Identification and Expression of the Multidrug and Toxic Compound Extrusion (MATE) Gene Family in *Capsicum annuum* and *Solanum tuberosum*

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**Abstract:** Multidrug and Toxic Compound Extrusion (MATE) proteins are essential transporters that extrude metabolites and participate in plant development and the detoxification of toxins. Little is known about the MATE gene family in the Solanaceae, which includes species that produce a broad range of specialized metabolites. Here, we identified and analyzed the complement of MATE genes in pepper (*Capsicum annuum*) and potato (*Solanum tuberosum*). We classified all MATE genes into five groups based on their phylogenetic relationships and their gene and protein structures. Moreover, we discovered that tandem duplication contributed significantly to the expansion of the pepper MATE family, while both tandem and segmental duplications contributed to the expansion of the potato MATE family, indicating that MATEs took distinct evolutionary paths in these two Solanaceous species. Analysis of ω values showed that all potato and pepper MATE genes experienced purifying selection during evolution. In addition, collinearity analysis showed that MATE genes were highly conserved between pepper and potato. Analysis of cis-elements in MATE promoters and MATE expression patterns revealed that MATE proteins likely function in many stages of plant development, especially during fruit ripening, and when exposed to multiple stresses, consistent with the existence of functional differentiation between duplicated MATE genes. Together, our results lay the foundation for further characterization of pepper and potato MATE gene family members.

**Keywords:** MATE; Expression profile; Solanaceae; *Capsicum annuum*

1. **Introduction**

The Multidrug and Toxic Compound Extrusion (MATE) protein family (also named Detoxification Efflux Carriers, DTXs) consists of essential multidrug transporters that can dispose and detoxify exogenous and endogenous toxins in development and response to various stresses [1]. Four other protein families function as multidrug transporters: the ATP-binding cassette (ABC) family, major facilitator superfamily (MFS), resistance-nodulation-division (RND) family, and small multidrug resistance (SMR) transporters [2]. MATE members play important roles as secondary drug resistance transporters, which extrude toxins using Na$^+$ or H$^+$ electrochemical gradients [3].

MATE proteins have been identified in bacteria, archaea, fungi, plants, and mammals; however, plant genomes encode far more MATE proteins than genomes from other kingdoms, possibly because of the wide range of metabolites that occur in plants [4]. A typical MATE protein is 440–550 amino acids...
in length and contains a conserved PF01554 domain, consisting of 12 alpha-helical transmembrane domains (TMs), as confirmed by an X-ray crystallography structure analysis of the NorM protein from the Gram-negative bacterium Vibrion parahaemolyticus [5,6] and the PROTEIN DETOXIFICATION 14 (DTX14) protein from Arabidopsis (Arabidopsis thaliana) [7].

To date, research about the function and regulation of plant MATE genes has been relatively limited. Several MATE transporters have been shown to transport diverse noxious compounds or secondary metabolites in a number of plant species, thereby regulating plant development and stress tolerance. Many MATE proteins have been confirmed to transport or accumulate secondary metabolites, including transport of alkaloids by tobacco (Nicotiana tabacum) jasmonate-inducible alkaloid transporter 1 (NtJAT1) and AtDTX1 [8–10]; anthocyanins and the epicatechin 3′-O-glucoside by Arabidopsis TRANSIENT TESTA 12 (TT12)/DTX41 [11,12]; anthocyanins by grapevine (Vitis vinifera) anthoMATE1 (VvAM1) and VvAM3 [13,14]; proanthocyanidin precursors by barley (Medicago truncatula) MtMATE1 [12]; nicotine or flavonoid by NtMATE1 [15], MtMATE2 [16]; AtDTX35/FLOWER FLAVONOIOD TRANSPORTER (FFT) [17]; salicylic acid (SA) by Arabidopsis ENHANCED DISEASE SUSCEPTIBILITY 5 (EDS5) [18–21]; abscisic acid (ABA) by AtDTX50/ABNORMAL SHOOT (ABS) 3-LIKE 2 (ABS3L2) [22]; hydroxycinnamic acid amides by AtDTX18 [23]. In addition, several MATE proteins are involved in aluminum detoxification or iron translocation, including Arabidopsis MATE [24,25], Brassica oleracea BoMATE [26], Eucalyptus camaldulensis EcMATE1 [27], Arabidopsis FERRIC REDUCTASE DEFECTIVE 3 (FRD3) [28,29], soybean (Glycine max) GmFRD3b [30], barley (Hordeum vulgare) HvAACT1 [31–33], rice (Oryza sativa) FRD3-LIKE 4 (FRDL4)/OsMATE4 [34,35], OsFRDL1/OsMATE10 [36–38], OsFRDL2/OsMATE40 [37], sorghum (Sorghum bicolore) SbMATE [39,40], rye (Secale cereale) ScFRDL1 and ScFRDL2 [41], wheat (Triticum aestivum) TaMATE1B [42], maize (Zea mays) ZmMATE1 [43,44], and Arabidopsis ZF14/AtABS4 [45–47].

With the advent of new sequencing technologies, the genomes of many organisms have been sequenced, which allowed the identification of many additional MATE genes. To date, a few MATE genes have been identified in species from other kingdoms; for instance, the human genome only has two MATE genes, Solute carrier 47A1 (SLC47A1)/MATE1 and SLC47A2/MATE2-K [48,49]. However, plant genomes contain large MATE gene families; for example, 45 or 55 have been identified in rice [50,51] and 49 in maize [52], which belong to the Poaceae family in the monocots. From the Leguminosae family in the eudicots, 71 were identified in poplar (Populus trichocarpa) [56], 73 in flax (Linum usitatissimum) [57], 67 in Citrus sinensis [58], 56 in Arabidopsis [50], and 79–130 in four Brassicaceae species [59].

The Solanaceae family, also named nightshades, is one of the most economically and agronomically important plant families, including tomato (Solanum lycopersicum), tobacco, pepper (Capsicum annuum), potato (Solanum tuberum) and other plants. Species in this family produce numerous secondary metabolites and toxic compounds, such as nicotine, capsicain, hyoscyamine, and solanine; these compounds are used for foods or drugs [60]. The genomes of tomato [61], potato [62], and pepper [63,64] have been sequenced as high-quality assemblies and annotations and are frequently updated. An analysis of the tomato and potato genomes identified 67 MATE [4] and 48 MATE genes [65], respectively. However, little is known about the MATE family in pepper, and none have been experimentally characterized.

Here, our genome-level analysis of MATE gene families in the Solanaceae identified 42 MATE genes in pepper and 60 in potato. We classified plant MATE genes into five groups, based on their phylogenetic clustering, motif organization and exon–intron structures. In addition, we discovered that tandem duplications were the main drivers of gene family expansion in the pepper CaMATE family, and tandem duplications and segmental duplications drove expansion in the potato StMATE family, but both families were under purifying selection. Moreover, collinearity analysis showed that the pepper and potato MATE families were highly conserved. A detailed expression profile analysis
revealed that pepper CaMATE genes exhibited functional diversity during development and opposite behavior in roots and leaves in response to different stresses. Our findings provide foundational information for the further validation of MATE genes in the Solanaceae.

2. Results

2.1. Identification of MATE Genes in the Pepper and Potato Genomes

To gain a comprehensive genome-wide overview of the MATE gene families in pepper and potato, we performed a Basic Local Alignment Search Tool for Protein (BLASTP) analysis using 56 Arabidopsis AtMATE proteins as queries [50], as well as an HMMER search for the Pfam MATE domain (PF01554). We focused our efforts on the pepper and potato genomes, as both plants are of great economic and agronomic value and have well-sequenced genomes with good annotation. We then filtered candidate genes according to the position of their MATE domain and the number of transmembrane domains (TMs), which we detected using the SMART, InterProscan and CDD databases. This analysis identified 42 putative CaMATE genes in pepper and 60 candidate StMATE genes in potato. According to their chromosomal locations, we designated these genes CaMATE1–CaMATE42 and StMATE1–StMATE60 (Supplementary Materials Table S1).

Table S1 lists the 42 CaMATE and 60 StMATE genes and their encoded proteins, including gene name and length, isoelectric point (pI) value, predicted molecular weight (MW), number of TMs and subcellular localization (Table S1). Pepper CaMATE proteins varied in length from 298 to 752 amino acids, contained 6 to 12 TMs, had a predicted MW from 32.4 to 82.5 kDa, and had pI values from 4.98 to 9.06. Likewise, potato StMATE proteins varied in length from 337 to 615 amino acids, contained 9 to 12 TMs, had a predicted MW from 37.1 to 65.9 kDa, and had pI values from 5.29 to 9.44. For comparison, the 56 Arabidopsis AtMATE proteins were 470 to 561 amino acids in length, contained 6 to 12 TMs, with predicted MW values ranging from 50.8 to 60.0 kDa, and pI values from 4.93 to 9.64. These results indicated that MATE proteins were more variable in size within the pepper and potato MATE family than in Arabidopsis. We predicted the subcellular location of each MATE protein using the WoLF PSORT database, which predicted that 36 out of the 42 pepper CaMATE proteins localize to the plasma membrane, with another three localizing to the vacuolar membrane, and three to the nucleus, a distribution that was similar to that of potato StMATE proteins (Table S1).

2.2. Phylogenetic Analysis and Structural Characterization of Pepper MATE Genes

To explore the phylogenetic relationships and evolutionary history of the plant MATE gene family, we used MEGA X software to align the MATE domains from pepper, potato and Arabidopsis MATE family members along with another 25 functionally characterized plant MATE proteins with MUSCLE (Multiple Sequence Comparison by Log-Expectation), followed by phylogenetic analysis using the Maximum Likelihood method (Figure 1). We classified the 183 MATE proteins into five groups (Groups I–V), according to the topology of the phylogenetic tree, with high bootstrap values of 61.3, 88.3, 100, 98.3, and 98.9 (Figure 1), respectively, above the significance cutoff of 50. Intra-group bootstrap values were higher than between-group values (Figure 1). All five groups contained pepper, potato, and Arabidopsis MATE proteins, suggesting that the five groups formed before the divergence of the Brassicaceae and Solanaceae. The number of MATE proteins associated with each group was uneven in pepper and potato. Group I and Group II contained the largest number of MATE proteins, with 31 in pepper and 32 in potato.

We analyzed MATE proteins for conserved motifs using the multiple EM for motif elicitation (MEME) suite, revealing ten conserved protein motifs whose organization within MATE proteins largely agreed with the phylogenetic tree (Figure 2a and Figure S1). In addition, motifs identified by MEME in pepper and potato MATE protein belonging to the same groups shared a high degree of conservation, as evidenced by their lower E-values, and similar motif numbers and organization. Groups I, II, IV and V shared similar protein domain composition and organizations that were distinct
from those of Group III. For example, Group III members only had two motifs in common, but Group I, II, IV and V had 9–10 in common (Figure 2a). This observation suggests that Group III may have followed a different evolutionary trajectory compared to members of the other four groups.

Figure 1. Phylogenetic relationship of 42 CaMATE, 60 StMATE, and 56 AtMATE proteins, along with another 25 functional published MATE proteins. The phylogenetic tree was constructed using MEGA X with the Maximum Likelihood method, and visualized with FigTree software. MATE proteins were classified into five distinct groups, as indicated by the different colors.

To determine the extent of genomic structural diversity of MATE genes, we analyzed the exon–intron organization of the pepper and potato MATE genes, with the help of the Gene Structure Display Server (GSDS 2.0) website. The MATE gene family in both species showed a similar exon–intron structure within the same groups (Figure 2b), further validating the classification of MATE genes. Group I contained 48 MATE genes, with 44 (91.7%) having 5–7 introns; Group II contained 54 MATE genes, with 49 (91.7%) having 6–8 introns; Group III contained 19 MATE genes with 12–14 exons; Group V contained 10 MATE genes, with 4–6 introns (Figure 2b). Notably, the 27 MATE genes belonging to Group IV had 0–2 introns, suggesting a very different genomic structure for these genes (Figure 2b).

Our analysis demonstrated that pepper, potato and Arabidopsis MATE genes that belonged to the same group shared the same or a very similar arrangement of their functional motifs, intron patterns, and exon–intron structures, consistent with the phylogeny. Gene structures varied greatly among different groups, supporting the classification of the MATE family members.
Figure 2. Conserved motifs in pepper and potato MATE proteins and their associated MATE gene structures. Left: phylogenetic tree of 42 CaMATE and 60 StMATE proteins replotted from Figure 1. (a) Conserved motifs of pepper and potato MATE proteins. Each colored box represents a protein motif identified by multiple EM for motif elicitation (MEME), and the box is placed at the appropriate position within the protein. MATE proteins are ordered according to the phylogenetic tree. (b) MATE gene structures in pepper and potato.

2.3. Chromosomal Distribution and Duplication of Pepper and Potato MATE Genes

To explore the relationship between pepper and potato MATE genes, we determined their chromosomal locations and whether they originated from gene duplication events. MATE loci were unevenly distributed in the pepper and potato genomes. We identified pepper CaMATE genes on all chromosomes, except chromosome 09. Several pepper chromosomes had four to six CaMATE genes (chromosomes 00, 01, 02, 03, 04, 07, and 10), while other chromosomes had one to three CaMATE genes (chromosomes 05, 06, 07, 11, and 12) (Figure 3a). In addition, we observed clusters of CaMATE genes on chromosomes 02, 07, and 10 (Figure 3a). Similarly, potato StMATE genes mapped to all 12 potato chromosomes, with six to nine StMATE genes on chromosomes 01, 02, 03, 04, 07, and 10 (Figure 3b).
Figure 3. Chromosomal distribution of pepper and potato MATE genes. (a,b) pepper (a) and potato (b) locations of MATE genes on the chromosomes. The chromosome number is indicated above each chromosome (vertical bar). Chromosome lengths are based on information from the Ensembl database. Tandem-duplicated genes are connected by red arcs. MATE genes are color-coded according to the MATE group they belong to, in accordance with Figure 1.

Tandem-duplicated genes are defined as two paralogous genes that are separated by fewer than 10 intervening genes [50]. Using MCScanX, we identified 16 (38.1%) pepper MATE genes in five clusters.
that correspond to tandem duplications events (Figure 3a) and may have contributed to the expansion of the gene family. Among these tandem-duplicated genes, five pairs belonged to Group I, two pairs belonged to Group II, and three pairs belonged to Group V (Figure 3a). In potato, we identified 21 (35%) tandem-duplicated StMATE genes, comprising 12 gene pairs (Figure 3b). Of these, seven pairs belonged to Group I, with three, one, and one pairs that belonged to Groups II, III, and V, respectively (Figure 3b). These results suggested that tandem duplication played important roles in the expansion of the MATE gene family in both pepper and potato, but affected different MATE gene groups differently.

Interestingly, we failed to identify segmental duplication events among pepper CaMATE genes, in sharp contrast to potato, for which we identified 12 StMATE gene pairs resulting from segmental duplications (Figure 3b). Segmental-duplicated potato StMATE genes were observed in Groups I–IV, with six pairs in Group I, three pairs in Group II, one pair in Group III, and two pairs in Group IV. These results suggested that the MATE gene family has expanded by different mechanisms in pepper and potato. While tandem duplications contributed to MATE gene family expansion in both pepper and potato, segmental duplications only contributed to the expansion of the MATE family in potato, illustrating the similarities and differences between the two Solanaceous species.

We then used the Ka (non-synonymous distance), Ks (synonymous distance), and ω, (Ka/Ks ratio) values to evaluate selective pressure exerted on the MATE family during evolution [66]. The neutral theory posits that ω values below 1 indicate purifying selection, while values around 1 represent neutral evolution and ω values above 1 indicate positive selection [67]. To explore the selective pressure imposed on tandem-duplicated MATE genes and their possible functional diversification in pepper and potato, we calculated the ω values for tandem- and segmental-duplicated MATE gene pairs. ω values were below 1 for all 34 MATE gene pairs, indicating that tandem and segmental-duplicated pepper and potato MATE genes experienced purifying selection (Figure 4a, Table S2).

**Figure 4.** Collinearity analysis of MATE genes between pepper and potato. (a) Average value of Ka, Ks, and ω (Ka/Ks ratio). The x-axis indicates tandem duplication (TD) events in pepper (Ca) and potato (St), segmental duplication (SD) events in potato, and collinear pairs between pepper and potato (Ca-St). The y-axis shows Ka, Ks and Ka/Ks ratios of MATE genes for each pair. Boxplots were generated in R. (b) The potato chromosomes are in brown (top) and pepper chromosomes in green (bottom). Putative orthologous genes in their genomes are connected by lines and were identified using MCScanX. The innermost grey solid lines show collinear relationships between MATE genes. We identified 30 orthologous MATE gene pairs, indicated by magenta lines.
We also calculated the mean $\omega$ value for tandem-duplicated \textit{MATE} genes in pepper within each group: the $\omega$ value of Group II was 0.144, 0.228 for Group I and 0.227 for Group V, suggesting that \textit{MATE} genes within Group II experienced much stronger purifying selection than those in Groups I and V. Likewise, we obtained similar $\omega$ values for potato tandem-duplicated pairs: the mean $\omega$ value was 0.144 for Group II pairs, 0.275 for Group I, 0.428 for Group III and 0.282 for Group V. However, potato segmental-duplicated \textit{StMATE} gene pairs showed a mean $\omega$ value within Group I of 0.176, slightly lower than for tandem-duplicated pairs of the same group, suggesting that segmental-duplicated \textit{StMATE} genes experienced much more relaxed selection than tandem-duplicated gene pairs in Group I. We saw no evidence of positive selection for any \textit{MATE} gene pairs identified in either pepper or potato.

2.4. Collinearity Analysis of \textit{MATE} Genes between Pepper and Potato

To assign orthologous gene pairs between pepper and potato, we performed a collinearity analysis using MCScanX [68] and TBtools software [69]. We identified 30 putative orthologous \textit{MATE} gene pairs (Figure 4b). We detected 21 \textit{CaMATE} genes on 10 of the 12 chromosomes of the pepper genome, which formed pairs with 27 potato \textit{StMATE} genes mapping to 11 of the 12 chromosomes of the potato genome. Notably, pepper \textit{CaMATE12} on chromosome 02 showed synteny with five collinear \textit{StMATE} pairs in potato (Figure 4b). In terms of the five groups defined earlier, 12 putative orthologous pairs belonged to Group I and 10 pairs to Group II, with the remaining two, four, and one orthologous pairs belonging to Groups III, IV, and V, respectively (Figure 4b). We hypothesize that these putative orthologous pairs of \textit{MATE} genes may share the same function in these two Solanaceous species.

2.5. Analysis of Cis-Regulatory Elements in \textit{MATE} Promoters

\textit{MATE} genes take part in plant development and defense responses [2,4,50]. To investigate their potential functions during plant development and upon exposure to various stresses, we performed an analysis of 2 kb of sequence upstream of each pepper and potato \textit{MATE} gene. To this end, we used the PlantCARE website to predict the \textit{cis}-regulatory elements (CREs) in this 2-kb region. This analysis identified 12 distinct CREs in the \textit{MATE} promoters, including two development-related CREs, five phytohormone-responsive CREs, and five plant defense response-related CREs (Figure 5). The number of CREs was quite variable across the potato and pepper \textit{MATE} genes, with the highest number seen in the pepper \textit{CaMATE24} (39 CREs) and \textit{CaMATE28} (36 CREs) promoters, and only three CREs in the potato \textit{StMATE22} promoter (Figure 5). We identified 922 potential CREs in the pepper \textit{CaMATE} promoters, including 523 elements related to development, 271 related to phytohormone responses, and 128 related to plant defense responses (Figure 5). The 523 development-related CREs consisted of 512 light-responsive and 11 circadian elements. The phytohormone-responsive CREs consisted of 91 abscisic acid (ABA)-responsive elements in 32 pepper \textit{MATE} promoters, 34 auxin-responsive elements in 21 pepper promoters, 41 gibberellic acid (GA)-responsive elements in 24 pepper promoters, 80 methyl jasmonate (MeJA)-responsive elements in 27 pepper promoters, and 25 salicylic acid (SA)-responsive elements in 18 pepper promoters.

Of the 128 defense response-related CREs, we identified 24 drought-inducible sites (MYB-binding site or MBS) in 16 pepper promoters, 19 low-temperature-responsive elements (LTREs) in 16 pepper promoters (Figure 5), 7 AT-rich motifs (TAAAATACT) responsible for elicitor-mediated activation in seven pepper promoters, and 3 WUN-motifs (AAATTTCCCT), responsible for wound-responsive expression, in the promoters of pepper \textit{CaMATE2}, \textit{CaMATE14} and \textit{CaMATE38}. In addition, we identified 75 ARE and GC-motifs, mediating anaerobic or anoxic responses, in the promoters of 35 pepper \textit{CaMATE} genes (Figure 5), suggesting that these genes may participate in responses to hypoxia.

The above results strongly suggested that pepper \textit{CaMATE} genes participate in plant responses to multiple stresses. A similar analysis identified 1125 potential CREs in the promoters of the potato \textit{StMATE} genes, including 641 related to development, 350 related to phytohormone responses, and 134 related to plant defense responses (Figure 5), underscoring the similar distribution of CREs between pepper and potato \textit{MATE} genes. Many pepper and potato \textit{MATE} promoters contained various CREs.
related to development, phytohormones, and plant defense (Figure 5), suggesting that these genes may play important roles during plant growth and response to environmental stresses.

**Figure 5.** Predicted cis-regulatory elements in the promoters of pepper and potato *MATE* genes. The phylogenetic tree of the pepper CaMATE family is replotted from Figure 1. The cis-regulatory elements (CREs) in the 2 kb upstream regions of the 42 pepper CaMATE and 60 potato *StMATE* genes were predicted using the PlantCARE database. These CREs were divided into three types: development (including circadian-related and light-responsive elements), phytohormone (including ABA-responsive, auxin-responsive, GA-responsive, MeJA-responsive, and SA-responsive elements), and stress-responsive (including drought inducibility, low-temperature-responsive, elicitor-mediated activation, and wound-responsive elements).
2.6. Analysis of Pepper CaMATE Gene Expression Patterns

To assess the role of pepper and potato MATE genes in plant development, we turned to transcriptome deep-sequencing (RNA-seq) datasets from the PepperHub database [70], which we collected and analyzed as previously described [71]. We compiled the expression profiles of all pepper CaMATE genes across 54 different tissues and organs and ordered the genes according to their positions within the phylogenetic tree. We then visualized transcript levels as a heatmap, which illustrated the differences in expression patterns observed for CaMATE genes (Figure 6a). When applying a selection criterion of fifty Reads Per Kilobase of transcript, per Million mapped reads (RPKM) in at least one tissue, we identified 16 highly expressed CaMATE genes from all five phylogenetic groups. Of note was the observation that highly expressed genes exhibited different expression patterns in different pepper tissues (Figure 6a). Within the same group, CaMATE genes showed distinct expression profiles in different tissues (Figure 6a), suggesting subfunctionalization or functional diversification.

![Figure 6](image-url)

**Figure 6.** Expression of pepper CaMATE genes during plant development and in response to various stresses. (a) Tissue-specific expression data for pepper CaMATE genes were obtained from published data (http://www.hnivr.org/pepperhub). Red and green colors represent higher and lower expression, respectively. Pepper samples: L: leaf, F: flower, P: petal, O: ovary, STA: anther, FST: whole fruit, G: pericarp, T: placenta, ST: placenta, and seed, S: seed. Heat map of the expression data for MATE genes in the selected 54 pepper tissues. The heat map with phylogenetic tree was drawn with R. (b) Venn diagrams of CaMATE expression in response to phytohormones and stress. A: ABA-treated; S: SA-treated; J: JA-treated; I: IAA-treated; G: GA-treated; F: freezing-treated; R: H2O2-treated; N: NaCl-treated; M: mannitol-treated; H: heat-treated; Up: up-regulated genes; Down: down-regulated genes. L: leaves; R: roots. The two-letter code lists the treatment first, then the tissue. The numbers given in the Venn diagram represent the numbers of up-/down-regulated genes.
Our analysis also highlighted a set of five genes (CaMATE13/17/27/29/40), from Group I with expression levels below 1 RPKM in all samples, suggesting that these may be pseudogenes. Interestingly, four of these low-expressed genes (CaMATE13/27/29/40), were derived from tandem duplication, indicating that they may be undergoing pseudogenization. We identified 37 CaMATE genes with preferential expression in a single pepper tissue such as leaf, flower, pericarp, placenta, or seed (Figure 6a), hinting at their involvement in growth and development of the corresponding tissues. We detected seven highly expressed CaMATE genes in leaves, eleven in flowers, four in pericarp, eight in placenta and seven in seeds (Figure 6a), suggesting a function as tissue- or organ-specific regulators. Further investigation indicated that tandem-duplicated CaMATE genes were differentially expressed in the selected samples (Figure 6a), suggesting their functional differentiation.

2.7. Expression Analysis of Phytohormone- and Stress-Responsive Pepper CaMATE Genes

MATE genes take part in plant response to abiotic and biotic stresses [2]. To explore whether CaMATE genes are involved in plant responses to environmental stresses, we analyzed the expression pattern of CaMATE genes in pepper roots and shoots exposed to five phytohormones (ABA, GA, indole-3-acetic acid (IAA), JA, and SA) and five stress conditions (freezing, H$_2$O$_2$, salt, mannitol and heat stress), obtained from the PepperHub database [70,72]. Our analysis showed that 35 CaMATE genes were regulated by phytohormones and the stresses tested here (Figure 6b). The majority of CaMATE genes were regulated by more than one phytohormone or stress treatment, with only CaMATE16 being induced by JA in roots, and CaMATE05 being repressed by JA in shoots (Figure 6b). We identified 25–28 MATE genes that were regulated by phytohormones in roots, compared to 20–28 MATE genes in leaves (Figure 6b). In roots, 10–21 genes were down-regulated, and 7–10 genes were up-regulated compared with untreated controls. We saw the opposite pattern in shoots, where more MATE genes were repressed than induced in response to phytohormone treatments (Figure 6b).

In roots, we observed the induction of CaMATE genes that belonged to each of the five phylogenetic groups: five genes from Group I (CaMATE01/03/04/14/17), three genes from Group IV (CaMATE06/20/26), two genes from Group II (CaMATE19 and CaMATE24) and one gene from Group III (CaMATE09) in response to all phytohormones. In addition, two Group III (CaMATE07/32), one Group I (CaMATE41), and one Group II (CaMATE18) gene were repressed by all phytohormones in roots (Figure 6b). In leaves, only two Group I MATE genes (CaMATE01/28) and one Group II gene (CaMATE18) were induced by treatment with all phytohormones. Finally, three Group I (CaMATE03/17/14), two Group II (CaMATE19/24), two Group IV (CaMATE23/26) genes, and one Group III (CaMATE33) gene were repressed by treatment with the phytohormones in leaves (Figure 6b). These results showed that pepper CaMATE genes are differentially expressed in response to phytohormones and in a tissue-specific manner.

We also determined the gene expression profile of pepper CaMATE genes in response to stress such as freezing (F), H$_2$O$_2$ (R), salt (NaCl, N), mannitol (M), and heat (H), leading to the identification of 35 CaMATE genes that were differentially expressed in response to these treatments. As with the phytohormones above, the majority of CaMATE genes were regulated by more than one treatment, with only CaMATE32 and CaMATE38 being repressed by heat and salt stress in shoots, and CaMATE36 being repressed by freezing in roots (Figure 6b). There were 23–30 CaMATE genes that were regulated by various stresses in roots, compared to 22–29 MATE genes regulated in leaves (Figure 6b). In roots, 16–19 genes were down-regulated, while 7–10 CaMATE genes were up-regulated in response to stress treatments. Again, this pattern was opposite in shoots, with more MATE genes being repressed (16–25 MATE genes) than induced (3–6 MATE genes) (Figure 6b). In roots, five Group I genes (CaMATE01/03/04/12/14), three Group IV genes (CaMATE06/09/20), two Group II genes (CaMATE19/24) and one Group III (CaMATE09) gene were induced in all stress conditions. Likewise, three Group II genes (CaMATE05/18/39), two Group III genes (CaMATE07/32), and one Group I (CaMATE41) gene were repressed by all stresses in roots (Figure 6b). In leaves, only the group I gene CaMATE28 was induced by all stresses. In addition, four Group IV genes (CaMATE06/21/23/26), three Group I genes.
which are of high economic and agronomic importance. Here, we performed a comprehensive when pepper produces many metabolites during fruit ripening. CaMATE (Figure 6). These observations suggested that (phytohormones and stresses (Figure 6b) suggests a shared response brought upon by phytohormones Plantes 2020, 9, x FOR PEER REVIEW 13 of 24functions during pepper fruit ripening, we selected several CaMATE genes for RT-qPCR analysis, which demonstrated that CaMATE02/05/12/25/30 showed preferential expression in green and red fruit tissues (Figure 7), consistent with the data from the PepperHub RNA-seq datasets (Figure 6). These observations suggested that CaMATE genes participate in plant fruit development, when pepper produces many metabolites during fruit ripening.

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Expression of CaMATE genes during development. Quantitative reverse transcription PCR (qRT-PCR) analysis of six CaMATE genes in leaf and fruit tissues. CaMATE transcript levels were normalized using CaUBI-3 as the internal reference. Each data point represents the average of three biological repeats. * p < 0.05; ** p < 0.01 by Student’s t-test.

3. Discussion

MATE proteins are ubiquitous in nearly all kingdoms. In plants, they participate in diverse functions that regulate plant development and adaptation to stresses, by transporting and sequestering harmful substances and secondary metabolites. Although MATE genes have been identified in several plant genomes [50–59], little is known about the function of MATE transporters in Solanaceous species, which are of high economic and agronomic importance. Here, we performed a comprehensive genome-wide identification of MATE genes in pepper and potato, two members of the Solanaceae, identifying 42 CaMATE and 60 StMATE genes. Subsequently, we combined phylogenetic analysis, gene structure characterization, and expression pattern analysis to elucidate the evolution of MATE genes and their potential functions, which will contribute to our understanding of MATE transporter functions in the Solanaceae.
3.1. MATE Gene Family Conservation in the Solanaceae

Our analysis revealed 42 CaMATE genes in pepper and 60 StMATE genes in potato (Table S1). The StMATE gene family has previously been reported to consist of 48 members [65], but we attribute the higher numbers identified here to the recent update of the potato genome annotation. The number of MATE genes in pepper and potato was comparable to that in tomato, which has 60 MATE genes [4]. The MATE gene family has greatly expanded in plants relative to other kingdoms [48,49], suggesting their diverse and vital roles in plants.

Pepper CaMATE proteins varied from 298 to 752 amino acids, while potato StMATE proteins consisted of 337 to 615 amino acids, much longer than the range seen in Arabidopsis, with AtMATE proteins ranging from 470 to 561 amino acids [50], suggesting a higher diversity and complexity in the Solanaceae. Our analysis predicted that most MATE proteins localize to the plasma membrane, which would be consistent with their roles as transporters of toxic compounds [7], thereby conferring resistance to the toxin.

A phylogenetic tree constructed using 42 CaMATE, 60 StMATE, 56 AtMATE, and another 25 functionally characterized MATE proteins from other plant species classified MATE family members into five groups (Figure 1), which we validated based on their gene structures and the organization of their encoded functional motifs (Figure 2). In agreement with the phylogenetic analysis, gene structures, the number of exons, the number of TM domains, and the predicted subcellular locations showed higher similarity within each group than between groups, supporting our classification of MATE members. MATE family members from Group III only displayed two conserved motifs, but had the most exons relative to all other groups (Figure 2), indicating large structural differences in the MATE genes and variation in the function of the encoded proteins.

3.2. Tandem Duplications Contributed to MATE Gene Expansion between Pepper and Potato

The pepper and potato MATE genes were unevenly distributed across the chromosomes of their respective genomes, as already observed with the tomato SLIMATE family [4], indicating a possible aneuploidy event, in addition to the whole genome triplication event that occurred in these Solanaceous species [61,64,73].

Intra- and inter-synteny and collinearity analysis suggested that pepper CaMATE genes may have expanded by tandem duplication, as in tomato [4], resulting in the tight linkage of MATE genes in clusters in the Solanaceae (Figure 3 and Figure S1), and implying that tandem duplications may have contributed to the expansion of the MATE gene family in the Solanaceae. The ω values for tandem-duplicated pepper CaMATE genes ranged from 0.113 to 0.321, comparable to the 0.097 to 0.440 range seen for potato StMATE genes (Figure 4, Table S2). Importantly, these values were all less than 1, indicating purifying selection during evolution of the MATE gene family in pepper and potato. Notably, we detected no obvious segmental duplications among pepper CaMATE genes (Figure 1), but identified 12 such segmental duplication pairs of StMATE genes in potato (Figure 3b) and tomato [4], suggesting that expansion of MATE gene families may be driven by distinct mechanisms among Solanaceous species. In addition, there was a non-uniform number of tandem-duplicated MATE genes within groups (Figure 3 and Figure S1). Indeed, we detected tandem duplication in Groups I, II and V MATE genes in pepper (Figure 4, Table S2), and in Groups I, II, III and V for potato, while potato segmentally duplicated MATE genes belonged to Groups I, II, III and IV (Figure 4, Table S2). These results suggest that different groups have undergone diversification in pepper and potato, especially genes belonging to groups III and IV.

We classified pepper and potato MATE paralogous genes, which were derived from tandem and segmental duplications and showed similar motif organization and exon–intron structures, into the same group, with strong support from phylogenetic bootstrapping values (Figures 1 and 2). This suggested that they experienced purifying selection, without any domain gain or loss. After duplication, the duplicated genes will undergo possible subfunctionalization, neo-functionalization, or non-functionalization [74]. In Group I, we identified four CaMATE genes (CaMATE13/27/29/40) originating from tandem-duplicated
pairs with CaMATE12/14/28/41 being expressed below 1 RPKM across all tissues tested (Figure 6), making them good candidates for pseudogenes that experienced non-functionalization after duplication. In Group II, the two duplicated CaMATE gene pairs (CaMATE10/11, CaMATE18/19) were expressed at their highest levels in flowers and the placenta, respectively, but at different developmental stages (Figure 6), suggesting subfunctionalization after duplication. In Group V, a cluster of duplicated CaMATE genes CaMATE34/35/36/37 were preferentially expressed in flowers or the placenta (Figure 6), suggesting their subfunctionalization during evolution. Overall, MATE gene duplications drove diversification of gene expression; this may have affected plant development and helped plants adapt to changes in the environment in the Solanaceous species studied here.

3.3. MATE Function and Gene Expression

Changes in gene expression are routinely used to assess gene function during development and after exposure to stress conditions [75]. Accordingly, we used the preferential expression of pepper CaMATE genes in various tissues and stressed samples to predict their functional roles [71]. MATE proteins have been reported to extrude primary and secondary metabolites, such as organic molecules, terpenoids, alkaloids, phenols, and phytohormones [2]. In addition, several plant MATE genes have been shown to take part in plant development and response to stresses, by means of excreting toxic compounds. An analysis of RNA-seq data across 54 tissues or organs from PepperHub [70] established that many CaMATE genes were preferentially expressed in reproductive tissues, including flowers, pericarp, placenta, and seeds (Figure 6), suggesting that these genes may be involved in reproductive development. The Group II CaMATE genes, CaMATE18 and CaMATE39, showed high expression in flowers, while CaMATE30 expression was high during the G11 stage of pericarp development (Figure 6), indicating that these CaMATEs may have specific and narrow functions in reproductive tissues. Finally, CaMATE37 from Group V reached a peak in expression at the F7 stage in flowers, with almost no expression in other tissues or organs (Figure 6), suggesting that CaMATE37 may participate in the specification of floral organs, especially at the F7 stage.

We also screened the promoters of all pepper CaMATE genes for cis-regulatory elements, resulting in the identification of 922 CREs, with 523 CREs related to development, and 399 CREs related to phytohormones or stress (Figure 5), suggesting that CaMATE genes may play important roles in plant development and adaptation to environmental conditions. We observed strong connections between plant responses to phytohormones and stress [76,77]. Our results showed that phytohormone- and stress-related CREs were abundant across CaMATE promoters, suggesting that CaMATE genes contribute to plant responses to environmental stresses. A more detailed analysis of gene expression profiles across the 54 developmental stages and 24 treatments available at PepperHub will lay a solid foundation for the functional characterization of pepper CaMATE genes. Overall, CaMATE genes exhibited various and highly diversified expression profiles, especially in reproductive tissues (Figure 7), implying that CaMATE genes may have significant and complex functions in pepper development and in response to environmental stimuli.

Plants are continuously exposed to many environmental stresses; they have therefore evolved multiple stress response mechanisms to deal with a changing environment [78]. The excretion of metabolites and toxic compounds by transporters is one such regulatory mechanism that leads to improved plant stress resistance. Phylogenetic relationships between the pepper and potato MATE gene family may be used to predict putative gene functions, according to published functional characterization of plant MATE genes [4]. To date, the function of only a few MATE genes has been experimentally validated.

Based on phylogeny, Group I contained 14 CaMATEs, 17 StMATEs, 17 AtMATEs, tobacco (Nicotiana tabacum) NtJAT1s, and rice (Oryza sativa) OsMATE2 (Figure 1), which was the biggest MATE gene subfamily in plants. To date, NtJAT1 and AtDTX1 have been confirmed to transport alkaloids from the cytosol to the vacuole, regulating plant development and disease resistance [8–10], suggesting that Group I MATE genes may participate in the transport of alkaloids and plant response
to disease. Group II contained 11 CaMATEs, 21 StMATEs, 22 AtMATEs, and another 10 MATE genes (Figure 1). Several Group II MATE genes have been reported to transport secondary metabolites, such as proanthocyanin, flavonoids, and nicotine. Arabidopsis TT12/DTDX41 mediates anthocyanin [11] and epicatechin 3′-O-glucoside [12] transport, and was shown to control the sequestration of flavonoids in the vacuole, thereby affecting seed coat pigmentation [79]. BrTT12 from rapeseed (Brassica rapa) also plays a role in seed coat pigmentation [80]. Several Group II MATE genes have been reported to transport secondary metabolites, such as proanthocyanin, flavonoids, and nicotine. Arabidopsis TT12/DTDX41 mediates anthocyanin [11] and epicatechin 3′-O-glucoside [12] transport, and was shown to control the sequestration of flavonoids in the vacuole, thereby affecting seed coat pigmentation [79]. BrTT12 from rapeseed (Brassica rapa) also plays a role in seed coat pigmentation [80]. Grapevine (Vitis vinifera) VvAM1 and VvAM3 appear to transport anthocyanins [13,14].

Arabidopsis DTX35/FFT was experimentally confirmed to be a flavonoid transporter [17], while DTX33 serves as a chloride channel that plays important roles in turgor regulation during stomatal movement [81]. Although the pepper and potato MATEs have not been functionally characterized, their similarity to MATEs from other species suggests that Group II MATEs may mediate the transport and accumulation of secondary metabolites.

Group III contained 5 CaMATEs, 8 StMATEs, and 6 AtMATEs. The few functionally characterized MATE genes from this group have been suggested to be involved in aluminum detoxification or iron translocation [2], and included AtMATE [24,25] and AtFRD3 [28], BoMATE [26], EcMATE1 [27], GmFRD3b [30], HvAACT1 [31–33], OsFRDL1/OsMATE10 [36–38], OsFRDL4/OsMATE4 [34,35], and OsFRDL2/OsMATE40 [37], SbMATE [27,39,40], ScFRDL1 and ScFRDL2 [41], TaMATE1B [42], and ZmMATE1 [43,44]. The results above suggested that Group III MATE genes may constitute the best candidates for aluminum detoxification or iron translocation. By contrast, Group IV contained 8 CaMATEs, 10 StMATEs, and 9 AtMATEs. Arabidopsis BUSH-AND-CHLOROTIC-DWARF 1 (BCD1)/ZARIZ (ZRZ)/ABS4 is involved in organ initiation, iron homeostasis, and hypocotyl cell elongation [45–47], while ACTIVATED DISEASE SUSCEPTIBILITY 1 (ADS1)/ABS3/ALtered DEVELOPMENT PROGRAM 1 (ADPI) was reported to negatively regulate plant disease resistance and hypocotyl cell elongation [47,82–85]. Arabidopsis EARLY LEAF SENESCENCE 1 (ELS1)/ABS3L1 and DTX50/ABS3L2 were shown to regulate cell elongation [22,86]. Group V contained 4 CaMATEs, 4 StMATEs, and 2 AtMATEs genes. Arabidopsis DTX18 functions in the export of hydroxycinnamic acid amides to the leaf surface, inhibiting the germination of Phytophthora infestans spores [23], while ABERRANT LATERAL ROOT FORMATION 5 (ALF5) was reported to be an efflux transporter for the protection of roots from toxic compounds [87].

The above results provide valuable information for further functional characterization of MATE genes during development and under stress conditions.

4. Materials and Methods

4.1. MATE Gene Identification

To identify MATE gene family members in pepper (Capsicum annuum) and potato (Solanum tuberosum), we used the 56 Arabidopsis AtMATE proteins [30] as queries for Basic Local Alignment Search Tool for Protein (BLASTP) against the pepper and potato proteomes [62–64]. In addition, we downloaded the Pfam entry PF01554 for the MATE domain from the Pfam database [88] and used it to search for MATE candidates in the pepper and potato proteomes from the Ensembl database [89], using HMMER 3.0 software [90], with an E-value cutoff of 10^{-5}, as previously described [71,91]. Candidate MATE proteins were further examined for the presence of the complete MATE domain, followed by protein scans through the SMART [92], CDD [93], InterProscan [94], and Pfam [88] databases.

4.2. Gene Structure and Domain Combinations Analysis

We predicted the MATE gene exon–intron organization through the Gene Structure Display Server (GSDS 2.0) (http://gsds.cbi.pku.edu.cn) [95], and TBTools [69]. MATE protein sequences were also used to search the SMART [92], InterProscan [94], and MEME [96] databases to detect functional domains.
4.3. Gene Promoter Cis-Regulatory Elements and Protein Subcellular Predictions

The MATE gene promoters were retrieved as the 2-kb sequence upstream of the coding regions, and were scanned through the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html) [97] to predict putative cis-regulatory elements. We determined the predicted subcellular localization of MATE proteins using the WoLF PSORT database (https://psort.hgc.jp).

4.4. Phylogenetic Analysis

We aligned the protein sequence of all MATE domains using MUSCLE, followed by phylogenetic tree construction using the maximum likelihood method in MEGA X [98], with the most suitable substitution pattern (LG + G + F), estimated by 56 different amino acid substitution models (Table S3), pairwise deletions and 1000 bootstraps. The phylogenetic tree was visualized using FigTree software.

4.5. Gene Duplication Synteny Analysis

The determination of intra-genomic syntenic and inter-genomic collinearity blocks in the Solanaceae was performed by employing MCScanX software [68] with default parameters. Tandem and segmental duplications were detected as previously described [50,71]. Tbtools software was used to visualize and illustrate the results [69].

4.6. Gene Expression Analysis

The tissue- and stress-specific expression patterns of pepper MATE genes were obtained from previously published transcriptome deep sequencing (RNA-seq) datasets (http://pepperhub.hzau.edu.cn) [70]. We identified differentially expressed genes and clustered the results using R software as previously described [99–101].

4.7. Quantitative RT-PCR Analysis

We harvested leaf and fruit tissue from the pepper cultivar Capsicum 6421 and stored the tissue at −80 °C [102,103] until RNA extraction. We performed quantitative reverse transcription PCR (qRT-PCR) analysis as previously described [102,104,105]. Primers used for pepper MATE gene expression analysis were obtained from the qprimerDB database [106] (Table S4), with CaUBI-3 as the internal control.

5. Conclusions

This comprehensive analysis of the MATE gene family in two economically and agronomically important species from the Solanaceae identified 42 CaMATE genes in pepper and 60 StMATE genes in potato, with an uneven distribution across chromosomes. Our phylogenetic analysis and gene structure analysis grouped these plant MATE genes into five groups, which showed notable conservation within each group, validating our classification scheme. Intra-genome synteny analysis indicated that tandem duplications played an important role in shaping the evolution of the pepper CaMATE gene family, while inter-genome collinearity analysis revealed the putative orthologs between the two Solanaceous species. Estimation of $\omega$ values demonstrated that MATE genes were under purifying selection. The analysis of cis-elements in the MATE promoters and MATE gene expression patterns highlighted their functional diversification in plant reproductive development and adaptation to diverse environmental stimuli, with functional differentiation between tandem-duplicated genes. In particular, examination of their expression patterns suggested that CaMATE genes might participate in plant fruit development, as pepper produces many metabolites during fruit ripening. This study provides a comprehensive and systematic characterization of the pepper and potato MATE gene families and will assist the continuing investigation of MATE functions in the Solanaceae.
Supplementary Materials: Supplementary materials can be found at http://www.mdpi.com/2223-7747/9/11/1448/s1. Figure S1: Motif sequences of the pepper and potato MATE proteins. MATE protein motif sequences were identified using MEME database. E-value indicates motif statistical significance. Sites indicated the amount of sites contribution in each motif. Width indicates the amount of amino acid in each motif. Table S1: Characteristic features of 42 CaMATE and 60 StMATE genes. Table S2: Ka, Ks and Ka/Ks ratios of segmental duplication pairs between pepper and potato. Table S3: Amino acid substitution models estimation of the maximum likelihood. Table S4: List of CaMATE genes primers used for qRT-PCR.

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Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ABA          | abscisic acid |
| Ca           | Capsicum annuum |
| CRE          | cis-regulatory element |
| GA           | gibberellin |
| GSDS         | Gene Structure Display Server |
| HMM          | hidden Markov model |
| IAA          | indole-3-acetic acid |
| Ka           | non-synonymous distance |
| Ks           | synonymous distance |
| MATE         | Multidrug and Toxic Compound Extrusion |
| MeJA         | methyl jasmonate acid |
| MEME         | multiple EM for motif elicitation |
| MUSCLE       | Multiple Sequence Comparison by Log-Expectation |
| MW           | molecular weight |
| Os           | Oryza sativa |
| pI           | isoelectric point |
| qRT-PCR      | quantitative reverse transcription PCR |
| SA           | salicylic acid |
| St           | Solanum tuberosum |

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