 18, 2008 using a pressure chamber (PMS Instruments, Albany, OR, USA) and was used as an index of soil water availability. From mid-June to mid-August, Ψ_{pd} remained > -0.18 MPa in the irrigated regime while a progressive drop was recorded in drought condition with a peak reaching -0.52 MPa on July 24. Annual shoot fresh mass (biomass, g) and annual height growth increment (cm), along with other traits, were assessed in order to evaluate the effect of the drought stress on these trees (Chamaillard et al. 2011). During winter 2008–2009, due to sampling constraints, Shoot Apical Meristems (SAM) were only collected on 42 genotypes (11 NOH, 15 RAM and 16 SPM) in one block for each of the two watering regimes (irrigated and drought) as described by Lafon-Placette et al. (2013) and used to extract genomic DNA.

DNA methylation analysis

Genomic DNA was extracted from SAMs as previously described by Porebski et al. (1997). Briefly, sampled SAMs were ground into a fine powder in liquid nitrogen and genomic DNA was extracted using CTAB buffer. RNA digestion was realized by RNAse A (Sigma-Aldrich, Saint Quentin Fallavier, France) and precipitation in isopropanol buffer. DNA was then hydrolyzed in nucleosides and analyzed by high-performance liquid chromatography (HPLC) as previously described by Zhu et al. (2013). About 5 μg of the extracted genomic DNA was digested in 700 U of DNAse I (Roche Diagnostic, France). Phosphodiesterase I (0.05 U) (Pharmacia, France) and 0.5 U of alkaline phosphatase III (Sigma, France) were used in order to remove phosphate groups. The free nucleosides were then purified (Ethanol 96%), and global DNA methylation was assessed by HPLC. A hydrophobic column Gemini™ (150 × 4.6 mm, 5 μm, Phenomenex, France) was used. The solution of the free nucleosides was loaded onto the hydrophobic column containing an aqueous buffer with 0.5% of methanol (*v*/*v*) and 5 mM of acetic acid. The flow was set at 1.5 ml/min. Commercial standard of 2′-deoxycytidine (C) and 5-methyl-2′-deoxycytidine (mC) was used to set the standard curve allowing to identify and quantify the amount of C and mC. Global DNA methylation levels were then determined using the following formula: % mC = (mC / (mC + C)) × 100. To test possible contamination by RNAs, commercial standards were also injected. For each sample, two independent hydrolyses and two HPLC runs for each hydrolysis at least were realized.

Statistical analysis

Statistical analyses were realized with the R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Global DNA methylation changes, treatment, and population effects were estimated by analysis of variance (ANOVA). Genetic and residual correlations were calculated using the "sommer" package (Covarrubias-Pazaran 2016). Statistical tests were considered significant at $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$.

SNP data, genomic relationship, and population structure

SNP data on the 42 genotypes studied have been previously reported (Faivre-Rampant et al. 2016; Le Paslier et al. 2016). RAM and SPM samples were genotyped by using the BlackPoplar 12K Infinium BeadChip array and the NOH samples with a pilot multispecies-Plant Illumina BeadChip developed by the INRA-EPGV group. Briefly, they consisted in 7918 SNPs which specifically targeted candidate regions for quantitative traits of interest such as rust resistance, bud phenology, water use efficiency, and wood production and quality. Because the SNPs targeted specific regions, they were not evenly spaced on the 19 chromosomes of *Populus trichocarpa* genome, potentially resulting in uneven linkage disequilibrium between them and consequently bias in genomic heritability estimates (Speed et al. 2012). To account for such possible bias, we estimated a LD-adjusted genomic relationship matrix (GRM) using "LDAK" software as proposed by Speed et al. (2012). We further evaluated the population structure in our dataset by performing (i) a hierarchical ascendant clustering (Ward method) on the LD-adjusted GRM, converting relationships (k_{ij}) into dissimilarities (d_{ij}) as $d_{ij} = \max(\text{GRM}) - k_{ij}$) and (ii) a principal component analysis through an eigen decomposition of the LD-adjusted GRM. Finally, for further comparison with the phenotypic differentiation index (P_{ST}) (see below), the overall genetic differentiation index (F_{ST}) was computed over the populations under study using all SNPs with a minor allele frequency above 5% ($n = 7378$) and the Weir and Cockerham (1984) estimator as implemented in the R package "hierfstat" (Goudet and Jombart 2015).

Narrow sense heritability (h^2) and phenotypic differentiation index (P_{ST})

To estimate the genetic and residual variance-covariance matrices between all the traits measured across the two water regimes, we fitted the following multivariate linear mixed model with the mmer2 function of the R package "sommer" (Covarrubias-Pazaran 2016):

$$y = \begin{bmatrix} y_1 \\ \dots \\ y_n \end{bmatrix} = X\beta + Z_g g + \epsilon, \quad (1)$$

where β is the vector of fixed effects and **g** the random vector of additive genetic effects, with $\mathrm{var}(g) = \begin{bmatrix} \sigma^2_{g_1} & \cdots & \sigma_{g_{1n}} \\ \vdots & \ddots & \vdots \\ \sigma_{g_{n1}} & \cdots & \sigma^2_{g_n} \end{bmatrix} \otimes K_g$ and $\mathrm{var}(\epsilon) = \begin{bmatrix} \sigma^2_{\epsilon_1} & \cdots & \sigma_{\epsilon_{1n}} \\ \vdots & \ddots & \vdots \\ \sigma_{\epsilon_{n1}} & \cdots & \sigma^2_{\epsilon_n} \end{bmatrix} \otimes I$, $\mathrm{K_g}$ is the LD-adjusted GRM, and **I** an identity matrix.

The genetic covariance matrix enabled to compute for each trait the genomic narrow-sense heritability as well as the genetic and residual correlation matrices between all traits:

$h^2_i = \frac{\sigma^2_{g_i}}{\sigma^2_{g_i}+\sigma^2_{\epsilon_i}}$, $\rho_{g_{ij}} = \frac{\sigma^2_{g_i}}{\sqrt{\sigma^2_{g_i}\sigma^2_{g_i}}}$ and $\rho_{\epsilon_{ij}} = \frac{\sigma^2_{g_i}}{\sqrt{\sigma^2_{\epsilon_i}\sigma^2_{\epsilon_i}}}$, where $\sigma^2_{g_i}$ and $\sigma^2_{\epsilon_j}$ are estimates of additive genetic and residual variances for the trait i, and $\sigma_{g_{ij}}$, and $\sigma_{\epsilon_{ij}}$ are estimates of additive genetic and residual covariances between traits *i* and *j*.

Because of the low sample size, we could not perform a further decomposition of the genetic variation into within- and between-population variances which would enable an estimation of the genetic differentiation index (Q_{ST}) for each trait, even under a multivariate setting which would maximize the available information in the dataset. As an alternative, we computed the phenotypic divergence between populations as the P_{ST} using previous estimates of heritability together with variance components from the following multivariate mixed model fitted with the function mmer2 of the R package "sommer":

$$y = \begin{bmatrix} y_1 \\ \dots \\ y_n \end{bmatrix} = X\beta + Z_p p + \epsilon, \quad (2)$$

where β is the vector of fixed effects and p the random vector of cluster or population effects, with $\mathrm{var}(p) = \begin{bmatrix} \sigma^2_{p_1} & \cdots & \sigma_{p_{1n}} \\ \vdots & \ddots & \vdots \\ \sigma_{p_{n1}} & \cdots & \sigma^2_{p_n} \end{bmatrix} \otimes K_p$, and $\mathrm{K_p}$ is the LD-adjusted GRM between clusters or populations.

P_{ST_i} was calculated as follows: $P_{ST_i} = \frac{\sigma^2_{p_i}}{\sigma^2_{p_i}+2h^2_i\sigma^2_{ei}}$ with h^2_i estimated within Eq. 1 and where $\sigma^2_{p_i}$ and σ^2_{ei} are estimates of cluster or population and residual variances among and within-cluster or population respectively.

Results

DNA methylation variations among *P. nigra* populations and in response to water deficit

Global DNA methylation levels were evaluated from winter dormant buds 6 months after the summer period of water stress (Chamaillard et al. 2011; see Supplementary Table 1). The ANOVA model revealed no population × condition interaction effect. DNA methylation ranged from 15.9 to 55.7% between genotypes or treatments in the three populations. In both well-watered (irrigated) and water-deficit (drought) regimes, the three populations could not be distinguished according to their levels of DNA methylation. However, the global DNA methylation was significantly affected by water deficit (Fig. 1a). Biomass and height were significantly and positively correlated among them but not with levels of global DNA methylation (Fig. 1b; Supplementary Table 1).

Genetic structuration of the *P. nigra* populations

A hierarchical ascendant clustering on the genomic relationship matrix revealed four different clusters (Fig. 2a; Supplementary Tables 2 and 3). The first cluster was constituted of the NOH individuals ($n = 10$), the second of the RAM individuals ($n = 11$), and the third of the SPM individuals ($n = 10$). However, the fourth cluster was composed of both RAM and SPM individuals ($n = 11$). This group was likely due to introgression from the cultivated compartment (IT for InTrogressed). This genetic structure within our dataset was also clearly revealed by the PCA analysis carried out from the GRM (Fig. 2b). This underlying structure was therefore used in the following analyses by performing the F_{ST} and P_{ST} estimations on the four clusters defined by the clustering in addition to the three original populations (geographic). The average F_{ST} index over the four groups was equal to 5.48%. This value was slightly higher than the F_{ST} estimated on the three original populations (4.65%). DNA methylation variations among the four clusters were reassessed (Fig. 1c) but with no major change compared to the three original populations (Fig. 1a).

Heritability, P_{ST} estimates of DNA methylation, and genetic correlations

For all the traits studied, h^2 was higher when plants were grown under drought condition. Heritability estimates varied also between traits ranging from 0.15 for global DNA methylation in the irrigated condition to 0.56 for height in the drought stress. Narrow-sense heritability of global DNA methylation showed a twofold increase in drought compared to irrigated conditions ("0.32"; Fig. 3a). Phenotypic differentiation index estimates varied also depending on traits and growing conditions. They ranged from 0.01 (Global DNA methylation, irrigated) to 0.30

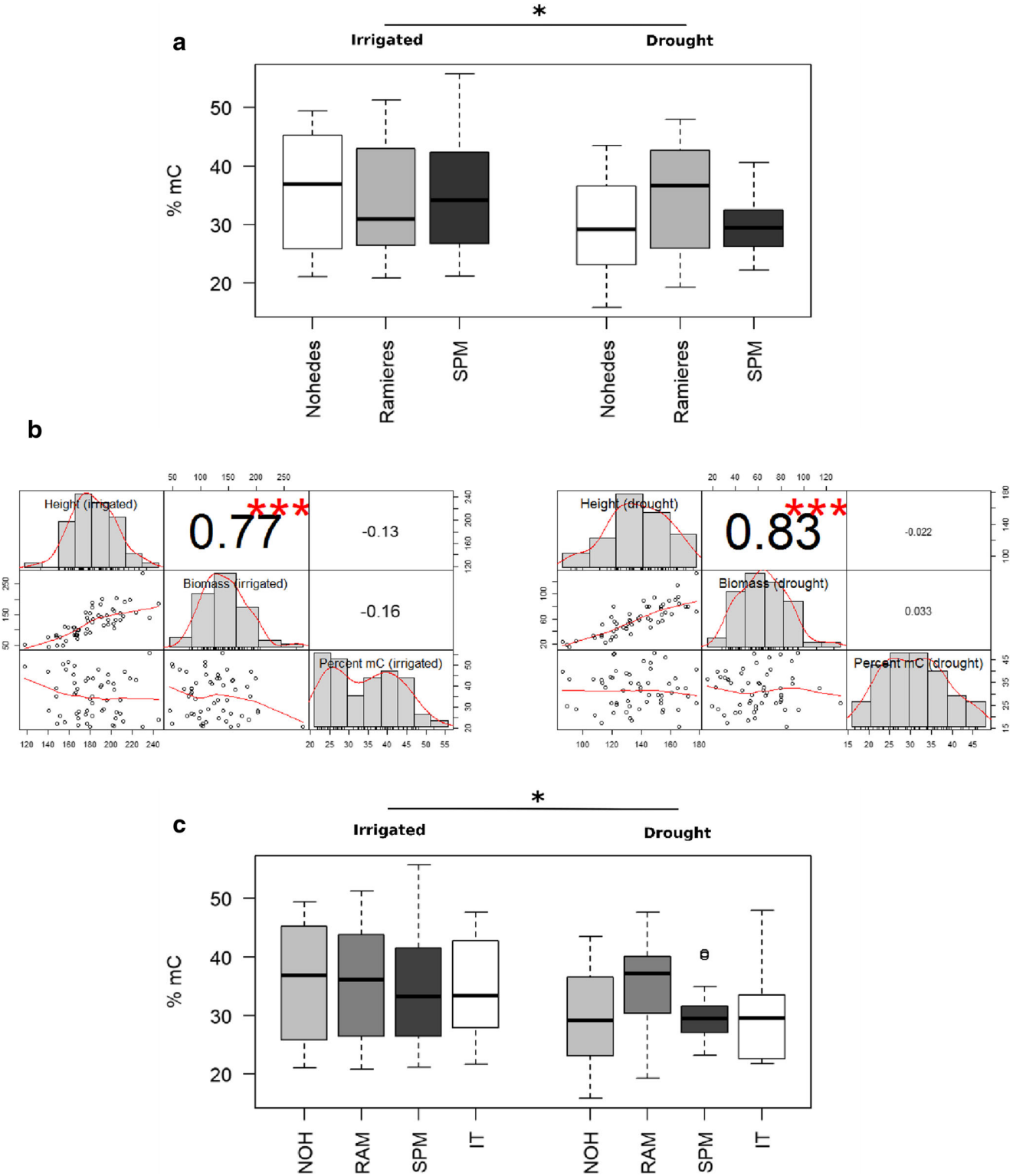

Fig. 1 Global DNA methylation variations and phenotypic correlations. **a** DNA methylation of SAM for three geographical populations of *P. nigra* (Nohèdes [NOH], Ramières [RAM], and Saint-Pryvé Saint-Mesmin [SPM]) grown in irrigated and drought regimes. NOH is represented by 11 genotypes, RAM by 15, and SPM by 16 genotypes. % mC percentage of methylcytosine. **b** Phenotypic correlations between global DNA methylation and biomass or height in irrigated (left) and drought (right) regimes. Significant differences are indicated: $*P < 0.05$, $**P < 0.01$, or $***P < 0.001$. **c** DNA methylation variations of SAM among the four groups defined by the clustering on the genomic relationship matrix (NOH, RAM, SPM, IT) grown in irrigated and drought regimes. NOH is represented by 10 genotypes, RAM by 11, SPM by 10, and IT by 11 genotypes

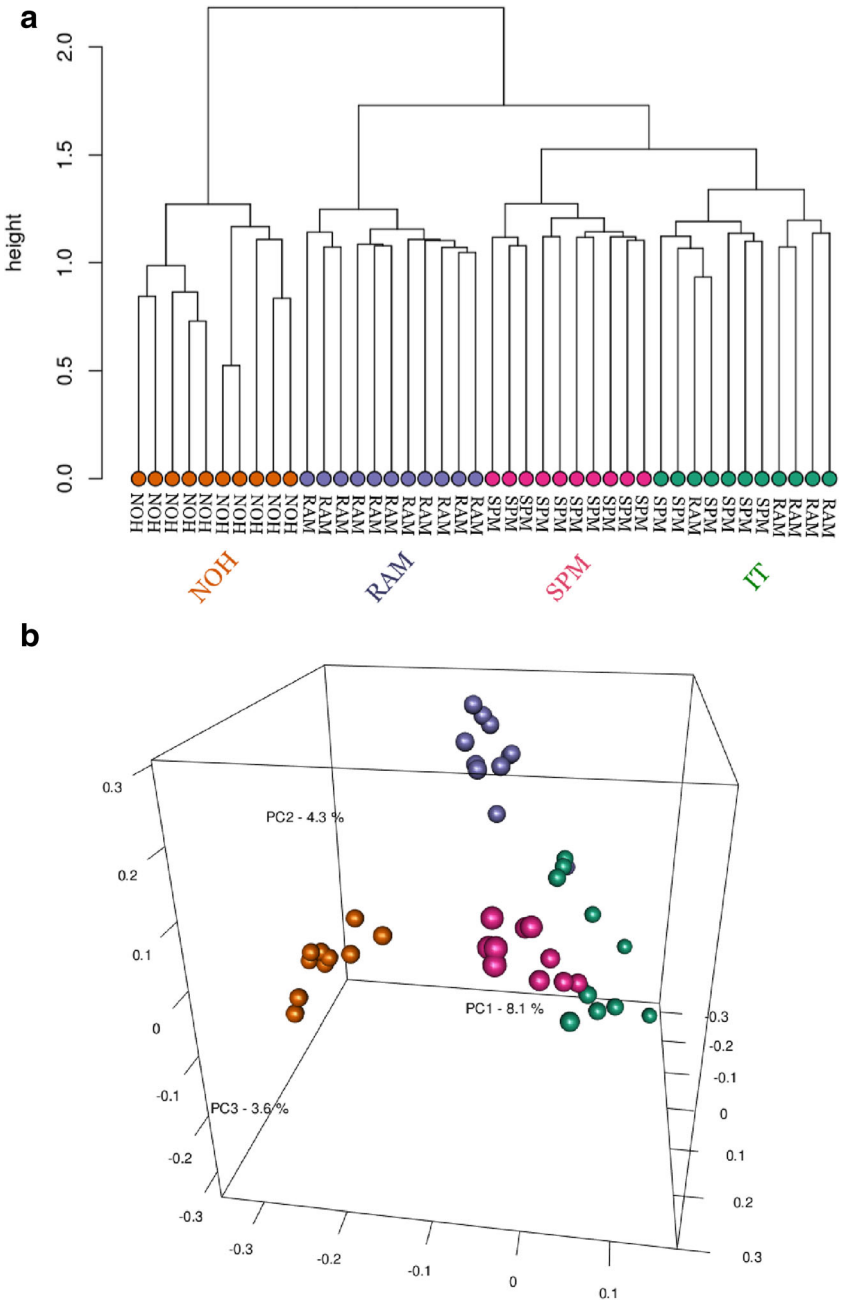

Fig. 2 Genetic architecture of the populations. **a** Genetic similarities and cluster tree were generated using ward hierarchical classification from genome-wide SNPs. The clustering on the GRM shows four different groups (NOH, RAM, SPM and IT) where IT refers to introgression of the cultivated compartment. **b** Principal component analysis of the four groups identified in the genomic kinship matrix. PC1 (8.1%), PC2 (4.3%), and PC3 (3.6%). NOH is represented by 10 individuals (yellow), RAM by 11 (blue), SPM by 10 (red), and IT by 11 individuals (green)

(Height, drought) with a global F_{ST} of 0.05. However, The P_{ST} of global DNA methylation exhibited a strong increase in drought stress (0.26) compared to the irrigated condition (0.01) or the global F_{ST} (0.05). These P_{ST} estimates obtained by taking in account our genetic structure (i.e., by considering the four groups) were slightly higher to the ones determined using the three original populations (Fig. 3b).

Finally, genetic and residual correlations between global DNA methylation and height or biomass were computed (Fig. 4). In non-irrigated regime only, biomass and height were negatively correlated to global DNA methylation level (-0.45 and -0.54, respectively; Fig. 4a). Positive residual correlations between DNA methylation level and biomass or height were detected under drought stress only (0.62 and 0.72 respectively; Fig. 4b).

Discussion

Global DNA methylation, a simple widely used epigenetic indicator

In plants, DNA methylation is associated with transposon silencing and complex interactions with gene expression that

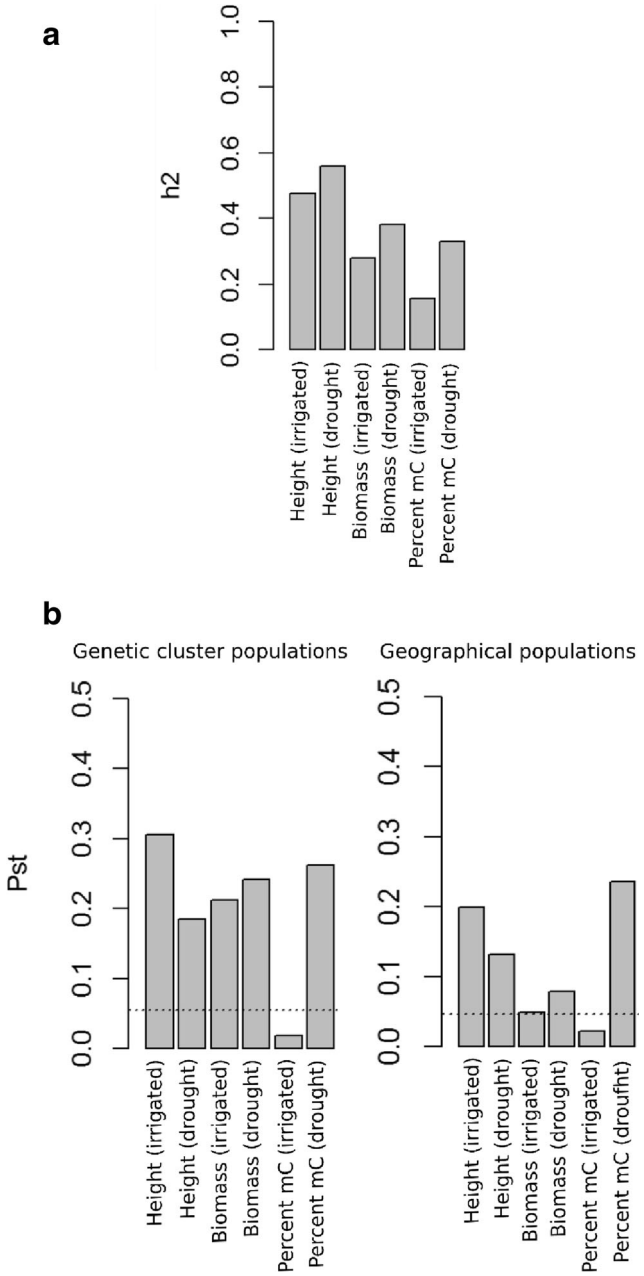

Fig. 3 Narrow-sense heritability and P_{ST} estimates calculated using sommer package in a multivariate analysis with genome-wide SNPs. **a** Narrow-sense heritability estimates. **b** P_{ST} estimates computed using the four groups defined by the clustering (left) or the three original populations (right). Horizontal dot lines indicate the average F_{ST} index. The F_{ST} over the four groups was slightly higher (0.0548) than in the three original populations (0.0456)

can modify phenotype in response to environmental variations (Lande 2009; Bräutigam et al. 2013; Meyer 2015; Kawakatsu et al. 2016; Bewick and Schmitz 2017; Seymour and Becker 2017). Although epigenomics methods such as whole genome bisulfite sequencing (WGBS) can actually provide methylation at the cytosine level over the whole genome for sequenced species (Yong et al. 2016), global DNA methylation is still reported in many studies (Lambé et al. 1997; Trap-Gentil et al. 2011; Teyssier et al. 2014; Alonso et al. 2015; Alonso et al. 2016; Plomion et al. 2016). Global DNA methylation varies among plants species (Lambé et al. 1997; Alonso et al. 2015; Plomion et al. 2016), in relation to genome size in Angiosperms (Alonso et al. 2015), in extensive ploidy series in *Dianthus broteri* (Alonso et al. 2016), between individuals from a given population (Vaughn et al. 2007; Schmitz et al. 2013), as well as within a genotype in response to environmental constraints or during developmental processes (Causevic et al. 2005; Teyssier et al. 2008; Gourcilleau et al. 2010; Trap-Gentil et al. 2011; Teyssier et al. 2014; Condé et al.2017). In poplar, variations in global DNA methylation have been measured in response to biotic or abiotic stress, or according to geographic origin (Gourcilleau et al. 2010; Raj et al. 2011; Latzel et al. 2013; Garg et al. 2015; Song et al. 2016; Lafon-Placette et al. 2018). Here, we evidenced variations in global DNA methylation in winter-dormant SAMs among genotypes from three populations of black poplar in field conditions subjected to contrasting water availability during the growth period. The values and the range of variations were similar to those previously reported in poplar (Gourcilleau et al. 2010; Raj et al. 2011) or other plants (Trap-Gentil et al. 2011; Alonso et al. 2015 and 2016). Altogether, epigenetic variations, such as global DNA methylation, among populations and in response to stress, can be a determinant of population phenotypic variations in addition to genetic variations (Latzel et al. 2013; Cortijo et al. 2014; Kooke et al. 2015; Kawakatsu et al. 2016; Richards et al. 2017).

Drought induces stable changes of global DNA methylation in populations

Chamaillard et al. (2011) previously reported a broader ecophysiological analysis of genotype drought responses, including the 42 genotypes studied here. The moderate drought reduced growth performance-related traits such as annual shoot fresh mass, but the effect of drought was neither genotype- nor population-dependent. Trait variations such as water-use efficiency (assessed from bulk leaf carbon isotope discrimination, $\Delta^{13}C$), growth performance, and leaf traits were larger within than among populations. Altogether, Chamaillard et al. (2011) proposed that the large variations found within populations combined with the consistent differences among populations could suggest a large adaptive potential for *P. nigra*.

Here, the geographical (three populations) or genetically based clusters (four populations) did not differ in global DNA methylation, but drought was shown to significantly decrease global DNA methylation in all clusters. In addition, the SAMs (winter dormant) were collected six months after the summer drought, suggesting a stable epigenetic effect of water deficit probably maintained through cell division in the meristem at least until winter and dormancy. Many studies in

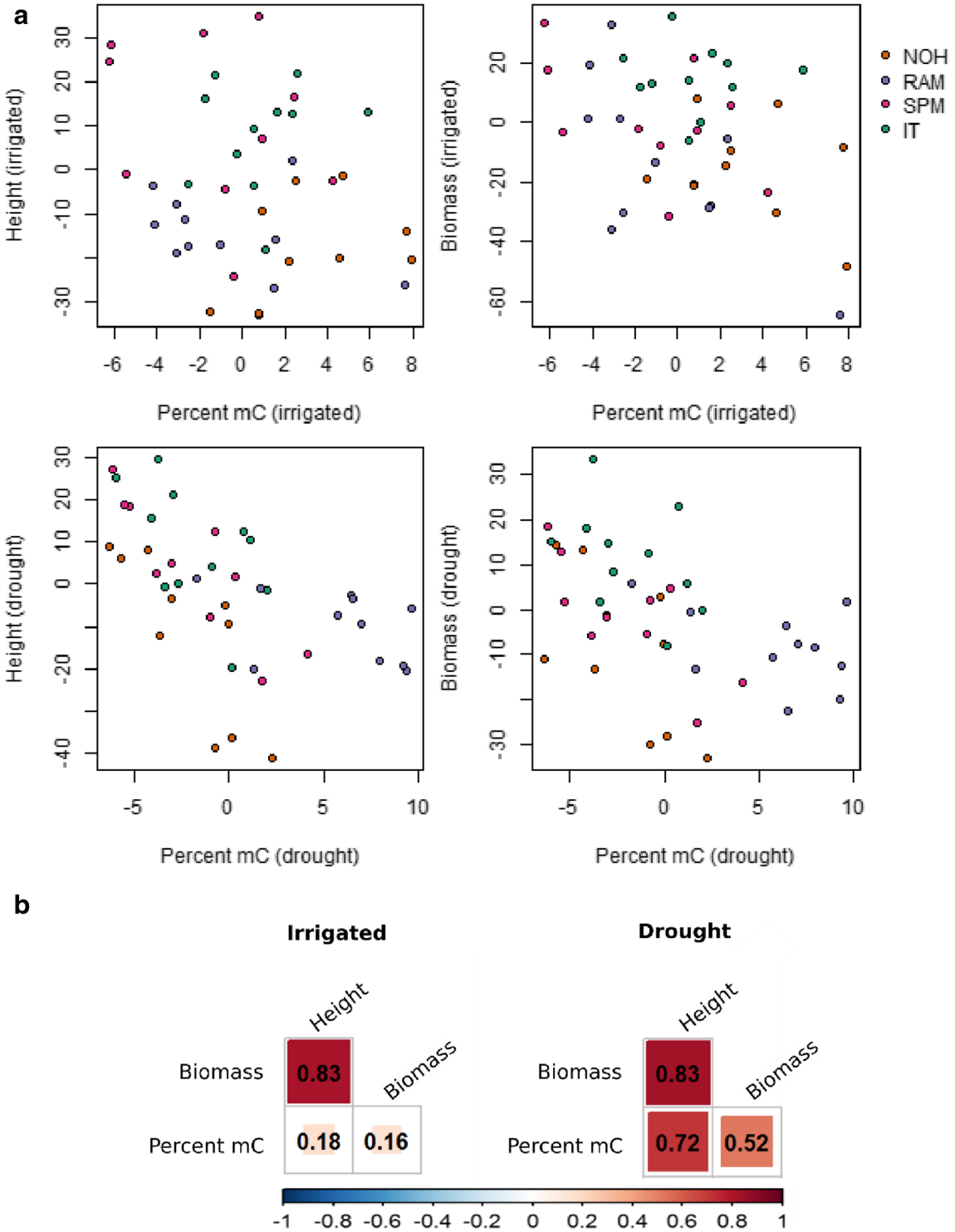

Fig. 4 Genetic and residual correlations between global DNA methylation and height or biomass calculated using variance estimates of the mixed model of Eq. 1. **a** Illustration of the genetic correlations. **b** Residual correlations

poplar have highlighted the importance of DNA methylation in response to environmental changes (Gourcilleau et al. 2010; Raj et al. 2011; Bräutigam et al. 2013; Liang et al. 2014; Conde et al. 2017; Lafon-Placette et al. 2018). Recently, Lafon-Placette et al. (2018) have shown that variations in soil water availability induce changes in DNA methylation patterns (differentially methylated regions) preferentially for genes of the phytohormone pathways. The concepts of "priming" and "stress memory" in plants have been recently applied to abiotic stress responses such as water deficit (Sultan

et al. 2009; Ding et al. 2012; Fleta-Soriano and Munné-Bosch 2016; Mauch-Mani et al. 2017; Lämke and Bäurle 2017). Only few reports are actually available for trees that are long-living organisms subjected to repeated environmental constraints (Yakovlev et al. 2010; Raj et al. 2011; Yakovlev et al. 2011; Bräutigam et al. 2013; Schönberger et al. 2016; Yakovlev et al. 2016; Yakovlev and Fossdal 2017). Here, we showed that the winter dormant SAMs of individuals from distinct natural black poplar populations exhibited variations of global DNA methylation six months after a summer drought suggesting an epigenetic memory in field conditions that could possibly participate in acclimation or local adaptation (Richards et al. 2017).

Global DNA methylation as a genetic marker of black poplar population differentiation under drought stress

For the first time, genetic parameters (h^2, $P_{ST,}$ genetic correlations) were estimated for DNA methylation level in natural tree populations using genome-wide SNPs (Supplementary Table 1). Unexpectedly, genomic relatedness matrix revealed four different groups with a mixed group composed of RAM and SPM individuals and F_{ST} showing that RAM and SPM were more similar than NOH population. This group was likely due to the introgression from the very few poplar cultivars deployed on French poplar stands (as already hypothesized in Faivre-Rampant et al. 2016). Estimates of DNA methylation h^2 were similar to the ones obtained for biomass and increased when populations (geographically or genetically clustered ones) were subjected to drought. This suggests that under environmental constraint, the DNA methylation variations are likely linked to genetic variations in these populations. In line with this, Dubin et al. (2015) have shown in *Arabidopsis thaliana* that an increase in temperature was associated to increased DNA methylation levels in transposable elements and that these variations were strongly associated to genetic variations close to the DNA methyltransferase CMT2 gene involved in DNA de novo methylation in CHH context.

We also assessed population differentiation using P_{ST} (an estimation of phenotypic differentiation Q_{ST}) estimates and found that P_{ST} for DNA methylation levels exhibit a strong increase under drought stress suggesting a population specific DNA methylation status under drought. In irrigated condition, the P_{ST} of global DNA methylation was lower than the F_{ST} ($P_{ST} < F_{ST}$) while in drought, the P_{ST} became much higher than the F_{ST} ($P_{ST} > F_{ST}$). Using $P_{ST} - F_{ST}$ approach to detect indirect signals of divergent selection on dorsal plumage coloration in pied flycatcher males, Lehtonen et al. (2009) have found that P_{ST} and F_{ST} were positively correlated suggesting that genetic drift may have contributed to the observed phenotypic differentiation. The higher P_{ST} recorded under drought stress in our study as compared to the F_{ST} could be suggestive of a directional selection influencing the DNA methylation level (Merilä and Crnokrak 2001; Leinonen et al. 2008). To go further, genetic correlations between global DNA methylation and height or biomass were significantly negative only in drought condition. For the residual correlations, a positive correlation between global DNA methylation and height or biomass in drought was detected indicating an effect of the environment. This suggests that the negative genetic correlations between growth and global DNA methylation are compensated by environmental positive correlations resulting in no significant phenotypic correlations.

Altogether, we showed that winter-dormant SAMs of black poplar genotypes from natural populations grown in field condition keep an epigenetic memory of a drought summer episode through modifications of global DNA methylation levels. It will be interesting to see if the variations of global DNA methylation observed in winter-dormant SAMs after a drought are held through the following summer, with or without another round of induced summer drought. In addition, global DNA methylation levels in these populations are genetically and environmentally determined and may be used as a genetic marker for population differentiation. This work opens perspectives that should be addressed in future projects to analyze the role of DNA methylation in stress memory and G × E interactions in natural tree populations using epigenomic approaches at the whole genome level.

Data archiving statement The raw data containing the full list of genotypes, global DNA methylation levels, biomass, and height are included as Supplementary Table 1. The SNP data used in order to reconstruct genomic relationships between genotypes, within and between populations, and to estimate h^2, P_{ST}, and F_{ST} for global DNA methylation are in Supplementary Tables 2 and 3.

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