Cloning and functional characterization of 4-Diphosphocytidyl-2-C-Methyl-D-Erythritol kinase (*LiCMK*) gene in oriental lily (*Lilium* 'Sorbonne') Func. Char. of *CMK* in *Lilium* 'Sorbonne'

Wuhua Zhang[#], Chen Chen[#], Yidi Qin, Jiaxuan Yu, JinZhu Zhang, Jie Dong, Tao Yang and Jinping Fan[*] [iD]

College of Horticulture and Landscape, Northeast Agricultural University, Harbin 150030, China
[#] Authors contributed equally: Wuhua Zhang, Chen Chen
[*] Correspondence: Jinpingfan2020@neau.edu.cn (Fan J)

Abstract

Oriental lily (*Lilium* spp.) has emerged as a model system for floral fragrance research due to its distinctive scent profile. Terpenoid compounds, major constituents of its floral volatiles, are primarily synthesized through the methylerythritol phosphate (MEP) and mevalonic acid (MVA) pathways. 4-Diphosphocytidyl-2-C-Methyl-D-Erythritol Kinase (CMK), a key enzyme in the MEP pathway, is involved in monoterpene biosynthesis. However, the function of *CMK* in lily remains uncharacterized. In this study, the role of *LiCMK* in *Lilium* 'Sorbonne' is investigated. Gene cloning revealed that *LiCMK*, previously identified in the present transcriptome analysis, has a full-length coding sequence of 1,191 bp encoding 396 amino acids, and the protein localizes to the chloroplast. Tissue-specific expression analysis showed that *LiCMK* is highly expressed in outer whorl petals during the full-blooming stage. Virus-induced gene silencing (VIGS) of *LiCMK* led to a significant reduction in volatile terpenoid emissions and downregulation of downstream genes (*LiLIS*, *LiMYS*, *LiOCS*), confirming *LiCMK* as a key rate-limiting enzyme in floral fragrance biosynthesis. Functional validation in model plants showed that overexpression of *LiCMK* in Arabidopsis led to enlarged leaves, increased photosynthetic pigments, and upregulation of terpenoid pathway genes. In transgenic tobacco, *LiCMK* overexpression caused delayed flowering, elevated levels of hormones (GA and ABA), and specific accumulation of (*E*)-*β*-ocimene and linalool, with activation of genes like *NtDXS*. Collectively, the findings of this study indicate that *LiCMK* enhances floral fragrance synthesis in *Lilium* 'Sorbonne' by activating MEP pathway genes. This study provides molecular insights into the mechanism of fragrance biosynthesis in lilies and will facilitate future breeding and application-oriented research.

Introduction

Floral scent compounds represent a crucial class of plant volatile secondary metabolites, primarily consisting of volatile organic compounds (VOCs) synthesized and released by floral organs[1]. These compounds play essential roles in plant–environment interactions, not only attracting pollinators but also defending against pathogens and herbivores, and facilitating adaptation to environmental stresses[2,3]. Owing to their low molecular weight and high vapor pressure, VOCs can permeate cellular membranes and diffuse into the extracellular matrix and surrounding environment, enabling rapid dissemination and release[4]. Floral VOCs are chemically diverse, primarily comprising terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives, and nitrogen-containing compounds[5]. Based on biosynthetic pathway specificity, volatile metabolites are systematically classified into three major categories: terpenoids, phenylpropanoid/benzenoid compounds, and fatty acid derivatives[6]. The composition and abundance of these VOCs vary significantly among flowering plants, defining their distinctive scent profiles[7].

The biosynthesis of floral terpenoids in plants is initiated by the condensation of two C_5 precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP)[8]. These central metabolic precursors are primarily synthesized via the mevalonic acid (MVA) pathway and the methylerythritol phosphate (MEP) pathway[9]. The MVA pathway, localized in the cytosol, is responsible for the production of volatile sesquiterpenes and triterpenes. In contrast, the MEP pathway operates in plastids and contributes to the formation of volatile monoterpenes and diterpenes, as well as carotenoids and chlorophylls[10–13]. Although these isoprenoid biosynthetic pathways are compartmentalized, they may be interconnected through metabolite transport mediated by specific transporters[14,15]. Such metabolic crosstalk enables the MEP pathway to support terpenoid biosynthesis in the cytosol[16]. The MVA pathway begins with the sequential condensation of three acetyl-CoA molecules and involves six enzymatic steps, whereas the MEP pathway starts from the condensation of D-glyceraldehyde 3-phosphate and pyruvate and requires seven enzymatic reactions[17,18]. The methylerythritol phosphate (MEP) pathway is central to the formation of volatile terpenoids and certain floral traits[19,20]. A key example is linalool, the most abundant monoterpene in tea, whose synthesis is governed by terpene synthase (TPS) enzymes within this pathway[21,22].

Lilium spp., a genus of significant ornamental value within the Liliaceae family, has attracted increasing research attention due to its floral fragrance. More than 100 wild species are known, distributed primarily across the Northern Hemisphere at latitudes between 10° and 60°, including regions of Asia, Europe, and North America[23]. The floral scent, a key aspect of lilies' ornamental appeal, is largely determined by monoterpenoids, with variation among cultivars attributed to differences in monoterpene emission levels[24]. Major terpenoid volatiles identified in lilies include (*E*)-*β*-ocimene, linalool, and myrcene[25]. Recent advances in molecular biology have greatly facilitated the study of fragrance biosynthesis in lilies. Terpene synthase genes, such as LhTPS, play essential roles in the production and emission of scent compounds and represent key genetic determinants underlying floral aroma in lilies[26].

In the Oriental lily (*Lilium* 'Sorbonne'), previous studies have shown that heterologous overexpression of the two monoterpene synthase genes, *LiLIS* and *LiMYS*, in *Arabidopsis thaliana* promotes monoterpene biosynthesis[27]. In addition, *LiDXS2*, which encodes the first key enzyme of the MEP pathway, has been implicated in the regulation of floral scent emission in 'Sorbonne'[28]. Despite these advances, the molecular mechanisms underlying floral fragrance synthesis in *Lilium* 'Sorbonne' remain largely elusive. Further investigation into the key genes and regulatory networks controlling scent production is essential to advance the understanding of floral aroma formation and facilitate lily breeding and biotechnological applications.

Materials and methods

Plant materials

The Oriental lily (*Lilium* 'Sorbonne'), along with seeds of *Nicotiana benthamiana*, tobacco (*Nicotiana tabacum* L. '95'), and wild-type *Arabidopsis thaliana* Columbia-0 (Col-0), were used in this study. All plant materials were preserved and cultivated in the Laboratory of Ornamental Plant Genetics and Breeding at Northeast Agricultural University. Plants were grown under the following controlled conditions: during the vegetative growth stage, a 12-h-light/12-h-dark photoperiod was applied; during the reproductive growth stage, the photoperiod was adjusted to 14-h-light/10-h-dark. The temperature was maintained at 22–24 °C, and the relative humidity was controlled between 40% and 60%.

RNA extraction and gene cloning

Total RNA was extracted from petals of *Lilium* Oriental Hybrid 'Sorbonne' using the FastPure Plant Total RNA Isolation Kit (Vazyme Biotech Co., Ltd, Nanjing, China). RNA concentration and purity were assessed using a microvolume spectrophotometer (Kaiao Science and Technology Development Co., Ltd, Beijing, China), and integrity was verified by 1.2% agarose gel electrophoresis. First-strand cDNA was synthesized from the extracted RNA using the EasyScript® All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) (TransGen Biotech Co., Ltd, Beijing, China). Specific primers were designed based on transcriptome data (primer sequences are provided in (Supplementary Table S1) to amplify the target fragment via PCR. The amplified product was purified by gel extraction, cloned into the pBZ plasmid vector, and sequenced by Sangon Biotech (Shanghai) Co., Ltd (China) to obtain the full-length sequence of *LiCMK*.

Multiple sequence alignment and phylogenetic analysis of *LiCMK*

To investigate the evolutionary relationships of *LiCMK*, a multiple sequence alignment of its amino acid sequence was performed using ClustalX. The alignment was further validated through domain prediction based on the SMART and Pfam databases. A maximum likelihood (ML) phylogenetic tree was constructed with MEGA5 software[29], and node support was assessed with 1,000 bootstrap replicates. These analyses provide insight into the evolutionary conservation and functional potential of *LiCMK* within the context of terpenoid biosynthesis in lily.

Analysis of *LiCMK* expression patterns

Total RNA was extracted from various tissues of *Lilium* Oriental Hybrid 'Sorbonne', including anthers, styles, stamens, inner petals, outer petals, and leaves, as well as from floral tissues at five developmental stages: green bud stage, pink bud stage, partially open stage, fully open stage, and senescent stage. The RNA was reverse-transcribed into cDNA. Quantitative real-time PCR (qRT-PCR)[30] was performed using ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd, Nanjing, China) according to the manufacturer's instructions. The reaction mixture (20 μL total volume) contained 1 μL cDNA template, 1 μL each of forward and reverse primers (10 μM), 10 μL of 2 × ChamQ Universal SYBR qPCR Master Mix, and 8.2 μL nuclease-free water. The amplification protocol consisted of an initial denaturation at 95 °C for 30 s, followed by forty cycles of 95 °C for 10 s and 58 °C for 30 s. A melting curve analysis was performed from 65 to 95 °C with a ramp rate of 0.5 °C/s to verify amplification specificity. Three biological replicates (independent plants) and three technical replicates were included for each sample. Relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method.

Subcellular localization

The open reading frame (ORF) of *LiCMK* was cloned into the pCAMBIA1300-GFP vector using a homologous recombination method to generate the fusion construct pCAMBIA1300-GFP-LiCMK. Both the recombinant plasmid and the empty vector control (35S::GFP) were introduced into *Agrobacterium tumefaciens* strain GV3101 competent cells[31]. The transformed agrobacteria were resuspended in an infiltration buffer (10 mM $MgCl_2$, 10 mM MES, 100 μM acetosyringone, pH 5.6). The bacterial suspensions were infiltrated into the leaves of *Nicotiana benthamiana* using a needle-less syringe. At 48 to 72 h after infiltration, GFP fluorescence signals were observed and imaged using a FV3000 laser scanning confocal microscope.

Virus-induced gene silencing of *LiCMK*

The TRV2-LiCMK silencing vector was constructed by cloning a fragment of *LiCMK* into the TRV2 vector via homologous recombination and then introduced into *Agrobacterium tumefaciens* strain GV3101. Flower buds of *Lilium* 'Sorbonne' were infected using a vacuum infiltration method (0.07 kPa, 5 min) with a mixed agrobacterial suspension containing TRV1 and TRV2-LiCMK (1:1 ratio). A control group was infiltrated with a mixture of TRV1 and empty TRV2 (1:1 ratio). After infiltration, plants were kept in darkness for 24 h and then transferred to a growth chamber set at 22 °C under a light cycle until full blooming. Total RNA was extracted from petals of silenced lilies[32] and reverse-transcribed into cDNA[33]. The silencing efficiency was evaluated by qRT-PCR using primers designed with Primer3 software, with *LrTIP41* (GenBank: KJ543466) as the internal reference gene. Expression changes of terpenoid biosynthesis-related genes were also analyzed.

Heterologous expression of *LiCMK* in *Arabidopsis*

The pCAMBIA1300-*LiCMK* expression vector was constructed using homologous recombination and introduced into *Agrobacterium tumefaciens* strain GV3101 via the freeze-thaw method[34]. *Arabidopsis thaliana* transformation was carried out using the floral dip method. Developing inflorescences were immersed in an infiltration solution containing 5% sucrose and 0.01%–0.05% (v/v) Silwet L-77. After infiltration, plants were grown under standard conditions until seed maturation. T_0 seeds were surface-sown on MS medium containing 30 mg/L hygromycin for selection of transformants[35]. Putative positive transformants were identified by RT-PCR. Seeds from confirmed T_1 plants (T_2 generation) were

collected. Total RNA was extracted from leaves of transgenic plants, reverse-transcribed into cDNA, and used as a template for PCR verification. Positive lines were advanced to the T₃ generation (The *Arabidopsis* mutant used in the subsequent experiments) through repeated screening under the same selection conditions for subsequent experiments.

Analysis of gene expression and phenotype in transgenic *Arabidopsis*

Total RNA was extracted from homozygous T3 transgenic *Arabidopsis* plants and reverse-transcribed into cDNA. Three independent transgenic lines with high *LiCMK* expression levels were selected by qRT-PCR using *AtActin2* (AT3G18780) as the internal reference gene[36]. Chlorophyll a, chlorophyll b, and carotenoid contents were measured in both wild-type and *LiCMK*-overexpressing plants. Phenotypic differences in seed development and leaf morphology were also analyzed. Furthermore, expression levels of key genes involved in terpenoid metabolism (*DXS*, *DXR*, *TPS14*, *TPS21*) were examined by qRT-PCR to elucidate the functional role of *LiCMK*.

Tobacco transformation and identification of *LiCMK* transgenics

Transgenic tobacco plants were generated via Agrobacterium-mediated leaf disk transformation[37]. After acclimatization, plants were transferred to a peat-vermiculite (1:1) substrate. One month later, they were repotted into a mixture of peat : vermiculite : perlite (1:1:1) and grown for three to four months until the flowering stage. Leaves were collected for RNA extraction, and PCR, along with qRT-PCR (using *NtActin7*; Gene ID: 107831145 as the internal reference) was performed to identify *LiCMK*-overexpressing lines for subsequent analysis of volatile compounds.

Phenotypic and gene expression analysis in transgenic tobacco

To investigate the effects of *LiCMK* overexpression on tobacco growth, key phenotypic traits—including leaf size, flower size, and flower color at full bloom—were compared between transgenic and wild-type plants. To confirm *LiCMK* overexpression, photosynthetic pigment contents were measured in leaves from one-month-old plants. Total RNA was extracted from pink petals of three independent transgenic lines with the highest *LiCMK* expression (as determined by RT-PCR) and wild-type controls. qRT-PCR was performed using established reaction conditions with gene-specific primers designed via NCBI for assessing the expression of terpenoid-related genes. Additionally, volatile compounds were analyzed in three selected high-expression transgenic lines and three wild-type plants using solid-phase microextraction (SPME)[38] coupled with gas chromatography–mass spectrometry (GC–MS)[39]. Automated high-throughput SPME was employed for extraction, and volatile identification was conducted by comparing mass spectra with the NIST 08 database, combined with manual interpretation.

Determination of phytohormone content in transgenic tobacco

Leaf samples (0.25 g) from one-month-old transgenic and wild-type tobacco plants were ground in liquid nitrogen and extracted with 1.5 mL of pre-cooled methanol (–20 °C) for 12 h at 4 °C. After centrifugation at 8,000 rpm for 10 min, the supernatant (A) was collected. The pellet was re-extracted with 0.5 mL of 80% methanol,

and the resulting supernatant (B) was combined with A. The combined supernatant was dried under a nitrogen stream at 35 °C. The extract was subsequently processed as follows: (1) defatted three times with petroleum ether (aqueous phase retained); (2) acidified to pH 2.5 with citric acid and extracted twice with ethyl acetate (2 mL each); the organic phases were combined after centrifugation at 3,000 rpm for 3 min; (3) concentrated under nitrogen at 35–40 °C, reconstituted in 200 μL methanol, sonicated (40 kHz, 2 min), and filtered through a 0.22 μm PVDF membrane. Quantification of GA₃ and ABA was performed using a Waters 2695 HPLC system equipped with a Compass C18(2) reversed-phase column.

Analysis of floral volatiles by GC–MS

Volatile organic compounds from lily and tobacco flowers were extracted using headspace solid-phase microextraction (HS-SPME). Following extraction, the SPME fiber was thermally desorbed in the injection port of a gas chromatograph–mass spectrometer (GC–MS). GC–MS analysis was performed under the following conditions: the initial oven temperature was set at 40 °C and held for 2 min, then increased to 250 °C at a rate of 5 °C/min, and held for 5 min. Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. Mass spectrometry detection was conducted in electron impact (EI) mode with an ionization energy of 70 eV. Ethyl decanoate (dissolved in methanol, 1:1,000, v/v) was used as an internal standard for quantification of target terpenoids, including *(E)-β*-ocimene, myrcene, and linalool[40,41].

Results

Cloning and characterization of *LiCMK* in oriental lily 'Sorbonne'

Gene cloning and sequencing revealed that *LiCMK* has a full-length coding sequence of 1,191 bp, encoding a protein of 396 amino acids. Multiple sequence alignment of the deduced LiCMK protein with CMK homologs from other plant species—including *Asparagus officinalis* L. (AoCMK), *Arabidopsis thaliana* (L.)Heynh.(AtCMK), *Artemisia annua* L. (AaCMK), *Canna indica* L. (CiCMK), *Nicotiana tabacum* L. (NtCMK), *Dioscorea zingiberensis* C.H.Wright(DzCMK), *Hevea brasiliensis* (HbCMK), *Pinus massoniana* Lamb.(PmCMK), and *Acorus calamus* L. (AcCMK)—showed that LiCMK shares 77.19% sequence identity with AoCMK from *Asparagus officinalis* L. (Fig. 1a). Phylogenetic analysis further indicated that LiCMK is closely related to DzCMK from Dioscorea zingiberensis (Fig. 1b), a species within the order Liliales known to be involved in terpenoid biosynthesis. Notably, DzCMK has been functionally implicated in the synthesis of the terpenoid diosgenin. These findings suggest that *LiCMK* may play a role in terpenoid metabolism in lilies.

Expression analysis of *LiCMK*

The expression dynamics of *LiCMK* were examined by qRT-PCR in petals of *Lilium* Oriental Hybrid 'Sorbonne' at five developmental stages (Fig. 2a). The results showed that *LiCMK* expression increased initially and then decreased, peaking at the full blooming stage. To further investigate the tissue-specific expression of *LiCMK*, total RNA was extracted from various organs—including filaments, stigma, anthers, leaves, inner petals, and outer petals—and analyzed by qRT-PCR. *LiCMK* expression was lowest in anthers and highest in outer petals, followed by inner petals; expression in outer petals was approximately 12-fold higher than that in anthers (Fig. 2b). Given its
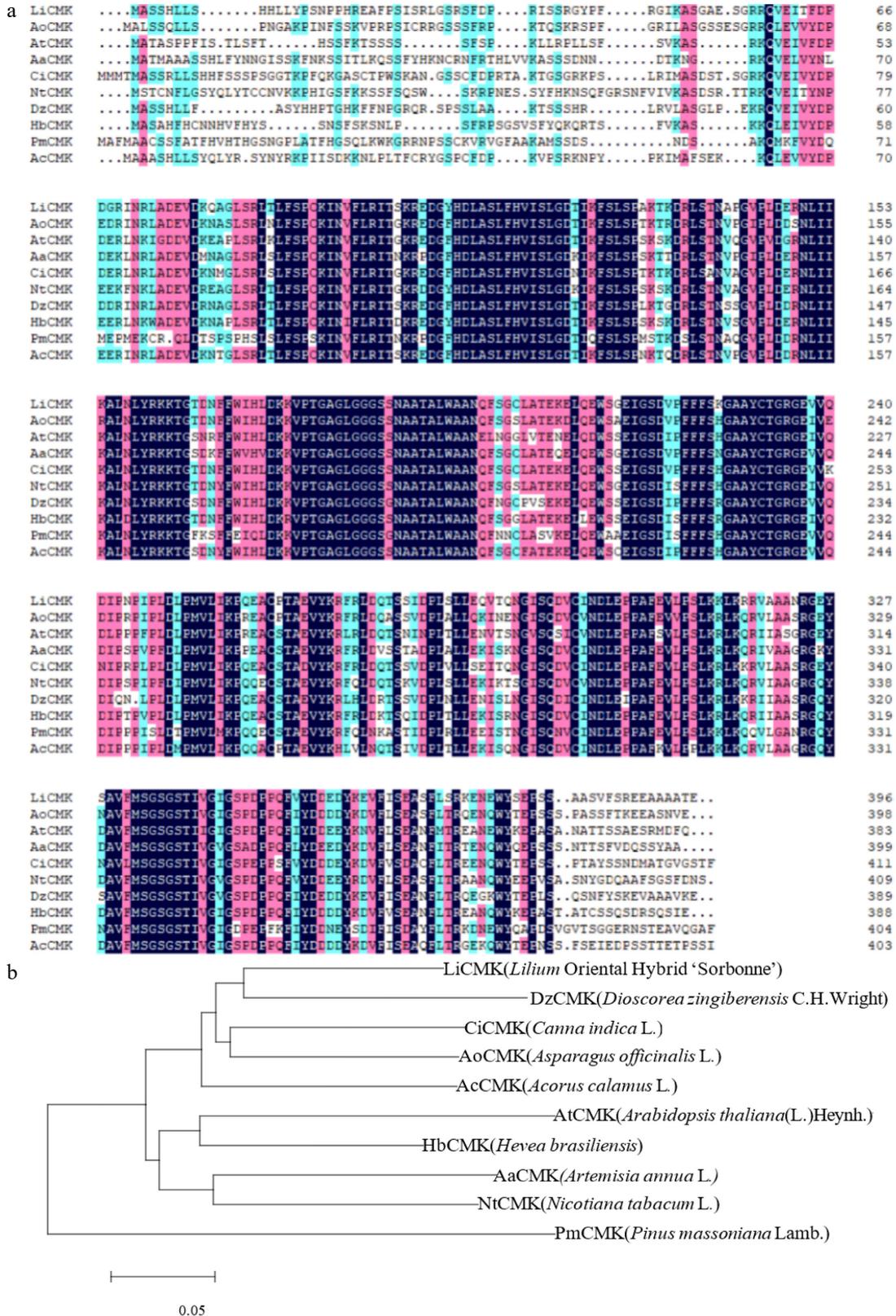
Fig. 1 Phylogenetic analysis and multiple sequence alignment of LiCMK with known homologs. (a) Multiple sequence alignment of the amino acid sequences of LiCMK and CMK proteins from other plant species. (b) Phylogenetic analysis of LiCMK from *Lilium* 'Sorbonne' and CMK homologs from other species.

specific expression in petals—the main site of fragrance emission in 'Sorbonne'—it is proposed that *LiCMK* plays a critical role in floral scent formation.

Subcellular localization of LiCMK protein

The constructed pCAMBIA1300-LiCMK-GFP fusion vector was transiently expressed in tobacco leaf epidermal cells via
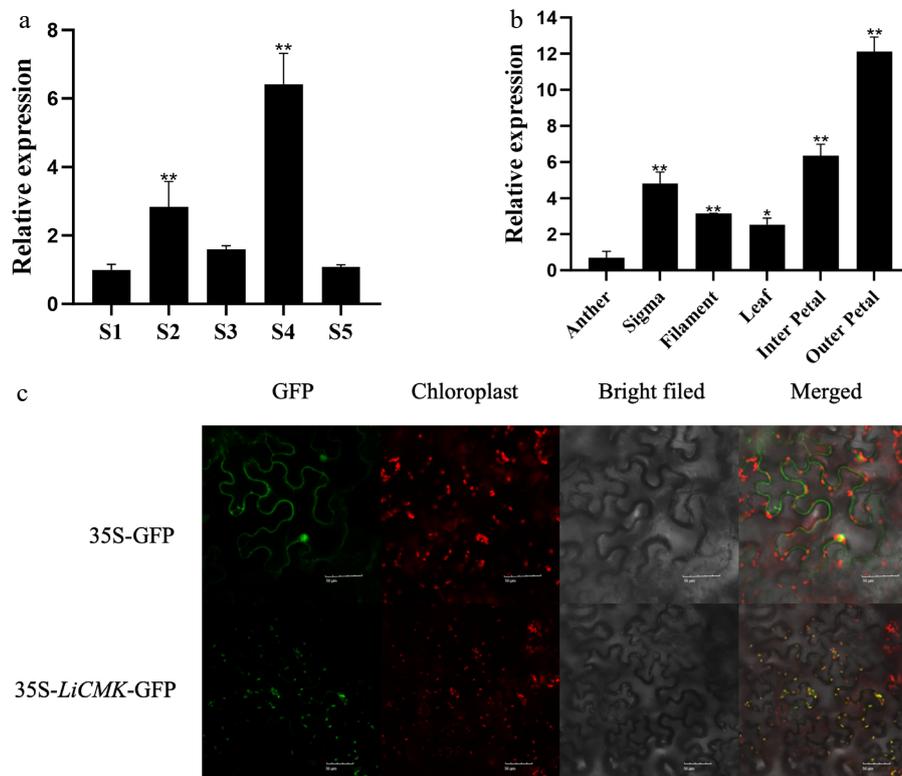
Fig. 2 Temporal-spatial characterization and subcellular localization of *LiCMK* in *Lilium* 'Sorbonne'. (a) Expression analysis of *LiCMK* at different developmental stages in *Lilium* 'Sorbonne'. (b) Tissue-specific expression analysis of *LiCMK* in *Lilium* 'Sorbonne'. (c) Subcellular localization of *LiCMK*. Asterisks denote statistically significant difference, * $p < 0.05$, ** $p < 0.01$.

Agrobacterium tumefaciens-mediated transformation, with the empty GFP vector serving as a control. The 35S:LiCMK-GFP fusion protein produced green fluorescent signals that colocalized with chloroplasts (Fig. 2c), indicating that LiCMK is localized to the chloroplast. This result supports its potential role in plastidial terpenoid biosynthesis via the MEP pathway.

Analysis of terpenoid-related gene expression in *LiCMK*-silenced *Lilium*

To elucidate the biological function of the *LiCMK* gene in *Lilium* 'Sorbonne', this study employed virus-induced gene silencing (VIGS) for functional characterization. The results (Supplementary Fig. S1) showed that *LiCMK* expression was significantly reduced in the experimental groups compared with the pTRV2 control group, indicating successful silencing of *LiCMK* in *Lilium* 'Sorbonne'. Among the silenced plants, lines 1#, 2#, and 5# exhibited high silencing efficiencies of 85.1%, 84.9%, and 59.6%, respectively, and were therefore selected for subsequent experiments.

Petals from these high-efficiency silencing lines (1#, 2#, and 5#) were sampled and compared with those of the pTRV2 control. Changes in the levels of the three major volatile terpenes—*(E)-β-ocimene*, linalool, and myrcene—in *Lilium* 'Sorbonne' are shown in Supplementary Fig. S2. Silencing of *LiCMK* led to a 2.8-fold decrease in *(E)-β*-ocimene content (Fig. 3a), and a 2.6-fold reduction in linalool (Fig. 3b). These results demonstrate that suppression of *LiCMK* expression impairs the accumulation and synthesis of monoterpenes in *Lilium* 'Sorbonne'.

Furthermore, silencing of *LiCMK* also influenced the expression of other terpenoid-related genes. qRT-PCR analysis revealed that the expression levels of key monoterpene synthase genes were significantly downregulated compared with the control. Specifically, *(E)-β*-ocimene synthase (*OCS*) expression decreased by approximately 66%, linalool synthase (*LIS*) by 62%, and myrcene synthase (*MYS*) by 52% (Fig. 3c). Thus, *LiCMK* plays a crucial role in regulating the accumulation and synthesis of major floral scent compounds in lilies.

Phenotypic comparison of transgenic and wild-type *Arabidopsis*

Seeds from T$_3$ transgenic lines were noticeably larger than those of wild-type plants (Fig. 4a). Determination of thousand-seed weight further confirmed a significant increase in seed weight in transgenic lines (Fig. 4b). Biometric analysis revealed that the average seed weight of transgenic plants increased by approximately 34% compared to the wild-type, supporting a role for *LiCMK* in influencing seed development. In addition, rosette leaves of *LiCMK*-overexpressing transgenic plants (T$_3$ generation) were significantly larger than those of wild-type plants under identical growth conditions (Fig. 4c).

Analysis of photosynthetic pigments in transgenic *Arabidopsis*

Based on expression levels, two-week-old leaves from three independent T$_3$ transgenic lines with high *LiCMK* expression and wild-type controls were subjected to spectrophotometric quantification of chlorophyll a, chlorophyll b, and carotenoid contents. The results indicated that the accumulation of all three photosynthetic pigments was significantly higher in transgenic lines compared with the wild-type (Fig. 4d), suggesting that overexpression of *LiCMK* promotes the biosynthesis of photosynthetic pigments.
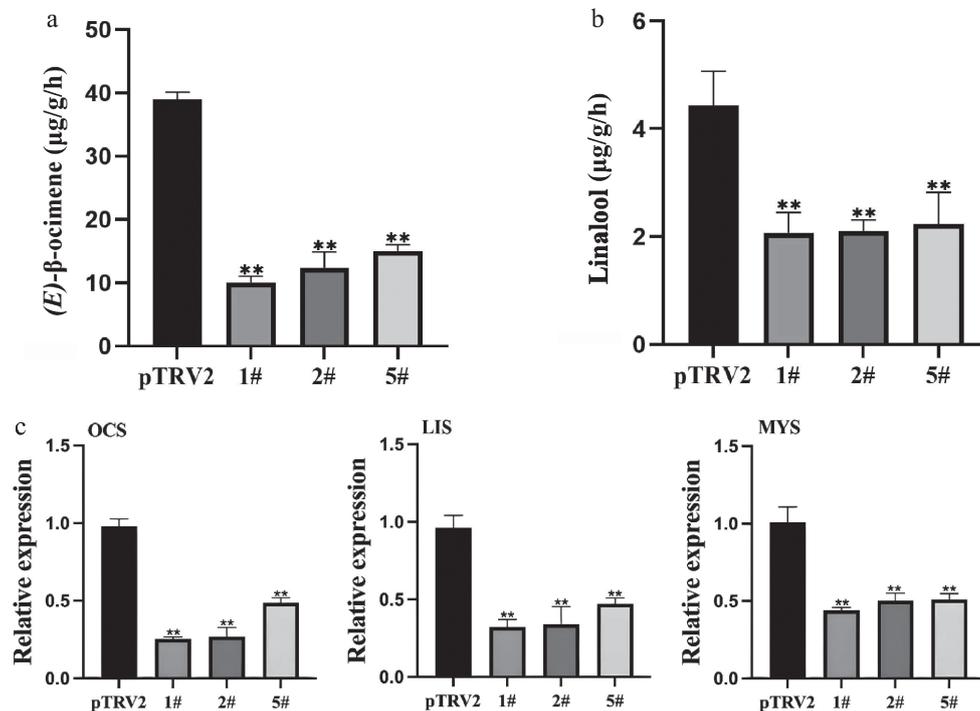
Fig. 3 Effects of *LiCMK* silencing on monoterpene accumulation and synthase gene expression in *Lilium* 'Sorbonne'. (a), (b) Contents of *(E)-β*-ocimene and linalool in petals after *LiCMK* silencing. (c) Relative expression levels of monoterpene synthase genes in response to *LiCMK* silencing. pTRV2: empty vector control; 1#, 2#, and 5#: *LiCMK*-silenced lines; *OCS*: *(E)-β*-ocimene synthase; *LIS*: linalool synthase; *MYS*: myrcene synthase. Data are presented as mean ± SD ($n = 3$). Asterisks indicate significant differences compared to the pTRV2 control group (one-way ANOVA followed by Duncan's test, * $p < 0.05$, ** $p < 0.01$).

Expression profiling of terpenoid biosynthetic genes in transgenic *Arabidopsis*

To investigate the regulatory effect of *LiCMK* on key genes involved in terpenoid metabolism, qRT-PCR was performed using flowers collected at full bloom from three independent overexpression lines (OE1, OE3, and OE4). As shown in Fig. 4e, within the MEP pathway, the expression of the upstream gene *AtMCT* was downregulated, whereas downstream genes *AtIPI1* and *AtIPI2* were significantly upregulated. This expression pattern is consistent with the enzymatic position of *CMK*, which functions downstream of *MCT* and upstream of *IPI* in the MEP pathway. Moreover, among terpene synthase genes, expression of the monoterpene synthase gene *AtTPS14* and the sesquiterpene synthase genes *AtTPS11* and *AtTPS21* was upregulated. In summary, these findings demonstrate that overexpression of *LiCMK*, a key enzyme catalyzing the fourth step of the MEP pathway, markedly alters the expression of downstream terpenoid biosynthetic genes.

Transformation and identification of *LiCMK* in tobacco

qRT-PCR analysis was performed to detect the expression level of *LiCMK* in transgenic tobacco lines (Supplementary Fig. S3). Three lines with high expression (3#, 6#, and 9#) were selected for further experiments. Phenotypic comparison between wild-type (WT) and transgenic plants from the seedling to flowering stages revealed no significant differences in floral organ morphology between the transgenic and WT plants (Fig. 5e, a). At one month old, leaf length (Fig. 5b) and fresh weight (Fig. 5c) were measured. The results showed that WT leaves were significantly larger than those of the transgenic lines. The average leaf length and fresh weight of transgenic plants were approximately 75% and 55% of the WT values, respectively. It was also observed that the WT controls exhibited

precocious flowering compared with the *LiCMK* transgenic lines, and the latter exhibited a slower growth rate (Fig. 5d).

Detection of photosynthetic pigments in transgenic tobacco

The contents of photosynthetic pigments in leaves of wild-type and three transgenic tobacco lines were measured, with WT serving as the control. The results indicated that the levels of chlorophyll a, chlorophyll b, and carotenoids in transgenic plants were higher than those in the control (Fig. 5f). Specifically, line 9# exhibited the highest chlorophyll a content, which was 3.30-fold that of the control, while line 6# showed the lowest level at 1.43-fold. For chlorophyll b, line 9# had the highest content (2.72-fold of the control), and line 6# had the lowest (1.20-fold). Regarding carotenoids, line 9# accumulated the highest level (2.84-fold of the control), and line 6# showed the lowest (1.79-fold). These results suggest that the introduction of the *LiCMK* gene promotes the synthesis and accumulation of photosynthetic pigments, which are downstream products of the MEP pathway.

Analysis of terpenoid biosynthesis gene expression in transgenic tobacco

To investigate the effect of *LiCMK* overexpression on genes involved in terpenoid biosynthesis in tobacco, RNA was extracted from flowers of WT and overexpression lines (3#, 6#, and 9#), followed by qRT-PCR analysis. Candidate genes included *NtDXS* and *NtMCT* from the MEP pathway, *NtGGPPS* from the GGPP biosynthesis branch, and *NtPSY* from the carotenoid biosynthesis pathway. As shown in Fig. 5g, compared with the WT control, the expression levels of *NtDXS*, *NtGGPPS*, and *NtPSY* were significantly up-regulated in the overexpression lines, whereas the expression
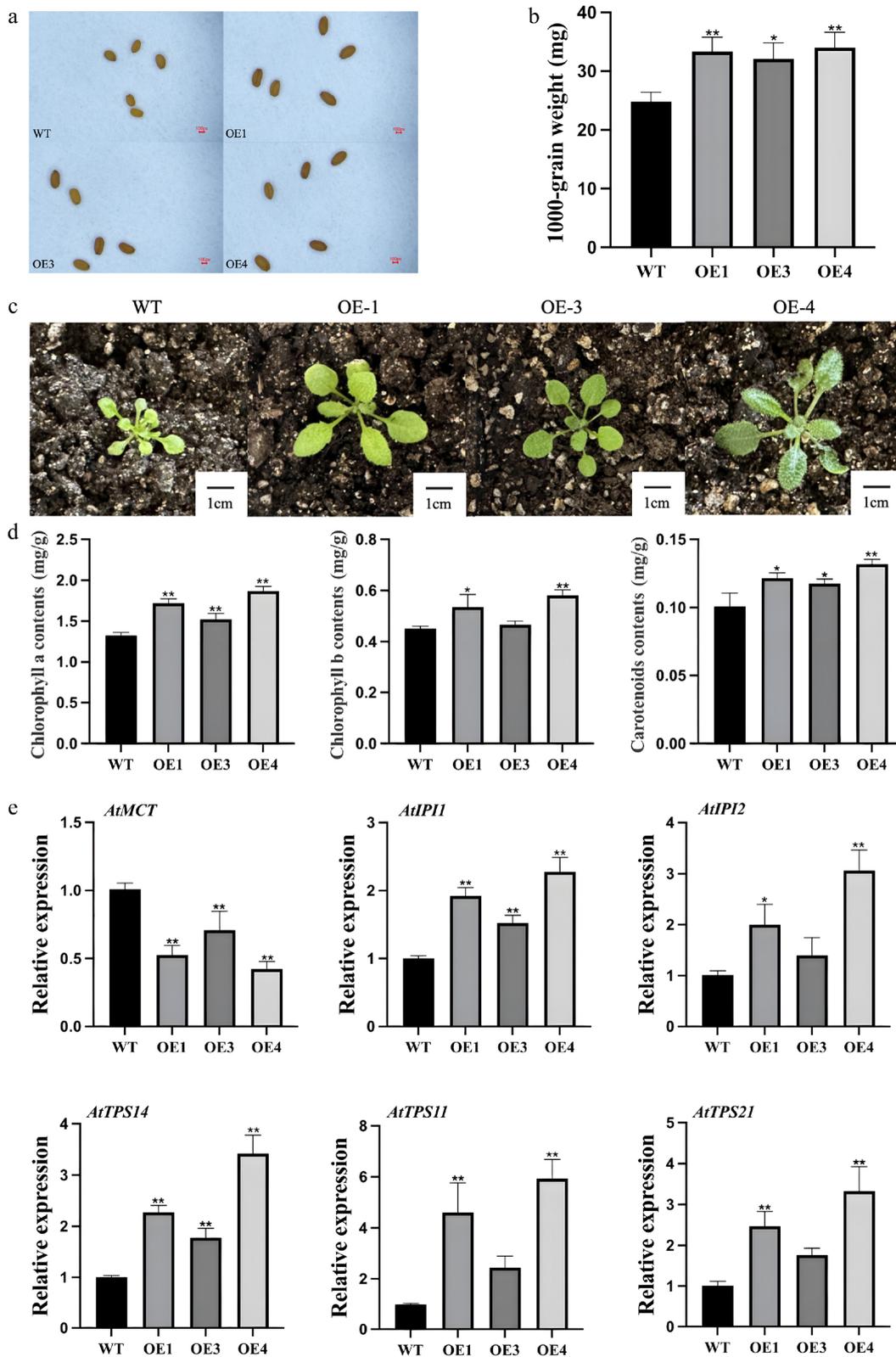
Fig. 4 Phenotypic and gene expression analysis of transgenic and mutant *Arabidopsis thaliana*. (a) Comparison of seed size between wild-type and transgenic Arabidopsis plants. (b) Thousand-seed weight analysis of wild-type and transgenic lines. (c) Leaf size comparison among different genotypes. (d) Photosynthetic pigment content in leaves of *LiCMK*-overexpressing Arabidopsis plants. (e) Expression levels of terpenoid biosynthesis-related genes in Arabidopsis. WT, wild-type; OE1, OE3, OE4, *LiCMK*-overexpression transgenic lines. Data are shown as mean ± SD (*n* = 3). Asterisks indicate significant differences between each transgenic line (OE), and the wild-type (WT) control (one-way ANOVA followed by Duncan's test, * *p* < 0.05, ** *p* < 0.01).
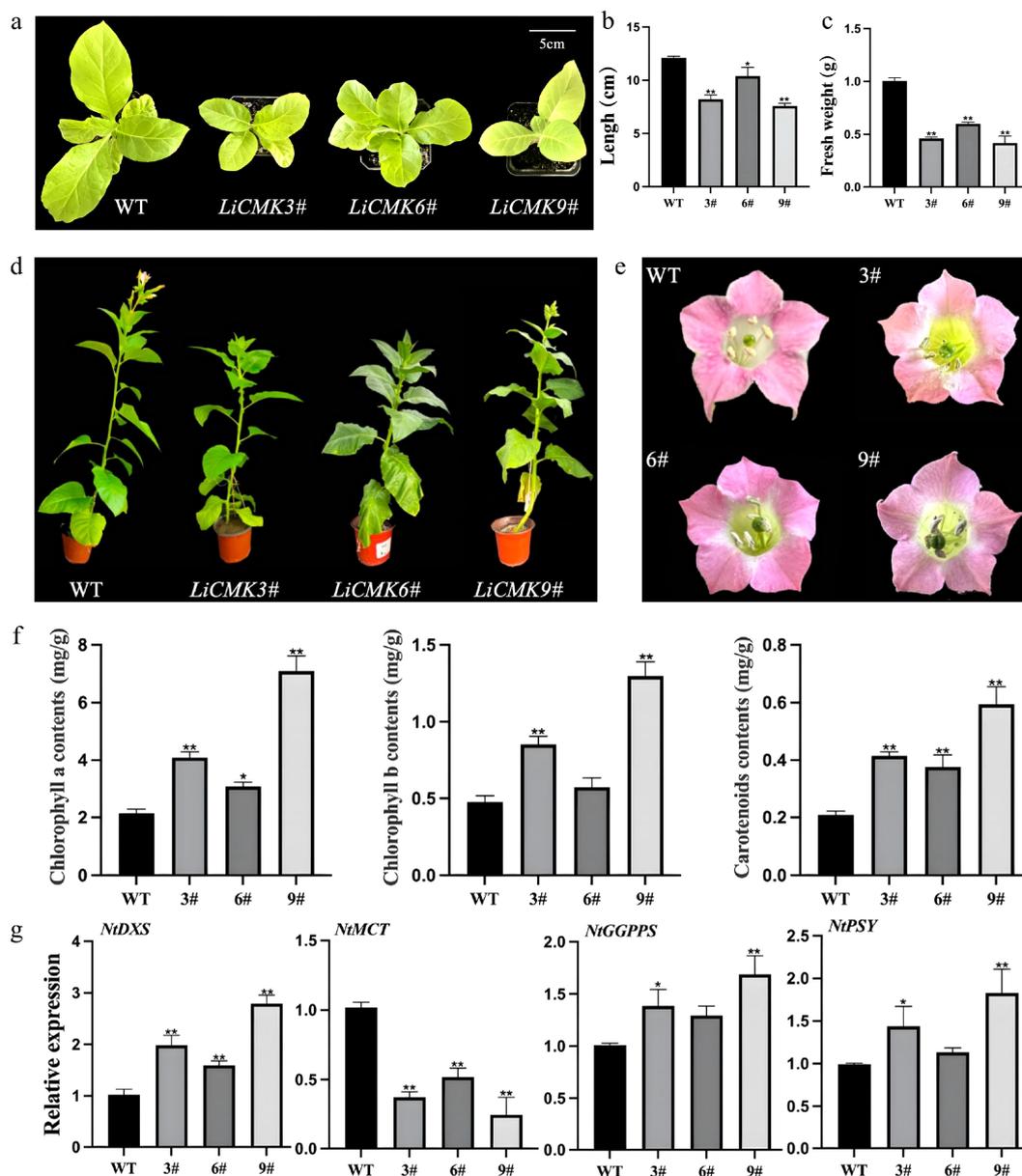
Fig. 5 Phenotypic and gene expression analysis of transgenic tobacco. Comparison of (a) one-month-old seedling phenotype, (b) leaf length, and (c) fresh weight between wild-type and transgenic lines. (d) Comparison of the phenotypes of wild type (WT) and *LiCMK* transgenic lines of tobacco. (e) Phenotypic comparison of flowers between wild-type and transgenic tobacco plants. (f) Analysis of photosynthetic pigments in leaves of transgenic tobacco plants. (g) Relative expression levels of genes involved in the terpenoid biosynthesis pathway in transgenic tobacco. WT, wild-type; 3#, 6#, 9#, transgenic tobacco lines. Data are presented as mean ± SD (*n* = 3). Asterisks indicate significant differences between the wild-type (WT) and transgenic lines (one-way ANOVA followed by Duncan's test, * *p* < 0.05, ** *p* < 0.01).

of *NtMCT*, a gene in the MEP pathway, was significantly down-regulated. These findings indicate that overexpression of *LiCMK* in tobacco suppresses the expression of its upstream gene *MCT*, while promoting the expression of genes related to terpenoid biosynthesis and the MEP pathway.

Analysis of volatile compounds in transgenic tobacco

To evaluate the effect of *LiCMK* gene overexpression on volatile profiles, floral volatiles from a mixture of high-expression transgenic lines (3#, 6#, 9#) and wild-type (WT) tobacco were analyzed using solid-phase microextraction (SPME) (Fig. 6a, b). The results showed significantly higher levels of *(E)-β*-ocimene and linalool in

transgenic plants compared to WT, while the caryophyllene content did not change significantly (Fig. 7a). Specifically, linalool content in transgenic tobacco was approximately 3.2-fold that of WT, indicating that *LiCMK* is a key gene in terpenoid biosynthesis.

Plant hormone analysis in transgenic tobacco

Analysis of hormone levels in WT and transgenic lines (3#, 6#, 9#) was also conducted (Fig. 6c, d). Since *LiCMK*-overexpressing lines exhibited a late-flowering phenotype and flowering time is hormonally regulated, the results measured the contents of gibberellin (GA₃) and abscisic acid (ABA) in one-month-old leaves. Both GA₃ and ABA levels were significantly elevated in the transgenic lines relative to WT (Fig. 7b, c).
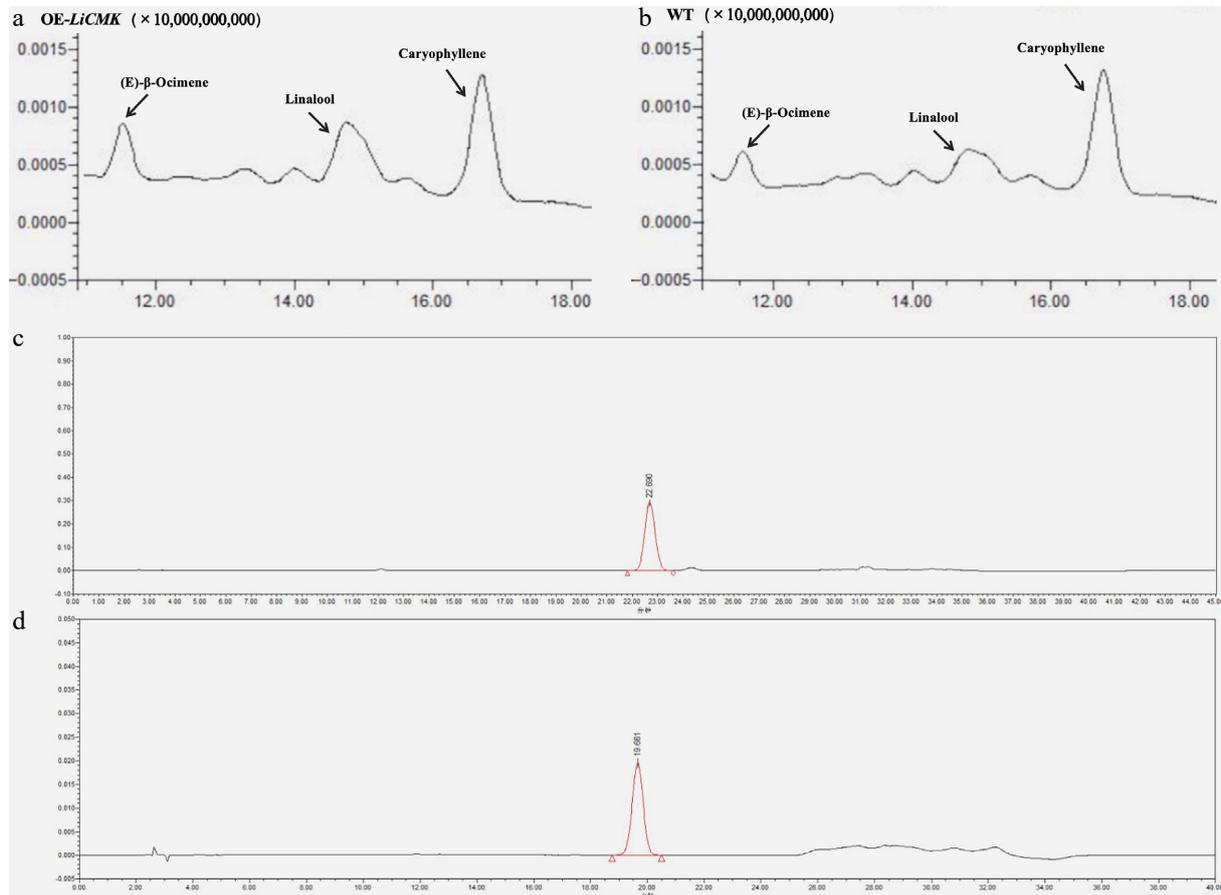
Fig. 6 Total ion chromatograms (TIC). (a) TIC of major volatile compounds in transgenic tobacco hybrid samples. (b) TIC of major volatile compounds in wild-type (WT) tobacco. (c) Chromatogram of GA$_3$ standard. (d) Chromatogram of ABA standard.
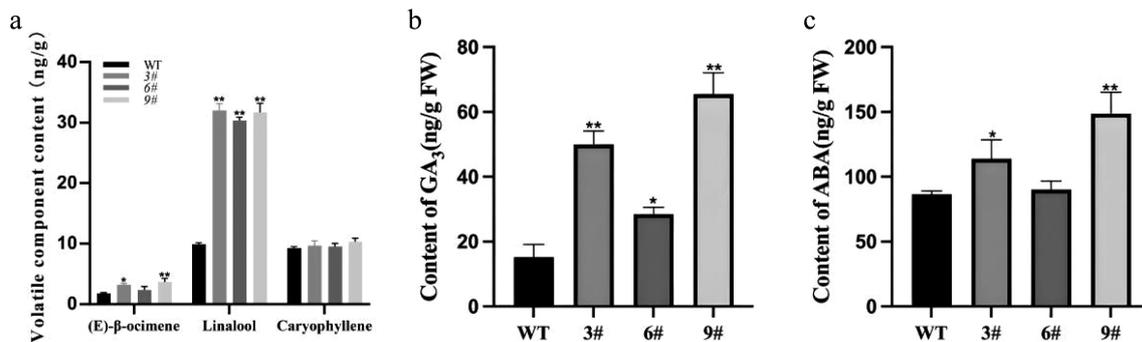

Fig. 7 Analysis of volatile compounds and phytohormones in transgenic tobacco. (a) Contents of major terpenes in flowers of wild-type and transgenic tobacco plants. (b),(c) Endogenous GA$_3$ and ABA contents in *LiCMK*-overexpressing transgenic tobacco lines. WT, wild-type; 3#, 6#, 9#, independent transgenic lines. Data are shown as mean ± SD (*n* = 3). Asterisks indicate significant differences between the wild-type (WT) and transgenic lines (one-way ANOVA followed by Duncan's test, * *p* < 0.05, ** *p* < 0.01).

Discussion

The main aromatic components of lily fragrance are terpenoid volatiles. Their biosynthetic precursors, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), are generated via both the cytosolic mevalonate (MVA) pathway and the plastidial methylerythritol phosphate (MEP) pathway. The MEP pathway primarily contributes to the synthesis of monoterpenes, diterpenes, and polyterpenes. In its fourth enzymatic step, 4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase (CMK) catalyzes the conversion of CDP-ME to CDP-ME2P, representing a key

rate-limiting reaction toward the formation of IPP and DMAPP[42]. In this study, the full-length open reading frame (ORF) of *LiCMK* was isolated. Bioinformatics analysis indicated that it encodes a hydrophilic protein lacking both transmembrane domains and a signal peptide. Subcellular localization predictions suggested a chloroplast-specific localization for *LiCMK*. As the chloroplast is a central site for the synthesis of various floral scent volatiles, such as linalool[43], these results imply a potential role for *LiCMK* in floral fragrance metabolism. Tissue-specific expression profiling demonstrated that *LiCMK* expression was highest in the outer petals of *Lilium* 'Sorbonne', correlating with terpenoid emission patterns.

Throughout floral development, *LiCMK* transcript levels peaked at the full-blooming stage (S4), consistent with the highest emission of monoterpenes at this phase. In contrast, the expression of *LiDXR*, another gene within the MEP pathway, reached its maximum at the half-opening stage (S3). This distinct temporal expression pattern suggests that *LiCMK* may specifically regulate monoterpene synthesis during the full-blooming stage in lilies.

Virus-induced gene silencing (VIGS) is a powerful and versatile reverse genetic tool that enables targeted suppression of gene expression without the need for stable genetic transformation. This technique has been widely employed to investigate the function of fragrance-related genes; for instance, silencing of *PsuLIS* in tree peony led to reduced linalool synthesis[44], and suppression of *LcTPS1* and *LcTPS14* expression in crape myrtle resulted in decreased volatile production[45]. In this study, VIGS was applied to *Lilium* 'Sorbonne' by vacuum infiltrating stem segments with a virus-derived construct targeting *LiCMK*, achieving efficient gene silencing. qRT-PCR analysis confirmed a significant downregulation of *LiCMK* transcripts in silenced plants, accompanied by reduced expression of downstream terpenoid synthase genes (*LiOCS*, *LiLIS*, and *LiMYS*). GC-MS profiling of floral volatiles further revealed markedly decreased emissions of key monoterpenes, including *(E)-β*-ocimene, linalool, and myrcene. These findings provide direct in planta evidence that *LiCMK* positively regulates terpenoid biosynthesis in lilies. While the infiltration-based method used differs from the needle injection approach used by Yang et al. to silence *LiSRM1* in *Lilium* 'Siberia'[46], both strategies effectively achieved silencing, demonstrating the adaptability of VIGS for functional genetic studies across lily cultivars.

To further investigate the function of *LiCMK*, heterologous overexpression assays were conducted in both Arabidopsis and tobacco. In Arabidopsis, overexpression of *LiCMK* significantly increased the contents of chlorophyll a/b and carotenoids and complemented the albino phenotype of the IspE mutant[47], indicating a role in promoting photosynthetic pigment and terpenoid production[48,49]. Transgenic lines also produced larger and heavier seeds, a phenotype consistent with previous reports concerning *ZmHAK1* overexpression[50]. At the molecular level, *LiCMK* overexpression upregulated key terpenoid biosynthetic genes, including *AtTPS14*, *AtTPS11/21*, and *AtIPI1/2*, but suppressed the expression of the upstream gene *AtMCT*, suggesting the presence of a feedback regulatory mechanism. Similarly, in tobacco, transgenic lines (3#, 6#, and 9#) overexpressing *LiCMK* exhibited notably elevated photosynthetic pigment levels, consistent with phenotypes reported in *PmCMK* transgenics[51]. qRT-PCR analysis revealed up-regulation of *NtDXS* and suppression of *NtMCT*, further supporting feedback inhibition at the transcriptional level[52]. The transgenic plants displayed developmental delay and late flowering, accompanied by elevated endogenous levels of GA_3 and ABA. Exogenous application of ABA or GA_3 promoted terpenoid accumulation[53−55]. Volatile profiling confirmed significantly increased emissions of *(E)-β*-ocimene and linalool, resembling the effects of *LiDXS* overexpression. These results demonstrate that *LiCMK* contributes to monoterpene biosynthesis, likely through phytohormone-mediated pathways.

This study reveals that heterologous overexpression of the lily *LiCMK* gene leads to contrasting growth phenotypes in *Arabidopsis* and tobacco, underscoring the significant impact of species-specific metabolic regulatory networks. Research in *Arabidopsis* and tomato reveals that species-specific architectures of the interconnected polyamine-ethylene network underlie their distinct stress responses[56]. In this present study, heterologous overexpression of the lily *LiCMK* gene in *Arabidopsis* enhanced the metabolic flux of the MEP pathway, promoting the accumulation of photosynthetic pigments and the expression of terpenoid biosynthesis-related genes, thereby increasing plant biomass. In tobacco, although photosynthetic pigment synthesis was similarly enhanced, carbon flux was reprogrammed toward terminal terpenoid products, accompanied by abnormal accumulation of GA_3 and ABA, collectively leading to the suppression of vegetative growth. These findings indicate that while the catalytic function of *LiCMK* is conserved, the system-level responses triggered by its overexpression are strongly dependent on the host's inherent metabolic allocation and regulatory mechanisms[57]. In alignment with findings from studies on the lily *LiMCT* gene[58], this research further confirms that while core enzymatic functions of MEP pathway genes are maintained in heterologous systems, the ultimate phenotypic outcomes are governed by the distinct metabolic and regulatory contexts of the host species.

Conclusions

This study aimed to characterize the role of the *CMK* gene in terpenoid biosynthesis in lily, identified *LiCMK* (1,191 bp, encoding 396 amino acids), and confirmed its subcellular localization to the chloroplast. qRT-PCR analysis revealed that *LiCMK* is preferentially highly expressed in outer petals at full bloom, consistent with the emission pattern of monoterpenes. Silencing of endogenous *LiCMK* via a TRV2-based VIGS system led to downregulation of monoterpene synthase genes (*LiOCS*, *LiLIS*, and *LiMYS*) and significantly reduced petal contents of *(E)-β*-ocimene, linalool, and myrcene, as determined by SPME-GC-MS. Furthermore, heterologous overexpression of *LiCMK* in Arabidopsis and tobacco enhanced photosynthetic pigment accumulation, upregulating terpenoid biosynthetic genes and increasing the emission of linalool and *(E)-β*-ocimene in tobacco. These results demonstrate that *LiCMK* participates in floral scent formation by modulating terpenoid biosynthesis and influences plant development.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design, methodology: Dong J; investigation, draft manuscript preparation: Zhang W, Chen C; software: Zhang W, Chen C, Qin Y; formal analysis: Zhang W, Chen C, Yu J; data curation: Zhang W, Chen C, Yu J, Dong J, Zhang J; visualization: Yang T; resources: Qin Y; supervision: Fan J. All authors reviewed the results and approved the final version of the manuscript.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Acknowledgments

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] Mostafa S, Wang Y, Zeng W, Jin B. 2022. Floral scents and fruit aromas: functions, compositions, biosynthesis, and regulation. *Frontiers in Plant Science* 13:860157

[2] Schiestl FP. 2010. The evolution of floral scent and insect chemical communication. *Ecology Letters* 13:643–656

[3] Gross K, Sun M, Schiestl FP. 2016. Why do floral perfumes become different? region-specific selection on floral scent in a terrestrial orchid. *PLoS One* 11:e0147975

[4] Pichersky E, Noel JP, Dudareva N. 2006. Biosynthesis of plant volatiles: nature's diversity and ingenuity. *Science* 311:808–811

[5] Knudsen JT, Eriksson R, Gershenzon J, Ståhl B. 2006. Diversity and distribution of floral scent. *The Botanical Review* 72:1

[6] Abbas F, Zhou Y, O'Neill Rothenberg D, Alam I, Ke Y, et al. 2023. Aroma components in horticultural crops: chemical diversity and usage of metabolic engineering for industrial applications. *Plants* 12(9):1748

[7] Farré-Armengol G, Filella I, Llusià J, Peñuelas J. 2017. *β*-ocimene, a key floral and foliar volatile involved in multiple interactions between plants and other organisms. *Molecules* 22(7):1148

[8] Fujihashi M, Sato T, Tanaka Y, Yamamoto D, Nishi T, et al. 2018. Crystal structure and functional analysis of large-terpene synthases belonging to a newly found subclass. *Chemical Science* 9:3754–3758

[9] Huang Y, Xie FJ, Cao X, Li MY. 2021. Research progress in biosynthesis and regulation of plant terpenoids. *Biotechnology & Biotechnological Equipment* 35:1799–1808

[10] Lange BM, Croteau R. 1999. Isoprenoid biosynthesis via a mevalonate-independent pathway in plants: cloning and heterologous expression of 1-deoxy-D-xylulose-5-phosphate reductoisomerase from peppermint. *Archives of Biochemistry and Biophysics* 365:170–174

[11] Eisenreich W, Bacher A, Arigoni D, Rohdich F. 2004. Biosynthesis of isoprenoids via the non-mevalonate pathway. *Cellular and Molecular Life Sciences CMLS* 61:1401–1426

[12] Chaurasiya ND, Sangwan NS, Sabir F, Misra L, Sangwan RS. 2012. Withanolide biosynthesis recruits both mevalonate and DOXP pathways of isoprenogenesis in Ashwagandha *Withania somnifera* L. (Dunal). *Plant Cell Reports* 31:1889–1897

[13] Yadav RK, Sangwan RS, Sabir F, Srivastava AK, Sangwan NS. 2014. Effect of prolonged water stress on specialized secondary metabolites, peltate glandular trichomes, and pathway gene expression in *Artemisia annua* L. *Plant Physiology and Biochemistry* 74:70–83

[14] Bick JA, Lange BM. 2003. Metabolic cross talk between cytosolic and plastidial pathways of isoprenoid biosynthesis: unidirectional transport of intermediates across the chloroplast envelope membrane. *Archives of Biochemistry and Biophysics* 415:146–154

[15] Flügge UI, Gao W. 2005. Transport of isoprenoid intermediates across chloroplast envelope membranes. *Plant Biology* 7:91–97

[16] Ward JL, Baker JM, Llewellyn AM, Hawkins ND, Beale MH. 2011. Metabolomic analysis of *Arabidopsis* reveals hemiterpenoid glycosides as products of a nitrate ion-regulated, carbon flux overflow. *Proceedings of the National Academy of Sciences of the United States of America* 108:10762–10767

[17] Guirimand G, Guihur A, Phillips MA, Oudin A, Glévarec G, et al. 2012. A single gene encodes isopentenyl diphosphate isomerase isoforms targeted to plastids, mitochondria and peroxisomes in *Catharanthus roseus*. *Plant Molecular Biology* 79:443–459

[18] Chappell J. 2002. The genetics and molecular genetics of terpene and sterol origami. *Current Opinion in Plant Biology* 5:151–157

[19] Qin W, Li Y, Liu H, Yan X, Hu X, et al. 2024. Integrated multi-omics profiling reveals a landscape of dramatic metabolic defect in *Artemisia annua*. *Horticulture Research* 11:uhae174

[20] Yeon JY, Kim WS. 2021. Biosynthetic linkage between the color and scent of flowers: a review. *Horticultural Science and Technology* 39:697–713

[21] Feng Z, Li Y, Li M, Wang Y, Zhang L, et al. 2019. Tea aroma formation from six model manufacturing processes. *Food Chemistry* 285:347–354

[22] Yue R, Li Y, Qi Y, Liang X, Zheng Z, et al. 2025. Divergent MYB paralogs determine spatial distribution of linalool mediated by JA and DNA demethylation participating in aroma formation and cold tolerance of tea plants. *Plant Biotechnology Journal* 23:1455–1475

[23] Chen Y, Lu X, Gao T, Zhou Y. 2025. The scent of lily flowers: advances in the identification, biosynthesis, and regulation of fragrance components. *International Journal of Molecular Sciences* 26(2):468

[24] Hu Z, Tang B, Wu Q, Zheng J, Leng P, et al. 2017. Transcriptome sequencing analysis reveals a difference in monoterpene biosynthesis between scented *Lilium* 'Siberia' and unscented *Lilium* 'Novano'. *Frontiers in Plant Science* 8:1351

[25] Kong Y, Bai J, Lang L, Bao F, Dou X, et al. 2017. Variation in floral scent compositions of different lily hybrid groups. *Journal of the American Society for Horticultural Science* 142:175–183

[26] Wang T, Fan J, Zhang H, Fan W, Du F. 2018. Correlation of monoterpenes release and LhTPS expression in *Lilium* plants. *Journal of Henan Agricultral Sciences* 47:88–108

[27] Cao L, Jiang F, Liu D, Zhang J, Yang T, et al. 2025. Genome-wide characterization of differentially expressed scent genes in the MEP control network of the flower of *Lilium* 'Sorbonne'. *Molecular Biotechnology* 67:510–526

[28] Yu J, Wu N, Fu R, Xue L, Zhang J, et al. 2024. Cloning and expression characterisation of *LiDXS2* gene in Oriental Lily (*Lilium* 'Sorbonne'). *Folia Horticulturae* 36:463–474

[29] Liu C, Zhou X, Li Y, Hittinger CT, Pan R, et al. 2024. The influence of the number of tree searches on maximum likelihood inference in phylogenomics. *Systematic Biology* 73:807–822

[30] Álvarez-Urdiola R, Bustamante M, Ribes J, Riechmann JL. 2023. Gene expression analysis by quantitative real-time PCR for floral tissues. In *Flower Development*, eds. Riechmann JL, Ferrándiz C. New York, NY: Humana. pp. 403–428 doi: 10.1007/978-1-0716-3299-4_20

[31] Carlson ED, Rajniak J, Sattely ES. 2023. Multiplicity of the *Agrobacterium* infection of *Nicotiana benthamiana* for transient DNA delivery. *ACS Synthetic Biology* 12:2329–2338

[32] Sun G, Ju Y, Zhang C, Li L, Jiang X, et al. 2024. *Styrax japonicus* functional genomics: an efficient virus induced gene silencing (VIGS) system. *Horticultural Plant Journal* 10:252–258

[33] Tong Z, Dong T, Li Q, Wang R, He J, et al. 2024. R2R3-type LoMYB21 affects jasmonate-regulated development and dehiscence of anthers in lily (*Lilium* oriental hybrids). *Horticultural Plant Journal* 10:239–251

[34] Li M, Yang Y, Raza A, Yin S, Wang H, et al. 2021. Heterologous expression of *Arabidopsis thaliana* rty gene in strawberry (*Fragaria × ananassa* Duch.) improves drought tolerance. *BMC Plant Biology* 21:57

[35] Mase K, Kamiya Y, Sakaoka S, Morikami A, Tsukagoshi H. 2025. Root growth control by negative regulation of MYB50 under ABA signaling in *Arabidopsis*. *Bioscience, Biotechnology, and Biochemistry* 89:362–370

[36] Wang F, Liang Z, Ma X, He Z, Li J, et al. 2024. LcMPK3 and LcMPK6 positively regulate fruitlet abscission in litchi. *Molecular Horticulture* 4:29

[37] Ershova NM, Sheshukova EV, Kamarova KA, Alimova AR, Savchenko YY, et al. 2025. Xyloglucan endotransglycosylase/hydrolase downregulation increases *Nicotiana benthamiana* tolerance to tobacco mosaic virus infection. *International Journal of Molecular Sciences* 26(22):11183

[38] Ilbeigi V, Valadbeigi Y, Slováková L, Matejčík Š. 2022. Solid Phase microextraction–multicapillary column–ion mobility spectrometry (SPME–MCC–IMS) for detection of methyl salicylate in tomato leaves. *Journal of Agricultural and Food Chemistry* 70:15593–15601

[39] Rontani JF. 2022. Use of gas chromatography-mass spectrometry techniques (GC-MS, GC-MS/MS and GC-QTOF) for the characterization of photooxidation and autoxidation products of lipids of autotrophic organisms in environmental samples. *Molecules* 27(5):1629

[40] Zhou Y, Wang F, Wang Q, Chen Y, Dong J, et al. 2024. Diversity of chemical compounds in lily fragrance and identification of key sensory markers. *Industrial Crops and Products* 222:120034

[41] Liu Z, Wang Y, Xu H, Zhu J, Zhai H, et al. 2025. Integrative analyses of metabolome and transcriptome reveal the regulatory network underlying flavor differences in lychee pulp from different varieties. *Plant Physiology and Biochemistry* 229:110594

[42] Lipko A, Pączkowski C, Perez-Fons L, Fraser PD, Kania M, et al. 2023. Divergent contribution of the MVA and MEP pathways to the formation of polyprenols and dolichols in *Arabidopsis*. *Biochemical Journal* 480:495−520

[43] Zhang T, Guo Y, Shi X, Yang Y, Chen J, et al. 2020. Overexpression of LiTPS2 from a cultivar of lily (*Lilium* 'Siberia') enhances the monoterpenoids content in tobacco flowers. *Plant Physiology and Biochemistry* 151:391−399

[44] Song C, Ma H, Li R, Zhao G, Niu T, et al. 2024. Analysis of the emitted pattern of floral volatiles and cloning and functional analysis of the *PsuLIS* gene in tree peony cultivar 'High Noon'. *Scientia Horticulturae* 326:112750

[45] Xu Y, Zhang H, Wu Y, Xu W, Cai M, et al. 2024. Functional characterization of *LcTPS1* and *LcTPS14* explains the biosynthesis of citronellol, citronellal and linalool in *Lagerstroemia caudata*. *Industrial Crops and Products* 209:118033

[46] Yang Y, Liu X, Hu Z, Zhang Y, Yu H, et al. 2024. Negative regulatory role of LiSRM1 in monoterpene biosynthesis in *Lilium* 'Siberia'. *Plant Physiology and Biochemistry* 216:109167

[47] Hsieh MH, Chang CY, Hsu SJ, Chen JJ. 2008. Chloroplast localization of methylerythritol 4-phosphate pathway enzymes and regulation of mitochondrial genes in *ispD* and *ispE* albino mutants in *Arabidopsis*. *Plant Molecular Biology* 66:663−673

[48] Yang X, Chen H, Chen C, Gu G, Zhang B, et al. 2025. Analysis of mechanisms underlying accelerated plant growth induced by NtGLK85 overexpression in tobacco. *Scientific Reports* 15:40584

[49] Jang S, Li HY. 2018. Overexpression of *OsAP2* and *OsWRKY24* in *Arabidopsis* results in reduction of plant size. *Plant Biotechnology* 35:273−279

[50] Fan B, Cong D, Sheng H, Zhao K, Zhang T, et al. 2019. Phenotype and potassium uptake of transgenic ZmHAK1 *Arabidopsis thaliana*. *Journal of Jilin Agricultural University* 41:149−153 (in Chinese)

[51] Jiang F, Liu D, Dai J, Yang T, Zhang J, et al. 2024. Cloning and functional characterization of 2-C-methyl-D-erythritol-4-phosphate cytidylyltransferase (*LiMCT*) gene in oriental lily (*Lilium* 'Sorbonne'). *Molecular Biotechnology* 66:56−67

[52] Estévez JM, Cantero A, Reindl A, Reichler S, León P. 2001. 1-deoxy-D-xylulose-5-phosphate synthase, a limiting enzyme for plastidic isoprenoid biosynthesis in plants. *Journal of Biological Chemistry* 276:22901−22909

[53] Khozaei M, Fisk S, Lawson T, Gibon Y, Sulpice R, et al. 2015. Overexpression of plastid transketolase in tobacco results in a thiamine auxotrophic phenotype. *The Plant Cell* 27:432−447

[54] Cao J, Jin Q, Kuang J, Wang Y, Xu Y. 2021. Regulation of flowering timing by ABA-NnSnRK1 signaling pathway in lotus. *International Journal of Molecular Sciences* 22(8):3932

[55] Yaish MW, El-Kereamy A, Zhu T, Beatty PH, Good AG, et al. 2010. The APETALA-2-like transcription factor OsAP2-39 controls key interactions between abscisic acid and gibberellin in rice. *PLoS Genetics* 6:e1001098

[56] Cermanová K, Bublavá P, Darbandsari M, Fellner M, Novák O, et al. 2025. Metabolic network divergence: polyamine and ethylene biosynthesis dynamics in *Arabidopsis thaliana* and *Solanum lycopersicum*. *Plant Stress* 18:101124

[57] Bénédetti H, Vallée B. 2023. LIM kinases: from molecular to pathological features. *Cells* 12(12):1649

[58] Hu Q, Zhang Y, Tu Z, Wen S, Wang J, et al. 2024. The identification and functional characterization of the *LcMCT* gene from *Liriodendron chinense* reveals its potenatial role in carotenoids biosyanthesis. *Gene* 902:148180