TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2021) 45: 411-433

doi:10.3906/tar-2102-5

# Identification and expression profiling of toxic boron-responsive microRNAs and their targets in sensitive and tolerant wheat cultivars

**Doğa Selin KAYIHAN[1], Emre AKSOY[2], Ceyhun KAYIHAN[3,*]**

[1]Department of Molecular Biology and Genetics, Gebze Technical University, Kocaeli, Turkey
[2]Department of Agricultural Genetic Engineering, Faculty of Agricultural Sciences and Technologies, Niğde Ömer Halisdemir University, Niğde, Turkey
[3]Department of Molecular Biology and Genetics, Başkent University, Ankara, Turkey

**Received:** 02.02.2021 • **Accepted/Published Online:** 26.05.2021 • **Final Version:** 18.08.2021

**Abstract:** Post transcriptional gene regulation in wheat cultivars caused by boron (B) toxicity has not been reported to date. In this study, two wheat cultivars Bolal-2973 (B-tolerant) and Atay-85 (B-sensitive) were compared with regard to the transcriptional regulation of miR319, miR172 and miR398 and their plausible target genes in order to address differences in their performances under high B levels. The expression levels of *Cu/ZnSOD* gene were found to be greater in Atay exposed to toxic B compared to Bolal, as verified by stable expression level of miR398. In wheat cultivars, both toxic B concentrations might cause an induction of leaf senescence mechanism due to stable level of JA and ethylene-related miRNAs, miR172 and miR319. miR172 targeting transcription factor TOE1 was only induced under B toxicity in sensitive cultivar Atay. However, MYB3 as target of miR319 was significantly upregulated under toxic B in both cultivars. Also, computational prediction of these miRNA targets in wheat was performed and their orthologs in *Arabidopsis thaliana* were determined. Functional protein association networks of proteins encoded by miRNA targets and gene ontology enrichment analyses of these genes were identified. We identified new sets of genes that are targets of miR172, miR319 and miR398 in *T. aestivum*. In addition, miR172, miR319 and miR398 are responsive to different nutrient deficiencies or toxicities such as Fe, P, B, S and Cu, suggesting crosstalk between the post-transcriptional regulatory mechanisms involving miRNAs in plants.

**Key words:** *Arabidopsis thaliana*, boron toxicity, microRNA, *Triticum aestivum*, post-transcriptional regulation

## 1. Introduction

Boron (B) toxicity has negative impacts on crop yield and quality in agricultural lands, especially in arid and semi-arid regions. Rising temperatures caused by global warming and alteration of precipitation patterns can cause an increase in desertification rates, thereby, favoring B accumulation to the toxic levels in the soil worldwide (Landi et al., 2019). B toxicity alters plant metabolism, causes growth impairments, leads to chlorotic and necrotic patches in leaves and stems (Reid et al., 2004), and negatively affects the root growth in major crops (Choi et al., 2007; Aquea et al., 2012). One of the widely-accepted tolerance mechanisms against B toxicity is the enhancement of the antioxidative systems allowing the plant to combat B-triggered oxidative stress occurring due to overproduction of reactive oxygen species (ROS) (Landi et al., 2012). Effects of B toxicity and accompanying oxidative stress have been investigated in many plants. However, our knowledge about how plants respond to B toxicity at the molecular level is still limited. Also, contradictory information related to physiological and biochemical responses of plants to excess B was reported (Liu et al., 2005). The generation of osmotic stress by toxic B levels was not precisely proven as stated by different researchers (Reid et al., 2004; Landi et al., 2019). This statement cannot be valid and should be polished since the discrepancy between studies related to excess B levels might be dependent on experimental design and plant genotype. One of the reasons for insufficient comprehension of B stress is that the examinations have mostly been conducted at only one biological organization level (Kayıhan et al., 2016). However, stress is expressed simultaneously at different sub-organismal levels in a plant body including physiological, biochemical, cellular, and molecular levels (Zhang, 2015). For this reason, in our previous study, we evaluated the changes in antioxidative mechanisms with an integrative approach at physiological, biochemical, transcriptional, and post-transcriptional levels under B toxicity conditions in *Arabidopsis thaliana* as a model plant (Kayıhan et al., 2016). Accordingly, B toxicity strongly

* Correspondence: ckayihan@baskent.edu.tr

stimulated the expression levels of genes encoding for superoxide dismutases (SOD) and its respective activity, which led to the over-accumulation of hydrogen peroxide ($H_2O_2$). This change was due to the expression of *miR398* that provides post-transcriptional regulation of *Cu/ZnSOD* (*CSD1*) in *Arabidopsis thaliana*. Recently, Landi et al., (2019) suggest that an antioxidant apparatus is one of the critical features for tolerant species/genotypes coping with the effects of B toxicity. Therefore, it is essential to study the regulation of important genes involved in antioxidant responses at molecular level in plants against to B toxicity. In addition to its effects on antioxidant mechanisms, toxic B can disrupt metabolic processes related to photosynthesis (Landi et al., 2013) due to the decrease in electron transport rate, $CO_2$ efficiency, and photosystem II efficiency (Papadakis et al., 2004; Landi et al., 2014). Also, toxic B led to alterations of photosynthetic pigment content and the ratio of chlorophyll a and b (Huang et al., 2014; Kayıhan et al., 2017) and carotenoid content (Sarafi et al., 2018). These effects are related to the ability of B to form complexes with molecules such as ATP and NADPH (Cervilla et al., 2009). This interaction limits the availability of free energy required for carbohydrate biosynthesis in the Calvin–Benson cycle. Thus, alterations in sugar content and partitioning (Roessner et al., 2006; Papadakis et al., 2018), and carbon skeleton devoted to amino acids can be observed under B toxicity (Guo et al., 2014; Sang et al., 2015). Therefore, the mechanism of early senescence can be promoted by an imbalance of the C/N ratio under B toxicity (Sotiras et al., 2019). Possible activation of leaf senescence might be verified by altered expression of the genes involved in jasmonic acid (JA) and ethylene metabolisms under high B in barley (Öz et al., 2009) and wheat (Kayıhan et al., 2017). Also, in our previous report, the changes in this mechanism were investigated in more detail by determining the expression levels of microRNAs regulating the JA- and ethylene-related transcription factors in *A. thaliana* under high B conditions (Kayıhan et al., 2019). As expected, we found that the expression levels of *miR172* and *miR319* were dramatically induced by B toxicity in *Arabidopsis thaliana*. miR172 regulates flowering time and floral organ identity in *Arabidopsis thaliana* by targeting *APETALA2/ETHYLENE RESPONSE FACTORS* (*AP2/ERFs*) (Zhao et al. 2007). miR319 and its target genes, *TEOSINTE BRANCHED1*, *CYCLOIDEA*, and *PROLIFERATING CELL NUCLEAR ANTIGEN BINDING FACTOR* (*TCP*), play pivotal roles in leaf development (Fang et al., 2021). Therefore, understanding the post-transcriptional regulation of key genes controlling the leaf and flower development is important in plants tolerant to B toxicity.

After determining the possible role of post-transcriptional regulation of *CSD1* by miR398 and post-transcriptional regulation of JA- and ethylene-related transcription factors by miR172 and miR319 in *Arabidopsis thaliana* against B toxicity (Kayıhan et al., 2016; Kayıhan et al., 2019), in this study, we aimed to identify the potential targets of miR172, miR319, and miR398 in wheat, and their orthologs in *Arabidopsis thaliana* were determined. We identified new sets of genes that are targets of miR172, miR319 and miR398 in wheat with interesting functions that have not been studied before under B toxicity. Also, functional protein association networks of miRNA targets were identified. Gene ontology (GO) enrichment analyses of genes encoding for the proteins involved in PPI networks and the expression levels of these genes identified in PPI network analysis were performed. Finally, we determined the expression levels of these miRNAs as well as their selected targets in B-sensitive (Atay-85) and -tolerant (Bolal-2973) Turkish wheat cultivars under B toxicity. To the best of our knowledge, this is the first report that demonstrates the possible correlation of transcriptional and post-transcriptional regulations caused by B toxicity in wheat cultivars with already found changes in *Arabidopsis thaliana* as a model plant.

## 2. Materials and methods

### 2.1. miRNA target prediction and homology analysis

To determine the targets of miR172, miR319 and miR398 in *T. aestivum*, mature miRNA sequences were determined from *Arabidopsis thaliana* in miRBase database (version 22.1, http://www.mirbase.org/) (Kozomara et al., 2019). Then, the miRNA targets were identified by using psRNATarget software (version 2, http://plantgrn.noble.org/psRNATarget/) (Dai et al., 2018) with miRNA-target pairing stringency of 3, complementarily scoring length (HSP size) of 20, allowed maximum energy to unpair the target-miRNA site (UPE) of ≤ 25, and range of central mismatch leading to translational inhibition of 9-11 nt, according to Kumar et al., (2015). Target mRNA sequences in *T. aestivum* (for miR172, miR319) or in all plant species (for miR398) were downloaded and protein sequences were identified by blastx against *T. aestivum* genome in National Center for Biotechnology Information (NCBI, Johnson et al., 2008) with default settings. Then, homologous targets with the highest significant similarity were identified in *A. thaliana* by using pBLAST in NCBI and The Arabidopsis Information Resource (TAIR, Lamesch et al., 2012) databases with default settings.

### 2.2. Protein-protein interaction (PPI) network analysis

To determine the proteins that potentially interact with the miRNA targets in *T. aestivum*, functional protein association networks of miRNA targets in *T. aestivum* were determined via STRING protein-protein interaction networks functional enrichment and network drawing analysis (version 11, https://string-db.org/) (Szklarczyk et

al., 2019) with a confidence level of 0.7 and excluding text-mining as the source of interaction. Then, homologous targets with the highest significant similarity were identified in *A. thaliana* and *Hordeum vulgare* by using pBLAST in NCBI, TAIR, and The Barley Genome Explorer (BARLEX, Colmsee et al., 2015) databases with default settings.

**2.3. Gene ontology (GO) enrichment analysis**

GO enrichment analyses of genes encoding for the proteins involved in PPI networks were done by PANTHER version 14 (Mi et al., 2019) against the GO database released on 2019-12-09. The reference list included all available *T. aestivum* genes. Results of GO biological process and molecular function were given after Fisher's exact testing followed by Bonferroni correction for multiple testing ($p < 0.05$).

**2.4. In silico analysis of microarray experiments and gene set enrichment analysis (GSEA)**

To determine the expression levels of genes identified in PPI network analysis of miR172, miR319 and miR398 target genes, microarray CEL. files were downloaded from the Gene Expression Omnibus database (Edgar et al., 2002) for *A. thaliana* (GSE32659 - Aquea et al., 2012) and *H. vulgare* (GSE14521 - Öz et al., 2009) or obtained from our previous study for *T. aestivum* (Kayıhan et al., 2017) under B toxicity. Then, the expression levels of each gene were determined by Genespring GX (Agilent) according to the user's manual, and a heat map was generated in TBtools (Chen et al., 2020).

GSEA was performed using GeneTrail (Backes et al., 2007; Schuler et al., 2011) according to our previous study (Aksoy et al., 2013). GSEA was performed for each sorted dataset using gene sets created from B toxicity (Kayıhan et al., 2017) or heat stress in *T. aestivum* (Aprile et al., 2013), *A. thaliana* (Aquea et al., 2012 or Kilian et al. 2007, respectively) and *H. vulgare* (Öz et al., 2009 or Pacak et al., 2016, respectively). The gene sets not identified in the PPI network but enriched in heat stress were used as negative controls in all three organisms. False discovery rate was used as the *P*-value adjustment, and the values are presented (Benjamini and Hochberg, 1995).

**2.5. Plant material and stress treatment**

Wheat seeds of Atay-85 (B-sensitive) and Bolal 2973 (B-tolerant) cultivars were provided by the Republic of Turkey Ministry of Agriculture and Forestry. Sensitive and tolerant characters of these wheat cultivars were previously determined according to B accumulation in their leaf tissues (Kalaycı et al., 1998; Kayıhan et al., 2017). Seeds were imbibed for 24 h and sterilized in 40% of NaOCl by agitation for 20 min and then rinsed with sterile distilled water X3. Seeds were sown in vitro Vent containers (Duchefa Biochemie, NL) containing sterile perlite and directly irrigated by liquid Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) including sufficient (100 μM $H_3BO_3$) and toxic levels of B (1 and 3 mM $H_3BO_3$) in 2 days interval. They were grown at 22 ± 2 °C with an irradiance of 300 μmol $m^{-2}$ $s^{-1}$ at 16-h of light photoperiod and 60 % relative humidity in a growth chamber for 10 days.

**2.6. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis**

Leaf samples from 10-day-old seedlings of wheat cultivars were ground in 2 mL-Eppendorf tubes by Retsch MM400 grinder. Total RNAs were isolated from the ground tissue samples by the Trizol method (Chomczynski and Sacchi, 1987). Total RNA integrity was determined by running on 1% agarose gel electrophoresis, and the quantity was determined by a nano-spectrophotometer (data not shown). Primers for miRNA target genes were specifically designed according to the coding sequence of genes in *T. aestivum* using NCBI and Primer3 primer designing tool (Untergasser et al., 2012). miRNA sequences were obtained from miRBase and stem-loop (SL) RT, and forward primers were specifically designed according to the protocol of Varkonyi-Gasic et al. (2007). Primer sequences for miRNAs and their targets are given in Table 1.

The first-strand cDNAs were synthesized from 1 μg of total RNAs by using Maxima First Strand cDNA Synthesis Kit (Thermo Scientific). cDNA synthesis was confirmed by reverse transcription PCR(RT-PCR). Briefly, 1.5 μL of cDNA product was amplified with 5× FIREPol Master Mix (Solis BioDyne) containing 12.5 mM $MgCl_2$, and specific primers (0.4 μM) in a 20 μL total volume of PCR reaction. The PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 40 s, 55 °C for 30 s and 72 °C for 45 s, and a final extension at 72 °C for 5 min. PCR products were run on 1% agarose gel and visualized by Image Lab (Biorad, USA). After confirmation of correct cDNA synthesis, qRT-PCR was conducted using Maxima SYBR Green qPCR Master Mix (2X) (Thermo Scientific) on StepOnePlus Real-Time PCR System (Applied Biosystems). For qRT-PCR analysis, 1 μL of cDNA product, 7 μL of 2X Master Mix, 0.3 μM final concentration of forward and reverse primers were supplied to 15 μL of total volume with nuclease-free water. The qRT-PCR conditions were initiated with denaturation at 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 59 °C for 30 s, and 72 °C for 30 s. The melting curve was analyzed at 60–95 °C after 40 cycles. Normalization was made by using the actin gene (*ACT2*) (Kayıhan et al., 2016) and $2^{-\Delta\Delta Ct}$ was used for the determination of fold change of each comparison.

The expression of wheat miRNAs was detected using the stem-loop qRT-PCR method of Varkonyi-Gasic et al. (2007). Mixtures of 12 μL involving 1 μg of RNA, RNase-free water, and 2 μM SL primer mix were prepared.

**Table 1.** List of primers used in RT-qPCR studies.

| Primer name | Sequence 5' to 3' |
|---|---|
| Tae_miR398_SL | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGGGGG |
| Tae_miR398_F | CGGTGGTGTGTTCTCAGGTCG |
| Tae_miR172_SL | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACATGCAG |
| Tae_miR172_F | CGGCGGAGAATCTTGATGATG |
| Tae_miR319_SL | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGAG |
| Tae_miR319_F | CGGATATTGGACTGAAGGGAG |
| Universal_R | GTGCAGGGTCCGAGGT |
| Tae_Actin_F | AGGTTCAGGTGCCCTGAGGT |
| Tae_Actin_R | CAACATCACACTTCATGATGGA |
| Tae_MYB3_F | AAACTGCGGCTTCATGGATC |
| Tae_MYB3_R | TGAGAACTGATCGACCGAGG |
| Tae_TOE1_F | CCGGTGGCCAAGAAGAGC |
| Tae_TOE1_R | TTGCCGCAATCCCAGATATG |
| Tae_CSD_F | GCACCATCTTCTTCACCCAG |
| Tae_CSD_R | AGGGTTGAAGTGTGGTCCAG |

Then, they were incubated for 5 min at 65 °C followed by incubation on ice for 2 min. Subsequently, 5X reaction buffer, RiboLock RNase inhibitor (20 U/μL), 10 mM dNTP, and reverse transcriptase (Thermo Scientific) were added to the mixtures. They were incubated at 16 °C for 30 min followed by pulsed reverse transcription of 60 cycles at 30 °C for 30 s, 42 °C for 30 s, and 50 °C for 1 s. The tubes were subsequently incubated for 5 min at 70 °C. The cDNAs of miRNAs obtained from stem-loop pulsed RT reactions were used for qRT-PCR. Mixture, conditions, and normalization procedures for miRNA expression were similar to the target genes mentioned above.

### 2.7. Statistical analyses

Gene expression experiments were performed as four biological replicates (n = 4). The data of qRT-PCR were statistically analyzed by using a non-parametric version of the t-test in SPSS statistical programme. They were shown as mean with standard error (SE). Statistics used in individual bioinformatic analyses were given in suitable sections and figure/table legends.

## 3. Results

### 3.1. Determination of miRNA target sequences and their orthologs

Known sequences of Arabidopsis mature miR172, miR319 and miR398 were determined from the miRBase database, and their targets were identified by psRNATarget software (Supplemental Dataset). Among all potential targets from different organisms, target mRNA sequences were selected in *T. aestivum* (for miR172, miR319) for more species-specific target discovery. mRNA target sequences of miR398 were determined in all plant species since there was no predicted wheat target identified to date in the database. After the determination of proteins encoded by the target mRNAs in *T. aestivum*, their orthologs were identified in *A. thaliana*.

miR172 targets six mRNAs encoding for three floral homeotic proteins (QEL52102.1, ANW09544.1 and AFV46170.1), one asynapsis protein (ABR20128.1), and two proteins involved in alternative splicing (pre-mRNA processing) (AAY84884.1 and AAY84871.1) in wheat (Table 2). Arabidopsis orthologs of floral homeotic proteins included RELATED TO AP2.7 / TARGET OF EARLY ACTIVATION TAGGED (EAT)1 (RAP2.7/TOE1), APETALA2 / FLOWER1 / FLORAL MUTANT2 (AP2/FL1/ FLO2) and TARGET OF EARLY ACTIVATION TAGGED (EAT)3 (TOE3). Arabidopsis orthologs of ABR20128.1 are ASYNAPTIC1 (ASSY1) and ASYNAPTIC2 (ASY2). Orthologs of AAY84884.1 and AAY84871.1 in *Arabidopsis thaliana* are the proteins found in SERINE/ARGININE-RICH PROTEIN SPLICING FACTOR family.

miR319 targets six mRNAs encoding for one histone protein (CAA42530.1), two R2R3-MYB transcription factors (AEV91140.1 and AAT37169.1), one ribosomal protein (DAA01149.1), one lipid transfer protein (ABE99813.1), and one ubiquitin-activating protein (AAA34265.1) in wheat (Table 3). Arabidopsis ortholog

**Table 2.** miR172 target sequences in *Triticum aestivum* and their orthologs in *Arabidopsis thaliana*.

| *Triticum aestivum* | | *Arabidopsis thaliana* orthologs | | | | | |
|---|---|---|---|---|---|---|---|
| NCBI number | Protein name | NCBI number | AGI number | Protein name | Protein Code | Percent Identity (%) | E value |
| QEL52102.1 | floral homeotic protein | NP_001189625.1 | AT2G28550 | RELATED TO AP2.7 / TARGET OF EARLY ACTIVATION TAGGED (EAT) 1 | RAP2.7/TOE1 | 47.98 | $2 \times 10^{-98}$ |
| | | OAO99308.1 | AT4G36920 | APETALA 2 / FLOWER 1 / FLORAL MUTANT 2 | AP2/FL1/ FLO2 | 62.77 | $3 \times 10^{-89}$ |
| | | NP_001332132.1 | AT5G67180 | TARGET OF EARLY ACTIVATION TAGGED (EAT) 3 | TOE3 | 61.63 | $1 \times 10^{-84}$ |
| ANW09544.1 | target of EAT1-B1 | NP_001189625.1 | AT2G28550 | RELATED TO AP2.7 / TARGET OF EARLY ACTIVATION TAGGED (EAT) 1 | RAP2.7/TOE1 | 49.29 | $7 \times 10^{-109}$ |
| | | OAO99308.1 | AT4G36920 | APETALA 2 / FLOWER 1 / FLORAL MUTANT 2 | AP2/FL1/ FLO2 | 60.91 | $2 \times 10^{-99}$ |
| | | NP_001332132.1 | AT5G67180 | TARGET OF EARLY ACTIVATION TAGGED (EAT) 3 | TOE3 | 75.12 | $1 \times 10^{-93}$ |
| AFV46170.1 | spelt factor protein | NP_001189625.1 | AT2G28550 | RELATED TO AP2.7 / TARGET OF EARLY ACTIVATION TAGGED (EAT) 1 | RAP2.7/TOE1 | 47.98 | $2 \times 10^{-98}$ |
| | | OAO99308.1 | AT4G36920 | APETALA 2 / FLOWER 1 / FLORAL MUTANT 2 | AP2/FL1/ FLO2 | 62.77 | $3 \times 10^{-89}$ |
| | | NP_001332132.1 | AT5G67180 | TARGET OF EARLY ACTIVATION TAGGED (EAT) 3 | TOE3 | 61.63 | $1 \times 10^{-84}$ |
| ABR20128.1 | asynapsis 1 | OAP18558.1 | AT1G67370 | ASYNAPTIC 1 | ASY1 | 55.96 | 0.0 |
| | | AAG00246.1 | AT4G32200 | ASYNAPTIC 2 | ASY2 | 55.79 | 0.0 |
| | | - | - | - | - | - | - |
| AAY84884.1 | alternative splicing regulator | NP_001320500.1 | AT1G09140 | SERINE/ARGININE-RICH PROTEIN SPLICING FACTOR 30 | SR30 | 72.96 | $3 \times 10^{-96}$ |
| | | NP_001318908.1 | AT1G02840 | SERINE/ARGININE-RICH PROTEIN SPLICING FACTOR 34 | SR34 | 66.17 | $4 \times 10^{-94}$ |
| | | NP_001078264.1 | AT3G49430 | SERINE/ARGININE-RICH PROTEIN SPLICING FACTOR 34A | SR34A | 71.21 | $9 \times 10^{-94}$ |
| AAY84871.1 | pre-mRNA processing factor | OAO99960.1 | AT2G24590 | RS-CONTAINING ZINC FINGER PROTEIN 22A | RSZ22A | 63.59 | $3 \times 10^{-52}$ |
| | | NP_001078474.1 | AT4G31580 | RS-CONTAINING ZINC FINGER PROTEIN 22 / SERINE/ARGININE-RICH 22 | RSZ22 / SR22 | 63.08 | $2 \times 10^{-50}$ |
| | | NP_001117342.1 | AT1G23860 | RS-CONTAINING ZINC FINGER PROTEIN 21 | RSZ21 | 66.96 | $2 \times 10^{-40}$ |

**Table 3.** miR319 target sequences in *Triticum aestivum* and their orthologs in *Arabidopsis thaliana*.

| *Triticum aestivum* | | *Arabidopsis thaliana* orthologs | | | | | |
|---|---|---|---|---|---|---|---|
| NCBI number | Protein name | NCBI number | AGI number | Protein name | Protein Code | Percent Identity (%) | E value |
| CAA42530.1 | histone H2B | NP_190184.1 | AT5G22880 | HISTONE B2 | H2B | 82.47 | $2 \times 10^{-74}$ |
| | | NP_180440.1 | AT5G59910 | HISTONE B4 | H4B | 81.17 | $2 \times 10^{-73}$ |
| | | AAM66958.1 | AT1G07790 | HISTONE B1 | H1B | 80.52 | $3 \times 10^{-72}$ |
| AEV91140.1 | R2R3-MYB protein | NP_196228.1 | AT5G06100 | MYB DOMAIN PROTEIN 33 | MYB33 | 39.24 | $2 \times 10^{-63}$ |
| | | NP_001077993.1 | AT2G32460 | MYB DOMAIN PROTEIN 1 / MYB DOMAIN PROTEIN 101 / ABNORMAL SHOOT 7 | MYB1/MYB101/ ABS7 | 63.95 | $1 \times 10^{-60}$ |
| | | NP_001327043.1 | AT3G11440 | MYB DOMAIN PROTEIN 65 | MYB65 | 41.28 | $7 \times 10^{-60}$ |
| AAT37169.1 | transcription factor Myb3 | NP_196228.1 | AT5G06100 | MYB DOMAIN PROTEIN 33 | MYB33 | 43.00 | $7 \times 10^{-86}$ |
| | | NP_001327043.1 | AT3G11440 | MYB DOMAIN PROTEIN 65 | MYB65 | 43.00 | $1 \times 10^{-84}$ |
| | | NP_001077993.1 | AT2G32460 | MYB DOMAIN PROTEIN 1 / MYB DOMAIN PROTEIN 101 / ABNORMAL SHOOT 7 | MYB1/MYB101/ ABS7 | 68.75 | $3 \times 10^{-67}$ |
| DAA01149.1 | ribosomal protein L3A-1 | NP_001031146.1 | AT1G43170 | EMBRYO DEFECTIVE 2207 / ARABIDOPSIS RIBOSOMAL PROTEIN 1 | EMB2207 / ARP1 | 85.83 | 0.0 |
| | | NP_176352.1 | AT1G61580 | ARABIDOPSIS RIBOSOMAL PROTEIN 2 / R-PROTEIN L3 B | ARP2 / RPL3B | 83.68 | 0.0 |
| | | OAP15395.1 | AT3G17465 | ARABIDOPSIS RIBOSOMAL PROTEIN L3 PLASTID | ARPL3P | 83.42 | 0.0 |
| ABE99813.1 | lipid transfer protein | NP_181387.1 | AT2G38530 | LIPID TRANSFER PROTEIN2 / CELL GROWTH DEFECT FACTOR3 | LTP2 / CDF3 | 44.95 | $9 \times 10^{-25}$ |
| | | NP_195807.1 | AT5G01870 | Unknown (Belongs to the lipid transfer protein (PR-14) family) | - | 43.36 | $1 \times 10^{-23}$ |
| | | NP_187489.1 | AT3G08770 | LIPID TRANSFER PROTEIN6 | LTP6 | 48.94 | $2 \times 10^{-23}$ |
| AAA34265.1 | ubiquitin activating enzyme | NP_568168.1 | AT5G06460 | UBIQUITIN ACTIVATING ENZYME 2 | UBA2 | 77.10 | 0.0 |
| | | NP_565693.1 | AT2G30110 | UBIQUITIN-ACTIVATING ENZYME 1 / MODIFIER OF SNC1 5 | UBA1 / MOS5 | 76.26 | 0.0 |
| | | NP_001189570.1 | AT2G21470 | SUMO-ACTIVATING ENZYME 2 / EMBRYO DEFECTIVE 2764 | SAE2 / EMB2764 | 27.76 | $7 \times 10^{-40}$ |

of CAA42530.1 encodes for histone proteins, namely HISTONE B2, HISTONE B4 and HISTONE B1. Arabidopsis orthologs of AEV91140.1 and AAT37169.1 encode for different MYB domain proteins, namely MYB33, MYB1/MYB101 and MYB65. The closest orthologs of DAA01149.1 include Arabidopsis ribosomal proteins, namely EMB2207/ARP1, ARP2/RPL3B and ARPL3P. Arabidopsis orthologs of ABE99813.1 are LIPID TRANSFER PROTEIN2 / CELL GROWTH DEFECT FACTOR3 (LTP2/CDF3) and LTP6. Finally, the Arabidopsis orthologs of AAA34265.1 include UBIQUITIN ACTIVATING ENZYME2 (UBA2), UBA1 and SUMO-ACTIVATING ENZYME2 (SAE2).

miR398 targets three mRNAs encoding for one ethylene response factor (QBC41000.1), one fasciclin-like protein (ABI95405.1), one lipoxygenase (QBG67102.1), one heat shock protein (AGO59086.1) and two superoxide dismutases (AFF27608.1 and ACO90194.1) in wheat (Table 4). It also targets three unknown proteins (CDM81914.1, SPT16818.1 and SPT19866.1). Arabidopsis orthologs of QBC41000.1, ETHYLENE RESPONSE FACTOR71 (ERF71), ERF72 and ERF73, are members of the ERF (ethylene response factor) subfamily B-2 of the plant-specific ERF/AP2 transcription factor family. Arabidopsis orthologs of ABI95405.1 encode for FASCICLIN-LIKE ARABINOGALACTAN-PROTEIN11 (FLA11), FLA6 and FLA13. Arabidopsis orthologs of QBG67102.1 include LIPOXYGENASE1 (LOX1), LOX3 and LOX5 that encode for dioxygenases involved in the production of traumatin, jasmonic acid, oxylipins, and volatile aldehydes (Baysal and Demirdöven, 2007). SPT16818.1 has Arabidopsis orthologs of DExH-box RNA helicases, namely HUA ENHANCER2 (HEN2), HOMOLOG OF YEAST MTR4 (MTR4) and SUPERKILLER2 (SKI2). Arabidopsis orthologs of SPT19866.1 encode for zinc finger transcription factors, namely FLORAL DEFECTIVE10 / FLORAL ORGAN NUMBER1 / SUPERMAN (FLO10 / FON1 / SUP), ZINC-FINGER PROTEIN10 (ZFP10) and ZFP11. The closest orthologs of AGO59086.1 in Arabidopsis were encoding for members of the RETICULATA-RELATED (RER) gene family, namely RER6, RER5 and RER6. Arabidopsis orthologs of AFF27608.1 and ACO90194.1 include SUPEROXIDE DISMUTASE family members SOD1, SOD2 and SOD3. Overall, we identified new sets of genes whose mRNAs are targets of miR172, miR319 and miR398 in *T. aestivum* with interesting functions, which has not been studied before under B toxicity.

### 3.2. PPI network and GO enrichment analyses of miRNA target sequences

As the miRNA targets represented genes with different biological processes that are not connected, we investigated the proteins that can potentially interact with the proteins translated from miRNA targets via STRING protein-protein interaction (PPI) network functional enrichment and drawing analysis. Among the targets of miR172, AAY84871.1, AAY84884.1, ABR20128.1, AFV46170.1, ANW09544.1 and QEL52102.1 encoded for proteins that interact with 6, 5, 6, 8, 7 and 8 other proteins, respectively (Supplementary Figure 1 and Supplementary Table 1). Gene ontologies of these proteins were enriched in biological processes including regulation of RNA splicing/processing and RNA metabolism while they were highly related to recombinase activity and RNA binding (Table 5). miR319 targets AAA34265.1, AAT37169.1, ABE99813.1, AEV91140.1, CAA42530.1 and DAA01149.1 encoded for proteins that form protein interaction networks composed of 5, 3, 8, 3, 5 and 9 proteins, respectively (Supplementary Figure 2 and Supplementary Table 2). GO enrichment analysis revealed that these proteins were positively enriched in biological processes of translation, peptide biosynthesis, protein metabolism and gene expression as they represented molecular functions in ribosomes (Table 5). Finally, PPI network of proteins encoded by miR398 targets in *T. aestivum* included 7 (with ABI95405.1), 5 (with CDM81914.1), 5 (with QBC41000.1), 6 (with QBG67102.1), 4 (with STP16818.1), 7 (with SPT19866.1), 6 (with AGO59086.1) and 7 (with AFF27608.1) proteins (Supplementary Figure 3 and Supplementary Table 3). Proteins involved in the PPI network of miR398 targets were positively enriched in biological processes of superoxide metabolism, response to superoxide and ROS as well as oxylipin biosynthesis, which functions in jasmonic acid synthesis (Table 5). Respective barley homologs of proteins identified in PPI network analyses were also searched in NCBI BLAST and were given in supplemental tables. Interestingly, the number of members in each PPI network was higher in the Ensembl Genome Browser, which is used by STRING curation, than the ones identified in NCBI suggesting a discrepancy between different genome browsers. Moreover, some wheat and/or barley proteins were represented by the same proteins in Arabidopsis, suggesting a potential duplication in cereals. Taken together, PPI network and GO enrichment analyses showed that the proteins interacting with miR172, miR319, and miR398 targets in *T. aestivum* are involved in processes that have not been linked with B toxicity responses and/or tolerance mechanisms before.

### 3.3. In silico expression levels of miRNA targets and GSEA under B toxicity

As the proteins interacting with miR172, miR319 and miR398 targets were involved in processes that were not been linked with B toxicity responses and/or tolerance mechanisms before, expression levels of the genes encoding for the proteins in the interaction network were determined in B toxicity microarray experiments available in public databases. For this purpose, we determined the

**Table 4.** miR398 target sequences in *Triticum aestivum* and their orthologs in *Arabidopsis thaliana*.

| *Triticum aestivum* | | *Arabidopsis thaliana* orthologs | | | | | |
|---|---|---|---|---|---|---|---|
| NCBI number | Protein name | NCBI number | AGI number | Protein name | Protein Code | Percent Identity (%) | E value |
| QBC41000.1 | ethylene response factor | OAP04118.1 | AT3G16770 | ETHYLENE RESPONSE FACTOR 72 / RELATED TO AP2 3 | ERF72 / RAP2.3 | 49.65 | $6 \times 10^{-37}$ |
| | | NP_001077812.1 | AT1G72360 | ETHYLENE RESPONSE FACTOR 73 / HYPOXIA RESPONSIVE ERF 1 | ERF73 / HRE1 | 50.00 | $9 \times 10^{-36}$ |
| | | OAP10407.1 | AT2G47520 | ETHYLENE RESPONSE FACTOR 71 / HYPOXIA RESPONSIVE ERF 2 | ERF71 / HRE2 | 38.12 | $3 \times 10^{-34}$ |
| ABI95405.1 | fasciclin-like protein FLA15 | AAM62616.1 | AT5G03170 | FASCICLIN-LIKE ARABINOGALACTAN-PROTEIN 11 | FLA11 | 44.44 | $5 \times 10^{-39}$ |
| | | NP_565475.1 | AT2G20520 | FASCICLIN-LIKE ARABINOGALACTAN-PROTEIN 6 | FLA6 | 40.08 | $6 \times 10^{-39}$ |
| | | CAA0407418.1 | AT5G44130 | FASCICLIN-LIKE ARABINOGALACTAN-PROTEIN 13 | FLA13 | 38.83 | $2 \times 10^{-36}$ |
| QBG67102.1 | lipoxygenase 1 | OAP12239.1 | AT1G55020 | LIPOXYGENASE 1 | LOX1 | 42.54 | 0.0 |
| | | OAP05851.1 | AT3G22400 | LIPOXYGENASE 5 | LOX5 | 40.69 | 0.0 |
| | | NP_564021.1 | AT1G17420 | LIPOXYGENASE 3 | LOX3 | 40.28 | $2 \times 10^{-172}$ |
| CDM81914.1 | - | NP_001190691.1 | AT4G08460 | - | - | 54.64 | $8 \times 10^{-71}$ |
| | | VYS50362.1 | AT1G68140 | zinc finger/BTB domain-containing protein (putative) | - | 45.80 | $1 \times 10^{-66}$ |
| | | CAA0340205.1 | AT1G77770 | forkhead box protein (putative) | - | 49.28 | $2 \times 10^{-62}$ |
| SPT16818.1 | - | NP_565338.1 | AT2G06990 | HUA ENHANCER 2 / SUPPRESSOR OF PAS2 3 | HEN2 / SOP3 | 74.45 | 0.0 |
| | | NP_176185.1 | AT1G59760 | ORTHOLOG OF YEAST MTR4 | MTR4 | 47.55 | 0.0 |
| | | VYS59595.1 | AT3G46960 | SUPERKILLER 2 | SKI2 | 34.80 | 0.0 |
| SPT19866.1 | - | NP_188954.1 | AT3G23130 | FLORAL DEFECTIVE 10 / FLORAL ORGAN NUMBER 1 / SUPERMAN | FLO10 / FON1 / SUP | 73.08 | $5 \times 10^{-22}$ |
| | | OAP09364.1 | AT2G37740 | ZINC-FINGER PROTEIN 10 | ZFP10 | 77.08 | $1 \times 10^{-19}$ |
| | | OAP09867.1 | AT2G42410 | ZINC-FINGER PROTEIN 11 | ZFP11 | 91.67 | $8 \times 10^{-19}$ |
| AGO59086.1 | putative heat shock transcription factor | OAP01610.1 | AT3G56140 | RETICULATA-RELATED6 | RER6 | 66.12 | 0.0 |
| | | VYS60557.1 | AT2G40400 | RETICULATA-RELATED5 / BRZ-INSENSITIVE-PALE GREEN3 | RER5 / BPG3 | 65.89 | 0.0 |
| | | OAP09379.1 | AT5G12470 | RETICULATA-RELATED4 | RER4 | 63.55 | 0.0 |

**Table 4.** (Continued).

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| AFF27608.1 | cytosolic Cu/Zn superoxide dismutase | NP_001077494.1 | AT1G08830 | COPPER/ZINC SUPEROXIDE DISMUTASE 1 / SUPEROXIDE DISMUTASE 1 | CSD1 / SOD1 | 80.92 | $2 \times 10^{-83}$ |
| | | NP_565666.1 | AT2G28190 | COPPER/ZINC SUPEROXIDE DISMUTASE 2 / SUPEROXIDE DISMUTASE 2 | CSD2 / SOD2 | 67.57 | $5 \times 10^{-67}$ |
| | | NP_197311.1 | AT5G18100 | COPPER/ZINC SUPEROXIDE DISMUTASE 3 | CSD3 / SOD3 | 64.67 | $1 \times 10^{-66}$ |
| ACO90194.1 | superoxide dismutase (mitochondrion) | NP_001077494.1 | AT1G08830 | COPPER/ZINC SUPEROXIDE DISMUTASE 1 / SUPEROXIDE DISMUTASE 1 | CSD1 / SOD1 | 80.26 | $7 \times 10^{-83}$ |
| | | NP_565666.1 | AT2G28190 | COPPER/ZINC SUPEROXIDE DISMUTASE 2 / SUPEROXIDE DISMUTASE 2 | CSD2 / SOD2 | 66.89 | $5 \times 10^{-66}$ |
| | | NP_197311.1 | AT5G18100 | COPPER/ZINC SUPEROXIDE DISMUTASE 3 | CSD3 / SOD3 | 64.67 | $7 \times 10^{-66}$ |

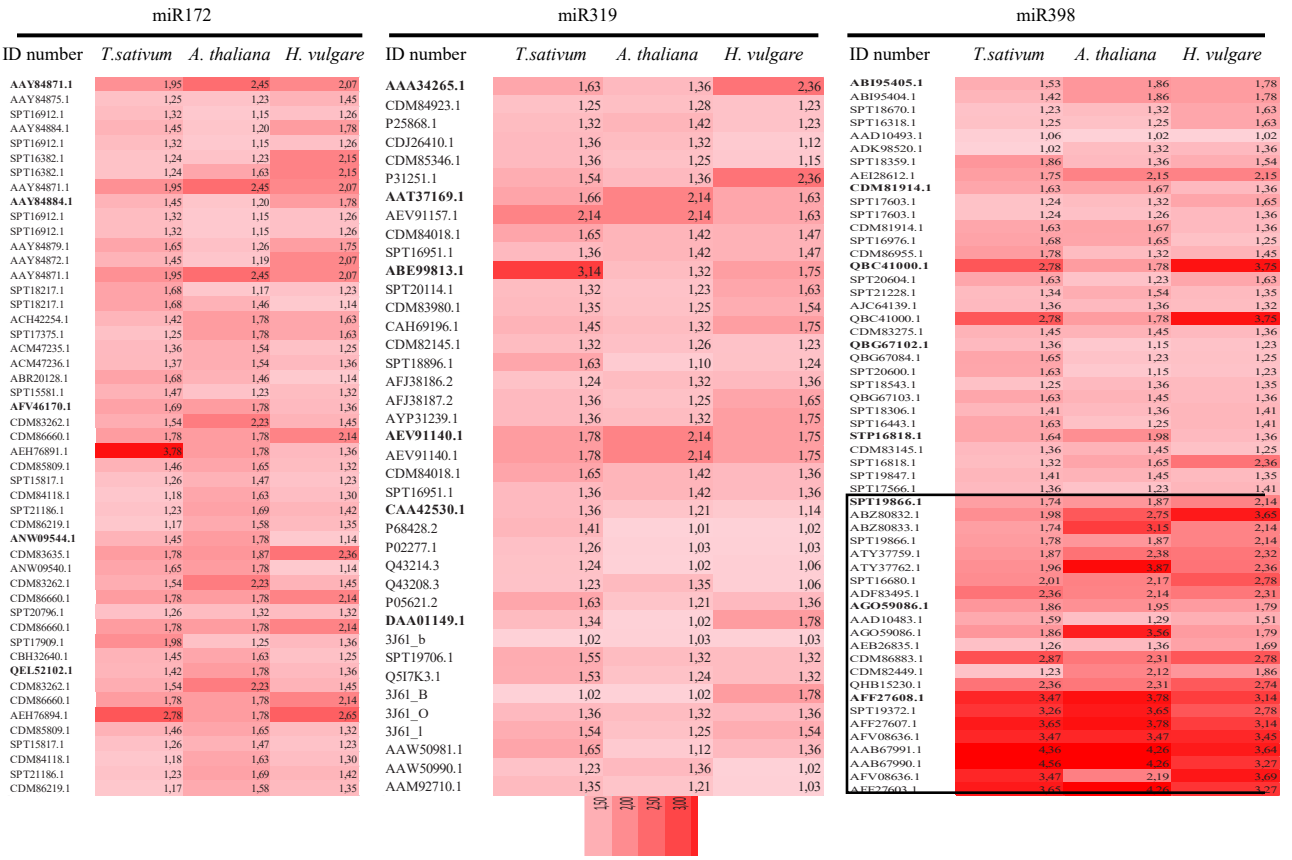


**Figure 1.** Expression levels of genes identified in PPI network analysis of miR172, miR319 and miR398 targets in *T. aestivum*, *A. thaliana* and *H. vulgare* under B toxicity. miRNA targets are indicated in bold. Color bar represents expression levels between 1 and 4. Genes in black box represent the cluster 1, which is highly induced in all three species under B toxicity.

expression levels of genes in wheat, barley, and *Arabidopsis thaliana* from three different datasets generated under B toxicity (Figure 1). The majority of the genes were expressed more than 1.5 times compared with the control samples while some of them reached further higher levels. As expected, some genes were specifically induced more in wheat (e.g. AEH76891.1) or wheat and barley together (e.g. AEH76894.1), while their Arabidopsis homologs were not that much induced under B toxicity. On the contrary, some genes (e.g. AGO59086.1) were induced much higher in *Arabidopsis thaliana* than did in the cereals, suggesting the evolution of different regulatory mechanisms in dicots and monocots responding to B toxicity. Interestingly, induction levels of genes that code for the proteins of SPT19866.1, AGO59086.1 and AFF27608.1, and their interacting partners were more pronounced in all three plant species under B toxicity (Cluster 1 in Figure 1). All these genes were targeted by miR398 and were related to ROS scavenging and stress tolerance (Supplementary Table 4). Overall, these results suggest the involvement of different sets of genes that were not been characterized before in B toxicity responses and/or tolerance in wheat.

To prove that the proteins identified in PPI analyses are specifically affected under B toxicity, we performed a Gene Set Enrichment Analysis (GSEA) of the genes encoding for the proteins in PPI networks of miR172, miR319 and miR398 targets in *T. aestivum*, *A. thaliana* and *H. vulgare* in B toxicity. To this end, we generated unique sets of genes identified in connection to the PPI analyses by discarding the replicates. Then, we performed GSEA by using GeneTrail against all genes in three organisms. As seen in Table 6, the gene sets identified via PPI network of all three miRNA targets in *T. aestivum* (31, 35 and 49 genes in miR172, miR319 and miR398 targets, respectively), *A. thaliana* (24, 29 and 45 genes in miR172, miR319 and miR398 targets, respectively) and *H. vulgare* (26, 30 and 45 genes in miR172, miR319 and miR398 targets, respectively) were significantly enriched under B toxicity in respective plant species. To demonstrate that the gene set enrichments were specific to the B toxicity treatment, separate sets of genes were identified from heat stress microarray experiments in each organism. Since these gene sets do not encode for the proteins identified in PPI network analyses but enriched only in heat stress, they

**Table 5.** Gene ontology (GO) enrichment of PPI networks of miR172, miR319 and miR398 target genes in *T. aestivum*. *

| | Expected | Fold Enrichment | Enrichment Representation | p-value |
|---|---|---|---|---|
| **miR172** | | | | |
| **GO: Biological process** | | | | |
| regulation of alternative mRNA splicing, via spliceosome | 0.03 | > 100 | Over | $2.05 \times 10^{-09}$ |
| regulation of RNA splicing | 0.04 | > 100 | Over | $1.13 \times 10^{-08}$ |
| regulation of mRNA processing | 0.04 | > 100 | Over | $2.26 \times 10^{-08}$ |
| regulation of mRNA metabolic process | 0.06 | > 100 | Over | $7.42 \times 10^{-08}$ |
| mRNA processing | 0.35 | 22.73 | Over | $3.91 \times 10^{-06}$ |
| **GO: Molecular function** | | | | |
| recombinase activity | 0.00 | > 100 | Over | $1.88 \times 10^{-02}$ |
| RNA binding | 1.10 | 7.31 | Over | $1.25 \times 10^{-02}$ |
| nucleic acid binding | 3.20 | 4.37 | Over | $5.13 \times 10^{-04}$ |
| **miR319** | | | | |
| **GO: Biological process** | | | | |
| translation | 0.60 | 13.36 | Over | $2.87 \times 10^{-04}$ |
| peptide biosynthetic process | 0.60 | 13.25 | Over | $3.06 \times 10^{-04}$ |
| peptide metabolic process | 0.69 | 11.58 | Over | $8.41 \times 10^{-04}$ |
| cellular protein metabolic process | 3.56 | 3.65 | Over | $4.09 \times 10^{-02}$ |
| gene expression | 1.75 | 5.71 | Over | $1.28 \times 10^{-02}$ |
| **GO: Molecular function** | | | | |
| protein heterodimerization activity | 0.12 | 40.40 | Over | $2.98 \times 10^{-04}$ |
| structural constituent of ribosome | 0.32 | 25.21 | Over | $1.37 \times 10^{-06}$ |
| structural molecule activity | 0.44 | 18.50 | Over | $1.77 \times 10^{-05}$ |
| **miR398** | | | | |
| **GO: Biological process** | | | | |
| cellular response to superoxide | 0.02 | > 100 | Over | $1.98 \times 10^{-05}$ |
| superoxide metabolic process | 0.02 | > 100 | Over | $2.64 \times 10^{-05}$ |
| response to superoxide | 0.02 | > 100 | Over | $3.45 \times 10^{-05}$ |
| oxylipin biosynthetic process | 0.04 | > 100 | Over | $2.93 \times 10^{-04}$ |
| cellular response to reactive oxygen species | 0.05 | 79.70 | Over | $8.35 \times 10^{-04}$ |
| **GO: Molecular function** | | | | |
| linoleate 9S-lipoxygenase activity | 0.00 | > 100 | Over | $2.45 \times 10^{-02}$ |
| superoxide dismutase activity | 0.01 | > 100 | Over | $4.37 \times 10^{-06}$ |
| oxidoreductase activity, acting on single donors with incorporation of molecular oxygen | 0.06 | 83.89 | Over | $1.16 \times 10^{-05}$ |
| oxidoreductase activity | 2.39 | 5.02 | Over | $4.63 \times 10^{-03}$ |
| ion binding | 8.68 | 2.53 | Over | $1.07 \times 10^{-02}$ |

* GO enrichment was done by using the tool at http://geneontology.org/ with Fisher's Exact test, and the results were corrected with the Bonferroni correction for multiple testing (Mi et al., 2019). Top 5 results were listed according to the fold enrichment.

were used as negative controls in GESA. Therefore, they were only enriched in heat stress but not under B toxicity. Interestingly, targeted by miR398, the Cluster 1 genes were related to the ROS scavenging and stress tolerance and were enriched in both B toxicity and heat stress, suggesting activation of some common mechanisms in both stresses

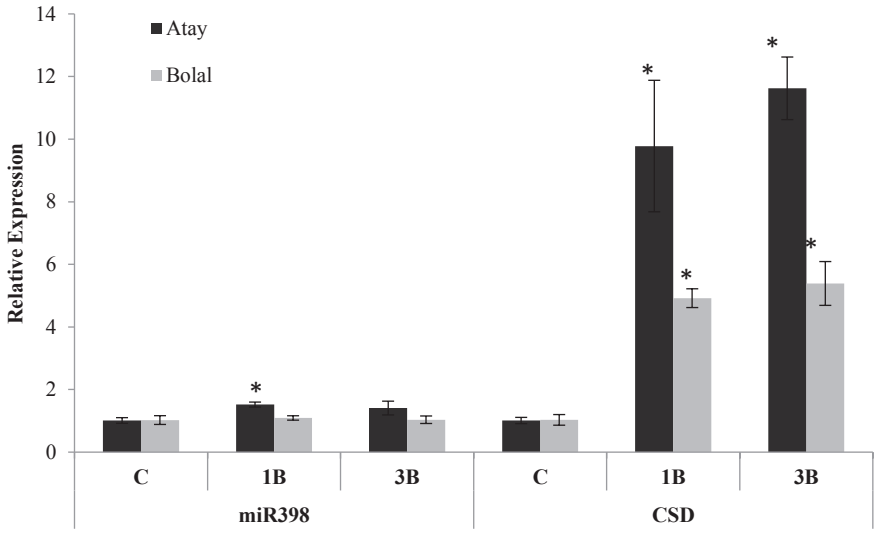


**Figure 2.** Changes in relative expression levels of miR398 and *CSD* in Atay and Bolal cultivars in response to toxic B treatments. C: Control, 1B: 1 mM $H_3BO_3$ treatment, 3B: 3 mM $H_3BO_3$ treatment. The mean and SEM from four independent experiments are shown ($p < 0.05$).

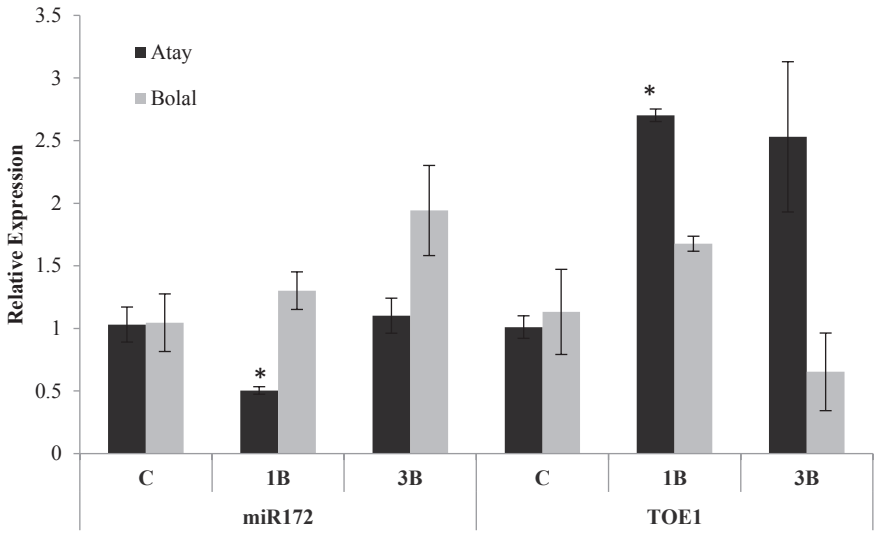


**Figure 3.** Changes in relative expression levels of miR172 and TOE1 in Atay and Bolal cultivars in response to toxic B treatments. C: Control, 1B: 1 mM $H_3BO_3$ treatment, 3B: 3 mM $H_3BO_3$ treatment. The mean and SEM from four independent experiments are shown ($p < 0.05$).

to prevent devastating effects of ROS under environmental stresses.

### 3.4. Expression levels of miRNAs and their targets in wheat cultivars under B toxicity

At the end of 10 d, chlorosis was observed in the leaves of the B-sensitive wheat cultivar Atay, but not in the B-tolerant cultivar Bolal under 1B condition. However, 3B caused a chlorosis in leaves and necrosis in leaf apexes of both sensitive and tolerant cultivars. Moreover, 3B led to decreases in germination rates and the shoot growth of both cultivars (Supplementary Figure 4).

Both 1B and 3B conditions led to remarkable increases in the expression levels of *CSD* in both sensitive and

**Table 6.** Gene set enrichment analysis (GSEA) of PPI networks of miR172, miR319 and miR398 targets in *T. aestivum*, *A. thaliana* and *H. vulgare* in B toxicity.

| | B toxicity in *T. aestivum* [a] | Heat stress in *T. aestivum* (GSE45563) [b] | B toxicity in *A. thaliana* (GSE32659) [c] | Heat stress in *A. thaliana* (GSE5628) [d] | B toxicity in *H. vulgare* (GSE14521) [e] | Heat stress in *H. vulgare* (GSE82134) [f] |
|---|---|---|---|---|---|---|
| p-value | | | | | | |
| PPI network of miR172 targets in *T. aestivum* (31 genes) | $2.0 \times 10^{-5}$ * | 0.278 | | | | |
| PPI network of miR319 targets in *T. aestivum* (35 genes) | $1.4 \times 10^{-2}$ * | 0.145 | | | | |
| PPI network of miR398 targets in *T. aestivum* (49 genes) | $2.6 \times 10^{-4}$ * | 0.105 | | | | |
| Cluster 1 genes in *T. aestivum* (20) | $7.8 \times 10^{-7}$ * | $1.3 \times 10^{-10}$ * | | | | |
| Non-network gene set in *T. aestivum* (35 genes) [g] | 0.345 | $2.1 \times 10^{-3}$ * | | | | |
| PPI network of miR172 targets in *A. thaliana* (24 genes) | | | $8.5 \times 10^{-4}$ * | 0.121 | | |
| PPI network of miR319 targets in *A. thaliana* (29 genes) | | | $2.4 \times 10^{-3}$ * | 0.069 | | |
| PPI network of miR398 targets in *A. thaliana* (45 genes) | | | $1.8 \times 10^{-4}$ * | 0.091 | | |
| Cluster 1 genes in *A. thaliana* (18) | | | $3.5 \times 10^{-5}$ * | $4.8 \times 10^{-7}$ * | | |
| Non-network gene set in *A. thaliana* (30 genes) [g] | | | 0.178 | $5.0 \times 10^{-8}$ * | | |
| PPI network of miR172 targets in *H. vulgare* (26 genes) | | | | | $7.3 \times 10^{-4}$* | 0.547 |
| PPI network of miR319 targets in *H. vulgare* (30 genes) | | | | | $4.6 \times 10^{-2}$ * | 0.423 |
| PPI network of miR398 targets in *H. vulgare* (45 genes) | | | | | $3.7 \times 10^{-3}$* | 0.131 |
| Cluster 1 genes in *H. vulgare* (19) | | | | | $1.7 \times 10^{-6}$ * | $2.9 \times 10^{-9}$ * |
| Non-network gene set in *H. vulgare* (35 genes) [g] | | | | | 0.326 | $4.2 \times 10^{-3}$ * |

[a] 5 mM $H_3BO_3$ for 5 days in *T. aestivum*, tissue: leaf (Kayıhan et al., 2017).
[b] GSE45563: 30/22 °C (day/night) for 2 days, 34/26°C (day/night) for 2 days, 40°C/ 32°C (day/night) for 1 day in *T. aestivum*, tissue: leaf (Aprile et al., 2013).
[c] GSE32659: 5 mM $H_3BO_3$ for 5 days in *A. thaliana*, tissue: roots (Aquea et al., 2012).
[d] GSE5628: 38 °C for up to 24 h in *A. thaliana*, tissue: leaves and roots (Kilian et al., 2007).
[e] GSE14521: 5 or 10 mM $H_3BO_3$ for 5 days in *H. vulgare*, tissue: leaves (Öz et al., 2009).
[f] GSE82134: 35.5 °C for 1 h in *H. vulgare*, tissue: leaves and roots (Pacak et al., 2016).
[g] A set of genes not involved in PPI networks of miR172, miR319 and miR398 targets in *T. aestivum*, *A. thaliana* and *H. vulgare* were used as control in GSEA.
* Indicates a specific gene set significantly enriched ($p < 0.05$) in top-ranked genes.

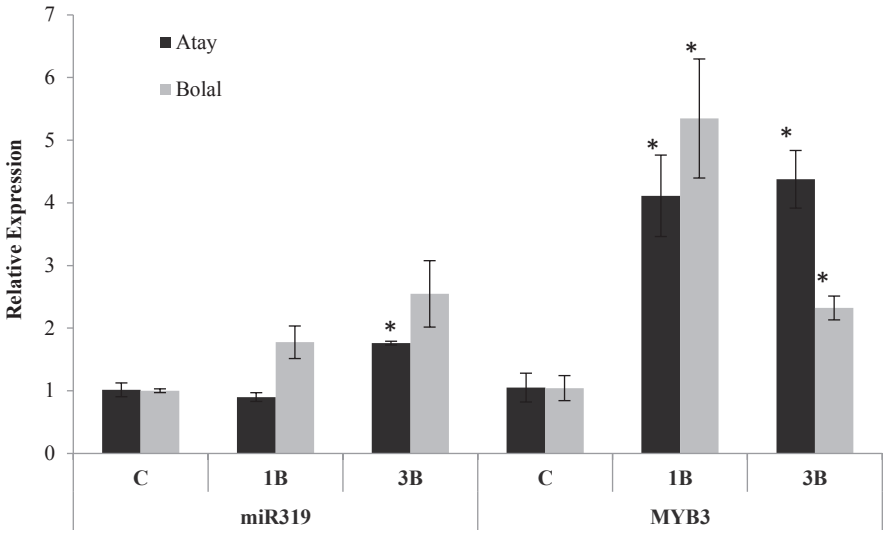


**Figure 4.** Changes in relative expression levels of miR319 and MYB3 in Atay and Bolal cultivars in response to toxic B treatments. C: Control, 1B: 1 mM $H_3BO_3$ treatment, 3B: 3 mM $H_3BO_3$ treatment. The mean and SEM from four independent experiments are shown ($p < 0.05$).

tolerant cultivars (Figure 2). These increases were more remarkable in Atay by approximately ten and twelve folds under 1B and 3B conditions, respectively, whereas five-fold increases were obtained in Bolal after these conditions (Figure 2). However, the expression level of *miR398*, as a miRNA targeting *CSD*, did not change significantly in Bolal under both toxic B conditions and in Atay after 3B treatment (Figure 2). Also, 1B caused a slight increase of *miR398* expression in Atay (Figure 2).

The expression level of *miR172* was decreased significantly in Atay exposed to 1B condition, whereas it stayed stable after 3B (Figure 3). On the other hand, 1B did not affect the expression level of *miR172* in Bolal, while 3B caused a non-significant increase in *miR172* expression (Figure 3). The target of *miR172*, *TOE1* was induced by both 1B and 3B conditions in Atay, but only 1B induction was found statistically significant (Figure 3). In Bolal, *TOE1* expression did not significantly change after both 1B and 3B treatments (Figure 3).

In Bolal, *miR319* expression was induced in both B toxicity conditions, but the induction was not significant (Figure 4). Likewise, 1B did not induce the expression level of *miR319* in Atay. However, its expression level was increased slightly by 3B in this cultivar (Figure 4). In contrast to *miR319*, its target *MYB3* was induced significantly by B toxicity in both sensitive and tolerant cultivars. Indeed, the *MYB3* expression level was increased more than four folds by both B toxicity conditions in Atay (Figure 4). Furthermore, it was increased approximately six-folds by 1B and more than two folds by 3B condition in Bolal (Figure 4).

## 4. Discussion

### 4.1. miR172, miR319 and miR398 targets in wheat and their Arabidopsis orthologs present novel sets of genes that can function in B tolerance

In our previous studies, we have shown the induction of *miR172* and *miR319* (Kayıhan et al., 2019) together with *miR398* and its target *CSD1* under B toxicity conditions in *A. thaliana* (Kayıhan et al., 2016). Therefore, in this study, we identified the targets of miR172, miR319 and miR398 in *T. aestivum* and their orthologs in *A. thaliana* to elucidate their potential functions in B toxicity tolerance in plants. According to our homology search, miR172 targets six mRNAs in wheat (Table 2), and their Arabidopsis orthologs have known functions in flowering (Zhang et al., 2015), cell division (Morgan et al., 2020), and alternative splicing (Xiang et al., 2019). miR172 targets were known to be involved in flowering time and floral organ identity in Arabidopsis (Zhao et al., 2007). For instance, TOE1 and its paralogs inhibit the CONSTANS (CO) activity by interacting with both CO and FLAVIN-BINDING KELCH REPEAT F-BOX1 (FKF1) whereby repressing the FLOWER LOCUS T (FT) and early flowering (Zhang et al., 2015). Moreover, APETALA2-LIKE (AP2-LIKE) gene family regulates the adult plant development time (Wu et al., 2009). B deficiency and toxicity lead to several anatomical abnormalities in flowers decreasing the crop yields (Rerkasem et al., 2020). Moreover, B deficiency reduced the number of flowers and delayed the flowering time in black gram (Pandey and Gupta, 2013).

Although miR172 was shown to regulate the flowering by targeting *APETALA2/ETHYLENE RESPONSE*

*FACTORS* (*AP2/ERFs*) (Zhao et al., 2007), our homology search identified new targets of miR172 in wheat. Arabidopsis orthologs of ABR20128.1, ASYNAPTIC1 (ASY1) and ASYNAPTIC2 (ASY2) are involved in the formation of chromosome axes along sister chromatids in meiosis and are essential for recombination, chromosome segregation, and fertility (Morgan et al., 2020). The most sensitive stage of microsporogenesis to B deficiency is around meiosis while a second, less sensitive stage is found between mitosis I and II during which starch accumulation occurs in the pollen grain (Huang et al., 2000). Therefore, B levels are critical for proper chromosome segregation in meiosis, and male and female sterility in plants (Agarwala et al., 1981; Rerkasem et al., 1997).

Orthologs of AAY84884.1 and AAY84871.1 in *A. thaliana* function in spliceosome assembly and regulation of the pre-mRNA splicing (Xiang et al., 2019). PPI network analysis also identified interacting proteins enriched in the regulation of RNA splicing/processing and RNA metabolism (Table 5). B toxicity alters the nucleic acid metabolism in *A. thaliana* (Kasajima and Fujiwara, 2007; Sakamoto et al., 2011) and citrus (Guo et al., 2014; Guo et al., 2016). Moreover, the involvement of RNA metabolism, via miRNA biosynthesis and DNA demethylation, in abiotic stress tolerance (Chang et al., 2020) and iron deficiency tolerance (Aksoy et al., 2013) has been shown in plants, suggesting their potential connection to the B toxicity tolerance. Therefore, the potential roles of the RNA metabolism regulators should be investigated under B deficiency and toxicity in plants. These results expand the possible targets of miR172 and their potential functions in plant growth and development as well as RNA metabolism under B toxicity in plants.

We found that miR319 targeted six mRNAs in wheat, and their Arabidopsis orthologs encode for histone proteins, namely HISTONE B2, HISTONE B4 and HISTONE B1, which have known functions in chromatin structure (Fojtová and Fajkus, 2020), and their regulation is essential for chromosomal stability, and DNA repair and replication as well as proper transcription (Zhu et al., 2012; Venkatesh and Workman, 2015). HISTONE H4 was induced in *Citrus sinensis* roots under B toxicity (Guo et al., 2016), and in rice roots under aluminum toxicity (Mao et al., 2004). It was shown that the histone hyperacetylation was induced, whereby increasing the formation of double-strand DNA breaks under B toxicity in *A. thaliana* (Sakamoto et al., 2018), suggesting that miR319 represents an essential post-transcriptional regulatory mechanism by controlling the mRNA availability of histone proteins, and in turn, limiting the chances of DNA damage under B toxicity.

Arabidopsis orthologs of AEV91140.1 and AAT37169.1 encode for different MYB domain proteins, namely MYB33, MYB1/MYB101 and MYB65. They are highly conserved (Jiang and Rao, 2020), and control the downstream genes function in leaf (An et al., 2014), root (Xue et al., 2017) and pollen tube development (Liang et al., 2013), and plant–nematode interactions (Jaubert-Possamai et al., 2019). The importance of MYB transcription factors under B tolerance has been shown in different plants (Lu et al., 2015; Jyothi et al., 2018). The induction of *miR319* in *Citrus grandis* roots represses the *MYB* genes under B toxicity, and this leads to the formation of fewer root tips (González-Fontes and Fujiwara, 2020). This, in turn, was linked to limited uptake of B to develop B toxicity tolerance in citrus roots. Moreover, miR319 is also induced in maize roots under nitrogen limitation (Xu et al., 2011), suggesting the crucial functions of MYB transcription factors in metal uptake in the roots by manipulating the root system architecture. Induction of a calmodulin-like protein under B deficiency was associated with the induction of MYB transcription factors in Arabidopsis (Quiles-Pando et al., 2013). Therefore, post-transcriptional regulation of *MYB33*, *MYB1/MYB101* and *MYB65* via miR319 could be essential for calcium-mediated manipulation of root system architecture under B toxicity.

The closest homologs of DAA01149.1 include Arabidopsis ribosomal proteins, namely EMB2207/ARP1, ARP2/RPL3B and ARPL3P. They have functions in actin polymerization and plasma membrane alteration during endocytosis in yeast (Toshima et al., 2016), autophagosome formation (Wang et al., 2016), and epidermal cell morphology in plants (Djakovic et al., 2006). Arabidopsis homologs of ABE99813.1 are LIPID TRANSFER PROTEIN2 / CELL GROWTH DEFECT FACTOR3 (LTP2/CDF3) and LTP6, and they play a role in maintaining the integrity of the cuticle-cell wall interface (Jacq et al., 2017). Several studies have shown the importance of B in cross-linking the apiose residues in cell wall pectin structure, whereby retaining the cell wall architecture (Bolaños et al., 2004). The expression of several genes involved in the biosynthesis of cell wall components as well as cell wall development was shown to be repressed under B deficiency (Camacho-Cristóbal et al., 2008). The expression of some genes involved in cell wall structure was also altered under B toxicity (Kayıhan et al., 2017). Moreover, B availability can also modify the plasma membrane composition (Brown et al., 2002); thus, it affects the mineral and water uptake (Wimmer and Eichert, 2013). A plasma membrane B exporter, BOR2, is required for proper boric acid/borate transportation from symplast to apoplast and effective cross-linking of rhamnogalacturonan II in the cell wall (Miwa et al., 2013). BOR2 was shown to be essential for root cell elongation under B deficiency. Therefore, miR319 can potentially regulate the cell expansion via cell wall- and

plasma membrane-associated proteins, indicating the essential post-transcriptional control of these components depending on the B availability.

Finally, the Arabidopsis homologs of AAA34265.1 include UBIQUITIN ACTIVATING ENZYME2 (UBA2), UBA1, and SUMO-ACTIVATING ENZYME2 (SAE2). UBA1 and UBA2 are involved in the first step in conjugating multiple ubiquitins to proteins targeted for degradation (Bachmair et al., 2001), while SAE2 encodes one of the two subunits of the SUMO activation enzyme required during sumoylation (Castaño-Miquel et al., 2013). Upon sufficient B supply, BOR1 is ubiquitinated at lysine 590 (Kasai et al., 2011) and targeted to the vacuole for 26S proteasomal degradation via multi-vesicular body/late endosome (MVB/LE) complexes (Viotti et al., 2010). Mutation of *BOR1* at lysine 590 abolishes both the ubiquitination and the degradation via the endocytic pathway (Takano et al., 2010). Interestingly, expression of *BOR1* was not affected under B deficiency in *A. thaliana* (Takano et al., 2005) but was significantly enhanced in a Turkish B-tolerant barley cultivar (Tarm-92) under mild B toxicity (Oz, 2012), indicating the regulation of stress responses at different biological levels. Similar degradation patterns have been shown for other metal and amino acid transporters in plants (Yates and Sadanandom, 2013). To support our results, the proteins or the genes involved in the 26S proteasome are significantly induced under B toxicity in B-hyperaccumulators *Gypsophila perfoliata* (Unver et al., 2008), *G. sphaerocephala* (Tombuloglu et al., 2017), and *Puccinellia distans* (Öztürk et al., 2018). Our PPI network analysis identified interacting proteins enriched in the regulation of translation and protein metabolism (Table 5), thus, supporting the critical roles of miR319 in B toxicity responses in plants.

We found that miR398 targeted nine mRNAs in wheat (Table 4). Among their Arabidopsis orthologs, AFF27608.1 and ACO90194.1 include SUPEROXIDE DISMUTASE family members SOD1, SOD2 and SOD3, which are involved in detoxification of superoxide radicals in the cytosol (Dreyer and Schippers, 2018). Recently, we have shown that the expression of *MnSOD* and *Cu/ZnSOD* genes and total SOD activity was enhanced in Arabidopsis seedlings under B toxicity (Kayıhan et al., 2016). This indicates that miR398-based post-transcriptional regulation of *SOD* genes may be critical for ROS scavenging and plant tolerance in wheat under B deficiency.

Arabidopsis orthologs of QBC41000.1, ETHYLENE RESPONSE FACTOR71 (ERF71), ERF72 and ERF73, are members of the ERF (ethylene response factor) subfamily B-2 of the plant-specific ERF/AP2 transcription factor family. They function in the ethylene signaling pathway under oxidative stress responses (Mizoi et al., 2012), and contribute to the integration of the jasmonic acid and ethylene signal transduction pathways (Pré et al., 2008). *ERF71*, *ERF75* and *ERF83* were upregulated in B-treated poplar leaves (Yıldırım and Uylaş, 2016). Some ERF/AP2 family proteins were also enriched in B-tolerant *Gypsophila sphaerocephala* roots under B-toxicity (Tombuloglu et al., 2017). Moreover, several genes encoding for ERF transcription factors were induced in Arabidopsis (Peng et al., 2012) and *Brassica napus* (Zhou et al., 2016) under B deficiency, suggesting an essential role of ethylene-based signaling in the regulation of downstream genes responding to alteration in B levels.

Orthologs of ABI95405.1 in Arabidopsis encode for FASCICLIN-LIKE ARABINOGALACTAN-PROTEIN11 (FLA11), FLA6 and FLA13 that are known to play roles in plant growth, development, and adaptation to environmental conditions by participating in cell-matrix adhesion (Costa et al. 2019), thereby interacting with the cytoskeleton (Sardar et al., 2006). FLAs are included in the Arabinogalactan-protein (AGP) family (Schultz et al., 2002), which are embedded in the plasma membrane with the help of a glycosylphosphatidylinositol (GPI) anchor or found in the extracellular matrix attached to the cell wall (Showalter, 2001). Expression of *AGP*s and *FLA*s are altered under B (Camacho-Cristóbal et al., 2008) or potassium (Armengaud et al., 2004) deficiencies in Arabidopsis. Moreover, one *FLA* gene was down-regulated under B deficiency in citrus together with other genes involved in cell wall metabolism (Zhou et al., 2015), indicating the essential requirement for these proteins in the maintenance of cell structure under B alterations. Alterations in the cell wall structure and cytoskeletal proteins under B deficiency (Goldbach et al., 2001) suggest that miR398 may be an important post-transcriptional regulator of genes involved in the formation of cell ultrastructure under B alterations.

Arabidopsis orthologs of QBG67102.1 include LIPOXYGENASE1 (LOX1), LOX3 and LOX5 that encode for dioxygenases involved in the production of traumatin, jasmonic acid, oxylipins, and volatile aldehydes (Baysal and Demirdöven, 2007). Higher LOX activity coupled by lipid peroxidation is linked to increased hydrogen peroxide levels under B toxicity in tomato and cucumber (Alpaslan and Gunes, 2001), onion (Inal and Tarakcioglu, 2001), barley (Karabal et al., 2003; Inal et al., 2009), apple rootstock (Molassiotis et al., 2006), grapevine (Gunes et al., 2006), wheat (Gunes et al., 2007) and lentil (Tepe and Aydemir, 2011). Recently, the lipid peroxidation rate was shown to be regulated by the antioxidant systems in soybean (Hamurcu et al., 2013) and Arabidopsis (Kayıhan et al., 2016) such that the lipid peroxidation increased under mild B toxicity while it decreased under higher levels. Since LOX functions in jasmonic acid biosynthesis in plants (Vick and Zimmerman, 1983), and the jasmonate levels are altered by sub-optimal B concentrations (Zhou

et al., 2016), miR398 may control the cellular antioxidant status and, in turn, jasmonic acid production under B toxicity.

SPT16818.1 has Arabidopsis orthologs of DExH-box RNA helicases, namely HUA ENHANCER2 (HUA2/ HEN2), HOMOLOG OF YEAST MTR4 (MTR4), and SUPERKILLER2 (SKI2). HUA2 acts redundantly with HEN1, HUA1, and HUA2 in the specification of floral organ identity in Arabidopsis (Shan et al., 2019). It is well-known that an optimal B level is required for proper pistil and stamen development as well as pollen tube growth (Dell and Huang, 1997; Rerkasem et al., 1997). Moreover, HEN2 is involved in the degradation of polyadenylated nuclear exosome substrates such as snoRNA and miRNA precursors, incompletely spliced mRNAs, and spurious transcripts produced from pseudogenes and intergenic regions (Lange et al., 2014), while MTR4 functions in the degradation of rRNA precursors and rRNA maturation by-products (Lange et al., 2011). Therefore, miR398 may regulate the floral organ identity via RNA helicase-based post-transcriptional regulation of genes.

Arabidopsis orthologs of SPT19866.1 encode for zinc finger transcription factors, namely FLORAL DEFECTIVE10 / FLORAL ORGAN NUMBER1 / SUPERMAN (FLO10 / FON1 / SUP), ZINC-FINGER PROTEIN10 (ZFP10), and ZFP11, that function in root hair development (Han et al., 2020), or controlling the boundary of the stamen and carpel whorls via hormonal regulations (Nibau et al., 2011; Breuil-Broyer et al., 2016). *ZFP4* was induced in *Citrus sinensis* roots under B deficiency (Lu et al., 2015). It is known that root hair formation and elongation are induced under B deficiency with the help of ETHYLENE-INSENSITIVE2 (EIN2) (Martín-Rejano et al., 2011). Recently, the necessity of the interaction between auxin and ethylene was shown to control the root cell elongation and hair formation under B deficiency (González-Fontes et al., 2016). Taken together, miR398 may regulate the root hair development via targeting *ZFP10* and *ZFP11* transcription factors under B toxicity.

The closest homologs of AGO59086.1 in Arabidopsis were encoding for members of the RETICULATA-RELATED (RER) gene family, namely RER6, RER5, and RER6. Although the function of these proteins has not been studied yet, the mutants develop leaf reticulation (Pérez-Pérez et al., 2013). Interestingly, RER5/BPG3 is localized in chloroplasts and is involved in brassinosteroid (BR) signaling, chloroplast movement and leaf greening (Yoshizawa et al., 2014). Under B toxicity, a substantial decrease in chlorophyll content (Chen et al., 2014) and structural changes in thylakoid membranes (Papadakis et al., 2004) suggest that RERs may be involved in the development of B toxicity symptoms in plants. Surprisingly, the primary root growth is inhibited under B deficiency due to the down-regulation of BR biosynthesis genes and reduction in the active BR levels in Arabidopsis roots (Zhang et al. 2021). BRs are also responsible for the inhibition of shoot growth under B deficiency (Eggert and von Wirén, 2017). Thus, our physiological observations may be explained by the modulation of BR signaling through RER proteins. Our PPI network results identified a set of proteins interacting with the miR398 target genes involved in ROS scavenging, JA production, and lipid peroxidation, indicating an important role of miR398 in regulating the antioxidative systems allowing the plant to combat B-triggered oxidative stress (Landi et al., 2012).

Overall, we identified new sets of genes that are targets of miR172, miR319 and miR398 in *T. aestivum* with interesting functions that have not been studied before under B toxicity. It is worth noting that some common miRNAs including miR172, miR319 and miR398 are responsive to different nutrient deficiencies or toxicities such as Fe, P, B, S and Cu, suggesting crosstalk between the post-transcriptional regulatory mechanisms involving miRNAs (Lu et al., 2014; Zeng et al., 2014; Lu et al., 2015; Paul et al., 2015). Therefore, the functions of these common miRNAs should be studied under diverse nutrient stresses.

**4.2. Expression levels of miR172, miR319, miR398 and their targets might indicate different levels of B tolerance in wheat cultivars**

In this study, we determined transcriptional and post-transcriptional regulations of CSD in wheat cultivars having a difference in B tolerance. The most remarkable induction of expression was observed in CSD with ten-fold and approximately twelve-fold increases in Atay in 1B and 3B conditions, respectively. Similarly, a lesser but still remarkable increase of CSD expression was determined in Bolal exposed to 1B (four-fold) and 3B (five-fold). Expectedly, miR398 expression did not dramatically change under 1B and 3B conditions in both cultivars. Stable levels of miR398 can contribute to the induction of *CSD*. More induction in the expression level of the *CSD* gene might be related to the mechanism of toxic B sensitivity in Atay. Also, a dramatic increase in *CDS* expression and a stable level of *miR398* expression were compatible with our previous findings in Arabidopsis exposed to B toxicity (Kayıhan et al., 2016).

Furthermore, we investigated expressional regulations of two miRNAs, *miR319* and *miR172* and their targets *MYB3* and *TOE1*, in wheat cultivars because they were most significantly induced miRNAs in Arabidopsis under B toxicity conditions in our previous work (Kayıhan et al., 2019). Accordingly, a moderate level of toxic B (1B) causes a dramatic increase in the expression level of *miR172* in Arabidopsis, however, in this study, the same concentration led to a significant decrease in *miR172* expression in Atay,

whereas it stayed stable after 3B in Atay and Bolal (Figure 3) similar to Arabidopsis. However, TOE1 expression was increased under both conditions in Atay. Both miR172 and TOE1 expressions were not altered significantly in Bolal. miR172 seems likely to be regulated species-specific and even, cultivar specific, under B stress. miR172 promotes flowering primarily by post-transcriptionally repressing a set of APETALA2 (AP2)-like genes, including TARGET OF EAT1 (TOE1), TOE2, and TOE3 (Aukerman and Sakai, 2003). Overexpression of TOE1 causes late flowering, whereas miR172-overexpressing plants exhibit early flowering under both LDs and SDs. Variation of the expression profile of miR172 and its target might be related to those specific regulations of miRNA-mediated responses participate in varying tolerance capacity of species and also cultivars of the same plant against abiotic stresses (Zhang, 2015). miR172 was induced under B (Ozhuner et al., 2013), mercury (Zhou et al., 2012) and cadmium toxicities (Mendoza-Soto et al., 2012) in different plants, suggesting that a common response mechanism is evolved against metal toxicities to regulate the flowering time in plants.

In this study, miR319 expression did not change after 1B but increased significantly after 3B in Atay, whereas its target MYB3 (Kumar et al., 2015) was remarkably induced in both conditions. On the other hand, increasing levels of B led to higher induction of miR319 and lower induction of MYB3 in Bolal. Contrarily, in our previous report, miR319 expression was induced under 1B more than under 3B in *Arabidopsis thaliana* (Kayıhan et al., 2019). Tae-MYB3 has a high similarity with AtMYB59 in *Arabidopsis thaliana* (Rahaie et al., 2013), whose expression is induced by JA and ethylene (Li et al., 2006). Furthermore, higher miR319 expression accompanying low MYB3 in the early stage of wheat was attributed to a higher developmental rate (De Paola et al., 2016). Also, transgenic creeping bentgrass plants overexpressing miR319 exhibited an impairment of the function of the miR319 target, TCP, and thus, this caused a lesser accumulation of JA and delay in leaf senescence. It has been suggested that miR319 regulates positively leaf senescence via the jasmonic acid (JA) biosynthesis pathway (Schommer et al., 2008). Also, the expression level of AtMYB59 increases in response to JA and ethylene which inhibit plant cell growth. Similarly, in our case, the remarkable induction of MYB3 may imply inhibition of development and induction of leaf senescence mechanism due to B toxicity. Conversely, the increase in the expression level of ttu-miR319 and the decrease in the expression level of its target gene MYB3 might be a consequence of the higher cell growth, cell division, and DNA synthesis (De Paola et al., 2016).

In conclusion, differential regulation of miRNA-mediated responses against B toxicity might be associated with varying tolerance capacity in plants. Also, post-transcriptional regulation of JA and ethylene metabolisms might coordinately be regulated with antioxidant machinery in plants exposed to toxic B (Kayıhan et al., 2019). Similarly, in this study, induced expression levels of *CSD* were found in two wheat cultivars under B toxicity. Both toxic B conditions caused higher inductions in *CSD* levels in sensitive cultivar than in the tolerant cultivar, suggesting more ROS accumulation and oxidative damage in the sensitive cultivar. Supportively, 1B condition caused yellowing in leaves of sensitive cultivar whereas it did not cause any visible symptom in the tolerant cultivar. Coordinately, B accumulated higher in leaves of Atay than Bolal following toxic B treatment (Kayıhan et al., 2017). On the other hand, between two cultivars, B toxicity conditions caused the induction of TOE1 in only sensitive cultivar Atay. Thus, TOE1 regulation via miR172 may contribute to B tolerance in wheat.

**Author contributions**

CK planned the project. DSK and CK designed the experiment and searched the database and collected the data and performed the experiments for expressions of miRNAs and their targets and wrote the manuscript. EA performed computational analysis for miRNAs, targets and their networks and wrote the manuscript. CK and EA finalized the manuscript.

## References

Agarwala SC, Sharma PN, Chatterjee C, Sharma CP (1981). Development and enzymatic changes during pollen development in boron deficient maize plants. Journal of plant Nutrition 3 (1-4): 329-336.

Aksoy E, Jeong IS, Koiwa H (2013). Loss of function of Arabidopsis C-terminal domain phosphatase-like1 activates iron deficiency responses at the transcriptional level. Plant physiology 161 (1): 330-345.

An R, Liu X, Wang R, Wu H, Liang S et al. (2014). The over-expression of two transcription factors, ABS5/bHLH30 and ABS7/MYB101, leads to upwardly curly leaves. PloS One 30; 9 (9): e107637.

Aprile A, Havlickova L, Panna R, Marè C., Borrelli GM et al. (2013). Different stress responsive strategies to drought and heat in two durum wheat cultivars with contrasting water use efficiency. BMC Genomics 22; 14 (1): 821.

Aquea F, Federici F, Moscoso C, Vega A, Jullian P et al. (2012). A molecular framework for the inhibition of Arabidopsis root growth in response to boron toxicity. Plant, Cell & Environment 35: 719-734.

Armengaud P, Breitling R, Amtmann A (2004). The potassium-dependent transcriptome of Arabidopsis reveals a prominent role of jasmonic acid in nutrient signaling. Plant Physiology 136 (1): 2556-2576.

Aukerman MJ, Sakai H (2003). Regulation of flowering time and floral organ identity by a MicroRNA and its APETALA2-like target genes. The Plant Cell 15: 2730-2741.

Bachmair A, Novatchkova M, Potuschak T, Eisenhaber F (2001). Ubiquitylation in plants: a post-genomic look at a post-translational modification. Trends in Plant Science 6: 463-470.

Backes C, Keller A, Kuentzer J, Kneissl B, Comtesse N et al. (2007). GeneTrail—advanced gene set enrichment analysis. Nucleic Acids Research 35 (suppl_2): W186-W192.

Baysal T, Demirdöven A (2007). Lipoxygenase in fruits and vegetables: A review. Enzyme and Microbial Technology 40: 491-496.

Benjamini Y, Hochberg Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal statistical society: series B (Methodological) 57 (1): 289-300.

Bolaños L, Lukaszewski K, Bonilla I, Blevins D (2004). Why boron?. Plant Physiology and Biochemistry 42 (11): 907-912.

Breuil-Broyer S, Trehin C, Morel P, Boltz V, Sun B et al. (2016). Analysis of the Arabidopsis superman allelic series and the interactions with other genes demonstrate developmental robustness and joint specification of male-female boundary, flower meristem termination and carpel compartmentalization. Annals of Botany 117: 905-923.

Brown PH, Bellaloui N, Wimmer MA, Bassil ES, Ruiz J et al. (2002). Boron in plant biology. Plant Biology 4 (2): 205-223.

Camacho-Cristóbal JJ, Herrera-Rodríguez MB, Beato VM, Rexach J, Navarro-Gochicoa MT et al. (2008). The expression of several cell wall-related genes in Arabidopsis roots is down-regulated under boron deficiency. Environmental and Experimental Botany 63 (1-3): 351-358.

Castaño-Miquel L, Seguí J, Manrique S, Teixeira I, Carretero-Paulet L et al. (2013). Diversification of SUMO-activating enzyme in Arabidopsis: implications in SUMO conjugation. Molecular Plant 6: 1646-1660.

Cervilla LM, Blasco B, Ríos JJ, Rosales MA, Rubio-Wilhelmi MM et al. (2009). Response of nitrogen metabolism to boron toxicity in tomato plants. Plant Biology 11: 671-677.

Chang YN, Zhu C, Jiang J, Zhang H, Zhu JK et al. (2020). Epigenetic regulation in plant abiotic stress responses. Journal of Integrative Plant Biology 62: 563-580.

Chen C, Chen H, Zhang Y, Thomas HR, Frank MH et al. (2020). TBtools-an integrative toolkit developed for interactive analyses of big biological data. BioRxiv, 289660.

Chen M, Mishra S, Heckathorn SA, Frantz JM, Krause C (2014). Proteomic analysis of Arabidopsis thaliana leaves in response to acute boron deficiency and toxicity reveals effects on photosynthesis, carbohydrate metabolism, and protein synthesis. Journal of Plant Physiology 171 (3-4): 235-242.

Choi EY, Kolesik P, McNeill A, Collins H., Zhang Q et al. (2007). The mechanism of boron tolerance for maintenance of root growth in barley (*Hordeum vulgare* L.). Plant, Cell & Environment 30: 984-993.

Chomczynski P, Sacchi N (1987). Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem 162: 156-159.

Colmsee C, Beier S, Himmelbach A, Schmutzer T, Stein N et al. (2015). BARLEX-the barley draft genome explorer. Molecular Plant 8: 964-966.

Costa M, Pereira AM, Pinto SC, Silva J, Pereira LG et al. (2019). In silico and expression analyses of fasciclin-like arabinogalactan proteins reveal functional conservation during embryo and seed development. Plant Reproduction 32: 353-370.

Dai X, Zhuang Z, Zhao PX (2018). psRNATarget: a plant small RNA target analysis server (2017 release). Nucleic Acids Research 46: W49-W54.

De Paola D, Zuluaga DL, Sonnante G (2016). The miRNAome of durum wheat: isolation and characterisation of conserved and novel microRNAs and their target genes. BMC Genomics 17: 505.

Dell B, Huang L (1997). Physiological response of plants to low boron. Plant and Soil 193 (1-2): 103-120.

Djakovic S, Dyachok J, Burke M, Frank MJ, Smith LG (2006). BRICK1/HSPC300 functions with SCAR and the ARP2/3 complex to regulate epidermal cell shape in Arabidopsis. Development 133: 1091-1100.

Dreyer BH, Schippers JH (2018). Copper-Zinc superoxide dismutases in plants: evolution, enzymatic properties, and beyond. Annual Plant Reviews Online 933-968.

Edgar R, Domrachev M, Lash AE (2002). Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic acids research 30 (1): 207-210.

Eggert K, von Wirén N (2017). Response of the plant hormone network to boron deficiency. New Phytologist 216 (3): 868-881.

Fang Y, Zheng Y, Lu W, Li J, Duan Y et al. (2021). Roles of miR319-regulated TCPs in plant development and response to abiotic stress. The Crop Journal 9: 17-28.

Fojtová M. Fajkus J (2020). Chromatin, Epigenetics and Plant Physiology. International Journal of Molecular Sciences 21 (8): 2763.

Goldbach HE, Yu Q, Wingender R, Schulz M, Wimmer M et al. (2001). Rapid response reactions of roots to boron deprivation. Journal of Plant Nutrition and Soil Science 164 (2): 173-181.

González-Fontes A, Fujiwara T (2020). Advances in Plant Boron. International Journal of Molecular Sciences 21(11): 4107.

González-Fontes A, Herrera-Rodríguez MB, Martín-Rejano EM, Navarro-Gochicoa MT, Rexach, J et al. (2016). Root responses to boron deficiency mediated by ethylene. Frontiers in plant science 6: 103.

Gunes A, Inal A, Bagci EG, Coban S, Sahin O (2007). Silicon increases boron tolerance and reduces oxidative damage of wheat grown in soil with excess boron. Biologia Plantarum 51 (3): 571-574.

Gunes A, Soylemezoglu G, Inal A, Bagci EG, Coban S et al. (2006). Antioxidant and stomatal responses of grapevine (*Vitis vinifera* L.) to boron toxicity. Scientia Horticulturae 110 (3): 279-284.

Guo P, Qi YP, Yang LT, Ye X, Huang JH et al. (2016). Long-term boron-excess-induced alterations of gene profiles in roots of two citrus species differing in boron-tolerance revealed by cDNA-AFLP. Frontiers in Plant Science 7: 898.

Guo P, Qi YP, Yang LT, Ye X, Jiang HX et al. (2014). cDNA-AFLP analysis reveals the adaptive responses of citrus to long-term boron-toxicity. BMC Plant Biology 14 (1): 284.

Han G, Wei X, Dong X, Wang C, Sui N et al. (2020). Arabidopsis ZINC FINGER PROTEIN1 Acts Downstream of GL2 to repress root hair initiation and elongation by directly suppressing bHLH genes. The Plant Cell 32: 206-225.

Huang J, Cai Z, Wen S, Guo P, Ye X et al. (2014). Effects of boron toxicity on root and leaf anatomy in two Citrus species differing in boron tolerance. Trees 28: 1653-1666.

Inal A, Pilbeam DJ, Gunes A (2009). Silicon increases tolerance to boron toxicity and reduces oxidative damage in barley. Journal of Plant Nutrition 32 (1): 112-128.

Jacq A, Pernot C, Martinez Y, Domergue F, Payré B et al. (2017). The Arabidopsis Lipid Transfer Protein 2 (AtLTP2) Is Involved in Cuticle-Cell Wall Interface Integrity and in Etiolated Hypocotyl Permeability. Frontiers in plant science 8: 263.

Jaubert-Possamai S, Noureddine Y, Favery B (2019). MicroRNAs, New Players in the Plant-Nematode Interaction. Frontiers in plant science 10: 1180.

Jiang CK, Rao GY (2020). Insights into the Diversification and Evolution of R2R3-MYB Transcription Factors in Plants. Plant Physiology 183: 637-655.

Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S et al. (2008). NCBI BLAST: a better web interface. Nucleic acids research 36: W5-W9.

Jyothi MN, Usha S, Suchithra B, Ulfath TS, Devaraj VR et al. (2018). Boron toxicity induces altered expression of miRNAs in French bean (*Phaseolus vulgaris* L.). Journal of Applied Biology & Biotechnology 6 (06): 001-010.

Kalayci M, Alkan A, Çakmak I, Bayramoğlu O, Yilmaz A et al. (1998). Studies on differential response of wheat cultivars to boron toxicity. Euphytica 100: 123-129.

Kasai K, Takano J, Miwa K, Toyoda A, Fujiwara T (2011). High boron-induced ubiquitination regulates vacuolar sorting of the BOR1 borate transporter in Arabidopsis thaliana. Journal of Biological Chemistry 286 (8): 6175-6183.

Kayıhan DS, Kayıhan C, Çiftçi YÖ (2016). Excess boron responsive regulations of antioxidative mechanism at physio-biochemical and molecular levels in Arabidopsis thaliana. Plant Physiology and Biochemistry 109: 337-345.

Kayıhan C, Öz MT, Eyidoğan F, Yücel M, Öktem HA (2017). Physiological, biochemical, and transcriptomic responses to boron toxicity in leaf and root tissues of contrasting wheat cultivars. Plant Molecular Biology Reporter 35: 97-109.

Kayıhan DS, Kayıhan C, Çiftçi YO (2019). Moderate level of toxic boron causes diferential regulation of microRNAs related to jasmonate and ethylene metabolisms in *Arabidopsis thaliana.* Turk Journal of Botany 43: 167-172.

Kilian J, Whitehead D, Horak J, Wanke D, Weinl S et al. (2007). The AtGenExpress global stress expression data set: protocols, evaluation and model data analysis of UV-B light, drought and cold stress responses. The Plant Journal: For Cell And Molecular Biology 50: 347-363.

Kozomara A, Birgaoanu M, Griffiths-Jones S (2019). miRBase: from microRNA sequences to function. Nucleic Acids Research 47: D155-D162.

Kumar RR, Pathak H, Sharma SK, Kala YK, Nirjal MK et al. (2015). Novel and conserved heat-responsive microRNAs in wheat (*Triticum aestivum* L.). Functional & Integrative Genomics 15: 323-348.

Lamesch P, Berardini TZ, Li D, Swarbreck D, Wilks C et al. (2012). The Arabidopsis Information Resource (TAIR): improved gene annotation and new tools. Nucleic acids research 40: D1202-D1210.

Landi M, Degl'Innocenti E, Pardossi A, Guidi L (2012). Antioxidant and photosynthetic responses in plants under boron toxicity: a review. American Journal of Agricultural and Biological Sciences 7: 255-270.

Landi M, Guidi L, Pardossi A, Tattini M, Gould KS (2014). Photoprotection by foliar anthocyanins mitigates effects of boron toxicity in sweet basil (*Ocimum basilicum*). Planta 240: 941-953.

Landi M, Margaritopoulou T, Papadakis IE, Araniti F (2019). Boron toxicity in higher plants: an update 250: 1011-1032.

Landi M, Remorini D, Pardossi A, Guidi L (2013). Boron excess affects photosynthesis and antioxidant apparatus of greenhouse *Cucurbita pepo* and *Cucumis sativus*. Journal of plant research 126 (6): 775-786.

Lange H, Sement FM, Gagliardi D, (2011). MTR4, a putative RNA helicase and exosome co-factor, is required for proper rRNA biogenesis and development in Arabidopsis thaliana. The Plant Journal 68: 51-63.

Lange H, Zuber H, Sement FM, Chicher J, Kuhn L et al. (2014). The RNA helicases AtMTR4 and HEN2 target specific subsets of nuclear transcripts for degradation by the nuclear exosome in Arabidopsis thaliana. PLoS genetics 10: e1004564.

Lewis DH (2019). Boron: the essential element for vascular plants that never was. The New phytologist 221: 1685-1690.

Li J, Li X, Guo L, Lu F, Feng X et al. (2006). A subgroup of MYB transcription factor genes undergoes highly conserved alternative splicing in Arabidopsis and rice. Journal of Experimental Botany 57: 1263-1273.

Liang Y, Tan ZM, Zhu L, Niu QK, Zhou JJ et al. (2013). MYB97, MYB101 and MYB120 function as male factors that control pollen tube-synergid interaction in Arabidopsis thaliana fertilization. PLoS genetics 9: e1003933.

Liu P, Yang YS, Xu GD, Fang YH, Yang YA (2005). The response of antioxidant enzymes of three soybean varieties to molybdenum and boron in soil with a connection to plant quality. Plant Soil and Environment 51: 351-359.

Lu YB, Yang LT, Qi YP, Li Y, Li Z et al. (2014). Identification of boron-deficiency-responsive microRNAs in *Citrus sinensis* roots by Illumina sequencing. BMC Plant Biology 14 (1): 1-16.

Lu YB, Qi YP, Lee J, Guo P, Ye X et al. (2015). Long-term boron-deficiency-responsive genes revealed by cDNA-AFLP differ between Citrus sinensis roots and leaves. Frontiers in Plant Science 6: 585.

Lu YB, Qi YP, Yang LT, Guo P, Li Y et al. (2015). Boron-deficiency-responsive microRNAs and their targets in Citrus sinensis leaves. BMC Plant Biology 15 (1): 271.

Mao C, Yi K, Yang L, Zheng B, Wu Y et al. (2004). Identification of aluminium-regulated genes by cDNA-AFLP in rice (Oryza sativa L.): aluminium-regulated genes for the metabolism of cell wall components. Journal of Experimental Botany 55 (394): 137-143.

Martín-Rejano EM, Camacho-Cristóbal JJ, Herrera-Rodríguez MB, Rexach J, Navarro-Gochicoa MT et al. (2011). Auxin and ethylene are involved in the responses of root system architecture to low boron supply in Arabidopsis seedlings. Physiologia Plantarum 1422: 170-178.

Mendoza-Soto AB, Sánchez F, Hernández G (2012). MicroRNAs as regulators in plant metal toxicity response. Frontiers in Plant Science 3: 105.

Mi H, Muruganujan A, Ebert D, Huang X, Thomas PD (2019). PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. Nucleic Acids Research 47: D419-D426.

Miwa K, Wakuta S, Takada S, Ide K, Takano J et al. (2013). Roles of BOR2, a boron exporter, in cross linking of rhamnogalacturonan II and root elongation under boron limitation in Arabidopsis. Plant Physiology 163 (4): 1699-1709.

Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012). AP2/ERF family transcription factors in plant abiotic stress responses. Biochimica et Biophysica Acta 1819: 86-96.

Morgan C, Zhang H, Henry CE, Franklin FCH, Bomblies K (2020). Derived alleles of two axis proteins affect meiotic traits in autotetraploid Arabidopsis arenosa. Proceedings of the National Academy of Sciences 117: 8980-8988.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum 15: 473-497.

Nibau C, Di Stilio VS, Wu HM, Cheung AY (2011). Arabidopsis and Tobacco SUPERMAN regulate hormone signalling and mediate cell proliferation and differentiation. Journal of experimental botany 62: 949-961.

Ozhuner E, Eldem V, Ipek A, Okay S, Sakcali S et al. (2013). Boron stress responsive microRNAs and their targets in barley. PloS one 8 (3): e59543.

Öz MT, Yilmaz R, Eyidoğan F, De Graaff L, Yücel M et al. (2009). Microarray analysis of late response to boron toxicity in barley (*Hordeum vulgare* L.) leaves. Turkish Journal of Agriculture and Forestry 33: 191-202.

Öz MT (2012). Microarray based expression profiling of barley under boron stress and cloning of 3H boron tolerance gene. Middle East Technical University, The Graduate School of Natural and Applied Sciences, Ankara, Turkey (PhD Thesis).

Öztürk SE, Göktay M, Has C, Babaoğlu M, Allmer J et al. (2018). Transcriptomic analysis of boron hyperaccumulation mechanisms in *Puccinellia distans*. Chemosphere 199: 390-401.

Pacak A, Barciszewska-Pacak M, Swida-Barteczka A, Kruszka K, Sega P et al. (2016). Heat stress affects pi-related genes expression and inorganic phosphate deposition/accumulation in barley. Frontiers in plant science 7: 926.

Pandey N, Gupta B (2013). The impact of foliar boron sprays on reproductive biology and seed quality of black gram. Journal of Trace Elements in Medicine and Biology 27 (1): 58-64.

Papadakis I, Dimassi K, Bosabalidis A, Therios I, Patakas A et al. (2004). Effects of B excess on some physiological and anatomical parameters of 'Navelina'orange plants grafted on two rootstocks. Environmental and Experimental Botany 5: 247-257.

Papadakis IE, Dimassi KN, Bosabalidis AM, Therios IN, Patakas A et al. (2004). Boron toxicity in 'Clementine'mandarin plants grafted on two rootstocks. Plant Science 166 (2): 539-547.

Papadakis IE, Tsiantas PI, Tsaniklidis G, Landi M, Psychoyou M et al. (2018). Changes in sugar metabolism associated to stem bark thickening partially assist young tissues of Eriobotrya japonica seedlings under boron stress. Journal of Plant Physiology 231: 337-345.

Paul S, Datta SK, Datta K (2015). miRNA regulation of nutrient homeostasis in plants. Frontiers in Plant Science 6: 232.

Peng L, Zeng C, Shi L, Cai H, Xu F (2012). Transcriptional Profiling Reveals Adaptive Responses to Boron Deficiency Stress in Arabidopsis. Zeitschrift für Naturforschung C 67 (9-10): 510-524.

Pérez-Pérez JM, Esteve-Bruna D, González-Bayón R, Kangasjärvi S, Caldana C et al. (2013). Functional redundancy and divergence within the Arabidopsis RETICULATA-RELATED gene family. Plant Physiology 162: 589-603.

Pré M, Atallah M, Champion A, De Vos M, Pieterse CM et al. (2008). The AP2/ERF domain transcription factor ORA59 integrates jasmonic acid and ethylene signals in plant defense. Plant Physiology 147 (3): 1347-1357.

Quiles-Pando C, Rexach J, Navarro-Gochicoa MT, Camacho-Cristóbal JJ, Herrera-Rodríguez MB et al. (2013). Boron deficiency increases the levels of cytosolic $Ca^{2+}$ and expression of $Ca^{2+}$-related genes in Arabidopsis thaliana roots. Plant Physiology and Biochemistry 65: 55-60.

Rahaie M, Xue GP, Schenk PM (2013). The role of transcription factors in wheat under different abiotic stresses. Abiotic stress-plant responses and applications in agriculture 367-385.

Reid RJ, Hayes JE, Post A, Stangoulis JCR, Graham RD (2004). A critical analysis of the causes of boron toxicity in plants. Plant, Cell & Environment 27: 1405-1414.

Rerkasem B, Jamjod S, Pusadee T (2020). Productivity limiting impacts of boron deficiency, a review. Plant and Soil: 1-18.

Rerkasem B, Lordkaew S, Dell B (1997). Boron requirement for reproductive development in wheat. In Plant Nutrition for Sustainable Food Production and Environment (pp. 69-73). Springer, Dordrecht.

Roessner U, Patterson JH, Forbes MG, Fincher GB, Langridge P et al. (2006). An investigation of boron toxicity in barley using metabolomics. Plant Physiology 142: 1087-1101.

Sakamot T, Tsujimoto-Inui Y, Sotta N, Hirakawa T, Matsunaga TM et al. (2018). Proteasomal degradation of BRAHMA promotes Boron tolerance in Arabidopsis. Nature Communications 9 (1): 1-16.

Sang W, Huang ZR, Qi YP, Yang LT, Guo P et al. (2015). An investigation of boron-toxicity in leaves of two citrus species differing in boron-tolerance using comparative proteomics. Journal of Proteomics 123: 128-146.

Sarafi E, Siomos A, Tsouvaltzis P, Therios I, Chatzissavvidis C (2018). Boron toxicity effects on the concentration of pigments, carbohydrates and nutrient elements in six non-grafted pepper cultivars (*Capsicum annuum* L.). Indian Journal of Plant Physiology 23: 474-485.

Sardar HS, Yang J, Showalter AM (2006). Molecular interactions of arabinogalactan proteins with cortical microtubules and F-actin in Bright Yellow-2 tobacco cultured cells. Plant Physiology 142 (4): 1469-1479.

Schommer C, Palatnik JF, Aggarwal P, Chételat A, Cubas P et al. (2008). Control of jasmonate biosynthesis and senescence by miR319 targets. PLoS biology 6: e230.

Schuler M, Keller A, Backes C, Philippar K, Lenhof HP et al. (2011). Transcriptome analysis by GeneTrail revealed regulation of functional categories in response to alterations of iron homeostasis in *Arabidopsis thaliana*. BMC Plant Biology 11 (1): 87.

Schultz CJ, Rumsewicz MP, Johnson KL, Jones BJ, Gaspar YM et al. (2002). Using genomic resources to guide research directions. The arabinogalactan protein gene family as a test case. Plant Physiology 129 (4): 1448-1463.

Shan H, Cheng J, Zhang R, Yao X, Kong H (2019). Developmental mechanisms involved in the diversification of flowers. Nature plants 5 (9): 917-923.

Showalter AM (2001). Arabinogalactan-proteins: structure, expression and function. Cellular and Molecular Life Sciences CMLS, 58(10): 1399-1417.

Sotiras M, Papadakis I, Landi M, Tsaniklidis G, Tsiantas P et al. (2019). Allocation pattern, photosynthetic performance and sugar metabolism in hydroponically grown seedlings of loquat (Eriobotrya japonica Lindl.) subjected to salinity. Photosynthetica 57: 258-267.

Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S et al. (2019). STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Research 47: D607-D613.

Takano J, Miwa K, Yuan L, von Wirén N, Fujiwara T (2005). Endocytosis and degradation of BOR1, a boron transporter of *Arabidopsis thaliana*, regulated by boron availability. Proceedings of the National Academy of Sciences 102 (34): 12276-12281.

Takano J, Tanaka M, Toyoda A, Miwa K, Kasai K et al. (2010). Polar localization and degradation of Arabidopsis boron transporters through distinct trafficking pathways. Proceedings of the National Academy of Sciences 107 (11): 5220-5225.

Tepe M, Aydemir T (2011). Antioxidant responses of lentil and barley plants to boron toxicity under different nitrogen sources. African Journal of Biotechnology 10 (53): 10882-10891.

Tombuloglu H, Tombuloglu G, Sakcali MS, Turkan A, Hakeem KR et al. (2017). Proteomic analysis of naturally occurring boron tolerant plant *Gypsophila sphaerocephala* L. in response to high boron concentration. Journal of Plant Physiology 216: 212-217.

Toshima JY, Furuya E, Nagano M, Kanno C, Sakamoto Y et al. (2016). Yeast Eps15-like endocytic protein Pan1p regulates the interaction between endocytic vesicles, endosomes and the actin cytoskeleton. ELife 5: e10276.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC et al. (2012). Primer3-new capabilities and interfaces. Nucleic acids research 40: e115-e115.

Unver T, Bozkurt O, Akkaya MS (2008). Identification of differentially expressed transcripts from leaves of the boron tolerant plant *Gypsophila perfoliata* L. Plant Cell Reports 27 (8): 1411.

Varkonyi-Gasic E, Wu R, Wood M, Walton EF, Hellens RP (2007). Protocol: a highly sensitive RT-PCR method for detection and quantification of microRNAs. Plant methods 3: 1-12.

Venkatesh S, Workman JL (2015). Histone exchange, chromatin structure and the regulation of transcription. Nature reviews Molecular Cell Biology 16 (3): 178-189.

Vick BA Zimmerman DC (1983). The biosynthesis of jasmonic acid: a physiological role for plant lipoxygenase. Biochemical and Biophysical Research Communications 111 (2): 470-477.

Viotti C, Bubeck J, Stierhof YD, Krebs M, Langhans M et al. (2010). Endocytic and secretory traffic in Arabidopsis merge in the trans-Golgi network/early endosome, an independent and highly dynamic organelle. The Plant Cell 22 (4): 1344-1357.

Wang P, Richardson C, Hawes C, Hussey PJ (2016). Arabidopsis NAP1 regulates the formation of autophagosomes. Current Biology 26: 2060-2069.

Wimmer MA, Eichert T (2013). Mechanisms for boron deficiency-mediated changes in plant water relations. Plant Science 203: 25-32.

Wu G, Park MY, Conway SR, Wang JW, Weigel D et al. (2009). The sequential action of miR156 and miR172 regulates developmental timing in Arabidopsis. Cell 138: 750-759.

Xiang Y, Wang H, Yan H, He T, Wang Y et al. (2019). Identification, evolution and alternative splicing profile analysis of Serine/Arginine-Rich Protein Splicing Factors (SR Proteins) in poplar, Arabidopsis, grape, and papaya. Research Square, preprint.

Xu Z, Zhong S, Li X, Li W, Rothstein SJ et al. (2011). Genome-wide identification of microRNAs in response to low nitrate availability in maize leaves and roots. PloS one 6 (11): e28009.

Xue T, Liu Z, Dai X, Xiang F (2017). Primary root growth in Arabidopsis thaliana is inhibited by the miR159 mediated repression of MYB33, MYB65 and MYB101. Plant Science 262: 182-189.

Yates G, Sadanandom A (2013). Ubiquitination in plant nutrient utilization. Frontiers in Plant Science 4: 452.

Yıldırım K, Uylaş S (2016). Genome-wide transcriptome profiling of black poplar (*Populus nigra* L.) under boron toxicity revealed candidate genes responsible in boron uptake, transport and detoxification. Plant Physiology and Biochemistry 109: 146-155.

Yoshizawa E, Kaizuka M, Yamagami A, Higuchi-Takeuchi M, Matsui M et al. (2014). BPG3 is a novel chloroplast protein that involves the greening of leaves and related to brassinosteroid signaling. Bioscience, Biotechnology, and Biochemistry 78: 420-429.

Zeng H, Wang G, Hu X, Wang H, Du L et al. (2014). Role of microRNAs in plant responses to nutrient stress. Plant and Soil 374 (1-2): 1005-1021.

Zhang B (2015). MicroRNA: a new target for improving plant tolerance to abiotic stress. Journal of Experimental Botany 66: 1749-1761.

Zhang B, Wang L, Zeng L, Zhang C, Ma H (2015). Arabidopsis TOE proteins convey a photoperiodic signal to antagonize CONSTANS and regulate flowering time. Genes & development 29: 975-987.

Zhang C, He M, Wang S, Chu L, Wang C et al. (2021). Boron deficiency-induced root growth inhibition is mediated by brassinosteroid signalling regulation in *Arabidopsis*. The Plant Journal, doi:10.1111/tpj.15311.

Zhao L, Kim Y, Dinh TT, Chen X (2007). miR172 regulates stem cell fate and defines the inner boundary of APETALA3 and PISTILLATA expression domain in Arabidopsis floral meristems. The Plant Journal: For Cell And Molecular Biology 51: 840-849.

Zhou GF, Liu YZ, Sheng O, Wei QJ, Yang CQ et al. (2015). Transcription profiles of boron-deficiency-responsive genes in citrus rootstock root by suppression subtractive hybridization and cDNA microarray. Frontiers in Plant Science 5: 795.

Zhou T, Hua Y, Huang Y, Ding G, Shi L et al. (2016). Physiological and transcriptional analyses reveal differential phytohormone responses to boron deficiency in *Brassica napus* genotypes. Frontiers in Plant Science 7: 221.

Zhou ZS, Zeng HQ, Liu ZP, Yang ZM (2012). Genome-wide identification of Medicago truncatula microRNAs and their targets reveals their differential regulation by heavy metal. Plant, Cell & Environment 35 (1): 86-99.

Zhu Y, Dong A, Shen WH (2012). Histone variants and chromatin assembly in plant abiotic stress responses. Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms 1819 (3-4): 343-348.