Comprehensive genome wide identification and expression analysis of MTP gene family in tomato (Solanum lycopersicum) under multiple heavy metal stress

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Abstract

Plant metal tolerance proteins (MTPs) play major roles in enhancing resistance to heavy metal tolerance and homeostasis. However, the role of MTPs genes in tomato, which is one of the most popular crops, is still largely limited. Hence, we investigated genome-wide study of tomato MTPs, including phylogenetic, duplication, gene structure, gene ontology and previous transcriptomic data analysis. Moreover, the MTPs expression behaviour under various heavy metals stress has rarely been investigated. In the current study, eleven MTP candidate genes were genome-wide identified and classified into three major groups; Mn-cation diffusion facilitators (CDFs), Fe/Zn-CDFs, and Zn-CDFs based on the phylogeny. Structural analysis of \textit{SlMTP}s showed high gene similarity within the same group with cation\_efflux or \textit{ZT}\_dimerdomains. Evolutionary analysis revealed that segmental duplication contributed to the expansion of the \textit{SlMTP} family. Gene ontology further showed the vital roles of \textit{MTPs} in metal-related processes. Tissue-specific expression profiling exhibited similar expression patterns in the same group, whereas gene expression varied among groups. The \textit{MTPs} expression was evaluated after tomato treatments by five divalent heavy metals (Cd$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Zn$^{2+}$, and Fe$^{2+}$). \textit{SIMTP} genes displayed differential responses in either plant leaves or roots under heavy metals treatments. Nine and ten \textit{SIMTP}s responded to at least one metal ion treatment in leaves and roots, respectively. In addition \textit{SIMTP}1, \textit{SIMTP}3, \textit{SIMTP}4, \textit{SIMTP}8, \textit{SIMTP}10 and \textit{SIMTP}11 exhibited the highest expression responses in most of heavy metals treatments. Overall, our findings presented a standpoint on the evolution of \textit{MTPs} and their evolution in tomato and paved the way for additional functional characterization under heavy metal toxicity.

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1. Introduction

Metals act as co-factor, which has essential implications inactivating enzymes in plant cells to perform the specific biological reaction (Thomine and Vert, 2013). The essential metals such as zinc (Zn), manganese (Mn), iron (Fe), cobalt (Co), and copper (Cu) at low level play an essential role in plants, but excessive amounts of these ions lead to toxic effects (Kolaj-Robin et al., 2015). Moreover, a deficient concentration of non-essential metals, including mercury (Hg), silver (S), cadmium (Cd), and lead (Pb), can also cause plant cell toxicity (Clemens, 2001). Interestingly, plants are natural bioaccumulators for various heavy metals from the water and soil for appropriate plant growth and development activities (Ali et al., 2013).

Plants overcome heavy metal stress by various physiological and molecular mechanisms, including genomic-level and complex biochemical processes (Liu et al., 2019). Some of these mechanisms
are part of the homeostatic process and are constitutive (Rai et al., 2019). Other mechanisms are exclusively related to counter-specific metal toxicity (Gupta et al., 2019). All responses can be widely classified as being tolerant or avoidance types (Krzesłowska, 2011). Metal uptake, trafficking, storage, chelation, and efflux are plant mechanisms to maintain metal homeostasis (Montanini et al., 2007). Several studies indicated the essential roles of various protein families with their specific transporters in these regulatory processes (Gao et al., 2020). The cation diffusion facilitator (CDF) family genes are integral membrane divalent cation transporters involved in divalent metal ions efflux from the cytoplasm either into subcellular compartments or outside the cell (Gustin et al., 2011). The CDF transporters have been widely identified in many organisms since the first identification in the bacterial cell (Nies and Silver, 1995), which further classified into three major groups; Mn-CDF, Zn/Fe-CDF, and Zn-CDF based on either confirmed or hypothesized transported substrate specificities (Montanini et al., 2007).

The CDF transporters are considered as metal-tolerance proteins (MTPs) in plants, which were classified into seven distinct groups (1, 5, 6, 7, 8, 9, and 12) based on annotation and phylogenetic analysis in Arabidopsis (Gustin et al., 2011). Many MTP proteins were identified in several plants species, including Arabidopsis thaliana (van der Zaal et al., 1999), Vitis vinifera (Shirazi et al., 2019), Populus trichocarpa (Gao et al., 2020), Triticum aestivum (Vatansever et al., 2017), Nicotiana tabacum, Nicotiana sylvestris and Nicotiana tabacum (Liu et al., 2019). Many Zn-CDF proteins have been studied from the first identified AtMTP1 in Arabidopsis (van der Zaal et al., 1999). Zn-CDF genes play an essential role in plant Zn2+ tolerance. For instance, AtMTP1 and AtMTP3 of tonoplastic involved in Zn and Co tolerance through the excess transport of Zn2+ and Co2+ ions to the vacuole (Arraïault et al., 2006; Dräger et al., 2004; Kawachi et al., 2008; Kobae et al., 2004). Furthermore, two more genes of Zn-CDF family, including AtMTP5 and AtMTP12 were identified to form a functional complex during Zn2+ transportation into Golgi (Fujiiwara et al., 2015).

The Mn-CDF family members, such as AtMTP8, play an essential role in the transportation of Mn2+ besides its role in the localization of Fe2+ and Mn2+ in seeds (Chu et al., 2017; Delhaize et al., 2007; Eroglu et al., 2016). In Oryza sativa, the OsMTPs (OsMTP8.1 and 8.2) of tonoplastic participate in the transport of Mn2+ within the plant (Takemoto et al., 2017; Tsunemitsu et al., 2018). Furthermore, the ShMTP gene in Stylosanthes hamata was the first group of MTPs to be identified that enhances the tolerance against Mn when overexpressed in Arabidopsis (Delhaize et al., 2007). Also, the OsMTP8 gene of cucumber confers Mn2+ tolerance when over-expressed in yeast and the Arabidopsis (Migocka et al., 2014).

Tomato (Solanum lycopersicum L.) is a common and economically essential crop worldwide (El-Sappah et al., 2019). Its consumption increases annually due to the fruit attractiveness (several sizes, colors, flavors, and shapes), multiple utilization, and production of therapeutic compounds (Cheng et al., 2020). A recommended healthy human diet consists of fruits and vegetables, and tomatoes are important because they contain carbohydrates, proteins, vitamins, minerals, dietary fibers, and antioxidants (Liu et al., 2020). However, tomato fruits are a potential mediator for heavy metal’s entrance into the food chain (Cobb et al., 2000), influencing human health.

Tomato roots grown in contaminated soil or irrigated with sewage water accumulated a higher content of Cu2+, Zn2+, and Ni2+, while the shoots had a higher Cd2+, Mn2+, Fe2+, and Pb2+ (Singh et al., 2010). However, only a few tomato MTP proteins have been studied and characterized. In recent years, genome sequencing of model plants and commercially essential plants were performed and provided opportunities to screen candidate genes (Edwards et al., 2017). However, due to the limit of the integrity of the tomato genome sequence, a few MTP members were not identified at that time, and the expression patterns, especially those in response to heavy metal stresses and the metal transport features of SIMTP genes, are unknown. In the current study, we successfully identified 11 SIMTPs in the tomato genome and comprehensively analyzed their structures and sequences. We comprehensively characterized the proteins at the sequence level and performed bioinformatics analyses of putative SIMTP genes to explore phylogenetic relationships, chromosomal distributions, gene structure, conserved motifs, and synteny analysis. In addition, five different heavy metals (Cd2+, Co2+, Fe2+, Mn2+, and Zn2+) were applied to tomato seedlings, and expression profiling was presented. Taken together, the results in this study would lay a theoretical and practical foundation for the functional characterization of SIMTP genes in future studies.

2. Materials and methods

2.1. Sequence retrieval of MTP genes in tomato

Tomato sequences were obtained from the Solanaceae Genomics Network (https://solgenomics.net/), and then BioEdit 7.0 software was used for the local database construction. The candidate tomato MTP genes were confirmed using the hidden Markov model (HMM) profile of two MTP domains (PF16916 and PF01545) from Pfam (http://www.sanger.ac.uk/Software/Pfam). The blast search of putative MTP protein sequences was performed on the NCBI (http://blast.ncbi.nlm.nih.gov/blast.cgi), SPud DB tomato Solanaceae Genomics Network (https://solgenomics.net), and phytomzome (https://phytozome.jgi.doe.gov/).

The validation of all obtained protein sequences was done at E-value < 1 × 10−5 for identification of the MTP domain, using SMART (http://smart.embl-heidelberg.de/) tools (Letunic et al., 2004). All genomic information about the selected MTP gene family, such as chromosomes location and CDS, were obtained from the phytomzome website database (https://phytozome.jgi.doe.gov/). The MTP proteins were analyzed to obtain their characteristics, such as molecular weight, amino acid number, isoelectric point, the theoretical pl, molecular weight and instability index using EXPASY ProtParam Tool (http://www.expasy.org/tools/protparam.html) (Gasteiger et al., 2003). The subcellular localization data was predicted using the MTP amino acid sequences by protein subcellular localization prediction tools (https://wolfpsort.hgc.jp/).

2.2. Phylogenetic analysis

In addition to tomato, Arabidopsis (http://arabidopsis.org), Cucumis sativus (http://cucurbitgenomics.org/), Populus trichocarpa (http://plantgdb.org/PGDB), Oryza sativa, (https://rapdb.dna.affrc.go.jp), and Triticum aestivum (https://www.wheatgenome.org/) MTP amino acid sequences were used for the phylogenetic tree of evolutionary MTPs relationship. Next, CLUSTALX 2.0 software with default parameters has been used for multiple alignments. The alignment was utilized as an input file to MEGA 6.0 software. A phylogenetic tree was constructed by the Neighbor-Joining (NJ) method with the following parameters: 1000 bootstrap replications, pairwise deletion, and Poisson model (Tamura et al., 2011).

2.3. Chromosomal locations, synteny analysis, and protein–protein interactions

Tomato gene database (https://phytozome.jgi.doe.gov/) supports us by the chromosomal position information of MTP genes, which were used to generate the genetics map by MapChart software.
After that, two genes in the same species, located in the same clade of the phylogenetic tree, were defined as coparalogs to identify whether tandem and segmental duplication events had occurred. On the other hand, the tomato gene database (https://phytozome.jgi.doe.gov/) was further used with target genes for detecting the coordinates of the segmental duplications. The paralogs were regarded as tandem results duplicated when two genes separated by five or fewer genes in a 100 kb region (Tang et al., 2008). Additionally, coparalogs were considered segmental duplications if they were located on duplicated chromosomal blocks (Wei et al., 2007). Smith-Waterman algorithm (http://www.ebi.ac.uk/Tools/psa/) was used to calculate the local alignments of two protein sequences. The synteny relationship with the chromosomal distribution for each SlMTP genes was introduced using circos (http://circos.ca/) (Krzynowski et al., 2009). Furthermore, for more knowledge about the cellular function of the MTP protein family, the functional interactions between all expressed studied proteins were obtained. The amino acid sequences of all the family members used for protein-protein interaction studies using the STRING database (https://string-db.org/).

2.4. Gene structures and motif analyses

The structure of all SIMTP gene family members was analyzed to detect the intron/exon and their organization, using both genomic and cDS sequences with the online tools of the Genes Structure Display Server program (GSDS, http://gds.cbi.pku.edu.cn/index.php) (Hu et al., 2015). The conserved motif was detected for the gene family members using a Multiple EM for motif elicitation (MEME) (http://meme.nbcr.net/meme3/meme.html) online server with default parameters setting a maximum number of motifs to 10 and motif width to 6–200 (Bailey et al., 2006).

2.5. Protein modeling, prediction, and gene ontology annotation (GO)

The Phyre2 online webserver was used for protein modeling, prediction, and analysis of the SIMTPs at the intensive mode (sbg.bio.ic.ac.uk/phyre2/) (Kelley et al., 2015). Blast2GO v3.0.11 (https://wwwblast2go.com) and OmicsBox software were used to identify MTP protein sequences for GO annotation (Conesa and Götz, 2008).

2.6. Digital data expression analysis

Our research analysed the previous RNA-based digital data to obtain the global expression of MTP gene family members during normal growing conditions. The expression data were downloaded from tomato functional genomics databases (http://red.bti.cornell.edu/pgsc_download.shtml) for some of the tomato organs such as leaves and roots, and flowers. Then the gene expression was analyzed using the cufflinks (version: 2.2.1). Finally, FPKM expression values were divided with mean and transformed to log2. The MeV 4.5 was used to cluster the expression data as a heat map (http://heatmapper.ca/) (Babicki et al., 2016; Saeed et al., 2006).

2.7. Growth conditions and heavy metal treatments

In this study, the tomato M82 line was cultivated during the Autum of 2020 at the experimental greenhouse of Yibin University (China). First, the seeds were washed with 10% hypochlorous acid and distilled water. The seeds have been germinated using water-stabilized filtered paper and then transferred to fertilized pitmoss soil with germination conditions of 16 h light (27 °C) and 8.0 dark (18 °C) with a relative humidity of 70%. Four seeds were planted in each plastic pot. After emergence, thinning was performed to maintain two uniform seedlings per pot. Thirty-day-old tomato was placed in 1/2 Hoagland solutions (pH 6.0) with different heavy metal concentrations 0.1 mM CdCl2, 0.1 mM CoCl2, 0.5 mM FeSO4, 1 mM MnSO4, and 0.5 mM ZnSO4, respectively, while normal 1/2 Hoagland solutions was used as the control (CK) (Desoky et al., 2019; Gao et al., 2020). The experimental pots were positioned in a complete randomized block design. The experiment was composed of 6 treatments, as shown above, and each treatment was repeated with three pots. Then, 24 h later, the leaves and roots of tube plantlets were collected and used as RNA extraction materials. Three biological replicates of expression analyses have been performed for each treatment.

2.8. qRT-PCR analysis

The Trizol reagent (Invitrogen, USA) was used to isolate the RNA from all plant samples (leaf and root), and the cDNA synthesis was performed using SuperMix Kit (Transgen, Beijing). (El-Sappah et al., 2017). The specific primers of all selected genes were designed using Primer Premierv5.0 (Table S1) with β-actin as a housekeeping gene. The real-time PCR was performed with the following reagents volumes: 10 μL SYBR Premix Taq (2 ×) mixture, 1 μL of cDNA, 0.5 μL of each primer, and 8 μL of ddH2O for a total volume of 20. The PCR cycles were adapted as follows: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 60 s. The relative gene expression levels were calculated based on the 2−ΔΔCT method (Livak and Schmittgen, 2001).

2.9. Statistical analysis

Three biological replicates of expression analyses were performed with ± standard deviation (SD) at p < 0.05. The significant variations between means were compared at p < 0.05 (Student's t-test).

3. Results

3.1. Identification of MTP genes in tomato

A complete set of 18 putative genes were identified in the tomato genome using the homologous sequences of Arabidopsis as queries and after excluding the sequences with an incomplete or missing domain. We finally selected 11 candidate genes for further evaluation and study. The genes were designated new names from SlMTP1 to SlMTP11. The different physicochemical characters of these 11 genes were presented in Table 1. The majority of tomato chromosomes harbored the MTP genes, except chromosomes number 1, 4, 8, and 11, which do not carry any of these genes. Furthermore, the molecular weight varied between all genes, ranging from 41197.34 to 54954.83 Da. The number of introns varied mainly among the group, and all genes contained introns, ranging from 41 to 58 introns. Furthermore, most of SIMTPs, according to subcellular localization analysis, were located as secreted proteins in tonoplast, except three genes (SIMTP4, 8, and 11) in cytoplasm while SIMTP6 in the chloroplast.

3.2. Phylogenetic analysis of MTP gene families

The phylogeny of the MTP gene families showed that MTP proteins are divided into seven groups named: groups 1, 5, 6, 7, 8, 10, and 12 (Fig. 1). Group 10 harboured 21 of MTPs, which represent the biggest group. Groups 8 and 10 included most of the studied SIMTP, with three genes in each. Furthermore, all of the selected 11 SIMTP grouped under three clusters of Zn-CDFs (3 genes), Fe/Zn-CDFs (2 genes), and Mn-CDFs (6 genes).
lies (A, B, C, D, E, and F). Subfamilies A and B were the largest with (-), (+), MW, aa, GRAVY, and PI denote to a total number of negatively charged residues (Asp + Glu), the total number of positively charged residues (Arg + Lys), molecular weight, amino acid number, Grand average of hydropathicity, and isoelectric points, respectively.

Furthermore, our investigation showed no obvious tandem duplication events except SlMTP7, SlMTP8, and SlMTP9, which had no pair with any other gene.

SlMTPs gene family members as they carry six whole gene members were divided into six subfamilies. Members of subfamilies C, D, and E each had only one gene (Fig. 3a). There was variation in exon–intron among different subfamilies (Fig. 3c), supporting the close evolutionary relationships of tomato MTP gene family members. Our analysis showed that most MTP family members contain an incredibly varied intron number except for group F genes, which had a lack of intron. The complexity of gene structure often indicates the largest intron. The analysis of the conserved motifs of MTP using MEME depends on the amino acid sequences with ten motifs (Fig. 3b, and Table S3). Most of the studied motifs contain 50 amino acids, except motif 9 contains 49 amino acids, while motif 18 contains 15 amino acids. The largest common motifs were 2, which was noticed within all subfamilies except for C, D, and E, followed by motifs 3, 15, and 16, while the subfamily E contains only four motifs. It is primarily observed that the number, type, or order of motifs is similar within the same subfamily in addition to that among different families.

3.4. Gene structures and motif analyses of the tomato MTP gene family

The tomato MTP gene members were divided into six subfamilies (A, B, C, D, E, and F). Subfamilies A and B were the largest with six members, followed by subfamily F (2 genes), whereas subfamilies C, D, and E each had only one gene (Fig. 3a). The complexity of gene structure often indicates the largest intron. The analysis of the conserved motifs of MTP using MEME depends on the amino acid sequences with ten motifs (Fig. 3b, and Table S3). Most of the studied motifs contain 50 amino acids, except motif 9 contains 49 amino acids, while motif 18 contains 15 amino acids. The largest common motifs were 2, which was noticed within all subfamilies except for C, D, and E, followed by motifs 3, 15, and 16, while the subfamily E contains only four motifs. It is primarily observed that the number, type, or order of motifs is similar within the same subfamily in addition to that among different families.

3.5. Protein modeling, prediction, protein–protein interactions and gene ontology annotation (GO)

The eleven predicted models of the SlMTPs were generated based on c6xpdB, c3j1zP, c2qfiB, and d2qfia2 templates with a 100% identification ratio (Fig. 4a). The protein–protein interactions assessment showed the physical (direct) and the functional (indirect) associations (Fig. 4b). The result showed different interactions within the studied proteins where the total number of nodes was 11 with an average of 7. 09. The STRING database analysis showed 39 edges without any expected numbers and showed six representative local network clusters, which were CL:28212, CL:28208, CL:28384, CL:28201, CL:28198, and CL:28199 (Table S4). Moreover, our protein analysis showed two common domains within the MTP family, which were PF16916 (7 proteins), and PF01545 (10 proteins).

Similarly, sub-cellular localization, molecular function, and biological process were predicted by GO enrichment analysis (Fig. 5). In sub-cellular localization analysis, the predicted distribution scores of MTP proteins were as following; 11/69% in all membranes, 2/12% in the plasma membrane, vacuole, and Golgi apparatus.

Noticeably, SlMTP4 gene was localized in 19 sub-cellular compartments out of all 23, which underlined its significant role in metal stress resistance. Collective scores of MTP protein molecules during biological processes were as following; trans-membrane transport of Zn⁺ was 2/18%, and Mn⁺ ions were 4/36%, while trans-membrane transport of Fe was 1/9%. The molecular function and biological processes analysis revealed that SlMTP1 and SlMTP3...
Fig. 2. Genome-wide synteny analysis for MTP gene family at 12 tomato chromosomes. The blue lines represented the syntenic orthologs and paralogs and showed the segment duplication.

Fig. 3. Phylogenetic relationship, gene structure, and conserved motif analysis of SlMTP genes; a) The neighbor-joining phylogenetic tree was constructed with MEGA6.0 using SlMTP amino acid sequences with 1000 times replicate. b) The motif composition of SlMTP proteins using ten conserved motifs is represented by the unique colour mentioned in the box on the top left. c) Exon-intron structure of MTP tomato proteins where dark green boxes presented the exons, and the black lines represent the introns. The blue boxes represented the untranslated regions (UTRs), with size scales detailed at the bottom.
genes play a key role in the transmembrane transport of Zn⁺, while SlMTP4, SlMTP8, SlMTP10, and SlMTP11 play a crucial role in transmembrane transport of Mn²⁺. Moreover, SlMTP4 is an essential factor affecting Fe ion transport.

3.6. Gene expression profiling based on the digital data

The tissue expression patterns of SlMTPs were investigated depended on the public transcriptome data. As shown in Fig. 6 and Table S5, all 11 SlMTP genes were expressed in the ten examined tissues (log₂(FPKM + 1) > 0), except for SlMTP2 (which showed lower expression only in root tissue), SlMTP4 (only expressed in flower buds, opened flowers, and 3 cm fruits) and SlMTP8 (expressed only in flower buds and roots).

Furthermore, SlMTP1 had the significantly higher transcript accumulation compared with other SlMTPs in all detected tissues, except for in flower buds, mature green fruits, and leaves, whereas two gene SlMTP2, SlMTP4, and SlMTP8 exhibited the lowest or no expression levels in most tissues (0 < log₂(FPKM + 1) < 1). Furthermore, some genes exhibited tissue-specific expression. For instance, one gene (SlMTP1) in the 3 cm fruits, two genes (SlMTP1, and SlMTP9) in root, three genes (SlMTP1, SlMTP10, and SlMTP11) in leaves, one gene (SlMTP1, and SlMTP10) in opened flower showed the highest transcript abundances.

3.7. qRT-PCR analysis of the SlMTPs under different treatments

The 11 SlMTP genes showed differential expression pattern against different types of heavy metal stress in root and leaf (Fig. 7). In roots, Cd²⁺ enhanced the expression of SlMTP2 and SlMTP3, but down-regulated the expression of SlMTP1, SlMTP5, and SlMTP9, while it up-regulated the expression of SlMTP4. Co²⁺ decreased the expression levels of SlMTP2, but up-regulated the expression levels of SlMTP7 and SlMTP9. The SlMTP10 recorded the highest expression under Mn²⁺ contamination, but the expression of other three genes (SlMTP1, SlMTP7, and SlMTP9) down-regulated. Zn²⁺ increased the expression of SlMTP1 and SlMTP3. Fe²⁺ up-regulated the expression levels of SlMTP4 and SlMTP10, but down-regulated the expression of SlMTP8. However, Co²⁺ up-regulated the expression of SlMTP2, SlMTP4, SlMTP10, and SlMTP11, but decreased the expression of SlMTP9. Moreover, Fe²⁺ up-regulated the expression of SlMTP1, SlMTP3, SlMTP4, SlMTP10, and SlMTP11, but down-regulated the expression of SlMTP6, SlMTP8, and SlMTP9. Mn²⁺ down regulated the expression of SlMTP7, but enhanced the expression of SlMTP4, SlMTP8, SlMTP10, and SlMTP11. Zn²⁺ decreased the expression of SlMTP2 but up-regulated the expression of SlMTP1, SlMTP3, and SlMTP4.

4. Discussion

Heavy metals are the most effort on the ecosystem and make it unfit for human consumption (El-Sappah Et Al., 2012). Once released into the environment, they accumulate into plants then into other living tissues via the food chain and cause toxicity even at lower concentrations (Elrys et al., 2018). MTP genes (membrane divalent cation transporters) are essential for transporting various heavy metals and enhancing plant tolerance against heavy metals stress (Ricachenevsky et al., 2013). They also have an expected role in plant mineral nutrition maintenance (Liu et al., 2019). Moreover, these metal-binding proteins are now being utilized as bio-environmental markers for predicting heavy metal contamination based on their expression levels (Samuel et al., 2021). The MTP family has previously been studied in several plants, such as Arabidopsis thaliana (van der Zaal et al., 1999), Nicotiana tabacum, Nicotiana sylvestris and Nicotiana tomentosiformis (Liu et al., 2019), Triticum aestivum (Vatansever et al., 2017), and Populus trichocarpa (Gao et al., 2020), while this is the first genomic identification study of MTPs family in tomato. We successfully identified 11 MTP genes in tomato and named based on the sequence similarities and orthologous relationships between them and AtMTPs. The phylogeny of MTP proteins between tomato and other five species include Arabidopsis, Nicotiana, Triticum, Populus, and Populus trichocarpa.
Fig. 5. Gene Ontology analysis of tomato SlMTP genes. Gene ontology showed the distribution of every SlMTP gene in the plant, where a red colour column mentioned the cellular component. In contrast, the biological processes in which the MTP family participate were mentioned by the blue colour column, and the molecular function was mentioned by move colour.
in specific mechanisms, and this information could provide clues to predict their function in different species. The broad range of basic physicochemical properties of SlMTP gene was consistent with previous studies indicating huge probabilities of amino acid in metal tolerance (Ricachenevsky et al., 2013; Consortium, 2010; Eroglu et al., 2017). Consistently with the previous study (Vatansever et al., 2017), the subcellular localization analysis revealed that most genes are localized to tonoplast (the vacuole membrane), whereas some might also be localized in cytoplasm or chloroplast, suggesting that SlMTPs might function as the vacuole-localized cation transporters.

In our study, to obtain more knowledge about the gene annotation and the expansion mechanism of the MTP gene family in tomatoes, we investigated the gene synteny and duplication analysis (Fig. 1 and Table S2). Two or more genes on the same chromosome are often related to tandem duplication, while segmental duplication often occurs on different chromosomes (Schlueter et al., 2007). Our study did not show any of the tandem duplication pair, while there were 28 segmental duplications, such pairs are SIMTP1/SIMTP5, SIMTP3/SIMTP5, and SIMTP5/SIMTP6. During the evolution process of a plant gene, family duplication events occur, followed by divergence considering standard features and more related to secondary plant metabolism genes (Ober, 2005).

Almost all subfamilies contained the same numbers of introns and motif sequences which are consistent with the previous studies in where a similar gene structure was found within the same subfamilies (Liu et al., 2018). For example, all of the gene members of subfamily A contain five introns. However, subfamily F members do not contain any introns. These outcomes indicated that during the tomato evolution events of SIMTPs, some intron gain and loss occurred. Some genes have no intron but have one exon cause the lower ability of exons in the gain/loss rate due to higher selection pressure in the exons sequences (Harrow et al., 2006). Thus, with all these observations, it is probably that the placement divergences in intron number consider shared events related to the gene family evolution (Babicki et al., 2016; Jeffares et al., 2006; Rogozin et al., 2012). In a detailed evaluation of the MTP proteins, we predicted their 3D configuration, which considers supportive tools for expecting their function (Büyükköroğlu et al., 2018). The four temples in tomato MTP proteins indicated that these proteins with heavy metal where these transport proteins are in pant are classified into metal-uptake proteins that transport essential and toxic heavy metals to the cytoplasm and metal-uptake proteins. At the same time, the other is metal-efflux proteins that help the cell remove any excess heavy metals (Mani and Sankaranarayanan, 2018). On the other hand, protein–protein interaction analysis provided us with more knowledge about the plant developmental processes with their interactions with the environment (Struk et al., 2019). Our 11 nodes (MTPs) with 39 nodes indicate the significant interactions more than expected, reflecting that the MTP proteins are at least partially biologically connected as a group.

On the other hand, gene ontology is a fundamental analysis to predict putative functional contributions across living organisms (Consortium, 2010). Moreover, gene ontology classes and concepts have been used to define the relationships and gene functions existing between these concepts (Purwantini et al., 2014).
gene ontology analysis revealed the significant role of the tomato SlMTP genes with heavy metals (Fig. 5). Furthermore, the GO showed the molecular functions, where more than 8 of them participate in metal-related processes such as transmembrane transporter activity, cation transmembrane transporter activity, transporter activity, and ion transmembrane transporter activity.

The previous transcriptomic is a proper tool for detecting the existence, structure, and quantity of the RNA in any abiological sample under certain conditions (Zambounis et al., 2020). Thus, we investigated the expression profile of all members of the MTP gene family from previously published RNA-sequencing data, which showed the expression of all gene members in all selected tomato tissues (Fig. 6 and Table S5). Digital data analysis showed that the significant roles of the MTP gene could contribute significantly to growth and development. Worth evidence has been obtained about the essential roles of tomato MTPs after tissue expression evaluation. For instance, the exclusive expression of the three genes SlMTP3, SlMTP4, and SlMTP6 were in the young flower, whereas SlMTP7 was most plentiful in mature fruits, indicating that they might be involved in early flower and fruit development. Besides the vital expected roles of SlMTP3 in fruit maturation and development, its expression has increased. However, only SlMTP2 and SlMTP8 were rarely expressed in all examined tissues from all SlMTPs. The documented down-regulation in some gene expressions is essential for maintaining the gene duplicates and ancestral functions (Qian et al., 2010). Hence in our study, the down-regulation of SlMTP2 and SlMTP8 expression is expected to be vital for keeping their biological functions and maintain them from losing during the cell evaluation. The reliability of the transcriptome data was further validated by qRT-PCR; however, the minor asymmetry between both analyses may be due to different growth conditions and tomato varieties, which finally affected the spatial expression. We examined the expression behaviour of MTP genes under five divalent metals (Mn\(^{2+}\), Cd\(^{2+}\), Co\(^{2+}\), Fe\(^{2+}\), and Zn\(^{2+}\)). Numerous studies in other plants indicated the significant roles of the MTP gene family to enhance the plant tolerance against these metals (Gao et al., 2020; Montanini et al., 2007) as it was described as metal efflux transporters from the cytoplasm, mainly transporting Zn\(^{2+}\), but also transports Ni\(^{2+}\), Co\(^{2+}\), Cd\(^{2+}\), Fe\(^{2+}\), and Mn\(^{2+}\) (Ricachenevsky et al., 2013).

The transcript accumulation transcription of MTPs in response to various heavy metals was varied and complicated, although the gene expression response to different stresses is usually reflected in corresponding gene roles. In Arabidopsis, the tonoplast-localized Zn\(^{2+}\) transporter AtMTP1 showed slight changes in its expression with excess Zn\(^{2+}\) exposure at both
scription and translation levels (Dräger et al., 2004; Kobae et al., 2004). Moreover, although the high expression of CsMTP1 encoded protein, the gene expression was steady under the high concentration of Zn\(^{2+}\) in cucumber (Migocka et al., 2015).

As mentioned before, the up-regulation of AtMTP12 does not depend on Zn concentration, but it could transport Zn\(^{2+}\) by combining AtMTP5 in heterodimeric complex form (Fujiwara et al., 2015), similarly to findings of Liu et al. (2019) publication in tobacco. Moreover, Mn\(^{2+}\) different supplies have a slight effect on the expression of Mn-CDFs (AtMTP8, 9, 10, and 11) (Delhaize et al., 2007). Recently, similar findings were further described in tobacco (Liu et al., 2019). All Zn-CDF members except for SIMTP3 recorded slight changes in their expression with excess Zn\(^{2+}\) exposure in our study. Besides, the up-regulation of SIMTP6 of Zn/Fe-CDFs exceeds Zn\(^{2+}\), while it down-regulated by Fe\(^{2+}\) in different tomato tissues. Furthermore, only SIMTP10 of Mn-CDF class was highly affected by the accumulation of Mn\(^{2+}\). Therefore, our studies would be essential for investigating MTPs molecular roles in tomatoes under various heavy metals stresses.

Generally, these results would provide essential clues for clarifying the roles of SIMTPs in heavy metal tolerance and the mechanism of heavy metal transport mediated by SIMTP proteins. Taken together, these results would lay a theoretical and practical foundation for the functional characterization of SIMTP genes in future studies. Furthermore, the highest expressed MTPs (SIMTP1, SIMTP3, SIMTP4, SIMTP8, SIMTP10, and SIMTP11) can be used as bioenvironmental markers for predicting heavy metal contamination based on their expression levels.

5. Conclusion

We provided the first genome-wide study of the MTP gene family in tomatoes, providing important comparative data for evolutionary relationships. Eleven identified MTP genes were phylogenetically divided into three major subfamily-specific clusters (Zn/Fe-CDFs, Mn-CDFs, and Zn-CDFs), and seven groups seemed to have undergone expansion and gene loss after polyploidization through segmental duplication. Gene ontology further seemed to have undergone expansion and gene loss after polyploidization. Tissue-specific expression patterns exhibited similar expression patterns in the same group, whereas gene expression varied among groups. The expression patterns of SIMTP members in response to various heavy metals at different tissues indicated the significant role of these genes in tomato growth and development. Furthermore, our gene expression analysis of various heavy metals revealed the important parts of the MTPs, especially, SIMTP1, SIMTP3, SIMTP4, SIMTP8, SIMTP10, and SIMTP11 in-plant tolerance to heavy metals stresses. .

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CRediT authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.07.073.

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