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Genome-wide identification of the PSK family and the association between SlPSK7 and auxin signaling in tomato

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Abstract

Background

Phytosulfokine (PSK) is a tyrosine-disulfated pentapeptide with the core sequence YIYTQ that acts as a peptide hormone regulating plant growth, development, and stress responses by mediating intercellular communication. However, the PSK gene family has not been systematically characterized in tomato (*Solanum lycopersicum*). In this study, we performed a genome-wide identification of the PSK family in tomato and investigated the relationship between *SlPSK7* and auxin signaling during fruit set.

Results

Eight *SlPSK* genes were identified in the tomato genome, and their conserved domains were predominantly located near the C-terminus. Phylogenetic analysis showed that PSK genes from evolutionarily related species clustered together, supporting the conserved roles of this family during plant evolution. Tissue-specific expression analysis revealed distinct expression patterns among *SlPSK* members, with *SlPSK7* predominantly expressed in floral buds. Under exogenous treatments with the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D), the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG), and the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA), the expression pattern of *SlPSK7* was highly consistent with that of auxin response factor (ARF) genes but opposite to that of *IAA9*. In transient overexpression assays, elevated *SlPSK7* expression at anthesis (0 d) was accompanied by increased transcript levels of ARF family genes, particularly *ARF7* (up to 5.85-fold, $P < 0.01$); conversely, VIGS-mediated silencing of *SlPSK7* significantly downregulated ARF family gene expression (to 0.34-fold, $P < 0.05$).

Conclusions

Eight PSK family members were identified in the tomato genome, and *SlPSK7* was shown to be closely associated with ARF family genes. The expression pattern of *SlPSK7* parallels that of the ARF family, the core positive regulators of auxin signaling, suggesting that *SlPSK7* may act as a positive regulator of the auxin pathway. These findings provide a basis for further functional characterization of *SlPSK7* in tomato fruit set regulation.

Background

Fruit set in tomato is governed by a complex polygenic network. Accumulating evidence indicates that the initiation of fruit set is inseparable from the regulation of endogenous hormones, among which auxin plays a particularly critical role. When auxin levels in floral buds exceed a specific threshold, parthenocarpic fruit development can be induced [1]. However, the molecular mechanisms underlying this regulatory process remain poorly understood.

Phytosulfokine (PSK) was first isolated from conditioned medium of *Asparagus officinalis* mesophyll cell cultures [2]. Numerous studies have demonstrated that PSK functions as a plant growth regulator in various biological processes [3]. Systematic investigations by the Matsubayashi group provided early evidence that PSK possesses the properties of a plant peptide hormone. For example, studies using *Asparagus* cell cultures showed that PSK-α synthesis depends on the presence of both auxin and cytokinin, and that these three components synergistically initiate cell cycle progression [4]. PSK can also eliminate the density-dependent limitation on cell division rate, serving as a key intercellular signal that coordinates with these two hormones to regulate plant cell dedifferentiation and redifferentiation. Furthermore, Feng et al. [5] constructed an interaction network of 11 plant hormones, including PSK, reflecting the cross-regulatory relationships between PSK and multiple phytohormones in diverse physiological processes.

PSK is widely distributed across most plant species and plays important roles in promoting cell division and differentiation, cell elongation, pollen germination and pollen tube guidance, floral organ abscission, and resistance to pathogens and drought [6, 7]. With advances in genomics, the functional characterization of PSK gene family members has been reported in major crops, including rice [8–10], wheat [11, 12], and tomato [13–17].

As a globally important horticultural crop with significant economic value, tomato has been a major focus of research on the molecular mechanisms underlying yield-related traits. To date, functional studies of *SlPSK* genes in tomato remain limited, and the relationship between PSK signaling and the auxin pathway during fruit set has not been explored. Previous studies of PSK in tomato focused mainly on immunity and stress responses, such as auxin-dependent immunity, drought-induced flower drop, and flower abscission [13, 14, 16, 17]. By contrast, the present study provides a systematic genome-wide identification of the *SlPSK* family in tomato and reveals a previously unreported association between *SlPSK7* and the auxin signaling pathway during fruit set through integrated bioinformatics, transcriptome, and transient transformation analyses. These findings provide a theoretical foundation for further elucidation of the molecular regulatory network of tomato fruit set and for genetic improvement and breeding applications targeting yield traits.

Results

Identification and characterization of the SlPSK gene family in tomato

A genome-wide search of the tomato reference genome database, followed by sequence validation using the core conserved PSK motif YIYTQ, identified a total of eight *SlPSK* gene family members (Table 1). All eight *SlPSK* genes contained a relatively small number of exons (2–3). The encoded proteins ranged from 81 (*SlPSK7*) to 138 (*SlPSK6*) amino acids in length, with molecular weights ranging from 9.18 (*SlPSK7*) to 16.08 (*SlPSK6*) kDa, and all isoelectric points (pI) were below 7. The conserved PSK domains were predominantly located near the C-terminal region (Fig. 1B, red line).

Table 1
Characteristics of the *SlPSK* genes and their encoded proteins in tomato

Gene	Gene ID	Genomic position	Exons	Protein length (aa)	MW (kDa)	pI	Domain location
SlPSK1	Solyc09g009130	Chr9:2,464,872-2,466,169	3	90	10.47	5.02	18–87
SlPSK2	Solyc11g066880	Chr11:52,972,207 – 52,973,439	3	98	11.43	5.75	12–97
SlPSK3	Solyc02g092110	Chr2:53,960,693 – 53,961,812	2	82	9.35	5.51	11–82
SlPSK3L	Solyc02g092120	Chr2:53,963,296 – 53,964,278	2	83	9.46	5.35	11–83
SlPSK4	Solyc01g106830	Chr1:94,471,478 – 94,472,067	2	83	9.57	6.27	12–81
SlPSK5	Solyc10g083580	Chr10:63,490,602 – 63,492,575	3	92	10.48	5.21	13–89
SlPSK6	Solyc06g074540	Chr6:46,263,736 – 46,265,513	3	138	16.08	5.82	54–137
SlPSK7	Solyc04g077580	Chr4:62,626,878 – 62,627,839	2	81	9.18	6.07	47–80

MW, molecular weight; pI, isoelectric point; aa, amino acids.

Regarding chromosomal distribution (Fig. 1A), the eight genes were mapped to seven chromosomes, with chromosome 2 harboring two adjacent genes, *SlPSK3* and *SlPSK3L*. All genes were located in the distal regions of their respective chromosomes, suggesting high transcriptional activity of this gene family.

Phylogenetic and synteny analysis of the SlPSK gene family

To elucidate the evolutionary relationships of PSK family genes, a phylogenetic tree was constructed using MEGA software with 36 PSK genes from tomato (8), potato (9), *Arabidopsis thaliana* (7), and rice (12). Based on bootstrap values and tree topology, the 36 PSK genes were clustered into nine groups (Fig. 2A). Group C contained the largest number of members, including the tomato genes *SlPSK2*, *SlPSK6*, *SlPSK1*, and *SlPSK5*. Group I contained the tomato homologs *SlPSK3* and *SlPSK3L*, whereas *SlPSK7* and *SlPSK4* were placed in groups H and F, respectively. Notably, all eight tomato *SlPSK* members clustered in the same branches as potato PSK genes, showing the highest homology with potato, a fellow dicotyledon, whereas the lowest homology was observed with rice (a monocotyledon) PSK genes. This pattern indicates that PSK genes from evolutionarily closer species share more similar gene structures and conserved motifs, and that the PSK family is highly conserved within dicotyledons and monocotyledons, suggesting important roles during long-term plant evolution.

Synteny analysis among tomato, potato, *Arabidopsis thaliana*, and rice revealed the closest syntenic relationship between tomato and potato, with 15 syntenic pairs (48.4%), consistent with their close phylogenetic affinity within the Solanaceae. Rice-tomato and *Arabidopsis*-tomato each had 8 syntenic pairs (25.8%), indicating that the PSK gene family existed prior to the divergence of monocotyledons and dicotyledons. *SlPSK5* showed the highest number of syntenic genes with potato (4), suggesting tandem duplication events in the Solanaceae ancestor. *SlPSK3* and *SlPSK3L* had syntenic counterparts in tomato, potato, *Arabidopsis*, and rice, showing the broadest cross-species distribution and implying that they are ancient core members of the PSK family. Notably, *SlPSK7* was the only *SlPSK* gene without syntenic counterparts in potato; it had five syntenic genes in *Arabidopsis* (3) and rice (2), suggesting possible gene loss or extensive sequence divergence in potato after the divergence of the Solanaceae.

Collectively, these results indicate that the PSK gene family is evolutionarily conserved within the Solanaceae but has undergone lineage-specific expansion following the divergence of dicotyledons and monocotyledons. The core functional domains have remained highly conserved throughout long-term evolution, suggesting that this gene family plays important roles in plant growth and development.

Cis-acting element analysis of SlPSK gene promoters

Cis-acting elements do not encode proteins but interact with trans-acting factors to regulate gene expression. Analysis of the promoter regions of *SlPSK* family genes revealed hormone-related, stress-related, and growth/development-related elements (Fig. 3). All eight genes contained abscisic acid (ABA) response elements (ABRE). Four genes contained methyl jasmonate (MeJA) response elements (CGTCA-motif and TGACG-motif), three contained auxin response elements (TGA-element), two contained salicylic acid (SA) response elements (TCA-element), and only one gene contained a gibberellin response element (TATC-box). Regarding stress responses, three genes contained defense and stress response elements (TC-rich repeats), and two contained drought-inducible MYB transcription factor binding sites (MBS). Notably, only *SlPSK7* (Solyc04g077580) contained an O2-site element associated with endosperm-specific/seed storage protein regulation.

These results suggest that *SlPSK* family genes harbor multiple hormone-responsive cis-elements, indicating that they may regulate their growth factor functions through interactions with hormone signaling pathways and may also play important roles in stress responses. The exclusive presence of the O2-site element in *SlPSK7* implies a specialized function in reproductive growth.

Tissue-specific expression patterns of SlPSK genes

The *SlPSK* family genes exhibited varying expression levels across different tissues, with distinct expression patterns (Fig. 4). Among the eight homologs, *SlPSK2* (Solyc11g066880), *SlPSK3L* (Solyc02g092120), and *SlPSK6* (Solyc06g074540) were highly expressed in roots. *SlPSK3L* (Solyc02g092120) and *SlPSK5* (Solyc10g083580) showed high expression during fruit development at the breaker stage. *SlPSK1* (Solyc09g009130), *SlPSK6* (Solyc06g074540), and *SlPSK7* (Solyc04g077580) were highly expressed in floral buds; however, *SlPSK1* and *SlPSK6* also showed relatively high expression

in leaves. Notably, only *SlPSK7* exhibited specifically high expression in floral buds, suggesting a potential role in promoting fruit set.

Expression profiling of SlPSK family genes during tomato fruit set based on transcriptome data

Previous studies have shown that auxin is the primary hormone regulating parthenocarpic fruit set in tomato 'R35-P' and that the critical period determining whether flowers abscise or proceed to fruit set occurs before anthesis [1]. To explore the role of *SlPSK* genes in tomato fruit set, we analyzed transcriptome data from the parthenocarpic line 'R35-P' and its non-parthenocarpic sister line 'R35-N' during fruit set. As shown in Fig. 5, at the T1 stage, all SlPSK genes except *SlPSK2* (Solyc11g066880) showed significant expression differences between the parthenocarpic and non-parthenocarpic lines. *SlPSK5* showed significant differences across all three stages (T1, T2, and T3). Notably, *SlPSK7* (Solyc04g077580) was the only gene that showed significant differences at both the T1 and T2 stages but not at T3. Combined with its tissue-specific expression pattern in floral buds, *SlPSK7* was selected for further functional investigation.

Response relationship between SlPSK7 and auxin signaling

To verify the response pattern of *SlPSK7* to auxin, we performed exogenous hormone regulator treatments and transient expression assays to clarify the relationship between *SlPSK7* and auxin signaling.

Effects of exogenous hormone regulators on SlPSK7 and auxin-related gene expression

Previous studies have indicated that auxin acts as a positive regulator of parthenocarpic fruit set, whereas ethylene generally plays a negative regulatory role, with an antagonistic relationship between the two hormones [18]. To investigate the hormone response characteristics of *SlPSK7* during tomato fruit set, we treated pedicels with the synthetic auxin analog 2,4-D, the auxin transport inhibitor NPA, and the ethylene biosynthesis inhibitor AVG from 2 days before anthesis to 2 days after anthesis. Floral buds were collected at anthesis (0 d) and 3 days after anthesis (3 d) for qRT-PCR analysis of *SlPSK7* and auxin-related genes.

ARF5, *ARF7*, *ARF8A*, *ARF8B*, and *IAA9* are key genes in the auxin signaling pathway that play important roles in tomato fruit set regulation [15, 19]. *ARF5*, *ARF7*, *ARF8A*, and *ARF8B* are core members of the auxin response factor (ARF) family that mediate auxin signal transduction and directly regulate the expression of ovary development-related genes, functioning as important positive regulators of parthenocarpy. *IAA9* is an early auxin-responsive gene encoding an Aux/IAA repressor protein that negatively regulates the transcriptional activity of ARF family genes; its silencing or downregulation is a prerequisite for parthenocarpy in tomato [19].

As shown in Fig. 6, both 2,4-D and AVG treatments significantly induced the expression of ARF family genes and *SlPSK7* while suppressing *IAA9* expression, with 2,4-D showing a stronger effect than AVG. Conversely, NPA treatment showed the opposite trend. These results indicate that elevating auxin levels

(2,4-D treatment) or reducing ethylene levels (AVG treatment) in tomato ovaries significantly induces *SlPSK7* expression, whereas reducing auxin levels (NPA treatment) significantly suppresses it, indicating a positive correlation between *SlPSK7* expression and auxin levels in tomato ovaries.

Effects of transient SlPSK7 expression on auxin-related gene expression

In the parthenocarpic tomato line 'R35-P', auxin pathway and fruit set-regulating genes are actively expressed at 2 days before anthesis. At this stage, direct injection of expression vectors into the target tissue enables precise intervention in target gene expression.

To investigate the regulatory relationship between *SlPSK7* and the auxin signaling pathway, we constructed transient overexpression and silencing vectors for *SlPSK7* and delivered them into tomato floral buds at 2 days before anthesis (-2 d) via ovary injection. Ovary samples were collected at -2 d (injected in the morning and sampled in the afternoon), 0 d (anthesis), and 3 d (post-anthesis), covering the entire dynamic regulatory process from treatment to fruit set. qRT-PCR was performed to detect the expression levels of *SlPSK7* and auxin-related genes (*IAA9*, *ARF5*, *ARF7*, *ARF8A*, and *ARF8B*).

As shown in Fig. 7, at all three time points, *SlPSK7* expression in the overexpression group was significantly higher than that in the empty vector control, whereas expression in the silencing group was significantly lower than that in its corresponding control, indicating that the transient transformation system achieved stable and effective *SlPSK7* overexpression and silencing.

The four ARF family genes showed highly consistent expression patterns. In the *SlPSK7* overexpression group, all four ARF genes showed higher expression levels than the control at all three time points, with some differences reaching statistical significance; ARF7 showed the most pronounced response, reaching up to 5.85-fold at anthesis (0 d) ($P < 0.01$). In the *SlPSK7* silencing group, all four ARF genes were significantly downregulated compared with the control, with *ARF7* again showing the most pronounced response (to 0.34-fold, $P < 0.05$). These results are consistent with the exogenous hormone regulator experiments, supporting a positive regulatory role for *SlPSK7* in the auxin signaling pathway during tomato fruit set.

However, the expression pattern of *IAA9* was contrary to expectations and inconsistent with the results of the exogenous hormone regulator experiments: in both the *SlPSK7* overexpression and silencing groups, *IAA9* expression changed in the same direction as that of the ARF family genes (Fig. 7); possible explanations for this unexpected pattern are discussed below.

Discussion

Evolutionary conservation and functional diversity of the tomato SlPSK gene family

In this study, we identified eight *SlPSK* family members in tomato through genome-wide identification. Phylogenetic and synteny analyses revealed that tomato *SlPSK* genes share the highest synteny and

homology with those of potato, a fellow dicotyledon, indicating that this family was established early in plant evolution and has maintained a relatively stable gene structure.

Cis-acting element analysis revealed that the promoter regions of *SlPSK* family genes are enriched with various hormone-responsive elements. Notably, the ABA response element ABRE was present in all members, consistent with previous reports of PSK involvement in abiotic stress responses [3, 7]. In addition, auxin response elements (TGA-element) were identified in three *SlPSK* genes, providing molecular evidence for cross-regulation between PSK and auxin signaling pathways. Of particular interest, *SlPSK7* was the only member containing the O2-site element associated with endosperm-specific/seed storage protein regulation. Combined with its floral bud-specific high expression, this suggests a specialized role for *SlPSK7* in tomato reproductive development and fruit set.

Positive regulatory relationship between SlPSK7 and auxin signaling

Through exogenous hormone regulator treatments and transient expression assays, we systematically investigated the association between *SlPSK7* and the auxin signaling pathway. Exogenous hormone treatment experiments showed that 2,4-D and AVG significantly induced the expression of *SlPSK7* and ARF family genes, whereas NPA suppressed their expression. This pattern is consistent with the characteristics of positive regulators of auxin signaling. Transient overexpression and silencing experiments further indicated that changes in *SlPSK7* expression significantly affect the expression of ARF family genes, with *ARF7* being the most sensitive responder. Collectively, these results support a positive regulatory role for *SlPSK7* in the auxin signaling pathway and suggest that *SlPSK7 participates in* tomato fruit set, at least in part, by modulating the expression of ARF family genes. However, because the present evidence is based on gene expression correlations, causal relationships—for example, direct regulation of ARF gene expression by *SlPSK7* or protein-level interactions—remain to be established in future studies using stable transgenic lines and phenotypic analyses.

The unexpected response pattern of *IAA9* in the transient expression experiments merits discussion. In the classical auxin signaling model, Aux/IAA proteins function as transcriptional repressors of ARFs, and their expression is typically negatively regulated by auxin [19]. However, in this study, *SlPSK7* overexpression led to *IAA9* upregulation, whereas silencing led to *IAA9* downregulation, a pattern similar to that of ARF family genes. We speculate that a feedback balance mechanism involving "coordinated induction of negative regulators" may exist within the auxin pathway. Specifically, when *SlPSK7* and ARF family genes are upregulated to initiate the fruit set program, the negative regulator *IAA9* is simultaneously induced to prevent excessive activation of auxin signaling, which could otherwise lead to abnormal fruit set (e.g., fruit malformation). Alternatively, this discrepancy may reflect differences between pharmacological (exogenous auxin) and genetic (transient *SlPSK7* perturbation) interventions, tissue- and cell-type-specific effects of the injection-based transformation, or feedback regulation operating over different timescales. Direct measurement of *IAA9* protein abundance and endogenous auxin content would help to distinguish these possibilities; this point warrants further investigation.

Multifaceted roles of PSK in plant growth and potential applications

As a class of plant peptide hormones, PSK plays important regulatory roles in multiple biological processes, including cell proliferation, organ development, and stress responses [7]. The positive regulatory relationship between *SlPSK7* and auxin signaling identified in this study provides new evidence for the involvement of PSK in fruit set regulation. Previous studies have shown that PSK promotes seed vigor in rice [9] and grain development in wheat [11]. Our findings further suggest that *SlPSK7* may function as a positive regulator during tomato fruit set.

As a class of small-molecule peptides, PSK offers potential advantages including low dosage requirements and environmental friendliness, making it promising for agricultural applications [6]. The synergistic regulatory relationship between *SlPSK7* and auxin identified in this study provides new insights for regulating tomato fruit set and improving yield through genetic engineering or exogenous PSK application.

Limitations and future perspectives

Several limitations of this study should be acknowledged. First, PSK is produced from precursor proteins through proteolytic processing and tyrosine sulfation; thus, SlPSK transcript levels may not directly reflect the abundance of the mature active peptide, and the present conclusions are based on transcriptional responses. Second, the tissue-specific expression patterns were derived from the Tomato eFP Browser, and qRT-PCR validation in the experimental line 'R35-P' is warranted. Third, the relationship between SlPSK7 and auxin signaling was inferred from expression correlations in transient assays; phenotypic analyses (e.g., fruit set rate and ovary development) and stable transgenic lines are required to establish causality. Fourth, whether the absence of potato syntenic counterparts of SlPSK7 reflects gene loss or rapid sequence divergence could be resolved by TBLASTN searches against the potato genome. Finally, the unexpected co-regulation of IAA9 with ARF family genes in the transient expression assays was not fully resolved at the mechanistic level; direct measurement of IAA9 protein abundance and endogenous auxin content would help to clarify the underlying mechanism. Addressing these points will help to clarify the precise role of SlPSK7 in tomato fruit set.

Conclusions

This study systematically identified and characterized the tomato *SlPSK* gene family, identifying eight members distributed across seven chromosomes. Through comprehensive bioinformatics analysis, including phylogenetic reconstruction, synteny analysis, cis-acting element characterization, and tissue expression profiling, we established a foundational understanding of the *SlPSK* family. Functional investigation of *SlPSK7*, which showed floral bud-specific expression, suggested that this gene positively regulates auxin signaling, with its expression pattern closely aligned with that of ARF family genes. Exogenous hormone treatments and transient expression assays consistently supported a positive association between *SlPSK7* and the auxin signaling pathway. These findings provide a theoretical basis

for further functional characterization of *SlPSK7* and for elucidating the molecular mechanisms underlying parthenocarpic fruit set in tomato.

Methods

Plant materials and growth conditions

The tomato (*Solanum lycopersicum* L.) parthenocarpic inbred line 'R35-P' and its non-parthenocarpic sister line 'R35-N' were used in this study. Both lines were isolated from the parthenocarpic tomato line 'R35' [1] by selfing and selection at the College of Horticulture, Ludong University, Yantai, China. Seeds were germinated and sown in pots under standard greenhouse conditions.

For transcriptome analysis, the stigmas of 'R35-P' flower buds were removed two days before anthesis to prevent self-pollination, and the emasculated flowers were denoted Pe; 'R35-P' flowers self-pollinated at anthesis were denoted Ps; and 'R35-N' flowers self-pollinated at anthesis were denoted CK. Ovaries were collected at three stages of fruit set (-2 d, 0 d, and 3 d after anthesis, as defined in [1]), frozen in liquid nitrogen, and stored at -80°C.

For exogenous hormone treatments, pedicels were treated with auxin regulators 2 days before anthesis. For transient silencing and overexpression assays, *Agrobacterium tumefaciens* suspensions were injected into ovaries. Total RNA was extracted from developing ovaries at different stages, reverse-transcribed into cDNA, and analyzed by qRT-PCR.

No specific permissions were required for the collection of plant materials in this study, as all materials were obtained from cultivated greenhouse-grown plants. The lines were formally identified by the authors at the College of Horticulture, Ludong University; voucher specimens were not deposited because the materials are cultivated inbred lines maintained by the research group, and seed stocks are available from the corresponding author upon request. All experimental procedures complied with institutional, national, and international guidelines. As all plant materials were cultivated greenhouse-grown plants, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) is not applicable to this study.

Identification of PSK gene family members in tomato

Arabidopsis thaliana PSK protein sequences were obtained from the TAIR database (https://www.arabidopsis.org/). A hidden Markov model (HMM) for PSK proteins was constructed using HMMbuild in HMMER 3.0. Tomato protein sequences and genomic data were obtained from the Ensembl Plants database. The HMM was used to search the tomato protein database via HMMsearch. Retrieved protein sequences were further validated using Pfam and NCBI Batch CD-Search to exclude sequences lacking complete PSK conserved domains.

Protein physicochemical properties and chromosomal localization

Physicochemical properties of the *SlPSK* proteins were analyzed using the ExPASy tool. Chromosomal location information was extracted from the tomato genome GFF3 annotation file, and genes were mapped to chromosomes using TBtools (Gene Location Visual function).

Phylogenetic and synteny analysis

Phylogenetic trees were constructed using the neighbor-joining method with 1,000 bootstrap replicates in MEGA software, using PSK protein sequences from tomato, potato, *Arabidopsis thaliana*, and rice. Multiple sequence alignments were performed, and the phylogenetic tree was visualized using iTOL (https://itol.embl.de/). Synteny analysis of PSK family genes among tomato, potato, *Arabidopsis thaliana*, and rice was performed and visualized using TBtools.

Cis-acting element analysis

The 2000-bp sequences upstream of the translation start sites of *SlPSK* family genes were extracted using TBtools. Cis-acting elements were identified using the PlantCARE database and visualized using TBtools (Basic BioSequence Viewer).

Tissue expression pattern analysis

Tissue expression patterns of *SlPSK* family genes were analyzed using the Tomato eFP Browser (http://bar.utoronto.ca/efp_tomato/cgi-bin/efpWeb.cgi).

Transcriptome-based expression analysis during fruit set

Transcriptome data from our previous study [1] were analyzed using FPKM values as indicators of gene expression levels. Expression levels of *SlPSK* family genes were compared between the parthenocarpic line 'R35-P' and the non-parthenocarpic control line 'R35-N' at three stages of fruit set (T1, T2, and T3, corresponding to 2 days before anthesis, anthesis, and 3 days after anthesis, respectively, as defined in [1]). For 'R35-P', flowers were either emasculated by stigma removal (Pe) or pollinated (Ps) at anthesis, and pollinated 'R35-N' flowers served as the non-parthenocarpic control (CK).

Exogenous hormone regulator treatments

The ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG), the auxin transport inhibitor N-1-naphthylphthalamic acid (NPA), and the synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) were used for treatments, with water as the control. Treatments were applied to pedicels with a brush once daily in the morning from 2 days before anthesis (-2 d) to 2 days after anthesis. AVG and NPA were prepared at a final concentration of 0.3 μg/μL and 2,4-D at 0.2 μg/μL; these concentrations were selected on the basis of preliminary dose-response experiments, and no visible phytotoxicity was observed. Emasculation was performed at anthesis (0 d). Floral buds were collected at 0 d and 3 d (three plants pooled per sample), flash-frozen in liquid nitrogen, and stored at -80°C for subsequent qRT-PCR analysis.

Construction of SlPSK7 transient silencing and overexpression vectors and injection treatment

Target fragments for transient silencing of *SlPSK7* (forward primer: 5'-CGGGATCCATGTCTAAATTGTGCACT-3'; reverse primer: 5'-CGAGCTCTGAGCAGCCATTGTCCTCCT-3') and transient overexpression (forward primer: 5'-GGAATTCATGTCTAAATTGTGCAC-3'; reverse primer: 5'-CGAGCTCTCATGGATGATTGTTAT-3') were cloned. The fragments were ligated into the pTRV2-based virus-induced gene silencing (VIGS) vector and the overexpression vector pSuper1300-GFP, respectively, and transformed into *Agrobacterium tumefaciens* strain LBA4404. Because the bacterial suspension was injected directly into ovaries, the silencing effect was expected to be local rather than systemic.

Two days before anthesis, young ovaries of floral buds were injected with the bacterial suspension (empty vector as control). Inoculated plants were kept in darkness for 24 h and then transferred to standard growth conditions (25–28°C, 16 h light/8 h dark). Ovary samples were collected on the day of injection (-2 d; injected in the morning and sampled in the afternoon), at anthesis (0 d), and 3 days after anthesis (3 d), flash-frozen in liquid nitrogen, and stored at -80°C for subsequent qRT-PCR analysis.

RNA extraction and qRT-PCR analysis

Total RNA was extracted from developing tomato ovaries using TRIzol reagent. Reverse transcription and qRT-PCR were performed using the Takara PrimeScript™ RT Master Mix (RR092A) with three biological replicates. The tomato *Actin* gene was used as the internal reference, and relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method [20]. The primers used for qRT-PCR are listed in Table 2.

Table 2
Primer sequences used for qRT-PCR analysis

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
Actin	GAGAGAAAGCAAGTCGGCCA	CCAGCAAATCCTGCCTTAACC
SlPSK7	CTCTCACATTTGCTGTTCGTCC	AGCAGCCATTGTCCTCCTCT
ARF5	AGAGTGGGATGAGCCTGGAT	TCCCACTCAGTTTGTGCTCC
ARF7	CCAAGTTATCCTAATCTTCCTTCC	GTAAAGCCTCCTGGTCATATTTG
ARF8A	GGATGTGCAGAAACTGGGGA	AGAACGCCAAGCTCTTGTGA
ARF8B	GTGCAGTTCGACCACAGACT	GGCTAGCCCTTGGGTTAAAA
IAA9	GAGAGGCCTTCTGCTGTGAA	CAACCAACAACCTGTGCCTT
qRT-PCR, quantitative real-time PCR.		

Statistical analysis

All qRT-PCR data are presented as mean ± SD from three biological replicates. Relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method with Actin as the internal reference; the SD for each group was calculated from the ΔCt values of the three biological replicates and is presented on the relative-

expression scale. For each gene and time point, differences between treatment and control groups were assessed by Student's t-test (*P < 0.05, **P < 0.01, ***P < 0.001). Because the analyses were hypothesis-driven and focused on predefined candidate genes, P values are reported without correction for multiple testing; the results should therefore be interpreted with appropriate caution.

Abbreviations

PSK
Phytosulfokine
ARF
Auxin response factor
2,4-D
2,4-Dichlorophenoxyacetic acid
AVG
Aminoethoxyvinylglycine
NPA
N-1-Naphthylphthalamic acid
VIGS
Virus-induced gene silencing
qRT-PCR
Quantitative real-time PCR
ABA
Abscisic acid
MeJA
Methyl jasmonate
SA
Salicylic acid
FPKM
Fragments per kilobase of transcript per million mapped reads
HMM
Hidden Markov model
MW
Molecular weight
pI
Isoelectric point
SD
Standard deviation.

Declarations

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

Not applicable.

Competing interests:

The authors declare that they have no competing interests.

Authors' contributions

Zhang Shaoli and Xie Lu contributed equally to this work (co-first authors). Xie Lu and Song Yuanyuan performed the experiments. Li Haidong, Yi Pengcheng and Yang Shude performed the data analysis. Li Haidong and Zhu Luying participated in the experiments and data collection. Zhang Shaoli contributed to material preparation. Zhang Shaoli and Pan Kai conceived and designed the study, supervised the research. Zhang Shaoli, Xie Lu and Wang Guiyun wrote the manuscript, and Wang Guiyun translated the manuscript. All authors read and approved the final manuscript.

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Author Contribution

Xie Lu and Zhang Shaoli contributed equally to this work (co-first authors). Xie Lu and Song Yuanyuan performed the experiments. Li Haidong, Yi Pengcheng and Yang Shude performed the data analysis. Li Haidong and Zhu Luying participated in the experiments and data collection. Zhang Shaoli contributed to material preparation. Zhang Shaoli and Pan Kai conceived and designed the study, supervised the research. Zhang Shaoli, Xie Lu and Wang Guiyun wrote the manuscript, and Wang Guiyun translated the manuscript. All authors read and approved the final manuscript.

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Not applicable.

Availability of data and materials:

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. Genome and protein sequence data were obtained from public databases (TAIR and Ensembl Plants). The transcriptome datasets analyzed in this study are from our previous publication [1].

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Figures

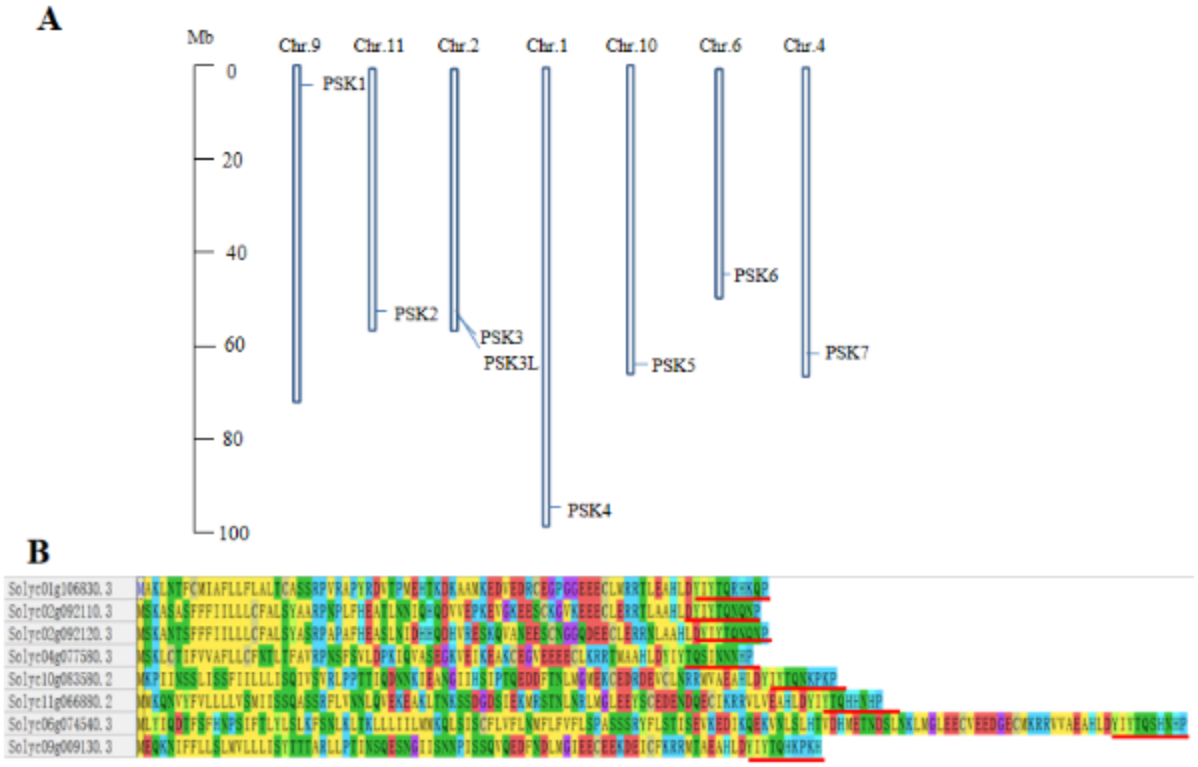


Figure 1

Chromosomal distribution and amino acid sequence analysis of the PSK family genes in tomato. **A** Chromosomal localization of the eight *SlPSK* genes. **B** Multiple sequence alignment of the *SlPSK*proteins; the conserved PSK domain is indicated by the red line. PSK, phytosulfokine.

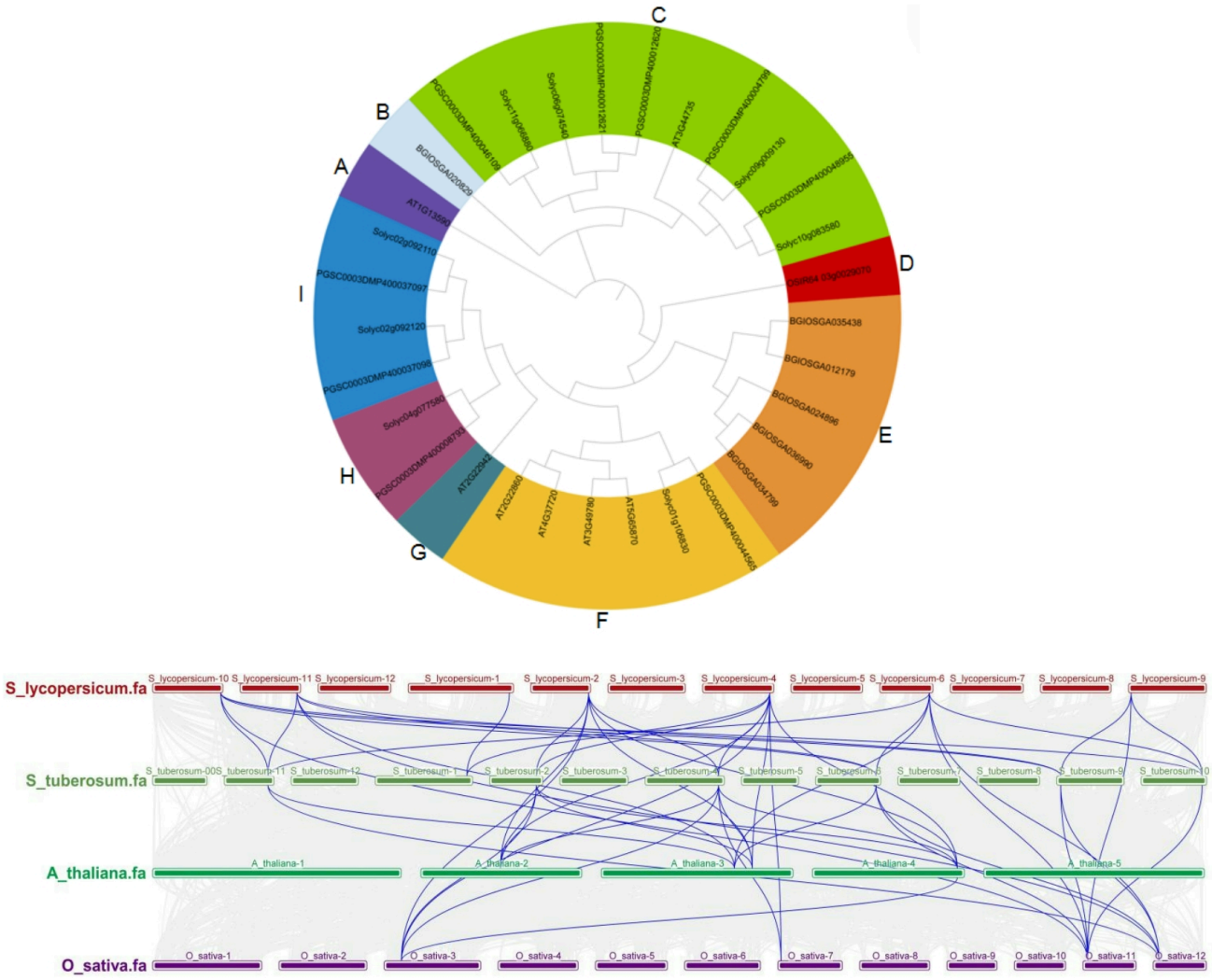


Figure 2

Phylogenetic and synteny (collinearity) analysis of the PSK gene family. **A**Phylogenetic tree of 36 PSK proteins from tomato (8), potato (9), *Arabidopsis thaliana* (7), and rice (12), constructed with MEGA; the nine groups are indicated. **B** Synteny analysis of the PSK genes among tomato, potato, *Arabidopsis thaliana*, and rice. PSK, phytosulfokine.

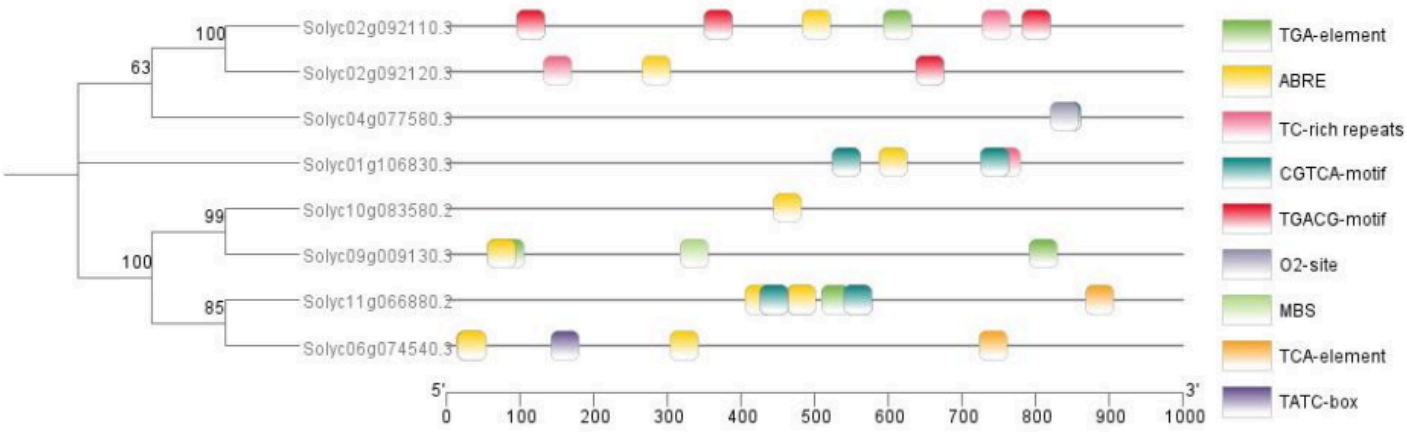


Figure 3

Locations of cis-acting elements in the promoters of the *SlPSK* genes. The 2000-bp sequences upstream of the translation start sites of the eight *SlPSK* genes were analyzed using PlantCARE. Different cis-acting elements are indicated by colored boxes. ABA, abscisic acid; MeJA, methyl jasmonate; SA, salicylic acid; PSK, phytosulfokine.

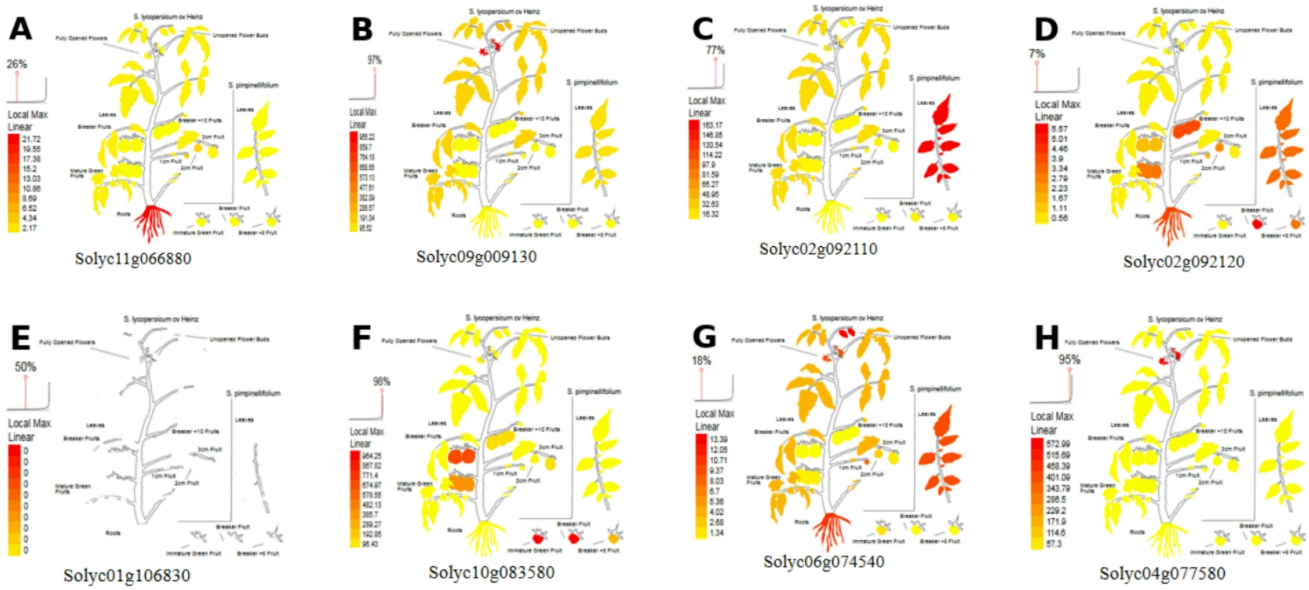


Figure 4

Tissue-specific expression patterns of the *SlPSK* genes in tomato. Expression data were obtained from the Tomato eFP Browser. Panels A–H show the expression of the eight *SlPSK* genes in different tissues; panel labels were added to the original database screenshots for clarity (see Table 1 for the corresponding gene list). PSK, phytosulfokine.

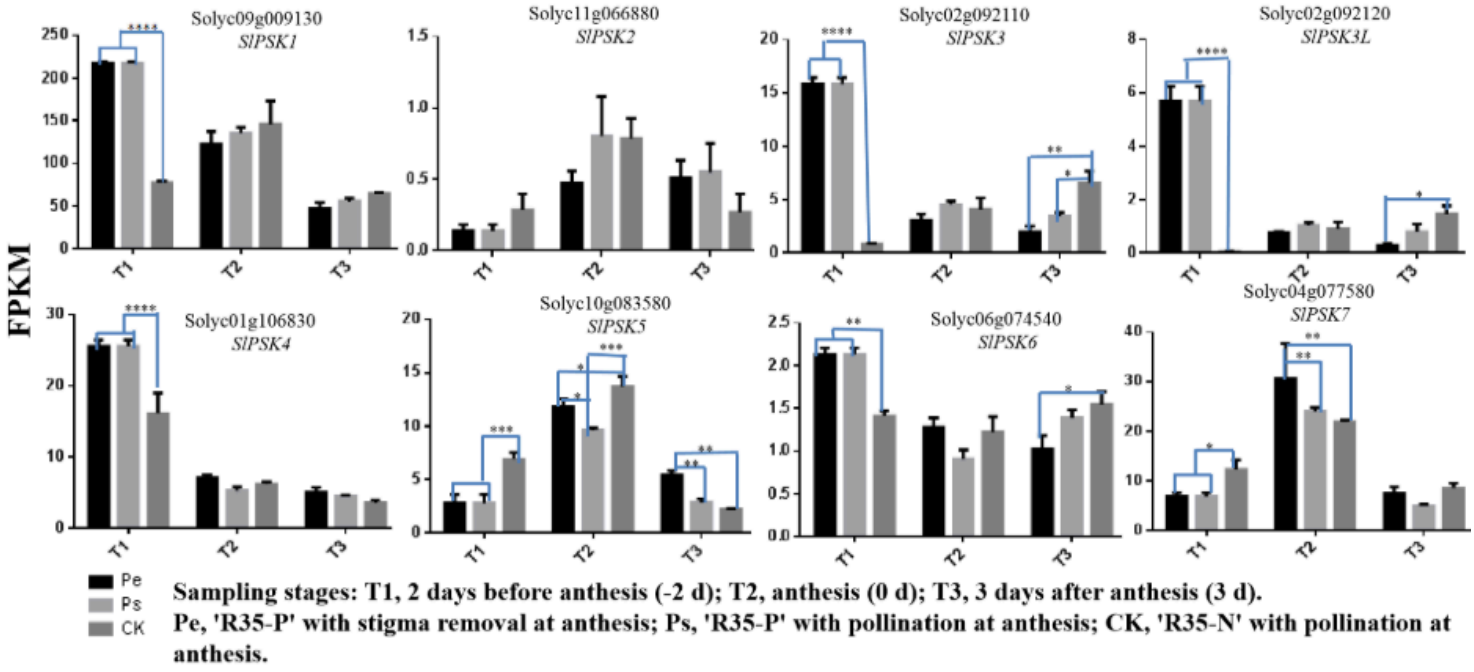


Figure 5

Expression levels of the *SlPSK* gene family during tomato fruit set based on transcriptome data. Expression levels of the eight *SlPSK* genes in the parthenocarpic line 'R35-P' and the non-parthenocarpic line 'R35-N' at three stages of fruit set (T1: 2 days before anthesis; T2: anthesis; T3: 3 days after anthesis, as defined in [1]). Pe, 'R35-P' with stigma removal at anthesis; Ps, 'R35-P' with pollination at anthesis; CK, 'R35-N' with pollination at anthesis. *P < 0.05; **P < 0.01; ***P < 0.001 (Student's t-test). PSK, phytosulfokine.

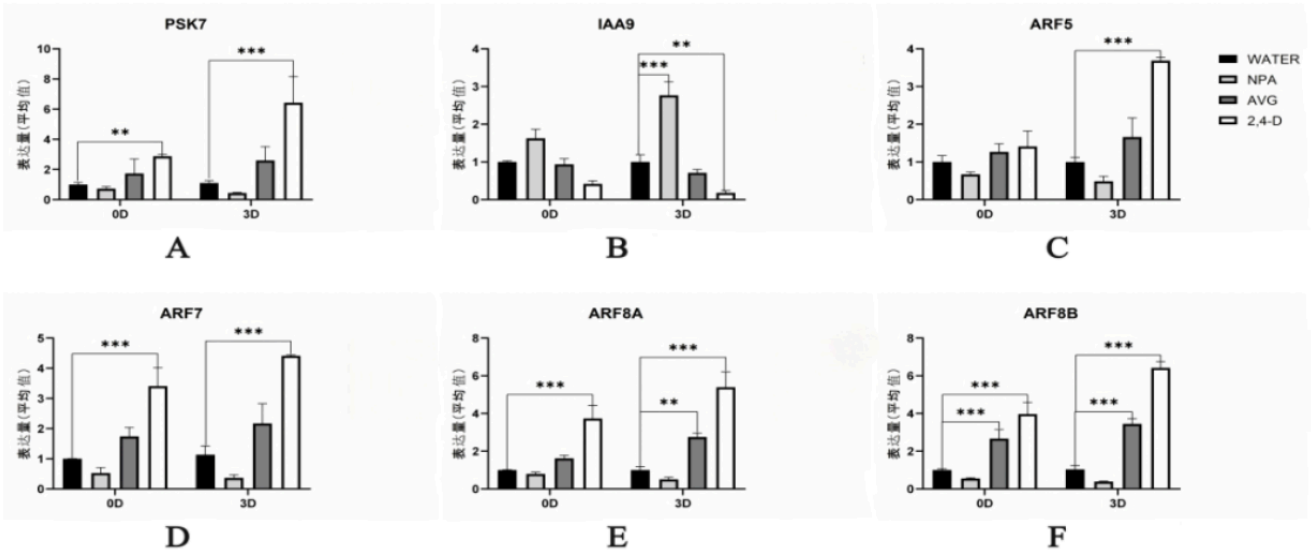


Figure 6

Expression levels of *SlPSK7* and auxin-related genes under exogenous hormone treatments. **A** *SlPSK7*; **B** *IAA9*; **C** *ARF5*; **D** *ARF7*; **E** *ARF8A*; **F** *ARF8B*. Floral buds of 'R35-P' were collected at anthesis (0 d) and 3 days after anthesis (3 d) after treatment with 2,4-D (0.2 μg/μL), AVG (0.3 μg/μL), NPA (0.3 μg/μL), or water (control). Data are presented as mean ± SD of three biological replicates. *P < 0.05; **P < 0.01; ***P < 0.001 (Student's t-test). 2,4-D, 2,4-dichlorophenoxyacetic acid; AVG, aminoethoxyvinylglycine; NPA, N-1-naphthylphthalamic acid; qRT-PCR, quantitative real-time PCR; SD, standard deviation.

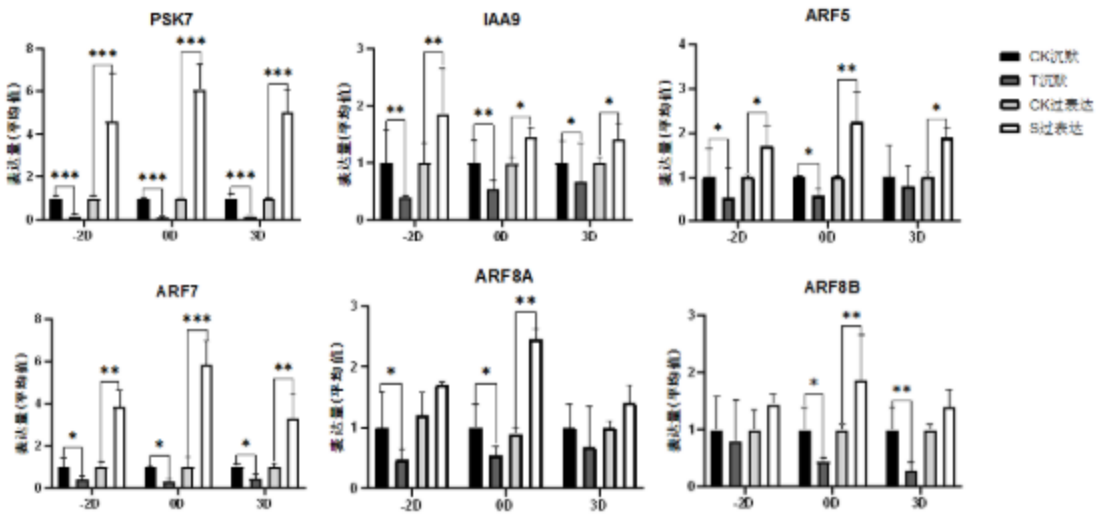


Figure 7

Expression levels of auxin-related genes after transient overexpression and silencing of *SlPSK7* in tomato ovaries. CK-silencing and CK-overexpression represent the corresponding empty vector controls; T-silencing represents *SlPSK7* silencing (VIGS); S-overexpression represents *SlPSK7*overexpression. Ovary samples were collected at -2 d, 0 d, and 3 d. Data are presented as mean ± SD of three biological replicates. *P < 0.05; **P < 0.01; ***P < 0.001 (Student's t-test). VIGS, virus-induced gene silencing; SD, standard deviation.