Characterization of *MATE* Gene Family And Its Role In Drought, Heat, And Salt Tolerance In Wheat (*Triticum Aestivum* L.)

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Research Article

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Abstract

To explore the response of multidrug and toxic compound extrusion (MATE) proteins to drought, heat, and salt stress in wheat, a genome-wide identification and expression study was performed. 20 *MATE* genes located on 4 of the 12 chromosomes were identified and categorized into four (I-1V) subfamilies, based on phylogenetic analysis. Wheat *MATE* family expansion was primarily driven by whole-genome duplication (WGD) and tandem events. In the same subfamily, gene exon-intron structures and motif composition are more similar. *TaMATE* genes had cis-acting elements that were implicated in stress and defense response. Tae-miR5175e was identified as the highly expressed miRNA that targets *TaMATEs* by miRNA prediction. When compared to controls, the relative expression patterns of seven *TaMATE* genes were substantially elevated during drought stress. TaMATE2, 10, 13, and 14 expression levels considerably elevated after 15 days (d) of heat stress, whereas *TaMATE2*, 14, 18, and 20 expression levels were highly upregulated following 15 d of salt stress treatment, indicating the crucial role of *TaMATEs* under these abiotic stress conditions. Furthermore, drought, heat, and salt stress decreased wheat water content, but increased malondialdehyde (MDA), electrolyte leakage (EL), and proline content, whereas the expression of the 7 putative MATE genes was correlated with physio-biochemical indicators of these stress conditions. The findings contribute to a better understanding of the complexities of *MATEs* and present a theoretical base for future *MATE* gene discovery and application in wheat and other crop species.

Introduction

Abiotic stresses that affect plant growth and development include drought, heat, and salt. These abiotic factors cause decreased plant water content, membrane damage, increased lipid peroxidation, osmolyte accumulation, and stress-responsive gene activation [1, 2]. Increased intensity of these stressed cause oxidative stress, which severely damages cell structure and integrity, resulting in organelle function loss, decreased metabolic function, electrolyte leakage, and programmed cell death [3]. Plants have evolved various physiological, biochemical, and molecular mechanism to combat the effect of abiotic stress. These mechanisms are largely enzymatic and non-enzymatic in nature. Among the enzymatic mechanisms, catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), and ascorbate peroxidase (APX) have been reported to increase during abiotic stress conditions across species, modulating plant response to drought and oxidative stresses [4–7]. Furthermore, abiotic stress causes transcriptional reprogramming, which activates mechanisms that protects plants stressful conditions [8]. AP2/ERF, MYB, WRKY, NAC, bZIP, bHLH, and DREB are among the transcription factors (TFs) known to regulate plant response to abiotic stress. These transcription factors control the regulation in order of a slew of downstream genes, eventually increasing plant tolerance to single or multiple stresses [9–13]

Secondary transporters of highly conserved cations, as well as multidrug and toxic chemical extrusion transporters (MATE), are present in archaea, bacteria, and eukaryotes [14]. Most members of this family contain 450–550 amino acid residues; a few individuals with 9–12 transmembrane helices in the sequence can exceed 700 amino acids [15]. MATE family members have been found to be involved in a wide range of activities in plants, including disease resistance, aluminum detoxification, toxic metal efflux, secondary metabolites and plant hormones, and abiotic stress tolerance [16]. They are also involved in root detoxification, the transport of harmful substances such as salicylic acid, alkaloids, and antibiotics, as well as the maintenance of iron ion balance in plants and the regulation of lateral organ growth [17–19]. Notably, MATE's specific functions in Arabidopsis thaliana have been identified and characterized, and the Arabidopsis thaliana MATE protein (AtDXT1) has been shown to be involved in the efflux of alkaloids, antibiotics, toxic compounds, and the detoxification of cadmium, a heavy metal [20]. AtDTX19 has been found to perform functions similar to AtDTX1. [21]. Plant development is also influenced by MATE protein, which regulates phytohormone transport. AtDTX50, for example, has the ability to efflux ABA, and both AtFRD3 (AtDTX43) and AtDTX50 have citric acid activity and the ability to transport metallic iron [22, 23]. By transferring hydroxycinnamic acid amide, AtDTX18, on the other hand, can improve plant defense against infections [24].

MATE protein functions have been characterized in other plants. Two maize MATE proteins (ZmMATE1 and ZmMATE2), as well as a rice MATE protein (OsFRDL4), have been shown to function in aluminum detoxification [25, 26]. Furthermore, whereas OsMATE2 controlled arsenic accumulation in rice and tobacco, NtMATE1 and NtMATE2 were engaged in alkaloids transport to the vacuole, decreasing their toxicity, while MtMATE67 facilitated symbiotic nitrogen fixation by regulating citrate transport into the symbiotic plastid [27–29]. Cotton MATE gene overexpression has recently been shown to improve tolerance to oxidative stress via reactive oxygen species (ROS) scavenging [30]. Extrusion protein for multidrug and toxic compound has been extensively investigated in various plants [6, 31–38]. From these studies, MATE genes were reported to be involved in plant growth, development, and stress tolerance. The functions of some of the MATE genes have been characterized, and all of these indicate that the MATE gene family plays an important role in plant growth, development, and stress resistance.

Wheat (*Triticum aestivum* L.) is a major staple crop that is grown all over the world. Wheat demand is expected to grow by 60% by 2050 [39]. MYB, NAC, and WRKY are among the gene families that have been identified and characterized in wheat [40–42]. However, the functions of MATE protein family in wheat (*T. aestivum* L.) remains unelucidated, especially under drought, heat, and salt stress conditions. The study aimed to identify and characterize MATE gene in wheat under different abiotic stress conditions. In order to accomplish this, the gene structure, chromosomal localization, phylogenetic relationship, gene ontology, miRNA targets, cis-acting element, and expression patterns of putative TaMATE genes were investigated. Following drought, heat, and salt stress, the physio-biochemical response and correlation between MATE gene expression and physio-biochemical indicators were also evaluated.

Materials And Methods

Plant materials and drought treatment conditions

The seeds of commercially grown wheat genotype ‘Geumgangmil’ was used in the study. Seeds were obtained from the Rural Development Administration (RDA), Korea's National Agrobiodiversity Centre. This wheat cultivar has been shown to be drought stress resistant [43, 44]. However, little is known about its response to heat and stress. Seeds were grown in pots (10 × 10 × 8 cm) containing soil (sunshine mix #2) under a photocycle of 13:11 h (day: night), 25 to 22°C (day to night), 80% relative humidity, and active photosynthetic radiation at 600 µmol $m^{-2}s^{-1}$ for 14 d before the drought, heat, and salt stress treatments were initiated. Stress treatment was initiated when plants were at the fully expanded 2nd to 3rd leaf stage, according to the Zadok’s scale (scale 12) of cereal growth [45]. Four plant treatment groups were made. The first set of plants, the control with no stress treatment, was watered daily for 15 d with a half strength Hoagland solution. The second group of plants was subjected to drought stress by withholding water for 15 d, the third group of plants was subjected to heat stress treatment (high temperature of 45 ^{O}C) for 15 d, and the fourth group of plants was subjected to half strength Hoagland solution containing varying concentrations (50 to 300 mM) of sodium chloride (NaCl) for 15 d. Leaf tissue samples were taken at 5, 10, and 15 days after stress treatment.

Relative water content (RWC) and electrolyte leakage measurement

The leaf relative water content (RWC) was measured as described by [44]. The fresh weight (FW), turgid weight (TW), and dry weight (DW) of leaves and roots were measured, and the RWC was calculated as follows:

RWC (%) = (FW – DW) / (TW – DW) × 100, (1)

For EL determination, the leaves were cut into 2 cm-long pieces, washed with distilled water to remove injured tissues, and 10 pieces were incubated in 15 mL of distilled water for 24 h at 25^{o}C. Subsequently, the initial conductivity (C_1) of the solutions was measured. The samples were boiled for 30 min, and the final conductivity (C_2) was measured after cooling to room temperature.

EL (%) = 1 - (C_1/C_2) x 100% (2)

Malondialdehyde (MDA) and proline content determination

The MDA content was determined using the method of [46]. Each 0.1 g leaf sample was homogenized in 1 mL 10% (w/v) trichloroacetic acid (TCA) solution on ice. The homogenate was centrifuged at 12 000 × *g* for 10 min at 4°C, and the supernatant was collected. Then, 1 mL 0.5% thiobarbituric acid was added to a 1 mL aliquot of the supernatant. The mixture was boiled for 25 min and immediately cooled on ice. After centrifugation at 5 000 × *g* for 10 min, the absorbance of the supernatant was measured at 532 and 600 nm. The concentration of MDA was calculated using an extinction coefficient of 155 mM/cm.

RNA isolation and gene expression analysis

Leaf total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). RNA was quantified spectrophotometrically, and the quality was evaluated by agarose gel electrophoresis. Synthesis of cDNA was performed using a Power cDNA Synthesis Kit (Intron Biotechnology Inc., South Korea). Quantitative real-time polymerase chain reaction (qPCR) was performed using a CFX 96 Real-Time system (Bio-Rad, Richmond, CA, USA) with SYBR-green fluorescence, and the results were analyzed using the ΔΔCT method. Gene-specific primers (Table S1) for qPCR were used to evaluate the genes' activity under progressive drought conditions. The thermal cycle employed was 95°C for 5 min and 40 cycles of 95°C for 15 s, 55°C for 15 s, and 72°C for 30 s. All experiments were conducted with three biological replicates, and the relative transcript levels were standardized using *Actin* as the internal control.

Statistical analysis

The data were analyzed using R (v.3.5.1). The data were analyzed by one-way analysis of variance (ANOVA). The differences between means were assessed via Tukey's multiple range test ($P < 0.05$) using SIGMAPLOT (v.14.0). Each result was summarized by the mean ± standard error (SE) of three independent experiments. systematic clustering, and GraphPad Prism 5 and TBtools [47] (v1.0971) were used for preparation of the figures.

Identification of the DUF569 genes in tomato

To identify *MATE* genes in wheat, whole-genome data from tomato (*Triticum aestivum v2.2*) were downloaded from the phytozom13 website (https://phytozome-next.jgi.doe.gov/blast-search). The Pfam (http://pfam.sanger.ac.uk/) hidden Markov model (HMMER) profile of the MATE domain (pfam01554) was used to search the wheat protein database at a standard E-value of $< 1\times10^{-5}$ [48]. A total of 20 putative MATE proteins were identified. Furthermore, the protein sequences of 10 rice (OsMATE), Arabidopsis (AtMATE), maize (ZmMATE), and potato (StMATE) proteins were BLAST-searched against the 20 putative MATE proteins of wheat to discover the best match sequences.

The *MATE* genes were non-uniformly distributed on the wheat chromosome and were named *TaMATE1* to *TaMATE20*, according to their position on the wheat chromosome. The NCBI conserved domain database (CDD) search was also used to evaluate the conserved domains of candidate (https://www.ncbi.nlm.nih.gov/cdd). The physicochemical propertie, such as including the theoretical isoelectric point (p*I*) and molecular weight (MW) of TaMATE proteins were analyzed through the ProtParam server (https://web.expasy.org/protparam/) [49]. The number of transmembrane domains (TMDs) was identified by the TMHMM server v2 (http://www.cbs.dtu.dk/services/TMHMM/) [50]. The sub-cellular localizations of the TaMATE proteins were predicted using the TargetP-2.0 Server (http://www.cbs.dtu.dk/services/TargetP/) [51].

Multiple sequence alignment, gene structure, conserved motifs, gene duplication, and phylogenetic analysis

The multiple sequence alignment and sequence identity matrix were generated using the BioEdit v7.2.5 software [52]. The Gene Structure Display Server GSDS 2.0 (http://gsds.cbi.pku.edu.cn/) online tool, was used to determine the structures of

these genes [53]. The Multiple EM for Motif Elicitation (MEME) software server v5.3.3 (https://meme-suite.org/meme/) [54] was used to identify the conserved protein motifs of these genes, with the following parameters: The optimum motif width was between 6 and 200 residues, allowing for any number of repeated motif sites, while the maximum number of motifs was 10. The *TaMATE* genes were mapped onto the chromosomes to identify their chromosomal positions. To analyze gene duplication, the TBtools program was utilized, and manual screening was done using Wang's mature method [55]. After that, the TBtools program was used to visualize the chromosomal localizations and duplicated regions of all *TaMATE* genes [47]. The diverse roles of duplicated genes are shaped by natural selection. Ka/Ks Calculator 2.0 was used to compute the non-synonymous (Ka) and synonymous (Ks) ratio (Ka/Ks) of each aligned codon in the pairs of duplicated *TaMATE* genes to examine the influence of sequence duplication on the function of *TaMATE* [56]. The divergence time was computed as $T = Ks/(2 \times 1.5 \times 10^{-8}) \times 10^{-6}$ million years ago (MYA) [57].

MATE gene family members from *Oryza sativa*, *Arabidopsis thaliana*, *Zea* mays, and *Solanum tuberosum*, were investigated using a phylogenetic tree with wheat *MATE* genes to explain the evolutionary relationship and identify their subfamilies. MUSCLE 3.8 was used to perform multiple-sequence alignment of the protein sequences during the process [58]. and the maximum likelihood (ML) phylogenetic tree was constructed using MEGA7 with the bootstrap of 1000 replicates [59].

Identification of cis-regulatory elements and prediction of three-dimensional modeling

The promoter regions for each gene was defined as the sequence 2000 bp upstream of the start codon, and the promoter sequences were retrieved from each genome using the SAMtools program [60]. The PlantCARE server, a database of plant cis-acting regulatory elements was used to predict the putative transcriptional response elements within these gene promoters (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [61]. The three-dimensional structure of a representative MATE protein from each subfamily was determined using the Phyre2 server to identify the variations in structure and their impact on functions (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index) [62].

Gene ontology (GO) analysis and miRNAs prediction

GO analyses was done using the Blast2GO software (v10.1) [63]. To obtain the predicted miRNA targets in the coding sequences of *TaMATEs*, 299 published miRNAs was analyzed using the psRNATarget online server (http://plantgrn.noble.org/psRNATarget/analysis?function=2) [64, 65].

Results

Identification and analysis of MATE gene family in wheat

A total of 20 MATE genes were discovered from the wheat genome using homology search and MATE domain analysis, and they were designated TaMATE1–20 depending on their chromosomal position. MATE proteins from wheat encoded by 20 *TaMATE* genes were sequenced, and the proteins were found to be 202–533 amino acids long, with molecular weights ranging from 21.45 kDa to 58.20 kDa, theoretical isoeletric points (p*I*) ranging from 5.46 to 9.19, GRAVY ranging from 0.449 to 0.783, and the number of TM regions in TaMATE proteins ranging from 3 to 12. (Table 1). The sequence alignment indicated the presence of a conserved domain (MatE, pfam01554) and numerous residues in the MATEs (Fig. 1 A-B), indicating that the MATE domain organization is highly conserved.

The results of subcellular localization prediction showed that MATE proteins of wheat are well distributed in the plasma membrane (PM), chloroplast (chlo), vacuolar membrane (vacu), nucleus (nucl), endoplasmic reticulum (ER), and mitochondria (mito), golgi body (golg), cytoskeleton (cyto), extracellular and (extr). Surprisingly, 50% of TaMATE proteins were localized to the plasma membrane, 28% to the golgi body, and 9% to the endoplasmic reticulum. However, TaMATE10 was specifically localized to the endoplasmic reticulum and extracellular (Fig. 2)

Chromosome distribution and MATE gene duplication in wheat

The 20 *TaMATE* genes were found on 3 of the 12 wheat chromosomes (Table 1). Chromosomes 1 and 5 each had six TaMATE genes and chromosome 7 had eight TaMATE genes. Two gene pairs, *TaMATE3- TaMATE5* and *TaMATE4 - TaMATE6* exhibited tandem duplication, whiles three gene pair, *TaMATE9 - TaMATE13*, *TaMATE10 - TaMATE12*, and *TaMATE14 -TaMATE19* showed segmental duplication (Table 2). The synonymous rate (Ks) for the segmental duplicated gene ranged from 0.114 to 0.228, with a and divergent time ranging from 8.67 to 17.35 Mya. The Ka/Ks value for the segmental duplicate gene ranged from 0.061 to 0.348, whereas that of the two tandem duplicate genes ranged from 0.093 to 0.095. The Ka/Ks values of the segmental and tandem duplicates were less than 1, indicating that purification selection occurred in these duplicates (Table 2).

Phylogenic analysis and classification

Following multiple sequence alignment, a phylogenetic tree was constructed using the MEGA-X software (v10.0.2) for the 20 *TaMATE* proteins, 10 *AtMATEs*, 10 *OsMATEs*, 10 *ZmMATEs*, and 10 *StMATEss*. *MATE* genes from wheat, rice, Arabidopsis, potato, maize, and rice were classified into four major subfamilies (groups), namely I-IV. Group I had 9 *TaMATE* genes with 3 *OsMATEs*, 7 *AtMATEs*, 3 *StMATEs*, and 4 *ZmMATEs*. Group II had 1 *TaMATE* with 3 *ZmMATEs*, 2 *StMATEs*, and 2 *OsMATEs*. Group III contained 8 *TaMATEs* with 2 *AtMATEs*, 4 *OsMATEs*, 2 *ZmMATEs*, and 4 *StMATEs*, while group IV had 2 *TaMATEs* with 1 *AtMATE* and 1 *StMATE* (Fig. 3).

Gene structure and motif analysis of MATE genes in wheat

From the results of the gene structure analysis, the twenty *TaMATE* genes could be divided into three separate groups (I-III) based on the similarity of their gene sequence. Group I had eight *TaMATE* genes, Group II had eight *TaMATE* genes, and Group III had four *TaMATE* genes (Fig. 4 A). Genes had one exon (5%, *TaMATE9*), two exons (9%, *TaMATE8* and *TaMATE17*), three exons (5*%, TaMATE16*), five exons (5%, *TaMATE9*), six exons (9%, *TaMATE15* and *TaMATE20*), seven exons (14%, *TaMATE2*, *TaMATE13*, and *TaMATE18*), eight exons (48%, *TaMATE1*, *TaMATE3*, *TaMATE4*, *TaMATE5*, *TaMATE10*, *TaMATE11*, *TaMATE12*, *TaMATE114*, and *TaMATE19*), nine exons (5%, *TaMATE6*) (Fig. 4A).

The conserved motifs of the *TaMATE* proteins were identified using the MEME program, and the motif sequences and annotations were further predicted by Pfam online server. The results showed that 10 putative conserved motifs were identified in most of the *TaMATE* proteins (Table 3, Fig. 4 B and C). The conserved motifs varied in length from 15 to 50 amino acids, with putative *TaMATE* domains predicted in conserved motifs 1, 2, 3, 4, 5, 6, and 8 of the *TaMATE* proteins (Table 4). Motifs 1, 2, 3, 4, 5, 6, and 8, which contained MATE domains, were found to be highly conserved in the 20 *TaMATE* proteins. Twelve (12) *TaMATEs* (60%) contained all ten motifs, 4 *TaMATEs* (20%) possessed nine motifs, 3 *TaMATEs* (15%) possessed five motifs, and 1 *TaMATE* (5%) possessed four motifs.

Promoter cis-acting regulatory element analysis and 3-dimensional modelling

Cis-acting regulatory elements (CAREs) serve as specific binding sites for transcription factors and so play a crucial role in regulating genes involved in the growth, differentiation, and development of organisms, including plants. The number of cis-acting regulatory elements found in the putative *TaMATE* genes ranged from 56 (2%, *TaMATE9*) to 173 (6%, *TaMATE6*) (Fig. 5 A). According to their functional annotations, the CAREs were divided into three groups: growth and development responsive elements (15%), phytohormone responsive elements (46%), and environmental stress responsive cis elements (39%) (Fig. 5 B). Furthermore, the growth and development responsive elements were CAT-box, ARE, AACA_motif, O_2-site, NON-box, RY-element, GCN4_motif, GC-motif, HD-Zip 1, which were mostly involved in meristem expression, anaerobic induction, endosperm-specific negative expression, zein metabolism regulation, meristem specific activation, seed-specific regulation, endosperm expression, anoxic specific inducibility, and differentiation of the palisade mesophyll cells, respectively (Fig. S1). CGTCA-motif, TGACG-motif, TGA-element, ABRE, TATC-box, TCA-element, P-box, GARE-motif, and

AuxRR-core elements in group II were implicated in the response to plant hormones such as gibberellin, auxin, abscisic acid, and methyl jasmonate (Fig. S1), whereas In group III, MBS, 3-AF1 binding site, G-box, G-Box, Sp1, CGTCA-motif, LTR, ACE, TC-rich repeats, GT1-motif, and MRE were implicated in low-temperature, defense and stress, and light responses (Fig. S1).

MATE proteins were predicted to have secondary structures, such as α-helix, TM helix, and coil structures (Fig. 6 and Table S2). The multiple α-helix structures ensured that MATE proteins were transported across the membrane in an efficient and stable manner. Most MATE proteins had comparable three-dimensional structures, and the closer the evolutionary relationship between genes, the more similar the three-dimensional (secondary) structures of the proteins were, as was the case with *TaMATE10*, 11, 12, 13, and 14 (Fig. 6 and Table S9).

Gene ontology (GO) analysis and miRNA targets

The analysis of *MATE* GO annotations showed three elements of functional classification: biological process (BP), molecular functions (MF), and cellular component (CC) (Fig. 7). The 20 *MATE* genes were involved in all the GO functional annotation. For BP, MF, the *MATE* genes were mainly involved in transmembrane transport, detoxification, response to toxic substances, and export of toxic substances across the plasma membrane (Fig. 8 A). For MF, the *MATE* genes were involved in activities such as transmembrane transport, xenobiotic transport, secondary active transport, and antiporter activity (Fig. 8 B). The 20 *MATEs* annotated to the CC were shown to function in cellular membrane (Fig. 8 C).

The predicted miRNA target regions of the *MATE* genes were identified using the psRNATarget online server, and 299 miRNAs targeting the *MATE* genes were identified (Fig. 8 A and Table S2). Tae-miR5175e was the miRNA that highly targeted *MATE* genes (Fig. 8 B). TaMATE11, 16, 13, and 14 are the genes highly targeted by the miRNAs (Fig. 8 B). The discovered miRNAs were involved in either cleavage (Fig. 8 C) or translation inhibition (Fig. 8 D). Among them, *TaMATE11* showed the highest number of predicted miRNAs involved in cleavage inhibition (11%), whereas *MATE7* showed the highest name of predicted miRNAs involved in translation inhibition (22%) (Fig. 8C-D).

Expression level analysis of MATE genes in wheat under drought, heat, and salinity stress

To investigate whether abiotic stress affects the expression levels of *TaMATE* genes, we chose seven *TaMATE* genes (from each subfamily, depending on gene structure and phylogenetic analysis) after analyzing the phylogenetic tree and promoters, and evaluated their relative expression levels in leaf tissue after drought, heat, and salinity stress for 5, 10, and 15 d using qRT-PCR (Fig. 9). The results of qRT-PCR analysis revealed that, when compared to control (CK), the time for TaMATE genes to reach a higher expression level in the same tissue was comparable, i.e., the period for most genes to reach the highest expression level in leaf tissues was 15 d, under these three abiotic stress conditions. *TaMATE1*, *TaMATE2*, and *TaMATE18* expression levels rose 3.8-fold, 3.4-fold, and 2.5-fold, respectively, after 15 days of drought stress treatment, compared to the control. Similarly, *TaMATE1* and *TaMATE18* were the most highly expressed genes under heat stress, with 3.7-fold and 2.9-fold increases in expression, respectively. Additionally, when exposed to salt stress, the expression of *TaMATE2* and *TaMATE18* increased 3.6-fold and 2.4-fold, respectively, compared to the control (Fig. 9). *TaMATE18*, on the other hand, was the most expressed gene after 15 days of drought, heat, and salt stress, indicating that the expression patterns of genes in the same subfamily might differ considerably even under the same stress treatment conditions. The findings revealed that some genes had a greater and distinct expression pattern when subjected to certain stress conditions and the expression pattern of most of *TaMATE* genes, for example, followed a trend of 5 d > 10 d > 15 d.

In addition, the relative expression levels of the six *TaMATE* genes were utilized in a clustering analysis, with the results shown in a heat map (Fig. 10). The six *TaMATE* genes were classified into distinct groups based on the findings under drought, heat, and salinity stress conditions. Under drought stress, group I exhibited a lowly upregulated gene (*TaMATE13*), but group II had upregulated genes (*TaMATE1*, 10, 18, 2, and 14). (Fig. 10 A). *TaMATEs* were categorized into

one distinct group under heat stress, and among the genes in this group, *TaMATE1* and *TaMATE14* were the most highly expressed (Fig. 10 B).

Furthermore, the relative expression values of the seven *TaMATE* genes were used in a clustering analysis and the results displayed in a heat map (Fig. 10). From the results, the seven *TaMATE* genes were classified into distinct groups under drought, heat, and salinity stress conditions. Under drought stress, group I had gene (*TaMATE13*), which was lowly upregulated, while group II had genes (*TaMATE1*, 10, 18, 2, and 14) which were upregulated (Fig. 10 A). Under heat stress, *TaMATEs* were classified into one distinct group and among the genes in this group, *TaMATE1* and *TaMATE14* were the highly expressed genes (Fig. 10 B). Similarly, under salt stress, TaMATE14, 1, and 13 comprised group I, and these genes were weakly expressed, whereas *TaMATE13*, *2*, *10*, and *18* comprised group II, and these genes were highly expressed (Fig. 10 C).

Physiological response to drought and heat stress in wheat

"Geumgangmil", a commonly cultivated wheat genotype was used to assess the impact of drought and heat stress on wheat. According to the findings, treatments (drought, heat, and salt stress) caused significant alterations in the physio-biochemical indicators evaluated when compared to the control. However, the magnitude of change in these parameters differed among stress treatment (Fig. 11). For example, after 15 d of stress treatment, a significant 16%, 6%, and 11% decrease in RWC was observed under drought, heat, and salt stress conditions, compared to control (Fig. 11 A). The EL values increased by 61% (under drought stress), 65% (under heat stress), and 75% (under salinity stress) (Fig. 11 B). Furthermore, the MDA levels increased by 50% (under drought stress), 55% (under heat stress), and 62% (under salt stress) (Fig. 11 C), where a significant 35%, 52%, and 73% increase in proline content were observed under drought, heat, and salt stress treatment conditions, respectively (Fig. 11 D). The RWC, EL, MDA, proline, and the expression pattern of putative MATE genes (*TaMATE1*, *TaMATE2*, *TaMATE10*, *TaMATE13*, *TaMATE14*, and *TaMATE18*) were significantly affected by genotype (G), treatments (T), and their interactions (G × T) under drought, heat, and salt stress conditions, according to the two-way analysis of variance (ANOVA) (Table S3-5).

Correlation analysis between TaMATE gene expression and physiological stress indicators

Pearson's correlation analysis was done to examine the relationship between physiological indicators and the expression of putative *TaMATE* genes. From the results, the expression of most of the *TaMATEs* was associated (negative or positive) with the physiological stress indicators (Table S6-S8). For example, under drought stress conditions, RWC, EL, and proline were strongly associated with *TaMATE1* and *TaMATE18* expression, while MDA was correlated with *TaMATE1* expression (Table S6). EL was strongly associated with *TaMATE1* and *TaMATE10* expression and proline with *TaMATE10* expression under heat stress conditions (Table S7), whereas under salt stress conditions, RWC and EL were associated with *TaMATE1* and *TaMATE18* expression, whereas proline level was correlated with *TaMATE18* expression (Table S8).

Discussion

To resist the invasion of harmful substances from the outside and to prevent the buildup of poisonous substances released in their own metabolism, organisms have evolved several defensive mechanisms [66]. Plants, as eukaryotic organisms, excrete toxins that accumulate in the cell via transmembrane transporter activity, aided by multidrug efflux pumps [67]. The multidrug and toxin extrusion (MATE) protein family is one of five families of multidrug efflux pumps found in plant cell membranes. It has been reported that MATE proteins play an important role in the efflux of plant secondary metabolites, acting as gatekeepers for cells by regulating the inflow of useful substances and the exudation of harmful ones [16]. MATE family genes have been studied in some plant species [33, 36, 68–70], but not in wheat. Wheat is a protein-rich grain crop. As a result, we anticipate that research into MATE transporters in wheat will play a significant

role in wheat breeding. Analysis of the MATE family in wheat will aid in clarifying the molecular genetic basis of wheat genetic improvement and provide a foundation for transgenic research.

We discovered 20 MATE-encoding genes from the wheat genome in this study, which can be classified into three main subfamilies, which is consistent with previous classification of MATE genes in [70] (Fig. 3). MATE proteins were found in several cellular compartments in tomato based on subcellular localization analysis (Fig. 1). For example, the 20 putative *MATE* genes were found to be strongly localized in the plasma and vacuolar membrane, suggesting that TaMATE may be a membrane-localized protein [69, 70]. Wheat MATE protein length ranged from 202 to 533 amino acids, compared to Arabidopsis thaliana and Populus protein lengths of 400 to 700 amino acids and 120 to 608 amino acids, respectively. The putative *TaMATEs* exhibited considerable regularity in gene structure and protein motifs, indicating that the wheat MATE family is highly conserved. Despite having a bigger genome (16 gigabytes), the number of MATE genes discovered in wheat was comparable to that in Arabidopsis [20]. The study of Arabidopsis thaliana's evolutionary history showed four rounds of segmental duplications, which explains this phenomenon.

TaMATE genes showed differential localization on the 4 of the 12 chromosomes of wheat, including five paralogous gene pairs (Tables 1–2). Two of these gene pairs, *TaMATE3 - TaMATE5* and *TaMATE4 - TaMATE6* exhibited tandem duplication, whiles three gene pairs, *TaMATE9 - TaMATE13*, *TaMATE10 - TaMATE12*, and *TaMATE14 - TaMATE19* showed segmental duplication (Table 2). The major driving factor for evolution, leading to functional speciation and diversity, is considered to be gene duplication [71]. The results of the gene duplication analysis showed that the *TaMATE* gene family has evolved rapidly, with segmental events largely contributing to the expansion, which was accompanied by tandem duplications. Previous research has shown that the tomato genome experienced two distinct large-scale genome and/or segmental duplication events. One of these ancient duplications happened approximately 170–235 Mya, just after the split of monocots and dicots. The other type of duplication was recent polyploidy duplication, which happened around 90 Mya and corresponds to the estimated separation period of tomato and Arabidopsis [72].

Additionally, the Ks values of segmental duplicates in wheat *MATE* genes ranged from 0.114 to 0.228, corresponding to a divergence time ranging from 8.679 to 12.502 (Table 2), indicating that gene duplication events occurred before the split of tomato and Arabidopsis. *TaMATE3 - TaMATE5* and *TaMATE4 - TaMATE6* in the wheat genome, on the other hand, grouped on chromosomes 2, formed a tandem duplicate cluster and the period of clustered divergence ranged from 6.708 Mya to 6.131 Mya. Tandem duplications are likely to occur than segment duplications, because tandem duplications in plants are likely to participate in stress responses, and these tandem duplicates were not preserved as long as non-tandem duplicates (Table 2) [73, 74].

Because phylogenetic analysis of genes can contribute to determining their function in a given species [75], we perform phylogenetic analysis on the 20 *TaMATE* genes and observed that they are divided into three major subfamilies (I–IV). Previous research classified rice and Arabidopsis *MATE* genes into four subfamilies, whereas cotton *MATE* genes were classified into three subfamilies based on the topological structure and self-priming value of the protein sequence [32, 76]. From our data, the putative 20 *TaMATE* genes were grouped with *MATE* genes from other species in all the four groups (subfamily). Among them, the Arabidopsis thaliana *AtMATE1* in group III, is thought to be localized in the plasma membrane and mediates the export of exogenous toxic compounds such as berberine, and flavonoid transporter, which regulates flavonoid levels, as well as growth and development under osmotic stress condition in A. thaliana [20, 77]. *OsMATE1* (subfamily III) and *OsMATE2* (subfamily I) have been reported to regulate plant growth and development as well as negatively affect disease resistance [31]. Furthermore, in subfamily I, *ZmMATE1* and *ZmMATE6* have shown to be highly upregulated, conferring tolerance to Aluminum (Al) treatment in maize [78]. Additionally, in subfamily II, *StMATE5* was highly induced, conferring tolerance to nickel (Ni^{2+}) stress in *S. tuberosum* [69]. Based on an assessment of the known protein functions in subfamilies (I-III), we discovered that *MATE* genes from the same subfamily have the same or similar functions, but MATE genes from other subfamilies have entirely distinct functions. This aligns with previous

findings [32, 70]. As a result, the grouping of MATE in this study set the basis for the study of the functions of *MATE* genes in wheat.

Gene structure and motifs of *TaMATEs* were examined, since protein functions are impacted by their structures [79]. The findings showed that *TaMATE* genes in the same subfamily had comparable exon–intron structure and shared conserved motifs. Intron gains and losses reveal the evolution of gene families [80], which shows that homologous genes have a comparable structure and function, such as in subfamily III, where TaMATE17, 9, 18, and 18 lacked or had only one intron, indicating that their amplification might be happening differently from genes in other subfamilies. The variation in the gene structure may be linked to dynamic regulation of MATEs in plant cell metabolism. Furthermore, the results of a motif analysis among all three (III) subfamilies showed that all *TaMATE* genes commonly possessed motifs 1, 2, 8, and 9 (Fig. 5 B-C). Conserved amino acid motifs help in compartmentalization of proteins into subfamilies and may have significance to the function of the proteins within the family [81].

Plant promoters are essential regulatory elements for transcription of plant gene and perform significant transcriptional regulatory functions [82]. Based on their functional annotations, 489 cis-acting elements were selected and classified, and 38% (71/21) of them were associated with plant growth and development, 46% (227/289) were involved in phytohormone response, and 39% (191/489) were involved in environmental stress responses (Fig. 6). Among the environmental stress response elements, MBS (predicted in TaMATE1, 3, 5, 6, 7, 8, 10, 11, 14, 16, 19, and 20) and TC-rich repeats (predicted in 10, 15, and 17) were the cis-elements identified to be involved in defense and stress response, which is consistent with previous findings [83]. The findings indicated that these genes may be associated with tomato drought resistance.

Gene ontology helps in the functional study of genes by assessing their similarities to other known function genes, as well as improve genes annotation. From the study, all *TaMATEs* were annotated and assigned GO terms (Fig. 7). In the cellular component (CC) category, *TaMATEs* showed enrichment by cell membrane (Fig. 8 B), which is consistent with the prediction of subcellular localization of *TaMATEs* (Fig. 1). In the molecular function category (MF), xenobiotic transmembrane transporter activity (GO:0042910) was the most enriched category followed by transmembrane transporter activity (GO:0022857) (Fig. 8 A). A xenobiotic is a substance that is alien to the organism that is exposed to it. The activity of xenobiotic transmembrane transporters allows for the controlled flow of substances from one side of a membrane to the other. This is important in helping plants to ensure membrane stability under abiotic stress conditions. The findings indicated that *TaMATEs* have numerous functions in plant cell metabolism.

Plant miRNAs are small-sized non-coding stranded RNAs that have been shown to be crucial in plant response to abiotic stresses through gene expression regulation at the post-transcriptional level [84]. In the study, the predicted miRNAs targeted the putative *MATE* genes in wheat (Fig. 9). Tae-miR5175e was the highly predicted miRNA in *TaMATE* genes. There is presently no information about Tae-miR5175e functions in wheat or other plant species. The findings support the post-transcriptional regulation of *MATE* genes during developmental stage and highlight the potential function of the highly targeted miRNAs (Tae-miR5175e) under drought, heat, and salt stress conditions.

According to the findings of the qRT-PCR study, the six selected *TaMATE* genes were differently expressed in leaf tissue under three abiotic stress conditions (Fig. 10). Notably, genes from the same subfamily have similar expression levels when subjected to the same stresses. It is consistent with the findings of gene structure and motif analysis (Fig. 4), which show that genes in the same subfamily have comparable structure and function. The mean expression level of genes followed a trend of 5 d > 10 d > 15 d under all stress conditions. Under various stress conditions, however, genes from different subfamilies respond to stresses at varying degrees. Under drought stress for 5 days, for example, the expression level of *TaMATE14* rose 9-fold compared to controls. *TaMATE10* was substantially increased by 5-fold and 4-fold under heat and salt stress conditions, respectively. *TaMATE14* and *TaMATE10*, interestingly, are in the same subfamily as *AtMATE1* on the phylogenetic tree (subfamily III). *AtDTX1 (AtMATE1)* improved tolerance to abiotic (drought, cold, and salt) stress in transgenic Arabidopsis plants [30]. As a result, we hypothesize that *TaMATE14* plays an important role in

wheat drought stress responses. The expression level of *TaMATE10* was consistently induced by salt and heat stress. The duration and severity of the three abiotic stresses (drought, heat, and salt) on wheat might be ascribed to this finding [85]. Salinity stress significantly increased the expression levels of *TaMATE1* and *TaMATE2* as compared to the control. *OsMATE2* improved rice tolerance to salt stress [86]. As a result, we expect *TaMATE1* and *TaMATE2*, which belong to the same subfamily (subfamily I), to be implicated in wheat salt stress response. In the same study, *OsMATE4* improved rice tolerance after 24 hours of salt stress treatment, which was consistent with the expression level of *OsMATE20* under salt stress conditions, indicating that *TaMATE20* may also be involved in wheat salt stress response. *TaMATE2*, 14, 18, and 20 expression levels were significantly upregulated after 15 d of drought treatment, and *TaMATE2*,10,13, and 14 expression levels were significantly increased after 15 d of heat stress, similar to a higher upregulation of *TaMATE2*, 14, 18, and 20 after 15 d of salt stress treatment. *TaMATE2*, 14, 18, and 20 had a synergistic impact under drought stress, *TaMATE2*, 10, 13, and 14 under heat stress, and *TaMATE2*, 14, 18, and 20 under salt stress. Drought, heat, and salt stress are the primary abiotic factors that influence plant growth and development, and plants have adapted drought candidate gene expression as one of the mechanisms for increasing their resistance to the effects of these stresses [87]. The findings showed that *TaMATE2*, 10, 13, 14, 18, 20 are crucial for plant growth under various abiotic stress condition.

Previous research demonstrated that *LuDTX71* and *LuDTX73* belong to the same evolutionary branch as AtMATE1 and that they play an essential role in the response to cadmium, cold, and salt stress [34]. This finding, when complemented with our findings, clearly suggest that the *TaMATE* genes are involved in drought, salt, and heat stress. In addition, *CaMATE1* and *CaMATE28* expression is particularly increased under hormone stress circumstances, and phylogenic study revealed that they belong to the same evolutionary branch as *AtMATE1* [88]. This demonstrates that cis-acting elements involved in phytohormone responsiveness are required for the MATE gene family to respond to diverse stresses, and that MATE transporters can increase the ability to adapt to stress by increasing phytohormone efflux [89].

Furthermore, stress treatment (drought, heat, and salt) resulted in substantial alterations in the physiological indicators examined (Fig. 11). For example, a significant decrease in RWC was found under these treatment conditions when compared to control. The findings are consistent with earlier studies [90, 91]. Moreover, during drought, heat, and salt stress, the EL values, MDA, and proline levels were significantly higher in the treated plant of Geumgangmil than in the control. The findings are consistent with previous research findings [90, 92, 93]. Genotype, treatments, and their interaction produce a significant effect on RWC, EL, MDA, proline, and the putative *TaMATE* genes (Table S3-S5). The findings show for the first time the response of the common wheat genotype “Geumgangmil” to heat and salt stress, despite the fact that this genotype has previously been studied under drought stress conditions. [43, 44]. Additionally, to the best of the authors' knowledge, no study has established a link between physiological stress indicators based on the literature examined. According to our findings, putative *TaMATE* gene expression was highly (positively or negatively) correlated with RWC, EL, MDA, and proline under drought, heat, and salt stress conditions (Table S6-S8). As a result, the findings shed light on the relationship between *TaMATE* gene expression and physio-biochemical indicators of drought, heat, and salt stress.

Conclusion

In conclusion, the study identified 20 *TaMATE* genes from the wheat genome, divided them into four subfamilies, analyzed their expression pattern levels of seven *TaMATE* genes, and their relationship with physio-biochemical indicators under drought, heat, and salt stress conditions. Under three abiotic stress conditions, qRT-PCR analyses showed significant changes in the expression levels of seven *TaMATE* genes. The majority of the genes were substantially and differently induced by abiotic stress treatments. Albeit *TaMATE2* (I), 14 (III), 18 (IV), and 20 (IV) levels were significantly upregulated under drought stress, *TaMATE2* (I), 10 (III), 13 (III), and 14 (III) levels were significantly upregulated under heat stress, and *TaMATE2* (I), 14 (III), 18 (IV), and 20 (IV) levels were significantly upregulated under salt stress. The study's

findings will serve as the foundation for future research into the molecular processes of *TaMATE* genes in response to drought, heat, and salt stress in wheat, a globally important cereal crop.

Declarations

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Conflicts of interest/Competing interests

No potential conflict of interest is reported by the authors.

Availability of data and material

Data and materials are available upon request from the corresponding author.

Code availability

Not Applicable.

Authors' contribution statement

JNA conceived and design the experiment, conducted the experiments, analyzed data, and wrote the manuscript with support from YWS. YWS contributed to valuable discussions. All authors have discussed the results and approved the final manuscript.

Ethics approval (include appropriate approvals or waivers)

Not applicable.

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Tables

Table 1

Information on identified MATE genes in wheat.

Gene locus	Gene name	Chrom pos.	CDS length (bp)	AA	MW (kDa)	pI	Intron/ exon	GRAVY	SL	TMD
Traes_2AL_5925A665E	TaMATE1	2	1602	533	58.21	8.71	7/8	0.449	PM	11
Traes_2AL_D6E9FFDC9	TaMATE2	2	1431	457	49.26	8.84	6/7	0.765	PM	10
Traes_2BL_05B60AB14	TaMATE3	2	1464	487	52.28	8.53	7/8	0.713	PM	12
Traes_2BL_FFA34DA53	TaMATE4	2	1443	480	51.98	8.68	7/8	0.725	PM	10
Traes_2DL_3F9CD32D8	TaMATE5	2	1482	493	52.56	7.86	7/8	0.754	PM	12
Traes_2DL_F714CB6DF	TaMATE6	2	1443	480	52.00	8.68	8/9	0.704	PM	10
Traes_5AL_7693D91E1	TaMATE7	5	1026	342	36.40	8.44	4/5	0.736	PM	8
Traes_5BL_0B4FFE3B5	TaMATE8	5	810	270	28.79	8.46	1/2	0.783	PM	6
Traes_5BL_1C01C0970	TaMATE9	5	783	260	27.95	9.19	0/1	0.592	PM	5
Traes_5BL_97BEC5636	TaMATE10	5	1437	478	51.24	8.2	7/8	0.768	PM	12
Traes_5DL_80A6FDB52	TaMATE11	5	1449	482	51.91	8.18	7/8	0.769	PM	12
Traes_5DL_8DFB259AB	TaMATE12	5	1449	482	51.72	8.65	7/8	0.767	PM	12
Traes_7AL_2C76D82CD	TaMATE13	7	1428	475	50.98	8.69	6/7	0.732	PM	8
Traes_7AL_970814850	TaMATE14	7	1413	470	50.95	7.54	7/8	0.728	PM	10
Traes_7BL_19C0AB139	TaMATE15	7	1413	470	50.85	8.03	5/6	0.714	PM	10
Traes_7BL_BB760666F	TaMATE16	7	765	253	27.44	8.22	23	0.576	PM	3
Traes_7BS_37AA60A70	TaMATE17	7	606	202	21.46	5.46	1/2	0.717	PM	5
Traes_7DL_1DF94E957	TaMATE18	7	1452	453	48.42	8.68	6/7	0.771	PM	11
Traes_7DL_4A9E55BEB	TaMATE19	7	1413	470	50.93	7.06	7/8	0.723	PM	8
Traes_7DL_C1A7BA0C7	TaMATE20	7	1218	406	44.07	8.47	5/6	0.684	PM	6

Chrom pos, chromosome position; CDS, coding sequence; bp, base pair, AA, amin acid sequence; MW, molecular weight, kDa, kilo-Dalton; GRAVY, Grand average of hydropathicity; SL, subcellular localization prediction with cello-life software; TMD, transmembrane domain; and PM, plasma membrane.

Table 2

Ks, Ka, and Ka/Ks calculation and divergent time of the duplicated wheat MATE gene pairs. Ka, nonsynonymous substitution; Ks, synonymous substitution; Ka/Ks, nonsynonymous substitution over synonymous substitution; and Mya, million years ago.

Duplicated gene pairs	Ka	Ks	Ka/Ks	Type of duplication	Purity selection	Time (Mya*)
TaMATE3 - TaMATE5	0.008	0.088	0.095	Tandem	Yes	6.708
TaMATE4 - TaMATE6	0.007	0.080	0.093	Tandem	Yes	6.131
TaMATE9 - TaMATE13	0.079	0.228	0.348	Segmental	Yes	17.358
TaMATE10 - TaMATE12	0.011	0.114	0.100	Segmental	Yes	8.679
TaMATE14 - TaMATE19	0.010	0.164	0.061	Segmental	Yes	12.502

Table 3

Ten different motifs observed in wheat MATE proteins.

Motif	Protein sequence	Length	Pfam domain
1	WAYTGQILLLFGQDPEIAMEAGSYIRWMIPALFVYGPLQCHVRFLQTQNI	50	MatE
2	MFVGHLGELDLSSASIATSFANVTGFSLLSGMASSLDTLCGQAFGAKQYH	50	MatE
3	NHILVCWLLVYKLGLGNKGAALANTISYLTNVSILALYIRLSPSCKRTWT	50	MatE
4	EAFHDIVSFMKLAVPSALMVCWEWWSFELLVLFSGFLPNPKLEASVLSIC	50	MatE
5	RNLWGYAYSNEKEVVEYIARMMPILAVTFIFDDMQCVLSGIVRGCGFQKI	50	MatE
6	NTISLVFRIPYGLGAAISTRVSNELGAGRPDAARLATRVIMVLGLVSGVS	50	MatE
7	ALCFAFVYHLGGMGLWFGITCGLVVQMVLFMAITMRTNWDKEALKAKDRV	50	-
8	VVIEVKKQLYLAGPLIAGCLLQNVVQMISV	30	MatE
9	LGIYKQRAILVLTPVSVVVAV	21	
10	GACVNLSAYYLVGIP	15	

Figures

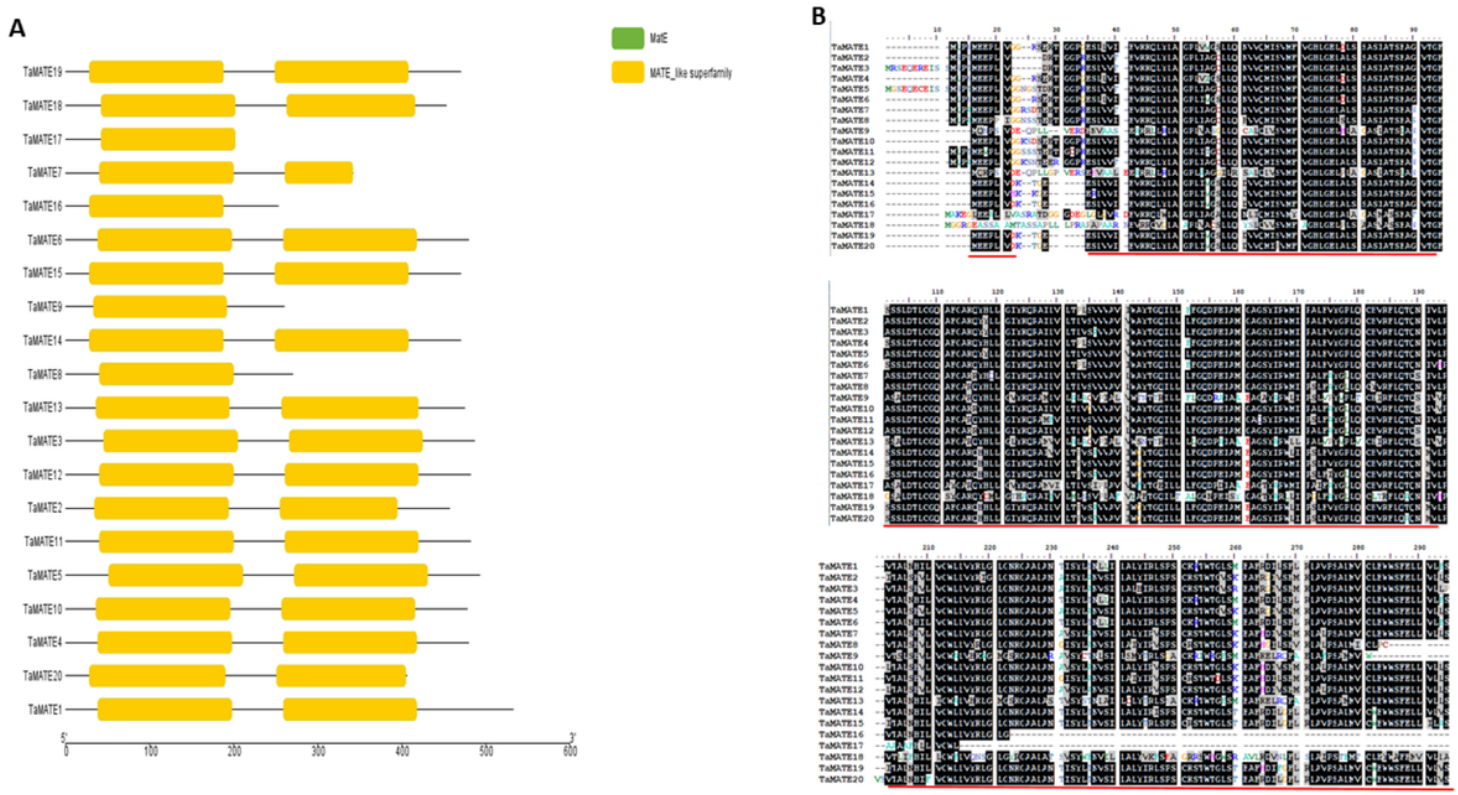

Figure 1

Domain structure features (A) and multiple sequences alignment of wheat MATE amino acid sequences (B). The red line shows the pfam01554 (transmembrane transporter, MatE) domain structure. In addition, the shading indicates the identity similarity with 50% and above.

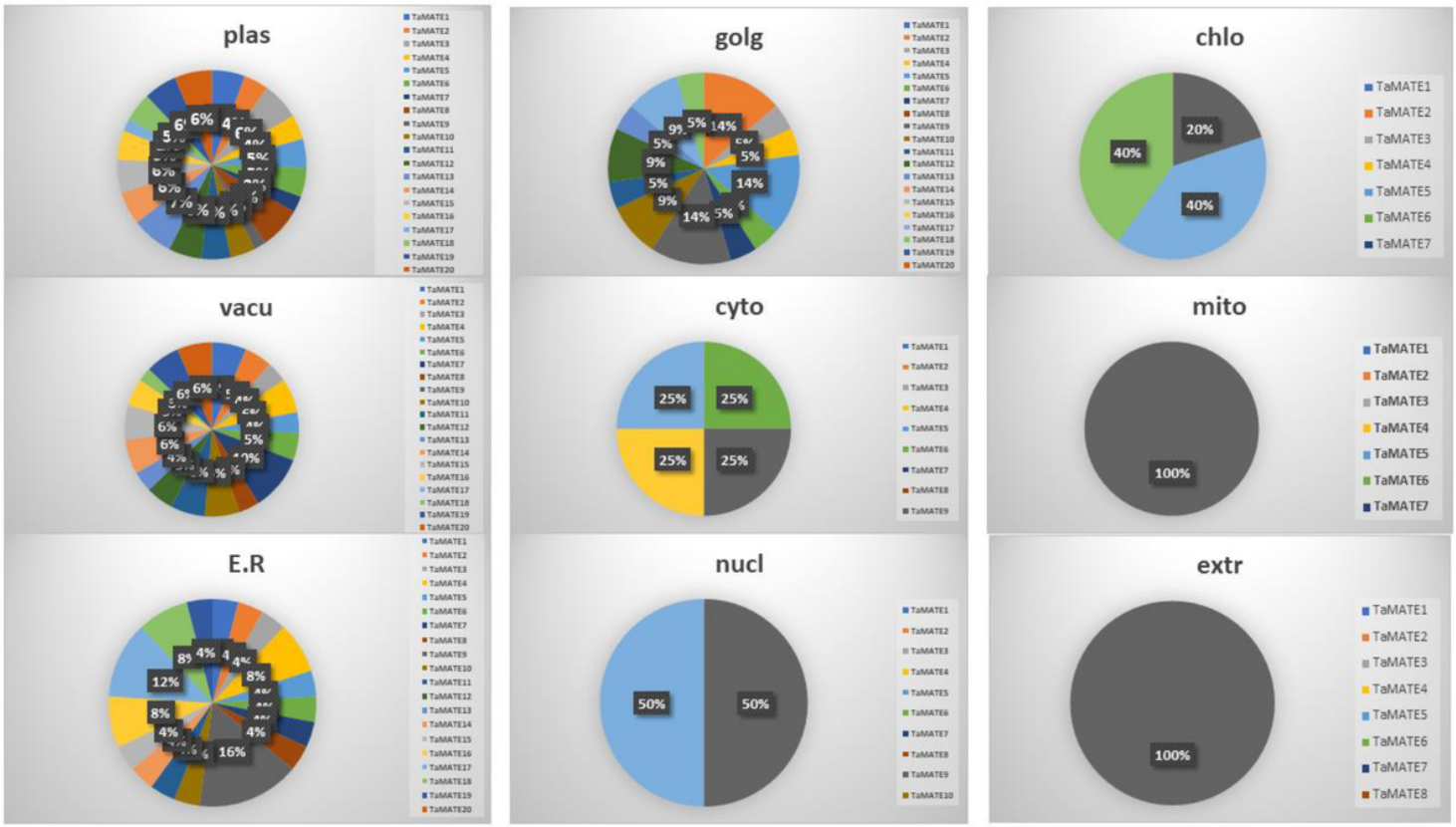

Figure 2

Subcellular localization prediction of putative MATE proteins using the Target P online sever. Nucl, nucleus; ER, endoplasmic reticulum; mito, mitochondria; chlo, chloroplast; golg, golgi body; plas, plasma membrane; extr, extracellular; vacu, vacuolar membrane; and cyto, cytoplams.

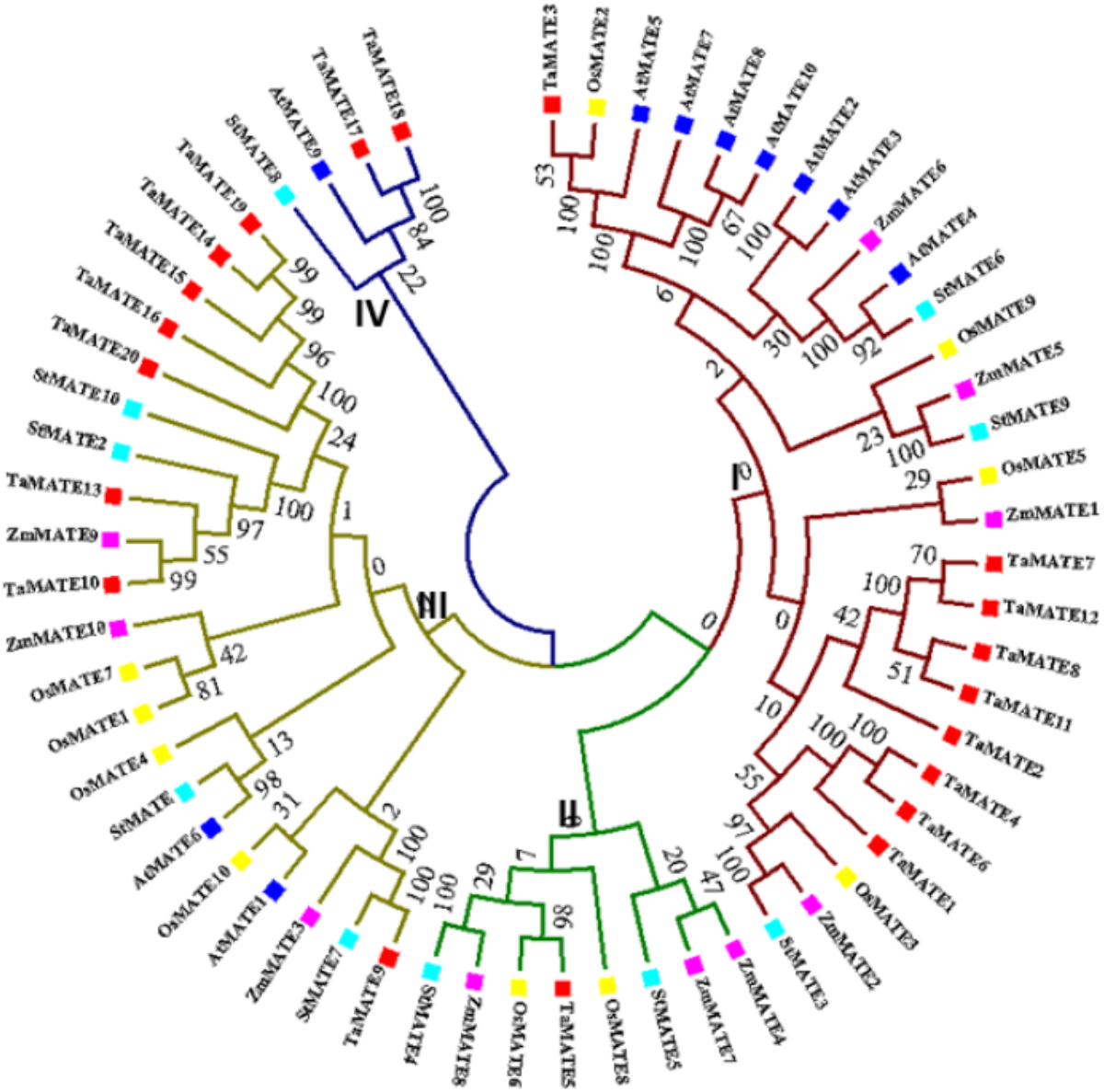

Figure 3

Phylogenetic relationship of TaMATE proteins in five plant species. The evolutionary relationship is presented using a phylogenetic tree. MATE proteins (TaMATE 1 TaMATE20) from tomato, Arabidopsis thaliana (AtMATE1-1-AtMATE10), maize (ZmMATE1- ZmMATE10), potato (StMATE1-StMATE10), and rice (OsMATE1-OsMATE10) are marked with red, blue, pink, cyan-blue, and yellow rectangles, respectively The neighbor-joining phylogenetic tree of the MATE protein sequences was constructed with 1000 bootstrap replicates by MEGA v10.0 (Pennsylvania State University, Philadelphia, PA, USA).

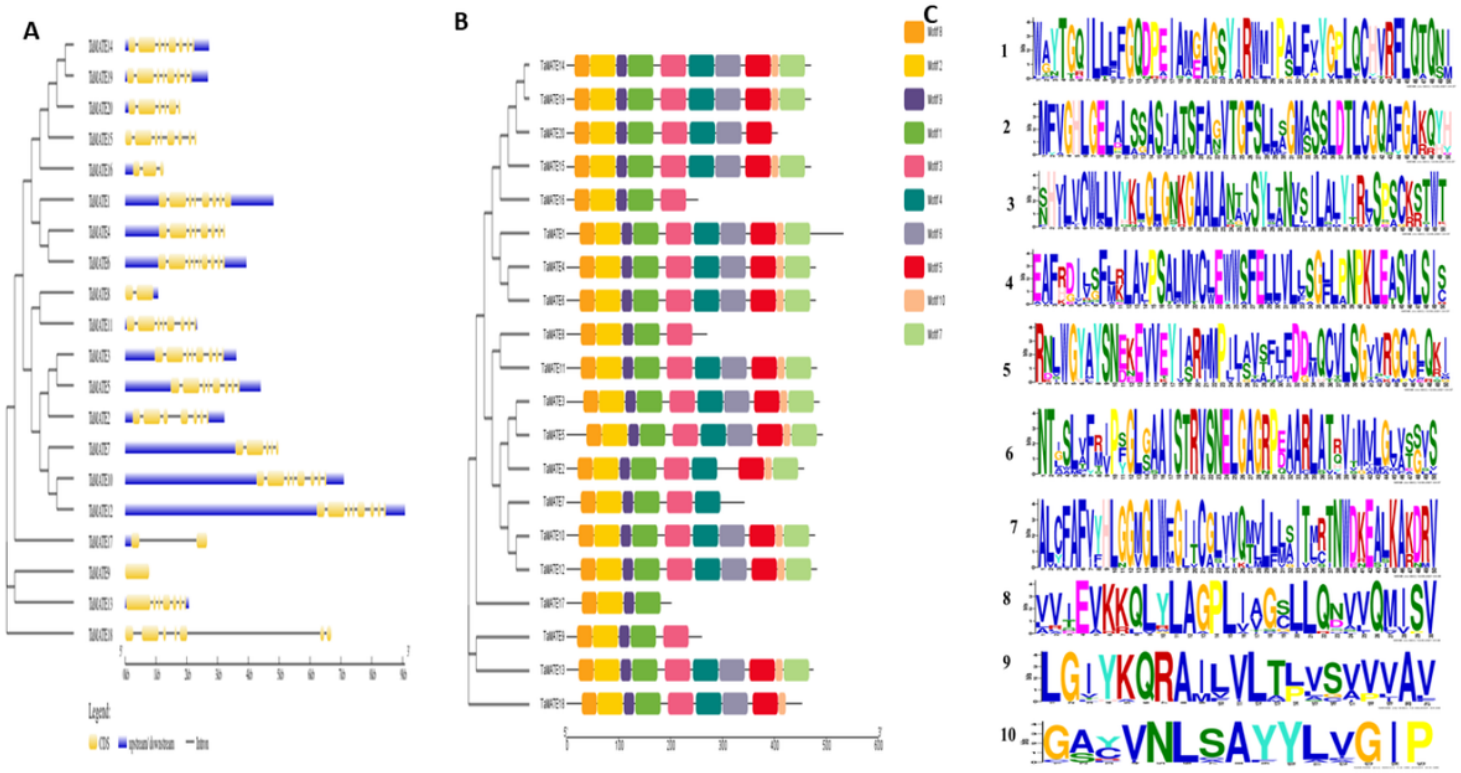

Figure 4

Gene (exon-intron) structure of TaMATE gene generated from the GSDS 2.0 (Gene structure display server) website (http://gsds.cbi.pku.edu.cn) (A). Conserved motifs of the TaMATEs identified by the MEME (Multiple Em for Motif Elicitation) (B) and motif structure (logos) (C). Each motif is indicated by colored box numbered at the bottom. The length of the motifs in each protein is proportional. The phylogenetic tree was constructed using the maximum likelihood method with 1000 bootstrap replicates by MEGA v10.0 (Pennsylvania State University, Philadelphia, PA, USA).

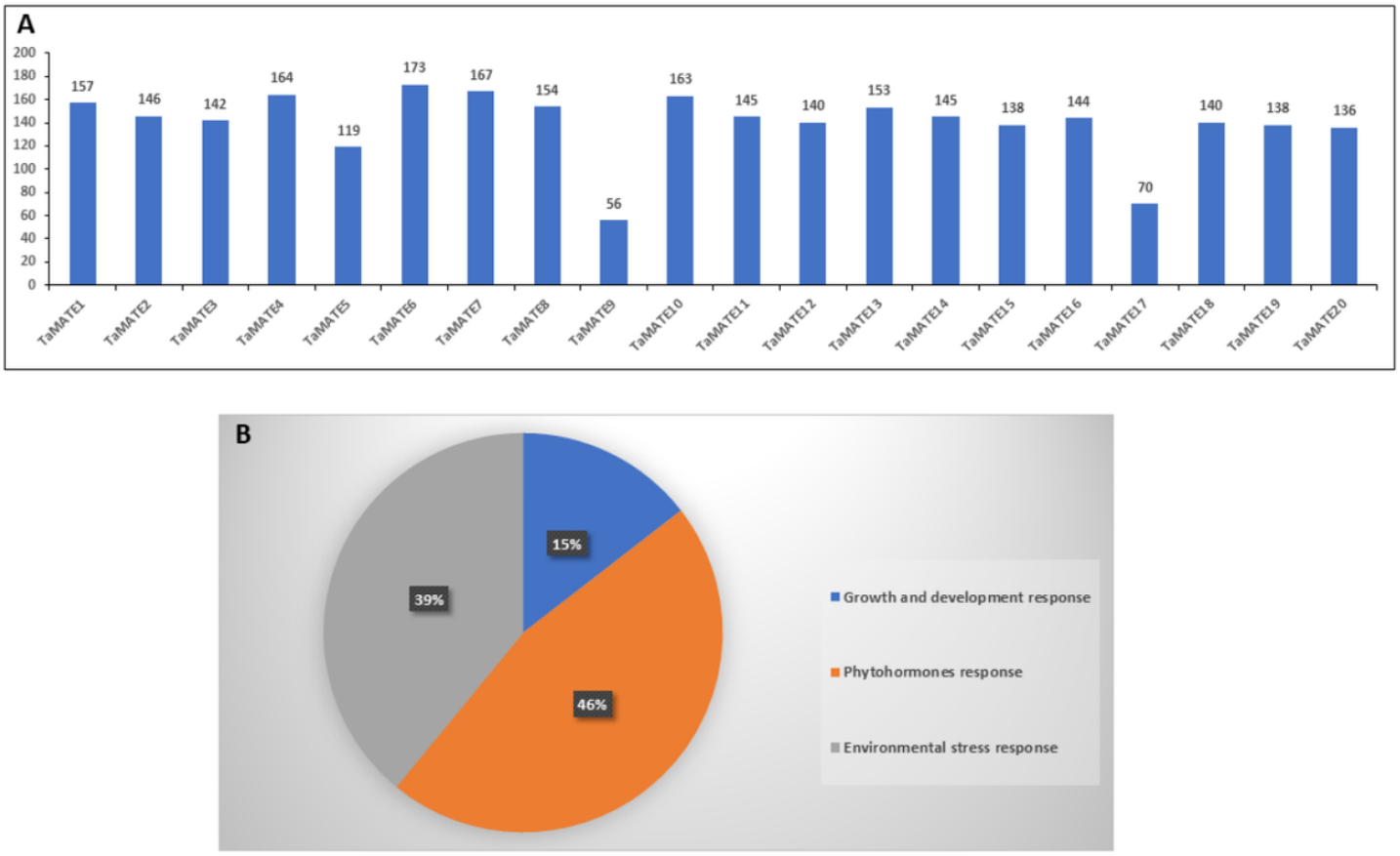

Figure 5

Cis-acting regulatory element (CREs) analysis of 20 TaMATE gene promoters in T. aestivum. The potential CREs in the promoter regions of 2,000 bp upstream of wheat were predicted by the PlantCARE software. Gene specific cis-acting regulatory elements (A) and classification of identified CREs (B).

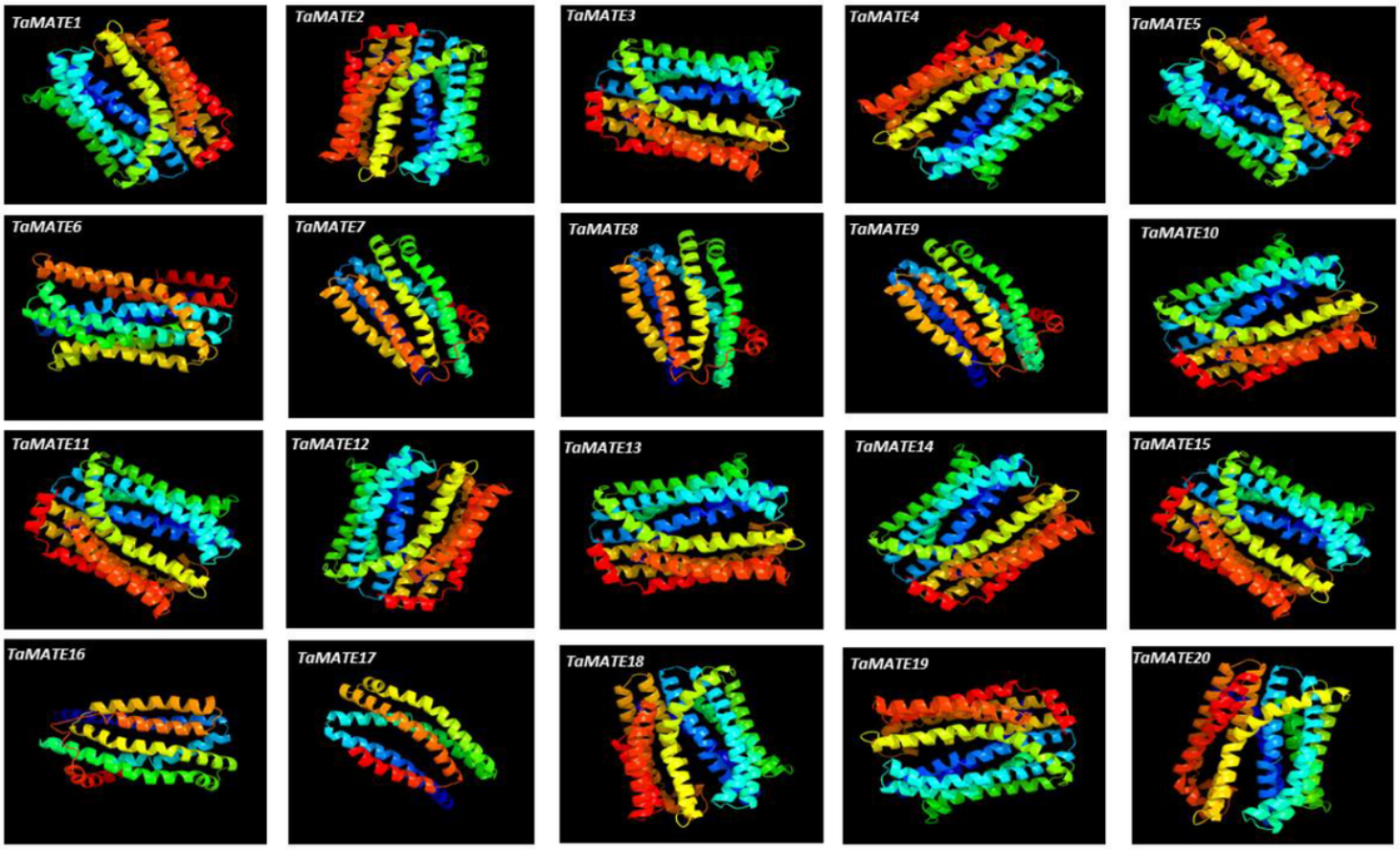

Figure 6

Three-dimensional modeling of domain of unknown function (MATE) proteins in tomato. The MATE proteins 3-D structures were predicted and displayed with a confidence level > 90%.

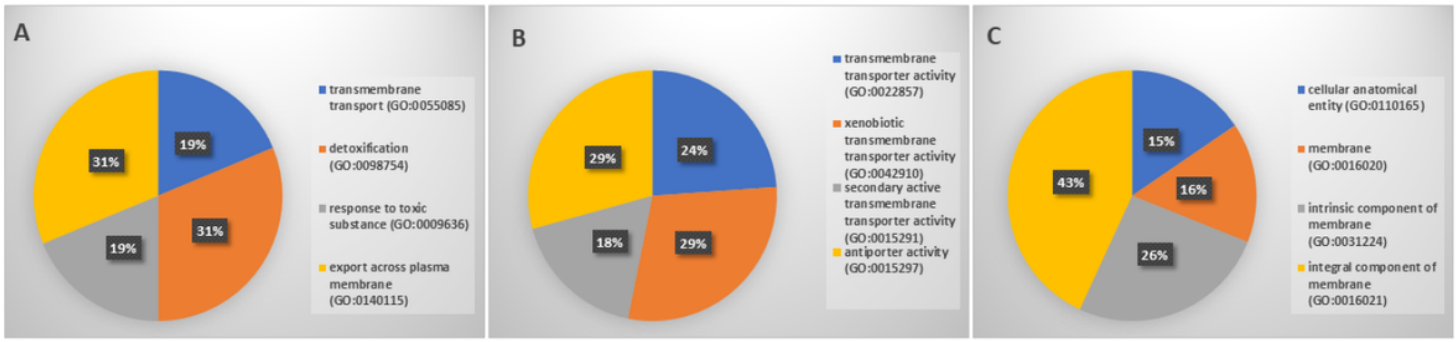

Figure 7

Gene ontology (GO) analyses of TaMATE genes in wheat. All annotated GO terms including molecular biological process (A), molecular function (B), and cellular component (C) of 20 TaMATEs.

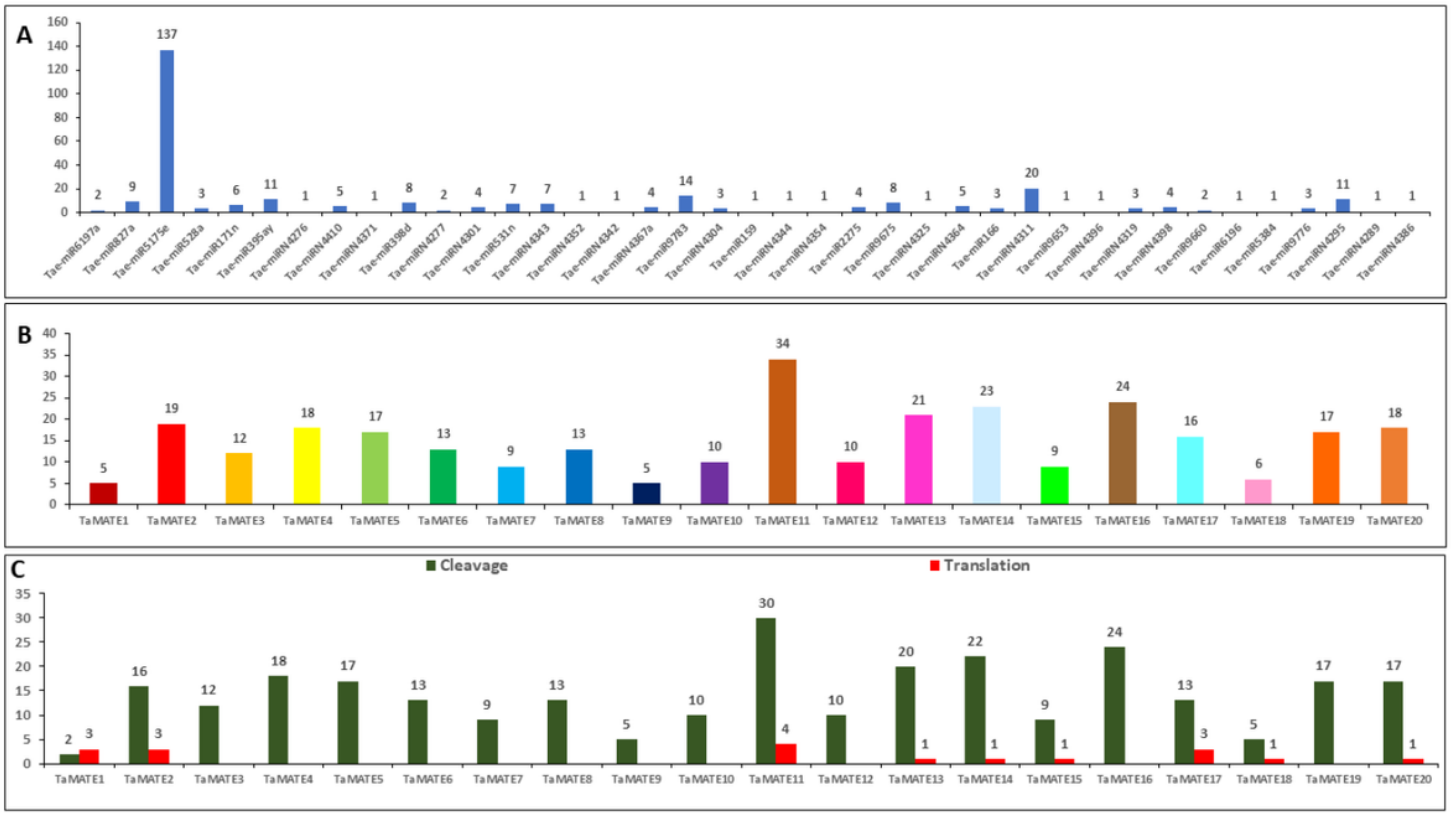

Figure 8

Predicted miRNAs targeting TaMATE genes based on 299 published miRNA sequences. Identified miRNAs (A), Gene specific miRNA targets (B), predicted miRNAs involved in cleavage and translation inhibition (C), and predicted miRNAs involved in translation inhibition (D). Details of the predicted miRNA and their targets are provided in Table S9.

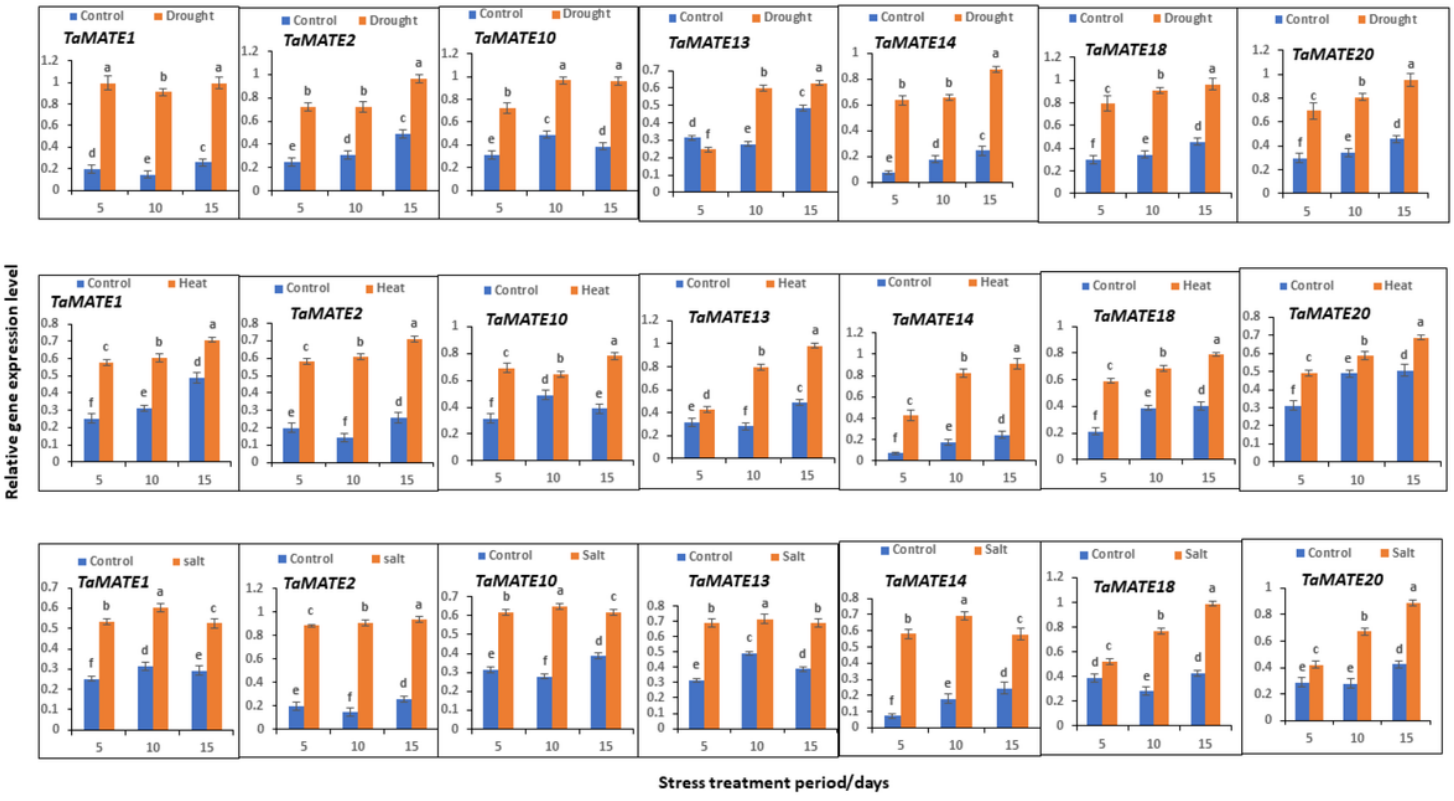

Figure 9

Expression patterns of 20 TaMATE genes in the leaf of wheat. qRT-PCR was conducted to analyze TaMATE gene expression in the leaves under control (CK), 5, 10, and 15 days of drought, heat, and salt stress treatment . Error bars depict standard deviation of the mean (n = 3). Different letters depict significant difference at P<0.05.

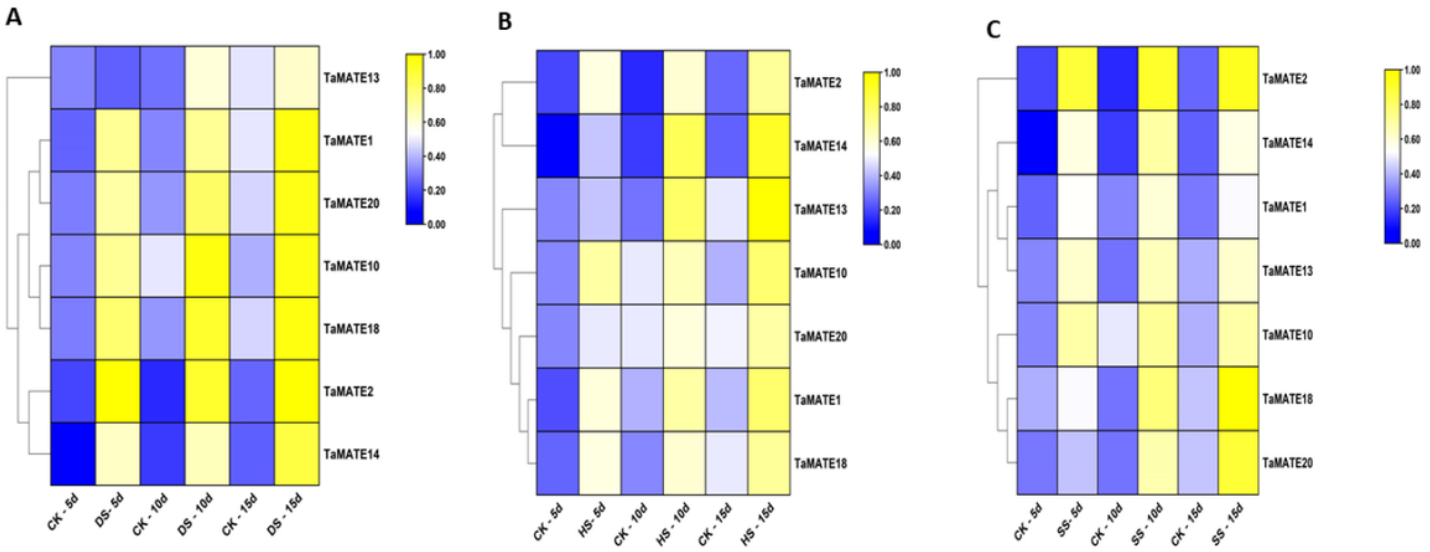

Figure 10

Heat map of the expression profiles of MATE genes under 5, 10, and 15 days of drought (A) heat (B), and salt (C) stress treatment in the leaves of Geumgangmil. Error bars depict standard deviation of the mean (n = 3). Different letters depict significant difference at P<0.05.

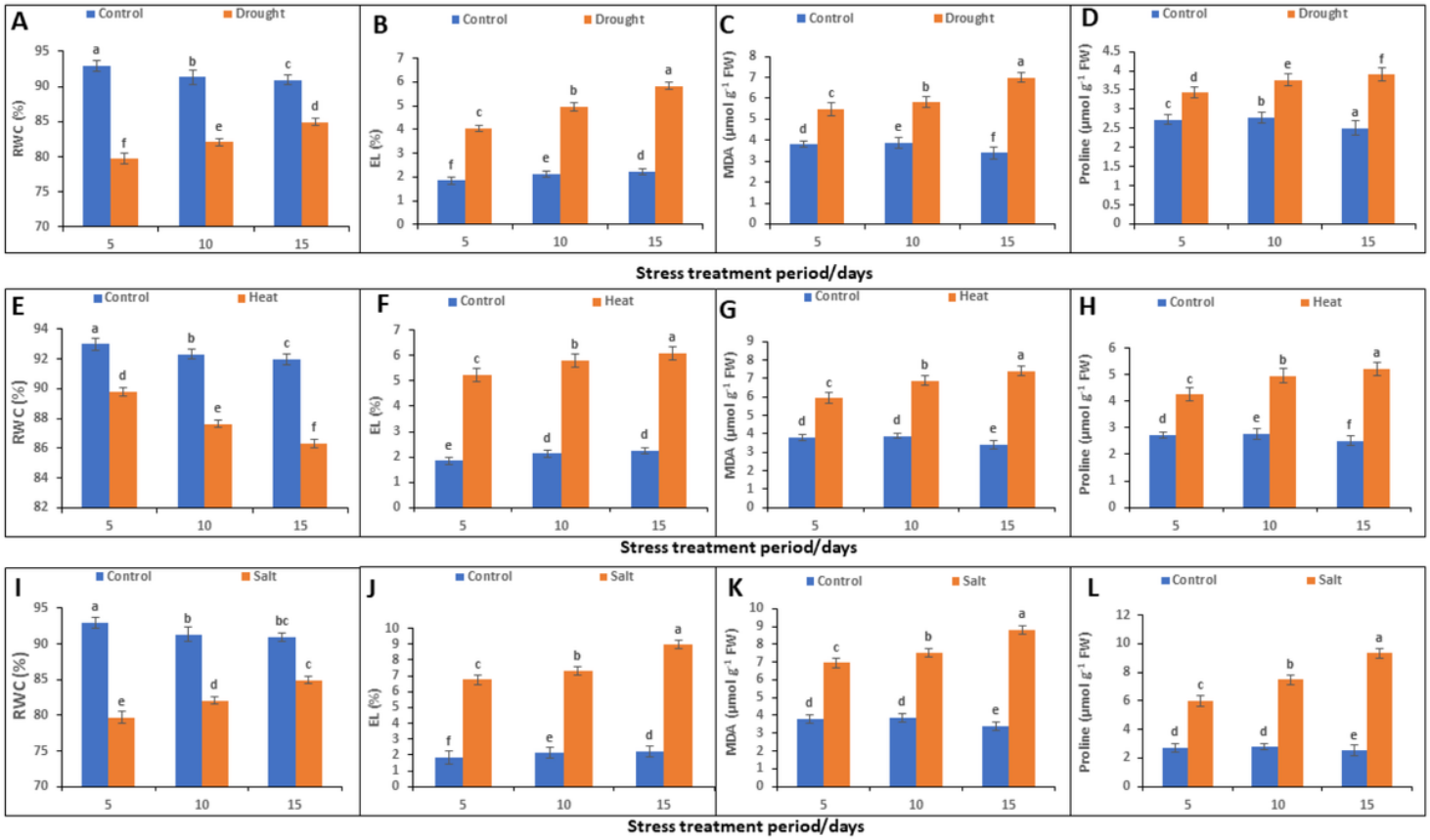

Figure 11

Effect of drought stress on relative water content, electrolyte leakage, malondialdehyde, and proline content of Geumgangmil wheat genotype under drought (A-D), heat (E-H), and salt (I-L) stress treatment. Values are means ± standard error (SE) of three independent samples. Different letters on vertical bars indicate significant differences between means at $P \leq 0.05$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure.docx
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- TableS9.xlsx