



Genome-Wide Identification of the *SCAMP* Gene Family in Apple and Their Expression Response to Simulated Hail and Post-Hail Treatments with Amino Acid and PGPR

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Abstract

Secretory Carrier Membrane Proteins (SCAMPs) are conserved components of intracellular membrane trafficking; however, their specific roles in perennial fruit crops and responses to mechanical injury remain largely uncharacterized. In this study, we conducted a genome-wide identification and characterization of the *SCAMP* gene family in apple (*Malus domestica*), identifying six *MdSCAMP* members distributed across the genome. Phylogenetic, structural, and promoter analyses indicated that *MdSCAMP* genes are evolutionarily conserved and possess *cis*-regulatory elements associated with stress- and hormone-responsive pathways. To investigate their function under mechanical stress, we integrated molecular analyses with a field experiment evaluating the effects of simulated hail damage and post-hail foliar applications of amino acids and the plant growth-promoting rhizobacterium (*Aeromonas hydrophila* *iehthiosmia* AE425) on ‘Golden Delicious’ apple trees. Hail injury significantly reduced fruit size, weight, and soluble solids, while post-hail biological treatments supported physiological activity but did not fully compensate for early growth losses. RT-qPCR analysis revealed that the *MdSCAMP* genes, particularly *MdSCAMP5*, were significantly downregulated in response to simulated hail damage. This repression suggests that mechanical injury may disrupt standard membrane trafficking processes, potentially redirecting cellular energy toward repair rather than *de novo* transcription. Although post-hail applications of amino acids and PGPR provided partial physiological support, they did not fully restore *MdSCAMP* expression levels or compensate for fruit growth losses. Overall, these findings provide novel insights into the transcriptional reprogramming of the *SCAMP* gene family under physical stress and positioning *MdSCAMP5* as a promising candidate for genetic improvement of mechanical stress resilience in perennial fruit systems.

Keywords Apple · Bioinformatics · Gene family · Hail damage · *Malus domestica* · PGPR

Introduction

Global climate change has significantly altered agricultural productivity by affecting crop growth cycles, increasing the frequency of extreme weather events, and intensifying pest and disease outbreaks (Yuan et al. 2024). Between 1961 and 2021, global agricultural productivity declined by approximately 21%, with the strongest reductions occurring in warmer regions (Ortiz-Bobea et al. 2021). Among agricultural crops, perennial fruit species are particularly vulnerable to climate-driven stresses because their long-life cycles expose them to multiple consecutive seasons of environmental fluctuations. Unlike annual crops, whose life cycle is confined to a single growing season and does not involve long-term investment costs as in perennial systems, fruit trees may experience lasting and cumulative damage when exposed to environmental stress at any stage of their life

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cycle, with consequences that can persist into subsequent years.

Extreme climatic events such as drought, frost, hail, heavy rainfall, and flooding are major causes of yield and quality losses in horticultural crops. Hailstorms are among the most destructive natural hazards for horticultural production worldwide (Fan et al. 2022). Hail consists of solid ice particles typically larger than 5 mm in diameter, formed in thunderstorms under conditions of sub-freezing temperatures and the presence of supercooled water droplets (Rana et al. 2022). Climate projections indicate that hailstorm frequency will increase in parts of Australia and Europe, while slightly declining in East Asia and North America (Raupach et al. 2021). By the end of the 21st century, the occurrence of severe convective storms-including hail, lightning, and strong winds-is expected to rise considerably across Europe (Radler et al. 2019). Consequently, annual agricultural losses caused by hail may increase by 25–50% by 2050 (Botzen et al. 2010).

Previous studies on hail injury have primarily focused on annual crops such as vegetables and cereals (Bueckert 2011; Lollato et al. 2018; Alzahib et al. 2021). In contrast, relatively few studies have investigated hail injury and recovery in perennial fruit crops such as grapevine or tree fruits (Tartachnyk and Blanke 2002; Kiprijanovski et al. 2016; Petoumenou et al. 2019). Several approaches have been proposed to mitigate hail damage, including anti-hail nets, agricultural photovoltaics, chemical protectants, and crop-insurance systems (Kiprijanovski et al. 2016; Rana et al. 2022). However, biological and biochemical post-hail recovery strategies remain underexplored. Amino acids regulate metabolic pathways associated with abiotic stress tolerance and have been reported to improve yield and fruit quality following hail injury (Gökçe and Özbay 2019; Banița et al. 2020). In contrast, the potential role of PGPR, such as *Aeromonas hydrophila iehthiosmia* AE425, which produces ACC deaminase and growth-regulating hormones, has not been investigated in relation to hail damage (TÜBİTAK-TEYDEB project no: 315O426).

Apple is one of the most widely cultivated fruit crops worldwide, with global production reaching 97.9 million tons in 2024 (FAOSTAT 2026). Türkiye ranks third globally, with an annual production of 4.4 million tons, following China and the United States (FAOSTAT 2026). The present study was conducted in Isparta, Türkiye's principal apple-growing region, which accounted for 23% of national production in 2024. Within this region, 'Golden Delicious' (42%) and 'Red Delicious' (49%) are the predominant cultivars (TURKSTAT 2026). Hail damage in apple orchards often leads to partial defoliation, fruit bruising, and yield loss, which can reach up to 67% in sensitive cultivars such as 'Golden Delicious' (Chelaru et al. 2023). While these physiological effects are well documented, the molecular mechanisms

underlying cellular recovery after such mechanical trauma remain poorly understood. Hailstones cause rapid loss of membrane integrity and uncontrolled cellular leakage. Repairing these damaged membranes is an essential survival strategy that requires highly coordinated intracellular trafficking to deliver new lipids and proteins to the wound site. In this regard, *SCAMPs* appear to be critical candidates for maintaining plasma membrane balance during environmental stress.

Intracellular membrane trafficking plays a central role in wound repair and stress adaptation. *SCAMP* gene family represents a conserved group of proteins involved in intracellular trafficking, vesicle secretion, and stress response in plants (Lam et al. 2007; Obudulu et al. 2018; Wang et al. 2023). Although *SCAMP* genes have been characterized in *Arabidopsis*, rice, poplar, and soybean, they have not yet been comprehensively characterized in apples. *SCAMP* proteins localize to the trans-Golgi network, plasma membrane, endosomes, and secretory granules, where they regulate processes essential for cellular homeostasis and stress signaling (Jiang et al. 2025). While *SCAMPs* have been extensively studied in mammalian systems, their functions in plants remain comparatively less understood. Studies in *Arabidopsis* and rice indicate roles in endocytosis, growth, and developmental regulation (Lam et al. 2007). In lily, *SCAMPs* support pollen tube growth by coordinating secretory and endocytic pathways (Wang et al. 2010). In poplar, *SCAMP* proteins influence the composition of secondary cell walls by regulating the accumulation of polysaccharides and phenolic compounds (Obudulu et al. 2018).

Recent functional studies further highlight the involvement of plant *SCAMP* genes in abiotic stress responses. In *Arabidopsis*, *AtSCAMP4* enhances salt tolerance by interacting with the vacuolar Na/H antiporter AtNHX2, thereby contributing to ionic homeostasis under salt stress (He et al. 2024). In soybean, overexpression of *GmSCAMP5* improved salt tolerance by reducing Na accumulation and cellular damage, while silencing *GmSCAMP5* increased stress sensitivity (Wang et al. 2023). Additionally, *GmSCAMP4* has been identified as a key regulator of phosphate starvation response, exhibiting root-predominant expression and enhancing low-phosphate tolerance by interacting with GmZFP2 (Yang et al. 2025). In cotton, silencing *GhSCAMP2* and *GhSCAMP4* produced salt-sensitive phenotypes and reduced antioxidant enzyme activity (He et al. 2024).

Given the importance of mechanical injury in apple production, this study aimed to evaluate the effects of simulated hail damage and post-hail foliar applications of amino acids and *Aeromonas hydrophila iehthiosmia* AE425 on the pomological and biochemical characteristics of apple fruits. Furthermore, to our knowledge, this work presents the first genome-wide identification and RT-qPCR-based expression

analysis of the *MdSCAMP* gene family in apple under hail stress. The findings would provide novel insights into the roles of *SCAMPs* in membrane trafficking mediated wound responses and contribute to improving mechanical stress tolerance in perennial fruit crops.

Materials and Methods

Plant Material

Field experiments were conducted in 2024 at the experimental orchard of the Fruit Research Institute in Eğirdir, Isparta, Türkiye (37°48'52.07" N, 30°52'39.63" E; 919 m a.s.l.). The orchard was not equipped with a hail net. The plant material consisted of 'Golden Delicious' apple trees grafted onto M9 rootstock and planted at 3.5 × 1 m spacing in February 2005 using one-year-old whip trees. 'Red Delicious' served as the pollinator cultivar. Trees were trained on a trellis system and irrigated via drip irrigation with fertigation. No pollination problems were encountered during the experiment; no chemical blossom thinning was applied, and all cultural practices were performed according to local procedures. The bacterial strain *Aeromonas hydrophila* *iehthiosmia* AE425, known for its ability to produce ACC deaminase, synthesize the plant hormones auxin, gibberellin, and cytokinin, and fix nitrogen and solubilize phosphorus was used in the experiment. The strain was provided by the Department of Horticulture, Faculty of Agriculture, Selçuk University, applied at a final concentration of 10⁸ colony-forming units (CFU) per mL. A plant-derived solid organic fertilizer containing amino acids (vegetable protein and soybean pulp) was used (Lena, Aminostar, Türkiye). To improve adhesion of the amino-acid solution to the leaf surface, an organic silicon-based spreader-sticker (Catch®, 98% Polyether-Polymethylsiloxane Copolymer, Türkiye) was added at a concentration of 0.05%.

Experimental Design and Mechanically Simulated Hail Damage

The experiment was arranged in a randomized complete block design with three blocks. Each block consisted of four trees, and four main branches were selected per tree. Mechanical hail damage was simulated by puncturing each leaf on the selected branches twice using holes of 1.77 cm² to mimic hail injury (Fig. S1). Hail simulation was conducted on 27 May 2024, corresponding to the fourth week of May, when historical hail events are most frequent according to long-term (2007–2023) damage records from the Turkish Agricultural Insurance Pool (TARSIM 2025a, b). These records indicate that hail damage in Eğirdir typically

occurs between late May and early June, with damage intensity ranging from 11 to 100% (average 48%) (data not shown).

Five treatments were applied: control (C, no treatment), hail (H, simulated hail damage), hail + amino acid (H + A, simulated hail damage followed by foliar application of amino acids one day later), hail + bacteria (H + B, simulated hail damage followed by foliar application of the bacterial suspension one day later), and hail + amino acid + bacteria (H + A + B, simulated hail damage followed by foliar applications of both amino acids and the bacterial suspension one day later).

Fruit Quality Analyses

Fruit quality analyses were performed on ten fruits per branch, corresponding to forty fruits per tree. Fruit diameter and length were measured using a digital calliper with 0.01 mm precision, while fruit weight was determined using a digital balance (Ragwag, PS 4500.R2.M, Poland) sensitive to 0.01 g. Flesh firmness was measured with a fruit texture analyzer (Güss, FTA-GS15, Germany) and expressed in newtons (N). Soluble solids content (SSC) was determined using a digital refractometer (Hanna, HI 96801, USA) and expressed as a percentage. pH and titratable acidity (TA) were measured with a digital titrator (Mettler Toledo T50, Switzerland) equipped with a glass electrode. Fruit skin color was evaluated using a colorimeter (Minolta, CR-400, Japan), which provided L* (100 = white, 0 = black), a* (+ = red, − = green), and b* (+ = yellow, − = blue) values, as well as chroma and hue angle.

Identification and Characterization of SCAMP Gene Family in the Apple Genome

Five *Arabidopsis* SCAMP protein sequences, (AT1G61250, AT1G11180, AT2G20840, AT1G03550, and AT1G32050) were retrieved from The Arabidopsis Information Resource (TAIR) (<https://www.arabidopsis.org/>) (Swarbreck et al. 2008). These sequences were used as queries for BLASTp (E-value ≤ 10^{−5}) searches of the protein sequence datasets of *Malus × domestica* GDDH13 v1.1 and other Rosaceae species in the GDR database (<https://www.rosaceae.org/>) (Jung et al. 2019) (Table S1). To confirm the presence of SCAMP domain, the putative SCAMP proteins were submitted to NCBI-CDD (Conserved Domain Database) (<https://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml>) (Marchler-Bauer et al. 2015) and SMART (https://smart.embl.de/smart/change_mode.cgi) (Letunic et al. 2021) databases. Gene names were renamed in ascending order according to their chromosomal position and similarity to AtSCAMP genes. The physicochemical properties of MdSCAMP proteins (including amino acid num-

ber, molecular weight, isoelectric point, instability index, aliphatic index, and grand average of hydropathicity) were analyzed using the ExPASy ProtParam (<https://web.expasy.org/protparam/>) (Duvaud et al. 2021). Subcellular localization prediction of MdSCAMP proteins was performed using the online tool and DeepLoc 2.1 (<https://services.healthtech.dtu.dk/services/DeepLoc-2.1/>) (Thummuluri et al. 2022).

Phylogenetic, Domain, Gene Structure and Conserved Motifs Analysis, and Promoter Analysis of SCAMP Genes

To decipher the evolutionary relationships of SCAMP genes in Rosaceae, we first extracted protein sequences from different Rosaceae species and identified SCAMP family genes using the same identification protocol used for MdSCAMP genes. The amino acid sequences of all SCAMPs were aligned using MEGA-MUSCLE software and the resulting alignments were used to construct a phylogenetic tree using the maximum likelihood method (ML), a bootstrap of 1000 replications, and the Jones-Taylor-Thornton (JTT) model and MEGA11 version 11.0.13 software (Kumar et al. 2018) and visualized with iTOL (<https://itol.embl.de>) (Letunic and Bork 2019). Positions of exons, introns, and untranslated regions (UTRs) of MdSCAMP genes were retrieved from the GFF3 files of the *Malus × domestica* GDDH13 v1.1 genome. Conserved motifs of MdSCAMP proteins were predicted using the online MEME tool (<https://meme-suite.org/meme/tools/meme>) with the following parameters: number of conserved motifs: 5; optimal width of each motif=6–50 amino acids. Domain, conserved protein motifs and gene structure of MdSCAMPs were visualized with TBtools software (Chen et al. 2020). The putative promoter region, defined as the 2000bp sequence upstream of the starting codon, was extracted from the apple genome. Subsequently, these sequences were analyzed using PlantCARE (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html>) (Lescot et al. 2002) for cis-element prediction. TBtools visualized *cis*-acting elements in their promoter regions of MdSCAMP genes.

Secondary and Three-dimensional Protein Modelling Analyses of the MdSCAMPs

The secondary structures of MdSCAMP proteins were predicted using the online tool SOPMA (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=NPSA/npsa_sopma_f.html) (Geourjon and Deléage 1995). Subsequently, homology modeling of three-dimensional (3D) structures was conducted for all members of MdSCAMP proteins via the online platform Phyre (<https://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index>) (Kelley et al.

2015). After downloading the Protein Data Bank (PDB) files, we used UCSF Chimera software (Yang et al. 2012) to visualize the secondary structure elements within the 3D conformations of these proteins.

Synteny Relationship and Duplication Event Analyses of the Rosaceae SCAMPs

The whole genome sequence files and annotation information files of Rosaceae SCAMPs were downloaded from GDR (<https://www.rosaceae.org/>) (Jung et al. 2019) and mapped by GFF file configuration information. TBtools MCScanX module was used to analyze and visualize synthetic blocks of Rosaceae SCAMPs. The homologous (Ks) and non-homologous (Ka) rates of the tandem and segmental duplications of Rosaceae SCAMPs were calculated using PAL2NAL server (<https://www.bork.embl.de/pal2nal/>) (Suyama et al. 2006). The divergence time (T) of each duplicated gene pair was calculated as $T = Ks / 2r$ ($r = 6.56 \times 10^{-8} \times 10^{-6}$ million years ago (MYA), with Ks being the synonymous substitutions per site and r being the rate of divergence for nuclear genes from Rosaceae SCAMPs (Koch et al. 2000).

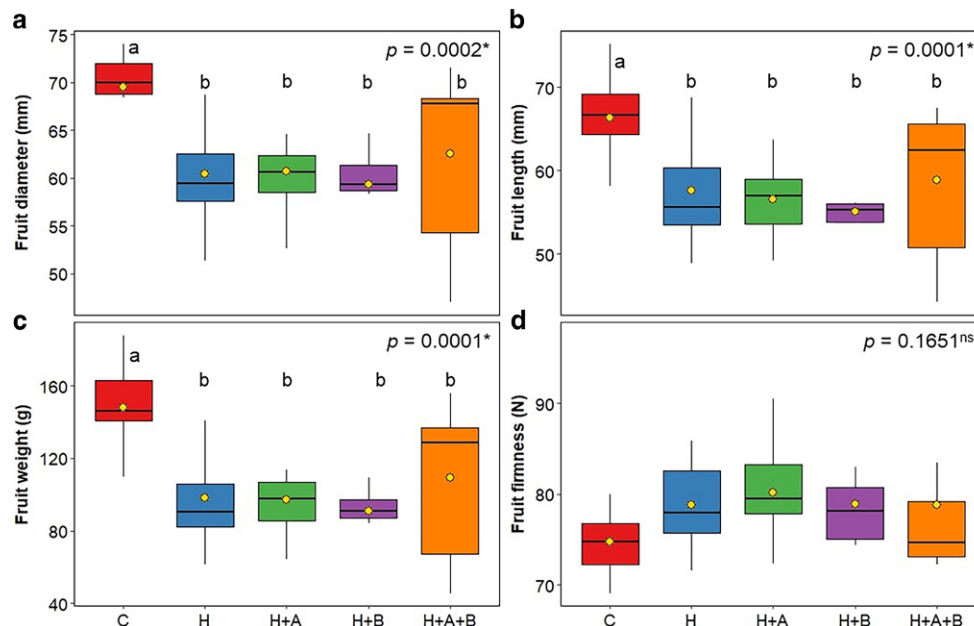
GO Annotation and Protein-protein Interaction and Digital Expression Pattern Analysis of MdSCAMPs

Go ontology (GO) of MdSCAMP proteins was predicted by predicted PANNZER2 (<http://ekhidna2.biocenter.helsinki.fi/sanspanz/>) (Törönen et al. 2018) tool and classified as molecular functions, biological process, and cellular component. Protein-protein interaction (PPI) among MdSCAMPs was detected by STRING v12.0 (<https://string-db.org/>) (Szklarczyk et al. 2021) with the confidence score >0.7 and an interaction network of MdSCAMPs proteins was visualized by Cytoscape v3.10.4 (Shannon et al. 2003). Gene expression data for apple growth stages and pathogen infections were retrieved from the Apple MDO database (<http://bioinformatics.cau.edu.cn/AppleMDO/index.php>) (Da et al. 2019) using the MdSCAMPs gene accession numbers. Expression levels were normalized as log2 (FPKM +1) and visualized using heatmaps and schematic diagrams generated with TBtools.

RNA Extraction and RT-qPCR Analysis

Total RNA was extracted from leaves subjected to five different treatments: control, hail, hail + amino acid, hail + bacteria, and hail + amino acid + bacteria. Leaf samples from each treatment were collected on days 0, 7, and 14, with three biological replicates per treatment. Leaves from the control and all treatment groups were excised from the stem end, wrapped in aluminum foil, and immediately frozen

Fig. 1 Effects of different treatments on physical fruit characteristics of ‘Golden Delicious’/M9 apple trees. **a** Fruit diameter, **b** fruit length, **c** weight, and **d** fruit firmness. In the boxplots, the thick horizontal line within each box represents the median, and gold circles denote the mean. The asterisk (*) indicates statistically significant differences among means labelled with different letters ($p < 0.05$). *ns* not significant ($p > 0.05$). C control; H hail damage; H + A hail damage + amino acid; H + B hail damage + bacteria; H + A + B hail damage + amino acid + bacteria



in liquid nitrogen under field conditions. They were then stored at -80°C until RNA extraction. Total RNA was isolated from the leaf tissues using GeneJET Plant RNA Purification Kit (Thermo Scientific) following the manufacturer's protocol. 500 ng of RNA was reverse transcribed using the VitaScript™ FirstStrand cDNA Synthesis Kit (Procomcare Biotech). The primers for the potential *MdSCAMP* genes were designed using the tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>). Details of the primers used for the RT-qPCR are given in Table S2. The reaction volume was 20 μL , including 10 μL of SYBR® Green Master Mix, 6 μL of ddH₂O, 2 μL of cDNA template, and 1 μL of forward and reverse primer. All reactions were performed with a CFX96 (Biorad) using SYBR® Green Master Mix (BioRad), following the protocol; 95°C for 5 mins, followed by 35 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 30 s. For each sample, three biological replicates and two technical replicates were analyzed. *MdEF1aF* (Zarei et al. 2015) was used as an internal reference gene. Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method (Livak and Schmittgen 2001), and the difference was analyzed by the LSD test of single-factor ANOVA ($p < 0.05$).

Data Analysis

Fruit quality data and RT-qPCR results were analyzed using R statistical software (version 4.1.2; R Core Team 2021). One-way analysis of variance (ANOVA) was used to test treatment effects, and significant differences among means were determined using the least significant difference (LSD) test within the ‘agricolae’ package. Boxplots and bar plots were generated using the ‘ggplot2’ package.

Correlation analyses were performed using the ‘Performance Analytics’ package to evaluate relationships among the measured fruit traits.

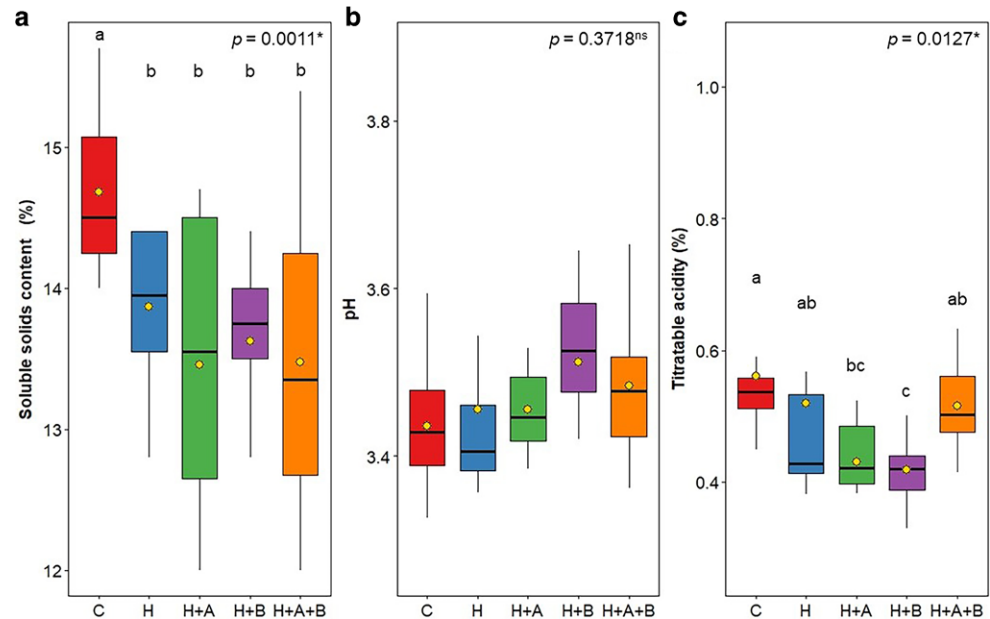
Results

Effects of Hail Damage and Post-hail Treatments On Fruit Physicochemical Quality

Hail simulation and subsequent foliar treatments significantly affected the physical attributes of ‘Golden Delicious’/M9 fruits. Control trees consistently produced larger fruits than those exposed to mechanical hail injury. Hail damage significantly reduced both fruit diameters (Fig. 1a) and fruit length (Fig. 1b) ($p < 0.001$), and all hail-affected treatments produced smaller fruits on average than the control. Likewise, fruit weight (Fig. 1c) declined significantly in all hail-treated groups compared with the control ($p < 0.001$). For fruit firmness (Fig. 1d), the difference between the control and hail treatment was not statistically significant ($p > 0.05$). Post-hail foliar applications of amino acids (H + A), bacteria (H + B), or their combination (H + A + B) did not significantly alter fruit size or firmness relative to the hail-only treatment. All pairwise contrasts between the hail and post-hail treatments were non-significant for fruit diameter, length, weight, and firmness (Table S7).

Soluble solids content (SSC) decreased markedly in all hail-damaged trees compared with the control ($p < 0.05$) (Fig. 2a). pH was not affected by the treatments (Fig. 2b), whereas titratable acidity was significantly influenced (Fig. 2c). When individual contrasts were examined,

Fig. 2 Chemical fruit characteristics of ‘Golden Delicious’/M9 apple trees. **a** Soluble solids content, **b** pH, **c** titratable acidity. In the boxplots, the thick horizontal line within each box represents the median, and gold circles denote the mean. The asterisk (*) indicates statistically significant differences among means labelled with different letters ($p < 0.05$). *C* control; *H* hail damage; *H + A* hail damage + amino acid; *H + B* hail damage + bacteria; *H + A + B* hail damage + amino acid + bacteria



differences among hail-affected groups were minimal. Only the H + B treatment showed slightly higher acidity than H ($p < 0.05$), whereas all other pairwise comparisons were non-significant (Table S8).

Among the evaluated color parameters, L^* was significantly affected by the treatments (Fig. S3A), whereas a^* (Fig. S3B), b^* (Fig. S3C), chroma (Fig. S3D), and hue angle (Fig. S3E) were not. Comparisons among the hail-affected groups (H, H + A, H + B, and H + A + B) showed few statistical differences for any of the color parameters (Table S9).

Identification and Characterization of SCAMP Gene Family in the Apple Genome

Protein sequences of the *Arabidopsis thaliana* SCAMP family were used as references to identify SCAMP genes in the Rosaceae family. A BLASTp search was performed against the genomic protein datasets of 32 Rosaceae species. Candidate SCAMP proteins were predicted by CDD, and SMART was employed to remove incomplete or absent structural domains. In total, 194 Rosaceae SCAMP genes were identified across the Rosaceae family and designated based on sequence homology (Table S3). In *M. domestica*, six SCAMP genes were identified and distributed across chromosomes 0, 5, 6, 9, 10, and 17. As shown in Table S3, the amino acid counts encoded by MdSCAMPs ranged from 273 aa (MdSCAMP5-MdSCAMP6) to 290 aa (MdSCAMP2). The estimated molecular weights varied between 30.9 kDa (MdSCAMP6) and 32.7 kDa (MdSCAMP1). The isoelectric point (pI) varied from 7.67 (MdSCAMP4) to 8.66 (MdSCAMP1-MdSCAMP2), in-

cluding six basic proteins ($pI > 7.5$). The instability index ranged from 40.28 (MdSCAMP6) to 45.07 (MdSCAMP1).

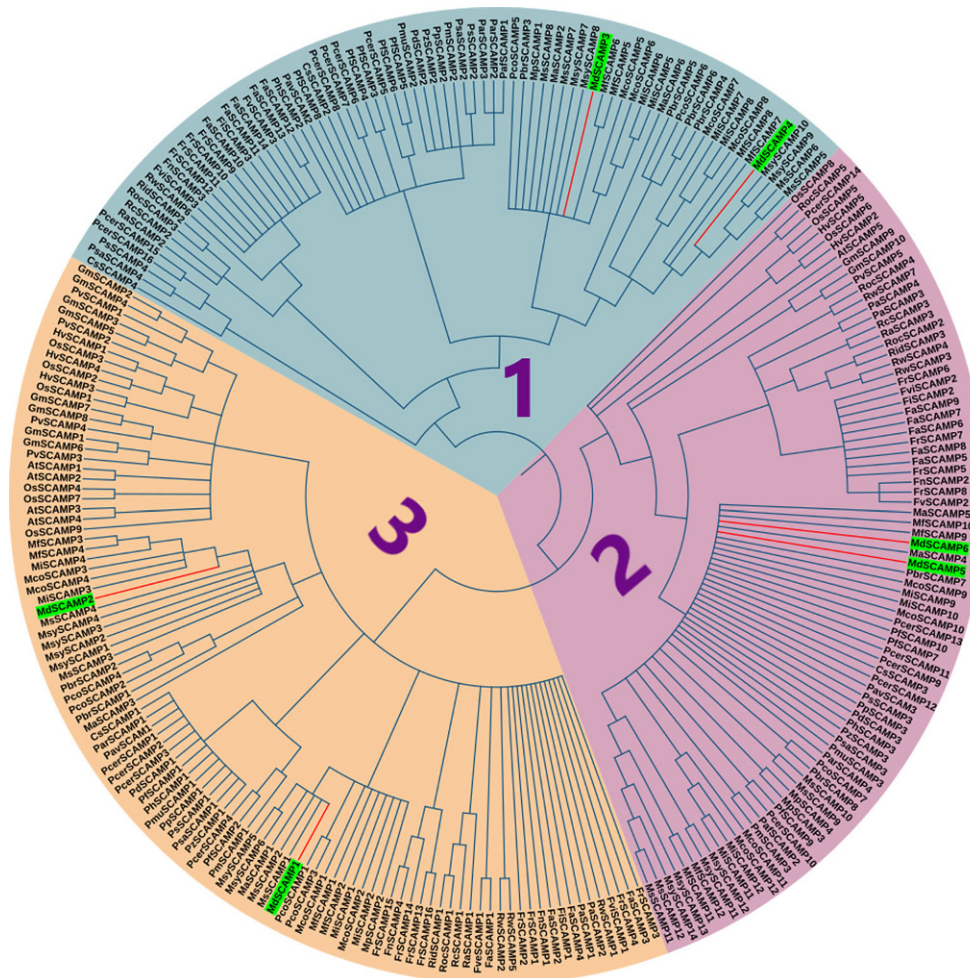
The aliphatic index values varied from a low of 96.62 in MdSCAMP2 to a high of 108.20 in MdSCAMP4. The grand average of hydropathicity (GRAVY) ranged from 0.188 (MdSCAMP1) to 0.245 (MdSCAMP3). Subcellular localization predictions consistently indicate plasma membrane localization for all MdSCAMP proteins.

Phylogenetic Tree, Domain, Gene Structure and Conserved Motifs Analysis, and Promoter Analysis of SCAMP Genes

To reveal the evolutionary relationship of SCAMP genes within the Rosaceae family, a maximum likelihood (ML) tree was constructed using SCAMP protein sequences from Rosaceae species (226), as well as from *A. thaliana* (5), *Hordeum vulgare* (5), *Oryza sativa* (9), and *Glycine max* (10). Based on phylogenetic clustering, the 240 SCAMPs were categorized into three subfamilies (Fig. 3). The majority of SCAMPs belong to Class 3, which contained 101 members, while Class 1 and Class 2 had 76 and 83 SCAMP members, respectively. The six MdSCAMP genes were distributed among different subfamilies, consistent with the taxonomic classification. Several MdSCAMPs located closely with AtSCAMP genes, suggesting potential functional conservation, but further experimental validation is required to confirm these functional relationships. These results may be due to the number of branches depending on the number of monocotyledonous and dicotyledonous SCAMP members.

Motif analysis revealed that motifs 1–5 were conserved among all proteins, indicating a high degree of structural

Fig. 3 Phylogenetic analysis of SCAMP genes. The SCAMP genes were divided into three groups. Different background colors indicate the different groups of the SCAMP proteins and the colors in the red nodes and green background represent MdSCAMPs



conservation (Fig. 4). Each MdSCAMP protein contained five conserved motifs, ranging in length from 29 (Motif 5) to 50 (Motif 1–2). Conserved domain analysis was performed using the NCBI Conserved Domain Database (CDD) and SMART. Domain analysis revealed the MdSCAMP proteins have SCAMP superfamily domain searched against the database (PF04144) in NCB-CDD. Gene structure analysis demonstrated that all *MdSCAMP* genes contain 12 exons, indicating a highly conserved exon-intron organization.

To investigate potential regulatory mechanisms underlying *MdSCAMP* gene expression, *cis*-acting element analysis was performed on the region 2000bp upstream of the *MdSCAMPs* using the PlantCARE database (Table S4). In this study, *cis*-elements were classified into four categories: light-responsive, hormone-responsive, stress related and development-related elements (Fig. 5). Within the light-responsive category, eleven types of *cis*-elements were identified in the majority of *MdSCAMPs* promoters, suggesting that *MdSCAMPs* may participate in light-mediated regulatory networks. Among the *cis*-elements related to phytohormones, the *cis*-active element ABRE was identified for the ABA response, the *cis*-active element ERE for the ethylene

response, the *cis*-regulatory element CGTCA-motif for the methyl jasmonate response, the *cis*-active elements GARE and P-box for the gibberellin response, and the *cis*-active element TCA-element for the salicylic acid response. Four types of *cis*-elements were identified in the environmental stress-related category, including MBS (MYB binding site involved in drought inducibility), ARE (anaerobic induction), TC-rich repeats (defense and stress response), and LTR (low temperature response). In the plant growth and development category, MSA-like, circadian, GCN4 and O₂ site *cis*-elements were detected. Overall, the *cis*-element composition of *MdSCAMP* promoters suggests potential regulatory involvement in light-responsive, hormone-related, stress-associated, and developmental processes.

Secondary and Three-dimensional Protein Modelling Analyses of the MdSCAMPs

Secondary structure prediction was performed to characterize the structural features of MdSCAMP proteins. The results revealed that MdSCAMP proteins are primarily composed of α -helices, extended strands, and random coils

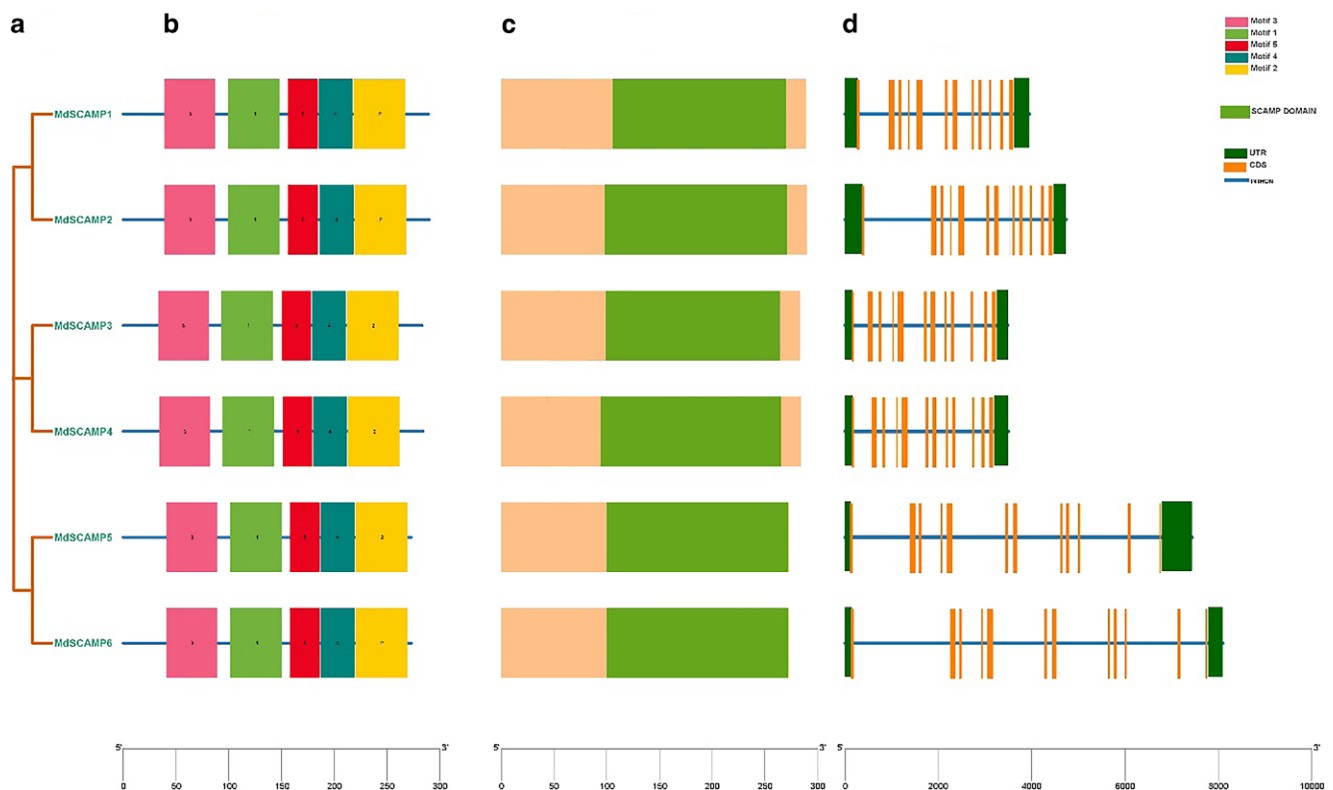
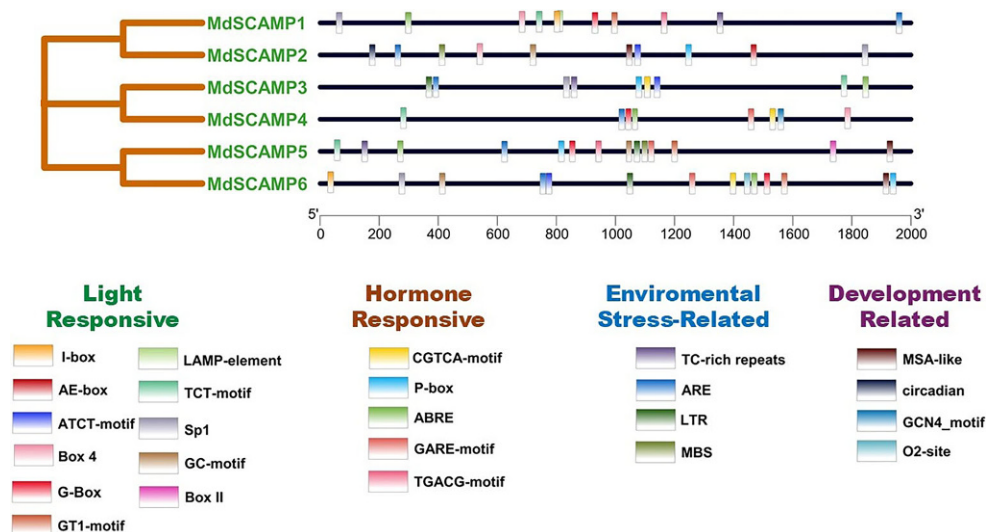


Fig. 4 **a** Phylogenetic relationship, **b** conserved protein motifs, **c** SCAMP domain locations, **d** gene structure of the MdSCAMPs. In the conserved protein motif section, motifs 1-2-3-4-5 are represented by green, yellow, pink, dark blue, and red, respectively. SCAMP domain location is represented by green box. In the gene structure section, UTRs are represented by green boxes, CDSs by orange boxes, and introns by blue lines

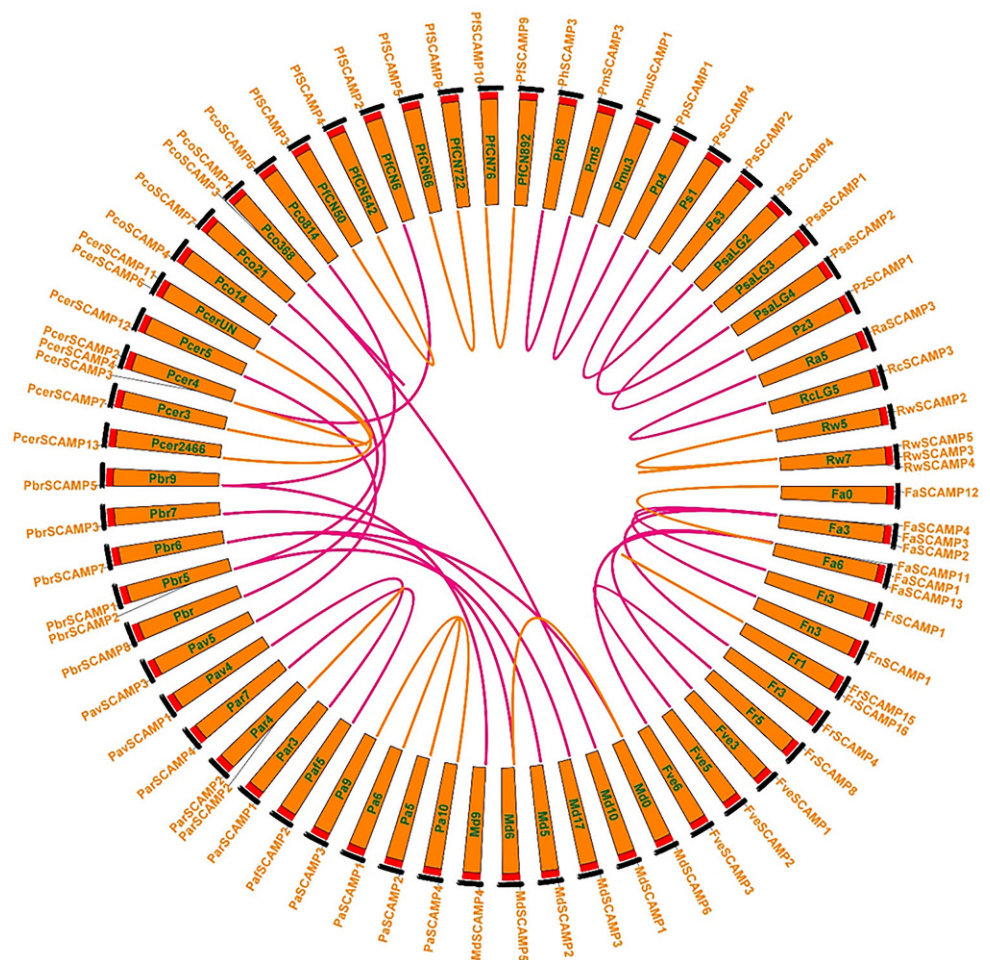
Fig. 5 *Cis*-acting regulator elements in the putative promoter regions of *MdSCAMPs*



(Table S5). Between these elements, the proportion of α -helices ranged from 45.79% (MdSCAMP6) to 54.48% (MdSCAMP2); the proportion of extended strands ranged from 4.84% (MdSCAMP1) to 8.79% (MdSCAMP5); and the proportion of random coils ranged from 40% (MdSCAMP2) to 46.15% (MdSCAMP6).

Three-dimensional (3D) structures of MdSCAMP proteins were predicted using the homology-based modeling server Phyre2. The modelled structures exhibited root mean square deviation (RMSD) values of less than 1 Å when superimposed on corresponding homologous templates, indicating high structural similarity and model reliability. A total of three templates (c5z1fA, c4n4wA, and c7r89A) were

Fig. 6 Chromosomal distribution, intraspecific and interspecific collinearity analysis of SCAMP family genes in Rosaceae. Different chromosome numbers with different colors labeled different species: Fa (*F. ananassa*), Fi (*F. iinumae*), Fn (*F. nilgerrensis*), Fr (*F. moupinensis*), Fve (*F. vesca*), Md (*M. domestica*), Pa (*P. anserina*), Par (*P. armeniaca*), Pav (*P. avium*), Pbr (*P. bretschneideri*), Pcer (*P. cerasus*), Pco (*P. communis*), Pf (*P. fruticosa*), Ph (*P. humilis*), Pm (*P. mongolica*), Pmu (*P. mume*), Pp (*P. persica*), Ps (*P. salicina*), Psa (*P. salicina* V2), Pz (*P. zhengheensis*), Ra (*R. argutus*), Rc (*R. chingii*), Rw (*R. wichuraiana*). Orange and red lines connect intraspecific and interspecific synthetic pairs, revealing linear relationships between duplicated SCAMP genes in different chromosomal regions



used to model the complete MdSCAMP protein set. In our analysis, Phyre2 confidence levels ranged from 39.5% to 90.2%, with most residuals in each model being modeled at confidence levels above 75%. Overall, these findings support the reliability of the predicted tertiary structures. Three-dimensional visualizations of MdSCAMP proteins are presented in Fig. S2.

Synteny Relationship and Duplication Event Analyses of the Rosaceae SCAMPs

To detect functionalization differences among SCAMP gene pairs in the Rosaceae family and to determine the nature and extent of selection pressure on the gene pairs, we calculated Ka/Ks values. The Ka/Ks value of 1 indicates neutral selection, a value greater than 1 indicates positive selection, and a value less than 1 indicates negative (purifying) selection. According to this phenomenon, most Rosaceae SCAMP gene pairs exhibited Ka/Ks values < 1, suggesting that these genes have primarily evolved under purifying selection, consistent with their conserved cellular functions (Table S6). Finally, we observed that the duplication of

these genes continued from 0.44 to 88.54 Mya, reflecting the long evolutionary history and conservation of SCAMP genes within the Rosaceae family.

To assess the evolutionary conservation of SCAMP genes within the Rosaceae family, synteny and duplication analyses were performed based on MdSCAMP genes in 21 different Rosaceae species. According to the comparative syntenic maps of Rosaceae SCAMP genes, 14 intraspecific and 27 interspecific collinear relationships were determined. Comparative synteny analysis between *M. domestica* and 21 other Rosaceae species (Fig. 6) revealed that six MdSCAMP genes showed syntenic relationships with those in *P. bretschneideri* and *P. communis*. This higher degree of linearity between MdSCAMPs and their homologs in other Rosaceae species suggests that there would be a large functional similarity among Rosaceae SCAMP genes. These results have provided important insights into the selective influence of SCAMP genes during the origin and evolution of Rosaceae.

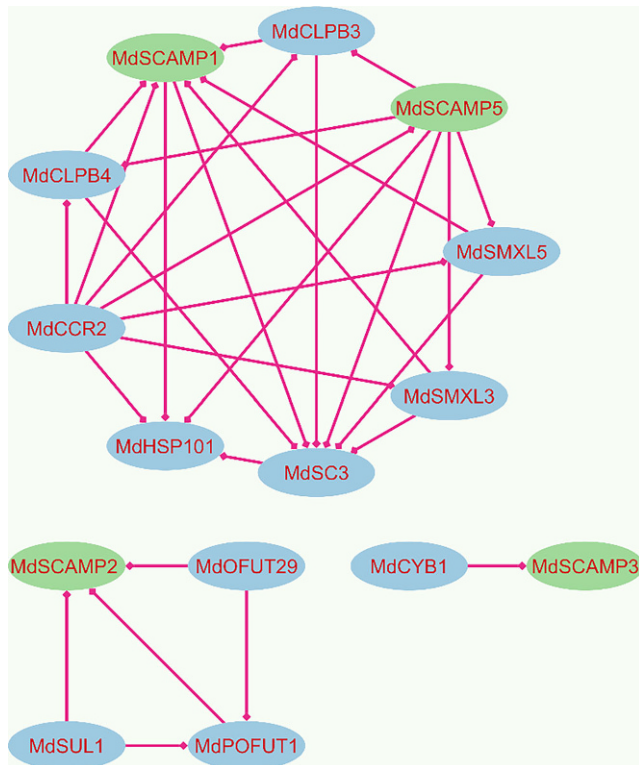


Fig. 7 The prediction of the interaction network of MdSCAMP proteins

GO Annotation and Protein-protein Interaction Analysis of MdSCAMPs

Gene Ontology (GO) annotation predicted that MdSCAMP proteins are primarily associated with protein transport processes within the biological process category (GO:0015031). In the molecular function category, the MdSCAMP proteins were distributed into two subcategories, including kinase activity (GO:0016301) and transferase activity (GO:0016740). Trans-Golgi network membrane (GO:0032588), recycling endosome membrane (GO:0055038), transport vesicle membrane (GO:0030658), plasma membrane (GO:0005886), cytosol (GO:0005829), nucleus (GO:0005634), and mitochondrion (GO:0005739) were in the cellular components.

In plants, proteins form complex protein-protein interaction (PPI) networks to regulate essential biological processes and physiological activities (Zhang et al. 2025). Protein-protein interaction (PPI) network analysis using STRING identified two major interaction clusters involving MdSCAMP proteins, except MdSCAMP4 (Fig. 7). In the first network, MdSCAMP1 and MdSCAMP5 were predicted to interact with stress-related proteins, including MdHSP101, MdCCR, and MdCLPB, which are associated with thermotolerance and stress adaptation. In second network, MdSCAMP2 was observed to interact tightly with

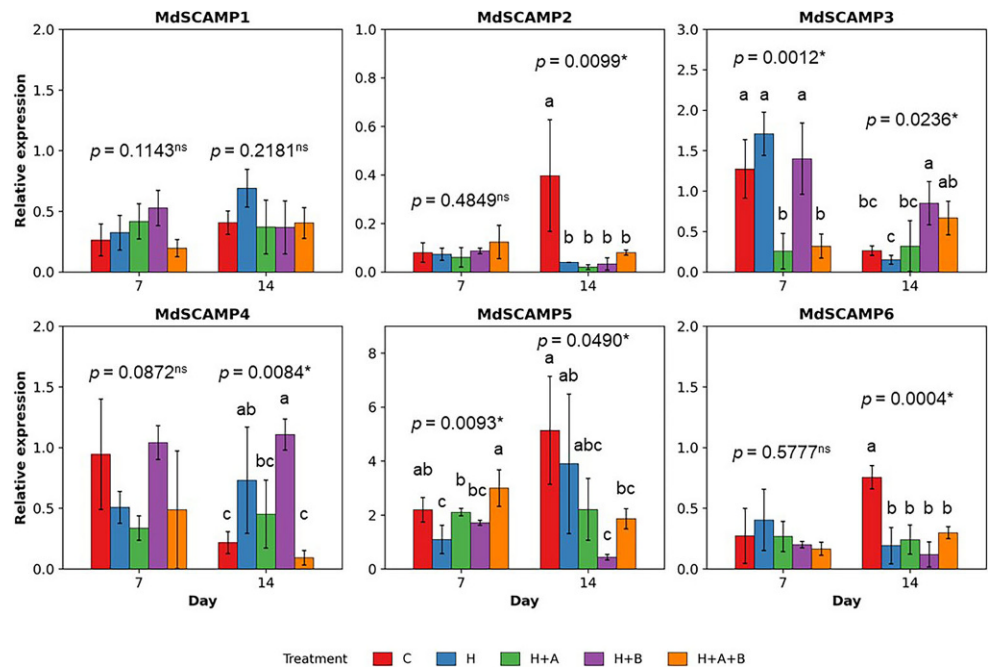
MdOFUT29, which binds directly to serine or threonine residues in specific protein domains; MdPOFUT1, which plays a critical role in pollen tube-to-pistil migration; and MdSUL1, a calcium ion-binding protein. Finally, MdSCAMP3 was predicted to interact with MdCYB1, which is localized in the endomembrane system and has carbon monoxide oxygenase activity.

Expression Pattern of MdSCAMP Genes in Different Tissues and Pathogen Infection

The diverse expression patterns of MdSCAMPs across tissues and their responses to pathogen infections further reinforced the hypothesis that this gene family is a key player in apple stress physiology, leading us to investigate their specific role during recovery from hail-induced mechanical injury. For more insight into the potential functions of MdSCAMPs, we performed an *in-silico* analysis of gene expression across 45 organs, tissues, and developmental stages, as well as under two pathogen treatments; *Penicillium expansum* and *Venturia inaequalis*. The MdSCAMP genes with FPKM values below 1 across all tissues were considered weakly expressed. Based on this criterion, MdSCAMP3 and MdSCAMP4 exhibited minimal expression across most tissues (Fig. S4, Table S10). Among other MdSCAMP genes, MdSCAMP1 displayed consistently high expression across the entire plant, including all floral organs, seeds, developing fruit, and vegetative tissues. MdSCAMP2 showed moderate expression pattern, detected in floral structures (petals, stamens, receptacle tissues), during the initial weeks of post-anthesis, and throughout the early stages of seed development. MdSCAMP5 is highly expressed across most fruit developmental stages, indicating that this gene may participate in fruit growth, ripening, and storage-related cellular processes. MdSCAMP6 also showed high expression across leaves, stems and fruit tissues.

The pathogens, *P. expansum* and *V. inaequalis* affect the quality and yield of apples, causing a lot of economic losses and waste (Zhang et al. 2021). The heat-map visualization of pathogen infected fruits revealed partially overlapping expression signatures among the MdSCAMP genes. After *P. expansum* infection, MdSCAMP1 and MdSCAMP2 showed a clear increase in expression, whereas MdSCAMP5 and MdSCAMP6 exhibited more subtle shifts (Fig. S5, Table S11). The FPKM values of MdSCAMP3 and 4 were 0, indicating that these two genes were inactive. In contrast, the *V. inaequalis* infection displayed a more gradual and dynamic progression (Fig. S6, Table S12). For example, MdSCAMP1 steadily increased across the 14-day infection period, and MdSCAMP5 showed a characteristic biphasic pattern with early and late activation. The expression pattern of MdSCAMP2 was modest but consistent over time. MdSCAMP3 and MdSCAMP4 again remained

Fig. 8 Relative expression levels of *MdSCAMP* genes in response to simulated hail damage and biological treatments measured by RT-qPCR. Bars represent means $\pm$ standard deviation (SD). The asterisk (*) indicates statistically significant differences among means labelled with different letters ($p < 0.05$). ns not significant ($p > 0.05$). C control; H hail damage; H + A hail damage + amino acid; H + B hail damage + bacteria; H + A + B hail damage + amino acid + bacteria



nearly inactive throughout all stages. Overall, *P. expansum* induces a rapid activation of *MdSCAMP1* and *MdSCAMP2*, whereas *V. inaequalis* triggers a slower, but more complex transcriptional response, particularly for *MdSCAMP1* and *MdSCAMP5*. These contrasting patterns show pathogen-specific regulatory roles for *SCAMP* family members in apple fruit defense mechanisms.

RT-qPCR Expression Profiling of *MdSCAMPs* Under Different Treatments

RT-qPCR analysis revealed member specific responses for *MdSCAMP* genes in response to simulated hail damage and biological treatments (Fig. 8). The expressions of *MdSCAMP1*, *MdSCAMP2*, and *MdSCAMP6* remained low across all treatments and time points, showing only slight fluctuations in response to hail or post-hail applications, with maximum expression levels showing minimal deviation (less than 1.5-fold) from the day 0 baseline, suggesting these genes are not primarily involved in the immediate transcriptomic response to wounding. *MdSCAMP3* and *MdSCAMP4* exhibited a modest but consistent induction under hail damage. For the *MdSCAMP3*, control, H and H+B samples displayed the highest expression, whereas H+A and H+A+B showed the lowest values on day 7. Although no significant differences were detected at Day 7 for the *MdSCAMP4*, clear differences appeared on Day 14. Interestingly, amino acid treatment applied to hail-damaged leaves downregulated the expression of both genes (*MdSCAMP3* and *MdSCAMP4*) at both 7 and 14 days. A similar downregulated pattern was also observed in H+

A+B treated leaves. *MdSCAMP5* showed the strongest overall expression among all family members. On Day 7, H+A+B samples exhibited higher expression than other treatment groups including C. On day 14, control samples showed higher expression than hail alone samples. Post-hail applications of amino acids (H+A) and the combined treatment (H+A+B) moderately supported the induction of gene expression. Bacterial treatment of hail-damaged leaves (H+B) resulted in a marked reduction in the expression level of *MdSCAMP5* on Day 14.

The RT-qPCR results demonstrate that control plants generally exhibited higher relative expression, confirming strong basal transcriptional activity before external disturbance. Hail damage and subsequent treatments mostly led to either stable or decreased expression, particularly noticeable for *MdSCAMP2*, *MdSCAMP3*, *MdSCAMP5*, and *MdSCAMP6*. These observations suggest that, under the conditions tested, mechanical injury does not consistently enhance *MdSCAMP* expression beyond basal levels and may instead downregulate several members of the gene family. Notably, although post-hail treatments showed a trend toward stabilizing expression levels, none of the applications fully restored *MdSCAMP* expression to control levels by Day 14, highlighting the long-lasting impact of mechanical injury at the transcriptional level.

Discussion

Fruit Quality and Physiological Responses to Hail Injury

Understanding how apple trees respond to mechanical injury is crucial for improving resilience in hail-prone orchards. In the present study, the marked reductions in fruit diameter, length, and weight following simulated hail damage are consistent with the mechanical disruption of fruit- and leaf-tissue integrity. The lack of effect of the amino acid and bacterial foliar treatments on physical fruit traits indicates that post-damage applications could not compensate for the loss of growth potential caused by early tissue injury. Following hail injury, photosynthesis is expected to decline during the first two weeks due to the destruction of active leaf area and stomatal dysfunction; the plants may gradually recover afterwards as new tissues develop. Under such circumstances, amino acids primarily serve as carbon and nitrogen sources that support metabolic recovery rather than directly affecting hormonal regulation. In contrast, *Aeromonas hydrophila* *iehlthiosmia* AE425, which produces ACC deaminase, may influence ethylene metabolism and stress-related signaling, potentially contributing to longer-term physiological stability. Nevertheless, the mild effects observed in this study suggest that the intensity of tissue injury and the timing of application limit the overall recovery response. The decrease in soluble solids content in hail-damaged fruits likely reflects a reduced source capacity caused by leaf damage, diminished carbohydrate accumulation, and possibly altered sugar partitioning into the developing fruit.

Promoter analysis revealed multiple stress-related *cis*-elements, including ABRE, MYB/MYC, W-box, and low-temperature or defense motifs, suggesting potential responsiveness to ABA, drought/osmotic stress, and pathogen or wound signals. Similar promoter architectures have been reported for *SCAMP* genes in soybean and cotton, where enrichment of light and stress responsive elements has been linked to their induction by salt and other abiotic stresses (Wang et al. 2023; He et al. 2024). These features support the idea that *MdSCAMPs* would also be involved in stress related regulatory networks.

While *SCAMP* genes have been implicated in abiotic stress tolerance in several crops, their role in rapid mechanical wounding remains largely unexplored. This study provides the first comprehensive analysis of the *MdSCAMP* gene family in apple and demonstrates that their transcriptional responses to hail injury differ markedly from those observed in classical salt or osmotic stress studies.

Previous studies have established that plant *SCAMP* genes play key roles in abiotic stress tolerance, primarily by regulating ion transporters and stabilizing membranes.

For example, *GmSCAMP5* in soybean (Wang et al. 2023) and *GhSCAMP2/4* in cotton (He et al. 2024) are known to be upregulated under salt and osmotic stress, enhancing tolerance by maintaining ionic homeostasis. These findings suggest that *SCAMPs* act as positive regulators during sustained environmental stress. Unlike a gradual increase in salinity, our results reveal that acute mechanical injury induced by simulated hail predominantly downregulates *MdSCAMP* genes, particularly *MdSCAMP2–6*. This divergent transcriptional profile indicates the existence of stress specific regulatory mechanisms that distinguish acute physical trauma from gradual environmental stressors like salinity. Under such acute conditions, cells may temporarily suppress energy-demanding vesicle trafficking pathways to prioritize immediate wound sealing and metabolic stabilization. Rather than triggering *SCAMP* mediated transport, the tissues seem to adopt a resource reallocation strategy in which transcription of trafficking components is reduced while existing protein pools may support rapid membrane repair. This interpretation aligns with findings in rubber tree (*Hevea brasiliensis*), where wounding similarly led to a decline in *SCAMP* expression (Yang et al. 2024). Based on this, it is reasonable to think that cells reduce the transcription of energy demanding trafficking pathways and instead make use of existing *SCAMP* protein reserves to ensure rapid membrane repair. Moreover, the inability of amino acid and PGPR treatments to fully restore *MdSCAMP* expression levels suggests that the molecular recovery of membrane trafficking machinery is a slower process than the immediate physiological improvements observed in fruit quality.

These findings suggest that *MdSCAMPs* may exhibit functional diversification associated with their phylogenetic relationships. *MdSCAMP5* showed the strongest overall transcriptional response among the family members, suggesting that it may be particularly responsive to membrane-damaging events such as hail. Unlike their salt-inducible counterparts in soybean and cotton, *MdSCAMP* genes are predominantly downregulated following hail damage, suggesting that *SCAMP*-mediated trafficking is largely repressed at the transcriptional level during the early wound response. In parallel, hail-induced reductions in fruit growth and soluble solids content underline the strong impact of mechanical injury on source sink relations and fruit quality, which could not be reversed by post-hail amino acid or PGPR treatments.

Conclusion

Artificially induced hail damage on apple trees significantly reduced fruit size, weight, and soluble solids, indicating serious physiological disruption. Although amino acid and

PGPR treatments supported molecular activity, they had limited capacity to restore, indicating that biological treatments provide only partial physiological support after severe tissue injury. At the molecular level, most *MdSCAMPs* exhibited reduced expression under hail damage, suggesting that SCAMP mediated membrane trafficking is not transcriptionally activated during early mechanical injury. Given its strong basal expression, stress-related promoter architecture and clear repression under hail, *MdSCAMP5* emerges as a promising target for future functional analyses. These findings advance our understanding of membrane trafficking dynamics under mechanical stress and highlight *MdSCAMP5* as a potential target for improving resilience to physical damage in perennial fruit crops.

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Author contributions Mustafa Koparan and Ersin Atay designed the study, completed the horticultural analysis, and analyzed the data. Ali Kiyak contributed all bioinformatic analysis. Selman Uluisik performed RT-qPCR analysis and drafted the initial version of the manuscript. All authors contributed to experiment planning and provided critical revisions to the manuscript. All authors reviewed and approved the final version of the manuscript.

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Data Availability All data supporting the findings of this study are available within the paper and its Supplementary Information.

Competing interests The authors declare no competing interests.

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References

- Alzahib R, Migdadi H, Ghamdi A, Alwahibi M, Afzal M, Elharty E, Alghamdi S (2021) Exploring genetic variability among and within hail tomato landraces based on sequence-related amplified polymorphism markers. *Diversity* 13:135. <https://doi.org/10.3390/D13030135>
- Banița S, Sestras A, Olteanu I, Calugar A, Bunea C (2020) The influence of hail and treatments with amino-acids on the fertility and biologic balance of grapevines. *Rom J Hortic*. <https://doi.org/10.51258/rjh.2020.15>
- Botzen W, Bouwer L, Van Den Bergh J (2010) Climate change and hailstorm damage: empirical evidence and implications for agriculture and insurance. *Resour Energy Econ* 32:341–362. <https://doi.org/10.1016/J.RESENEECO.2009.10.004>
- Bueckert RA (2011) Simulated hail damage and yield reduction in lentil. *Can J Plant Sci* 91(1):117–124
- Chelaru S, Manolache A, Gherghel M, Perju I, Turcu C (2023) Observations on the effects of the hail on some apple varieties under the conditions of 2021. *Bull UASVM Hortic*. <https://doi.org/10.15835/buasvmcn-hort:2022.0015>
- Chen C, Chen H, Zhang Y, Thomas HR, Frank MH, He Y, Xia R (2020) TBtools: an integrative toolkit developed for interactive analyses of big biological data. *Mol Plant* 13(8):1194–1202
- Da L, Liu Y, Yang J, Tian T, She J, Ma X, Su Z (2019) AppleMDO: a multi-dimensional omics database for apple co-expression networks and chromatin states. *Front Plant Sci* 10:1333
- Duvaud S, Gabella C, Lisacek F, Stockinger H, Ioannidis V, Durinx C (2021) Expasy, the Swiss bioinformatics resource portal, as designed by its users. *Nucleic Acids Res* 49(W1):W216–W227. <https://doi.org/10.1093/nar/gkab225>
- Fan J, Zhang Y, Wang J, Jeong J, Chen X, Zhang S, Lin Y, Feng Z, Adams-Selin R (2022) Contrasting responses of hailstorms to anthropogenic climate change in different synoptic weather systems. *Earths Future*. <https://doi.org/10.1029/2022EF002768>
- FAOSTAT (2026) FAOSTAT database collections. Food and Agriculture Organization of the United Nations, Rome. <http://www.fao.org/faostat/en/#data/>. Accessed 28 Feb 2026
- Geourjon C, Deléage G (1995) SOPMA: significant improvements in protein secondary structure prediction by consensus prediction from multiple alignments. *Bioinformatics* 11(6):681–684. <https://doi.org/10.1093/bioinformatics/11.6.681>
- Gökçe GF, Özbay M (2019) The effect of different amino acids on the development of cucumber (*Cucumis sativus* L.) plant species growing in Düzce region. *J Anatol Environ Animal Sci* 4(3):575–580
- He Z, Ma X, Zhu QH, Cheng S, Liu F, Zhang T, Zhang C, Li J, Xiong X, Sun J (2024) Genome-wide analysis of cotton SCAMP genes and functional characterization of GhSCAMP2 and GhSCAMP4 in salt tolerance. *BMC Plant Biol* 24:870
- Jiang Q, Vandrope M, Fox AR, Hdedeh O, Vermeersch M, Mylle E, Furones Cuadrado A, Kraus J, Liu H, Eeckhout D, Navarre C, Courtoy A, Kocourková DAB, Podmanická TK, Jacobs TB, Dragwidge JM, De Smet I, Pleskot R, Chaumont F, Van Damme D (2025) Secretory carrier membrane proteins assist with aquaporin trafficking in Arabidopsis. <https://doi.org/10.1101/2025.07.03.662988>
- Jung S, Lee T, Cheng CH, Buble K, Zheng P, Yu J, Humann J, Ficklin SP, Gasic K, Scott K, Frank M, Ru S, Hough H, Evans K, Peace C, Olmstead M, DeVetter LW, McFerson J, Coe M, Main D (2019) 15 years of GDR: new data and functionality in the genome database for Rosaceae. *Nucleic Acids Res* 47(D1):D1137–D1145. <https://doi.org/10.1093/nar/gky100>

- Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJE (2015) The Phyre2 web portal for protein modeling, prediction and analysis. *Nat Protoc* 10:845–858
- Kiprijanovski M, Gjamovski V, Arsov T (2016) The effects of anti-hail net in protection of pear orchard after hailstorm occurrence. *Acta Hortic* 1139:529–534. <https://doi.org/10.17660/ActaHortic.2016.1139.91>
- Koch MA, Haubold B, Mitchell-Olds T (2000) Comparative evolutionary analysis of chalcone synthase and alcohol dehydrogenase loci in Arabidopsis, Arabis, and related genera (Brassicaceae). *Mol Biol Evol* 17(10):1483–1498. <https://doi.org/10.1093/oxfordjournals.molbev.a026248>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* 35(6):1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lam SK, Siu CL, Hillmer S, Jang S, An G, Robinson DG, Jiang L (2007) Rice SCAMP1 defines clathrin-coated, trans-golgi-located tubular-vesicular structures as an early endosome in tobacco BY-2 cells. *Plant Cell* 19(1):296–319
- Lescot M, Déhais P, Thijs G, Marchal K, Moreau Y, Van de Peer Y, Rouzé P, Rombauts S (2002) PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res* 30(1):325–327. <https://doi.org/10.1093/nar/30.1.325>
- Letunic I, Bork P (2019) Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 47(W1):W256–W259. <https://doi.org/10.1093/nar/gkz239>
- Letunic I, Khedkar S, Bork P (2021) SMART: recent updates, new developments and status in 2020. *Nucleic Acids Res* 49(D1):D458–D460. <https://doi.org/10.1093/nar/gkaa937>
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* 25(4):402–408
- Lollato R, Silva A, Maeoka R, Bavia G, Bonassi L, Jaenisch B (2018) Timing and positioning of simulated hail damage effects on wheat yield in Kansas: 2015–2016 and 2016–2017 growing seasons. *Kans Agric Exp Stn Res Rep* 4:12. <https://doi.org/10.4148/2378-5977.7613>
- Marchler-Bauer A, Derbyshire MK, Gonzales NR, Lu S, Chitsaz F, Geer LY, Geer RC, He J, Gwadz M, Hurwitz DI, Lanczycki CJ, Lu F, Marchler GH, Song JS, Thanki N, Wang Z, Yamashita RA, Zhang D, Zheng C, Bryant SH (2015) CDD: NCBI's conserved domain database. *Nucleic Acids Res* 43(D1):D222–D226. <https://doi.org/10.1093/nar/gku1221>
- Obudulu O, Mähler N, Skotare T, Bygdell J, Abreu IN, Ahnlund M, Tuominen H (2018) A multi-omics approach reveals function of secretory carrier-associated membrane proteins in wood formation of populus trees. *BMC Genomics* 19:373
- Ortiz-Bobea A, Ault T, Carrillo C, Chambers R, Lobell D (2021) Anthropogenic climate change has slowed global agricultural productivity growth. *Nat Clim Chang* 11:306–312. <https://doi.org/10.1038/s41558-021-01000-1>
- Petoumenou DG, Biniari K, Xyrafis E, Mavronasios D, Daskalakis I, Palliotti A (2019) Effects of natural hail on the growth, physiological characteristics, yield, and quality of *Vitis vinifera* L. cv. Thompson Seedless under Mediterranean growing conditions. *Agronomy* 9(4):197
- R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna (<https://www.R-project.org/>)
- Radler A, Groenemeijer P, Faust E, Sausen R, Pucik T (2019) Frequency of severe thunderstorms across Europe expected to increase in the 21st century due to rising instability. *Npj Clim Atmos Sci* 2:30. <https://doi.org/10.1038/s41612-019-0083-7>
- Rana VS, Sharma S, Rana N, Sharma U, Patiyal V, Banita, Prasad H (2022) Management of hailstorms under a changing climate in agriculture: A review. *Environ Chem Lett* 20(6):3971–3991
- Raupach T, Martius O, Allen J, Kunz M, Lasher-Trapp S, Mohr S, Rasmussen K, Trapp R, Zhang Q (2021) The effects of climate change on hailstorms. *Nat Rev Earth Environ* 2:213–226. <https://doi.org/10.1038/s43017-020-00133-9>
- Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T (2003) Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 13(11):2498–2504. <https://doi.org/10.1101/gr.1239303>
- Suyama M, Torrents D, Bork P (2006) PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. *Nucl Acids Res* 34(W):W609–W612. <https://doi.org/10.1093/nar/gkl315>
- Swarbreck D, Wilks C, Lamesch P, Berardini TZ, Garcia-Hernandez M, Foerster H, Li D, Meyer T, Muller R, Ploetz L, Radenbaugh A, Singh S, Swing V, Tissier C, Zhang P, Huala E (2008) The Arabidopsis information resource (TAIR): gene structure and function annotation. *Nucleic Acids Res* 36(Suppl 1):D1009–D1014. <https://doi.org/10.1093/nar/gkm965>
- Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ, von Mering C (2021) The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res* 49(D1):D605–D612. <https://doi.org/10.1093/nar/gkaa1074>
- TARSIM (2025a) Corporate history. Tarım Sigortaları Havuz İşletmesi A.Ş., Ankara. https://www.tarsim.gov.tr/subPage_en/corporate-history. Accessed 26 Jan 2026
- TARSIM (2025b) Annual reports. Tarım Sigortaları Havuz İşletmesi A.Ş., Ankara. https://www.tarsim.gov.tr/subPage_en/annual-reports. Accessed 26 Jan 2026
- Tartachnyk I, Blanke MM (2002) Effect of mechanically-simulated hail on photosynthesis, dark respiration and transpiration of apple leaves. *Environ Exp Bot* 48(2):169–175
- Thumhuri V, Almagro Armenteros JJ, Rosenberg Johansen A, Nielsen H, Winther O (2022) DeepLoc 2.0: multi-label subcellular localization prediction using protein language models. *Nucleic Acids Res* 50(W1):W228–W234. <https://doi.org/10.1093/nar/gkac278>
- Törönen P, Medlar A, Holm L (2018) PANNZER2: a rapid functional annotation web server. *Nucleic Acids Res* 46(W1):W84–W88. <https://doi.org/10.1093/nar/gky350>
- TURKSTAT (2026) Population, education, agriculture. Turkish Statistical Institute, Ankara (<http://www.tuik.gov.tr/>). Accessed 28 Feb 2026
- Wang H, Tse YC, Law AHY, Sun SSM, Sun YB, Xu ZF, Hillmer S, Robinson DG, Jiang L (2010) Vacuolar sorting receptors (VSRs) and secretory carrier membrane proteins (SCAMPs) are essential for pollen tube growth. *Plant J* 61(5):826–838. <https://doi.org/10.1111/j.1365-313X.2009.04111.x>
- Wang M, He C, Shi G, Yin Q, Zhang H, Yang W, Du W (2023) Genome-wide analysis of the SCAMPs gene family of soybean and functional identification of GmSCAMP5 in salt tolerance. *BMC Plant Biol* 23:628
- Yang B, Huang X, Zhang Y, Gao X, Ding S, Qi J, Wang X (2024) Genome-Wide Identification of Rubber Tree SCAMP Genes and Functional Characterization of HbSCAMP3. *Plants* 13(19):2729
- Yang Y, Shen A, Zou L, Guan X, Wang Y, Wang L, Zhang J, Xu X, Huang Z (2025) Overexpression of GmSCAMP4 enhances the tolerance of soybeans to low phosphorus stress. *Plant Sci* 359:112680. <https://doi.org/10.1016/j.plantsci.2025.112680>
- Yang Z, Lasker K, Schneidman-Duhovny D, Webb B, Huang CC, Pettersen EF, Goddard TD, Meng EC, Sali A, Ferrin TE (2012)

- UCSF chimera, MODELLER, and IMP: an integrated modeling system. *J Struct Biol* 179(3):269–278
- Yuan X, Li S, Chen J, Yu H, Yang T, Wang C, Huang S, Chen H, Ao X (2024) Impacts of global climate change on agricultural production: a comprehensive review. *Agronomy* 14(7):1360. <https://doi.org/10.3390/agronomy14071360>
- Zarei A, Trobacher CP, Shelp BJ (2015) NAD-aminoaldehyde dehydrogenase candidates for 4-aminobutyrate (GABA) and β -alanine production during terminal oxidation of polyamines in apple fruit. *FEBS Lett* 589(19):2695–2700
- Zhang W, Liao L, Xu J, Han Y, Li L (2021) Genome-wide identification, characterization and expression analysis of MATE family genes in apple (*Malus × domestica* Borkh). *BMC Genomics* 22:632. <https://doi.org/10.1186/s12864-021-07943-1>
- Zhang ZQ, Li MM, Tian RM, Cheng X, Wang ZW, Li KL, Yang YY (2025) Genome-wide identification, expression profile and evolution analysis of Importin α gene family in *Glycine max*. *Agronomy* 15(11):2603

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