Genome-Wide Analysis of Serine Hydroxymethyltransferase Genes in Triticeae Species Reveals That TaSHMT3A-1 Regulates Fusarium Head Blight Resistance in Wheat

Ping Hu1, Puwen Song1†, Jun Xu2, Qichao Wei1, Ye Tao1,3, Yueming Ren1, Yonggang Yu1, Dongxiao Li4, Haiyan Hu1* and Chengwei Li1,4*

1 Henan Engineering Research Center of Crop Genome Editing, Henan International Joint Laboratory of Plant Genetic Improvement and Soil Remediation, College of Life Science and Technology, Henan Institute of Science and Technology, Xinxiang, China, 2 School of Horticulture and Landscape Architecture, Henan Institute of Science and Technology, Xinxiang, China, 3 Department of Plant Protection, Sumy National Agrarian University, Sumy, Ukraine, 4 College of Biological Engineering, Henan University of Technology, Zhengzhou, China

Serine hydroxymethyltransferase (SHMT) plays a pivotal role in cellular one-carbon, photorespiration pathways and it influences the resistance to biotic and abiotic stresses. However, the function of SHMT proteins in wheat remains largely unexplored. In the present study, SHMT genes in five Triticeae species, Oryza sativa, and four dicotyledon species were identified based on whole genome information. The origin history of the target gene was traced by micro-collinearity analysis. Gene expression patterns of TaSHMTs in different tissues, various biotic stresses, exogenous hormones, and two biotic stresses were determined by Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR). The function of the selected TaSHMT3A-1 was studied by barley stripe mosaic virus-induced gene silencing in common wheat Bainong207. A total of 64 SHMT members were identified and further classified into two main classes based on the structure of SHMT proteins. The gene structure and motif composition analyses revealed that SHMTs kept relatively conserved within the same subclasses. Interestingly, there was a gene, TdSHMT7B-1, on chromosome 7B of Triticum dicoccoides, but there was no SHMT gene on chromosome 7 of other analyzed Triticeae species; TdSHMT7B-1 had fewer exons and conserved motifs than the genes in the same subclass, suggesting that the gene of TdSHMT7B-1 has a notable evolutionary progress. The micro-collinearity relationship showed that no homologs of TaSHMT3A-1 and its two neighboring genes were found in the collinearity region of Triticum urartu, and there were 27 genes inserted into the collinearity region of T. urartu. Furthermore, qRT-PCR results showed that TaSHMT3A-1 was responsive to abiotic stresses (NaCl and cold), abscisic acid, methyl jasmonate, and hydrogen peroxide. Significantly, upon Fusarium graminearum infection, the expression of TaSHMT3A-1 was highly upregulated in resistant cultivar Sumai3. More importantly,
silencing of TaSHMT3A-1 compromises Fusarium head blight resistance in common wheat Baimong207. Our new findings suggest that the TaSHMT3A-1 gene in wheat plays an important role in resistance to Fusarium head blight. This provides a valuable reference for further study on the function of this gene family.

Keywords: genome-wide analysis, SHMT gene family, expression pattern, Fusarium head blight, virus-induced gene silencing, evolutionary progress

HIGHLIGHTS
- 64 SHMT genes were systematically analyzed in 10 species
- Wheat TaSHMT evolution was analyzed with micro-collinearity analysis
- Some TaSHMTs responded to abiotic, biotic, and hormone treatments
- TaSHMT3A-1 in wheat plays an important role in resistance to Fusarium head blight

INTRODUCTION
Wheat (Triticum aestivum) is one of the most important staple crops in the world, and is a heterologous hexaploid composed of three subgenomes of A, B, and D (IWGSC, 2018). Wheat is subjected to various biotic and abiotic stresses throughout its life cycle. Fusarium head blight (FHB) and powdery mildew seriously affect the yield and quality of wheat (Hu et al., 2018; Wang et al., 2020). Deoxynivalenol (DON) mycotoxins, produced by Fusarium graminearum in infected grains seriously affect the safety of human food and animal feed (Li et al., 2019; Wang et al., 2020). Changes in climate and crop planting systems have made FHB increasingly serious, even in regions where it has not been reported before (Del Ponte et al., 2009; Chakraborty and Newton, 2011; McMullen et al., 2012; Zhang et al., 2014). Mining disease-resistant genes and cultivating disease resistant varieties are the most economical and effective strategies to reduce the losses caused by disease (Bai and Shaner, 2004; Dean et al., 2012; Xing et al., 2018).

Serine hydroxymethyltransferase (SHMT), a pyridoxal phosphate-dependent enzyme, can catalyze the glycine/serine and tetrahydrofolate (THF)/5,10-methyleneTHF interconversion and it plays a vital role in one-carbon metabolism and photorespiration Gly-into-Ser conversion in higher plants (Schirch and Szebenyi, 2005; Lakhssassi et al., 2019). The mutant shm1-1 showed a lethal photorespiratory phenotype caused by photorespiration deficiency when grown at ambient CO2 (Voll et al., 2006), and showed a more susceptible phenotype than the wild type when infected with biotrophic and necrotrophic pathogens, as well as enhanced susceptibility to salt, drought, and high light stress (Moreno et al., 2005; Liu et al., 2019; Mishra et al., 2019). AtSHM2 is a functional mitochondrial SHM, and the expression of SHM2 is restricted to the vasculature of leaves (Engel et al., 2011). AtSHM2 and AtSHM1 operate synergistically in photorespiration, but AtSHM2 cannot substitute for AtSHM1 in photorespiratory metabolism. Overexpression of SHM2 cannot complement the shm1 allele, although the amino acid sequences of SHM1 and SHM2 are very similar (Voll et al., 2006; Engel et al., 2011). AtSHMT3 is targeted to plastids, and biochemical experiments show that SHMT activity in plastids of both Arabidopsis and Hordeum vulgare, and AtSHMT3 is also involved in one-carbon metabolism in plants (Zhang et al., 2010). In rice, OsSHMT1 is an ortholog of AtSHM1, and has been identified in photorespiratory mutant osshm1 (Wang et al., 2015). Studies indicate a conserved function of SHM1 in photorespiration in rice and Arabidopsis. Rice SHMT3 confers tolerance to salinity stress in heterologous Arabidopsis (Mishra et al., 2019). Overexpression of the halotolerant cyanobacteria Aphanothece halophytica gene ApSHMT in Escherichia coli induced an enhanced tolerance to salinity-stress (Waditee-Sirisattha et al., 2012). The expression level of wheat SHMT was significantly reduced when wheat plants were exposed to soil drought and PEG-induced stresses (Cui et al., 2019). In soybean, the GmSHMT gene showed a lack of functional redundancy in resistance to soybean cyst nematode (Liu et al., 2012; Kandoth et al., 2017; Lakhssassi et al., 2019). These studies suggest that SHMT genes are not only involved in photorespiration but also salt, drought, and disease resistance in different plant species.

In previous research, most investigations of the SHMT gene have focused on the photorespiration of dicotyledons, and there have been only a few studies on the systematic analysis of the SHMT gene family in monocotyledons, especially in biotic stress of Triticaceae species. Based on the whole genome information of wheat and its related species, this study defined the gene structure and evolutionary relationship of SHMT genes in Triticaceae species and three dicotyledonous horticultural crops. Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) was used to systematically analyze the expression pattern of SHMT genes in different wheat varieties under biotic and abiotic stresses, and hormone treatment, such as F. graminearum, powdery mildew, drought, NaCl, cold (4°C), abscisic acid (ABA).
and hydrogen peroxide (H₂O₂). To further verify the function of TaSHMT3A-1, which is in response to F. graminearum infection in Sumai3, the technology of barley stripe mosaic virus-induced gene silencing (BSMV- VIGS) was used to verify its function in wheat, and the results showed that silencing of TaSHMT3A-1 could increase the susceptibility of Bainong207 to FHB. This study will provide a foundation for further functional studies of the SHMT gene family.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The common wheat Chinese cultivar Sumai3 which carries Fhb1 and has consistently shown a major effect on resistance to FHB (Li et al., 2019; Su et al., 2019) was collected and maintained by Henan Institute of Science and Technology (HIST), Xinxiang, China. FHB susceptible common wheat Jimai22 was collected from the Shandong Academy of Agricultural Sciences, and preserved by HIST. Bainong207 was developed and maintained from the Shandong Academy of Agricultural Sciences, and has consistently shown a major effect on resistance to FHB inoculation method. The spore concentration was 10⁶ conidia mL⁻¹. Three spikelets of Sumai3 and Jimai22 were inoculated at the early flowering stage with 20 µL fresh spores of F. graminearum in the middle of the heads by the single-floret inoculation method. The spore concentration was 10⁶ conidia mL⁻¹. Three spikelets (from three inoculated spikes of different individuals) were collected at 0, 24, 48, and 72 hpi. All these three materials of Bainong207, Sumai3, and Jimai22 were grown in a greenhouse at 23°C/18°C, with a 14 h light/10 h dark cycle, and 70% relative humidity.

Expression Analysis of TaSHMTs by Quantitative Real-Time Reverse Transcription Polymerase Chain Reaction

The qRT-PCR procedure was performed as described by Hu et al. (2018). The first-strand cDNA was synthesized using the HiScript Q RT SuperMix for qRT-PCR Kit (Vazyme, Nanjing, China). The qRT-PCR was performed using the SYBR Green detection kit AceQ qPCR SYBR Green Master Mix (Vazyme, Nanjing, China) on LC 480II (Roche, German). The program used was as follows: 5 min at 95°C, followed by 40 cycles at 95°C for 10 s and 60°C for 20 s. The relative gene expression was calculated by comparative 2⁻ΔΔCT method. The primers used in this study are listed in Supplementary Table 1 and the wheat TaTubulin gene was used as an internal control.

Identification of SHMT Gene

The genome-wide data for T. aestivum (Chinese Spring) from IWGSC² was downloaded (IWGSC, 2018). Data for Triticum urartu (Tu 2.0) were downloaded from the MBKBase database⁴ (Ling et al., 2018). Triticum dicoccoides (WEWSeq_v.1.0), Aegilops tauschii (Aet_v4.0), Hordeum vulgare (IBSC_v2), Arabidopsis thaliana (TAIR10), Solanum lycopersicum (SL3.0), Cucumis sativus (ASM407v2), and Vitis vinifera (12X) were downloaded from the Ensemble Plants⁵ to construct a local database. The typical SHMT domain (PF00464) was downloaded from the Pfam database as the search model⁶ (El-Gebali et al., 2019). As described in Xu et al. (2021), a new hidden Markov model (HMM) was established to ensure the reliability of search results. The high-quality protein set was obtained using the raw SHMT HMM (E-value < 1 x 10⁻20 and manual validation of an intact SHMT domain), and then used to build a specific SHMT HMM by the hmmbuild from the HMMERv3 suite (Lozano et al., 2015). The specific HMM was used, and proteins with an E-value lower than 0.001 were retained. The longest transcript was included for the following analysis when a gene has multiple transcripts. Both The SMART (Simple Modular Architecture Research Tool)⁴ (Letunic et al., 2021) and Conserved Domains⁶ (Lu et al., 2020) were used to check the candidate SHMT protein sequences again. The proteins containing complete SHMT conserved domains were reserved for further analysis, and named sequentially to their species and location on the chromosomes; all gene names are listed in Supplementary Table 2.

Phylogenetic, Gene Structure, and Conserved Motif Analysis

Multiple sequence alignment of all these full-length SHMT proteins was performed with ClustalW using the default options.

¹http://smart.embl-heidelberg.de/
²http://www.wheatgenome.org/
³http://www.mbkbase.org/Tu/
⁴http://plants.ensembl.org/index.html
⁵http://pfam.xfam.org/
⁶https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi
in MEGA-X (Kumar et al., 2018). Phylogenetic trees were constructed using the Maximum likelihood method of MEGAX with 1000 bootstrap replicates (Kumar et al., 2018; Yu et al., 2020). The phylogenetic tree was visualized with EvolView² (He et al., 2016). The exon-intron structure was generated using TBtools based on the full-length genome sequence and the corresponding coding sequences (Chen C. et al., 2020). Conserved motifs analysis was performed using the MEME program³. The parameters were as follows: the maximum number of motifs was set to 20 and the optimum width was 6–50 residues (Xie et al., 2018; Xu et al., 2021). The gene structure with motif composition was visualized by the TBtools (Chen C. et al., 2020).

**Micro-Collinearity and Functional Diversification Analysis**

A micro-collinearity analysis is of great value for understanding gene evolutionary history and TGT (Triticeae-Gene Tribe⁴) was used to trace the origin history of the target gene, and gene pair was also analyzed with TGT (Chen Y. et al., 2020). DIVERGE v3.0 software was used to analyze the functional diversification among the subgroups based on the selected protein sequences (Gu et al., 2013).

**BSMV-VIGS**

BSMV-VIGS was performed as described by Wang et al. (2010) with some modifications. The fragment of TaSHMT3A-1 with the length of 249 bp was amplified with primer pair VIGS-TaSHMT3A-1 (Supplementary Table 1), and the target fragment was inserted into the γ-strain of BSMV to construct BSMV: TaSHMT3A-1 vector. An in vitro transcription kit (mMESSAGEmMACHINE T7, Invitrogen, Waltham, MA, United States) was used to produce the virus RNA. The common wheat Bainong207 was used for the gene silencing assay. When the second leaves were fully extended, the leaves infected with the virus BSMV: TaSHMT3A-1, with BSMV:TaPDS- and BSMV:γ- infected leaves as controls. The fourth leaves fully expanded with clear virus symptoms were used for disease resistance evaluation, and the inoculation method was performed as described in Xiang et al. (2011) with some modifications. The fourth leaves were detached from Bainong207, wounded on the adaxial surface, and then placed on the PCR board to form arch bridges. For *F. graminearum* inoculation, the inoculum comprised 1.5 μL of conidial suspension with a concentration of 1 × 10⁶ conidia mL⁻¹. The conidial suspension of *F. graminearum* was applied to the fresh wound, and then the PCR board with leaves was placed in 25 mL benzimidazole water, at the same time ensuring that the cut of the leaves contacted the water, and then sealed for moisturizing. The inoculated leaves were cultured in an incubator with 14 h light/8 h dark at 23°C. The lesion size was observed 3 and 5 days after inoculation. Target gene silencing efficiency was evaluated by qRT-PCR using the primer pair TaSHMT3A-1-Q (Supplementary Table 1).

**RESULTS**

**Identification and Classification of SHMT Gene Family Members in Triticeae and Four Dicotyledon Species**

In this study, a total of 64 genes with SHMT conserved domain (PF00464) were identified from 10 sequenced plant species, 37 from monocotyledon, including *T. aestivum* (12), *T. urartu* (3), *Ae. Tauschii* (4), *T. dicoccoides* (9), *H. vulgare* (4) and *O. sativa* (5), and 27 from dicotyledons, including *Arabidopsis* (7), *S. lycopersicum* (7), *C. sativus* (6), and *V. vinifera* (7). To investigate the evolutionary relationships of the SHMT proteins, all the above 64 proteins were used to construct a Maximum likelihood phylogenetic tree (Figure 1). According to the classification of *Arabidopsis* and soybean SHMT (Lakhssassi et al., 2019), SHMT is divided into two main classes (classes I and II) and four subclasses (classes I a–b, II a, and II b). Among them, Class II a subgroup only contains proteins from dicotyledons, and the other subgroups include proteins from monocotyledons and dicotyledons. Within the same subgroup, the SHMT members of monocotyledons and dicotyledons were clustered together respectively, indicating that the SHMT genes of monocotyledons and dicotyledons had experienced great differentiation in the process of evolution. SHMT members within the same subgroup of *Triticeae* species had high protein sequence similarity, and the evolutionary process in *Triticeae* species was relatively conservative.

The numbers of SHMT in each subgroup of all analyzed species are listed in Table 1. Compared with monocotyledons, dicotyledons have more SHMT genes, mainly reflected in class I b, class II a, and class II b subgroups (Table 1). In *Triticeae* species, *T. dicoccoides* and *T. aestivum* had 9 and 12 SHMTs, which was about two and three times that of diploid species, respectively; moreover, the number of SHMTs in each subgroup was almost 2–3 times that of the diploid species (Table 1). This indicated that the increased number of *TaSHMTs* in polyploid wheat was primarily due to genome polyploidization. Interestingly, there was no SHMT gene of *T. urartu* in class I b, and the SHMT genes of barley and *Ae. Tauschii* were distributed on chromosomes 1, 2, 3, and 4, while *T. urartu* lacked the SHMT gene on chromosome 3; moreover, the SHMT genes on chromosome 3 of *Ae. tauschii* and barley were classed in class I b (Figure 1 and Table 2). This may be due to either a poor reference genome sequence of *T. urartu* or gene loss events that occurred during the evolution of *T. urartu*. The proportion of SHMT gene and copy number in class I a of *T. dicoccoides* was higher than that of other *Triticeae* species and there was an SHMT gene, *TaSHMT7B-1*, on chromosome 7B, but there was no SHMT gene on chromosome 7 of other *Triticeae* species. From the perspective of evolutionary relationships, the number and evolution of SHMT genes have differentiated between monocotyledons and dicotyledons, and within *Triticeae* species. Therefore, gene structure and conserved

---

1. https://www.evolgenius.info/
2. http://memesuite.org/tools/meme
3. http://wheat.cau.edu.cn/TGT/
4. http://memesuite.org/tools/meme

motifs of SHMT in different species were further analyzed to help explore the evolutionary process.

**Gene Structure and Conserved Motif Composition Analysis**

To further understand SHMT functional divergence, the conserved motifs of these SHMT proteins were identified using MEME software. Twenty individual motifs were identified (Figure 2). Our results show that, among all the analyzed 60 SHMT proteins contained motif 4, motif 7 (except for TdSHMT7B-1), motif 10, and motif 18. All the analyzed SHMTs in Class I contained motif 9 (except for TdSHMT7B-1) and motif 17 (except for TdSHMT7B-1 and CsSHMT4-2); however, motif 9 and motif 17 lacked in the protein sequence of class II a (Figures 2A,B). Exon–intron structure divergence plays an important role during the evolution of duplicate genes and different compositions of the motif are important for their functional diversity (Yu et al., 2020; Xu et al., 2021). The intron–exon structure was analyzed by the aligning the full-length cDNA with genomic DNA sequence (Figure 2). The structure analysis of SHMT genes indicated that closely related members had a similar exon-intron structure. In class I, the number of exons ranged from three to five; among the 38 analyzed SHMT genes, 35 genes had four exons, and only TdSHMT7B-1 and AtSHMT4 in class I a had three exons, TaSHMT3B-1 in class I b had five exons (Figures 2A,C). Compared with class I, there were great differences in the intron–exon structure between class I and class II, each SHMT gene in class II had multiple short exons. Class II a, which is only composed of dicotyledon genes, contained 11 exons. Class II b contained 17 analyzed genes in total, and the number of exons from 13 to 16, especially, TdSHMT4A-1, had 13 exons. TdSHMT4B-1 and ActSHMT4D-1 had 14 exons, CsSHMT2-2 had 16 exons, and all the other 13 genes had 15 exons (76.47%). These data suggest that the exon-intron structure and motifs in SHMT were highly correlated with phylogenetic relationships and special motifs in SHMT may play critical roles in specific functions.

**Micro-Collinearity Analysis of Triticeae**

The micro-collinearity analysis helps to understand the replication or loss events of specific genes in the process of evolution or domestication; provides an opportunity to explore the inheritance and variation of genes in a local region, and can trace the origin history of a gene (Chen Y. et al., 2020).
and its two neighboring genes was found in TaSHMT3A-1 micro-collinearity relationship showed that no homolog of the collinearity region of T. urartu inserted into the collinearity region of TaSHMT3A-1. However, when and it mainly enters common wheat through polyploidy. collinearity regions of common wheat is relatively conservative, the genome of T. urartu may have been lost. Some duplication events in the local region also occurred during the evolution of T. urartu, or a poor reference genome sequence of T. urartu caused this phenomenon.

Based on the fact that there was an SHMT gene, TdSHMT7B-1, which was on chromosome 7B of T. dicoccoides, while other analyzed Triticeae species did not contain the SHMT gene in the homologous group 7, and the number of exons and conserved motifs of this gene was less than that of other genes in the same subclass, TdSHMT7B-1 was used as query gene for micro-collinearity analysis. However, there was no micro-collinearity of TdSHMT7B-1 and its neighboring genes with other analyzed Triticeae species; furthermore, TdSHMT7B-1 was a gene pair with TRITD7Bv1G144890 of Triticum turgidum. The above results suggest that SHMT in tetraploid wheat may have experienced different evolution processes from other SHMT genes.

Functional Diversification Analysis
DIVERGE v3.0 software was used to analyze the functional divergence of SHMT proteins in different subclasses (Table 3). The results showed that the $\theta$ value of Type -I ranged from 0.3337 to 0.6333, the degree of functional differentiation among subclasses fluctuated wildly, and the standard error was 0.06. The $P$-values of different subclasses were lower than 0.01, indicating that functional differentiation was caused by the change of evolution rate among subclasses (Gu et al., 2013; Xu et al., 2021). The Type -II analysis showed that $\theta$ ranged within 0.1584–0.3326 (Table 3), except class I a/I b. The differences among other subclasses reached a significant level ($P < 0.01$), indicating that there was functional differentiation caused by the constant evolution rate, but the change of corresponding amino acid characteristics among these subclasses meant that there were some changes of critical amino acid sites which caused the functional divergence among the above subclasses. The above results indicate that gene functional divergence of different classes (except class I a/I b) came from both changing of some critical amino acid sites and evolution rate.

Expression Patterns Analysis of TaSHMTs
The expression patterns of gene family members are helpful in predicting their potential biological functions. To elucidate

---

**Table 1** Numbers of SHMT homologs encoded by the surveyed genomes in total and individual subclasses.

| Genome       | Total number | Subgroup |
|--------------|--------------|----------|
|              | Class Ia     | Class Ib | Class Ia | Class Ib |
| H. vulgare (HH) | 4            | 2        | 1        | 0        |
| T. urartu (AA) | 3            | 2        | 0        | 0        |
| Ae. Tauschii (DD) | 4            | 2        | 1        | 0        |
| T. dicoccoides (AABB) | 5 (9)       | 3 (5)    | 1 (2)    | 0 (1)    |
| T. aestivum (AABBDD) | 4 (12)     | 2 (8)    | 1 (3)    | 0 (1)    |
| O. sativa     | 5            | 2        | 2        | 0        |
| A. thaliana   | 7            | 2        | 2        | 1        |
| V. vinifera   | 7            | 1        | 2        | 1        |
| S. lycopersicum | 7           | 2        | 1        | 2        |
| C. sativus    | 6            | 1        | 2        | 1        |
| Total         | 52 (64)      | 19 (25)  | 13 (16)  | 5 (15)   |

Numbers in brackets indicate number of copies for polyploid genomes.

---

**Table 2** Number of SHMT from different Triticeae species in each of the chromosomes.

| Chromosome | T. aestivum | T. dicoccoides | T. urartu | Ae. tauschii | H. vulgare | Total |
|-----------|------------|---------------|-----------|--------------|------------|-------|
|           | A          | B | D   | A       | B | | A | A | D | H | | |
| Chr.1     | 1          | 1 | 1   | 1       | 1 | | 1 | 1 | 1 | 8 | |
| Chr.2     | 1          | 1 | 1   | 1       | 1 | | 1 | 1 | 1 | 8 | |
| Chr.3     | 1          | 1 | 1   | 1       | 1 | | 0 | 1 | 1 | 7 | |
| Chr.4     | 1          | 1 | 1   | 1       | 1 | | 1 | 1 | 1 | 8 | |
| Chr.5     | 0          | 0 | 0   | 0       | 0 | | 0 | 0 | 0 | 0 | |
| Chr.6     | 0          | 0 | 0   | 0       | 0 | | 0 | 0 | 0 | 0 | |
| Chr.7     | 0          | 0 | 0   | 0       | 1 | | 0 | 0 | 0 | 1 | |
| Total     | 4          | 4 | 4   | 5       | 3 | | 4 | 4 | 32 | | |
The potential role of TaSHMTs, their expression patterns were studied by qRT-PCR. Expression patterns of TaSHMTs under two biotic stresses (powdery mildew pathogen and F. graminearum), abiotic stresses (PEG, NaCl, and 4°C), in different tissues (roots, stems, and leaves of the seedling stage) and two hormone treatments (Abscisic acid: ABA and H2O2) were analyzed. Because the sequence similarity of the three copy genes in different subgenomes of wheat is high, qRT-PCR primers could not effectively distinguish the three copy genes; therefore, the gene of subgenomes A was used to represent the relative expression of the three copy genes. The expression of TaSHMT genes in different tissues, under various abiotic stresses and response to ABA and H2O2 treatments is shown in Figure 4. The expression of TaSHMTs on chromosomes 4A, 4B, and 4D could not be detected in different tissues, so the relative expression data of TaSHMTs gene in homologous group 4 were not provided in this study.

The expression pattern of TaSHMT genes in different tissues, under various abiotic stresses and response to ABA and H2O2 treatments is shown in Figure 4. The expression of analyzed TaSHMTs in stem and leaf were higher than that in root (Figure 4A). With PEG and NaCl treatments, the relative expression of TaSHMT2A-1 and TaSHMT3A-1 was down-regulated, and the difference was significant, but the expression of TaSHMT1A-1 did not change significantly (Figures 4B,C). Under the cold treatment, the relative expression of all TaSHMT genes was up-regulated (Figure 4D). With ABA treatment, the expression patterns of the three TaSHMT genes were different, the relative expression of TaSHMT1A-1 did not change significantly at different time points (Figure 4E); TaSHMT2A-1 was down-regulated, TaSHMT3A-1 showed a rapid increase and reached peak levels at 12 h, then returned to the original level at 24 h (Figure 4E). The transcriptional responses of TaSHMT genes to H2O2 showed that TaSHMT1A-1 (39-fold), TaSHMT2A-1 (10-fold), and TaSHMT3A-1 (8-fold) were highly upregulated at 12 h and then returned to the original level at 24 h (Figure 4F).

After Bgt inoculation, the relative expression levels of TaSHMT1A-1 and TaSHMT3A-1 were down-regulated at 6 h, then reached the expression peak at 24 h; the relative expression levels of TaSHMT2A-1 were up-regulated at 6 h and then reached the expression peak at 24 h (Figure 5A); however, the absolute times of relative expression change were small. The expression patterns of TaSHMTs in the FHB resistant cultivar Sumai 3 and the susceptible cultivar Jimai 22 at different times after infection with F. graminearum were further analyzed by qRT-PCR. For Sumai 3 and Jimai 22, all the relative expressions of the three TaSHMT1A-1, TaSHMT2A-1, and TaSHMT3A-1 were up-regulated in both materials (Figures 5B,C). Remarkably, the transcript levels of TaSHMT3A-1 rapidly reached levels at 48 and 72 h were 46- and 101-fold higher than at 0 h in Sumai 3, respectively; furthermore, although the expression
Hu et al. TaSHMT3A-1 Regulates Wheat FHB Resistance

FIGURE 3 | Micro-collinearity analysis by TGT to track the evolutionary history of TaSHMT3A-1 gene homologs. (A,B) TaSHMT3A-1 was used as the query gene. The micro-collinearity relationship showed that no homolog of TaSHMT3A-1 and its two neighboring genes was found in the collinearity region of T. urartu, but other genes were relatively conserved across other investigated genomes and there were 27 genes inserted into the collinearity region of T. urartu. The red arrow indicates TaSHMT3A-1 (A). In the micro-collinearity relationship analyze the genome of T. urartu was deleted. The neighboring genes of TaSHMT3A-1 were conserved across investigated genomes and homologs of TaSHMT3A-1 were found in all investigated genomes (B). Blackline, 1-to-1-mutual-best. Greenline, 1-to-its-best. Yellowline, 1-to-many. RBH, “reciprocal best hits”; SBH “single-side best hits”.

of TaSHMT3A-1 in the susceptible material Jimai22 was also up-regulated, the levels that at 48 and 72 h were threefold higher than at 0 h. Therefore, we deduced that TaSHMTs, especially the TaSHMT3A-1 might play an important role in the resistance response to FHB.

Silencing of TaSHMT3A-1 Compromises Fusarium Head Blight Resistance in Common Wheat Bainong207

Previous studies have shown that the Jasmonic Acid signaling pathway is related to wheat FHB resistance (Xiang et al., 2011), and exogenous ABA treatment can increase wheat sensitivity to FHB (Qi et al., 2016). In this study, TaSHMT3A-1 gene quickly responded to the induction of ABA and MeJA (Figure 5A and Supplementary Figure 2), and the relative expression of TaSHMT3A-1 in the FHB resistant cultivar Sumai 3 was significantly increased by 101 times at 72 h. Therefore, TaSHMT3A-1 was selected to further analyze its potential role in wheat resistance to FHB. The BSMV-VIGS system was used to further characterize the function of the TaSHMT3A-1 gene in common wheat Bainong207. BSMV: SHMT3A-1, which carries a 249-bp TaSHMT3A-1 fragment was used to induce target silencing. The fourth fully expanded leaves of
### TABLE 3 | The result of Type-I and -II functional divergence.

| Subgroup | MFE | MFE se | I :P | Theta-I | Theta SE | II :P |
|----------|-----|--------|------|---------|----------|------|
| I a/b    | 0.3337 | 0.0606 | 1.4905E-10** | 0.1699 | 0.0660 | 0.0101 |
| I a/I b  | 0.4038 | 0.0743 | 8.5050E-10** | 0.1584 | 0.0565 | 0.0051** |
| I a/II a | 0.4128 | 0.0670 | 6.8656E-13** | 0.2122 | 0.0573 | 0.0002** |
| I b/II a | 0.3833 | 0.0691 | 2.2837E-10** | 0.1940 | 0.0509 | 0.0001** |
| I b/II b | 0.6333 | 0.0694 | 2** | 0.3326 | 0.0499 | 2.5331E-11** |
| II a/II b| 0.4793 | 0.0757 | 4.0479E-13** | 0.2130 | 0.0404 | 1.3256E-07** |

MFE, model-free method. **Significance at p-values less than 0.01.

BSMV: TaSHMT3A-1-infected plants were detached, followed by inoculation with a fresh *F. graminearum* spore suspension and RNA extraction. Leaves of the same age from BSMV:γ-infected plants were collected and inoculated with fresh *F. graminearum* as controls. Three and five days after infection, BSMV:TaSHMT3A-1-infected leaves were more susceptible to FHB than those BSMV:γ-infected individuals (Figure 6A). The expression levels of TaSHMT3A-1 were significantly decreased, by 3–6-fold, as assessed by qRT-PCR (Figure 6B). Therefore, silencing the TaSHMT3A-1 gene could increase the susceptibility of Bainong207 to FHB.

### DISCUSSION

**Evolutionary Relationship of SHMT Genes in Triticeae Species**

Serine hydroxymethyltransferase is involved in the reversible interconversion of Ser and Gly, participates in amination and decarboxylation reactions, and plays an important role in the cell-carbon metabolic pathway (Bauwe and Kolukisaoglu, 2003; Voll et al., 2006). Previous studies mainly focused on the function of SHMT genes under abiotic stresses such as light intensity, salt, and drought (Voll et al., 2006; Cui et al., 2019; Mishra et al., 2019); however, studies on the role of the SHMT gene family in biotic stresses and its evolutionary relationship in important crops at the genomic level have been limited. Wheat is a heterologous hexaploid composed of three subgenomes A, B, and D. Studies reveal that hybridization of *T. urartu* (AA) and an *Ae. speltoides*-related grass (BB) produced a tetraploid ancestor *T. turgidum* (AABB). After the second hybridization of *Ae. tauschii* (DD) and *T. turgidum*, the hexaploid ancestor (AABBDD) of wheat was formed (Shewry, 2009; Allaby et al., 2017). With the related species of wheat being sequenced, the evolutionary history of wheat has been relatively clear (Avni et al., 2017; Luo et al., 2017; IWGSC, 2018; Ling et al., 2018), and it is an important goal of future research to infer when some wheat gene subfamilies expanded and occurred during the evolutionary process (Schilling et al., 2020).

In this study, bioinformatics methods were used to comprehensively analyze the SHMT gene family in wheat, and to investigate its potential role in development and biotic and abiotic stresses. A total of 64 genes containing the complete conserved domain of SHMT (PF00464) were screened from the genomic data of six monocotyledons and four dicotyledons and divided into four subclasses. The number of SHMT genes identified from the whole genome of *H. vulgare* (HH), *T. urartu* (AA), *Ae. tauschii* (DD), *T. dicoccoides* (BBAA) and wheat (BBAADD) were 4, 3, 4, 9, and 12, respectively. The proportion of SHMT number among species was consistent with the proportion of the corresponding genomic multiples; the number
of genes without copy genes was almost the same (Table 1). Gene replication is the most common mechanism for gene family extension (Cannon et al., 2004; Yu et al., 2020) and results in functional differentiation, which is critical for environmental adaptation and speciation (Conant and Wolfe, 2008). This study showed no tandem duplication or segmental replication in wheat SHMT gene. Meanwhile, macro-collinear and micro-collinear analysis showed that the SHMT gene of wheat was derived from a polyploidization process rather than self-replication (Figure 3 and Supplementary Figures 1, 3). Interestingly, there was a TdSHMT7B-1 on chromosome 7B of T. dicoccoides and there was no gene pair in wheat and other related species analyzed in this study; however, there was a homologous gene TRITD7Bv1G144890 on chromosome 7B of heterotetraploid species T. turgidum. We extended our focus to the incomplete domain SHMT of Triticeae species. The chromosomes 7B and 4B of wheat; 7A of T. dicoccoides; 4D of Ae. tauschii, and 4A and 7A of T. urartu all had one incomplete SHMT gene (Supplementary Table 2). Compared with the SHMT gene in the same subgroup, TdSHMT7B-1 lacked motifs 7, 9, and 17 (Figure 2B). Structural and conserved motif analysis revealed that motif, intron/exon loss or gain may occur during the evolution of Triticeae SHMT and similar events also appeared in SHMT of soybean (Lakhssassi et al., 2019). Some studies suggest that, in monocots, a special gene loss event occurred in class II a, and a gene duplication event in class I a compensated for this loss (Lakhssassi et al., 2019). However, this study does not support the above viewpoint. Instead, we speculate that the loss of SHMT gene did occur in class II a of monocotyledons, but there was no particular replication in class I a to make up for it. On the contrary, the loss or regression of SHMT gene occurred in class I a of monocotyledons during the process of evolution. This difference in genetic evolution may be because the effect of the circadian clock on daily growth rhythms is different between monocotyledons and dicotyledons (Campoli et al., 2012). However, this speculation needs further research.

**TaSHMT3A-1 Positively Regulates Fusarium Head Blight Resistance**

In the process of evolution, duplicated genes often undergo functional differentiation, leading to neofunctionalization,
The results of class I a/I b showed that the functional differences among the subgroups (except class I a/I b) came not only from changes of some key amino acid loci, but also from changes in evolution and chemical properties of amino acids (Yang et al., 2020). The differentiation represents the change in physical and chemical properties of amino acids (Yang et al., 2020). The results showed that the gene functional differences among the subgroups (except class I a/I b) came not only from changes of some key amino acid loci, but also from changes in evolution rate. The results of class I a/I b showed that the functional differentiation between the two subgroups was mainly Type I, and it was mainly determined by the sites conserved in one subclass and not conserved in the other subclass. Gene expansion provides conditions for the formation of new functions. The sequence of one gene remains relatively stable and the sequence of the other expanded gene changes, and functional differentiation occurs.

Since the sequence similarity of the three copy genes in different subgenomes of wheat is high (Pfeifer et al., 2014), we used the universal qRT-PCR primers to analyze the expression levels of corresponding three copy genes. The SHMT gene was shown to be closely related to photosynthesis, and with reduced activities of SHMT would usually show severe growth retardation (Heineke et al., 2001; Somerville, 2001). In the present study, the results showed that the expression level of TaSHMTs in stems and leaves were higher than in roots, which may be because SHMT plays an important role in plant one-carbon metabolism and photosynthesis, and green tissues are important parts of photorespiration. The expressions of TaSHMT2A-1 and TaSHMT3A-1 were down-regulated under the stress of PEG and NaCl and TaSHMT1A-1 did not respond to these two treatments. These results are consistent with previous research results in diploid wheat Triticum monococcum (Bhuiyan et al., 2007), speculating that SHMTs also have similar functions as in T. monococcum. With ABA treatment, the expression levels of TaSHMT2A-1 were down-regulated, and TaSHMT3A-1 showed a rapid increase (Figure 4E). Furthermore, TaSHMT1A-1, TaSHMT2A-1, and TaSHMT2A-1 significantly responded to H2O2 (Figure 4F). Previous studies showed that AtSHMT1 could prevent cell death and reduce reactive oxygen species accumulation during salt stress (Zhou et al., 2019; Su et al., 2019; Wang et al., 2020). In this study, we found that the expression of TaSHMT3A-1 in the FHB resistant cultivar Sumai 3 was significantly increased by 101 times after infection by F. graminearum at 72 h (Figure 5B). When the TaSHMT3A-1 was silenced in Bainong207, the results showed that silencing the TaSHMT3A-1 gene could increase the susceptibility of Bainong207 to FHB. Given that the link of the transcriptional response of TaSHMT3A-1 to F. graminearum, ABA, MeJA and the result of BSMV-VIGS (Figures 4E,F, 6 and Supplementary Figure 2), it is reasonable for us to conclude that TaSHMT3A-1 may be involved in H2O2 - and ABA-induced tolerance to abiotic stress.

Wheat FHB is a serious disease of wheat, which seriously affects the human food and animal feed security (Li et al., 2019; Su et al., 2019; Wang et al., 2020). In this study, we found that the expression of TaSHMT3A-1 in the FHB resistant cultivar Sumai 3 was significantly increased by 101 times after infection by F. graminearum at 72 h (Figure 5B). When the TaSHMT3A-1 was silenced in Bainong207, the results showed that silencing the TaSHMT3A-1 gene could increase the susceptibility of Bainong207 to FHB. Given that the link of the transcriptional response of TaSHMT3A-1 to F. graminearum, ABA, MeJA and the result of BSMV-VIGS (Figures 4E,F, 6 and Supplementary Figure 2), it is reasonable for us to conclude that TaSHMT3A-1 may be involved in H2O2 - and ABA-induced tolerance to abiotic stress.

Wheat FHB is a serious disease of wheat, which seriously affects the human food and animal feed security (Li et al., 2019; Su et al., 2019; Wang et al., 2020). In this study, we found that the expression of TaSHMT3A-1 in the FHB resistant cultivar Sumai 3 was significantly increased by 101 times after infection by F. graminearum at 72 h (Figure 5B). When the TaSHMT3A-1 was silenced in Bainong207, the results showed that silencing the TaSHMT3A-1 gene could increase the susceptibility of Bainong207 to FHB. Given that the link of the transcriptional response of TaSHMT3A-1 to F. graminearum, ABA, MeJA and the result of BSMV-VIGS (Figures 4E,F, 6 and Supplementary Figure 2), it is reasonable for us to conclude that TaSHMT3A-1 may be involved in H2O2 - and ABA-induced tolerance to abiotic stress.

Wheat FHB is a serious disease of wheat, which seriously affects the human food and animal feed security (Li et al., 2019; Su et al., 2019; Wang et al., 2020). In this study, we found that the expression of TaSHMT3A-1 in the FHB resistant cultivar Sumai 3 was significantly increased by 101 times after infection by F. graminearum at 72 h (Figure 5B). When the TaSHMT3A-1 was silenced in Bainong207, the results showed that silencing the TaSHMT3A-1 gene could increase the susceptibility of Bainong207 to FHB. Given that the link of the transcriptional response of TaSHMT3A-1 to F. graminearum, ABA, MeJA and the result of BSMV-VIGS (Figures 4E,F, 6 and Supplementary Figure 2), it is reasonable for us to conclude that TaSHMT3A-1 may be involved in H2O2 - and ABA-induced tolerance to abiotic stress.

Wheat FHB is a serious disease of wheat, which seriously affects the human food and animal feed security (Li et al., 2019; Su et al., 2019; Wang et al., 2020). In this study, we found that the expression of TaSHMT3A-1 in the FHB resistant cultivar Sumai 3 was significantly increased by 101 times after infection by F. graminearum at 72 h (Figure 5B). When the TaSHMT3A-1 was silenced in Bainong207, the results showed that silencing the TaSHMT3A-1 gene could increase the susceptibility of Bainong207 to FHB. Given that the link of the transcriptional response of TaSHMT3A-1 to F. graminearum, ABA, MeJA and the result of BSMV-VIGS (Figures 4E,F, 6 and Supplementary Figure 2), it is reasonable for us to conclude that TaSHMT3A-1 may be involved in H2O2 - and ABA-induced tolerance to abiotic stress.

Wheat FHB is a serious disease of wheat, which seriously affects the human food and animal feed security (Li et al., 2019; Su et al., 2019; Wang et al., 2020). In this study, we found that the expression of TaSHMT3A-1 in the FHB resistant cultivar Sumai 3 was significantly increased by 101 times after infection by F. graminearum at 72 h (Figure 5B). When the TaSHMT3A-1 was silenced in Bainong207, the results showed that silencing the TaSHMT3A-1 gene could increase the susceptibility of Bainong207 to FHB. Given that the link of the transcriptional response of TaSHMT3A-1 to F. graminearum, ABA, MeJA and the result of BSMV-VIGS (Figures 4E,F, 6 and Supplementary Figure 2), it is reasonable for us to conclude that TaSHMT3A-1 may be involved in H2O2 - and ABA-induced tolerance to abiotic stress.
In this study, the function of TaSHMT3A-1 on FHB was verified through a leaf BSMV-VIGS assay. The effects and contribution of TaSHMT3A-1 gene on wheat FHB need to be further verified through stable genetic transformation plants. Although the function of the wheat TaSHMTs needs to be elucidated, this study revealed that TaSHMTs might have diverged new functions during the course of evolution.

**CONCLUSION**

In the present study, 64 SHMT members were identified from six monocotyledon and four dicotyledon species, and phylogenetic relationship analysis of SHMT members classified them into two main classes. The gene structure and motif composition analyses revealed that SHMTs kept relatively conserved within the same subclasses; however, TdSHMT7B-1 on chromosome 7B of *T. dicoccoides* had a special gene structure and motifs. Combined with micro-collinearity analysis, TdSHMT7B-1, TaSHMT3A-1 and corresponding homologs in Triticeae species may have experienced a special evolutionary process. The expression pattern showed that TaSHMT3A-1 was responsive to some abiotic, and biotic stresses, and hormone treatments. Significantly, upon *F. graminearum* infection the expression of TaSHMT3A-1 was highly upregulated in resistant cultivar Sumai3. More importantly, silencing of TaSHMT3A-1 compromised FHB resistance in common wheat Bainong207. Our new findings suggest that TaSHMT3A-1 gene in wheat plays an important role in resistance to FHB. This study provides a valuable reference for further functional study of these genes.

**REFERENCES**

Allaby, R. G., Stevens, C., Lucas, L., Maeda, O., and Fuller, D. Q. (2017). Geographic mosaics and changing rates of cereal domestication. Philos. Trans. R. Soc. Lond B. Biol. Sci. 372:20160429. doi: 10.1098/rstb.2016.0429

Avni, R., Nave, M., Barad, O., Baruch, K., Twardziok, S. O., Gundlach, H., et al. (2017). Wild emmer genome architecture and diversity elucidate wheat evolution and domestication. Science 357, 93–97. doi: 10.1126/science.aan0032

Bai, G., and Shaner, G. (2004). Management and resistance in wheat and barley to fusarium head blight. Annu. Rev. Phytopathol. 42, 135–161. doi: 10.1146/annurev.phyto.42.040803.140340

Bauwe, H., and Kolukisaoglu, U. (2003). Genetic manipulation of glycine decarboxylation. J. Exp. Bot. 54, 1523–1535. doi: 10.1093/jxb/erg171

Bhuiyan, N. H., Liu, W., Liu, G., Selvaraj, G., Wei, Y., and King, J. (2007). Transcriptional regulation of genes involved in the pathways of biosynthesis and supply of methyl units in response to powdery mildew attack and abiotic stresses in wheat. Plant Mol. Biol. 64, 305–318. doi: 10.1007/s11103-007-9155-x

Campoli, C., Shtaya, M., Davis, S. J., and Von Korff, M. (2012). Expression conservation within the circadian clock of a monocot: natural variation at barley Ppd-H1 affects circadian expression of flowering time genes, but not clock orthologs. BMC Plant Biol. 12:97. doi: 10.1186/1471-2228-12-97

Cannon, S. B., Mitra, A., Baumgarten, A., Young, N. D., and May, G. (2004). The roles of segmental and tandem gene duplication in the evolution of large gene orthologs. BMC Genomics 5, 169. doi: 10.1186/1471-2164-5-169

Chakraborty, S., and Newton, A. C. (2011). Climate change, plant diseases and food security: an overview. Plant Pathol. 60, 2–14.

Chen, C., Chen, H., Zhang, Y., Thomas, H. R., Frank, M. H., He, Y., et al. (2020). Tbitools: an integrative toolkit developed for interactive analyses of big biological data. Mol. Plant. 13, 1194–1202. doi: 10.1016/j.molp.2020.06.009

Chen, Y., Song, W., Xie, X., Wang, Z., Guan, P., Peng, H., et al. (2020). A collinearity-incorporating homology inference strategy for connecting emerging assemblies in the triticeae tribe as a pilot practice in the plant pangenomic era. Mol. Plant. 13, 1694–1708. doi: 10.1016/j.molp.2020.09.019

Conant, G. C., and Wolfe, K. H. (2008). Turning a hobby into a job: how duplicated genes find new functions. Nat. Rev. Genet. 9, 938–950. doi: 10.1038/nrg2482

Cui, G., Zhao, Y., Zhang, J., Chao, M., Xie, K., Zhang, C., et al. (2019). Proteomic analysis of the similarities and differences of soil drought and polyethylene glycol stress responses in wheat (*Triticum aestivum* L.). Plant Mol. Biol. 100, 391–410. doi: 10.1007/s11103-019-00866-2

Dean, R., Van Kan, J. A., Pretorious, Z. A., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P. D., et al. (2012). The Top 10 fungal pathogens in molecular plant pathology. Mol. Plant Pathol. 13, 414–430.

Del Ponte, E. M., Fernandes, J. M. C., Pavan, W., and Baethgen, W. E. (2009). A model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in Southern Brazil. J. Phytopathol. 157, 675–681.

El-Gebali, S., Mistry, J., Bateman, A., Eddy, S. R., Luciani, A., Potter, S. C., et al. (2019). The Pfam protein families database in 2019. Nucleic Acids Res. 47, D427–D432. doi: 10.1093/nar/gky995

Engel, N., Ewald, R., Gupta, K. J., Zeenner, R., Hagemann, M., and Bauwe, H. (2011). The presupposition of Arabidopsis serine hydroxymethyltransferase SHM2 selectively prevents import into mesophyll mitochondria. Plant Physiol. 157, 1711–1720. doi: 10.1104/pp.111.184564

García-Canaveras, J. C., Lancho, O., Ducker, G. S., Ghergurovich, J. M., Xu, X. C., Da Silva–Díaz, V., et al. (2021). SHMT inhibition is effective and synergizes with

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

**AUTHOR CONTRIBUTIONS**

PH and JX conceived and designed the experiments, analyzed the data, and wrote the manuscript. PH, PS, JX, QW, YT, YR, and YY performed the experiments and collected the data. DL, HH, and CL revised the manuscript. All the authors read and approved the final manuscript.

**FUNDING**

This work was supported by the National Natural Science Foundation of China (Nos. 31901538 and 31872129), the Key Scientific and Technological Research Projects in Henan Province (Nos. 212102110052 and 212102110477), and the Natural Science Foundation of Henan Province (No. 212300410143).

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2022.847087/full#supplementary-material
methotrexate in T-cell acute lymphoblastic leukemia. *Leukemia* 35, 377–388. doi: 10.1038/s41375-020-0845-6

Gu, X., Zou, Y., Su, Z., Huang, W., Zhou, Z., Aresendee, Z., et al. (2013). An update of DIVERGE software for functional divergence analysis of protein family. *Mol. Biol. Evol.* 30, 1714–1719. doi: 10.1093/molbev/ms359

He, Z., Zhang, H., Gao, S., Lercher, M. J., Chen, W. H., and Hu, S. (2016). Evolv2 v2: an online visualization and management tool for customized and annotated phylogenetic trees. *Nucleic Acids Res.* 44, W236–W241. doi: 10.1093/nar/gkw370

Heinke, D., Bykova, N., Gardestrom, P., and Bawe, H. (2001). Metabolic response of potato plants to an antisense reduction of the P-protein of glycine decarboxylase. *Plant* 212, 880–887. doi: 10.1002/0022-0031(200010)212:9<880::AID-PLA205>3.0.CO;2-X

Hu, P., Liu, J. Q., Xu, J. F., Zhou, C. Y., Cao, S. Q., Zhou, W. H., et al. (2016). A malecin-like/leucine-rich repeat receptor protein kinase gene, RLK-V, regulates powdery mildew resistance in wheat. *Mol. Plant Pathol.* 19, 2561–2574. doi: 10.1111/mpp.12729

IWGSC (2018). Shifting the limits in wheat research and breeding using a fully annotated reference genome. *Nucleic Acids Res.* 34, D741–D744. doi: 10.1093/nar/gkx1049

Pfeifer, M., Kugler, K. G., Sandve, S. R., Zhan, B., Rudi, H., Hvistendal, T. R., et al. (2014). Genome interplay in the grain transcriptome of hexaploid bread wheat. *Science* 345, 7509. doi: 10.1126/science.1250091

Prabhhu, V., Chatson, K. B., Abrams, G. D., and King, J. (1996). 13C nuclear magnetic resonance detection of interactions of serine hydroxymethyltransferase with C1-tetrahydrofolate synthase and glycine decarboxylase complex activities in Arabidopsis. *Plant Physiol.* 112, 207–216. doi: 10.1104/pp.112.1.207

Prince, V. E., and Pickett, F. B. (2002). Splitting pairs: the diverging fate of duplicated genes. *Nat. Rev. Genet.* 3, 827–837. doi: 10.1038/nrg9298

Qi, P. F., Balcerzak, M., Rocheleau, H., Leung, W., Wei, Y. M., Zheng, Y. L., et al. (2016). Jasmonic acid and abscisic acid play important roles in host-pathogen interaction between *Fusarium graminearum* and wheat during the early stages of fusarium head blight. *Physiol. Mol. Plant Pathol.* 93, 39–48.

Schilling, S., Kennedy, A., Pan, S., Jermin, L. S., and Melzer, R. (2020). Genome-wide analysis of MIKC-type MADS-box genes in wheat: pervasive duplications, functional conservation and putative neo-functionalization. *New Phytol.* 255, 511–529. doi: 10.1111/nph.16122

Schrich, V., and Szefeny, D. M. (2005). Serine hydroxymethyltransferase revisited. *Curr. Opin. Chem. Biol.* 9, 482–487.

Shewry, P. R. (2009). Wheat. *J. Exp. Bot.* 60, 1537–1553.

Somerville, C. R. (2001). An early Arabidopsis demonstration. Resolving a few issues concerning photosynthesis. *Plant Physiol.* 125, 20–24.

Su, Z. Q., Bernardon, A., Tian, B., Chen, H., Wang, S., Ma, H. X., et al. (2019). A deletion mutation in TaHRC confers Fhb1 resistance to Fusarium head blight in wheat. *Nat. Genet.* 51, 1099–1105. doi: 10.1038/s41588-019-0425-8

Turner, S. R., Ireland, R., Morgan, C., and Rawsthorne, S. (1992). Identification and localization of multiple forms of serine hydroxymethyltransferase in pea (*Pisum sativum*) and characterization of a CDNA encoding a mitochondrial isoform. *J. Biol. Chem.* 267, 13528–13534.

Voll, L. M., Jamai, A., Renne, P., Voll, H., Mcclung, C. R., and Weber, A. P. (2006). The photosynthetic Arabidopsis shm1 mutant is deficient in SHM1. *Plant Physiol.* 140, 59–66. doi: 10.1104/pp.105.071399

Waditee-Sirisattha, R., Sittipol, D., Tanaka, Y., and Takabe, T. (2012). Overexpression of serine hydroxymethyltransferase from halotolerant cyanobacterium in *Escherichia coli* results in increased accumulation of choline precursors and enhanced salinity tolerance. *FEBS Microbiol. Lett.* 333, 46–53. doi: 10.1111/j.1574-6968.2012.02597.x

Wang, D., Liu, H., Li, S., Zhai, G., Shao, J., and Tao, Y. (2015). Characterization and molecular cloning of a serine hydroxymethyltransferase 1 (OsSHM1) in rice. *J. Integr. Plant Biol.* 57, 745–756. doi: 10.1111/jipb.12336

Wang, H. W., Sun, S. L., Ge, W. Y., Zhao, L. F., Hou, B. Q., Wang, K., et al. (2020). Horizontal gene transfer of Fhb7 from fungus underlies Fusarium head blight resistance in wheat. *Science* 368 (6493): eaab5435. doi: 10.1126/science.aba5435

Xing, L., Hu, P., Liu, J., Witek, K., Zhou, S., Xu, J., et al. (2018). Pm21 from *P. syringae* confers tolerance to salinity stress in *E. coli*. *Front. Plant Sci.* 9:1506. doi: 10.3389/fpls.2018.00217

Xing, L., Hu, P., Liu, J., Witek, K., Zhou, S., Xu, J., et al. (2018). Pm21 from *P. syringae* confers tolerance to salinity stress in *E. coli*. *Front. Plant Sci.* 9:1506. doi: 10.3389/fpls.2018.00217

Moreno, J. I., Martin, R., and Castresana, C. (2005). Arabidopsis SHMT1, a serine hydroxymethyltransferase that functions in the photosynthetic pathway inhibits resistance to biotic and abiotic stress. *Plant J.* 41, 451–463. doi: 10.1111/j.1365-313X.2004.02311.x

Neuburger, M., Rebelle, F., Jourdain, A., Nakamura, S., and Douce, R. (1996). Mitochondia are a major site for folate and thymidylate synthesis in plants. *J. Biol. Chem.* 271, 9466–9472. doi: 10.1074/jbc.271.16.9466

Ohyanagi, H., Tanaka, T., Sakai, H., Shigemoto, Y., Yamaguchi, K., Habara, T., et al. (2006). The rice annotation project database (RAP-DB): hub for *Oryza sativa* ssp. *japonica* genome information. *Nucleic Acids Res.* 34, D741–D744. doi: 10.1093/nar/gkj094

Xing, L., Hu, P., Liu, J., Witek, K., Zhou, S., Xu, J., et al. (2018). Pm21 from *P. syringae* confers tolerance to salinity stress in *E. coli*. *Front. Plant Sci.* 9:1506. doi: 10.3389/fpls.2018.00217
Xu, J., Hu, P., Tao, Y., Song, P., Gao, H., and Guan, Y. (2021). Genome-wide identification and characterization of the Lateral Organ Boundaries Domain (LBD) gene family in polyploid wheat and related species. *PeerJ* 9:e11811. doi: 10.7717/peerj.11811

Yang, J. F., Chen, M. X., Zhang, J. H., Hao, G. F., and Yang, G. F. (2020). Genome-wide phylogenetic and structural analysis reveals the molecular evolution of the ABA receptor gene family. *J. Exp. Bot.* 71, 1322–1336. doi: 10.1093/jxb/erz511

Yu, J., Xie, Q., Li, C., Dong, Y., Zhu, S., and Chen, J. (2020). Comprehensive characterization and gene expression patterns of LBD gene family in *Gossypium*. *Planta* 251:81. doi: 10.1007/s00425-020-03364-8

Zhang, X., Halder, J., White, R. P., Hughes, D. J., Ye, Z., Wang, C., et al. (2014). Climate change increases risk of fusarium ear blight on wheat in central China. *Ann. Appl. Biol.* 164, 384–395.

Zhang, Y., Sun, K. H., Sandoval, F. I., Santiago, K., and Roje, S. (2010). One-carbon metabolism in plants: characterization of a plastid serine hydroxymethyltransferase. *Biochem. J.* 430, 97–105. doi: 10.1042/BJ20100566

Zhou, H., Zhao, J., Yang, Y., Chen, C., Liu, Y., Jin, X., et al. (2012). Ubiquitin-specific protease16 modulates salt tolerance in *Arabidopsis* by regulating Na(+)/H(+) antiport activity and serine hydroxymethyltransferase stability. *Plant Cell* 24, 5106–5122. doi: 10.1105/tpc.112.106393

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.