Bulgarian Journal of Agricultural Science, 25 (No 5) 2019, 845–851

Expression profiling of DNA methyltransferase genes in wheat genotypes with contrasting drought tolerance

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Abstract

Vassileva, V., Vaseva, I. & Dimitrova, A. (2019). Expression profiling of DNA methyltransferase genes in wheat genotypes with contrasting drought tolerance. *Bulgarian Journal of Agricultural Science, 25*(5), 845–851

DNA methylation is a reversible epigenetic mechanism that affects important developmental processes and stress-related events in living organisms. The process of cytosine methylation is catalysed by DNA methyltransferases that are structurally and functionally conserved in all eukaryotes. This study assessed the effect of drought stress and subsequent rewatering on the transcription of *DNA* methyltransferase coding genes (*TaMET1*, *TaMET2a*, *TaMET2b* and *TaMET3*) in wheat genotypes with contrasting drought tolerance. The applied drought stress led to changes in leaf water deficit in a variety-specific manner. Two of the wheat genotypes, Farmer and Bojana, performed as sensitive to drought, the other two, Yoana and Guinness, were considered as drought tolerant. Under drought and after recovery, the drought-sensitive genotypes exhibited high variability in the expression level of *TaMET1*, *TaMET2b* and *TaMET3*. The *TaMET1* transcripts increased approximately three times under dehydration, compared to the controls. The drought-tolerant varieties had a relatively stable transcription of DNA methyltransferase genes. The *TaMET1* expression was higher in the controls of tolerant varieties, compared to the sensitive genotypes, and did not change under drought exposure and after rewatering. The lowest response to dehydration had *TaMET2a*, which only slightly varied in the sensitive genotypes under dehydration. The results point to unique biological functions and diverse roles of the wheat DNA methyltransferases in drought conditions that mainly correlated with the level of drought tolerance of the studied genotypes.

Keywords: DNA methyltransferases; wheat genotypes; drought tolerance; gene expression

Introduction

DNA methylation is a reversible epigenetic mechanism that has an important role in development, differentiation and maintenance of cellular identity. It preserves genome integrity and stability by repressing mobile *DNA* elements (Lisch, 2013) and affects gene expression without changing underlying DNA sequence (Saze, 2008). The methylation control is enabled by modulating binding of transcription factors to DNA molecule, which affects chromatin structure (Osabe et al., 2014). During development and environmental stress responses, level of DNA methylation is governed by the processes of DNA methylation and demethylation (Cao et al., 2014).

In mammalian genomes, DNA methylation occurs preferentially in the symmetric CG context. In plants, DNA methylation is found in three cytosine contexts: the symmetric CG and CHG contexts and the asymmetric CHH context (H = A, T, or C) (Meyer, 2011). Maintenance and distribution of DNA methylation is controlled by the activity of a family of DNA methyltransferases that add a methyl group to the fifth carbon of the cytosine ring to form 5-methylcytosine

(Edwards et al., 2017). The methyltransferase enzymes are structurally and functionally conserved in all eukaryotes. Three main classes DNA methyltransferases are responsible for plant DNA methylation: *de novo* methylation is established by DOMAINS REARRANGED METHYLTRANSFERASE2 (DRM2) via RNA-directed DNA methylation pathway, and maintained by DNA METHYLTRANSFERASE1 (MET1) and CHROMOMETHYLASE3 (CMT3) for CG and CHG contexts, respectively, and through persistent *de novo* methylation by DRM2 for CHH context (Meyer, 2011; Law & Jacobsen, 2010). MET1 maintains CG methylation through recognizing the hemimethylated CG sites and methylating the newly synthesized DNA strand during DNA replication (Finnegan & Dennis, 1993; Kankel et al., 2003; Law & Jacobsen, 2010). In *Arabidopsis*, one *MET1* gene has been identified (Finnegan et al., 1996). It regulates plant morphology and flowering (Kankel et al., 2003). Rice has two *MET1* genes, *MET1a* (*OsMET1-1*) and *MET1b/OsMET1-2* (Teerawanichpan et al., 2004; Yamauchi et al., 2008). The transcript levels of *MET1b* are higher than those of *MET1a* in all of the examined rice tissues, indicating that *MET1b* may play a more important role in maintaining DNA methylation (Yamauchi et al., 2008). Consistently, other studies have shown that *MET1b* is an essential gene and its loss of function causes genome-wide reduction of CG methylation in rice seedlings (Hu et al., 2014; Yamauchi et al., 2008). Null function of *MET1-2* leads to abnormal seed development and seedling lethality (Hu et al., 2014). Dai et al. (2005) have cloned five genes encoding putative wheat DNA methyltransferases (*TaMET1*, *TaMET2a*, *TaMET2b*, *TaCMT* and *TaMET3*) and observed varying transcript abundance in different organs and vegetative tissues of wheat plants. Despite the available information that cytosine methylation levels are not always directly related to the DNA methyltransferase activity (Teerawanichpan et al., 2004), the DNA methyltransferases are key mediators in the methylation process.

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated food crops, however, drought reduces its yield by more than 50% (Amiri et al., 2013). Understanding the molecular basis of plant responses to drought is critical in finding approaches to increase plant stress tolerance. Comparative analysis of drought-tolerant and drought-susceptible wheat genotypes revealed a number of molecular, biochemical and physiological mechanisms, conferring plant tolerance to dehydration (Vaseva et al., 2010; Vassileva et al., 2009; 2011; 2012). Plants have also evolved epigenetic strategies to cope with water stress, based on DNA methylation-associated changes in the expression level of specific genes involved in drought stress responses (Wang et al., 2010). Reduced DNA methylation often negatively affects plant's ability to tolerate environmental stresses.

In the present study, we analysed expression patterns of four genes encoding for DNA methyltransferases in wheat genotypes with contrasting drought tolerance after exposure to severe dehydration and subsequent rewatering. The relationship between the drought-induced gene expression changes and the level of drought tolerance of the genotypes studied was examined.

Material and Methods

DNA methyltransferase genes

The sequences of wheat methyltransferase genes (*TaMET1*, *TaMET2a*, *TaMET2b* and *TaMET3*) were previously published (Dai et al., 2005), and are publicly available in databases NCBI (National Center of Biotechnology Information), Plaza 4.0 (Monocots) and URGI (http://urgi.versailles.inra.fr).

Experimental layout and growth conditions

Experiments were performed with four varieties of winter wheat (*Triticum aestivum* L.) differing in their field drought tolerance: Farmer, Bojana, Yoana and Guinness. Plants were grown in pots containing enriched soil mixture (20 plants per pot) under controlled conditions in a growth chamber with a light intensity of 150 μmol m^{-2} s^{-1}, day/night temperatures 25/2°C, and a 14-h photoperiod. Drought was imposed on 10-day-old plants with a fully extended second leaf and an emerging third leaf by withholding watering for 7 days, followed by a subsequent recovery for 4 days. Control plants were watered every day. The experimental analyses were performed with material derived from the second fully expanded leaf. The degree of drought stress was determined as previously described (Demirevska et al., 2008).

Leaf water status

Relative water deficit (WD) of the second leaf was calculated using the formula (TW-FW)/TW.100, where TW is the turgid weight of the leaf after equilibration in distilled water for 24 h (at 4°C), FW is the leaf fresh weight. All samples were collected around midday.

RT-PCR Analysis

Total RNA was extracted using GeneJET Plant RNA Purification Kit (Thermo Scientific). The isolated RNA was quantified using the micro UV-VIS spectrophotometer, Nano Drop 2000 (Thermo Scientific). cDNA synthesis using 1 μg of RNA was performed with the iScript cDNA syn-

thesis kit (Bio-Rad) according to the manufacturer's instructions. Semi-quantitative RT-PCR analyses were performed in a *PCR* Eppendorf Mastercycler. α-*TUB* was used as a reference gene. The following primer sequences were used to perform gene expression analyses: *TaMET1* 5′-gcctgtactggcctgagaag-3′ and 5′-ttcaggccaatgtttcttcc-3′; *TaMET2a* 5′-tttatccttcagtttttgtgtgtc-3′ and 5′-gcacatgccacctaactttg-3′; *TaMET2b* 5′-agaaagcagaagcctacgagaac-3′ and 5′-gttgatgtcaaaggcctcca-3′; *TaMET3* 5′-catagccgacttgacggaat-3′ and 5′-gaggagttggtgggatcaga-3′; α-*TUB* 5′-agcgcctttgagccttcgtcc-3′ and 5′-tcatcgccctcatcaccgtcc-3′. The reactions were carried out at least in triplicate, and three biological repeats for each experiment were performed.

Gel image analysis

Gel images were exported for analysis as JPG files and analysed using Fiji/ImageJ software (Schindelin et al., 2012). The bands of interest were selected using the rectangular selection tool, the band intensity peaks were plotted and the base of each peak of interest was closed off by the straight line tool. After the measurement of peak areas, each measured peak was presented as a percentage of the total size of all measured peaks (Ferreira & Rasband, 2012). The expression level of each target gene was calculated versus a reference gene to give the relative percentage of expression for each assessed transcript. The relative expression was used in statistical analyses as described below.

Statistical analyses

Results are based on a completely randomized experimental design of the four varieties studied. All the statistical analyses of data were carried out by the OriginPro 8 SRO package software (version v8.0724, OriginLab Corporation, Northampton, MA, USA). One-way ANOVA with Dunnett's post-hoc test were used to evaluate statistical significance of the changes between control and treatment variants.

Results and Discussion

Results

Leaf water status

Four wheat varieties (Farmer, Bojana, Yoana and Guinness) were grown either under benign conditions or exposed to drought stress with a subsequent period of recovery. Well-watered control plants maintained water deficit between 1.9 and 4.9% (Fig. 1). After the applied drought, two of the varieties, Farmer and Bojana, showed higher levels of water deficit (around 69%), whereas the other two genotypes, Yoana and Guinness, maintained lower water scarcity levels (45 and 37%, respectively). At the end of the recovery period, the leaf water deficit of the drought tolerant Yoana and Guinness decreased and reached values close to the controls (10.5 and 5.9%). The drought-sensitive varieties were not able to achieve substantial recovery, exhibiting leaf water deficit of 44 and 41% (Fig. 1).

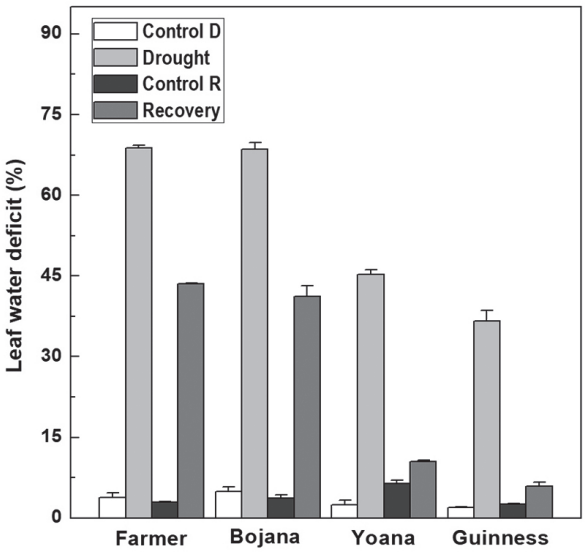

Fig. 1. Relative water deficit evaluated in the second fully expanded leaf of wheat genotypes exposed to drought stress (D) and after four days of recovery (R); vertical bars represent standard errors of the mean (n = 10)

Expression analysis of MT genes

Based on the sequences retrieved from the integrative genomic data resources and the information provided in previous studies (Dai et al., 2005; Thomas et al., 2014), we designed primers for the methyltransferase genes *TaMET1*, *TaMET2a*, *TaMET2b* and *TaMET3*, and analysed gene expression levels in the studied wheat varieties (Fig. 2). After plant exposure to drought and a subsequent recovery, the second fully developed leaf was sampled and used to quantify relative transcript abundance (Fig. 3).

Comparative gene expression profiling showed that most of the studied DNA methyltransferases had moderate levels of expression in the control plants (Fig. 2A-D). *TaMET1* showed the highest transcript abundance in Yoana and Guinness controls (Fig. 2C,D), while the levels in the non-treated Farmer and Bojana were lower (Fig. 2A,B). Bojana and Yoana varieties had the lowest expression of *TaMET2a* transcripts (Fig. 2B,C).

Exposure to dehydration led to variety-specific expression responses that mainly depended on the plant drought

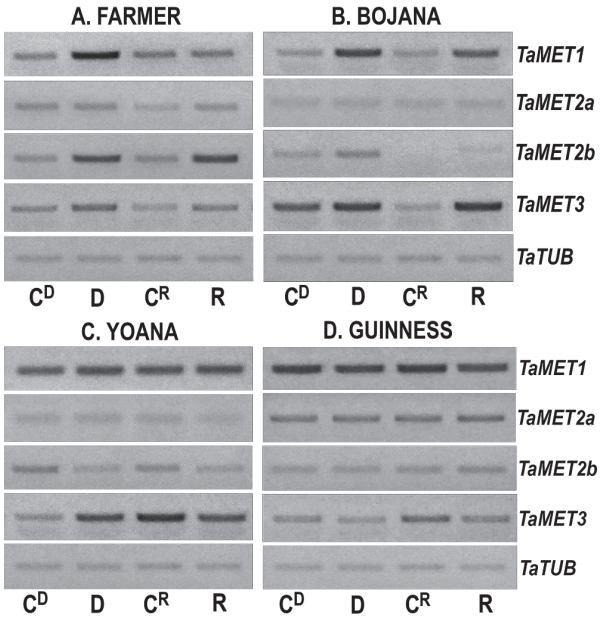

Fig. 2. Representative gel images of expression profiles of genes encoding DNA methyltransferases (*TaMET1*, *TaMET2a*, *TaMET2b* and *TaMET3*) and the reference gene *a-TUB* (*a-tubuline*) in different wheat genotypes (A-D) exposed to drought stress (D) and after four days of recovery (R); the corresponding controls are labelled as C^D (control drought) and C^R (control recovery)

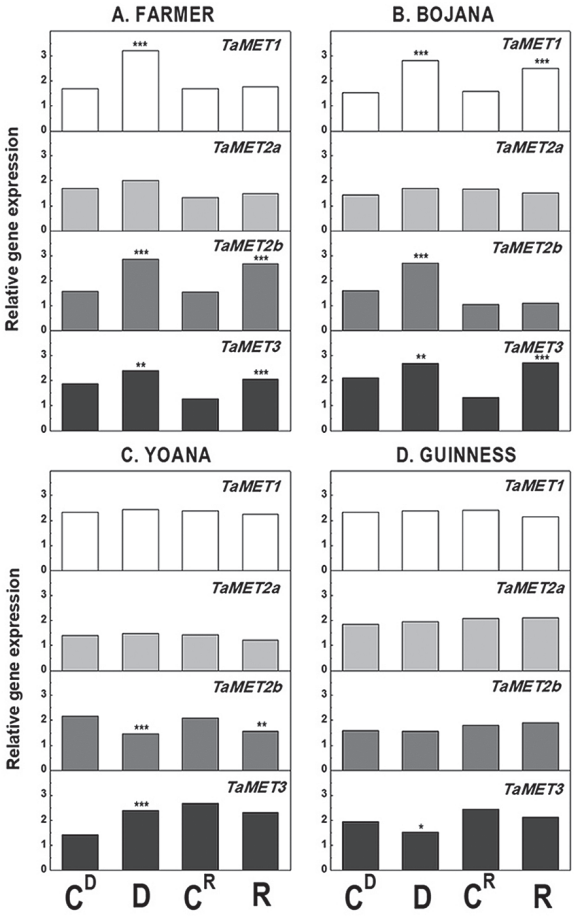

Fig. 3. Normalised expression levels of genes encoding DNA methyltransferases (*TaMET1*, *TaMET2a*, *TaMET2b* and *TaMET3*) in different wheat genotypes (A-D) exposed to drought stress (D) and after four days of recovery (R); the corresponding controls are labelled as C^D (control drought) and C^R (control recovery); expression ratio between the reference gene *a-TUB* and the studied DNA methyltransferasesis is represented in the graphs; significant differences are depicted by asterisks (*p < 0.05, **p < 0.01 and ***p < 0.001, one-way ANOVA with Dunnett's post hoc test)

vulnerability. Specifically, the drought-sensitive Farmer and Bojana genotypes displayed a high degree of variability in the expression level of *TaMET1*, *TaMET2b* and *TaMET3* (Fig. 2A,B), whereas in the drought-tolerant varieties Yoana and Guinness these methyltransferases had relatively stable transcript abundance (Fig. 2C,D). The accumulation of the *TaMET1* transcripts in Yoana and Guinness control samples was between 2.2 and 2.4 times higher than the levels measured in the controls of the drought-sensitive varieties, and did not change under drought stress exposure and after subsequent rewatering (Fig. 3C,D). Opposite responses were observed in the drought-sensitive Farmer and Bojana varieties, where dehydration led to approximately 3.0 times higher *TaMET1* transcripts levels, compared to the controls (Fig. 3A,B). After the recovery period, the *TaMET1* expression in Bojana was still higher, compared to the corresponding age control (Fig. 3B).

In general, the most unresponsive to drought stress was *TaMET2a*, which showed insignificant variations after drought stress treatment (Fig. 3A-D). The *TaMET2b* showed a nearly 2-fold increase after drought stress treatment in Farmer and Bojana (Fig. 3A-D), which was still evident following the recovery period in Farmer (Fig. 3A). *TaMET2b* was hardly detectable in Bojana's recovery samples (Fig. 2B).

Expression of the *TaMET3* gene was the most variable one in all tested wheat varieties. There was a significant drought-induced expression of *TaMET3* in the drought-sen-

sitive Farmer and Bojana, compared to controls (Fig. 3A,B). The changes of *TaMET3* expression in the drought-tolerant Yoana and Guinness varieties were rather age-related (Fig. 3C,D).

Discussion

A growing body of evidence indicates that epigenetic modifications, such as DNA methylation, histone modifications and small RNA interference, affect responses of plants to abiotic stress, modifying their growth and developmental processes, and minimizing the damaging stress effects (Yaish, 2013). The relationship between epigenetic changes and plant stress response has been widely discussed (Boyko & Kovalchuk, 2008; Chen et al., 2010; Alvarez et al., 2010; Chinnusamy & Zhu, 2009). However, the relation between DNA methylation and abiotic stress tolerance has been poorly investigated, especially in economically important crops.

In this study, we evaluated the effect of drought stress on the transcription of *DNA* methyltransferase coding genes in wheat genotypes with contrasting drought tolerance. The applied drought stress led to changes in leaf water deficit in a variety-specific manner. Two of the wheat genotypes (Farmer and Bojana) were considered as sensitive to drought. They reached a high degree of leaf water deficit and were not able to recover completely after rewatering. The other two genotypes (Yoana and Guinness) performed as drought tolerant since they displayed a lower level of water deficit and achieved substantial recovery after rewatering.

Comparative analysis of expression levels of the investigated DNA methyltransferases revealed genotype-specific variations, which could be associated with the general degree of plant drought tolerance. In previous studies, transcript profiling of drought-tolerant and drought-susceptible wheat genotypes has shown diverse global transcriptional responses under control and drought conditions. It has been found that more genes were repressed in the tolerant genotypes following the exposure to drought stress (Li et al., 2012; Kumar et al., 2018). Gene down regulation could, at least partially, be associated with epigenetic transcriptional repression, modulated by DNA methyltransferase activity.

The expression patterns of four putative wheat DNA methyltransferase genes upon drought and subsequent recovery showed that drought-tolerant varieties had more stable levels of DNA methyltransferase expression, than the drought-sensitive genotypes. The highest transcript accumulation was observed for*TaMET1*. Similarly, Dai et al. (2005) observed that the transcript level of *TaMET1* is higher than those of *TaMET2a*, *TaMET2b* and *TaMET3* in most wheat tissues. *MET1* encodes a putative methyltransferase that maintains CG methylation after DNA replication, silencing transposable elements and controlling gene expression (Kankel et al., 2003). More than 80% of the wheat genome consists of transposable elements and repeats (Choulet et al., 2010). Since active transposable elements can move to different locations and lead to a substantial increase in the rate of stress-induced mutations, the high expression of *TaMET1* in the genome of drought-tolerant varieties could contribute to genome stability. In the drought-sensitive varieties Farmer and Bojana, the moderate expression level of *TaMET1* was induced significantly by the applied drought stress, compared to the untreated control. After the recovery period, the increased *TaMET1* transcripts were still detectable in Bojana. Wang et al. (2010) demonstrated that drought stress triggers an increase in DNA methylation in rice genotypes, and only 70% of the total changes in DNA methylation could be reset to the normal level after recovery under favourable conditions. Low levels of DNA methylation can negatively impact the ability of plants to tolerate environmental stress (Baek et al., 2011; Yaish, 2013). According to Zhong and Wang (2007), different salt tolerance of wheat genotypes is related to methylation levels, demonstrating that the salt-tolerant wheat variety has a higher level of methylation than the salt-sensitive wheat.

Although *TaMET2a* and *TaMET2b* were expressed at significantly lower levels, compared to *TaMET1*, their transcript profiles did not change in the drought-tolerant varieties under drought and after rewatering. Water deprivation can cause extensive remodeling of DNA methylation patterns in different species (Liang et al., 2014; Yong-Villalobos et al., 2015; Wibowo et al., 2016; Chwialkowska et al., 2016), which could be a result of differential activity of *DNA* methyltransferases. The unchanged transcript abundance of methyltransferase genes under drought implies a precise control of the stress response that is required for genome stability in drought-tolerant varieties. On the contrary, in the drought-sensitive genotypes Farmer and Bojana, the *TaMET2b* transcripts increased almost twice under water stress treatment. In the recovery samples of Bojana, *TaMET2b* was hardly detectable. These observations suggest that variable transcript levels of *TaMET2b* during drought stress might be indicative for its involvement in the stress-induced DNA methylation changes. Since this instability was observed in the drought-sensitive varieties, it could be interpreted as a factor that rather negatively affected the degree of plant drought tolerance. Another DNA methyltransferase with varying transcript levels was *TaMET3*. Despite the observed drought-induced accumulation in the sensitive genotypes Farmer and Bojana, and the presence of *TaMET3* transcripts in their recovery samples, this methyltransferase showed variable expression profiles in the drought-tolerant varieties as well. It is interest-

ing to note however, that during the recovery experimental stage *TaMET3* transcripts in the control plants of the drought tolerant genotypes were at least twice higher, compared to the controls of the drought-sensitive varieties. Regardless the short time of the recovery stage, the higher expression level in the drought-tolerant varieties Yoana and Guinness could be interpreted as age-related.

Conclusions

In conclusion, the obtained results demonstrated that drought stress caused specific alterations in the expression of DNA methyltransferase genes in wheat genotypes with contrasting drought tolerance. Differential expression patterns under drought and after the recovery, point to unique biological functions and diverse roles of the studied DNA methyltransferases in plant responses to dehydration. Understanding the epigenetic mechanisms behind drought-induced gene regulation could additionally clarify the mechanisms that regulate drought stress tolerance in wheat. This knowledge could be implemented in future breeding programs for development of new wheat varieties with improved agronomical characteristics.

Acknowledgments

This study was supported by the National Science Fund of the Bulgarian Ministry of Education and Science, grant No. DN06/12/19.12.2016.

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Received: September, 19, 2018; *Accepted:* November, 29, 2018; *Published:* October, 31, 2019