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RESEARCH PAPER

Changes in the epigenome and transcriptome of the poplar shoot apical meristem in response to water availability affect preferentially hormone pathways

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Abstract

The adaptive capacity of long-lived organisms such as trees to the predicted climate changes, including severe and successive drought episodes, will depend on the presence of genetic diversity and phenotypic plasticity. Here, the involvement of epigenetic mechanisms in phenotypic plasticity toward soil water availability was examined in *Populus×euramericana*. This work aimed at characterizing (i) the transcriptome plasticity, (ii) the genome-wide plasticity of DNA methylation, and (iii) the function of genes affected by a drought–rewatering cycle in the shoot apical meristem. Using microarray chips, differentially expressed genes (DEGs) and differentially methylated regions (DMRs) were identified for each water regime. The rewatering condition was associated with the highest variations of both gene expression and DNA methylation. Changes in methylation were observed particularly in the body of expressed genes and to a lesser extent in transposable elements. Together, DEGs and DMRs were significantly enriched in genes related to phytohormone metabolism or signaling pathways. Altogether, shoot apical meristem responses to changes in water availability involved coordinated variations in DNA methylation, as well as in gene expression, with a specific targeting of genes involved in hormone pathways, a factor that may enable phenotypic plasticity.

Keywords: Differentially expressed genes, differentially methylated regions, DNA methylation, phenotypic plasticity, *Populus×euramericana*, shoot apical meristem, water availability.

Introduction

Drought is a significant threat to forest health and agro-ecosystem productivity (Hamanishi and Campbell, 2011). As sessile organisms with a long lifespan, trees deploy mechanisms to contend with heterogeneity in water availability

Abbreviations: ABA, abscisic acid; DEG, differentially expressed gene; DMR, differentially methylated region; JA, jasmonic acid; SA, salicylic acid; SAM, shoot apical meristem; WD, water deficit; WD-RW, water deficit followed by rewatering; WW, well-watered.

(Neale *et al.*, 2017). Phenotypic plasticity, the ability of a genotype to display different phenotypes under distinct environmental conditions, is likely to be a key mechanism for tree survival under rapid climate change (Lande, 2009; Nicotra *et al.*, 2010; Baulcombe and Dean, 2014). However, massive tree mortality events suggest that these responses are limited and may not prove adequate for the predicted climate change, such as unprecedented long and severe periods of drought (Allen *et al.*, 2010; IPCC, 2014). In addition, it is not only the response to drought, but also the recovery of growth after rewatering that is of extreme importance for plant survival. It depends, among other factors, on the drought intensity and the extent of damage suffered (Gallé *et al.*, 2007; Xu *et al.*, 2010).

With the availability of its genome, its fast growth, and the large genetic and phenotypic variations observed, poplar has became a model tree (Tuskan *et al.*, 2006; Jansson and Douglas, 2007). Poplars (*Populus* spp.) are among the fastest growing trees in temperate latitudes and their high productivity is associated with large water requirements. For cultivated plant species such as poplars, water deficit tolerance is defined as the ability to limit the decrease of biomass production in response to a moderate water deficit (Passioura, 2002). In poplars, the response to variations in water availability comprises several physiological and morphological traits leading to water saving and allowing the maintenance of growth (Gebre *et al.*, 1994; Tschaplinski *et al.*, 1998; Marron *et al.*, 2003; Hamanishi *et al.*, 2012; Guet *et al.*, 2015). At the molecular level, the response to drought results from a multihormonal cross-talk between abscisic acid (ABA; the key hormone of abiotic stress), ethylene, jasmonate (JA), brassinosteroids (BR), auxin, salicylic acid (SA), and gibberellins (Seki *et al.*, 2007; Wolters and Jürgens, 2009; Choudhary *et al.*, 2012; Riemann *et al.*, 2015; Khan *et al.*, 2015). Despite the exhaustive study of phytohormone responses, these have been mostly focused on roots and leaves, the organs responsible for water uptake and water loss. Studies of transcriptome and proteome responses in *Populus* have a similar focus (Street *et al.*, 2006; Plomion *et al.*, 2006; Bonhomme *et al.*, 2009; Cohen *et al.*, 2010; Raj *et al.*, 2011). The shoot apical meristem (SAM) has been disregarded, although this region is where new organs are formed and is therefore likely to control the shoot developmental response to variations in water availability. A few phytohormones have been studied for their role in SAM functioning, such as cytokinins and auxin controlling meristem maintenance and organ initiation, respectively (Shani *et al.*, 2006), or jasmonate inhibiting mitotic activity in meristems (Zhang and Turner, 2008). Nevertheless the SAM response of these phytohormones during a drought stress remains unknown.

In plants, epigenetic reprogramming, particularly DNA methylation, occurs at key developmental steps and in response to environmental stimuli (Feng *et al.*, 2010; Meyer, 2015). These epigenome modifications contribute to genome protection, control of gene expression, and inheritance of transcriptional states via changes in chromatin structure (Teixeira and Colot, 2010; Sahu *et al.*, 2013; Baulcombe and Dean, 2014; Kooke *et al.*, 2015). Epigenome changes may contribute to phenotypic plasticity but also to plant adaptation to new environments (Schmitz *et al.*, 2011; Becker *et al.*, 2011; Bräutigam *et al.*, 2013; Meyer, 2015; Kawakatsu *et al.*, 2016; Seymour and Becker, 2017). Among the epigenetic marks, DNA methylation shows high stability throughout mitosis and meiosis, and has been well investigated. In plants, methylated cytosines are found in CG dinucleotides, but also in CHG and CHH contexts (where H is A, T, or C), methylation of which is catalysed by specific DNA methyltransferases (Goll and Bestor, 2005; Teixeira and Colot, 2010; Meyer, 2015). In Arabidopsis, genome-wide surveys revealed that DNA methylation is concentrated in heterochromatin and repetitive regions but is also present in 25–33% of gene bodies (Zhang *et al.*, 2006; Cokus *et al.*, 2008; Kawakatsu *et al.*, 2016). This pattern is found in most eukaryotes and, despite this high conservation, the functional role of gene-body methylation still needs to be clarified (Zemach *et al.*, 2010; Liang *et al.*, 2014; Bewick and Schmitz, 2017).

Gene-body DNA methylation in *P. trichocarpa* is extensive in the open chromatin state, is linked to structural gene characteristics, and is correlated to tissue-specific gene expression (Vining *et al.*, 2012; Lafon-Placette *et al.*, 2013; Bräutigam *et al.*, 2013). In leaves, transcriptome changes in response to drought correlated with the differences in global DNA methylation (Raj *et al.*, 2011). Genetically identical poplar trees show different hemi-methylation status according to their living sites, a potential response to environmental and edaphic conditions, even though its impact on gene expression has not been studied (Guarino *et al.*, 2015). In the SAM, global DNA methylation was shown to vary across *P.*×*euramericana* hybrids and in response to water deficit (Gourcilleau *et al.*, 2010). However, the nature and the extent of loci affected by DNA methylation, as well as its connection with differential gene expression, especially in the SAM, are still largely unknown (Plomion *et al.*, 2016).

In this study, we tested whether the variation in water availability had an impact on the SAM transcriptome and methylome. We identified in the SAM the hormonal pathways that were affected by water availability changes and tested whether these pathways were especially controlled by epigenetic mechanisms. SAMs were collected on clonally propagated young poplar trees submitted to three water regimes (optimal, moderate water deprivation, and rewatering). The SAM response to water regimes was assessed both at the methylome and the transcriptome level using microarrays, as recently reported (Hébrard *et al.*, 2016). A focus was made on gene body DNA methylation, a still poorly understood mechanism (To *et al.*, 2015; Bewick and Schmitz, 2017). Overall, we found that the rewatering condition triggered massive changes in gene expression and gene body DNA methylation. In particular, hypomethylated genes tended significantly to be repressed. Among these, genes related to phytohormone metabolism or signaling pathways such as JA, SA or ethylene were significantly enriched. Altogether our data unravel a physiological connection in the SAM between gene DNA methylation, gene expression, and phytohormone pathways in response to variations of water availability.

Materials and methods

Plant material, growth conditions and treatments

SAMs were collected from *Populus*×*euramericana* (*P. deltoides* Batr.×*P. nigra* L.) 'Carpaccio' young trees for which ecophysiological data were previously published (Bizet *et al.*, 2015). Briefly, plants were grown in 10-liter pots in a greenhouse (Nancy, France, in April 2008) exposed to natural daylight (400–900 µmol m^{-2} s^{-1}). Temperature and humidity were maintained within the ranges of 19–26 °C and 50–75%, respectively. Plants were watered to field capacity (until the excess water held in the soil started to drain) three times a day over 5 weeks. Before the experiment started (day 0), plants were randomly assigned to three water supply regimes. For the well-watered treatment (WW), evaporative demand was compensated by waterings to field capacity, four times per day. For the water deficit (WD), the relative extractable water content was allowed to decrease to 20% during the first 4 days and then maintained within the range of 17–23%. Both conditions were kept stable for the last 10 days while for the rewatering treatment (WD-RW), the water deficit was applied for 8 days, after which the plants were rewatered to field capacity for 6 days (Fig. 1A). Leaf predawn water potential, leaf full turgor osmotic pressure, height growth rate and gas exchange were monitored as previously described (Bogeat-Triboulot *et al.*, 2007) to assess the effect of the treatments (Bizet *et al.*, 2015). At the end of the experiment (day 14), the active buds were collected and immediately frozen in liquid nitrogen. Shoot apices were then cleared from all visible differentiated tissues, as described previously (Lafon-Placette *et al.*, 2013).

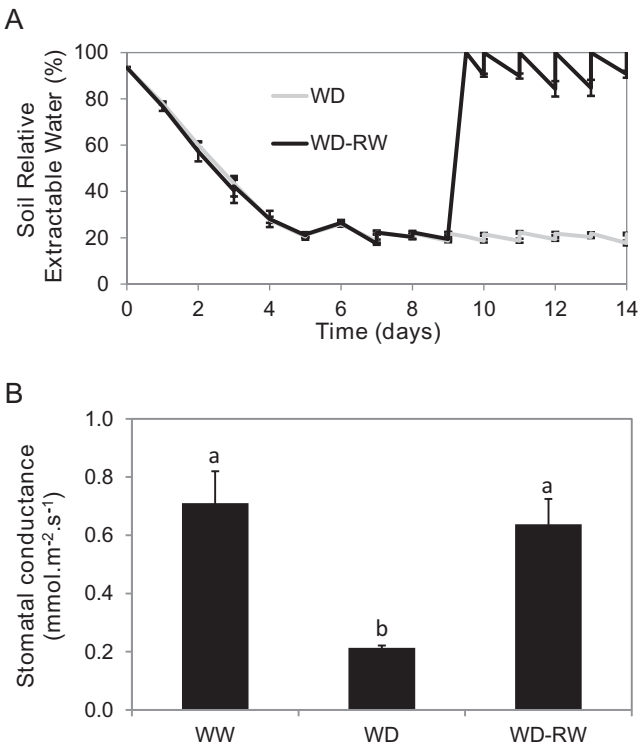

Fig. 1. Water deficit–rewatering cycle characterization. (A) Monitoring of the soil relative extractable water (REW) during the 14 days experiment for water deficit (WD) and rewatering after water deficit (WD-RW) conditions. REW is expressed as a percentage of well-watered treatment (WW) REW value. (B) Stomatal conductance for the *Populus deltoides*×*P. nigra* 'Carpaccio' genotype was measured for each water condition, at the end of the experiment. Values are means±SE (n=6). Values marked with different letters are significantly different between water treatments (P<0.05) as determined by one-way ANOVA.

RNA preparation

Total RNA was extracted separately from individual SAMs (50 mg fresh weight) using the Nucleospin® RNA Plant kit (Macherey-Nagel, Hoerdt, France). Two biological replicates were performed per water regime (WW, WD, WD-RW). Fluorescent complementary RNA was generated and quality controlled as described previously (Hébrard *et al.*, 2016). RNA hybridization was conducted according to the manufacturer's instructions (Agilent Technologies, Massy, France).

DNA extraction and methyl DNA immunoprecipitation

DNA was extracted separately from individual SAMs (50 mg fresh weight) with the CTAB DNA extraction protocol (Doyle and Doyle, 1987). Three biological replicates were performed per water regime. Chemicals were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). DNA was sonicated using a VC 505 Vibra-Cell sonicator (Fisher Scientific, Illkirch, France) to obtain fragments ranging from 0.2 to 0.8 kb. Methylated DNA was immunoprecipitated from 11 µg of sonicated DNA, according to Weng *et al.* (2009). For each extract, immunoprecipitated DNA and its corresponding input were labelled. Quality controls and DNA hybridizations were conducted according to Agilent Technologies' instructions as previously reported (Hébrard *et al.*, 2016).

Microarray probe design for transcriptome and methylome

Transcriptome and CH_3 microarray probe design, hybridization, data collection and normalization were performed by IMAXIO (Clermont-Ferrand, France).

Custom microarrays were designed based on *Populus trichocarpa* genome v2 (Tuskan *et al.*, 2006) with eArray software (https://earray.chem.agilent.com/earray/; Agilent Technologies).

The transcriptome microarray included one probe per *P. trichocarpa* transcript v2.0 (with a positive bias at the 3′ end of mRNA, limiting the GC content of the oligomers). When a probe did not efficiently discriminate transcripts (more than 70% identity on 50 residues), a second probe was added (designed in the 5′ end of the first probe). The microarray included 50 additional probes for reproducibility controls and internal Agilent control probes. Following the v3 improved assembly of reference genome, BLASTN was performed against *P. trichocarpa* v3 transcripts, using default parameters and probe sequences as queries. The best BLAST hits were used for v3 probe annotation.

The CH_3 microarray included five probes per *P. trichocarpa* gene v2.0 (one probe 0.5 kb upstream from the start codon, one probe 0.5 kb downstream from the stop codon, and three probes within the gene body). Additional probes covering genes and intergenic regions were also selected based on a previous study (Lafon-Placette *et al.*, 2013). These probes spanned from 2 kb upstream to 2 kb downstream from target loci (either genes or intergenic regions), with a distance between probes of 140 bp for genes and 780 bp for intergenic regions. The microarray contained 50 probes for reproducibility controls and internal Agilent control probes. Following the v3 *P. trichocarpa* genome release, probe information was updated by blasting their sequences to v3 genome. They were annotated as 'BODY' if matching with a v3 gene, 'PROMOTER' if spanning the 1 kb region upstream from a start codon of a v3 gene and 'TE' if falling within a transposable element as described by the RepeatMasker annotation of the *P. trichocarpa* v3 genome (http://www.phytozome.net/poplar.php). When a probe matched with a TE inserted into a gene or a promoter, it was annotated as 'BODY+TE' or 'PROM+TE', respectively. If a probe did not match with any of the previous categories, it was annotated as 'INTERGENIC'.

Transcriptome data collection, normalization and identification of differentially expressed genes

For the transcriptome analysis, 8 × 60K custom microarray slides were used for hybridization (Agilent Technologies). Microarray

slides were scanned and raw signal data were extracted and controlled using Feature Extraction 10.7 software (Agilent Technologies) as previously reported (Hébrard *et al.*, 2016).

Transcriptome data were normalized per chip to the 75th percentile. Data filtering was based on Feature Extraction flag criteria: non-outlier population; not saturated, uniform and significant probe signal; and probe signal above background (flag 'well-above background'). A probe passed the latter flag criterion if its signal was greater than 2.6×background standard deviation. To be included in further analysis, a probe needed to pass all flag criteria in the two biological replicates of at least one experimental condition (WW, WD, or WD-RW). Differentially expressed genes (DEGs) between the two water regimes were detected using the rank product method (Breitling *et al.*, 2004) as implemented in the Bioconductor RankProd Package (Hong *et al.*, 2006). The test was run with 100 permutations and corrected for multiple comparison errors. The DEG selection threshold was set at a false prediction rate <0.05 and a $\log_2$(fold-change) >|1|.

Methylome data collection, normalization and identification of differentially methylated regions

For the CH_3 microarray, 1 × 1 million custom microarray slides were used for hybridization (Agilent Technologies). Data normalization was performed by Feature Extraction software as already reported (Hébrard *et al.*, 2016). Data filtering was based on the same feature extraction flag criteria as the transcriptome analysis (signal above background, non-outlier, and ranks similar between the two Cy5/Cy3 dyes). To pass the filtering, a probe needed to pass all flag criteria for at least one experimental condition (WW, WD, or WD-RW). For each probe, Cy5/Cy3 log ratio was then calculated.

The signals of independent probes were grouped into contiguous 50 kb genomic windows. The methylation status of each window was determined independently for each experimental condition through the comparison of its methylation level to a reference mean per chromosome. Details regarding this approach are given in Supplementary Methods S1 at *JXB* online. Briefly, the reference mean under the null hypothesis was computed using 20% randomly selected probes for each scaffold and mixture model as implemented in the mixtools package (Benaglia *et al.*, 2009) for the R statistical software (R Core Team, 2015). The other 80% of the probes were grouped using 50 kb windows (7556 windows genome-wide). For each window, the difference between its mean and the previously determined reference mean was tested. For validation, this analysis was repeated with 10 kb windows and the results appeared to be similar (data not shown). A non-parametric one-sample Wilcoxon signed rank test was performed between each window mean and the corresponding reference mean, giving rise to a *P*-value of rejecting the null hypothesis. In order to control the global error risk of these multiple tests, the false discovery rate (FDR) was estimated using the R package fdrtool (Strimmer, 2008). Windows with a methylation level significantly lower (−1) or higher (+1) than the reference mean (0) at a FDR level of 5% were thus identified. Finally, differentially methylated regions (DMRs) were defined as a given window (locus) with different methylation status (0/1; 0/−1 or 1/−1) between at least two water conditions.

Determination of DNA methylation percentages by HPLC

The global percentage of methylation of genomic DNA was determined by high-performance liquid chromatography (HPLC) as described in Trap-Gentil *et al.* (2011). Three independent analyses and three replicates were performed for each measurement.

Real-time RT-PCR

Approximately 500 ng of total RNA was reverse transcribed using SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR kit (Invitrogen, France). Primers were designed with QuantPrime software (http://www.quantprime.de/) and are listed in Supplementary Table S1. Constitutively expressed genes, encoding phenylalanyl-tRNA synthetase (*Potri.004G155000*) and POLYUBIQUITIN 10 (*Potri.007G123300*), were selected as reference genes. PCR preparation, control, amplification and analysis were performed as previously reported (Hébrard *et al.*, 2016). Three biological and two technical replicates were performed for each gene and condition.

Bisulfite sequencing

Bisulfite conversion was performed using the Epitect bisulfite kit (Qiagen, France) and 500 ng of genomic DNA, according to the manufacturer's recommendations. Controls for bisulfite conversion efficiency and amplification as well as primer design, PCR, and sequencing procedures have been previously detailed (Trap-Gentil *et al.*, 2011; Lafon-Placette *et al.*, 2013; Hébrard *et al.*, 2016). Primers used for bisulfite sequencing are listed in Supplementary Table S1. Two biological and two technical replicates were performed for each sequence and genotype.

Additional bioinformatics and statistical analyses

Additional bioinformatics analyses have been summarized in Supplementary Methods S2.

Means are expressed with their standard error and compared by analysis of variance (one-way ANOVA; general linear model procedure). The relationship between gene expression and DNA methylation is not linear (Bewick and Schmitz, 2017). Thus, instead of correlations, hypergeometric tests were used to detect the significant co-occurrence of changes in gene expression and DNA methylation. The same test was used to detect whether such changes preferentially occur in specific sets of genes (e.g. phytohormone-responsive genes). Phytohormone effects on the distribution of DMRs were evaluated using the chi square (χ^2) homogeneity test. Statistical tests were considered significant at $*P<0.05$, $**P<0.01$ or $***P<0.001$.

Accession numbers

Microarray design, transcriptome (GSE46605) and methylome (GSE46624) data are available in the GEO database.

Results

Physiological responses to variations in water availability

Populus×euramericana 'Carpaccio' clone trees were submitted to three water regimes (Bizet *et al.*, 2015): (i) watered to field capacity (WW); (ii) 14-day-long moderate water deficit (WD); and (iii) the same water deficit during 8 days followed by full rewatering for 6 days (WD-RW; Fig. 1A). At the end of the experiment, stomatal conductance was significantly reduced under WD but not under WD-RW (Fig. 1B). Even if the water constraint was moderate and apical buds remained active with production of new leaves, growth was significantly reduced by WD (Bizet *et al.*, 2015). After days d of rewatering, leaf expansion was restored to a relative rate similar to that of well-watered plants (Bizet *et al.*, 2015). At the time of the SAM harvest, trees under the three water regimes exhibited clearly distinct physiological states.

The SAM transcriptome reveals distinct hormone signatures in response to variations in water availability

After microarray filtering and normalization, the expression of 22 398 transcripts out of a total of 41 335 *P. trichocarpa* v3 transcripts could be monitored in the SAM (Supplementary Fig. S1 and Supplementary Tables S2 and S3). Among these, transcripts corresponding to 1516 genes exhibited a differential expression (DEGs) in response to water regimes (Fig. 2A and Supplementary Tables S3 and S4). A qPCR test for a subset of genes confirmed the differential expression obtained in the microarray (Supplementary Fig. S2). Most DEGs were retrieved from the comparison between WD and WD-RW conditions (1065 DEGs; Fig. 2A). Among these 1065 DEGs (up- and down-regulated), stress signaling functions were over-represented compared with the reference (see Supplementary Methods S2 and Supplementary Table S4), such as salicylic acid (SA) and ethylene (Eth) response, protein phosphorylation, or cell communication (Supplementary Fig. S3). WW *vs* WD and WW *vs* WD-RW comparisons yielded a similar number of DEGs, 653 and 646, respectively (Fig. 2A). In the comparison WW *vs*. WD, 628 out of the total of 653 identified DEGs were up-regulated in WD. Half of these up-regulated DEGs were down-regulated upon rewatering (Fig. 2A, B and Supplementary Table S4). These DEGs transiently up-regulated during the water deficit were preferentially involved in the response to water deprivation, as confirmed by enriched functions (water homeostasis, response to SA, or protein phosphorylation; Supplementary

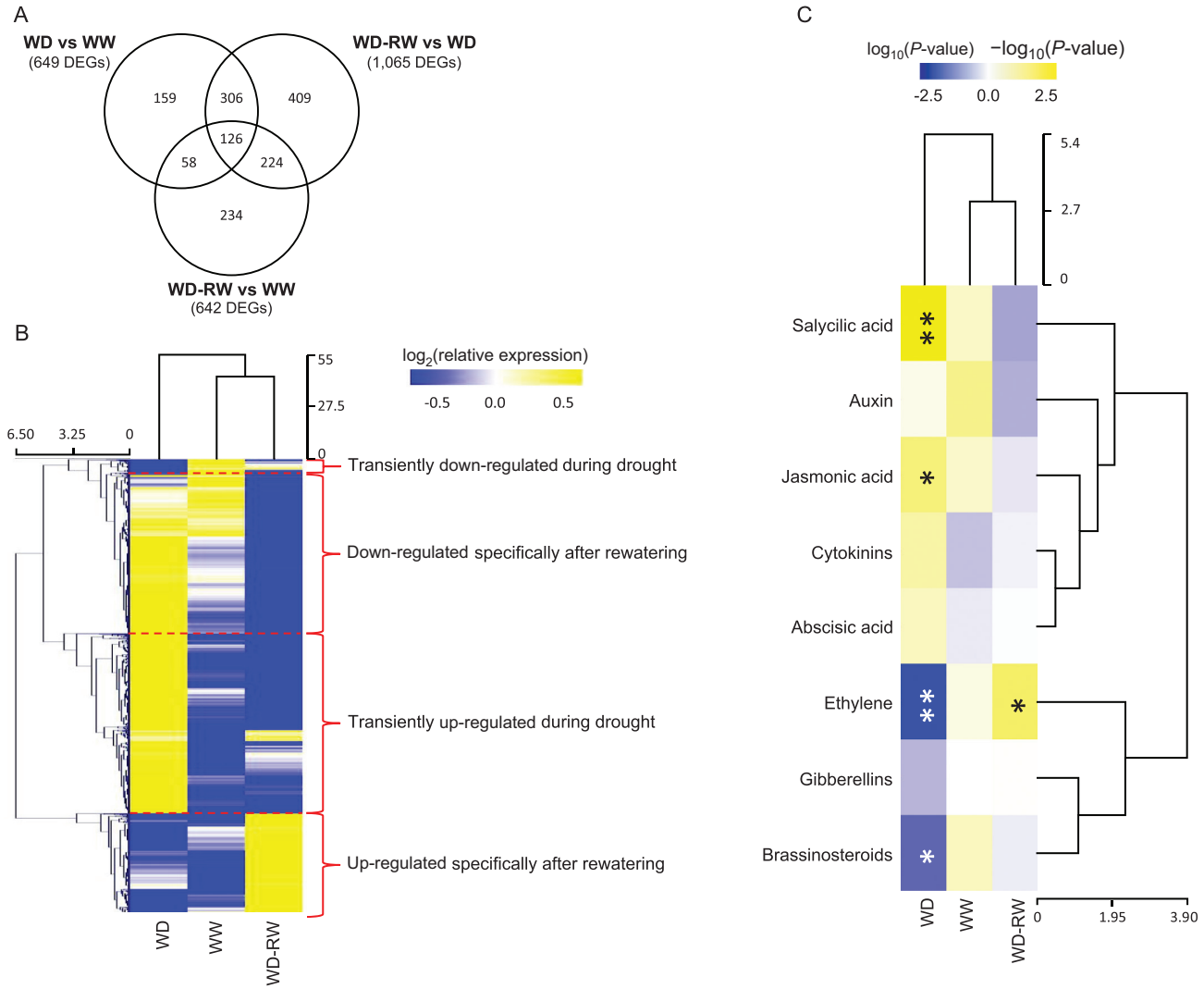

Fig. 2. DEGs between well-watered, water deficit and rewatering conditions are enriched in phytohormone-responsive genes. (A) Venn diagram showing the number of DEGs in pairwise comparisons. For criterion to define DEGs, see 'Material and methods'. (B) Clustered heatmap representation of DEG expression. Clustering was made according to Euclidian distances. Values used for the heatmap are $\log_2$-transformed relative expression (normalized per gene and per condition). (C) Clustered heatmap representation of the correlation between DEGs in each water condition (this study) and published DEGs in Arabidopsis phytohormonal experiments (see text for details). When a significant proportion (hypergeometric test) of up- and down-regulated genes in a water condition were also up- and down-regulated, respectively, in a hormone experiment in Arabidopsis, this was counted as positive association. If up- and down-regulated genes in a water condition were down- and up-regulated, respectively, in a hormone experiment in Arabidopsis, this was counted as negative association. The association significance is expressed as $-\log_{10}(P\text{-value})$ if the association is positive, and as $\log_{10}(P\text{-value})$ if the association is negative. WW: well-watered treatment; WD: water deficit; WD-RW: rewatering after water deficit. The *P*-value of the hypergeometric test is indicated: $^*P<0.05$, $^{**}P<0.01$.

Figs S4 and S5). More unexpectedly, genes differentially expressed between WW and WD-RW conditions showed not only enrichments in stress-responsive genes including abscisic acid (ABA) transport, but also morphogenesis functions such as secondary shoot formation (Supplementary Fig. S6). Finally, 346 DEGs were specific to the WD-RW condition (up- or down-regulated compared with both other conditions; Fig. 2A, B and Supplementary Table S4). These DEGs were enriched in a few significant functions such as ABA transport or programmed cell death (Supplementary Fig. S7).

Considering the recurrence of hormone response functions in the SAM transcriptome, we tested whether any specific hormone signatures could be inferred for each water condition. Using Arabidopsis identifiers, water condition-specific DEGs (up- or down-regulated in one condition compared with both other conditions) were compared with dysregulated genes after hormone treatments, and to dysregulated genes in specific Arabidopsis mutants (see Supplementary Methods S2). When a significant proportion of condition-specific DEGs showed a similar expression change to that observed in a given Arabidopsis hormone experiment (hypergeometric test), this was counted as a positive association. On the contrary, if a significant proportion of condition-specific DEGs showed an expression change opposite to that observed in an Arabidopsis hormone experiment, this was counted as a negative association. A significant positive association was found between the effects of WD and the transcriptomic response to JA and SA (Fig. 2C), while a negative association was detected with ethylene and BR. Conversely, a significant and positive association between the effects of WD-RW and the transcriptomic response to ethylene was observed. No significant association was found for the WW condition.

Altogether, the SAM transcriptome appeared not only to be responsive to moderate drought but also to remain affected after the end of the constraint. These sustained modifications could be ascribed to an indirect effect (such as modifications of tree structure and functioning), or to a direct rewiring of hormone signaling pathways. Indeed, the transcriptome analysis of the SAM revealed that the specific changes observed in watering conditions are correlated with distinct phytohormone signatures such as SA, JA during drought and ethylene during rewatering.

The SAM enters a specific epigenomic state after rewatering

After data filtering and normalization, 351 809 probes (35.1% of the designed probes) were available for analysis, and then mapped to the poplar reference genome v3 (Supplementary Table S3). To identify DMRs, we first computed the methylation status of consecutive 50 kb genomic windows within each experimental condition (Supplementary Methods S1). Manhattan plots, presented in Fig. 3A–C, showed variations of DNA methylation along the 19 scaffolds as $-\log_{10}(P\text{-values})$ for all tested windows. The DNA methylation status of each window was defined as the following: not significantly different from the average level of methylation (designated '0'; grey points in Fig. 3), significantly lower (hypomethylated, designated '−1'; blue points in Fig. 3), or higher (hypermethylated, designated '+1'; red points in Fig. 3, see 'Materials and methods'). The methylation status of some loci was confirmed by bisulfite sequencing (Supplementary Fig. S8). Among WW, WD, and WD-RW conditions, the number of hypomethylated loci ranged from 265 to 753, while the number of hypermethylated loci was between 1962 and 3213. Genomic profiles were globally conserved between WW and WD (r=0.99; P<0.001), while WD-RW exhibited a distinct profile (r=0.32 with both WW and WD; P<0.001) (Fig. 3D). Then the DMR was defined as a given window (locus) with a different methylation status ('0_+1'; '0_−1' or '+1_−1') between at least two conditions. Accordingly, 3736 DMRs (representing 201 180 probes, about 20% of the designed probes; Supplementary Table S5) were identified (Fig. 4A). The majority of non-DMR loci (2641 regions) exhibited an average level of methylation ('0') in all water conditions (Fig. 4A). DMRs were classified into mild (−1/0, 0/+1) and strong (−1/+1) DNA methylation changes, depending on the range of variation across water conditions. Most methylation changes were mild (3516 DMR regions) while strong changes affected only 220 regions (Fig. 4B, C).

Most of the mild DNA methylation changes involved WD-RW (2054 hypermethylated and 749 hypomethylated regions; Fig. 4B). Hypermethylation associated with the WD-RW was mainly found in gene bodies while the hypomethylation was notably found in transposable elements (Fig. 4D). As measured by an independent method (HPLC), the global trend of DNA methylation changes was hypermethylation in WD-RW compared with WD and WW (Fig. 4E). Consistently, genes involved in DNA methylation/demethylation reactions, namely *DEMETER*, *AGO2* and *AGO4*, *RDR1*, and the PolV subunit *NRPE1*, were found among DEGs (Supplementary Table S4), being down-regulated in WD-RW (Fig. 4E). In addition, the WD-RW condition triggered the highest number of strong methylation changes, with 57 (26%) hypermethylated and 87 (40%) hypomethylated regions, respectively (Fig. 4C). Finally, by focusing on the pattern of change rather than its intensity, we revealed 74 regions of interest, hereafter referred to as 'plasticity' DMRs, which exhibited a different DNA methylation status in each of the three water conditions (−1, 0 and +1; Fig. 4C). Altogether, the analysis of the SAM methylome revealed water-specific condition changes with a particularly dynamic situation in WD-RW conditions.

DNA methylation and gene expression changes reveal coordination during water conditions changes

A total of 639 genes, with a broad range of functions, overlapped between all DEGs and DMRs (Supplementary Fig. S1 and Supplementary Table S6), but this overlap was not significantly different from that expected by chance (hypergeometric test). A similar comparison was performed between the genes located in the 74 'plasticity' DMRs (different states of methylation in each water condition; Fig. 4C) and the DEGs (Supplementary Table S7). Few DEGs were retrieved in the 'plasticity' DMRs (17). Six of these genes

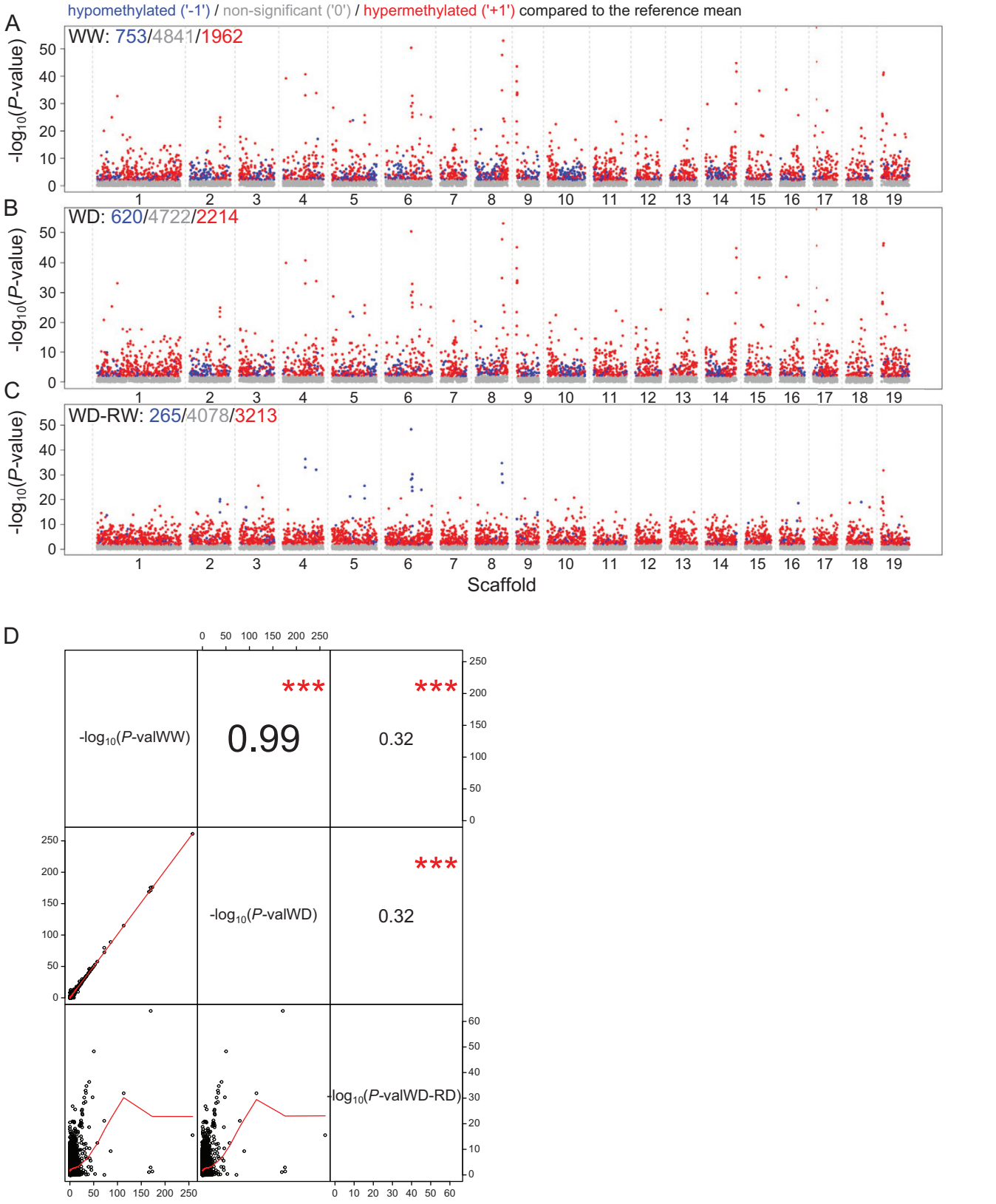

Fig. 3. Genomic features of DNA methylation in the shoot apical meristem in response to variations in water availability. (A) well-watered treatment (WW), (B) water deficit (WD), and (C) rewatering after water deficit (WD-RW). The three graphs are based on Manhattan plots for the significant windows at a false discovery rate (FDR) level of 5%. Those plots show $-\log_{10}$(*P*-values) on the *y*-axis, and the location of the different 50 kb windows in the genome with a gap in respect of the scaffold locations on the *x*-axis. Blue dots correspond to hypomethylated windows compared with the reference mean, red dots to hypermethylated windows compared with the reference mean and grey dots to non-significant windows compared with the reference mean. The numbers in blue, grey, and red indicate the number of hypomethylated, non-significant, and hypermethylated regions, respectively. (D) Pairwise correlation between $\log_{10}$(*P*-values) of each water conditions, represented as a graph (lower diagonal) and as the Pearson correlation coefficient together with its significance level (upper diagonal). ***$P<0.001$.

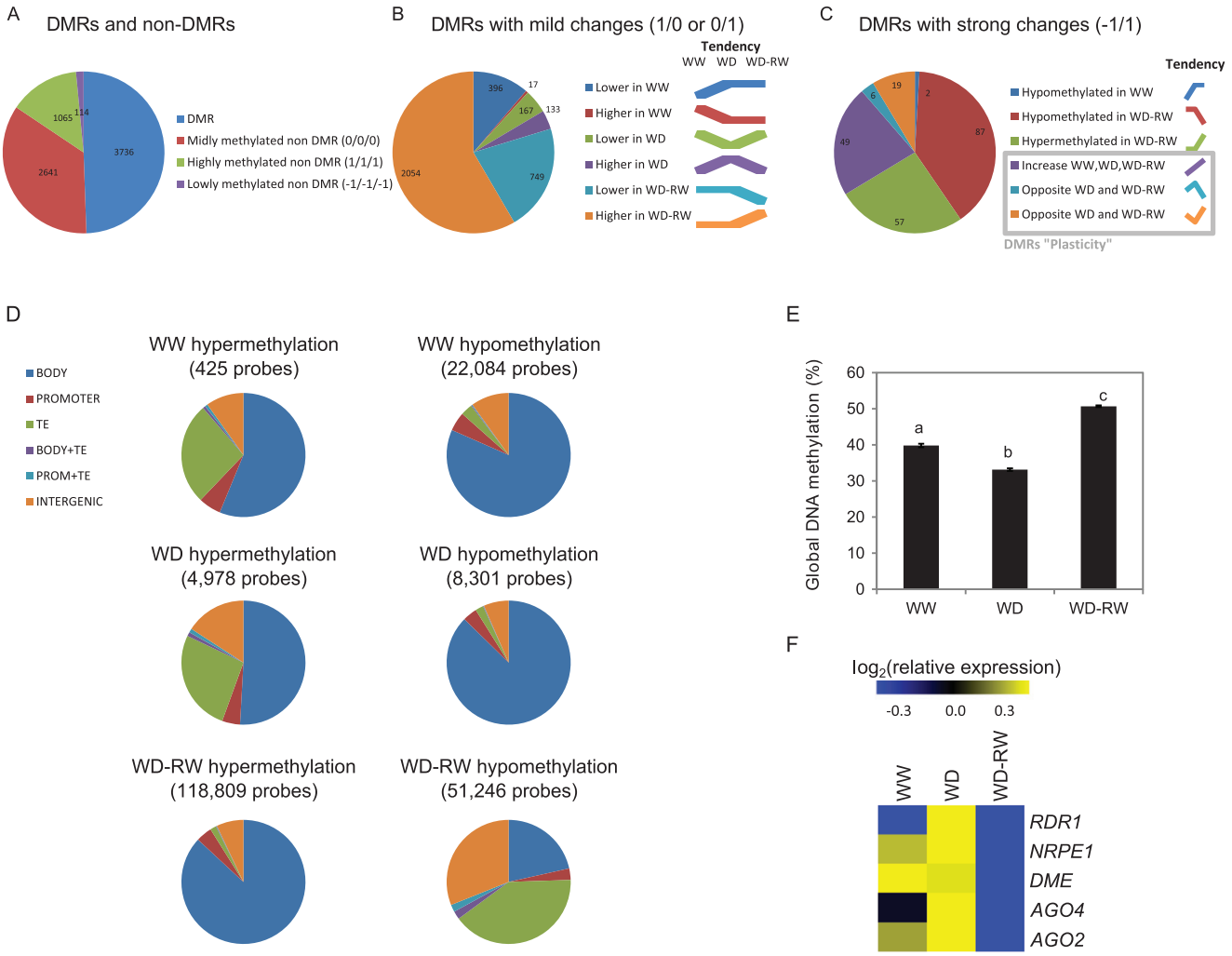

Fig. 4. Characterization of DNA methylation changes in response to different water conditions. (A) Number of genomic windows identified as DMRs or not. Unaffected regions were classified according to their methylation status: not significantly different (0) and significantly lower (–1) or higher (1) than the reference mean. (B) Mild DNA methylation changes involve a change from –1 (low methylation) to 0 (average methylation), 0 to 1 (high methylation) or vice versa among WW, WD, and WD-RW. (C) Strong DNA methylation changes involve a change from –1 (low methylation) to 1 (high methylation) or vice versa among the three water conditions. Regions with a DNA methylation level different for each water condition exhibited condition-dependent DNA methylation, and therefore called 'plasticity' DMRs. (D) Types of loci affected in the DMRs. For purposes of clarity, DMRs were classified as hypo- or hypermethylated in a given condition (lower or higher DNA methylation status, respectively, compared with the two other conditions). BODY: gene body; PROMOTER: 1 kb upstream region; TE: transposable element; BODY+TE: TE inserted in a gene body; PROM+TE: TE inserted in a promoter; INTERGENIC: any other loci. (E) Global DNA methylation level in the SAM for the three conditions, as determined by HPLC analyses. Values are means±SE (n=4). Values marked with different letters are significantly different between water treatments (P<0.05) as determined by one-way ANOVA. (F) Heatmap representation of expression of DEGs known to be involved in DNA methylation pathways. Values used for the heatmap are $\log_2$-transformed relative expression (normalized per gene and per condition). WW: well-watered treatment; WD: water deficit; WD-RW: rewatering after water deficit. *RDR1*: *RNA-DEPENDENT RNA POLYMERASE 1*, *Potri.008G135800*; *NRPE1*: *NUCLEAR RNA POLYMERASE D1B*, unique largest subunit of nuclear DNA-dependent RNA polymerase V, *Potri.T148900*; *DME*: *DEMETER*, DNA glycosylase, *Potri.010G234400*; *AGO2* and *AGO4*: *ARGONAUTE 2* and *4*, involved in siRNA mediated silencing, *Potri.015G117400* and *Potri.008G010500*, respectively.

had functions in relation to phytohormone signaling, such as *BRASSINOSTEROID-RESPONSIVE RING-H2*, or response to water stress, such as *MDIS1-INTERACTING RECEPTOR LIKE KINASE2* (Supplementary Table S7). Moreover, 3 of these 17 genes have previously been reported to exhibit polymorphism associated with quantitative traits related to growth and phenology (Supplementary Table S7; see Supplementary Methods S2). A cross-comparison between changes of DNA methylation and gene expression was further performed between each water condition, cross-comparing changes of DNA methylation and gene expression. Most of the comparisons did not yield any significant association (Fig. 5A). Nevertheless, genes down-regulated in WD-RW as compared with WD showed preferential loss of gene-body methylation (hypergeometric test, P<0.05; Fig. 5A). This association was also significant for genes having an inserted TE (hypergeometric test, P<0.05; Fig. 5A). Many of these genes encode functions involved in stress response such as receptor-like kinases or disease resistance (Supplementary Table S8). Low DNA methylation (<5%) and gene silencing, associated with the histone mark H3K27me3, is a hallmark of chromatin repressed by Polycomb complexes (Roudier *et al.*, 2011). To test whether the 127 genes exhibiting both hypomethylation and

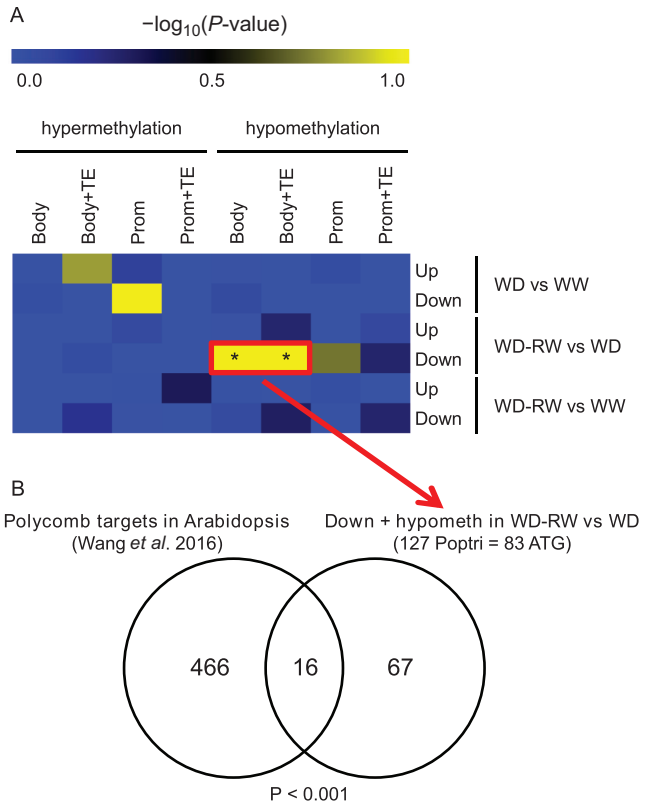

Fig. 5. Relationship between DNA methylation and gene expression changes in response to variations in water availability. (A) Heatmap representing the colocalization between expression and DNA methylation changes in pairwise comparisons between water conditions. The significance of the overlap between the two was estimated using a hypergeometric test and was expressed as $-\log_{10}(P\text{-value})$ to generate the heatmap. Body: gene body; promoter: 1 kb upstream region; TE: transposable element; body+TE: TE inserted in a gene body; prom+TE: TE inserted in a promoter. (B) Venn diagram representing the common genes between up-regulated genes in Arabidopsis polycomb mutants (Wang *et al.*, 2016; left circle) and repressed and hypomethylated genes after rewatering (right circle). WW: well-watered treatment; WD: water deficit; WD-RW: rewatering after water deficit. The *P*-value of the hypergeometric test is indicated: **P*<0.05.

down-regulation in WD-RW *versus* WD were putatively repressed by Polycomb complexes, a cross-comparison was carried with data obtained from the shoot of Arabidopsis Polycomb complex knockout mutants. This comparison revealed a significant overlap between our genes of interest and their Arabidopsis homologs up-regulated in the Polycomb complex mutants (hypergeometric test, $P<0.001$; Fig. 5B). Stress response functions were found among this gene overlap (Supplementary Table S9).

Altogether, most of the extensive DNA methylation changes in response to variations of water availability did not show any specific effect on gene expression. However, a significant association was revealed between hypomethylation and down-regulation of genes after rewatering. A significant proportion of these genes have their homologues in Arabidopsis up-regulated in Polycomb complex mutants.

Hormone-responsive genes are preferentially targeted by DNA methylation and expression changes in response to variations in water availability

Considering the over-representation of hormone-responsive genes in DEGs (Fig. 2C; Supplementary Figs S4–S8) and 'plasticity' DMRs (Supplementary Table S7), the patterns of DMRs were examined for hormone-responsive genes reported in Fig. 2C. In general, a majority of genes underwent a hypermethylation in WD-RW (Figs 4 and 6A). Nevertheless, genes activated by SA, JA, and ABA had a significantly different DNA methylation pattern as compared with all surveyed genes (Fig. 6A), undergoing less hypermethylation in WD-RW, but more hypomethylation in WD-RW and more hypermethylation in WD. Patterns for auxin-activated genes were significantly different from all genes and DEGs, mostly related to a large proportion of hypomethylation in WW. Similarly, genes repressed by BR and ethylene showed significantly more hypomethylation in WW and WD-RW conditions, respectively (Fig. 6A).

Due to the significant association between hypomethylation and down-regulation of genes in WD-RW (Fig. 5), we further tested whether hormone-responsive genes showed the same trend. A significant proportion of down-regulated DEGs and hypomethylated genes after rewatering corresponded to cytokinin-, JA-, SA-, and ABA-activated genes or auxin- and ethylene-repressed genes (hypergeometric test, $P<0.01$; Fig. 6B). Genes activated by JA and SA or repressed by auxin and ethylene were also significantly enriched in putative targets of Polycomb complexes (hypergeometric test, $P<0.01$; Fig. 6B). To conclude, hormone-responsive genes showed preferential expression and DNA methylation changes in response to variation in water availability. This suggests that upon short-term variations of water availability, SAM integrates hormone signals through epigenomic and transcriptomic imprints that could modulate shoot growth and morphogenesis as in the model proposed in Fig. 7.

Discussion

Many reports show that environmental conditions correlate with changes in DNA methylation profiles and in gene expression, offering a possible mechanism for stress memories and adaptation in plants (Kinoshita and Seki, 2014; Meyer, 2015; Kawakatsu *et al.*, 2016). However, we still lack clear evidence demonstrating that stress-specific epigenetic modifications control the expression of a gene network involved in plant acclimation (phenotypic plasticity) or long-term adaptation. In this work, we aimed at identifying DNA methylation changes in response to water conditions that could alter gene expression in the shoot apical meristem and ensure phenotypic plasticity towards the inducing conditions. To answer this question, we based our work on the following: (i) clonally propagated plants (no genetic variation), (ii) a drought-sensitive tree species (poplar) showing phenotypic plasticity under distinct water conditions (Bizet *et al.*, 2015),

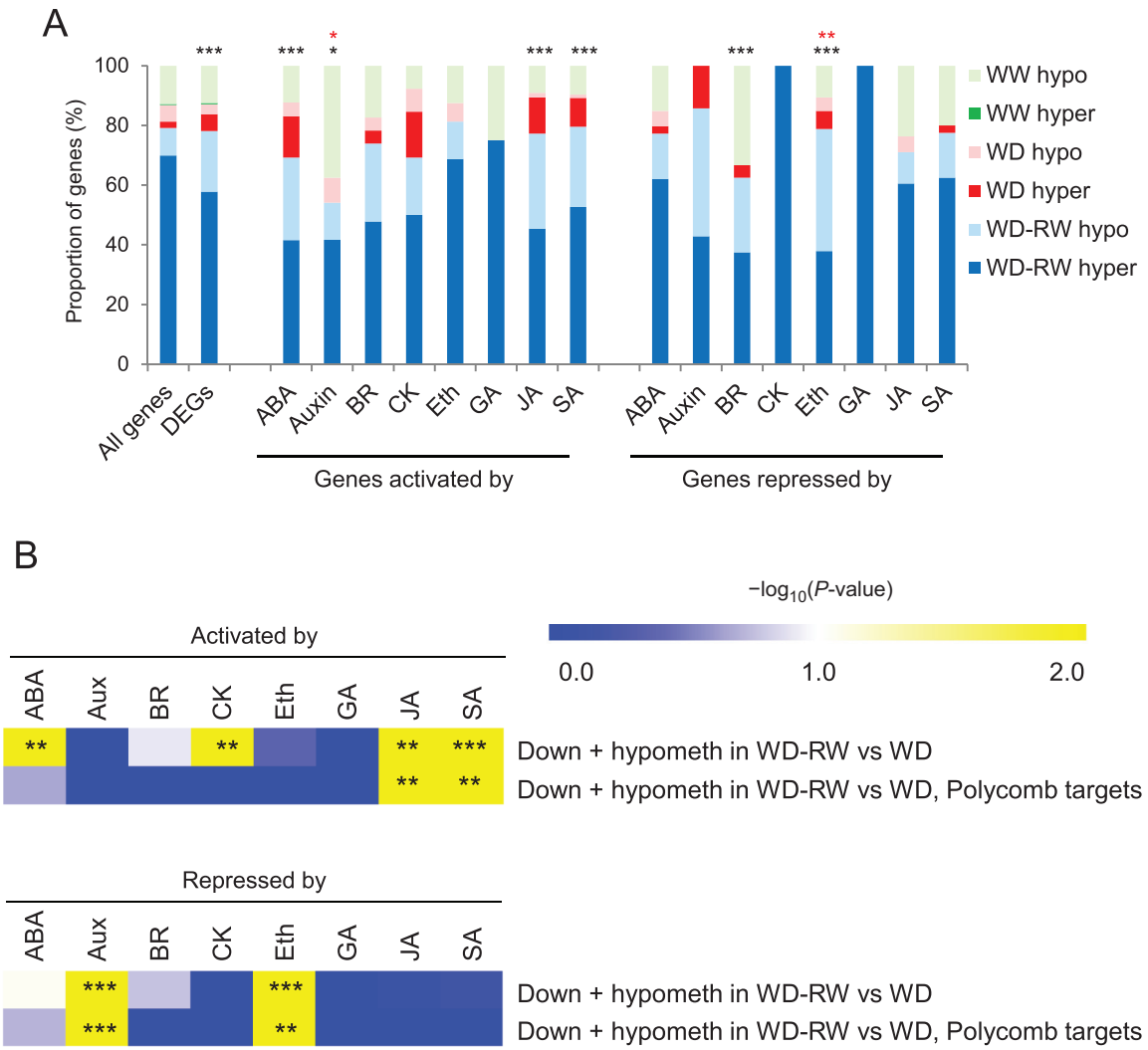

Fig. 6. DNA methylation changes affecting phytohormone-responsive DEGs. (A) Distribution of DMRs for each type of phytohormone-responsive DEG (responsive to hormone treatments in Arabidopsis, and DEGs in this study). For purposes of clarity, DMRs were classified as hypo- ('hypo') or hypermethylated ('hyper') in a given condition (lower or higher DNA methylation status, respectively, compared with the two other conditions). 'All genes' include all surveyed genes in the methylome analysis. 'DEGs' include all the 1516 DEGs presented in Fig. 2. A black star indicates a significantly different distribution of DMRs compared with all measured genes, while a red star is compared with DEGs (χ^2 test). (B) Heatmap representing the enrichment of phytohormone-responsive genes among the ones repressed, hypomethylated following rewatering and putative targets of Polycomb proteins in Arabidopsis. The significance of the enrichment was estimated using a hypergeometric test and expressed as $-\log_{10}(P\text{-value})$ to generate the heatmap. ABA: abscisic acid; BR: brassinosteroids; CK: cytokinins; Eth: ethylene; GA: gibberellic acid; JA: jasmonate; SA: salycilic acid. WW: well-watered treatment; WD: water deficit; WD-RW: rewatering after water deficit. The *P*-value of the hypergeometric test is indicated: **P*<0.05, ***P*<0.01, ****P*<0.001.

(iii) a SAM (the center of morphogenesis with continuously dividing cells) easy to collect for (epi)genomic analysis (Lafon-Placette *et al.*, 2013), and finally (iv) a SAM showing global DNA methylation variations in response to water conditions (Gourcilleau *et al.*, 2010).

Shoot apical meristem modulates its transcriptome and methylome in response to water conditions

Clonally propagated poplars submitted to a moderate water deficit showed an expected ecophysiological response (Cohen *et al.*, 2010; Gourcilleau *et al.*, 2010) but interestingly, stomatal conductance and leaf osmotic pressure came back to the control level after rewatering, while height and leaf growth did not (Bizet *et al.*, 2015). Underlying molecular mechanisms, particularly transcriptomics, have been well documented in poplar, but in leaves and roots (Bogeat-Triboulot *et al.*, 2007; Wilkins *et al.*, 2009; Hamanishi *et al.*, 2010; Raj *et al.*, 2011). Especially, previous reports in Arabidopsis shoot and *Populus* leaves showed that for most of the genes, the dysregulation caused by the drought disappeared after rewatering (Bogeat-Triboulot *et al.*, 2007; Huang *et al.*, 2008). Here, in the SAM, only half of the genes affected by the drought stress recovered an expression level similar to control after rewatering. In addition, a similar number of DEGs was found between the control and drought conditions (649 DEGs) and between the control and rewatering conditions (642 DEGs). Thus, the reversible drought effect previously observed in Arabidopsis shoots or *Populus* leaves (Bogeat-Triboulot *et al.*, 2007; Huang *et al.*, 2008) was only partially true in this study, suggesting a more dynamic response to rewatering in the SAM. While species- or tissue-specific responses could be suspected, different

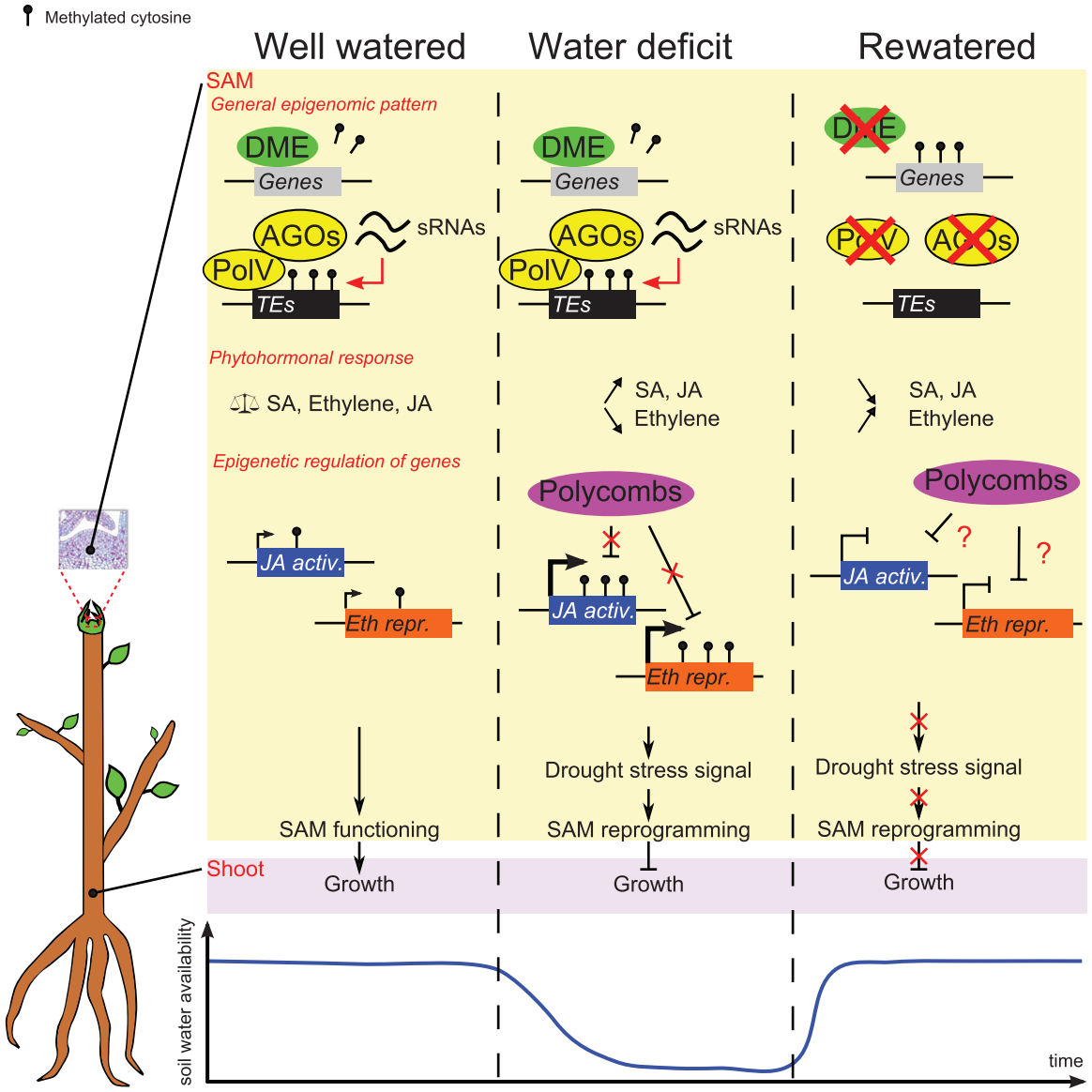

Fig. 7. Model for the epigenetic control of phytohormonal signal during water availability changes in the poplar shoot apical meristem. A poplar tree is schematized on the left side. 'General epigenomic pattern' panel: *DME*, *NRPE1* (PolV), and the *AGOs* are expressed in well-watered and water-deficit conditions (Fig. 4F) and are expected to fulfil their molecular function described in Arabidopsis, silencing genes or transposable elements (TEs) via production of small RNAs (sRNAs). After rewatering, these genes are down-regulated (Fig. 4F) and their molecular action is expected to be limited. 'Phytohormonal response' panel: in well-watered conditions, the balance symbol indicates that the genes specifically expressed or repressed in the well-watered condition did not belong to any particular hormonal pathway (Fig. 2C). The SAM transcriptomic response to drought is similar to a transcriptomic response to SA and JA treatments, suggesting activated JA and SA pathways in WD (shown by the upward arrow). In WD, the SAM transcriptome also exhibits a negative association with the transcriptomic response to ethylene, shown by a downward arrow (Fig. 2C). This association becomes positive in WD-RW, represented by the upward arrow. This suggests that the ethylene pathway is repressed during WD, and activated upon WD-RW. 'Epigenetic regulation of genes' panel: genes activated by jasmonate (*JA activ.*) or repressed by ethylene (*Eth repr.*) are expressed lowly in the well-watered condition. During drought stress, they are highly expressed (Fig. 2C), and the higher DNA methylation in genes activated by jasmonate (Fig. 6A) may prevent Polycomb complexes from repressing these genes. After the rewatering, the hypomethylation in genes activated by jasmonate and repressed by ethylene (Fig. 6) may allow Polycomb complexes to repress these genes. Sticks and filled circles represent methylated cytosines. ABA: abscisic acid; Eth: ethylene; JA: jasmonate; SA: salycilic acid.

drought treatments and rewatering kinetics could be also ascribed (Bizet *et al.*, 2015). Altogether, our data suggest that the SAM displays a dynamic and highly responsive molecular response in relation to the plant water status, possibly to ensure adjusted stress response, growth and morphogenesis.

DNA methylation has already been shown to be affected at the global level by water deficit in the poplar SAM (Gourcilleau *et al.*, 2010) and leaves (Raj *et al.*, 2011). Here, we confirm the DNA hypomethylation previously observed under water deficit (Gourcilleau *et al.*, 2010). This was mainly observed in gene bodies while transposable elements remained strongly methylated during drought. In agreement, Baubec *et al.* (2014) have shown in Arabidopsis that the SAM has specific expression of epigenetic regulators to ensure transposon silencing, providing a checkpoint for correct epigenetic inheritance. In contrast, following rewatering, a hypermethylation was observed at the

global level, occurring mainly in gene bodies and correlated with a decreased expression of the demethylase gene *DEMETER*. In parallel, hypomethylation specifically affected transposable elements, possibly explained by the decreased expression of key players of the RNA-directed DNA methylation (RdDM) pathway such as Pol V or AGOs (the key players of this pathway are reviewed in Matzke and Mosher, 2014). This suggests an extensive and dynamic chromatin remodeling occurs in the SAM after rewatering. Consistently, in rice, DNA methylation variations during salt stress mostly affected gene bodies (Karan *et al.*, 2012), suggesting that DNA methylation variations in response to water deficit are not only concentrated in pericentromeric regions (Pecinka and Mittelsten Scheid, 2012). This is in agreement with the predominant changes in the Arabidopsis methylome found in the promoters of genes encoding proteins that are suited to cope with environmental challenges (Colaneri and Jones, 2013). Nevertheless, other factors, such as mitotic activity, can contribute to the observed DNA methylation changes. It is also possible that DNA methylation changes observed after the 5 days of rewatering are transient and only long term kinetics could help to answer whether these changes are transient or not.

Specific variation of DNA methylation is correlated to gene expression

Changes in DNA methylation influence gene expression (Seymour and Becker, 2017), in particular transcription (Huettel *et al.*, 2007), splicing (Regulski *et al.*, 2013), and polyadenylation (Tsuchiya and Eulgem, 2013), but it is still difficult to differentiate between direct changes mediated by DNA methylation and secondary effects (Meyer, 2015). Indeed, gene body DNA methylation has been suggested to play a role in defining exon–intron borders or setting up paralog- and allele-specific expression (Takuno and Gaut, 2013; Wang *et al.*, 2015; Zhang *et al.*, 2016), but its biological significance still remains under debate (To *et al.*, 2015; Bewick and Schmitz, 2017). Interestingly, the correlation between gene-body methylation and transcription was more clearly observed in proliferating cells than in non-proliferating cells, suggesting that gene-body methylation might be connected to cell proliferation in mammals (Aran *et al.*, 2011). In addition, there is evidence for both DNA methylation affecting expression and conversely expression affecting DNA methylation, as recently shown during phosphate starvation in rice (Secco *et al.*, 2015).

While several studies showed the role of DNA methylation in the control of transcription of specific genes in response to water stress (González *et al.*, 2011; Bilichak *et al.*, 2012; Garg *et al.*, 2015; Ci *et al.*, 2016), few genome-wide scale data are available. Here, we observed that in *Populus* SAM, genes undergoing a hypomethylation of their body were significantly down-regulated during the rewatering process. This is not necessarily a causal relationship: if gene body methylation is a byproduct of transcriptional activity (Meyer, 2015), the observed hypomethylation could be merely a marker for lower transcriptional activity. However, we also found that these genes were putative targets of Polycomb proteins. Polycomb proteins are enzymes involved in transcriptional repression via the deposition of H3K27me3 histone marks (Mozgova *et al.*, 2015). These enzymatic complexes act in regions with low or no DNA methylation (Roudier *et al.*, 2011). In *met1* mutants, DNA hypomethylated regions are ectopically targeted by H3K27me3, suggesting that Polycomb complexes are actively excluded from DNA methylated regions (Weinhofer *et al.*, 2010; Deleris *et al.*, 2012). Here, the Arabidopsis homologues of genes hypomethylated and repressed during rewatering were targets of Polycomb complexes. This suggests that the hypomethylation undergone by this subset of genes may allow their repression via Polycomb complexes. Nevertheless, this result is correlative and the epigenetic landscapes in *Populus* and Arabidopsis may be different. Histone mark profiling would help in answering this question.

In most cases, a direct correlation between DNA methylation and gene expression changes could not be established. Especially upon rewatering, the extensive DNA hypermethylation affecting gene bodies, associated with *DME* down-regulation, could not be correlated to any global gene expression changes. Consistently, other studies have shown that the transcriptional activity of a subset of genes might be regulated through the alteration of gene-body methylation in response to abiotic stress (Karan *et al.*, 2012; Garg *et al.*, 2015; Chwialkowska *et al.*, 2016). This limited subset of DNA methylation-regulated genes can explain the difficulty in establishing correlations at the global scale. Thus, the ectopic DNA hypermethylation observed during rewatering, potentially caused by *DME* down-regulation, might still impact the expression of crucial genes. For example, the two *SHOOT MERISTEMLESS* homologues were found to be down-regulated and hypermethylated in their promoter during rewatering (data not shown). In Arabidopsis shoots, *DME* is expressed specifically in proliferating cells of the shoot and root apical meristems (Kim *et al.*, 2008). Nevertheless the role of *DME* in these cells remains unknown. In *Populus* SAMs, we show that *DME* expression is dependent on water availability, correlated with genome-wide DNA methylation changes. Whether and how such changes affect the functioning of proliferating cells in the SAM and impact shoot morphogenesis is an exciting question to address in the future.

Altogether, these reports confirm that gene-body DNA methylation is still a misunderstood process. Nevertheless, a specific association was found between hypomethylation and transcriptional repression of genes during the rewatering process, suggesting interplay between DNA methylation and other epigenetic factors influencing gene expression.

DNA methylation changes affect DEGs with phytohormone-related function in response to water conditions

In the SAM transcriptome affected by drought and rewatering treatments, responses to phytohormones were detected. According to these data, JA, SA, ethylene, and brassinosteroid pathways were activated. These hormones have been shown to be involved in the response to drought and rewatering in other organs (Oono *et al.*, 2003; Seki *et al.*, 2007; Wolters and Jürgens, 2009; Choudhary *et al.*, 2012; Riemann *et al.*, 2015; Khan *et al.*, 2015; Yamamuro *et al.*, 2016; Royer *et al.*, 2016). Interestingly, according to the transcriptome data, auxin and cytokinin pathways were mostly unaffected,

suggesting that the balance between meristem maintenance and organ differentiation remained similar across the water conditions (Shani *et al.*, 2006). Conversely, the activation of the JA pathway during drought suggests a reduction of the mitotic activity in the SAM (Zhang and Turner, 2008), consistent with the reduced growth observed during the drought stress. Similar results were found in a competition experiment leading to decreased leaf size in *Antirrhinum*: the cytokinin/auxin signature in the SAM transcriptome was unaffected while the JA pathway was activated (Weiss *et al.*, 2016).

In the present study, our major finding is that a part of these phytohormonal responses involve DNA methylation. Our data show that hormone-responsive genes are down-regulated and undergo a hypomethylation during the rewatering process. Our results suggest also that these genes could be targets of Polycomb proteins. In rice, *osfie2* polycomb mutant plants show increased levels of ABA, JA, and SA (Liu *et al.*, 2016), suggesting a role of *OsFIE2* in repressing genes involved in the biosynthesis of these hormones. In Arabidopsis, the Polycomb Repressive Complex 2 represses hormone-induced somatic embryogenesis in vegetative tissue (Mozgová *et al.*, 2017). While still unclear, these data suggest that the crosstalk between DNA methylation and Polycomb complexes could play a role in the developmental response to abiotic stresses via the repression of a specific set of hormone-responsive genes (Yamamuro *et al.*, 2016).

In conclusion, our data show that phenotypic plasticity in response to water conditions involved coordinated variations in SAM of DNA methylation and expression on genes related to phytohormone pathways. The role of DNA methylation in meristematic cells to acclimatize plant development to changing environments brings complementary views and perspectives to a recently published work supporting a role for DNA methylation in adaptation to geography and climate of origin (Kawakatsu *et al.*, 2016). Development of single-cell epigenomics will allow deciphering the role of DNA methylation. Future challenge will be to evaluate in the field the potential role of this coordinated response involving DNA methylation in stress memory and GxE interaction in trees, which are long living organisms submitted to repeated environmental fluctuations.

Supplementary data

Supplementary data are available at *JXB* online.

Methods S1. FDR estimation explanation.

Methods S2. Additional bioinformatics analysis.

Fig. S1. Venn diagram representing the overlap between transcriptome and methylome analyses.

Fig. S2. Fold-change expression using qRT-PCR validates transcriptome microarray data.

Fig. S3. Enriched GO terms in DEGs between water deficit and rewatering conditions.

Fig. S4. Enriched GO terms in DEGs between well-watered and water deficit conditions.

Fig. S5. Enriched GO terms in DEGs transiently deregulated in the water deficit condition.

Fig. S6. Enriched GO terms in DEGs between well-watered and rewatering conditions.

Fig. S7. Enriched GO terms in DEGs both between rewatering and water deficit and between rewatering and well-watered conditions (rewatering specific).

Fig. S8. Bisulfite sequencing confirmation of DNA methylation changes detected with the MeDIP-microarray.

Table S1. qPCR and bisulfite primers.

Table S2. List of all v3 *P. trichocarpa* genes for which the expression could be analysed using the microarray approach (i.e. all *P. trichocarpa* v3 genes for which a filtered probe exists).

Table S3. Microarray probes summary.

Table S4. Differentially expressed genes.

Table S5. Differentially methylated regions.

Table S6. Overlap between all DEGs and DMRs.

Table S7. Overlap between all DEGs and DMRs 'plasticity'.

Table S8. Overlap between down-regulated and hypomethylated genes in RW-WD *vs* WD.

Table S9. Overlap between down-regulated and hypomethylated genes in RW-WD *vs* WD and targets of Polycomb complexes in Arabidopsis.

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