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RESEARCH ARTICLE

# TaMIR1119, a miRNA family member of wheat (*Triticum aestivum*), is essential in the regulation of plant drought tolerance

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**Abstract**

Through regulating target genes *via* the mechanisms of posttranscriptional cleavage or translational repression, plant miRNAs involve diverse biological processes associating with plant growth, development, and abiotic stress responses. In this study, we functionally characterized TaMIR1119, a miRNA family member of wheat (*Triticum aestivum*), in regulating the drought adaptive response of plants. TaMIR1119 putatively targets six genes categorized into the functional classes of transcriptional regulation, RNA and biochemical metabolism, trafficking, and oxidative stress defense. Upon simulated drought stress, the TaMIR1119 transcripts abundance in roots was drastically altered, showing to be upregulated gradually within a 48-h drought regime and that the drought-induced transcripts were gradually restored along with a 48-h recovery treatment. In contrast, most miRNA target genes displayed reverse expression patterns to TaMIR1119, exhibiting a downregulated expression pattern upon drought and whose reduced transcripts were re-elevated along with a normal recovery treatment. These expression analysis results indicated that TaMIR1119 responds to drought and regulates the target genes mainly through a cleavage mechanism. Under drought stress, the tobacco lines with TaMIR1119 overexpression behaved improved phenotypes, showing increased plant biomass, photosynthetic parameters, osmolyte accumulation, and enhanced antioxidant enzyme (AE) activities relative to wild type. Three AE genes, *NtFeSOD*, *NtCAT1;3*, and *NtSOD2;1*, encoding superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) proteins, respectively, showed upregulated expression in TaMIR1119 overexpression lines, suggesting that they are involved in the regulation of AE activities and contribution to the improved cellular reactive oxygen species (ROS) homeostasis in drought-challenged transgenic lines. Our results indicate that TaMIR1119 plays critical roles in regulating plant drought tolerance through transcriptionally regulating the target genes that modulate osmolyte accumulation, photosynthetic function, and improve cellular ROS homeostasis of plants.

**Keywords:** wheat (*Triticum aestivum* L.), miRNA member, drought stress, plant growth, functional characterization

## 1. Introduction

Drought stress is one of the adverse environmental factors that negatively impacts on the agricultural production worldwide (Cattivelli *et al*. 2008). Water deficit derived from the drought stress affects a suite of physiological processes such as cellular membrane integrity, pigment content, osmotic adjustment, water relation, and photosynthesis,

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causing deteriorated plant growth and finally lowered yield potential (Benjamin and Nielsen 2006). Therefore, it is crucial to adopt drought-tolerant varieties in crop production that can promote the sustainable agriculture given the elite behavior of the varieties in water use efficiency.

Plants have evolved various physiological and molecular mechanisms to respond or cope with the drought stress (Munns 2002; Wang *et al*. 2003; Chaves *et al*. 2009). Recently, the regulatory networks underlying plant adaptation to drough stress have been largely elucidated. For example, a large set of drought response-associated regulatory components, including transcription factors, protein kinases, phosphatases, and the calmodulin-binding proteins, have been functionally characterized focusing the roles in the mediation of drought tolerance (Zhang *et al*. 2004; Shinozaki and Yamaguchi-Shinozaki 2007; Nakashima *et al*. 2009). The findings in this research have provided useful guidance in breeding crop cultivars with improved drought stress tolerance.

Plant miRNAs are a type of noncoding RNAs with generally 20 to 24 nt-long in length, involving the modulation of a large set of biological process through regulating target mRNAs *via* a base pair-specific action mode (Zhang *et al*. 2006; Shukla *et al*. 2008). Extensive investigations have indicated that the miRNAs act as critical meditors in regulating growth and development, growth phase transition and leaf morphogenesis, floral organ identity, and root architecture establishment (Lu and Huang 2008; Rubio-Somoza and Weigel 2011). Additionally, the plant miRNAs also modulate plant response to adverse abiotic stresses (Phillips *et al*. 2007; Lu and Huang 2008; Mazzucotelli *et al*. 2008; Shukla *et al*. 2008; Lewis *et al*. 2009). For example, miR319 members in *Arabidopsis* and other several species are responses to dehydration, high salinity, and low temperature and mediate plant adaptation to these stressors (Sunkar and Zhu 2004; Liu *et al*. 2008; Lv *et al*. 2010; Zhou *et al*. 2010; Thiebaut *et al*. 2012). Prediction on target genes suggested that miR319 interacts the *TCP* (for *TEOSINTE BRANCHED/CYCLOIDEA/PROLIFERATING CELL FACTORS* [*PCF*]) family members, which encode basic helix-loop-helix (bHLH) transcription factors (Palatnik *et al*. 2003; Ori *et al*. 2007; Nag *et al*. 2009). Likewise, a suite of other plant miRNA members are also responses to drought stress and contribute to plant water deficit tolerance (Zhou *et al*. 2010).

Wheat (*Triticum aestivum*) is one of the critical cereals planted worldwide. Thus far, a large set of wheat miRNA family members have been released in miRNA database (www.mirbase.org, release 21). Moreover, a line of investigations on the wheat miRNAs has been performed focusing on target gene prediction and expression analyses (Sun *et al*. 2014; Wang *et al*. 2014; Bakhshi *et al*. 2017). However, the functions of the wheat miRNAs remain to be further characterized. Previously, our expression analysis revealed that a wheat miRNA member designated as TaMIR1119 (accession no. MI0006181) is drought stress responsiveness (Lu *et al*. 2011). This finding suggests the potential role of this miRNA in regulating plant drought tolerance. In this study, we characterized the miRNA function in regulating drought stress adaptation based on transgene analysis. Our results indicate that TaMIR1119 is conserved in wheat and eudicot tobacco and essential in regulating plant drought adaptation *via* modulation of osmolyte accumulation, photosynthesis, and cellular ROS homeostasis.

## 2. Materials and methods

### 2.1. Characterization of the conserved nature of TaMIR1119

To characterize whether it possesses conserved nature, a reverse transcriptase-polymerase chain reaction (RT-PCR) was performed to amplify its homolog in tobacco, a model eudicot species, using the specific primers for TaMIR1119 precursor (Appendix A). During which, the cDNA from the drought-stressed roots of tobacco (*Nicotiana tabacum cv*. Wisconsin 35) was used as template. Sequence similarity between TaMIR1119 and the tobacco miR1119 homolog (designated as NtMIR1119) was determined based on an alignment analysis using DNAStar Software (verson 7.1; http://www.dnastar.com/).

### 2.2. Prediction of target genes of TaMIR1119

The TaMIR1119 target genes were predicted using psRNATarget (Plant MicroRNA Potential Target Finder; http://plantgrn.noble.org/psRNATarget/) Program. To this end, the mature sequence of TaMIR1119 was used as a query to search the Wheat cDNA Database (*Triticum aestivum* (wheat), cDNA, EnsemblPlants, released 31) as suggested. Biological functions of the target genes were annotated based on BLASTn search analysis.

### 2.3. Expression analysis of the miRNA and target genes

The expression patterns of miRNA and the TaMIR1119 target genes upon exposure to drought stress were characterized in detail. With this aim, wheat (*cv*. Shiyou 20) and tobacco (*cv*. Wisconsin 35) seedlings were hydroponically cultured in a standard MS solution to the third leaf-growth stage, then transferred to a modified MS solution supplemented with PEG 6000 (10%, w/w) for simulated drought treatment. After

48 h of treatment, aliquots of seedlings were retransferred to the standard MS solution for normal recovery treatment. Root tissues were collected at time points of 0 (before treatment), 6, 12, 24, and 48 h after drought stress as well as 6, 12, 24, and 48 h after the normal recovery treatment, respectively. Transcripts of TaMIR1119, its tobacco homolog NtMIR1119, and the target genes in the samples were assessed based on qRT-PCR as described previously (Guo *et al*. 2013). Two constitutive tubulin genes, including *Tatubulin* for wheat and *Nttubulin* for tobacco, were used as the internal references to normalize transcripts of the miRNAs and the target genes. Primer pairs used for the miRNAs, target genes, and the internal references are designed based on Primer Premier 5 Design Program and listed in Appendix A.

### 2.4. Generation of TaMIR1119 overexpression lines

Given the conserved nature of miR1119 in both wheat and eudicot tobacco, transgenic tobacco lines were generated to characterize the miRNA function due to the genetic transformation of this species to be much more convenient than that of wheat. With this aim, RT-PCR was performed to amplify the TaMIR1119 precursor using specific primers (Appendix A). The product was then integrated at the *Nco*I/*Bst*EII restriction sites in binary vector pCAMBIA3301 at position of downstream the constitutive CaMV35S promoter. After sequencing confirmation, the expression cassette was transferred into an *Agrobacterium tumefaciens* strain EHA105 using conventional heat shock approach. Genetic transformation of tobacco (*cv*. Wisconsin 35) and generation of tobacco lines with TaMIR1119 overexpression were carried out as described previously (Sun *et al*. 2012). Transcripts abundance of the target miRNA in transgenic lines was evaluated by qRT-PCR performed to be similar for assessment of TaMIR1119 as aforementioned.

### 2.5. Assays of growth features, biomass, and osmolytes amount in transgenic lines

Two lines designated as OE1 and OE3 possess much more transcripts of TaMIR1119 at $T_3$ generation (Appendix B). Therefore, they were selected for further functional analysis of miRNA in modulating plant drought response. For this, 10-day-old evenly seedlings of the transgenic lines and wild type (WT) were cultured in vermiculite supplied regularly with standard MS solution (normal growth, control) or the MS solution supplemented with 10% polyethylene glycol (v/v) for drought treatment. After five weeks of the treatment, phenotypes of the transgenic lines and WT were recorded using a digital camera. Plant biomass of the samples was obtained after the oven drying. Contents of proline and soluble sugar, two critical osmolytes functional in the osmoregulatory pathway, were assessed as reported previously (Du *et al*. 2013).

### 2.6. Assays of photosynthetic parameters, antioxidant enzyme (AE) activities, and AE gene expression in transgenic lines

Photosynthetic rate ($P_n$), PSII efficiency (φPSII), and nonphotochemical quenching (NPQ), three parameters reflecting effectively the photosynthetic function, were assessed using the upper expanded transgenic and WT leaves as previously described (Guo *et al*. 2013). To characterize whether the TaMIR1119-mediated drought response is associated with the modified cellular reactive oxygen species (ROS) homeostasis that drastically impacts on plant abiotic stress adaptation (Gill and Tuteja 2010), the superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) activities, and the malondialdehyde (MDA) content that acts as a cellular oxidation biomarker under adverse stresses, were assessed in TaMIR1119 overexpression lines and wild type as described previously (Huang *et al*. 2010). Moreover, to further understand the AE genes involving the mediation of AE activities in transgenic lines, expression patterns of an array of genes encoding SOD, CAT, and POD proteins were evaluated in the drought-stressed transgenic and wild type plants based on qRT-PCR. The SOD genes subjected to expression evaluation included *NtSOD1*, *NtSOD2*, *NtSOD3*, *NtMnSOD1*, and *NtMnSOD2*; the CAT genes included *NtCAT*, *NtCAT1*, *NtCAT3*, *NtCAT1;1*, *NtCAT1;2*, and *NtCAT1;3*; and the 11 POD genes included *NtPOD1;1* to *NtPOD1;7*, *NtPOD2;1*, *NtPOD2;2*, *NtPOD4*, and *NtPOD9*. Specific primers used for these AE genes are shown in Appendix A.

### 2.7. Statistics analysis

Averages of gene expression levels in qRT-PCR analysis, plant biomass, photosynthesis parameters, and proline and soluble sugar contents in transgenic lines and WT were derived from the results of four replicates. Standard errors of averages and significant differences were analyzed using Statistical Analysis System Software (SAS Corporation, Cory, NC, USA).

## 3. Results

### 3.1. miR1119 is conserved in both wheat and eudicot tobacco

TaMIR1119 precursor is 187 nt long containing a 21-nt mature sequence (5′-UGGCACGGCGUGAUGCUGAGUCAG-3′).

The stem-loop structure initiated by TaMIR1119 precursor is shown in Appendix B. Based on RT-PCR, the tobacco miR1119 homolog (NtMIR1119) was amplified, which shares an identical sequence to TaMR1119 (Appendix B). These results indicate the conserved nature of miR1119 in both wheat and tobacco as well as its possible conservation between monocots and eudicots, although this miRNA family member in tobacco has not been deposited in miRBase database (www.mirbase.org).

### 3.2. TaMIR1119 targets six genes with various biological functions

Online prediction analysis revealed that TaMIR1119 targets six genes, including Traes_5BL_71717F042.1, a bHLH type TF gene (*TabHLH49*, XM_020294420); Traes_7DL_C343BA649.2, a basic leucine zipper type TF gene (*TaLZ*, XM_020312111); Traes_3DL_27D1C646D.1, a CTP synthase encoding gene (*TaCS*, XM_003569426); Traes_7DL_E8584BFF8.1, a polyadenylation cleavage factor encoding gene (*TaPCF*, XM_010236026); Traes_6AS_3BAEA231B1.1, a metal-nicotianamine transporter gene (*TaMNT*, XM_020325679); and Traes_1DL_645A2ECC0.1, a glutathione *S*-transferase encoding gene (*TaGT*, XM_020302607). Fig. 1 shows the interaction characterization between TaMIR1119 and the target genes. Based on BLASTn search analysis, these target genes were shown to be categorized into various functional families, including two of which into transcriptional regulation (i.e., *TabHLH49* and *TaLZ*), two RNA or biochemical metabolism (*TaPCF* and *TaCS*), one trafficking (*TaMNT*), and one oxidative stress defense (*TaGT*). Therefore, TaMIR1119 possibly mediates distinct biological processes through regulating target genes that function as diverse roles.

### 3.3. Expression of miR1119 and the target genes as well as detection of target cleavage characterization

Expression analyses revealed that TaMIR1119, NtMIR1119, and most TaMIR1119 target genes are responses to the drought stress and the followed normal recovery condition. Across a 48-h of drought stress, the miRNA expression levels are gradually upregulated, reaching the highest value at 48 h. Once subjection to the normal recovery treatment, the drought-induced expression of miRNA is gradually reduced and restored to the similar level prior to treatment after 48 h of recovery (Fig. 2-A). Converse to the miRNAs, most target genes (i.e., *TabHLH49*, *TaLZ*, *TaPCF*, and *TaGT*) are downregulated in expression over a 48-h of drought treatment and whose drought-reduced transcripts are gradually elevated along with the recovery procession,


**Fig. 1** Base pairing characterization between TaMIR1119 and its target genes. *TabHLH49*, *TaLZ*, *TaPCF*, *TaCS*, *TaMNT*, and *TaGT* are putative target genes of TaMIR1119. *TabHLH49*, a bHLH type TF gene; *TaLZ*, a basic leucine zipper type TF gene; *TaCS*, a CTP synthase encoding gene; *TaPCF*, a polyadenylation cleavage factor encoding gene; *TaMNT*, a metal-nicotianamine transporter gene; *TaGT*, a glutathione *S*-transferase encoding gene.

albeit that the transcriptional amplitudes in responding to drought and the recovery treatment are different among them (Fig. 2-B and C). These results suggest that the miRNA regulates the target genes through a cleavage mechanism. Two target genes *TaCS* and *TaMNT* showed unaltered expression upon drought stress and the followed normal recovery treatment (Fig. 2-D), suggesting their regulation under TaMIR1119 possibly *via* the translation repression mechanism.

### 3.4. Phenotypes, biomass, and photosynthesis parameters are improved in transgenic lines after drought stress

Among the seven lines with TaMIR1119 overexpression, OE1 and OE3 possess much more target transcripts (Appendix C). They were then selected to characterize the miRNA function in mediating drought tolerance. Under the normal condition, the transgenic lines (OE1 and OE3) showed comparable phenotypes (Fig. 3-A), plant biomass (Fig. 3-C), and photosynthetic parameters (i.e., $P_n$, φPSII and NPQ) with WT (Fig. 4-A–C), suggesting that TaMIR1119 does not exert roles in the modulation of plant growth under the unstressed condition. In contrast, under the drought treatment, OE1 and OE3 displayed improved phenotypes (Fig. 3-B), increased plant biomass (Fig. 3-C), enhanced $P_n$ and φPSII (Fig. 4-A and B), and reduced NPQ (Fig. 4-C) compared with the wild type. Therefore, TaMIR1119 confers plants enhanced drought tolerance.

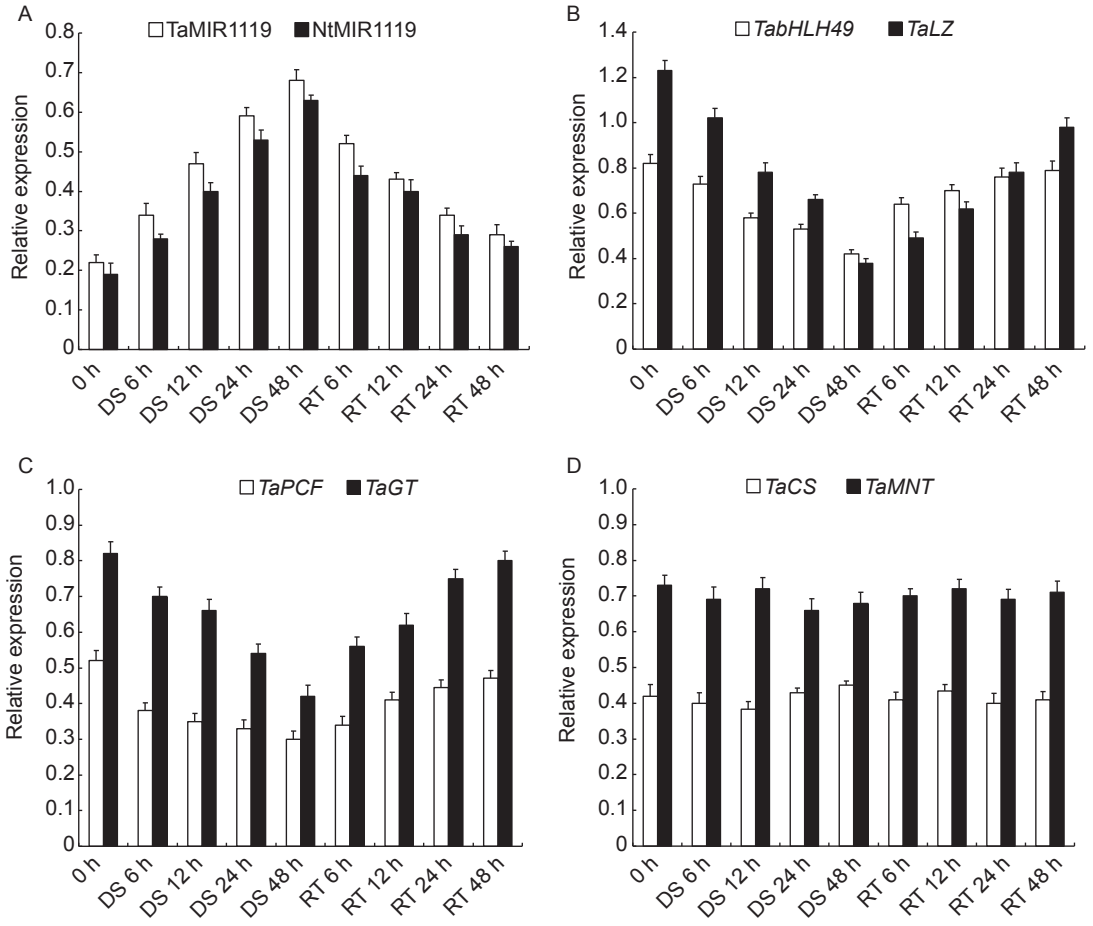


**Fig. 2** Expression patterns of TaMIR1119, NtMIR1119, and the target genes upon drought stress. 0 h, before drought stress; DS 6 h, DS 12 h, DS 24 h, and DS 48 h stand for 6, 12, 24, and 48 h after drought stress, respectively; RT 6 h, RT 12 h, RT 24 h, and RT 48 h stand for 6, 12, 24, and 48 h after recovery treatment, respectively. Data are normalized by internal standards and shown by average plus standard error.

### 3.5. Osmolytes accumulation and AE activities are increased in transgenic lines after drought stress

The contents of proline and soluble sugar, activities of SOD, CAT, POD, and the contents of MDA were assessed in the transgenic lines and wild type after drought treatment. In consistent with the phenotypes and plant biomass as described above, the transgenic lines displayed comparable proline and soluble sugar contents (Fig. 5-A and B), SOD, CAT, and POD activities, and contents of MDA with wild type under normal growth condition (Fig. 5-C–F), suggesting that the unaltered metabolism of osmolytes and cellular ROS in transgenic lines under this condition. Under drought treatment, however, OE1 and OE3 exhibited more osmolytes amounts, higher AE activities, and lower MDA contents than did WT plants (Fig. 5-A–F). These findings indicate that the TaMIR1119-mediated drought tolerance is closely associated with the miRNA role in modulating the osmolytes metabolism and the cellular ROS homeostasis.

### 3.6. Expression patterns of the AE genes in transgenic lines after drought stress

A suite of genes coding for the AE proteins, including five for SOD (*NtSOD1*, *NtSOD2*, *NtSOD3*, *NtMnSOD1*, and *NtMnSOD2*), six for CAT (*NtCAT*, *NtCAT1*, *NtCAT3*, *NtCAT1;1*, *NtCAT1;2*, and *NtCAT1;3*), and 11 for POD (*NtPOD1;1* to *NtPOD1;7*, *NtPOD2;1*, *NtPOD2;2*, *NtPOD4*, and *NtPOD9*), was subjected to expression evaluation in the transgenic and WT plants after drought stress treatment. Among them, *NtFeSOD*, *NtCAT1;3*, and *NtPOD2;1*, three genes encoding SOD, CAT, and POD proteins, respectively, showed significantly upregulated in expression in the transgenic lines with respect to wild type (Fig. 6-A–C), which are contrast to other genes that behave unaltered expression in the transgenic lines and wild type. Therefore, these differential SOD, CAT, and POD encoding genes (i.e., *NtFeSOD*, *NtCAT1;3*, and *NtPOD2;1*) posibly contribute to the enhanced AE activities in transgenic lines, TaMIR1119-

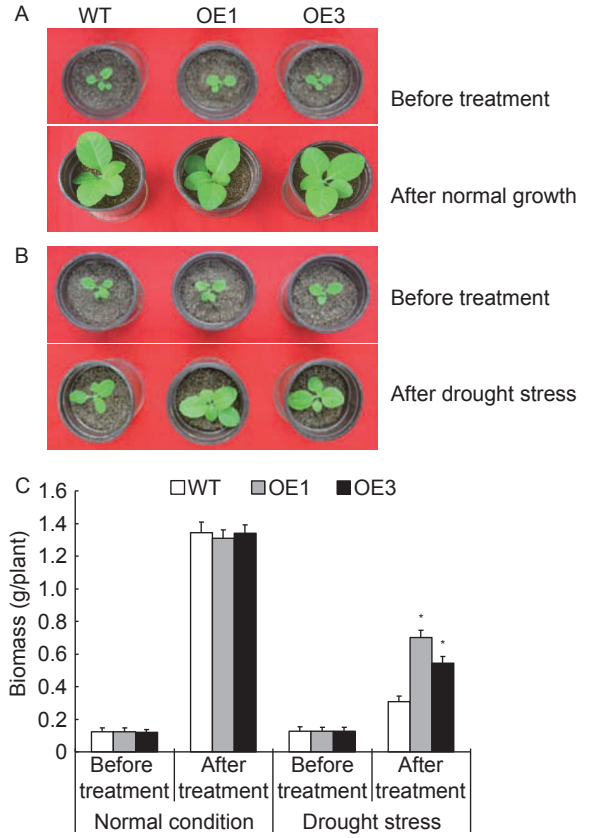


**Fig. 3** Phenotypes and biomass of the lines with TaMIR1119 overexpression under normal growth and drought treatment. A, phenotypes before and after normal growth. B, phenotypes before and after drought treatment. C, biomass. Data are shown by average plus standard error and * indicates statistically significant compared with WT ($P$<0.05). OE1 and OE3, two TaMIR1119 overexpression lines; WT, wild type.

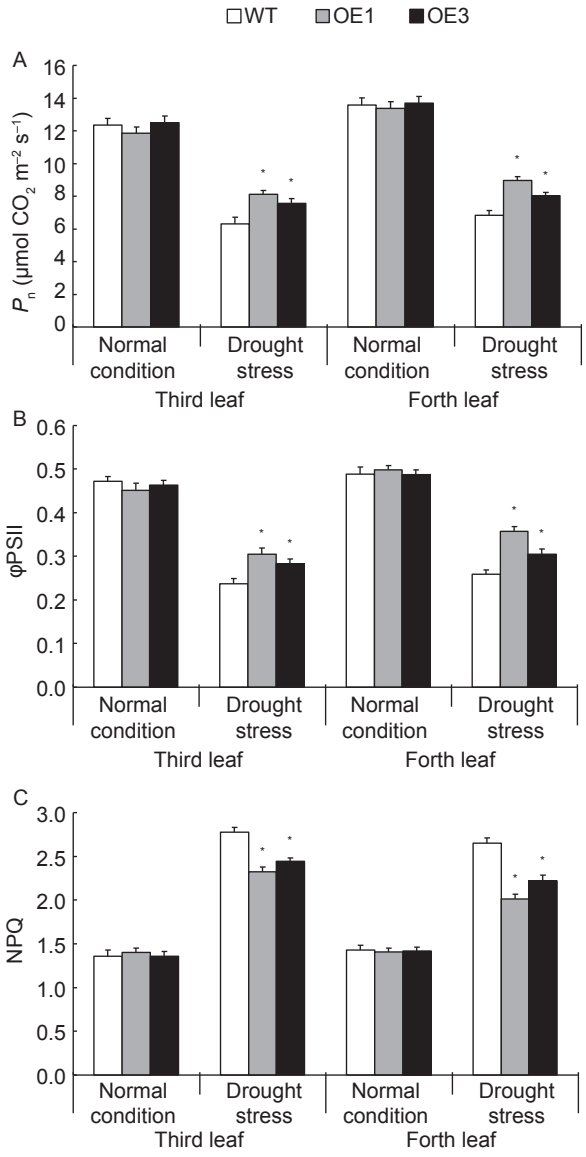


**Fig. 4** Photosynthesis parameters of the TaMIR1119 overexpressing lines under normal growth and drought treatment. A, photosynthetic rate ($P_n$). B, PSII efficiency (φPSII). C, nonphotochemical quenching (NPQ). OE1 and OE3, two TaMIR1119 overexpression lines; WT, wild type. Data are shown by average plus standard error and * indicates statistically significant compared with WT ($P$<0.05).

improved cellular ROS homeostasis, and the miRNA-mediated drought adaptaion response.

## 4. Discussion

### 4.1. TaMIR1119 mediates plant drought tolerance and displays drought stress-modified transcription

Understanding the miRNA-mediated regulatory network underlying drought stress tolerance can help further dissect the molecular pathways that plants acclimate to the water deficit stress. Recently, the miRNA-mediated plant drought adaptive response has also been functionally characterized. The miR319 transcripts are upregulated upon drought stress; the miR319 overexpression lines show modified morphological feature under osmotic stress treatments, displaying enhanced plant tolerance to drought and salt stress through regulating leaf wax content and water retention capacity. miR319 targets *AsPCF5*, *AsPCF6*, *AsPCF8*, and *AsTCP14*, four genes encoding the TCP TF family proteins. These target genes show downregulated expression under miR319 regulation and contribute to the miRNA-mediated drought tolerance (Nag *et al*. 2009). In this study, in comparison with wild type, the TaMIR1119 overexpression lines exhibited improved phenotypes and increased plant biomass after drought stress, these findings together with the enhanced photosynthetic function, increased osmolytes accumulation, and improved cellular ROS homeostasis in drought-challenged transgenic lines

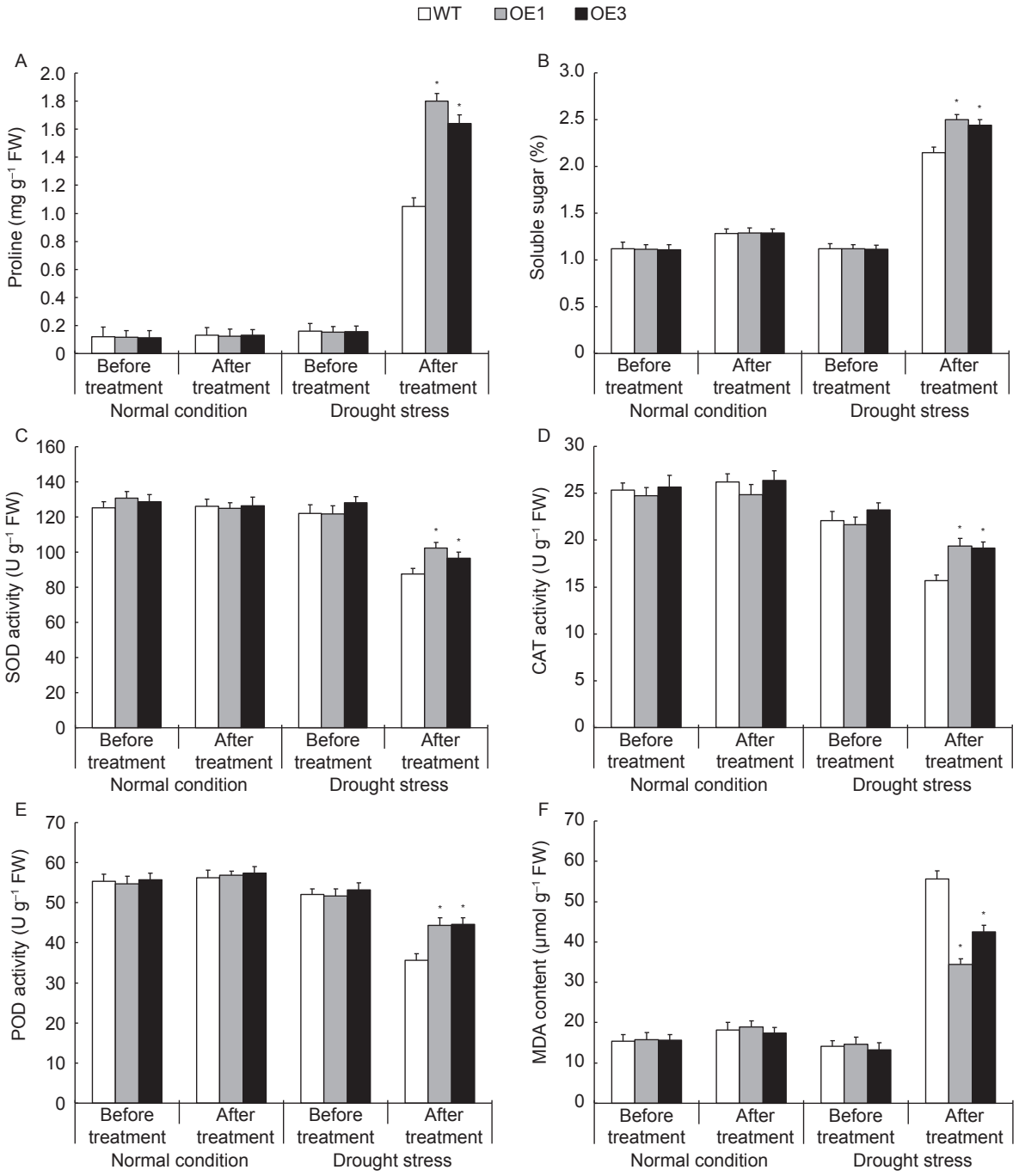


**Fig. 5** Osmolytes contents and reactive response species (ROS)-associated parameters of the TaMIR1119 overexpressing lines under normal growth and drought treatment. A, proline content. B, soluble sugar content. C, superoxide dismutase (SOD) activity. D, catalase (CAT) activity. E, peroxidase (POD) activity. F, malondialdehyde (MDA) content. OE1 and OE3, two TaMIR1119 overexpression lines; WT, wild type. FW, fresh weight. Data in A to F are shown by average plus standard error and * indicates statistically significant compared with WT ($P$<0.05).

indicate that TaMIR1119 is essential in mediating plant drought stress adaptation.

Transcription of miRNAs is similar to that of mRNAs, which involves a suite of regulatory factors, such as the PolII recruitment to promoter region mediated by transcriptional coactivators (Kim *et al*. 2011), conserved TATA box in promoter region (Xie *et al*. 2005), and distinct *cis*-acting regulatory elements in the promoter (Megraw *et al*. 2006; Hajdarpašić and Ruggenthaler 2012; Liang *et al*. 2012) that define the gene specific expression. Thus far, CRE motifs

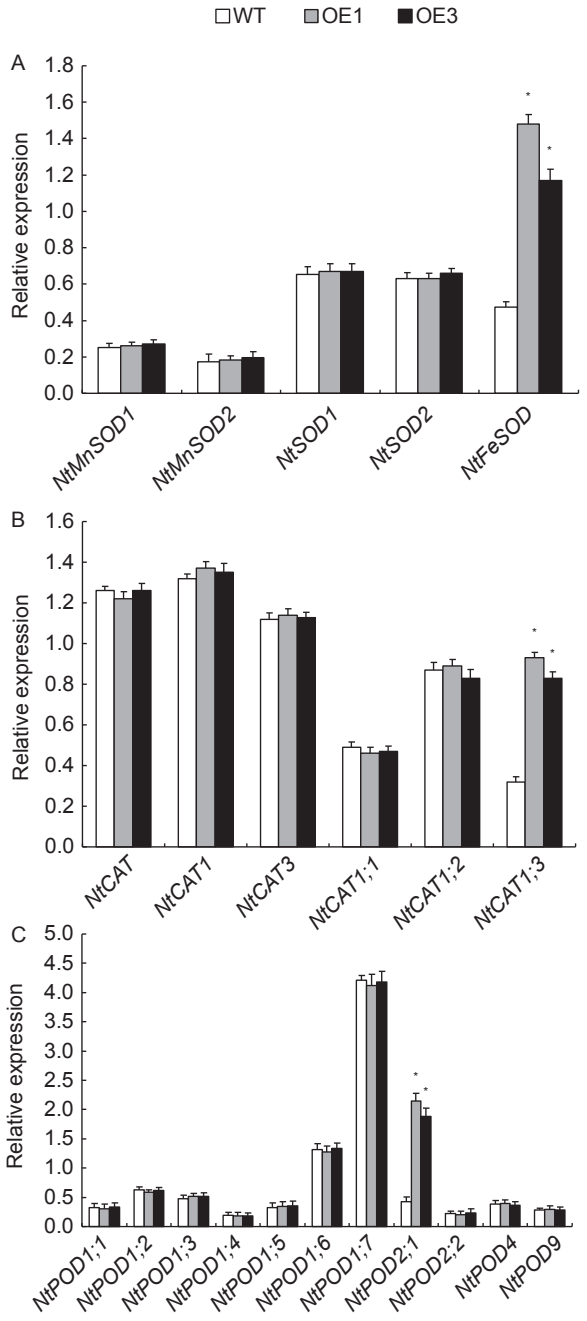


**Fig. 6** Expression patterns of the tobacco antioxidant encoding genes in drought-stressed TaMIR1119 overexpressing lines. A, superoxide dismutase (SOD) genes. B, catalase (CAT) genes. C, peroxidase (POD) genes. OE1 and OE3, two TaMIR1119 overexpression lines. WT, wild type. Data are shown by average plus standard error and * indicates statistically significant compared with WT ($P$<0.05).

(CCGCGT, CACGTGT, and AAGTCAA) enriched in gene promoters have been validated to impact on the transcription of the osmotic stress-responsive genes after their interaction with the bZIP TF proteins (Ma *et al*. 2012). In this study, TaMIR1119 and NtMIR1119 exhibited modified expression upon drought and followed normal recorevry treatment with a temporal-dependent manner, suggesting the situation of distinct drought response-associated *cis*-regulatory elements in the miRNA promoters, which control the miRNA response to drought stress. Further characterization of the elements, the element interaction mechanism with TFs, and distinct *Pol*II involved in transcription can provide novel insights underlying miR1119 transcription upon the drought stress.

### 4.2. TaMIR1119 targets genes categorized into diverse functional families

miRNAs mediate plant stress responses generally through regulating the TF genes (Palatnik *et al*. 2003). In this study, we found that TaMIR1119 targets the TF genes as well as other functional class genes. Expression analysis revealed that four of the target genes (*TabHLH49*, *TaZP*, *TaPCF*, and *TaGT*) are regulated by TaMIR1119 at the posttranscriptional level, given that their responses to the drought stress were converse to those of miRNAs. However, we found that two other target genes (*TaCS* and *TaMNT*) show unchanged expression upon drought stress, suggesting the regulation of them under miRNA through a translational repression mechanism. Further functional characterization of the target genes in mediating drought response can help establish the miRNA/target module(s) and dissect the module-mediated drought adaptive pathways.

### 4.3. TaMIR1119 mediates drought stress adaptation to be associated with its role in regulating ROS homeostasis

ROS is rapidly induced in plant tissues upon osmotic stresses, exerting negative roles on sustaining structure of proteins, lipids, and nucleic acid and causing cellular damage or cell death (Gill and Tuteja 2010). Meanwhile, AE proteins, such as SOD, CAT, and POD, act as positive regulators in plant tolerance to the osmotic stresses through detoxifying ROS and maintaining cellular ROS homeostasis (You and Chan 2015). For example, the alfalfa lines expressing *Mn-SOD* of *Nicotiana plumbaginifolia* show improved survival and vigor and increased yield upon water deficit stress compared with wild type (McKersie *et al*. 1996). Overexpression of an *Acanthioca marina* cytosolic copper-zinc SOD gene in rice endows plants improved drought adaptation capacity (Prashanth *et al*. 2008). In this study, the transgenic lines with TaMIR1119 overexpression exhibited enhanced SOD, CAT, and POD activities relative to WT under the drought stress treatment, suggesting the functions of them in the mediation of cellular ROS homeostasis in transgenic lines, which further impacts on the

miRNA-improved drought response. Based on expression analysis, we identified three AE encoding genes, including one SOD gene *NtFeSOD2*, one CAT gene *NtCAT1;3*, and one POD gene *NtPOD2;1*, are differentially upregulated in expression in the drought-stressed lines overexpressing TaMIR1119. These findings suggested that these AE genes are transcriptional regulated under miRNA by an indirect manner and they involve the regulation of AE activities and cellular ROS homeostasis in the transgenic lines. Further functional characterization of these AE genes can help understand the molecular mechanism underlying the miRNA-mediated drought tolerance.

## 5. Conclusion

miR1119 is conserved in both wheat and eudicot tobacco. TaMIR1119 transcriptionally responds to drought stress whereas most target genes of this miRNA show converse expression pattern upon the stressor. TaMIR1119 target the genes categorized into classes of transcriptional regulation, RNA and biochemical metabolism, trafficking, and oxidative stress defense. TaMIR1119 overexpression lines possess improved phenotypes, plant biomass, photosynthetic function, osmolytes contents, and AE activities relative to wild type under drought stress. The AE genes *NtFeSOD*, *NtCAT1;3*, and *NtPOD2;1* are upregulated in expression in drought-stressed TaMIR1119 overexpressors, suggesting their contribution to the AE activities and the cellular ROS homeostasis. TaMIR1119 mediates plant drought tolerance through regulating the target genes that modulate osmotic stress-associated biological processes including osmolytes accumulation and ROS homeostasis.

## Acknowledgements

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**Appendices** associated with this paper can be available on http://www.ChinaAgriSci.com/V2/En/appendix.htm

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