BMC Plant Biology

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Bioinformatics analysis of the tomato (*Solanum lycopersicum*) methylesterase gene family

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Abstract

Background Methylesterases (MESs) are a class of enzymes responsible for the demethylation of methylated compounds in plants, play a vital role in plant growth and development. However, studies on MES enzymes in tomato (*Solanum lycopersicum*) are limited.

Results This study systematically identified MES genes in tomatoes for the first time and studied their physicochemical properties, evolutionary relationships, and expression patterns. Sixteen *Solanum lycopersicum* methylesterase (*SlMES*) genes were identified through comprehensive bioinformatics analysis and were categorized into three subfamilies. Members of the same subfamily exhibited similar gene structures, structural domains, and conserved motifs. Chromosomal analysis revealed an uneven distribution of *SlMESs* across the five chromosomes, with evidence of gene duplication. Cis-acting element analyses suggested that the *SlMES* family may have important regulatory functions in tomato growth, development, and stress responses. Among them, *Solyc02g065260* was further examined for its role in tomato fruit ripening and stress responses. Its tissue-specific expression patterns, dynamic expression during fruit ripening, and responses to pathogens, low temperatures, and hormones, such as methyl jasmonate (MeJA), methyl salicylate (MeSA), abscisic acid (ABA), and ethylene (ET), were analyzed. The results provided further evidence towards understanding the roles of the SlMES family in the tomatoes.

Conclusions The results established a theoretical foundation for future investigations into the functional characterization of MES genes during tomato growth and development.

Keywords Tomato, MES gene family, Bioinformatics analysis, Expression analysis

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Introduction

Under specific environmental conditions, many plant hormones undergo demethylation, including methyl jasmonate (MeJA), methyl salicylate (MeSA), and indole-3-acetic acid methyl ester (MeIAA) [1]. The demethylation process of MeJA, MeSA, and MeIAA is catalyzed by different members of the methylesterase (MES) family, each exhibiting distinct hydrolytic activities [2]. The first identified MES protein was salicylic acid-binding protein 2 (SABP2) in tobacco, which functions as a MeSA esterase and is essential for plant stress responses, aiding in the development of systemic acquired resistance (SAR) [3]. Subsequently, Yang et al. [2] identified 20 proteins in *Arabidopsis* that share high sequence similarity with SABP2, collectively termed the MES family due to their specific hydrolytic activities towards MeSA, MeJA, and MeIAA [2]. All MES proteins belong to the α/β hydrolase superfamily, distinguished by a conserved catalytic triad of serine (Ser), aspartic acid (Asp), and histidine (His) residues [4, 5], with α/β hydrolase folding as their major structural motif.

Functional studies of the MES gene family have revealed its significant roles in stress responses and hormone regulation, primarily through the hydrolysis of ester bonds. Experimental evidence supports these functions in many plant species, including *Arabidopsis thaliana* [2], tobacco [6], grape [7], citrus [8, 9], and tomato (*Solanum lycopersicum*) [10].For example, silencing *SABP2* in tobacco leads to the loss of SAR and suppression of local defense responses [6]. In *Arabidopsis*, AtMES17 specifically hydrolyzes MeIAA to IAA, promoting the elongation of root hypocotyls [2]. In grapes, VvMJE1 targets MeJA hydrolysis, and its expression is significantly up-regulated following UV irradiation and cold treatment [7]. In citrus plants, overexpression of *SABP2* results in enhanced tolerance to Huanglongbing (HLB), with transcriptomic analysis showing upregulation of several genes related to plant defense mechanisms and the SAR pathway (e.g., *NPR1* and *PR1*) [8, 9]. Additionally, in tomato, members of the *Solanum lycopersicum* MES (SlMES) family (SlMES1-4) convert MeSA to salicylic acid (SA) in ripe fruits, enhancing the flavor of the fruit [10].

Tomatoes are a major horticultural crop cultivated worldwide [11] due to their rich nutrients and unique flavors [12]. Among the various flavor ester volatiles found in tomatoes, MeSA imparts a wintergreen oil flavor that can sometimes detract from the overall taste [13]. The SlMES family plays a crucial role in converting MeSA to SA, thereby improving the flavor profile [10]. Moreover, MeJA and MeSA, as volatile esters, serve as inactive signaling molecules that require hydrolysis by the MES family to be activated as JA and SA, which contribute to plant defense responses [14]. However, the biological features and specific roles of the *SlMES* family in tomatoes remain unclear, and systematic identification and analysis of the *SlMES* gene family using the latest genomic data from tomatoes is imperative.

Therefore, this study aimed to use the latest genomic data of tomatoes to identify and conduct a bioinformatics analysis of the members of the *SlMES* family. This study will provide a reference for further research on the function of this gene family in regulating tomato fruit ripening and stress tolerance processes.

Table 1 Physicochemical properties of 16 SlMES proteins identified in this study

Gene ID	Molecular weight (Da)	Isoelectric point	Grand average of hydropathicity	Instability index	Number of amino acids
Solyc03g070380	29932.80	5.64	-0.11	45.56	264
Solyc02g065240	29598.25	5.73	-0.17	37.02	264
Solyc02g065250	22395.80	5.48	-0.16	45.23	197
Solyc02g065260	19150.09	4.96	-0.05	47.86	170
Solyc01g108740	32133.47	9.39	0.10	32.36	288
Solyc01g108750	31591.92	7.13	0.15	45.00	286
Solyc03g044790	29526.96	5.52	-0.11	36.36	262
Solyc03g044740	34729.46	6.92	-0.21	41.81	301
Solyc02g065280	30191.02	5.56	-0.10	40.40	265
Solyc03g044820	29496.98	5.78	-0.14	37.43	262
Solyc06g048570	31028.76	5.42	-0.09	37.57	280
Solyc03g095550	30594.27	6.00	-0.22	53.99	273
Solyc06g064870	51029.79	7.17	-0.20	47.33	454
Solyc02g089060	41450.11	9.21	-0.32	52.54	379
Solyc05g012180	47213.69	9.57	-0.49	54.91	419
Solyc01g108780	29029.70	6.39	0.10	39.63	265

Results and analysis

Identification and analysis of *SlMES* family members

To identify members of the MES gene family in tomatoes, a BLAST search was conducted using 20 *Arabidopsis* MES protein sequences as queries in the relevant databases. Further screening was conducted using the Pfam database and NCBI CD-Search to validate conserved domains. After removing disqualified sequences, 16 *SlMES* genes were identified in the tomato genome (Table S1).

Predictions of the physicochemical properties of SlMES proteins (Table 1) indicated that their molecular weight ranged from 19,150.09 Da (Solyc02g065260) to 51,029.79 Da (Solyc06g064870). The isoelectric point varied from 4.96 (Solyc02g065260) to 9.57 (Solyc05g012180), with 11 SlMESs exhibiting an isoelectric point of less than 7 and 5 SlMESs exhibiting an isoelectric point greater than 7. This finding suggests that the majority of SlMES are acidic proteins. All SlMESs except for Solyc01g108740, Solyc01g108780, and Solyc01g108750 were hydrophilic, with average water solubility values below 0. The

instability coefficients of SlMESs ranged from 32.36 (Solyc01g108740) to 54.91 (Solyc05g012180). The majority of SlMESs (10) were unstable proteins, with an instability coefficient > 40. The number of amino acids in the SlMES proteins ranged from 170 (Solyc02g065260) to 454 (Solyc06g064870).

Phylogenetic tree analysis of SlMESs

To elucidate the functional and evolutionary relationships of MES proteins, multiple sequence alignments were performed using 36 MES protein sequences from *Arabidopsis thaliana* and *Solanum lycopersicum*, leading to a phylogenetic tree that classified the 36 MES members into three subfamilies: A, B, and C (Fig. 1).

Studies of the MES family in plants have demonstrated its central role in phytohormone demethylation. The classification of MES proteins in *Arabidopsis thaliana* provides a fundamental framework for studying the substrate specificity of the SlMESs. AtMESs can be categorized into four groups based on their substrate specificity [2]: the first group (AtMES1/2/3/7/9/16/17/18) exhibits hydrolase activity for MeIAA and is clustered in subfamilies A and C; the second group (AtMES1/2/4/7/9) exhibits MeSA hydrolase activity, with all five members belonging to subfamily A; the third group (AtMES1/2/3/9/10/16) possesses MeJA-hydrolyzing activity and is classified into subfamilies B and C; and the fourth group (AtMES5/8/11/12/14) does not demethylate any hormones and is clustered into subfamilies B and C.

Notably, Solyc03g44740 is closely related to AtMES10, indicating that it may also possess MeJA hydrolytic activity, while Solyc03g095550 and Solyc06g048570 are closely related to AtMES17, suggesting that they may function similarly to specifically hydrolyze MeIAA. Furthermore, Solyc02g089060 was most closely related to AtMES11, whereas Solyc06g064870 was most closely related to AtMES14, implying that neither was likely to function in hormone demethylation. Additionally, Solyc01g108740

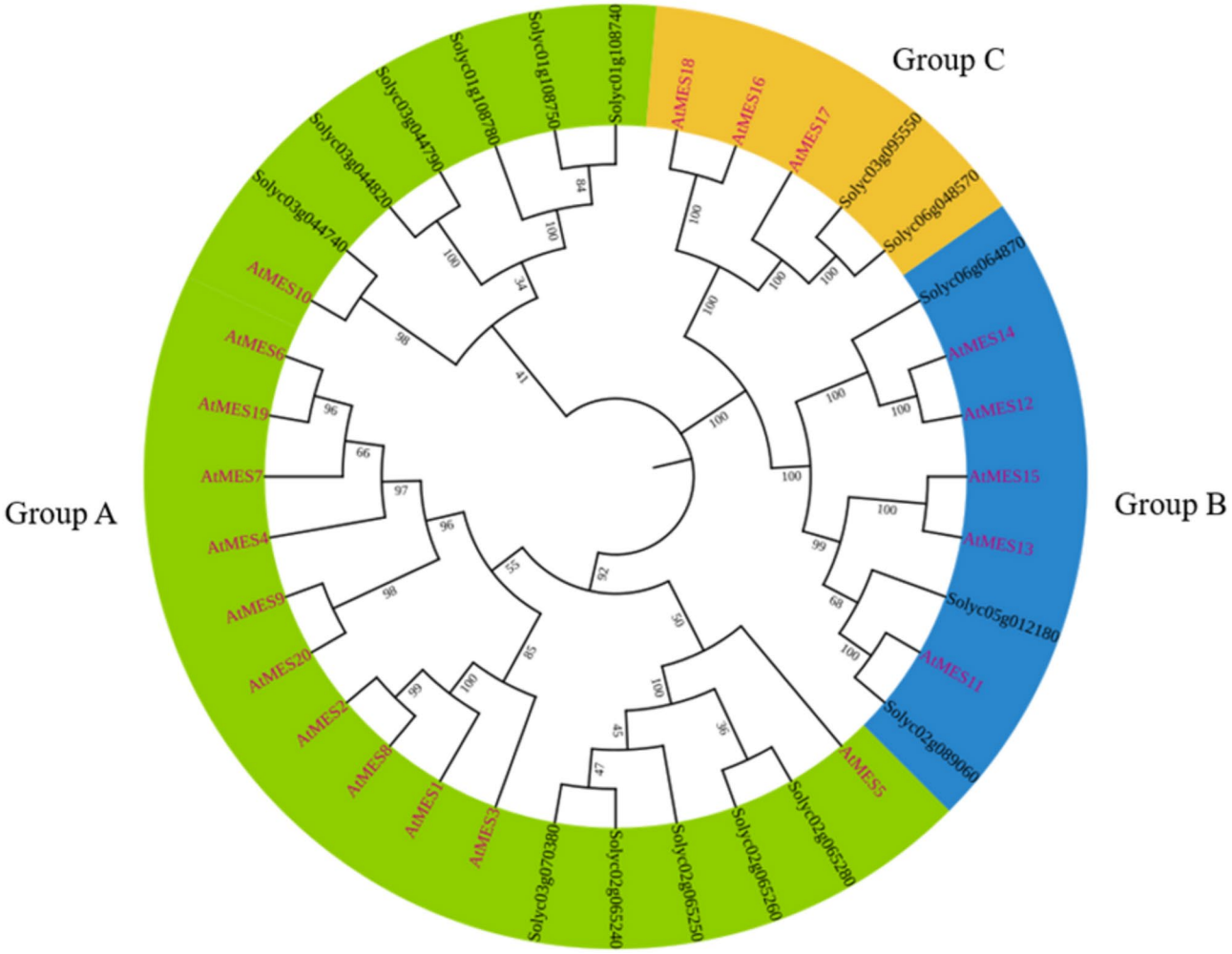

Fig. 1 Phylogenetic tree of SlMESs and AtMESs. Different background colors represent various subfamilies, with purple font indicating AtMESs and black font denoting SlMESs

and Solyc01g108750 did not cluster significantly with other members, which may indicate differences in their evolution, function, structure, and regulation compared to other SlMESs.

Chromosome localization and collinearity analysis of *SlMES* family members in tomato

Chromosomal localization analysis of *SlMESs* (Fig. 2) indicated that these genes were distributed across chromosomes 1, 2, 3, 5, and 6, with the highest concentrations found on chromosomes 2 and 3, each containing 5 *SlMES* members. Chromosome 5 had the lowest representation, containing only one *SlMES* (*Solyc05g12180*). The findings revealed that *SlMESs* were predominantly located at the chromosomal ends.

Gene duplication events were analyzed using TBtools (Fig. 2). Two pairs of fragment-duplicated genes were identified: *Solyc01g108750* and *Solyc03g095550*, *Solyc05g012180* and *Solyc02g089060*. The results indicate that segmental duplication significantly contributed to the expansion of the *SlMES* gene family members. To further explore the evolutionary patterns of *SlMES* genes, we assessed the synonymous substitution rates (Ks) between the duplicated gene pairs. All duplicated gene pairs exhibited Ka/Ks ratios of < 1, indicating that *SlMES* genes were generally under purifying selection (Table 2).

Additionally, MES genes from *Solanum lycopersicum*, *Oryza sativa*, and *Arabidopsis thaliana* were used to analyze interspecific collinearity (Fig. 3). The results showed that only 2 MES genes were collinear between *Solanum*

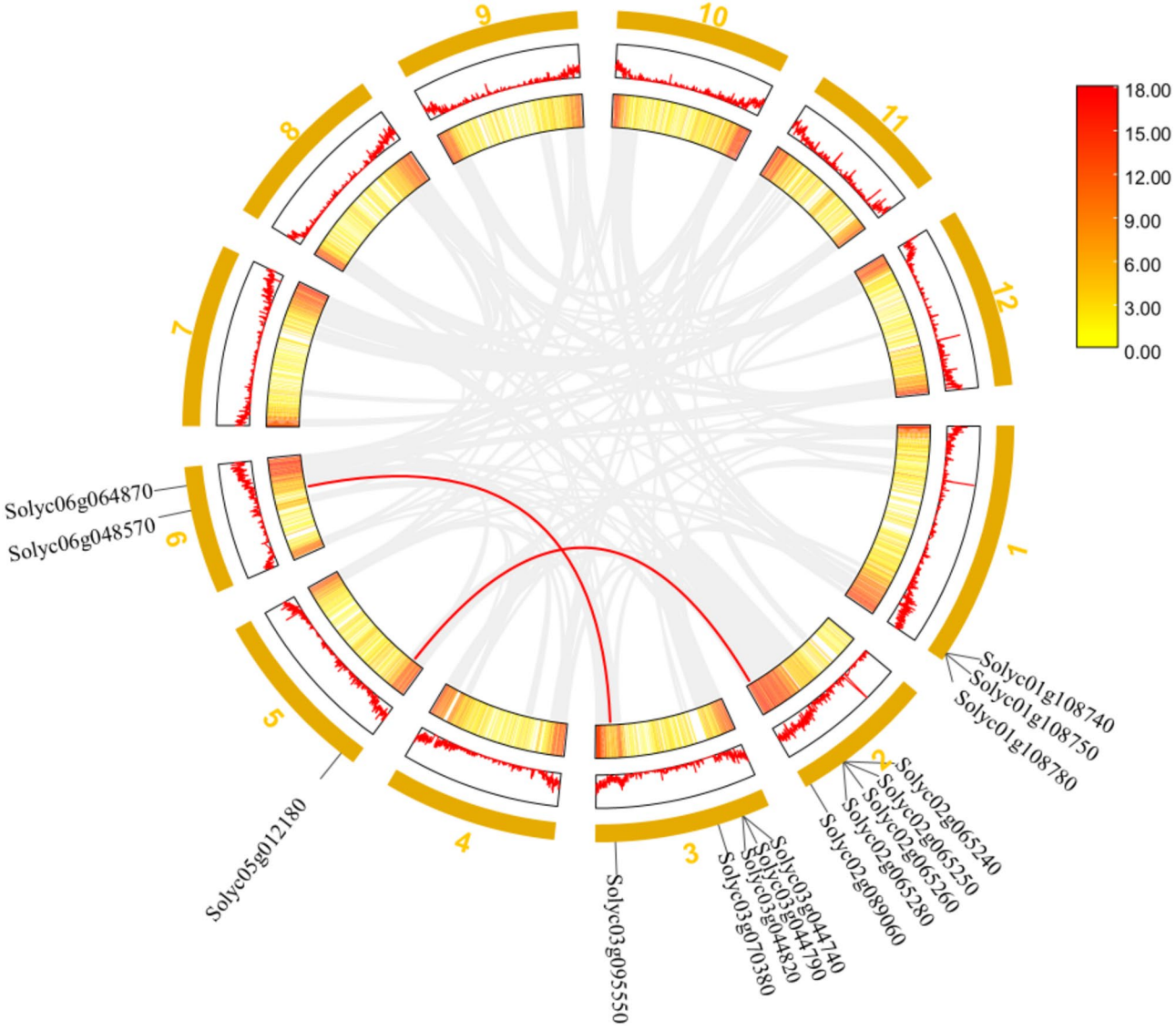

Fig. 2 Intraspecific synteny analysis of the *SlMES* gene family in tomato. Outer boxes represent the chromosome skeleton, and middle and inner boxes indicate gene density. The approximate distribution of each gene on the chromosome skeleton is marked by short black lines. Red and light grey lines represent the chain clusters with similarity of 0.98 ~ 0.99 and less than 0.95, and other genes, respectively

Table 2 Nonsynonymous (Ka), synonymous (Ks), and Ka/Ks ratio between 2 duplicate gene pairs

Gene pairs		Ka	Ks	Ka/Ks
Solyc06g048570	Solyc03g095550	0.18	0.89	0.21
Solyc02g089060	Solyc05g012180	0.32	1.77	0.18

lycopersicum and *Oryza sativa*, and that 11 MES gene family members exhibited collinearity between *Solanum lycopersicum* and *Arabidopsis thaliana*.

Conserved motifs and gene structure analysis of the *SlMES* family

As illustrated in Fig. 4, eight conserved motifs were identified, with motifs 1 and 3 representing conserved functional structural domains shared among the 16 SlMES protein sequences. However, except for Solyc02g065260, the remaining 15 proteins possessed motif 2, indicating the diversity in the evolutionary trajectory of the SlMES gene family (Fig. 4A).

Analysis of the gene structure of *SlMES* revealed that the number of exons in the genes varied from 2 to 7, with *Solyc06g064870* and *Solyc02g089060* exhibiting the highest exon counts. These structural differences suggest that *SlMESs* may have distinct functional roles or varying regulatory mechanisms for their expression (Fig. 4B).

Prediction of protein secondary, three-dimensional (3D), and transmembrane structures

Among the 16 SlMES proteins, four types of protein secondary structures were identified (Table 3), with quantitative ranges as follows: α-helices (72–123), β-turns (6–13), extended strands (27–41), and random coils (76–252).

The results of the protein structure analysis of the 16 SlMESs (Fig. 5) showed that the following templates accurately predicted the protein structures of SlMESs: 1y7h.2.A (Solyc03g070380, Solyc02g065240, Solyc02g065260, and Solyc02g065280), A0A3Q7EZJ3.1.A (Solyc02g065250), 3sty.1.A (Solyc01g108740,Solyc01g108750, and Solyc01g108780), Q6ED34.1.A (Solyc03g044790 and Solyc03g044820), A0A6N2APA5.1.A (Solyc03g044740), A0A3Q7HML6.1.A (Solyc06g048570), A0A6N2ASD2.1.A (Solyc03g09550), A0A3Q7GYM4.1.A (Solyc06g064870), A0A5D2ALH5.1.A (Solyc02g089060), and A0A6N2BFR9.1.A (Solyc05g012180). Notably, all 11 templates were hydrolases. The sequence identity ranged from 69.26 to 100.00%, and the GMQE values ranged from 0.96 to 0.70. These results indicate that the 3D model of the SlMES protein was accurately predicted. Additionally, prediction of the transmembrane structure of the 16 SlMESs showed that none contained transmembrane protein domains.

Predicted subcellular localization

The subcellular localization analysis revealed that the SlMES proteins are predominantly localized in the cytoplasm and chloroplasts, with a minor fraction detected in the plasma membrane (Table 3).

Cis-acting elements (CAEs) analysis

Sequences located 2000 bp upstream of the 16 *SlMES* genes were analyzed for CAEs using the Plant CARE online tool, with a focus on elements associated with hormone responses and abiotic stress. The results (Fig. 6) indicated that the hormone response- related CAEs

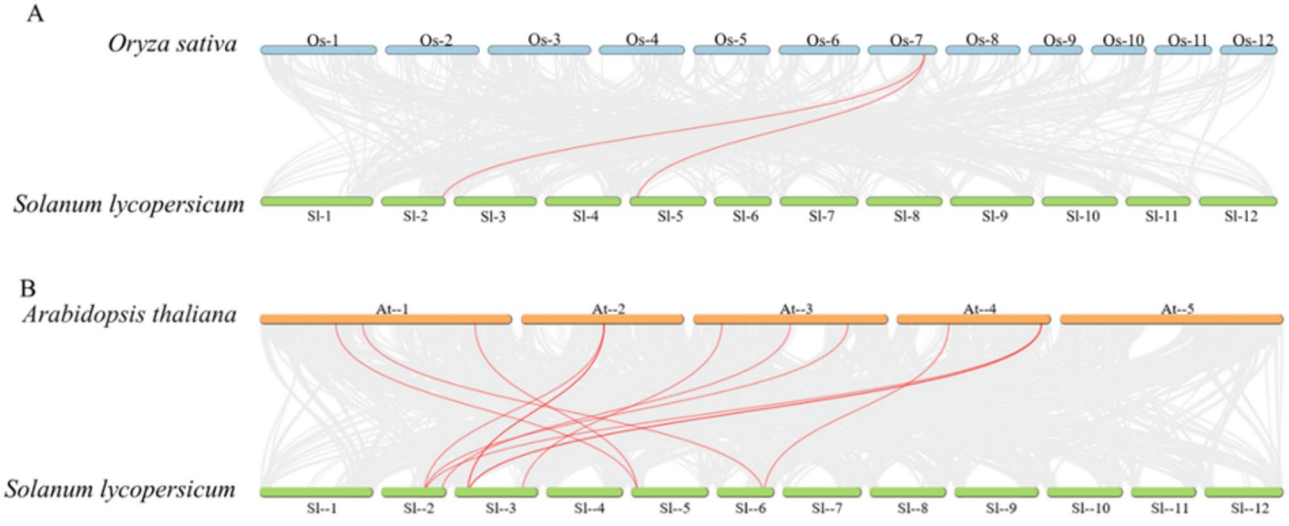

Fig. 3 Inter species collinearity analysis of MES gene family in *Solanum lycopersicum*, *Oryza sativa*, and *Arabidopsis thaliana*. Collinearity of SlMES genes between (**A**) *Oryza sativa* (blue) and *Solanum lycopersicum* (green) and (**B**) *Arabidopsis thaliana* (yellow) and *Solanum lycopersicum* (green). The grey lines represent collinear regions between different chromosomes, and the red lines represent collinear relationships between MES gene family members in the three species

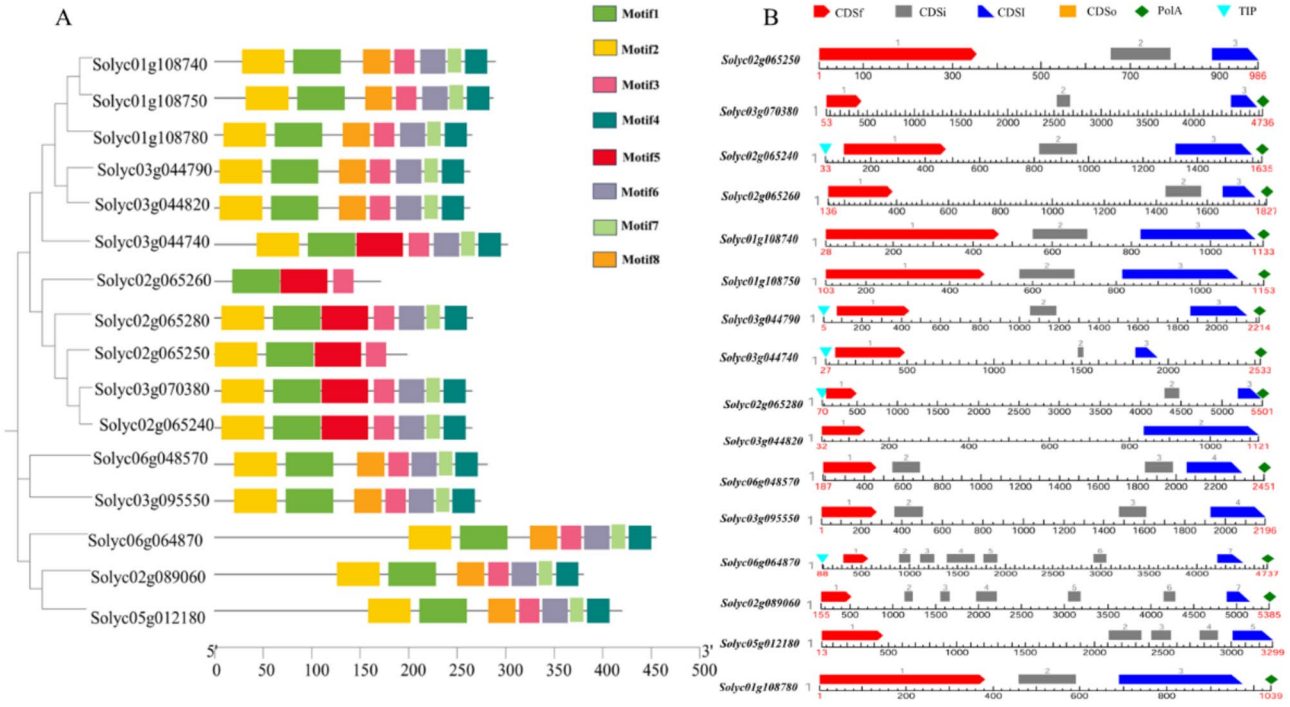

Fig. 4 Conserved motifs (**A**) and gene structures (**B**) of the *SlMES* family. (**A**) The different-colored boxes represent the eight prediction motifs. (**B**) TIP is the transcription initial position, CDSf is the start codon-containing first exon, CDSi are the intervening exons, CDSl is the stop codon-harboring final segment, CDSo is a single-exon coding region, and PolA is the 3′ polyadenylation site

Table 3 Secondary structure and subcellular localization of SlMESs

Gene ID	Sequence length	α- helix	β-turn	Extend strand	Random coil	Subcellular localization (WoLF PSORT)	Subcellular localization (CELLO)
Solyc03g070380	264	100	10	41	113	Cytoplasm	Cytoplasm
Solyc02g065240	264	102	12	38	112	Cytoplasm	Cytoplasm
Solyc02g065250	197	72	11	32	82	Cytoplasm	Cytoplasm
Solyc02g065260	170	61	6	27	76	Chloroplast	Cytoplasmic
Solyc01g108740	288	123	8	37	120	Chloroplast	Inner membrane
Solyc01g108750	234	88	9	36	101	Plasma membrane	Plasma membrane
Solyc03g044790	262	107	8	39	108	Cytoplasm	Periplasmic
Solyc03g044740	301	101	10	37	153	Nucleus	Cytoplasmic
Solyc02g065280	265	97	12	41	115	Cytoplasm	Cytoplasm
Solyc03g044820	262	108	8	35	111	Cytoplasm	Cytoplasm
Solyc06g048570	280	117	11	37	115	Cytoplasm	Cytoplasm
Solyc03g095550	273	102	8	41	122	Cytoplasm	Cytoplasm
Solyc06g064870	454	152	10	48	244	Chloroplast	Cytoplasmic
Solyc02g089060	379	126	12	43	198	Chloroplast	Nuclear
Solyc05g012180	419	112	13	42	252	Chloroplast	Periplasmic
Solyc01g108780	265	94	8	37	126	Chloroplast	Outer membrane

predominantly included those involved in salicylic acid responsiveness (TCA-element), gibberellin responsiveness (TATC-box and, P-box), and ABA responsiveness (ABRE), suggesting that *SlMES* genes may play a role in tomato development by interacting with multiple hormones. The primary regulatory elements associated with abiotic stress included those involved in low-temperature responsiveness (LTR), the MYB binding site for drought inducibility (MBS), anaerobic induction (ARE), and defense and stress responsiveness (TC-rich repeats). These findings implied that *SlMESs* respond to abiotic stresses via various pathways. In addition, CAEs related to meristem expression (CAT-box) and circadian regulation have been identified. Based on the number and distribution of promoter CAEs, most members contained response elements for gibberellins (GAs), MeJA, and SA,

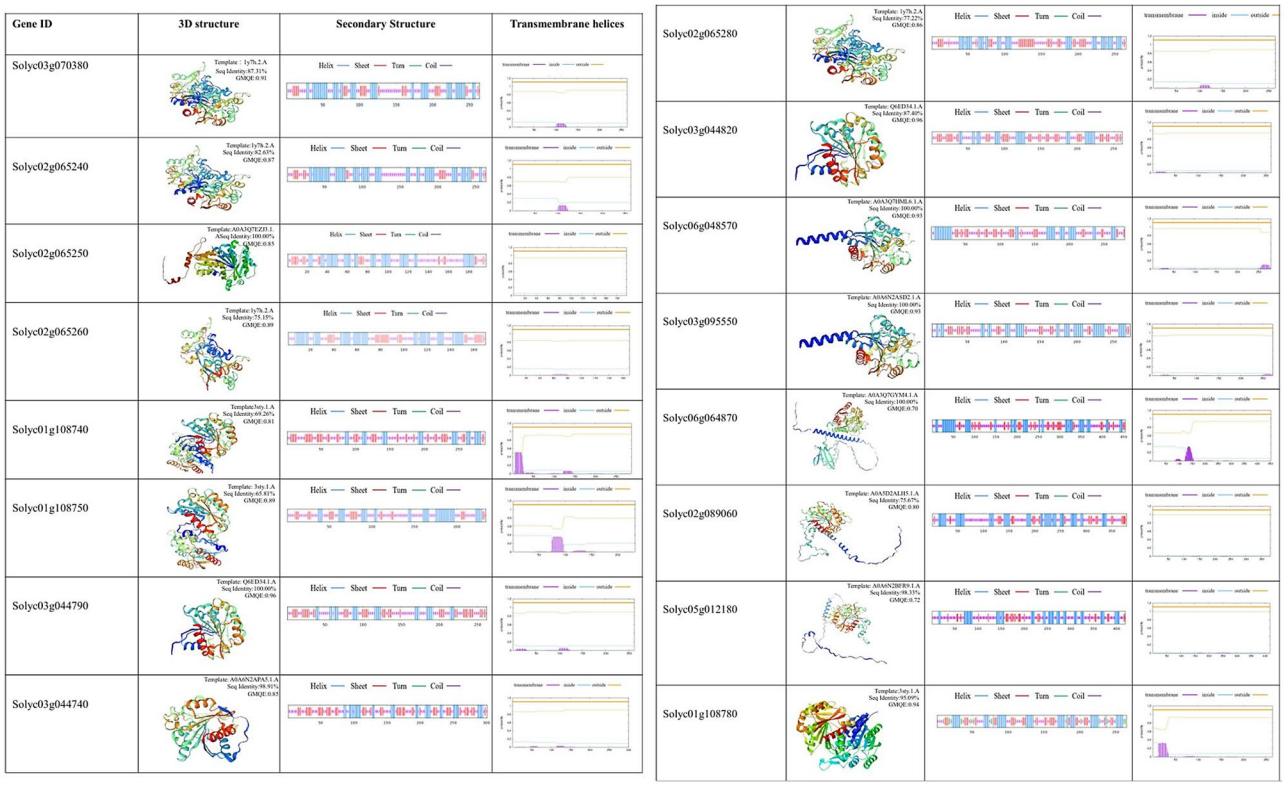

Fig. 5 Prediction of secondary, 3D, and transmembrane structures of SlMES proteins

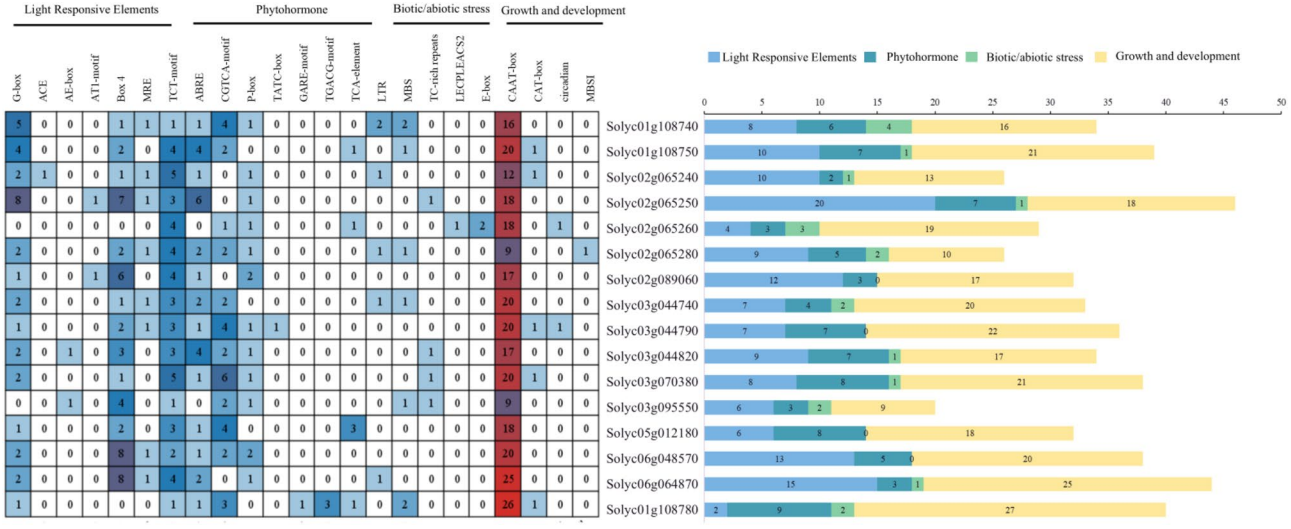

Fig. 6 Analysis of cis-acting elements (CAEs) of *SlMES*. (**A**) The different colors and numbers of the grid indicated the numbers of different promoter elements in these *SlMES*; (**B**) The histograms in different colors represent the sum of the CAEs in each category

suggesting that *SlMES* genes may respond to multiple hormonal regulations to facilitate tomato growth, development, and responses to adverse conditions.

Digital expression patterns of the *SlMES* family in tomato tissues

As illustrated in Fig. 7, the transcript levels of *SlMESs* varied significantly across tomato tissues, indicating that these genes may possess diverse functions during tomato growth and development. Notably, *Solyc02g065240*, *Solyc02g065280*, *Solyc02g065260*, and *Solyc02g065250* were highly expressed in ripening fruits, indicating their crucial roles during this stage. In contrast, *Solyc02g065240* and *Solyc02g065280* exhibited high expression in mature fruits, young flower buds, and leaves, suggesting their importance in flower bud differentiation and leaf development. In contrast, *Solyc03g095550* was consistently expressed at low levels in all tissues. Additionally, most genes exhibited lower expression levels in flowers and leaves.

Relative expression of *Solyc02g065260*

Analysis based on CAEs revealed that the *Solyc02g065260* promoter is enriched with elements associated with ABA response and ET synthesis (Fig. 8). Previous studies have demonstrated that *Solyc02g065260* exhibits specific hydrolytic activity towards MeSA [10]. Consequently, we analyzed the relative expression of *Solyc02g065260* under ABA/ET (Fig. 8A and B) and MeJA/MeSA (Fig. 8D and E) treatments. The results indicate that *Solyc02g065260* expression peaked 12 h after ABA treatment, reaching levels approximately 3-fold higher than those observed at 0 h. *Solyc02g065260* expression also significantly increased under ET treatment, peaking at 48 h post-treatment with a 4.8-fold increase compared with that at 0 h. In addition, *Solyc02g065260* expression was significantly upregulated in tomato fruit after 24 h of MeSA treatment. However, *Solyc02g065260* expression decreased following MeJA treatment. These findings suggest that ET and MeSA significantly induce *Solyc02g065260* expression.

To further explore the expression patterns of *Solyc02g065260* under biotic and abiotic stress, tomato fruits were subjected to *Botrytis cinerea* (*B. cinerea*) infection and cold treatment. The transcription levels of *Solyc02g065260* significantly increased with disease severity, with expression in fruit at disease level 4 showing an 8-fold increase compared to the baseline (disease level 0). Conversely, cold treatment significantly reduced the expression of *Solyc02g065260* (Fig. 8C and F).

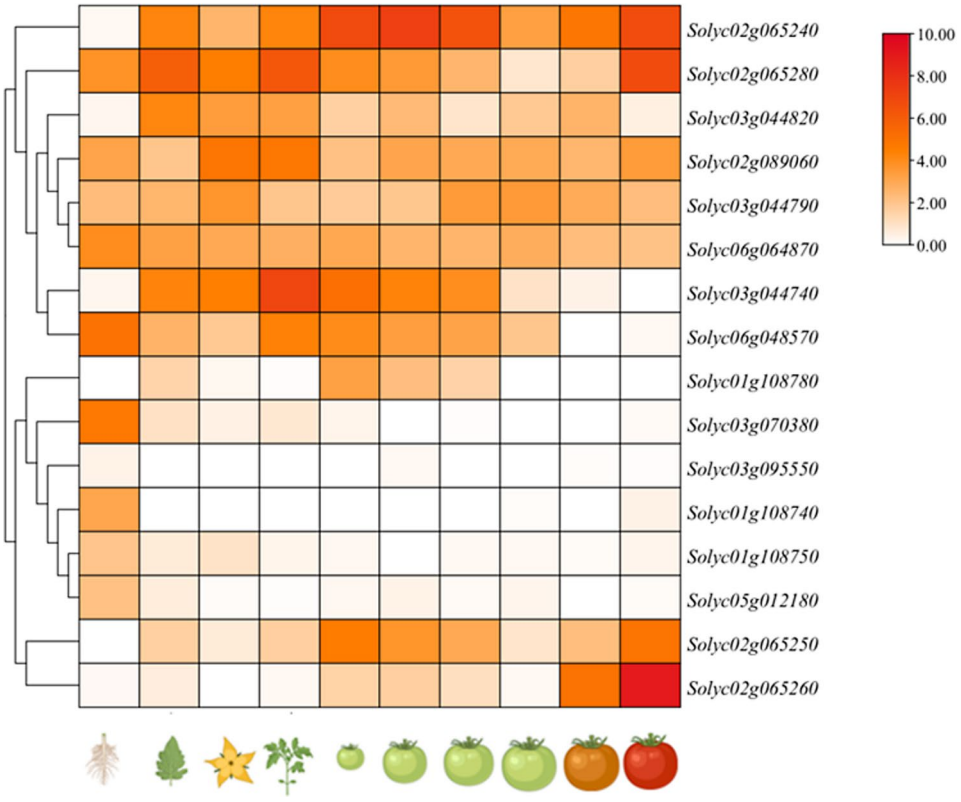

Fig. 7 Digital expression patterns of the *SlMES* family in different tomato tissues. From left to right: root, leaf, fully open flower, young shoot, 1 cm fruit, 2 cm fruit, 3 cm fruit, mature green fruit, break stage fruit, and break stage + 10 days fruit. Relative expression levels were normalized using the Z-score, with colors in the heatmap indicating expression levels; white represents low expression and red represents high expression

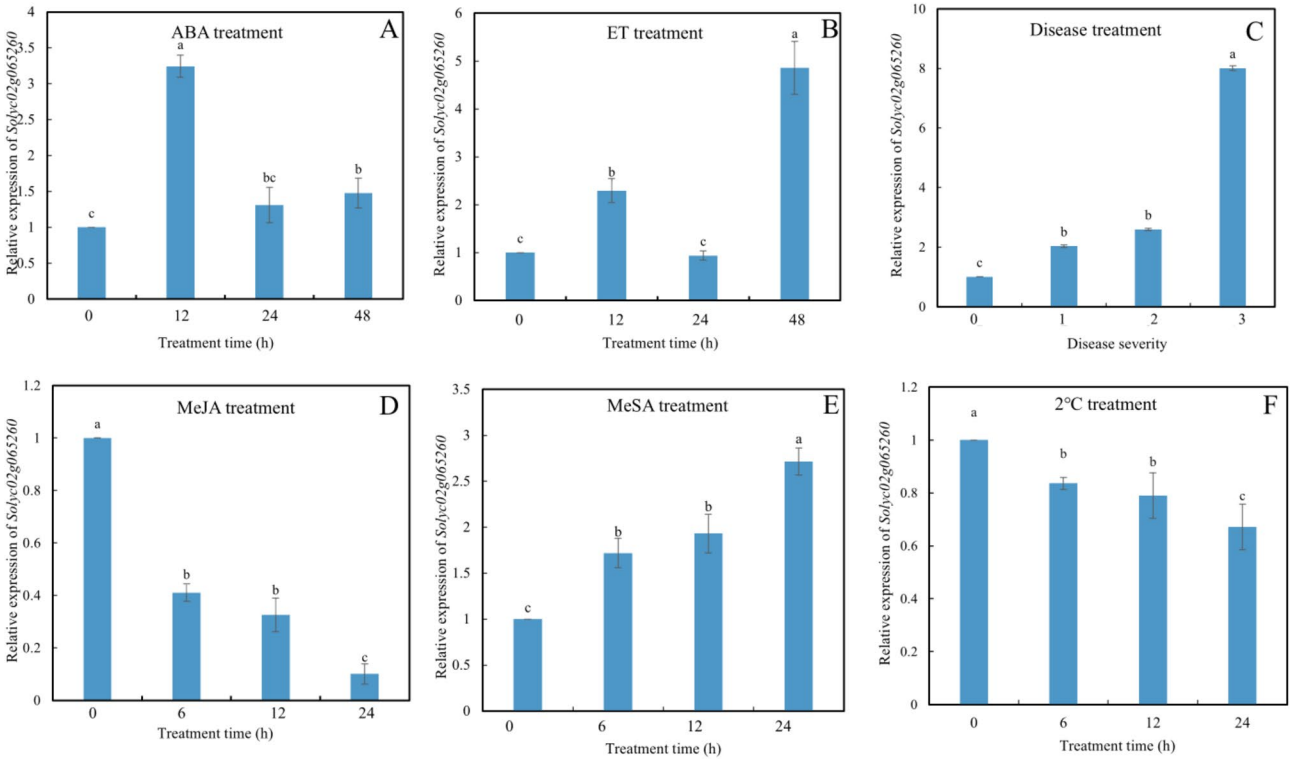

Fig. 8 Relative expression levels of the *Solyc02g065260* gene under different hormonal and abiotic stress treatments. Different lowercase letters denote significant differences ($p < 0.05$) among various time points for the same treatment

As shown in Fig. 9, the expression of *Solyc02g065260* in different tomato tissues remained relatively low from the roots until the Br stage. However, its expression increased significantly during the Pk stage, reaching its highest level during the RR stage, at 7.1-fold higher than that in MG fruit. This result aligns with the digital expression pattern observed in the "Heinz" tomato variety (Fig. 7).

Discussion

As members of the α/β superfamily of hydrolases, the MES family proteins are primarily involved in various hydrolysis reactions, particularly in catalyzing the hydrolysis of ester bonds [14]. MES hydrolyses MeJA, MeSA, and MeIAA to produce JA, SA, and IAA, respectively, thereby activating the expression of downstream defense genes. For example, in citrus, overexpression of *SABP2* enhances plant resistance to biotic stress and activates the transcription of genes related to plant resistance [8, 9]. Although the functions of MES family members have been well characterized in other plants, such as *Arabidopsis thaliana* [2], oilseed rape *(Brassica napus)* [15], and grapevine (*Vitis vinifera*) [7], there has been limited research on the MES family in tomatoes. This study comprehensively characterized the *SlMES* gene family in tomatoes, providing a theoretical foundation for future investigations into the role of the *SlMES* family in regulating tomato fruit ripening and stress tolerance. In this study, 16 members of the *SlMES* family were identified based on genomic information from tomatoes. Compared to other plant species, such as *Arabidopsis thaliana*s (20 members) [2], peaches (18 members) [14], and apples (19 members) [14], the number of *SlMES* members was relatively low. The variation in MES family size across species may be due to variations in genome size and gene duplication events that occur during evolutionary processes [16, 17]. Previous studies have shown that MES family members in various plant species exhibit specific hydrolytic activities towards different phytohormones [2]. To gain insights into the specific hydrolytic activity of SlMESs, we elucidated the homology between SlMESs and AtMESs by constructing a phylogenetic tree comprising 36 members classified into three subfamilies. In *Arabidopsis*, AtMJE (AtMES10) hydrolyzes MeJA to produce JA [18] and is the closest relative to Solyc03g044740. Additionally, AtMES9 [19] is associated with SlMES1-4 [10] and MeSA metabolism; these members cluster in subfamily C, which includes ten SlMES members. In addition to MeJA and MeSA, AtMES17 and AtMES18 in *Arabidopsis* can hydrolyze MeIAA to generate IAA [2]. These members are grouped in subfamily B, which contains two SlMES members.

The α/β superfamily of hydrolases is characterized by a conserved catalytic triad consisting of Ser, Asp, and His residues [20]. In this study, all identified SlMES members

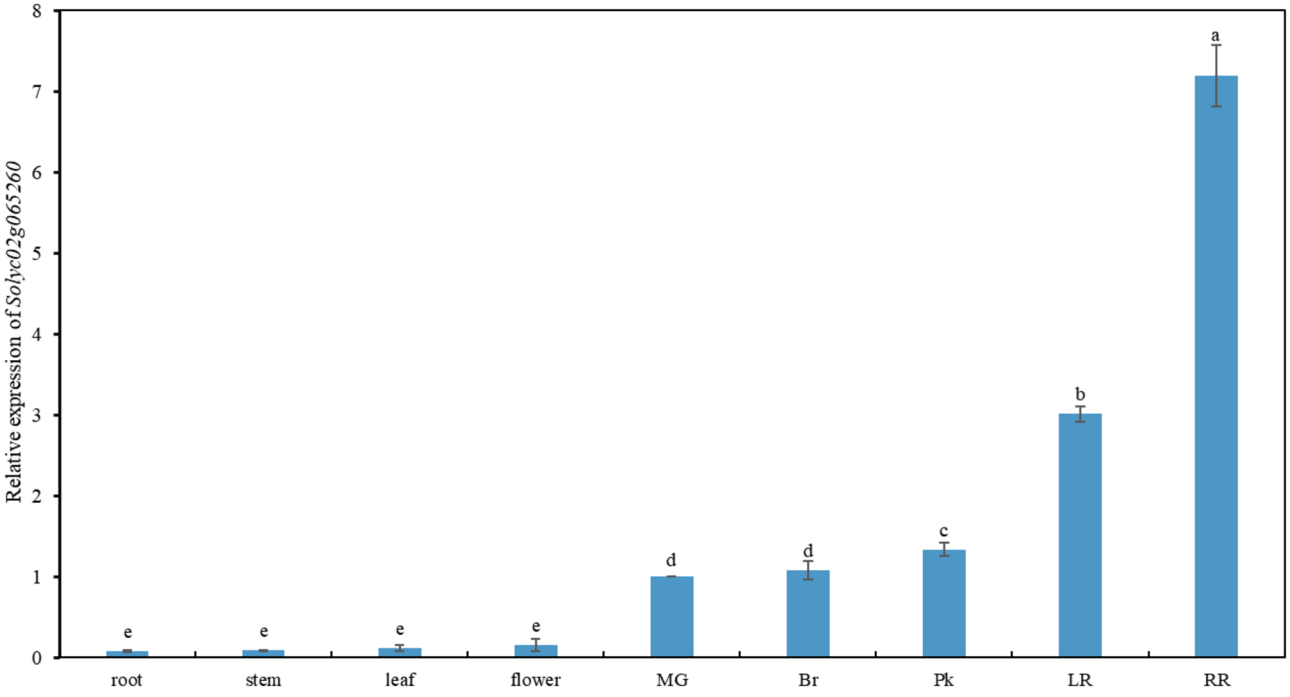

Fig. 9 Expression levels of *Solyc02g065260* in different tissues of tomato. MG: mature green; Br: breaker; Pk: pink; LR: light red; RR: red ripe. Different lowercase letters denote significant differences between treatments at the $p < 0.05$ level for the same period

contain the complete active site region, but not every member exhibits the highly conserved Ser-His-Asp catalytic triad. In the protein sequences of Solyc03g044740, Solyc06g048570, and Solyc03g095550, the conserved Ser residues in the catalytic triad were substituted with cysteine (Cys) and Asp (Figs. S1 and S2). Notably, *Solyc06g04870*, *Solyc02g089060*, and *Solyc05g012180* exhibited similar gene structures, with conserved motifs (Fig. 4), and were clustered within the same subfamily, providing a reasonable explanation for their clustering in the phylogenetic tree of the *SlMES* family. Chromosomal localization analysis revealed only two segmental duplication gene pairs among *SlMESs*; no tandem duplication genes were identified. This indicates that segmental duplication plays a more significant role than tandem duplication in the expansion of *SlMES* family members, which is consistent with previous finding [15]. Tandem duplication is a crucial mechanism for increasing gene number in an organism [21], which may explain the relatively low number of *SlMES* family members.

Based on the predicted structural models of 16 SlMESs, all proteins were classified into the α/β-hydrolase superfamily, with specific hydrolase activities observed in several members. Notably, Solyc03g070380, Solyc02g065240, Solyc02g065260, and Solyc02g065280 exhibited high structural similarity (>75% sequence identity) to the SABP2 protein template (PDB: 1y7h.2.A), which is a key methyl salicylate esterase [3]. Solyc01g108740, Solyc01g108750, and Solyc01g108780 showed significant homology (GMQE > 0.8) to the carboxylesterase template (PDB: 3sty 1. A) [22]. The remaining nine proteins were annotated as hydrolases; however, their specific catalytic substrates require further experimental validation.

CAEs in gene promoters are closely linked to their functions. The CAEs of the *SlMESs* were related to environmental stress and hormone responses, which is consistent with past findings. For instance, several stress-related, hormone-associated, and light-regulated CAEs were identified in the promoters of peach MES genes [14], while multiple TATA-boxes and CAAT-boxes were detected in every MES gene of silver birch [23]. The promoter regions of *SlMESs* were enriched with response elements for JA, GAs, MeJA, and SA, with the JA response element (CGTCA-motif) being the most prevalent. Numerous studies have demonstrated that JA and MeJA are crucial in various plant processes, such as pollen development [24], root growth [25], leaf senescence [26], and other physiological functions. Notably, JA and MeJA act as environmental signaling molecules that trigger plant defense responses against pathogens, herbivores, and abiotic stresses [27]. Therefore, the present findings suggest a potentially crucial role of SlMESs in tomato growth and stress responses. Furthermore, the digital expression patterns revealed that a substantial number of *SlMESs* were expressed across various tomato tissues, indicating their involvement in regulating the overall growth and development of tomato plant.

Fruit ripening is a critical stage in the development of fruit nutritional value and quality. To enhance both nutritional value and quality, it is essential to investigate the molecular regulatory mechanisms underlying fruit ripening. As the most widely cultivated dual-purpose crop globally, the ripening regulation of tomato has attracted significant attention [28, 29]. The expression analysis (Figs. 7 and 9) indicated that the expression of *Solyc02g065260* in tomato fruit gradually increased throughout the developmental and ripening stages, peaking ten days after the Br stage, it is evident that *Solyc02g065260* is predominantly expressed in tomato fruit tissues. The findings indicate that *Solyc02g065260* plays a crucial regulatory role in tomato fruit ripening. Frick et al. [10] demonstrated that *Solyc02g065260* can demethylate MeSA and convert it into SA. This process attenuates the wintergreen oil flavor associated with MeSA in tomatoes, thereby enhancing the flavor of ripe tomatoes and further confirming the role of *Solyc02g065260* in ripening. Promoter analysis (Fig. 6) revealed that *Solyc02g065260* contains multiple ET synthesis elements, including LECPLEACS2 (TAAAAT AT) and two E-boxes, implying that *Solyc02g065260* may play an important role in ET biosynthesis, with important implications for the ripening process. These results support those of Agarwal et al. [30]. Additionally, *Solyc02g065260* contains the AAAG motif, which serves as a binding site for the transcription factor Dof and participates in hormone signaling and multiple physiological processes such as seed formation and germination, secondary metabolite synthesis, and defense responses [31]. In the present study, the expression pattern of *Solyc02g065260* was investigated under various hormonal and stress conditions, revealing that its expression significantly increased in response to ET and MeSA (Fig. 8). Notably, *Solyc02g065260* expression increased in response to *B. cinerea* infection, whereas it was suppressed under low-temperature conditions. These findings provide valuable insights into the functions of *Solyc02g065260* and its family members.

Conclusions

This study presented a thorough analysis of the functional characteristics of the *SlMES* family. Sixteen *SlMES* genes were identified, which were distributed across five chromosomes and underwent gene duplication events, particularly segmental duplications. Through phylogenetic, motif distribution, and gene structure analyses, the *SlMES* genes were classified into three subgroups. In addition, the regulatory role of *Solyc02g065260* in tomato physiological metabolism and fruit ripening was explored using quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Materials and methods

Plant materials and treatments

Tomato fruits (*Solanum lycopersicum* L. cv. Badun) at the mature green (MG), breaker (Br), pink (Pk), light red (LR) and red ripe (RR) stages; roots; stems; leaves; and flowers were harvested from a greenhouse in Zibo City, Shandong Province. The fruits were transported to the laboratory for selection based on their size and the absence of mechanical injuries.

The fruits at the MG stage were treated as follows:

(1) MeJA: Fruits were placed in containers and fumigated with 0.05 mmol L^{-1} MeJA for 6, 12, and 24 h at 25 ± 1 °C;
(2) MeSA: Fruits were placed in containers and fumigated with 0.05 mmol L^{-1} MeSA for 6, 12, and 24 h at 25 ± 1 °C;
(3) 2 °C: Fruits were placed in a refrigerator at 2 °C for 6, 12, and 24 h;
(4) ABA: Fruits were soaked in a container containing 1 mmol L^{-1}ABA for 3 min and then stored at 25 ± 1 °C for 12, 24, and 48 h after drying;
(5) ET: Wild-type fruits were soaked in a container containing 200μL L^{-1} ET for 15 min and then stored at 25 ± 1 °C for 12, 24, and 48 h after drying;
(6) *B. cinerea* incubation: The fruits were inoculated with *B. cinerea* suspension as described in our previous study [32]. The degree of disease was evaluated according to the lesion diameter, classified as Level 0, lesion diameter = 0; Level 1, 0 < lesion diameter ≤ 0.5 cm; Level 2, 0.5 cm < lesion diameter ≤ 1 cm; and Level 3, lesion diameter > 1 cm.

After treatment, the mesocarp of the equatorial part of 8 fruits under each treatment and at various ripening stages was randomly selected, frozen in liquid nitrogen, and then stored at -80 °C for subsequent experiments. Each treatment was repeated three times.

Identification and physicochemical characterization of SlMESs

The genomic data for tomatoes were downloaded from the Sol Genomics Network website (https://solgenomics.net/organism/Solanum_lycopersicum/genome) [33]. *Arabidopsis* MES protein sequences were retrieved from the TAIR database (https://www.arabidopsis.org/) [34] and used as queries for the BLASTP homology search. The Hidden Markov Model (HMM) for the MES domain (PF00561) was obtained from the Pfam database (http://pfam.xfam.org) [35] and employed for HMMER (v3.3) domain searches within tomato protein sequences, with an E-value cutoff of ≤ $1e^{-5}$. After removing redundant sequences, the remaining candidates were further validated using the NCBI Conserved Domain Database (htt

ps://www.ncbi.nlm.nih.gov/cdd/) [36] and the SMART tool (http://smart.embl-heidelberg.de/) [37] to confirm the presence of conserved MES domains. The physicochemical properties of the SlMES proteins, including molecular weight and isoelectric point, were analyzed using the ExPASy online tool (https://web.expasy.org/compute_pi/) [38].

Phylogenetic analysis

The protein sequences of SlMESs were compared with *Arabidopsis thaliana*, and a phylogenetic tree was constructed using MEGA 11.0 software [39]. The NJ method was used for multiple sequence comparisons with a bootstrap coefficient of 1000 repetitions and default settings for other parameters. The resulting evolutionary tree was further enhanced and visualized using the online tool iTOL (https://itol.embl.de/) [40].

Chromosome localization and collinearity analysis of *SlMES* family members in tomato

Genome annotation files for tomatoes were obtained from the Sol Genomics Network website [33]. The TBtools software was used to map the chromosome distribution of *SlMESs* based on positional information provided in the genome annotation files [41]. Additionally, the genome sequences and gene structure annotation files of *Arabidopsis thaliana* and *Oryza sativa* were downloaded from the Ensemble Plants database (https://plants.ensembl.org) [42] for collinearity analysis.

Analysis of conserved motifs and gene structure

The conserved motifs of SlMES proteins were analyzed using the online tool MEME (https://meme-suite.org/meme/tools/meme), with parameters set to a maximum of 8 motifs and a motif width ranging from 6 to 50 amino acids [43]. The gene structure was analyzed with the online tool SoftBerry (http://www.softberry.com/), specifically the FGENESH module for exon-intron boundary analysis [44].

Prediction of protein secondary, 3D, and transmembrane structures

The online tool SWISS-MODEL (https://swissmodel.expasy.org/inter active) [45] was used to predict the 3D structure of SlMES proteins; The SOPMA website (http://npsa-pbil.ibcp.fr/cgibin/npsa_automat.pl?page=npsa_sopma.html) [46] predicted the secondary structure of SlMES proteins. Additionally, TMHMM-2.0 (https://services.healthtech.dtu.dk/service.php?TMHMM-2.0) [47] was used to predict the transmembrane structure of the sequence.

Predicted subcellular localization

Subcellular localization of SlMES was predicted using the WoLF PSORT (https://wolfpsort.hgc.jp/) [48] and CELLO (http://cello.life.nctu.edu.tw/) [49].

Analysis of promoter CAEs

The 2000 base pairs upstream of the transcription start site of *SlMESs* were obtained using TBtools software. The CAEs identified in the promoter regions of *SlMES* genes were subsequently analyzed using the Plant CARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) [50] and visualized using TBtools.

Digital expression patterns of the *SlMES* family in different tomato tissues

Tissue-specific expression data for the "Heinz" variety of tomato were obtained from the Tomato Functional genomics database (http://ted.bti.cornell.edu) [51]. This dataset includes information on whole roots, leaves, flowers, buds, and fruits at different developmental stages. Additionally, a heatmap illustrating the tissue-specific expression of *SlMES* was generated using TBtools.

Relative expression analysis of *Solyc02g065260*

Complementary DNA (cDNA) was synthesized as described previously [52]. The expression level of *Solyc02g065260* was measured [53]. Housekeeping gene *SlUbi3* served as an internal control. The specific primers used were as follows: *Solyc02g065260*-forward: 5′- TGC TGTTTTCTTGGCTGCTCTTATG-3′; *Solyc02g065260*-reverse: 5′-TTGTTGGCGTCCTCTCAAATTGC-3′; *SlUbi3*-forward: 5′-TCCATCTCGTGCTCCGTCT-3′; *SlUbi3*-reverse: 5′-CTGAACCTTTCCAGTGTCATC AA-3′. The relative expression levels were calculated according to the $2^{-\Delta\Delta Ct}$ method.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06625-4.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
JS: Writing–original draft, Software. FJL: Writing–review & editing, Formal analysis, Data curation. XHZ: Writing–review & editing, Supervision, Funding acquisition, Conceptualization. ZFRA： Writing–review & editing, Funding acquisition, Conceptualization. XRK: Data curation. SH: Data curation. LL Formal analysis, Data curation. XAL: Writing–review & editing. YJ: Data curation. YNL: Data curation.

Funding
This study was supported by the National Natural Science Foundation of China (grants 32172278, 32272384), the Natural Science Foundation of Shandong Province (grant ZR2024MC063), and the United Arab Emirates University (UAEU) Program for Advanced Research (UPAR) (grant 12R059).

Data availability
The data that support the findings of this study are available on request from the corresponding author, XHZ，upon reasonable request.

Declarations

Ethics approval and consent to participate
The authors declare that there are no known financial conflicts of interests or personal relationships that may have influenced the work presented in this paper.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 14 February 2025 / Accepted: 25 April 2025
Published online: 16 May 2025

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