Overexpression of Sesame Polyketide Synthase A Leads to Abnormal Pollen Development in *Arabidopsis*

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Abstract

Background: Sesame is a great reservoir of bioactive constituents and unique antioxidant components and is widely used for its nutritional and medicinal value. The expanding demands for sesame seeds are putting pressure on sesame breeders to develop reliable high-yielding varieties. Heterosis utilization is an efficient way to increase sesame yield. Polyketide synthases (PKSs) are critical enzymes in the biosynthesis of sporopollenin, a primary component of pollen exine. Their in planta functions are being investigated for application in crop breeding.

Results: In this study, we cloned the sesame POLYKETIDE SYNTHASE A (*SiPKSA*) and examined its function in male sterility. *SiPKSA* was specifically expressed in sesame flower buds, and its expression was significantly higher in sterile sesame anthers than in fertile anthers at the tetrad and microspore development stage. Further overexpression of *SiPKSA* in *Arabidopsis* caused transgenic plants male sterile. Ultrastructural observation showed that the pollen grains of *SiPKSA*-overexpressing plants contained few cytoplasmic inclusions and exhibited an abnormal pollen wall structure, with a thicker exine layer compared with wild type. In agreement with it, the expression of a set of sporopollenin biosynthesis-related genes and the contents of fatty acids and phenolics were significantly altered in anthers of *SiPKSA*-overexpressing plants compared with wild type during anther development.

Conclusion: These findings highlighted that overexpression of *SiPKSA* in *Arabidopsis* might cause excessive sporopollenin biosynthesis to influence pollen and pollen wall development, leading to male sterile, suggesting that its manipulation might improve hybrid breeding in sesame and other crop species.

Background

Sesame (*Sesamum indicum* L.) is one of the most ancient oil crops and is widely used for its high-quality products. Its seeds are a great reservoir of oil, protein, unsaturated fatty acids, lignans, vitamins, and minerals [1, 2]. Many studies revealed that sesame lignans were associated with various health-promoting effects against lifestyle diseases [3–6]. Accordingly, the sesame seeds market is expanding, giving a big pressure to sesame breeders to develop environment stable and high-yielding varieties. Hybrid breeding strategy based on male sterile lines is one of the most reliable techniques to increase crop yield. Therefore, deciphering the molecular mechanism of fertility-related genes in sesame might provide genetic resources and new ideas for its utilization of heterosis.

Pollen is a critical organ in angiosperms plants reproduction. Male reproductive organ development is a complex biological process that consists of various biosynthesis events, among which pollen wall formation is essential for pollen viability and fertility [7, 8]. Any defects during the pollen wall formation may lead to abnormal pollen and further male sterility [9]. The pollen wall is an important structure of the outer surface of pollen. It protects microspores against the harm of harsh environmental conditions [10], and is essential for species-specific pollen-stigma recognition [11]. It is principally comprised of three layers: the pollen coat, the outer exine, and the inner intine. The pollen exine patterning mainly includes

three developmental events: primexine formation, callose wall formation, and sporopollenin synthesis [7, 10, 12]. Sporopollenin, a complex of fatty acids and phenolic compounds, represents the principal component that constitutes the pollen exine architecture [13].

Studies in *Arabidopsis* revealed that the sporopollenin biosynthesis occurs in tapetal secretory cells after the temporary callose wall surrounding tetrads is degraded, and the primexine is deposited [14]. Other studies in rice, wheat, and barley showed that pollen development in all plant species involves identical key genes, stages and conserves similar regulatory pathways [7, 15–18]. Among genes that contribute to vibrant and fertile pollen formation, polyketide synthases are the key ones [15, 16]. The *Arabidopsis* *pkas/lap6* or *pkasb/lap5* mutant produces abnormal pollen exine, and the double mutant produced collapsed pollen without exine deposition and was male sterile [19, 20]. Kim et al. demonstrated that *Arabidopsis* *POLYKETIDE SYNTHASE A/PKSA* and *PKSB* (also known as *LAP6* and *LAP5*, respectively) interacted continuously with other pollen exine biosynthesis genes [19]. The integration of the increasing genomic information and advanced genetic tools is facilitating the transfer of information from *Arabidopsis* to other plants, including *Physcomitrella patens*, rice, and rapeseed, in which the orthologues of PKSA have been identified and characterized for application in hybrid breeding [21–23].

In sesame, although early studies reported strong heterosis [24–26], few nuclear male sterile lines have been used in hybrid breeding [27, 28]. Studies on sesame male sterility were limited to the identification of two genes, *Sesamum indicum* male sterility 1 (*SiMs1*) [29] and *Sesamum indicum* cell wall invertase 1 (*Sicwinv1*) [30]. In the present study, the sesame *polyketide synthase A* (*SiPKSA*) was cloned and functionally characterized to involve in male reproduction. Our results suggested that *SiPKSA* might be involved in sporopollenin biosynthesis to affect pollen and pollen wall formation.

Results

Clone and sequence analysis of SiPKSA

Sesame polyketide synthase, the orthologue of *Arabidopsis* *PKSA*, designated *SiPKSA* (*Sesamum indicum* Polyketide Synthase A), was cloned using the cDNA of sesame anthers at the tetrad stage as the template. The open reading frame (ORF) of *SiPKSA* was 1191 bp, encoding 396 amino acids, with an isoelectric point of 6.41, a fat index of 95.33, and a molecular weight of 43.6 kDa.

Multiple sequence alignment of *SiPKSA* with other PKSA proteins reported in *Arabidopsis* (*Arabidopsis thaliana*), rice (*Oryza sativa*), and rape (*Brassica napus*) showed that *SiPKSA* contained typical chalcone/stilbene synthase N-terminal domain (25–242 bp), C-terminal domain (252–396 bp) and various conserved active sites, such as polypeptide binding site, malonyl-CoA binding site, and product binding site (Fig. 1A). As well, it contained catalytic triad Cys-His-Asn, which is common and important in the PKSs family (Fig. 1A). Phylogenetic analysis further confirmed that *SiPKSA* clustered together with PKSA proteins in other species (Fig. 1B), confirming that it encoded a type III polyketide synthase A protein.

In addition, the promoter region of the *SiPKSA* was analyzed using the PLACE and PLANTCARE databases. It was found that a large number of formal elements were annotated as MYB recognition sites, light-responsive elements, and auxin-responsive elements. Notably, multiple copies of AGAAA, which was considered as an anther- and/or pollen-specific cis-regulatory motif, were predicted in the promoter region of *SiPKSA*. Other motifs, such as Q-element and GTGA-motif, related to pollen development were also predicted in the promoter region (Table S2). These results indicated that *SiPKSA* was a type III polyketide synthase A protein and might be involved in pollen development in sesame.

***SiPKSA* is predominantly expressed in reproductive tissue**

The expression profile of *SiPKSA* in different tissues was analyzed. It was shown that *SiPKSA* was especially expressed in flower buds (Fig. 2A and 2B), indicating its function in sesame might be related to plant reproduction. Further, to determine the potential role of *SiPKSA* in sesame male sterility, we compared the expression pattern of *SiPKSA* in sterile and fertile sesame anthers at different developmental stages. We observed that the expression of *SiPKSA* was significantly higher in sterile sesame anthers at the tetrad stage and microspore development stage compared with the fertile anthers (Fig. 2C and 2D), suggesting that *SiPKSA* might be related to sesame male fertility.

Overexpressing *SiPKSA* in *Arabidopsis* caused transgenic plants male sterile

To further assess the in vivo function of *SiPKSA* in the anther development, *SiPKSA* was overexpressed in *Arabidopsis*. Several T3 generation transgenic plants were harvested, and two homozygous lines, in which *SiPKSA* expression was highly overexpressed, were selected for phenotypic and functional analysis (Fig. 3A). The expression level of the *Arabidopsis* homologous gene *AtPKSA* was also detected. The results showed that there were no significant differences among *SiPKSA*-overexpressing plants, wild type and plants transformed with empty vector in terms of the expression level of *AtPKSA* both in seedlings and anthers at different developmental stages (Fig. S1A-C), suggesting that the phenotype of transgenic plants in the present study was caused by *SiPKSA*.

As shown in Fig. 3B, the siliques of *SiPKSA*-overexpressing plants were flat and short compared with those of the wild type (WT) and plants transformed with empty vector (EV) (Fig. 3C-E). The acetocarmine staining showed that the pollen grains of WT and EV were plump and darkly stained (Fig. 3F and 3H). While, the pollen grains of *SiPKSA*-overexpressing plants were uncolored and smaller compared with the controls, indicating the pollen grains of *SiPKSA*-overexpressing plants were poor vigor (Fig. 3G). Scanning electron microscope (SEM) further confirmed that the WT pollen grains were normal and those of the *SiPKSA*-overexpressing plants were abnormal. The WT pollen grains were full with a typical sculpture surface (Fig. 3I and 3J). In contrast, the pollen grains of the *SiPKSA*-overexpressing plants were shrunken

and collapsed (Fig. 3K and 3L). These results indicated that *SiPKSA*-overexpressing plants were male sterile.

Abnormal pollen and pollen wall development in *SiPKSA*-overexpressing *Arabidopsis* plants

Transmission electron microscopy (TEM) observation was further performed to investigate the ultrastructure of pollen development. According to the cytological events, *Arabidopsis* anther development was characterized into 14 stages, and the microspores were differentiated into mature pollen during stages 9–12 [8]. Our results showed that, during stages 9–10, the tapetum of WT contained small vacuoles and abundant plastids filled with plastoglobuli (Fig. 4A). The microspores of WT were rich in inclusions and embedded with a regular primary structure of the exine (Fig. 4B). However, in the tapetal cells of *SiPKSA*-overexpressing plants, large cavities and less substance were observed (Fig. 4C), the microspores of *SiPKSA*-overexpressing plants were obviously abnormal, with few cytoplasmic components, and the sporopollenin particles were abundant accumulated on the microspore surface (Fig. 4D). During stage 11–12, the pollen of WT exhibited a typical normal pollen wall with obvious exine and intine layers (Fig. 4E). In contrast, the pollen grains of *SiPKSA*-overexpressing plants were seriously collapsed, with irregular exine and intine (Fig. 4F). Further measurement of the thickness of the pollen exine during the stages 9–10 and 11–12 showed that, compared with the WT, the exine layer of *SiPKSA*-overexpressing plants was significantly thicker than WT (Fig. 5A–E).

Overexpression of *SiPKSA* disrupted the expression of sporopollenin biosynthesis-related genes

To further explore whether sporopollenin biosynthesis was affected in anthers of *SiPKSA*-overexpressing plants, the expression of several sporopollenin synthesis-related genes was analyzed by qRT-PCR, including *AtMS2*, *AtCYP704B1*, *AtTKPR1*, and *AtTKPR2*. As shown in Fig. 6, comparing to WT, the expression of *AtMS2*, *AtCYP704B1*, *AtTKPR1* and *AtTKPR2* were up-regulated in anthers of *SiPKSA*-overexpressing plants during stage 6–8. During stages 9–10, there were no significant differences among *SiPKSA*-overexpressing plants, WT, and EV in terms of the expression of *AtMS2*, *AtCYP704B1*, *AtTKPR1*, except for *AtTKPR2*, which expression was also up-regulated. These results indicated that the expressions of sporopollenin biosynthesis-related genes were influenced by the high expression of *SiPKSA* in transgenic *Arabidopsis*.

Disrupted fatty acids metabolism occurred in anthers of *SiPKSA*-overexpressing plants

Pollen exine is mainly composed of sporopollenin, which was a complex biopolymer constituted of fatty acids, phenols, and their derivatives [38]. Therefore, the contents of fatty acids and their derivatives were detected by GC-MS analysis. The results showed that, in comparison with the WT, the content of several long-chain fatty acids such as C15:1, C16:0, ethyl-C16, C18:1, C22:1, and C22:2 changed greatly in anthers of *SiPKSA*-overexpressing plants during the anther development compared with the WT (Fig. 7A). In addition, three phenolic compounds (C14-OH, C15-OH, and C25-2OH) and one fatty alcohol (Phytol) were increased significantly at stages 6–8 in *SiPKSA*-overexpressing plants (Fig. 7B). The results indicated that over-expression of *SiPKSA* affected fatty acid metabolism in anthers of transgenic *Arabidopsis*.

Discussion

SiPKSA is a typical chalcone synthase with a similar function as its homologous

Polyketide compounds are usually produced under the action of polyketide synthases (PKSs). PKSs can be divided into three types (I, II, and III) according to the protein structure [39, 40]. Type III PKSs, also known as a chalcone synthase (CHS) superfamily, are responsible for the biosynthesis of monocyclic or bicyclic aromatics polyketides compounds [41]. These type III PKSs had a highly conserved catalytic active center (Cys164-His303-Asn336), and their active amino acid residues, generally Thr197, Phe215, Gly256, and Ser338, were located in the cavity of the active center [42]. While the active site and amino acid residues of different functions of type III PKSs are slightly different, so they will ultimately produce diverse products [43]. A series of type III PKSs with different functions have been characterized in plants, such as chalcone synthase (CHS), stilbene synthase (STS), acibone synthase (ACS), and 2-pyrone synthase (2-PS). It has been reported that plant-specific CHS-like type III PKSs *LAP6/PKSA* and *LAP5/PKSB* were specifically expressed in anthers and critical for pollen development, and their mutant shows thinner exine compared with the WT [20]. *OsPKS1*, a homolog of *LAP6/PKSA*, also has a similar function. The *OsPKS1* knockout mutant generated by the CRISPR/Cas9 genomic editing tool exhibited a complete male-sterile phenotype with the anthers failed to produce any viable pollen grains, and moreover, the mutant exhibited defective pollen sexine formation [16]. Consistent with it, a single-nucleotide polymorphism (SNP) mutation of the *OsPKS1* gene also showed the same phenotype of male sterility caused by defective pollen wall [17]. Interference expression of *NtPKSA* also caused disorganized pollen surface sculptures in tobacco [22].

In our study, the phylogenetic analysis results showed that *SiPKSA* was clustered together with PKSAs in other species and multiple alignment results showed that *SiPKSA* contained a typical chalcone synthase structure and possessed highly conserved catalytic active centers and active amino acid residues in consistency with *AtPKSA*, *BnPKSA* and *OsPKSA/OsPKS1* (Fig. 1). These findings indicated that *SiPKSA* was a type III PKSs and was homologous to other PKSAs, suggesting that *SiPKSA* might produce similar products and have similar functions with these PKSAs. In agreement with it, *SiPKSA* was specifically

expressed in sesame flower buds, and the expression of *SiPKSA* was higher in sesame sterile anthers than in the fertile anthers during tetrad release and microspores development stages (Fig. 2). Besides, Overexpression of *SiPKSA* in *Arabidopsis* caused transgenic plants male sterile. The pollen grains of *SiPKSA*-overexpressing plants were collapsed and embedded with a defective pollen wall (Fig. 4). Further TEM observation showed that the pollen exine of *SiPKSA*-overexpressing plants was thicker than that of wild-type plants (Fig. 5), consistent with the phenotypes in mutants of other enzymes involved in sporopollenin biosynthesis, which lack exine or have much thinner exine layers, suggesting that *SiPKSA* function similar as its homologs.

SiPKSA is required for proper pollen exine formation and might be involved in sporopollenin biosynthesis

The main component of pollen exine is sporopollenin. It was noteworthy that an ancient biochemical pathway for the biosynthesis of sporopollenin had been widely proposed [7, 9, 22], and *CYP704B1*, *MS2*, *TKPR1*, *TKPR2*, *PKSA/LAP6*, and *PKSB/LAP5*, were considered as the key enzymes involved in sporopollenin biosynthesis [19, 20, 44–48]. Mutants of these genes all showed defective or absent pollen exine, suggesting that down-regulation of these genes causes decreased sporopollenin biosynthesis. In our study, the pollen exine layer of *SiPKSA*-overexpressing plants was thicker compared with WT (Fig. 4, Fig. 5), suggesting that the sporopollenin might be abundantly accumulated on the surface of microspores of *SiPKSA*-overexpressing plants. This was supported by the expression analysis of sporopollenin synthesis-related genes, such as *MS2*, *TKPR1*, *TKPR2*, and *CYP704B1*, which were up-regulated in *SiPKSA*-overexpressing plants during stage 6–8 compared with the controls (Fig. 6). Similar phenotypes were observed in male sterile line 1335A of cotton, which was male sterile due to thicker nexine, and the up-regulation of the expression levels of sporopollenin synthesis-related genes caused excessive sporopollenin accumulation, finally lead to male sterility [49].

Many studies have demonstrated that sporopollenin is a complex biopolymer composed mainly of fatty acids, phenolic and aliphatic compounds [7, 14]. With the revelation of some genes related to sporopollenin synthesis, more and more biochemical experiments in vitro have been conducted to explore their biochemical function. Studies have shown that *AtPKSA* and the closely related *AtPKSB* can both catalyze the condensation of long-chain fatty acid acyl-CoA and malonyl-CoA to produce α -pyrone products. Accordingly, *AtPKSA* is known as a multifunctional enzyme and vital for the synthesis of both pollen exine fatty acids and phenolics [19, 20]. Simultaneously, in rice, *OsLAP6/OsPKS1* shared similar products of enzymatic reaction with *AtPKSA/LAP6* [22]. Moreover, *PpASCL*, a homolog of *AtPKSA/LAP6* in moss, was encoding a sporophyte-specific enzyme that had similar catalytic activity to *AtPKSA/LAP6* in vitro [21]. In agreement with that, in our study, the contents of various long-chain fatty acids were affected in anthers of *SiPKSA*-overexpressing plants compared with the wild-type plants. Also, the contents of several phenolic compounds increased in the *SiPKSA*-overexpressing lines (Fig. 7). According to these results, we deduced that overexpression of *SiPKSA* might disrupt the sporopollenin biosynthesis

in transgenic *Arabidopsis*, leading to excessive accumulation of sporopollenin, thicker pollen exine layer, which cause male sterile. Further experiments were needed to explore the intricate regulatory mechanism.

Conclusion

In this study, we cloned and characterized the sesame polyketide synthase A (*SiPKSA*). Sequence analysis and tissue expression pattern analysis showed that *SiPKSA* is a polyketide synthase closely related to the development of sesame anthers. *SiPKSA*-overexpressing *Arabidopsis* plants showed an obvious male-sterile phenotype. Ultrastructure observation revealed that the pollen grains of transgenic *Arabidopsis* were shrunken and collapsed, and the pollen exine layer was thicker than WT. Moreover, the expression levels of sporopollenin biosynthesis-related genes and fatty acids metabolism were altered in anthers of *SiPKSA*-overexpressing plants. Our results highlighted that overexpression of *SiPKSA* might cause excessive sporopollenin biosynthesis, forming thicker pollen exine layer, and then leading to male sterile, suggesting that its manipulation might improve hybrid breeding in sesame and other crop species.

Materials And Methods

Plant materials and growth conditions

The male-sterile mutant 95ms-5A and its fertile segregant 95ms-5B [29] were cultivated under natural conditions at the experimental station of the Oil Crops Research Institute of the Chinese Academy of Agricultural Sciences (OCRI-CASS, Wuhan, China). *Arabidopsis* ecotype Colombia (Col-0) was grown under standard growth conditions, the temperature was maintained at approximately 22°C, the light intensity was 130 $\mu\text{E}/\text{m}^2\text{s}$, and the photoperiod was 16 h light/ 8 h dark. All the plant materials were provided by the OCRI-CASS.

Clone and sequence analysis

The ORF of *SiPKSA* was amplified with the specific gene primer SiPKSA-f (5'-ATGTCCAACATCATCATCAACAGC-3') and SiPKSA-r (5'-TCAAAGACTCCTAAGAAGAATGCCT-3') via PCR using the fertile sesame anthers at the tetrad stage as the template. The physical and chemical properties of *SiPKSA* were analyzed using the EXPASY database (<https://web.expasy.org/protparam/>). The PLANTCARE and PLACE databases were used to analyze the cis-acting elements of *SiPKSA* [31, 32].

For multiple alignment analysis, *SiPKSA* and three other *PKSAs* (*AtPKSA*, At1g02050; *OsPKSA*, NP_001064891; *BnPKSA*, XP_013701181) were aligned by the ClustalX program. For phylogenetic analysis, *SiPKSA* and selected *PKSs* from other species (*Arabidopsis thaliana*, At4g34850; *Brassica napus*, XP_013707052; *Helianthus annuus*, XP_022010182; *Glycine max*, XP_003537759, XP_003516799; *Populus trichocarpa*, XP_002302511; *Oryza sativa*, XP_015646301; *Zea mays*, PWZ54429; *Arachis hypogaea*, XP_015971059, XP_015952719; *Mesicago truncatula*, XP_024638419; *Brassica rapa*, XP_009108697) were subsequently constructed via MEGA-X with the neighbor-joining method as the default and 1000 bootstrap replicates [33]. All sequences were downloaded from the NCBI database.

Vector construction and plant transformation

First, the ORF of *SiPKSA* was inserted into TSV-007S vector (Tsingke, China) and sequenced. Subsequently, through double enzyme digestion (restriction site: *Nde*I and *Sma*III), the fragment was ligated into PRI101-AN (Takara, Japan) to generate the overexpression vector (35S::SiPKSA) according to previously described methods [34, 35]. The expression vector was then introduced into *Arabidopsis thaliana* (Col-0) through the *Agrobacterium tumefaciens* (strain GV3101). *Arabidopsis* plants transformed with empty vector (EV) and wild-type *Arabidopsis* (WT) were used as the control.

Plant morphology and pollen viability analysis

When the seedlings were eight weeks old, the phenotypes of transgenic plants were photographed using a Nikon D90 digital camera. Pollen grains from wild type plants transformed with empty vector and *SiPKSA*-overexpressing plants were stained with 0.5% acetyl carmine reagent, and the photographs were captured using an optical microscope (IX71, Olympus). All plump and darkly stained pollen grains were determined as fertile, and the collapsed and unstained pollen grains were considered sterile.

Ultrastructure observation

For Scanning electron microscope (SEM) observation, pollen grains sampled from freshly dehiscent anthers of WT, EV, and transgenic lines were dehydrated in a graded ethanol series. The dehydrated samples were dried to the critical point in liquid CO₂ and coated with gold particles. The images were acquired by a scanning electron microscope (VEGA3, Tescan).

For Transmission electron microscope (TEM) observation, anthers at different developmental stages collected from WT and *SiPKSA*-overexpressing plants were fixed in 2.5% glutaraldehyde at 4°C overnight. Next, the samples were post-fixed with 1% osmium tetroxide, and subsequently dehydrated twice in 30%, 50%, 70%, 80%, 90%, 95%, and 100% ethanol for 15–20 minutes each time. After that, the samples were infiltrated with a mixture of acetone and epoxy resin in different proportions. The dehydrated samples were then embedded in epoxy resin and polymerized at 60°C (incubator) for 48 hours. Further, they were sliced into the thickness of 80-100nm by an ultra-thin slicer (EMUC7, Leica), and stained with the uranium-lead double staining solution. The pictures were captured using the transmission electron microscope (Tecnaig²20 TWIN). The length of the baculum was measured using the Nano measurer 1.2 software (<http://emuch.net/html/201402/7022970.html>). Three biological replicates were performed, and three microspores were measured for each replicate. The measurements were repeated five times for each sample.

RNA isolation, RT-PCR and qRT-PCR analysis

Sesame root, stem, leaf, flower buds, capsules were collected for expression profile analysis of *SiPKSA* in sesame. To compare the expression difference of *SiPKSA* between sterile sesame plants (95ms-5A) and the fertile plants (95ms-5B), anthers at different developmental stages (tetrad stage, microspore development stage, and mature pollen stage) from sterile sesame plants (95ms-5A) and the fertile plants

(95ms-5B) were respectively sampled. Expression was normalized to *SiActin* (SIN_1009011) [36]. For expression analysis of sporopollenin biosynthesis-related genes in *Arabidopsis* anthers at different development stages (stage 6–8, 9–10, 11–12, 13–14) from wild type, plants transformed with empty vector and *SiPKSA*-overexpressing plants were sampled. Expression was normalized to *AtActin* (At3g18780) [19]. Total RNA was respectively extracted from each sample using the Trizol reagent (Invitrogen, USA). The first-strand cDNA was synthesized by the SuperScript II reverse transcriptase (Vazyme Biotech, Nanjing, China). qRT-PCR was carried out using the Roche Light Cyclers 480 system. The relative expression level was calculated using the $2^{-\Delta C_t}$ method [37]. The primers used in this study were listed in **Table S1**.

Fatty acid and its derivatives measurement

Anthers at different developmental stages sampled from WT and the *SiPKSA*-overexpressing plants were respectively extracted twice with 1mL of chloroform for 2 hours. Next, the mixed supernatant was re-extracted and dried with a nitrogen blower. Finally, 0.25mL of 5% potassium hydroxide-methanol solution was added, and the mixture was incubated at 60°C for 30 minutes and subsequently quenched with n-hexane. The final supernatant was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). The chromatographic conditions by GC-MS were as follows: inlet temperature 280°C, split ratio 20:1, carrier gas: He, carrier gas flow rate: 1.0ml/min, ion source temperature 230°C, scanning mass range 35 ~ 800_M. The injection volume was 1μL. The temperature rising program: The initial temperature was 50°C and kept for 1 minute; the temperature was raised to 200°C gradually (5°C/min); finally, the temperature was raised to 230°C and kept for 10 minutes.

Statistical analysis

All the experiments were performed by three biological replicates, and the presented values are mean ± SD. Statistical significance was determined using Student's t-test (*P < 0.05, **P < 0.01).

Abbreviations

ORF: Open Reading Frame

WT: Wild Type

EV: Transformed Plants with Empty Vector

OX: Overexpression Lines

RT-PCR: Reverse Transcription-Polymerase Chain Reaction

qRT-PCR: Quantitative Real-Time PCR

SEM: Scanning Electron Microscope

TEM: Transmission Electron Microscope

Declarations

Acknowledgments

Not application.

Author's Contributions

TYL, TZ, and YZZ conceived and designed the project and experiments. TYL and YXY performed the experiments. TYL analyzed the data and wrote the manuscript. HYL and FZ assisted in preparing the materials. YZZ, TZ, and DSSK revised the manuscript. All authors read and approved the manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

A

B

Figure 1

Multiple sequence alignment and phylogenetic analysis of SiPKSA with the plant-specific type III polyketide synthase (PKSs) in other species. (A) Protein sequence alignment of SiPKSA with anther developmental-related PKSA from other crops. The conservative sites are underlined and amino acid residues are framed. AtPKSA (At1g02050), BnPKSA (XP_013701181.1), OsPKSA (NP_001064891.1). (B) Phylogenetic analysis of SiPKSA with PKSs from other species. Sesame indium (Si); Arabidopsis thaliana (At); Zea mays (Zm); Glycine max (Gm); Populus trichocarpa (Pt); Oryza sativa (Os); Brassica napus (Bn); Medicago truncatula (Mt); Citrus sinensis (Cs); Helianthus annuus (Ha); Arachis hypogaea (Ah); Brassica rapa (Br).

Figure 2

Expression analysis of SiPKSA in sesame. (A-B) The expression profile of SiPKSA in different sesame tissues by RT-PCR (A) and qRT-PCR analysis (B). (C-D) RT-PCR (C) and qRT-PCR analysis (D) of the expression of SiPKSA in sesame fertile and sterile anthers at different developmental stages. Si-Actin (SIN_1009011) was used as the internal reference control.

Figure 3

Ectopic expression of SiPKSA in Arabidopsis caused transgenic plants male sterile. (A) RT-PCR analysis of SiPKSA in SiPKSA-overexpressing lines (OX-1, OX-2, OX-3), wild-type (WT) and plants transformed with empty vector (EV). At-Actin (At3g18780) was used as an internal standard. (B) Phenotype of 8-weeks-old SiPKSA-overexpressing Arabidopsis plants. (C-E) Magnified image of SiPKSA-overexpressing plant (C), WT (D), and EV (E). White arrow indicated the siliques of plants. (F-H) Detection of pollen vigor of WT (F), SiPKSA-overexpressing plant (G) and EV (H) with 0.5% aceto carmine reagent. (I, J) Pollen grains of WT observed by SEM. (K, L) Pollen grains of SiPKSA-overexpressing plant observed by SEM. Scale bars represent 10 μ m (I, K), 2 μ m (J, L), respectively.

Figure 4

Transmission electron micrographs (TEM) observation of the ultrastructure of microspore and tapetum development of wild-type and SiPKSA-overexpressing plants at different anther developmental stages. (A, D) Tapetal cells of the WT (A) and SiPKSA-overexpressing plant during stage 9-10 (D). (B, E) Microspores of the WT (B) and SiPKSA-overexpressing plant during stage 9-10 (E). (C, F) Pollen wall of WT (C) and SiPKSA-overexpressing plant (F) at stage 11-12. Msp, microspore; DMsp, degenerated microspore; T, tapetum; In, intine; Ex, exine; N, nucleus; P, plastid filled with plastoglobuli; Try, tryphine; PG, pollen grain. Scale bars were 2μm.

Figure 5

TEM observation of pollen wall from wild-type (WT) and SiPKSA-overexpressing plants at different anther developmental stages. (A-D) The ultrastructure of pollen wall of WT and SiPKSA-overexpressing plants at anther developmental stages 9-10 and 11-12. (E) The thickness of exine layer at stages 9-10 and 11-12. Ex, exine; In, intine. Scale bars were $1\mu\text{m}$. Three biological replicates were conducted and the value are mean $\pm$ SD. Statistically significant differences were assessed using student's t-test (* $p < 0.05$, ** $p < 0.01$).

Figure 6

qRT-PCR analysis of the pollen development-related genes in the controls and SiPKSA-overexpressing plants. Three biological replicates were performed and expression was normalized to *AtActin* (*At3g18780*). Statistically significant differences were assessed using student's t-test (* $p < 0.05$, ** $p < 0.01$). *AtMS2* (*At3g11980*), *AtCYP704B1* (*At1g69500*), *AtTKPR1* (*At4g35420*), *AtTKPR2* (*At1g62940*)

Figure 7

Measurement of fatty acids and their derivatives in anthers of SiPKSA-overexpressing plant and wild type by GC-MS. (A) The content of fatty acids in anthers at stages 6-8 and 13. (B) The content of Phenols and fatty alcohol in anthers at stages 6-8 and 13. C12:0, Lauric acid methyl ester; C14:0, Methyl tetradecanoate; C15:0, Pentadecanoic acid methyl ester; C15:1, 9-Octadecenoic acid methyl ester; C16:0, Hexadecanoic acid methyl ester; ethyl-C16:0, 9-Hexadecenoic acid methyl ester; C16:1w11, 11-Hexadecenoic acid methyl ester; C16:2, 7,10-Hexadecadienoic acid methyl ester; C16:3, 7,10,13-Hexadecatrienoic acid methyl ester; hexade-C16, Hexadecanedioic acid dimethyl ester; butyl-C16, butyl isobutyl phthalate; C17:0, Heptadecanoic acid methyl ester; C18:0, Methyl stearate; iso-C18:0, Methyl isostearate; C18:1, (Z)-9-Octadecenoic acid methyl ester; C18:1T, 9,12,15-Octadecatrienoic acid ethyl ester; C20:0, Arachidic acid methyl ester; C20:1, cis-11-Eicosenoic acid methyl ester; C20:2, 11,13-Eicosadienoic acid methyl ester; C22:1, 13-Docosenoic acid methyl ester; C22:2, cis-13,16-Docosadienoic acid methyl ester; C24:0, Tetracosanoic acid methyl ester; Phenol(C15-OH), Butylated Hydroxytoluene; Phenol(C14-OH), 2,4-bis(1,1-dimethylethyl)-Phenol; Phenol(C25-2OH), 2,2'-methylenebis[6-(1,1-dimethylethyl)]-4-methyl-Phenol.

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