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Full Length Article

TaMIR5086, a microRNA Member in *Triticum aestivum*, Confers Plants Drought Tolerance *via* Modulating Stomata Movement and Antioxidant Enzyme Activities

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Abstract

The microRNA (miRNA) members exert essential roles in regulating the growth and development of plants as well as plant response to various abiotic stresses. In this study, TaMIR5086, a member of the miRNA family in wheat (*Triticum aestivum* L.), was subjected to functional evaluation in plant drought tolerance. TaMIR5086 has six target genes that are involved in transcription, translation, protein degradation, and trafficking. The transcripts of TaMIR5086 were elevated in both aerial and underground tissues within a 48-h regime of drought treatment. Moreover, the drought-induced expression levels of which were restored following a normal recovery progression. In contrast to TaMIR5086, all the target genes reversed the expression modes upon drought stress. These results suggested that these target genes are regulated under control of TaMIR5086 at posttranscriptional level. Transgene analysis on TaMIR5086 validated its positive function in regulating drought stress adaptation; the transgenic lines overexpressing TaMIR5086 displayed improved plant growth, biomass, and photosynthetic function compared with wild type under drought treatment. Moreover, the stomata closing rates were promoted and the activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) were enhanced in the drought-treated transgenic lines. These results suggested that TaMIR5086 improves drought adaptation by regulating stomata movement and AE catabolism. *TaSOD3*, *TaCAT5* and *TaPOD9*, the genes in AE families, modified transcription in the drought-challenged transgenic lines, suggesting their contribution to reactive oxygen species (ROS) homeostasis and drought adaptation *via* modifying AE activities underlying the miRNA regulation. Therefore, TaMIR5086 acts as one useful index in evaluating drought adaptation and molecular breeding of drought-tolerant cultivars in *T. aestivum*. Our findings provide insight into understanding of drought-tolerant mechanisms underlying modulation of distinct miRNA members.

Keywords: Wheat (*Triticum aestivum* L.); miRNA; Drought stress; Plant growth; Physiological traits; Antioxidant enzymes

Introduction

Drought stress negatively impacts on the plant growth, development and yield formation for cereal crops (Cattivelli *et al*. 2008). Recent investigations have documented that water deficit leads to modification of numerous physiological processes, which are associated with root development, cellular membrane integrity, photosynthetic pigment metabolism, osmolytes biosynthesis, and tissue and organ establishment (Sallam *et al*. 2019). The alterations of them finally result in deterioration of plant growth traits and the yield formation potential (Benjamin and Nielsen 2006; Seleiman *et al.* 2021). Therefore, adoption of drought-tolerant varieties has been an effective strategy in enhancing crop productivity and water utilization ability of the cereal crops cultivated under water-saving conditions.

Under drought conditions, plants acclimate to the adverse stressor through various mechanisms evolved at physiological and molecular levels (Wang *et al.* 2016; Seleiman *et al.* 2021). Thus far, the molecular networks underlying plant drought adaptation have been extensively investigated. A quantity of regulators has been identified to modulate the biochemical pathways associated with transduction of the drought signaling. Among them, the protein receptors, mitogen-activated protein kinases (MAPK), calmodulin-binding proteins, phosphatases, and distinct transcription factors, act as the critical components

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in the drought signal transduction processes and regulate largely the drought response (Shinozaki and Yamaguchi-Shinozaki 2007; Mittal *et al.* 2017; Jagodzik *et al.* 2018; Cui *et al.* 2019). These findings suggested the complicate nature of drought signal transduction in plants and distinct regulatory components are valuable in efforts for generating the drought-tolerant crop germplasms.

The microRNA (miRNA) members constitute a large class of non-coding RNA in plant species. With nucleotide acids from 20 to 24 nt in length, the miRNA members are involved in the regulation of quantities of physiological processes, based on their roles in controlling the target mRNAs at posttranscriptional or translational level *via* a base pairing mechanism (Zhang *et al.* 2006; Lu *et al.* 2011). Thus far, it has been documented that miRNA members are functional in mediating diverse biological processes, such as growth phase transition, senescence, floret tissue establishment and root architecture establishment (Rubio-Somoza and Weigel 2011; Swida-Barteczka and Szweykowska-Kulinska 2019; Zhao *et al*. 2019). In addition, distinct members in plant miRNA family have also been recorded to impact on plant responses to abiotic stress conditions (Ji *et al*. 2018; Liu *et al*. 2019; Nadarajah and Kumar 2019). For example, miR319 in *Arabidopsis* responds to dehydration, salt, and chilling stresses at transcriptional level (Lv *et al*. 2010; Thiebaut *et al*. 2012). Functional characterization on this miRNA member indicated that it conferred plants increased adaptation to above stressors, *via* modulating transcription of the transcription factor (TF) genes in *TCP* family (for *TEOSINTE BRANCHED/CYCLOIDEA/PROLIFERATING CELL FACTORS* [*PCF*]) that encode basic helix-loop-helix (bHLH) transcription factors (Ori *et al*. 2007; Nag *et al*. 2009). Meanwhile, an aliquot of the miRNA members in cereal crops sensitively respond to the drought stress (Nadarajah and Kumar 2019). Rice miR393 has been documented to regulate plant development and adaptation to multiple abiotic conditions (Zhao *et al*. 2019). Thus far, although the relations between plant drought response and miRNA members are largely established, the mechanisms underlying miRNA-mediated drought acclimation are needed to be further characterized in plant species, especially in cereal ones.

As one of the important cereal crops, wheat (*T. aestivum*) is widely cultivated and contributes greatly to food security worldwide. However, a large quantity of water consumption is needed for wheat cultivation due to the long growth period together with less precipitation in most cultivation regions, which results in shortage of the water resource and limitation to the sustainable crop production. Therefore, improving WUE for wheat plants is an urgent issue for a long term of crop production. Thus far, a quantity of the miRNA family members of *T. aestivum* has been stored in the miRNA bank (www.mirbase.org). Moreover, a suite of investigations concentrated on characterizing the miRNA functions and predicting the corresponding target genes in *T. aestivum* species have also been documented (Sun *et al*. 2014; Wang *et al*. 2014; Bakhshi *et al*. 2017). However, the mechanisms underlying the miRNA-mediated physiological processes and stress responses in this species are largely unknown and need further elucidation. In this study, TaMIR5086, a miRNA member in wheat, was subjected to functional evaluation for the role in regulating plant drought response. Our investigation aimed at elucidating the drought-tolerant mechanisms mediated by miRNA members and providing essential regulators for generating crop germplasms to be drought defensiveness.

Materials and Methods

Characterization of TaMIR5086

Ourexpression evaluation on the miRNA members derived from wheat (*Trtitcum aestivum* L.) revealed that TaMIR5086 (accession number MI0017949) displayed upregulated transcripts upon drought stress (our unpublished data), which suggested the involvement of it in drought response at transcriptional level. The precursor sequence, stem-loop feature, and mature sequence of TaMIR5086 were derived from the miRNA database of *T. aestivum* (www.mira.org).

Evualution of the target genes of TaMIR5086

The genes interacted by TaMIR5086 were identified based on an online tool (psRNATarget, Plant microRNA Potential Target Finder; http://plantgrn.noble.org/psRNATarget/). The cDNA databases of *T. aestivum* used for scanning the target genes were *Triticum aestivum* (bread wheat), cDNA, EnsemblPlant, release 43. Putative roles of the target genes were predicted by performing BLASTn analysis supplemented in NCBI, using target cDNA sequences as queries.

Determination of transcripts of TaMIR5086 and its target genes

The transcripts of TaMIR5086 and its target genes in response to drought condition were determined based on qRT-PCR. With this aim, wheat (cv. Shimai 22) seedlings were cultured in a standard Murashige and Skoog (MS) solution under following condition: photoperiod of 12 h/12 h (day/night) with 300 μmolE$\cdot$m$^{-2}\cdot$s^{-1} light intensity during light phase, temperature of 28°C/23°C (day/night), and relative humidity from 65 to 70%. They were then subjected to the simulated drought treatment by growing in a MS solution containing PEG-6000 (10% concentration, w/v). At times of 6, 12, 24 and 48 h under drought treatment, the underground and aerial organs were sampled. In addition, an aliquot of the 48 h-treated seedlings were transferred to MS solution for a normal recovery treatment.

The tissues mentioned were sampled at 6, 12, 24 and 48 h under the recovery condition. The tissues collected prior to simulated drought treatment (referred to as 0 h) were used as control. qRT-PCR for the samples was conducted following the previous method (Guo *et al.* 2013). In brief, total RNA in samples was isolated using TRIzol reagents (Invitrogen, USA). After removal of putative genomic DNA using DNase (TaKaRA, Dalian, China), total RNA (~2 μg) in each sample was used for synthesis of cDNA using the RT-AMV transcriptase (TaKaRa, Dalian, China). qRT-PCR reaction was conducted using following components: 12.5 μL of SYBR Premix ExTaqTM (TaKaRa, Dalian, China), 0.5 μL of forward and reverse primers, 1 μL cDNA and 10.5 μL nuclease-free water. The transcripts of TaMIR5086 and its target genes were determined following the $2^{-\Delta\Delta CT}$ method, with wheat constitutive gene *Tatubulin* to normalize the target miRNA and its target genes (Wang *et al*. 2020). The primers used for TaMIR5086 and its targets are shown in Table S1.

Evaluations of growth feature, dry mass and photosynthetic parameters in transgenic lines

The lines with TaMIR5086 overexpression were established for determining function of this miRNA in mediating the drought response. With this aim, the precursor sequence of TaMIR5086 was RT-PCR amplified with specific primers (Table S1) and inserted into binary expression vector referred to as pCAMBIA3301 controlled underlying the CaMV35S promoter. The binary cassette was then integrated into *A. tumefaciens* (strain EHA105) using the conventional heat-shock approach and subjected to genetic transformation onto *T. aestivum* (cv. Shimai 22) as previous description (Guo *et al.* 2013).

Two lines at T3 generation with more TaMIR5086 transcripts (Fig. S1), OE 2 and OE 3 together with wild type (WT), were used to characterize the miRNA function in mediating plant drought tolerance. Briefly, the seedlings at the third leaf stage under normal condition as aforementioned were cultured in a MS solution containing PEG-6000 (5%, w/v). Three weeks later, phenotypes and dry mass of the transgenic lines and WT plants were analyzed. Of these, the plant phenotypes were recorded based on images taken with a digital camera; the dry mass was derived from the oven-dried samples as conventional approach. Several photosynthetic traits, including the photosynthetic rate (Pn), PSII efficiency (ΦPSII) and nonphotochemical quenching (NPQ), were determined as previous description (Guo *et al*. 2013).

Assays of stomata closing rate in transgenic lines

Stomata closing rates (SCR) representing the extent of plant drought response was evaluated using the drought-challenged transgenic lines as samples. Briefly, the plants of Sen 2 and WT cultured under normal growth condition were transferred into a modified MS solution containing 10% PEG (w/v). At times of 0.5 h, 1 h and 2 h under the treatment, leaf samples of transgenic and WT seedlings at indicated times together with that prior to treatment (0 h, control) were collected. The stomata nature of samples after collection was fixed using nail polish oil and subjected to observation under microscope as described previously (Wei *et al*. 2020). SCR values in the transgenic and WT were calculated based on stomata widths at the time points mentioned and those expressed at 0 h.

Assay of AE activities and AE family gene expression

Several cellular reactive oxygen species (ROS) traits, such as the activities of antioxidant enzymes (AE, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD)) and contents of MDA, were measured using the transgenic and WT plants after drought treatment as materials (Huang *et al*. 2010). To deepen understanding of molecular processes underlying the modified AE activities regulated by TaMIR5086, the genes in AE families (six SOD, six CAT and eleven POD) in *T. aestivum* were identified in NCBI GenBank database and were analyzed for transcripts in the drought-challenged transgenic lines, using qRT-PCR with the specific primer pairs (Table S1). *Tatubulin*, a wheat constitutive gene, was used for normalization of the transcripts of the target genes.

Statistics analysis

Gene expression levels, plant dry mass, photosynthetic traits, stomata closing rate, activities of AE, and contents of MDA in the experimental materials were determined using four replicates. The Statistical Analysis System software (SAS Corporation, Cory, NC, USA) was adopted for analysis of standard errors for averages and significant differences for the traits among the materials.

Results

Characterization of the target's genes of TaMIR5086

In total of six target genes putatively interacted by TaMIR5086 were identified, including those encoding eukaryotic translation initiation factor subunit C (*TaTIF*, TraesCS5B02G410800), transport protein section 16 (*TaTP*, TraesCS6D02G022300), DNA-RNA directed RNA polymerase subunit beta (*TaRPS*, TraesCS5D02G383900), mediator of RNA polymerase II transcription subunit 13 (*TaRPT*, TraesCS5D02G551300), sucrose 1-fructorsyltransferease (*TaSF*, TraesCS7D02G008700), and AP-1 complex subunit gamma (*TaAP*, TraesCS7D02G128900). The pairing features at nucleic acid level among the target genes and TaMIR5086 are shown in Fig. 1. Prediction analysis on the target genes

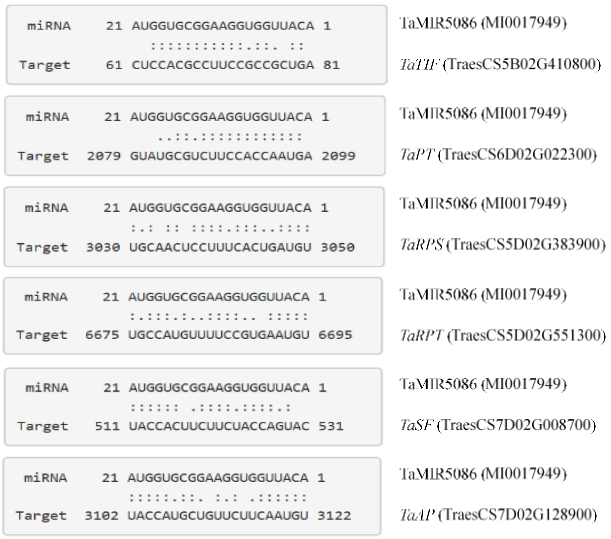

Fig. 1: Base pairing characterization among TaMIR5086 and its target genes

TaTIF (eukaryotic translation initiation factor s subunit C, TraesCS5B02G410800), *TaTP* (transport protein section 16, TraesCS6D02G022300), *TaRPS* (DNA-RNA directed RNA polymerase subunit beta, TraesCS5D02G383900), *TaRPT* (mediator of RNA polymerase II transcription subunit 13, TraesCS5D02G551300), *TaSF* (sucrose 1-fructorsyltransferase, TraesCS7D02G008700.1), and *TaAP* (AP-1 complex subunit gamma, TraesCS7D02G128900)

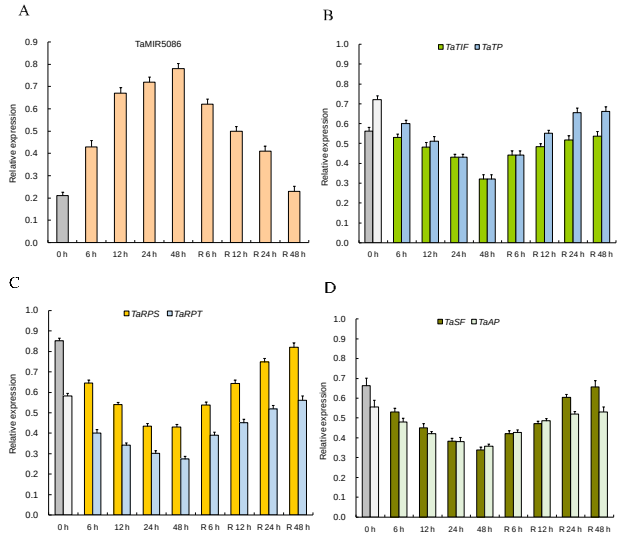

Fig. 2: Expression patterns of TaMIR5086 and the target genes under drought and recovery conditions

A, TaMIR5086; **B**, *TaTIF* and *TaTP*; **C**, *TaRPS* and *TaRPT*; **D**, *TaSF* and *TaAP*. 0 h, prior to treatment; 6 h, 12 h, 24 h, and 48 h, times after drought treatment; R6 h, R12 h, R24 h, and R48 h, times after normal recovery treatment. Data are normalized by internal standard *Tatubulin* and shown by averages plus standard errors

suggested that they are functional in diverse biological processes, including transcription (*i.e.*, *TaRPS* and *TaRPT*), translation (*TaTIF* and *TaABP*), protein degradation (*TaAP*), and trafficking (*TaTP* and *TaSF*). Therefore, TaMIR5086 exerts distinct biological functions in plants by regulating its target genes.

Expression behaviors of TaMIR5086 and its target genes

The transcripts of TaMIR5086 were modified under drought stress, which were elevated during a 48 h drought treatment (Fig. 2A). Moreover, the drought-upregulated expression of this miRNA was gradually decreased upon a normal recovery condition (Fig. 2A), suggesting that TaMIR5086 is sensitive upon drought signaling at transcriptional level. The target genes all displayed reverse expression patterns to the miRNA member; the transcripts of them were lowered under drought treatment (Fig. 2B–D) and whose repressed transcription efficiencies under drought gradually increased along with the normal recovery treatment, albeit that the modified extent on expression levels varied among them (Fig. 2B–D). Therefore, TaMIR5086 regulates the target genes *via* posttranscriptional mechanism.

TaMIR5086 confers plants improved growth and dry mass production capacities

OE 2 and OE 3, two lines overexpressing TaMIR5086, were selected for addressing the role of this miRNA in regulating plant drought response. Under the normal condition, the transgenic lines were comparable on phenotypes and dry mass with WT (Fig. 3A–C). Under drought treatment, OE 2 and OE 3 were much better on the growth features and biomass than wild type (Fig. 3A–C). Therefore, TaMIR5086 is an essential regulator in mediating the drought adaptation of plants.

Photosynthetic parameters and stomata closing rates in transgenic lines

Photosynthetic parameters and the stomata movement characterization in the transgenic lines were measured under drought condition. Like growth features and biomass shown in the transgenic and WT plants, the photosynthetic traits, namely, Pn, ΦPSII and NPQ, were similar each other among the transgenic lines and WT under normal condition and were modified drastically under drought treatment. Of which, Pn and ΦPSII were higher and NPQ lower in OE 2 and OE 3 than the WT plants under drought condition (Fig. 4A–C). Assays of the stomata closing rates (SCR) revealed that the stomata movement in OE 2 and OE 3 was sensitive in response to drought with respect to wild type; the SCR values were reduced swiftly in the transgenic lines following a 3 h-regime drought progression (Fig. 4D). Therefore, TaMIR5086 positively regulates the photosynthetic function and adjusts the stomata response to drought, by which to impact on the plant drought acclimation process.

Activities of AE proteins and AE family gene expression

Activities of SOD, CAT, and POD involving cellular ROS

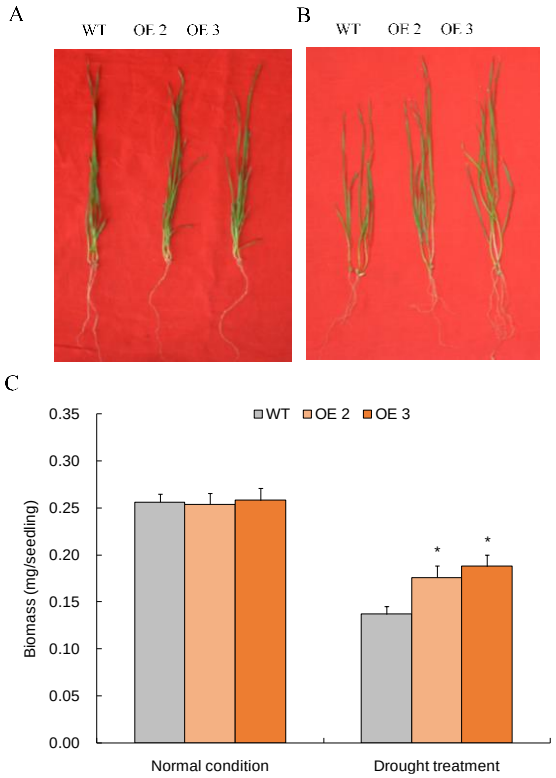

Fig. 3: Phenotypes and biomass of the transgenic lines with TaMIR5086 overexpression under drought treatment

A, phenotypes under normal condition; **B**, phenotypes under drought treatment; **C**, biomass. WT, wild type; OE 2 and OE 3, two transgenic lines with TaMIR5086 overexpression. In C, data shown are average plus standard error with symbol * to represent statistically significant compared with WT ($P < 0.05$)

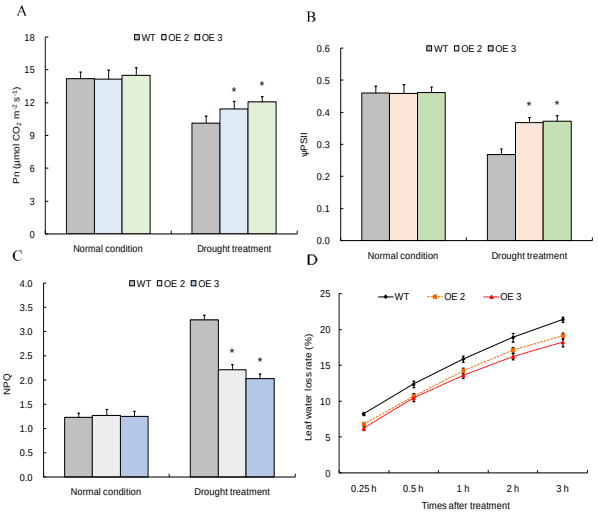

Fig. 4: Photosynthetic parameters and leaf water loss rates in the transgenic and wild type plants under drought treatment

A, Pn; **B**, ψPSII; **C**, NPQ; **D**, leaf water loss rates. WT, wild type; OE 2 and OE3, two transgenic lines with TaMIR5086 overexpression. Data shown are averages plus standard errors with symbol * to represent statistically significant compared with WT ($P < 0.05$)

homeostasis together with MDA amounts were assayed in the experimental materials. Under normal condition, OE 2

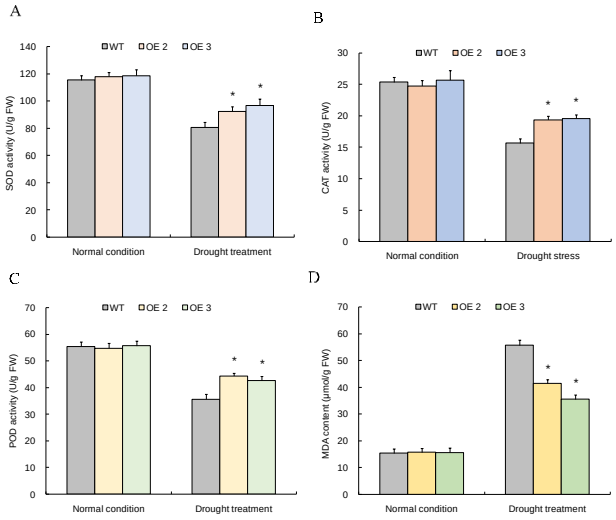

Fig. 5: Activities of antioxidant enzymes and contents of MDA in the transgenic and wild type plants under drought treatment

A, activities of SOD; **B**, activities of CAT; **C**, activities of POD; **D**, contents of MDA. WT, wild type; OE 2 and OE3, two transgenic lines with TaMIR5086 overexpression. Data shown are averages plus standard errors with symbol * to represent statistically significant compared with WT ($P < 0.05$)

and OE 3 displayed similar behaviors on the SOD, CAT, and POD activities and MDA amounts. Under drought treatment, the transgenic lines showed higher activities of SOD, CAT, and POD and lower MDA amounts than the WT plants (Fig. 5A–D). Therefore, the ROS-associated traits shown in transgenic lines were consistent with the photosynthetic function, biomass, and growth feature mediated by TaMIR5086. These results suggested that improvement of cellular ROS homeostasis underlying miRNA regulation positively modulates plant drought response.

The transcripts of the AE family genes in the samples were analyzed under drought condition. The SOD gene *TaSOD3*, CAT gene *TaCAT5* and POD gene *TaPOD9* displayed elevated transcripts in OE 2 and OE 3 compared with WT plants (Fig. 6A–C), whose modified patterns in expression contrasted with other ones that were unaltered on transcription in the assayed samples (Fig. 6A–C). These results indicated that distinct AE family genes, including *TaSOD3*, *TaCAT5* and *TaPOD9*, are transcriptional response to drought underlying regulation of TaMIR5086. These differential genes thus contribute to cellular ROS homeostasis and drought adaptation of plants by regulating the AE activities.

Discussion

The growth, development, and stress responses of plants underlying miRNA regulation are dependent on target genes interacted by the miRNA members, *via* posttranscriptional or translational regulation mechanisms (Ferdous *et al.* 2015; Basso *et al.* 2019). In this investigation, predicting target genes underlying TaMIR5086 revealed that this miRNA targets six genes,

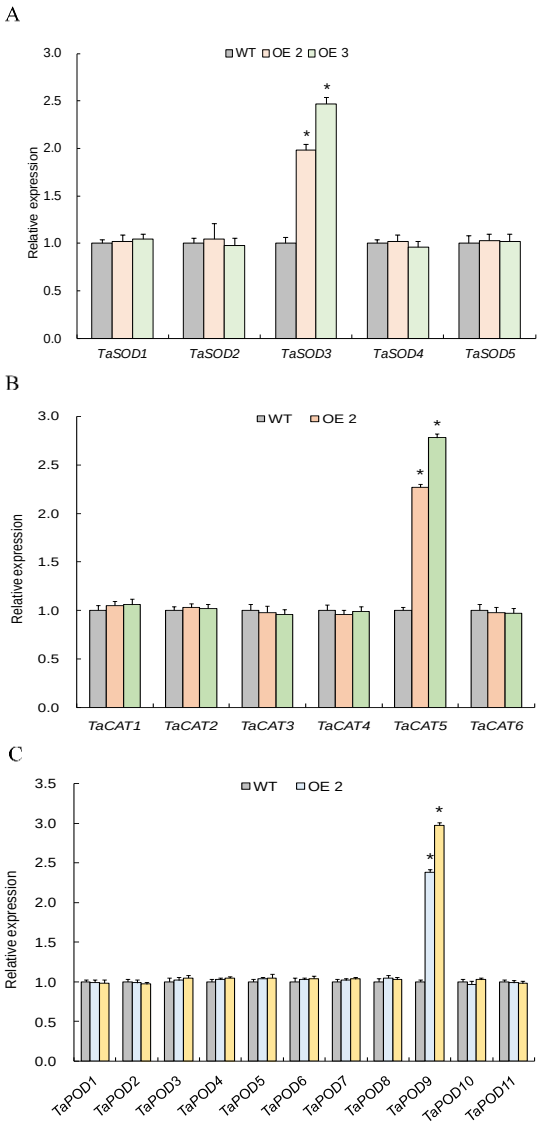

Fig. 6: Expression patterns of genes encoding antioxidant enzymes in the transgenic and wild type plants under drought treatment

A, genes of SOD family; **B**, genes of CAT family; **C**, genes of POD family. WT, wild type; OE 2 and OE3, two transgenic lines with TaMIR5086 overexpression. Data shown are averages plus standard errors with symbol * to represent statistically significant compared with WT ($P < 0.05$). The expression levels in wild type plants were set as 1

which are associated with diverse biological processes. Therefore, TaMIR5086 constitutes the miRNA/target modules to exert roles in distinct pathways impacting plant growth and stress responses. Previously, it has reported that the modified growth and stress responses underlying miRNA regulation are largely dependent on the target genes that encode transcription factors (Samad *et al*. 2017). In this study, six target genes interacted by TaMIR5086 are categorized into different functional classes, including transcription, translation, protein degradation and trafficking. Therefore, TaMIR5086 involves regulation of plant growth and stress response through modulating diverse biological processes. Further functional analysis of these target genes can benefit understanding of the regulation mechanisms of the miRNA members.

The transcription process of miRNA members is similar to that of mRNA molecules, which is involved in a suite of molecular processes mediated by various regulatory factors, such as enzyme Pol II recruit to promoter region mediated by distinct transcriptional coactivators (Kim *et al*. 2011; Megraw *et al*. 2016), the motif of TATA box situated in promoter location (Xie *et al*. 2005) and the regulatory elements located at distinct promoter positions (Hajdarpašić and Ruggenthaler 2012; Liang *et al*. 2012). Thus far, the *cis*-regulatory motif referred to as CRE (with motif CCGCGT, CACGTGT and AAGTCAA) has been confirmed to be enriched in gene promoters, playing critical roles in regulating transcription efficiency of the stress-associated genes, given their interaction with the transcription factors in the bZIP family (Ma *et al.* 2012). In this investigation, expression analysis on TaMIR5086 revealed that it is sensitive in response to drought stress, with a mode to be temporal-dependent across the drought regime and recovery transcripts following the normal recovery condition. Therefore, further investigation of the *cis*-acting elements, such as CRE and other ones involving modulation of miRNA transcription under drought stress, can provide insights in understanding of the transcription mechanisms of the miRNA members upon drought stressor.

The miRNA members have been documented in plant tolerance to various abiotic stressors. For example, miR319 member of Arabidopsis increased transcripts upon drought stress. The transgenic lines with miR319 overexpression improved growth traits and enhanced plant drought tolerance through enhancing leaf wax accumulation and water holding ability, given that it downregulates expression of *AsPCF5*, *AsPCF6*, *AsPCF8* and *AsTCP14* that encode the TCP TF proteins (Nag *et al*. 2009). In this investigation, transgene evaluation on TaMIR5086 confirmed its positive roles in regulating plant drought acclimation; the lines overexpressing the miRNA improved plant phenotypes, biomass, and photosynthetic traits under drought condition. These findings indicated that TaMIR5086 acts as one of molecular indices for evaluating drought tolerance across the wheat varieties, which acts as a valuable target for molecular breeding of the drought-tolerant cultivars in *T. aestivum*.

Stomata movement is one of the important acclimation mechanisms for plants to cope with drought stress (Kollist *et al*. 2014; He *et al*. 2018). Investigations have indicated that stomata closure is mediated byenhanced abscisic acid (ABA) levels due to promoted biosynthesis and reduced degradation of ABA under drought (Boursiac *et al*. 2013; Song *et al*. 2014; He *et al*. 2018). Thus far, the ABA signaling pathways associated with stomata closing upon drought signaling, such as characterizations of the ABA receptor members, namely RCAR, PYR1 and PYL, interaction mechanisms of ABA receptors with type 2C

protein phosphatases (PP2C), activation of the SNF1-related protein kinase OPEN STOMATA1 (OST1)/SnRK2, have been extensively investigated in the model plants (Park *et al*. 2009; Tan *et al*. 2018). In this study, analysis of the SCR behavior in lines with TaMIR5086 overexpression revealed its promoted stomata closing nature upon drought, which suggested that the modified stomata movement acts as one of the acclimation pathways for the miRNA-mediated drought response. Therefore, further investigation of the TaMIR5086-mediated ABA signaling pathway can help understanding of the molecular mechanisms of plant drought response underlying miRNA regulation.

Reactive oxygen species (ROS) have been documented to be over-accumulated in plants upon drought conditions, which negatively impact on plant drought tolerance due to deterioration of structures of protein, lipid, and nucleic acid and decrease of cell vigor (Gill and Tuteja 2010; Hasanuzzaman *et al*. 2020). The antioxidant enzymes (AE), including superoxide dismutase, catalase, and peroxidase, positively impact on plant adaptation to diverse stresses through improvement of cellular ROS homeostasis (You and Chan 2015; Lin *et al*. 2019). For example, the alfalfa lines overexpressing a MnSOD gene of *N. plumbaginifolia* endowed plants improved growth and yield under drought condition (McKersie *et al*. 1996). Likewise, overexpression of a cytosolic SuZnSOD gene of *A. marina* led to enhanced drought tolerance in rice plants (Prashanth *et al*. 2008). In this investigation, the transgenic lines overexpressing TaMIR5086 elevated the AE activities (*i.e*., SOD, CAT and POD ones) and reduced the contents of MDA under drought compared with WT, suggested that the AE enzymes improve plant drought response underlying miRNA regulation, *via* modulating the cellular ROS homeostasis. Moreover, expression analysis revealed that the SOD gene *TaSOD3*, CAT gene *TaCAT5* and POD gene *TaPOD9* upregulated expression levels in transgenic lines under drought stress. These findings suggested that the contribution of distinct genes in AE families to the improved antioxidant enzyme functions. Thus far, the internal relations between ROS and stomata movement under drought stress have been recorded. For example, the H_2O_2 production underlying ABA signaling pathway initiated by drought is promoted by plasma membrane-bound NADPH oxidase (RBOH), which promotes the hydrogen peroxide biosynthesis (Sirichandra *et al*. 2009; Czékus *et al*. 2020), by which to activate the calcium channels that elevate Ca^{2+} level in guard cells, promoting depolarization of membrane and the closure of stomata (Song and Matsuoka 2009). In this investigation, the modified stomata closing rate and ROS homeostasis under drought were shown to be regulated underlying TaMIR5086. It will be valuable to further elucidate the regulators to modulate the stomata movement and ROS homeostasis in the TaMIR5086 transgenic lines, which benefit to deepen understanding of the plant drought response mediated by distinct miRNA members.

Conclusion

TaMIR5086 has six target genes that are involved in diverse biological processes. The transcripts of TaMIR5086 were upregulated whereas its target genes downregulated upon drought stress, which suggested the regulation of the target genes underlying miRNA *via* posttranscriptional mechanism. TaMIR5086 conferred plants improved drought tolerance, being ascribed largely to improvement of stomata movement, ROS homeostasis and photosynthetic function. The AE genes *TaSOD3*, *TaCAT5*, and *TaPOD9* upregulate expression in the drought-challenged lines overexpressing TaMIR5086. Elevation of stomata closing and antioxidant enzyme functions contributes to plant drought adaptation and TaMIR5086 enhances plant drought tolerance and is useful for generating wheat cultivars sharing drought tolerance nature. Our findings provide insight into plant drought adaptation underlying miRNA members and elucidate valuable target for generating crop germplasms to be drought tolerant.

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Author Contributions

YZ, MZ, GS, LW, ZW, YZ, and HX planned the experiments, KX interpreted the results and made the write up, CN statistically analyzed the data and made illustrations.

Conflict of Interest

The authors declare there is no conflict of interest.

Data Availability

Not applicable.

Ethics Approval

This study is in accordance with the ethical standards.

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