Planta (2026) 263:54
https://doi.org/10.1007/s00425-026-04924-0

ORIGINAL ARTICLE

Functional and biosynthetic investigation of polyisoprenoids in roses leaves

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Received: 5 November 2025 / Accepted: 8 January 2026

Abstract

Main conclusion **In *Rosa chinensis*, four distinct polyisoprenoid families, including two very long-chain types, are synthesized by only three *cis*-prenyltransferases, challenging the traditional one-enzyme–one-family model. The presence of very long polyisoprenoids in leaves and young shoots is most probably involved in plant organ development.**

Abstract Although terpenoids in roses have been extensively studied, the polyisoprenoid fraction has remained unexplored. In this work, we provide the first characterization of polyisoprenoid diversity and biosynthesis in roses, revealing unexpected chemical and enzymatic complexity. Four distinct polyisoprenoid families (7–9, 15–25, 26–34, and 35–50 isoprene units) were identified in *Rosa chinensis*, with very long-chain compounds accumulated in leaves and young shoots. We functionally characterized three *cis*-prenyltransferases (CPTs) and a CPT-binding partner, RcNUS1, involved in their biosynthesis. The chloroplast-localized RcCPT2 synthesizes short-chain polyisoprenoids, whereas two endoplasmic reticulum-localized heteromeric enzymes, RcCPT1 and RcCPT3, require RcNUS1 as a partner to produce longer-chain compounds. Phylogenetic analysis revealed strong evolutionary conservation but notable species-specific diversification of these enzymes. Remarkably, the number of polyisoprenoid families exceeded the number of identified CPTs, challenging the long-standing one-enzyme–one-product paradigm and suggesting additional, yet unidentified mechanisms regulating chain length. To explore their potential functions, we analyzed the effects of temperature, light, and leaf age on polyisoprenoid accumulation. Environmental treatment had little effect, but leaf aging caused a marked increase in long-chain polyisoprenoids, suggesting roles in development and physiological stability. Our findings reveal new aspects of polyisoprenoid metabolism and highlight their potential functional diversity in plants.

Keywords Roses · Polyisoprenoids · Leaves · *cis*-Prenyltransferase · Terpenoids

Abbreviations

CPT	*cis*-Prenyl transferases
CPTB	*cis*-Prenyl transferases binding protein
DGDG	Digalactosyl diacylglycerol
DHDDS	Dehydrodolichol diphosphate synthetase
DMAPP	Dimethylallyl diphosphate
Dol	Dolichol
FPP	Farnesyl diphosphate
f.w.	Fresh weight
GGPP	Geranylgeranyl diphosphate
GPP	Geranyl diphosphate
IPP	Isopentenyl diphosphate
i.u.	Isoprene units
MeJA	Methyl jasmonate
MEP	Methylerythritol phosphate
MVA	Mevalonate acid
NUS1	Nuclear undecaprenyl pyrophosphate synthase 1 homolog
Pren	Polyprenol
Rer2	Dehydrodolichyl diphosphate synthase complex subunit

Communicated by Stefan de Folter.

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Published online: 16 January 2026

SRT *cis*-Prenyltransferase subunit related to the dolichol synthesis
UPPS Undecaprenyl pyrophosphate synthase

Introduction

Roses are vital for many industries due to their cultural value and use in producing essential oils and rose water (Hibrand Saint-Oyant et al. 2018; Soundararajan et al. 2019). Research focuses mainly on their genomics and flower development. The floral scent of roses contains many volatile molecules of different biochemical origins, with terpenoids being the most important and well characterized. The roses produce terpenoids derived from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) via the mevalonic acid (MVA) and methylerythritol phosphate (MEP) pathways (Magnard et al. 2015; Noh et al. 2023; Conart et al. 2023; Li et al. 2024). Terpenoids identified in roses include monoterpenes (C10), such as geraniol, citronellol, or nerol (Fig. 1), which are integral to the rose scent, as well as sesquiterpenes (C15) like germacrene D and *β*-caryophyllene. Additionally, triterpenes (C30), such as squalene and lupeol, are produced by certain rose species (Kong et al. 2022; Noh et al. 2023). A much less-studied group of terpenoids in roses are polyterpenoids (polyisoprenoids) (Fig. 1), a class of compounds still largely unexplored in this genus despite their potential biological significance.

Polyisoprenoids, linear polymers of isoprene residues, are ubiquitous components of cellular membranes found across all domains of life, from bacteria to humans. These compounds are classified into polyprenols and dolichols, differing in the saturation state of the alpha (α) isoprene residue (Fig. 1). Polyprenols possess an unsaturated alpha residue, while dolichols have a saturated one. Polyisoprenoid elongation is catalyzed by *cis*-prenyltransferases (CPTs), enzymes that sequentially condense isopentenyl diphosphate with an allylic primer, such as farnesyl diphosphate, to produce polyprenyl diphosphates of varying chain lengths (Swiezewska and Danikiewicz 2005; Kharel et al. 2006; Surmacz and Swiezewska 2011, 2013; Grabińska et al. 2016). Grabińska et al. (2016) found that the presence of the amino acid motif RXG is crucial for the proper functioning of CPTs in the enzymatic complex. Homomeric CPTs possess RXG motifs, while, instead, heteromeric *cis*-prenyltransferases require an additional binding protein (CPTB) with an RXG motif for their activity. It is accepted that dedicated CPTs synthesize polyisoprenoids in specific tissue or organ and accumulated as homologues mixtures ("families") with one dominant component or sometimes singles (Swiezewska and Danikiewicz 2005; Surmacz and Swiezewska 2011, 2013). The diverse chain lengths of polyisoprenoids are observed. In *Arabidopsis thaliana*, a single polyprenol (Pren-7) is found in roots, while leaves predominantly contain polyprenols ranging from Pren-9 to Pren-11. Additionally, dolichols in *A. thaliana* roots range from 13 to 16 i.u. (i.u., isoprene units), with longer Pren/Dol-20 to -23 observed in root hair cultures (Jozwiak et al 2013; Surmacz et al. 2014; Akhtar et al. 2017; Gawarecka et al. 2021). Many other plant species, such as *Taxus baccata* or *Juniperus communis*, accumulate in leaves polyisoprenoids of medium length (15–25 i.u.). However, some species, including *Hevea brasiliensis*, synthesize extra-long-chain polyisoprenoids in the form of natural rubber, reaching lengths of over 35,000 i.u. (Mooibroek and Cornish 2000). Interestingly, leaves of *Rosacea* species (e.g., *Rosa arvensis*, *Rosa multiflora*, *Rosa rugosa*, and *Rosa virginiana*) contain polyisoprenoids of up to 35 i.u. (Swiezewska et al. 1992, 1994), which are longer than those seen in most of other plants leaves, although still significantly shorter than natural rubber length. However, the biological function and subcellular localization of these compounds remain unknown, representing a significant gap in our understanding of rose metabolism.

To date, most insights into the biological functions of polyisoprenoids have been derived from non-plant systems. Among these, the best-characterized role is their involvement in glycosylation pathways. Polyisoprenyl phosphates—particularly undecaprenyl phosphate in bacteria and dolichyl phosphate in archaea and eukaryotes—serve as essential membrane-bound lipid carriers in glycan biosynthesis (Surmacz and Swiezewska 2011, 2013; Takahashi and Koyama 2006). For instance, undecaprenyl phosphate is required for the assembly and transport of the disaccharide-pentapeptide precursor in bacterial cell wall synthesis (Manat et al. 2014),

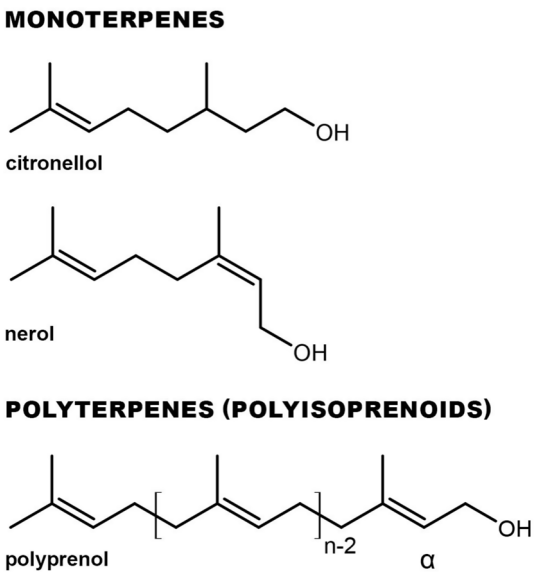


Fig. 1 Structural formulas of selected monoterpenes contributing to rose scent, and polyprenols found in e.g., rose leaves

whereas dolichyl phosphate facilitates N-linked glycosylation of proteins in eukaryotic cells by mediating the transfer of oligosaccharide precursors (Breitling and Aebi 2013). Moreover, the involvement of dolichyl phosphate phosphatase in recycling this lipid carrier after glycosylation has been postulated (Rush et al. 2008).

Beyond glycosylation, accumulating evidence suggests that polyisoprenoids may participate in diverse physiological processes in plants. Although the functions of free (non-phosphorylated) or esterified polyprenols remain incompletely understood, several studies point to their possible roles in plant responses to biotic and abiotic stressors (Bajda et al. 2009; Milewska-Hendel et al. 2017; Baczewska et al. 2014; Dmuchowski et al. 2020; Baczewska-Dąbrowska et al. 2021; Młodzińska-Michta et al. 2023; Weremczuk and Swiezewska (in revision)). Additionally, the expressions of *CPT* genes, and the accumulation of polyisoprenoids, appear to be regulated by environmental cues (Jozwiak et al. 2013; Jozwiak et al. 2017; Buszewicz et al. 2021).

Biophysical and physiological studies have also highlighted a potential role for polyisoprenoids in modulating membrane properties. Free polyprenols and their esters—often present in significant quantities within plant tissues—have been shown to affect membrane fluidity, permeability, and fusion in model systems (Janas et al. 2000; Ciepichal et al. 2011; Hartley and Imperiali 2012). In particular, short-chain Pren 9–11 i.u. have been implicated in regulating the fluidity of chloroplast thylakoid membranes in *A. thaliana* and tomato, also in response to stress factors (Akhtar et al. 2017; Van Gelder et al. 2018; Buszewicz et al. 2021).

Despite polyisoprenoids' structural diversity and potential functional relevance, their role in plant physiology remains largely uncharacterized. The identification of atypically long polyprenols (up to 35 i.u.) in rose leaves (Swiezewska et al. 1992, 1994) raises important questions about their biosynthesis and biological significance. This study addresses this knowledge gap by characterizing polyisoprenoid composition across organs of rose, identifying and functionally analyzing its CPTs for the first time, and investigating how environmental treatment influences their accumulation—laying the groundwork for understanding the specialized roles of these unique lipids in roses.

Materials and methods

Bioinformatics analysis

The nucleotide sequences of eleven *Roseace* genomes (*Rosa agrestis*, *Rosa canina*, *Rosa chinensis "Old blush"*, *Rosa damascena*, *Rosa hugonis*, *Rosa laevigata*, *Rosa lucieae*, *Rosa multiflora*, *Rosa. persica*, *Rosa rugosa*, *Rosa spinosissima*) were obtained from the NCBI Genome database. Genome numbers are listed below: *R. agrestis* (GCA_964662855.1; PacBio, Arima2), *R. canina* (GCA_965119335.1; PacBio, Arima2), *R. chinensis* (GCA_002994745.2; PacBio RSII), *R. damascena* (GCA_001662545.1; Illumina HiSeq), *R. hugonis* (GCA_045280955.1; PacBio), *R. laevigata* (GCA_049191275.1; PacBio), *R. lucieae* (GCA_024704745.1; PacBio Sequel, Oxford Nanopore PromethION, Illumina HiSeq), *R. multiflora* (GCA_002564525.1; Illumina HiSeq2000, Illumina MiSeq), *R. persica* (GCA_047833085.1; PacBio Revio), *R. rugosa* (GCA_958449725.1; PacBio, Arima2), and *R. spinosissima* (GCA_965112335.1; PacBio, Arima2). Since predicted proteome is available only for *R. chinensis* in the Uniprot database (Hibrand Saint-Oyant et al. 2018; Raymond et al. 2018), we decided to perform de novo gene calling for all analyzed *Roseace* for consistency. Gene calling was done with the use of Augustus (Stanke et al. 2008). Resulting protein sequences were scanned with the HMM profile for PFAM PF01255 family of undecaprenyl diphosphate synthases, and obtained results were assessed manually regarding identified domain completeness.

Multiple sequence alignments of *Roseace* CPTs, along with their homologs from *A. thaliana*, *Sacharomyces cerevisiae*, *Giardia lamblia*, *Homo sapiens*, and others, was prepared with MAFFT (local-pair, 1000 iterations) (Katoh and Standley 2013), and the maximum-likelihood phylogenetic tree was constructed using IQTREE2 (Minh et al. 2020) with ultrafast bootstrap (Hoang et al. 2017) and automatic model finder (Kalyaanamoorthy et al. 2017). The tree was visualized with TreeViewer (Bianchini and Sánchez-Baracaldo 2024).

The upstream regions (2.0 kb) of the translation initiation sites (ATG) of *RcCPT* genes, obtained from the Gramene database (Tello-Ruiz et al. 2022), were used as promoter sequences to analyze the *cis*-acting regulatory elements to query the PlantCARE database (Lescot 2002).

Plant materials and growth conditions

The material from English shrub roses "Abraham Darby" and "Goeff Hamilton" was collected from bushes growing in a natural environment in Warsaw, Poland. From "Abraham Darby" roses: leaves, leaf wax, young shoots, and flowers were collected. The flowers were divided into petals and the remaining parts (calyx, pistil, stamens, sepals, etc.). From "Goeff Hamilton" roses: leaves, young shoots, and fruits. The fruits were divided into seeds and the remaining part (pulp). The material was collected to determine the polyisoprenoid profiles.

The cuttings of *R. chinensis* 'Old Blush' (RcOB) plants, obtained from Guillot nursery owner (France), were cultivated in growth chambers under white fluorescent lamps

with a light intensity of 150–200 µmol m^{-2} s^{-1} with 50–60% relative humidity at 21–22 °C during the 16-h day period and at 17 °C during the 8-h night period. Two types of plants were used, 5–6 years old bushes grown indoor (calling later "older") and fresh cuttings of 4–5 weeks old (calling later "younger"). Bushes were cultivated in a soil mix containing 3/10 perlite, 1/10 pozzolan, and 6/10 Klasmann BP-substrate (Klasmann-Deilmann GmbH, Germany), while cuttings were grown only in the Klasmann BP-substrate (growing chamber/phytotron in St Etienne, France). *R. chinensis* plants were used to conduct experiments under altered temperature conditions; leaves and roots were collected for analysis.

The tobacco (*Nicotiana benthamiana*) plants used for transient transformation were grown in green house (Warsaw, Poland) in soil mixed with perlite, at 23 °C under 16-h light/8-h dark cycle until the leaves develop, but no longer than until the flower buds appear.

Treatments

Temperature variation: *R. chinensis* plants growing in the growth chamber were exposed to 4 °C or 30 °C for 72 h. Leaves and roots were collected after 4 h and 72 h of temperature exposure. The collected material was used for RNA and polyisoprenoid isolation.

Light limitation: to minimize environmental fluctuations affecting individual plants and thus potential changes in polyisoprenoid levels, leaves from a single rose shrub growing under natural conditions were used for this experiment. Based on Zhang et al. (2021), three levels of leaf shading of English shrub roses "Abraham Darby" were applied: (i) leaves wrapped in aluminum foil—complete darkness, (ii) leaves wrapped in a paper bag—light shading, and (iii) leaves without wrapping—full sunlight under natural conditions. The leaves were kept wrapped for 72 h, after which they were collected for polyisoprenoid isolation.

Preparation of *RcCPTs* and *RcNUS1* expression construct

Total RNA was isolated from *R. chinensis* leaves and purified using the GeneJET Plant RNA Purification Kit (ThermoFisher Scientific), transcribed to cDNA using RevertAid First Standard cDNA Synthesis Kit (ThermoFisher Scientific). *RcCPTs* and *RcNUS1* CDSs were amplified by PCR using specific primers (Suppl. Table 1). The purified PCR product of *RcCPTs* and *RcNUS1* was subcloned into the pENTR vector according to the manufacturer's instructions (pENTR D-TOPO; Invitrogen). The *RcCPTs* and *RcNUS1* CDS was recombined from the pENTR vector into the destination vectors plant expression Gateway binary vectors (Nakagawa et al. 2007) containing 35S promoter ImpGWB451 (C-terminal G3GFP tag) p651 or p2CA using the Gateway LR Clonase enzyme mix (Invitrogen) according to the manufacturer's protocol.

Transient expression of *RcCPTs* and *RcNUS1* in *N. benthamiana*

The S35::*RcCPTs/RcNUS1*-GFP constructs were introduced into *Agrobacterium tumefaciens* GV3101 strain. The *A. tumefaciens* cultures were grown in liquid LB medium supplemented with 100 mg l^{-1} spectinomycin at 28 °C to an OD_{600} of 1.5, diluted to an OD_{600} of 0.4 in infiltration medium containing 10 mM MES (pH 5.6) 10 mM $MgCl_2$ and 100 µM acetosyringone and infiltrated into the abaxial side of the *N. benthamiana* leaves. Infiltrated leaves were observed using a confocal microscope (2 or 3 days post-infiltration) or harvested for polyisoprenoid extraction (4 or 8 days post-infiltration), respectively.

Real-time PCR analysis of *RcCPTs*/*CPTB* expression level

Total RNA was isolated from younger and older *R. chinensis* roots and leaves using the GeneJET Plant RNA Purification Kit (ThermoFisher Scientific), and then transcribed to cDNA using RevertAid First Standard cDNA Synthesis Kit (ThermoFisher Scientific) according to the manufacturer's procedures. Real-time PCR analysis using *RcCPTs/CPTB* (Suppl. Table 1) *EF1α* (Li et al. 2020) and *GAPDH* (Hou et al. 2022) gene-specific primers and reaction mixture based on Fast SYBR™ Green Master Mix was performed in StepOnePlus™ Real-Time PCR System (Applied Biosystems) according to the manufacturer's instructions.

Polyisoprenoid extraction and purification

Rose leaf wax was removed by dipping the leaves in chloroform, three times for 30 s each. Plant material intended for polyisoprenoid extraction was homogenized using an Ultra-Turrax T25 apparatus (IKA Labortechnik) in a mixture of chloroform:methanol (1:1, *v/v*). Material from *R. chinensis* was additionally supplemented with internal standard of Pren-14 (12 µg) from the Collection of Polyprenols of the Institute of Biochemistry and Biophysics (Warsaw, Poland). Lipid extraction took place for at least 48 h in room temperature in the dark. The extract was filtered, evaporated, dissolved in 5 ml of a mixture of toluene:KOH:ethanol (20:17:3, *v/v/v*) and hydrolyzed for 1 h at 95 °C. Polyisoprenoids were extracted three times with hexane and purified on Silica gel columns (230–400 mesh, Merck Germany). Column was washed with increasing concentration of ether ethyl in hexane (0–20%). Fractions containing polyisoprenoids

(10%) were dissolved in propan-2-ol and analyzed by HPLC/UV as described before (Akhtar et al. 2017).

HPLC/UV analysis of polyisoprenoids

HPLC/UV analysis were performed on a Zorbax Eclipse XDB-C18 (4.6 × 75 mm, 3.5 µm) reverse-phase column (Agilent, USA) using Waters dual-pomp apparatus, Waters gradient programmer, and Photodiode Array detector. For elution of shorter polyisoprenoids, a combination of gradient (from 0 to 75% B for the initial 20 min according to the curve Waters no. 7. and then from 75 to 100% B during the following 11 min. according to the curve Waters no. 6.) was applied. In the last 6 min, re-equilibration back to 0% B was performed. As a solvent A methanol:water (9:1, *v*/*v*) and solvent B methanol:isopropanol:hexane (2:1:1, *v*/*v*/*v*) were used. For elution of longer polyisoprenoids, a combination of convex gradient Waters no. 5, from 0 to 70% B during 39 min and then linear gradient (Waters no. 6.) was applied. As a solvent A methanol:isopropanol:water (12:8:1, *v*/*v*/*v*) and solvent B hexane:methanol (7:3, *v*/*v*) was used. Polyisoprenoids were detected by absorption at 210 nm. The chain length and identity of lipids were confirmed by comparison with external standards of a polyisoprenoids mixture from the Collection of Polyprenols of the Institute of Biochemistry and Biophysics (Warsaw, Poland).

LC–MS analysis of polyisoprenoids

Liquid chromatography–mass spectrometry (LC–MS) analyses were performed using a Thermo Scientific Vanquish UHPLC system coupled to a Thermo Scientific Orbitrap IQ-X mass spectrometer equipped with a heated electrospray ionization (H-ESI) source operating in positive-ion mode.

Liquid chromatography

Metabolite separation was achieved on an Accucore Vanquish C18+ column (50 × 2.1 mm, 1.5 µm particle size) maintained at 35 °C. The autosampler temperature was set to 10 °C, and the flow rate was 0.4 ml min^{-1}. The mobile phases consisted of solvent A (90% methanol in water) and solvent B (methanol:isopropanol:hexane, 5:4:1, *v*/*v*/*v*). The needle wash solvent was isopropanol. The 25-min chromatographic gradient was as follows: 70% A and 30% B at 0 min; linear decrease to 0% A and 100% B over 15 min; held at 0% A and 100% B until 22 min; returned to initial conditions (70% A, 30% B) at 23 min; and equilibrated at 70% A, 30% B until 25 min. UV spectra were recorded with a photodiode array detector scanning from 190 to 800 nm.

Mass spectrometry

The Orbitrap IQ-X was operated in full-scan positive-ion mode with a resolving power of 60,000 (at *m*/*z* 200). The spray voltage was set to 3.5 kV, sheath gas to 50 a.u., auxiliary gas to 10 a.u., and sweep gas to 2 a.u. The ion transfer tube and vaporizer temperatures were both 350 °C. Internal mass calibration was performed using EASY-IC. Data were acquired in profile mode over an *m*/*z* range of 190–2000. The automatic gain control (AGC) target was 4×10^5, with a maximum injection time of 50 ms and one microscan per spectrum. The RF lens was set to 35%.

Confocal microscopy

For subcellular localization analysis of RcCPTs and RcNUS1, cd3-954 vector for endoplasmic reticulum localization was used (Nelson et al. 2007). Confocal images were taken under a Nikon C1 confocal system built on TE2000E with 488 and 543 nm laser excitations for ER marker (450/35 nm emission filter), and GFP (515/30 nm emission filter), respectively.

Results

Examination of the polyisoprenoid profile in rose organs

To verify the results obtained 30 years ago (Swiezewska et al. 1992, 1994), the polyisoprenoid composition in roses growing under natural conditions was analyzed. To assess whether the previously reported occurrence of very long-chain polyisoprenoids (up to 35 i.u.) in rose leaves could be confirmed using modern analytical techniques, HPLC/UV was applied—offering greater sensitivity and resolution than the methods available 3 decades ago. An English shrub rose cultivar was selected somewhat arbitrarily as a representative species to preliminarily test the presence of long-chain polyisoprenoids. It was found that very long-chain length polyisoprenoids (family: 27–50 i.u.) were most abundant in leaves and young shoots (Fig. 2a). Only trace amounts were detected in fruits. Medium-chain length polyisoprenoids (11–26 i.u.) were also found predominantly in leaves and shoots; however, their presence was also recorded in flowers, fruits, and seeds (Fig. 2a). No polyisoprenoids were detected in the leaf wax (Fig. 2a).

To determine whether the polyisoprenoids present in rose leaves are polyprenols or dolichols, an LC–MS analysis was performed. Unfortunately, due to the lack of appropriate standards, it was not possible to distinguish between polyprenols and dolichols in family of very long-chain polyisoprenoids. However, the analysis revealed that the

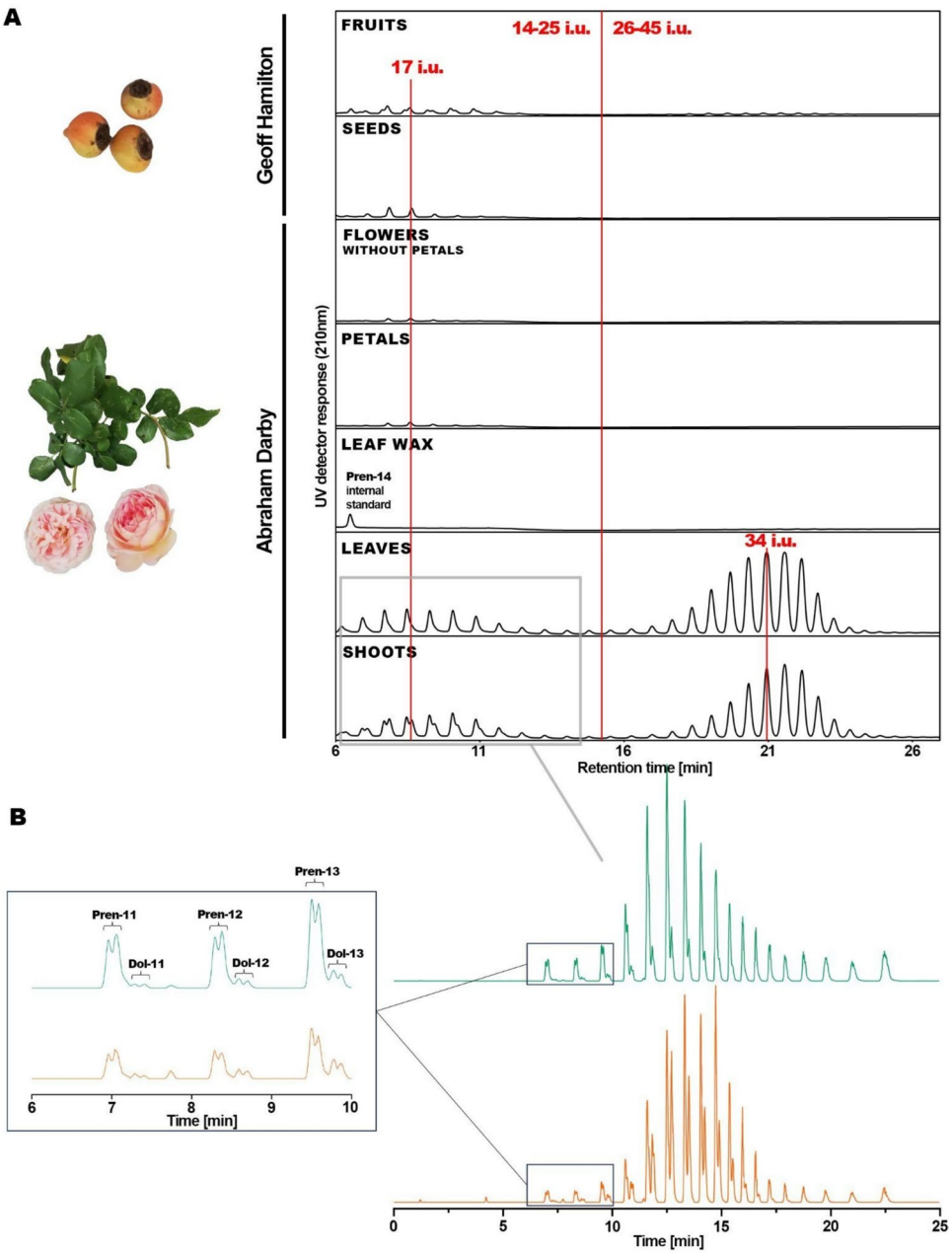


Fig. 2 HPLC/UV chromatograms of polyisoprenoids extracted from English shrub roses growing under natural conditions: **A** rosa 'Geoff Hamilton'—fruits and seeds; rosa 'Abraham Darby'—flowers (without petals), petals, leaf surface wax (Pren-14 indicated—internal standard added), leaves, and shoots. HPLC/UV method for longer compounds identification was used. Representative chromatograms are shown. **B** LC–MS analysis of selected polyisoprenoid families in leaves and shoots of the English shrub rose Rosa 'Abraham Darby'. Polyprenols and dolichols of the same chain length are indicated

polyisoprenoid family ranging from 11 to 26 i.u.—detected predominantly in leaves but also present in other organs—consists of polyprenol–dolichol pairs of identical chain length (Fig. 2b). The polyprenol-to-dolichol ratio varies depending on the polyisoprenoid chain length. The Dol-16 being the dominant species within the dolichol family. Interestingly, in polyprenol–dolichol pairs, a higher amount of dolichol was observed in shoots than in leaves (Fig. 2b).

Since *R. chinensis* was also used in the study, the polyisoprenoid profile in *R. chinensis* leaves and roots was also examined. In roots of the *R. chinensis* family of polyisoprenoids, 15–21 i.u. were detected (Fig. 3b). The following families in leaves were observed: 7–9 i.u. (Fig. 3a), 15–27 i.u., and, interestingly, two families of very long polyisoprenoids, 28–34 i.u. and 35–50 i.u. (Fig. 3b). The dominant polyisoprenoids within these families, as well as the ratios of families with different chain lengths, showed slight variation between individual plants these were vegetatively propagated offspring from the same parent plant (Fig. 3b). This analysis shows also that some polyisoprenoids cannot be rigidly assigned to a specific family. For instance, the 34 i.u. polyisoprenoid appears in family 28–34 i.u. in one chromatogram and in family 35–50 i.u. in another (Fig. 3b). This indicates that the number of family members is flexible and depends on the analyzed

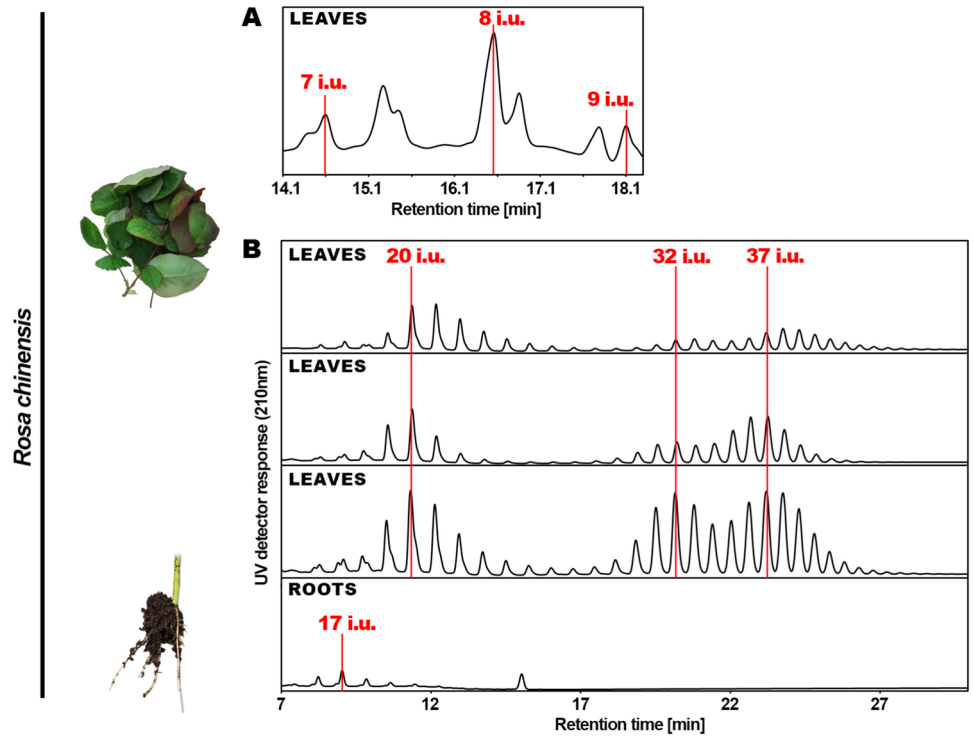


Fig. 3 HPLC/UV chromatograms of polyisoprenoids extracted from *R. chinensis*: **A** leaves—a fragment of the chromatogram is shown, presenting peaks corresponding to 7–9 i.u.; HPLC/UV method for shorter compounds identification was used (**B**) leaves of *R. chinensis* obtained from individual plants vegetatively propagated from the same parent plant. In *R. chinensis* leaves, the relative proportions among different polyisoprenoid families vary, as does the predominant polyisoprenoid within each family. Roots of *R. chinensis*—following the family dominated by the polyisoprenoid with 17 isoprene units. Additionally, an unidentified single peak is observed, characterized by a retention time of 15.031 min. HPLC/UV method for longer compounds identifications was used. Representative chromatograms are shown

material; thus, the chain-length ranges (i.u.) used in this study should be regarded as approximate.

The results concerning polyisoprenoids in rose leaves indicate that very long-chain polyisoprenoids (27–50 i.u.) are indeed present; however, depending on the species, polyisoprenoids with this chain lengths are synthesized either as a single homologous family (English rose) or as two distinct families (*R. chinensis*), each characterized by a different dominant polyisoprenoid.

Identification and phylogenetic analysis of *RcCPTs* in *R. chinensis*

The aim was to identify which CPT in rose are responsible for the formation of 27–50 i.u. long-chain length polyisoprenoids. At the time when these studies were initiated, *R. chinensis* was the only rose species with a sequenced genome. Based on similarity to all 9 *CPTs* from *A. thaliana*, three *RcCPTs* and one *RcCPTB*, named after *NUS1*, were identified in the *R. chinensis* genome (Table 1).

During the course of this project, additional genomic sequences of various rose species became available. Based on the newly obtained sequences, homologs of *cis*-prenyltransferases and *cis*-prenyltransferase-binding proteins were identified in other rose species with sequenced genomes. Homologs of CPT1, CPT2, CPT3, and NUS1 were identified in the genomes of all analyzed rose species, except for *R. damascena*, in which no CPT1 homolog was detected. Interestingly, while no CPT2 homolog was found in the genome of *R. lucieae*, two distinct NUS1 homologs were identified in this species. Based on sequence similarity, the rose CPT and CPTB were classified into three distinct groups, as shown in Fig. 4b.

The nucleotide sequences of the identified *CPTs* from roses were compared to previously described *CPTs* from other plant species, as well as to *CPTs* from *S. cerevisiae* (*Rer2*, *SRT*, *Nus1*), *G. lamblia* (*UPPS*), and *H. sapiens* (*NgBR*, *DHDDS*). The sequence relationships among these proteins are presented in the form of a phylogenetic tree (Fig. 4a). Notably, *RcCPT2* shows the highest sequence similarity to *AtCPT7* from *A. thaliana* (At5g58770). In contrast, *RcCPT1* and *RcCPT3* exhibit the greatest similarity to *CPTs* from *Solanum lycopersicum* (tomato), *Parthenium argentatum* (guayule), *Taraxacum* (dandelion), and *Lactuca sativa*

Table 1 The *RcCPTs* and *RcCPTB* (*RcNUS1*) genes/protein members identified and characterized in present study in *R. chinensis*

Gene name	Gene accession	Protein ID	Locus	CDS length (bp)	Prot. length (aa)	Chr	Cellular localization	*N. benthamia* poly-isoprenoids products	Potential products in *Rose* sp.
RcCPT1	XM_024312137.2	XP_024167905.1	LOC112174381	873	290	6	ER	13–16 i.u. co-expressed with RcNUS1	15–26 i.u.
RcCPT2	XM_024304706.2	XP_024160474.1	LOC112164067	933	310	5	Chloroplast	7–9 i.u.	7–9 i.u.
RcCPT3	XM_024326962.2	XP_024182730.1	LOC112187978	1146	381	2	ER	14–21 i.u. co-expressed with RcNUS1	> 26 i.u.
RcNUS1	XM_024305672.2	XP_024161440.1	LOC112168761	774	257	6	ER	–	–

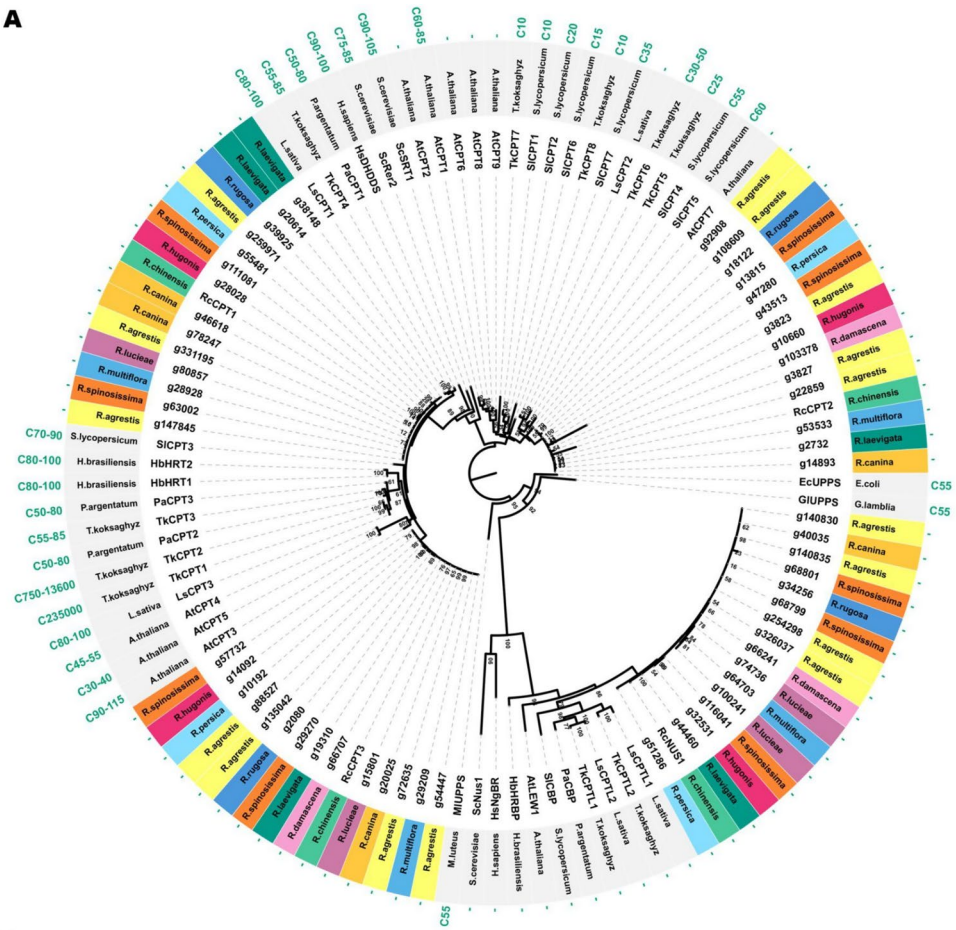


Fig. 4 **A** Maximum-likelihood phylogenetic tree of the Rosa CPT/CPTB (labeled in colors) with CPT/CPTB from other plant species. Bootstrap values are shown on the corresponding branch nodes; these are tree topology confidence estimates indicating how often a specific clade appears upon resampling the data. The lengths given in carbon atoms of the formed polyisoprenoids by a specific CPT were given. **B** A summary of the CPTs and CPTBs found in the rose genomes. The classification was based on similarity to the CPT/CPTB from *R. chinensis*. The numbers next to the plant names indicate the number of copies of the respective gene found in the genome of each rose species

(lettuce) producing medium-chain polyprenols. It is worth emphasizing that *RcCPT1* and *RcCPT3* do not cluster within the clade containing *CPTs* known to be involved in natural rubber-length polyisoprenoids biosynthesis, as depicted in the phylogenetic tree (Fig. 4a).

A protein domain analysis of rose CPTs was conducted. Five domains, defined for CPTs, were identified (Grabińska et al. 2017). The presence of the amino acid motif RXG was observed in RcCPT2 and RcNUS1 (Fig. 5b). No RXG motif was detected in CPT1 and CPT3, suggesting that these are heteromeric *cis*-prenyltransferases (Fig. 5b) (Grabińska et al. 2016).

Subcellular localization and polyisoprenoids' products of RcCPTs

The cellular localization of RcCPTs was determined based on transient transformation of tobacco leaves. The pattern of green fluorescence for CPT2-GFP matches the fluorescence pattern observed for chloroplasts (with an excitation wavelength of 543 nm and emission wavelengths of 605–675 nm, which correspond to chlorophyll autofluorescence), indicating that CPT2 is most likely localized in the chloroplasts. In contrast, the green fluorescence pattern for CPT1-GFP and CPT3-GFP closely resembles the blue fluorescence pattern of the ER marker, suggesting that these proteins are likely localized in the endoplasmic reticulum (Fig. 6). Both program ChloroP 1.1 and program TargetP-2.0 indicate that CPT2 contains a chloroplast-targeting signal peptide at the beginning of the sequence, whereas CPT1, CPT3, and NUS1 do not (Fig. 5a).

Potential polyisoprenoid products of rose CPTs were identified based on transient tobacco transformation. When RcCPT2 was used for tobacco leaf transformation, additional lipid products corresponding to polyprenols of 7–9 i.u. were obtained (Fig. 7c). No products were detected when RcCPT1 or RcCPT3 were transformed individually (Suppl. Fig. 1b, c). However, when pairs of RcCPT1 and RcNUS1 or RcCPT3 and RcNUS1 were used, additional lipid products were observed. For the CPT1–NUS1 pair, the polyisoprenoids had chain lengths of 13–19 i.u. (Fig. 7b), while for the CPT3–NUS1 pair, polyisoprenoids with chain lengths of 13–22 i.u. were detected (Fig. 7d). No additional products were detected when tobacco leaves were transformed solely with NUS1 (Suppl. Fig. 1d). No products corresponding to the 27–50 i.u. family were obtained for any of the RcCPTs upon transient expression in tobacco.

Characterization of *cis*-acting elements in promoter regions of *RcCPTs*

To further investigate the potential regulatory factors of polyisoprenoid biosynthesis in *R. chinensis*, *cis*-acting elements in the promoter regions of various *CPTs* were analyzed. The analysis revealed the presence of several stress-related, hormone-related, light-related, and development-related *cis*-elements in the promoter regions of *RcCPT1*, *RcCPT2*, *RcCPT3*, and *RcNUS1* (Fig. 8).

Fig. 5 **A** Predicted chloroplast-targeting peptide in RcCPT2; the numbers of first and last residue are provided. **B** Amino acid sequence alignment of the CPT/CPTB orthologs (*S. cerevisiae*: Rer2 P35196 and Srt1 Q03175, *A. thaliana*: CPT7 At5g58770 and LEW1 At1g11755, *R. chinensis* CPT1 XP_024167905.1, CPT2 XP_024160474.1, CPT3 XP_024182730.1, and NUS1 XP_024161440.1). Conserved protein domains are highlighted. The numbers of residues omitted from the alignment are given in parentheses. Residues conservation: yellow—uncharged, gray—polar. RXG motif and its NXG counterpart are marked in red. Predicted secondary structure elements are marked above the corresponding alignment blocks (*H* α-helices, *E* β-strands)

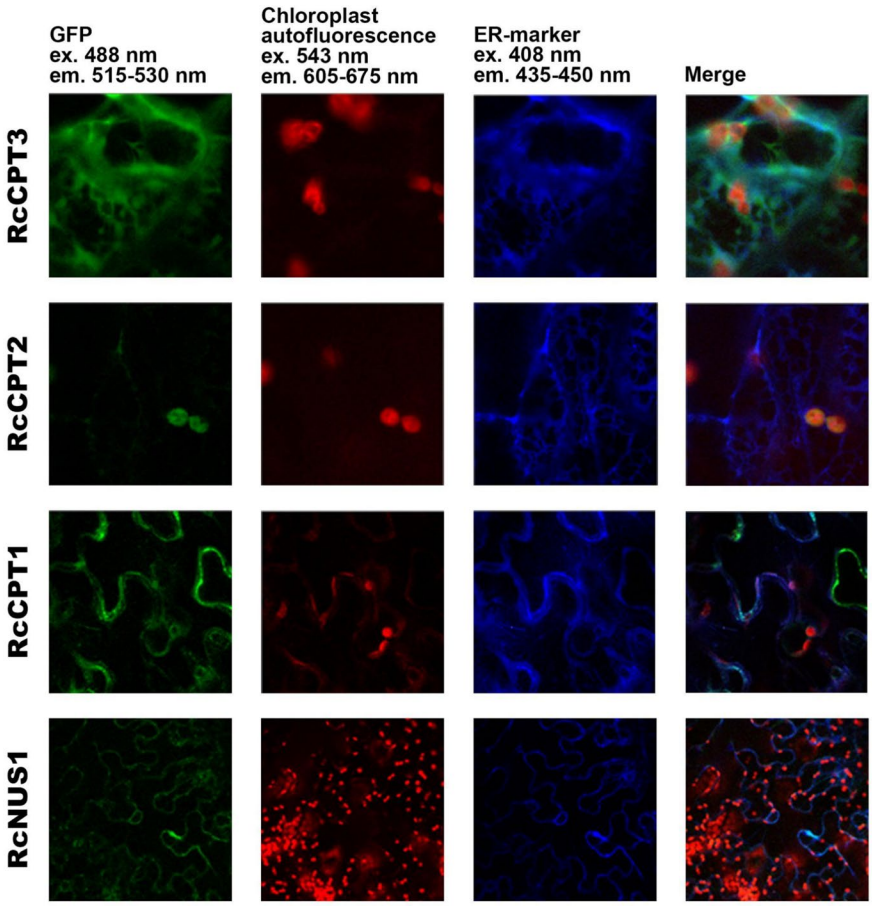


Fig. 6 Representative pictures of the subcellular localization of the RcCPT1, RcCPT2, RcCPT3, and RcNUS1 proteins containing GFP-tags. The green color in the images represents GFP fluorescence, which indicates the localization of the CPTs proteins. The red color corresponds to chlorophyll autofluorescence, thus indicating the localization of chloroplasts. The blue color represents the ER-cb/CD3-954, which points to the endoplasmic reticulum localization. Microscopic observations were made on the lower epidermis of transiently transformed tobacco leaves. Representative images were selected

In terms of stress-related *cis*-elements, several elements involved in plant responses to stress were detected. The TC-rich repeats, associated with defense and stress responsiveness, were identified in the promoters of *RcCPT2*, *RcCPT3*, and *RcNUS1*.

Hormone-related *cis*-elements were also abundant. The TGACG-motif, known for its role in methyl jasmonate (MeJA) responsiveness, was found in the promoters of *RcCPT1*, *RcCPT2*, and *RcCPT3*, with the highest number observed in *RcCPT3* (14 occurrences). Additionally, the ARE element (essential for anaerobic induction) was identified in *RcCPT1* and *RcNUS1*, and the ABRE (abscisic acid responsiveness) element was present in all four genes, though its frequency varied across the genes.

Development-related *cis*-elements were identified as well, with the TATA-box and CAAT-box being highly conserved in all promoters. The analysis reveals that the highest number of records is found in the "Development" category, indicating potential involvement of the *RcCPTs*.

Light-related *cis*-elements, such as the TCT-motif, ATC-motif, and GT1-motif, were notably highly present in the promoters of *RcCPT1*, *RcCPT2*, and *RcCPT3*, suggesting that these genes might be involved in the regulation of light response.

These findings suggest that the regulation of *RcCPTs* might be influenced by a combination of stress, hormonal, and light-related signaling pathways, with significant roles in plant development.

Expression patterns of *RcCPTs* under cold and heat treatments

Considering the results from promoter analyses and the evidence suggesting that polyisoprenoids may be involved in plant responses to temperature variation, the expression profiles of *RcCPT*s were examined in the leaves of *R. chinensis*. In younger plants exposed to low temperature, a decrease in the expression of *CPT3* and *NUS1* was observed 72 h after the onset of exposure (Fig. 9b, c), relative to optimal conditions, while no change was noted for *CPT1* (Fig. 9a). In plants subjected to 30 °C for 72 h, a slight increase in the expression of *CPT3* and *NUS1* was detected (Fig. 9b, c), but *CPT1* remained unchanged (Fig. 9a). Conversely, for the *CPT1* gene, an increase in expression was recorded in control conditions after 72 h, relative to the "zero time" measurement point (Fig. 9a).

In older *R. chinensis* plants, an increase in *CPT1*, *CPT3*, and *NUS1* expression levels was observed 4 h after the onset

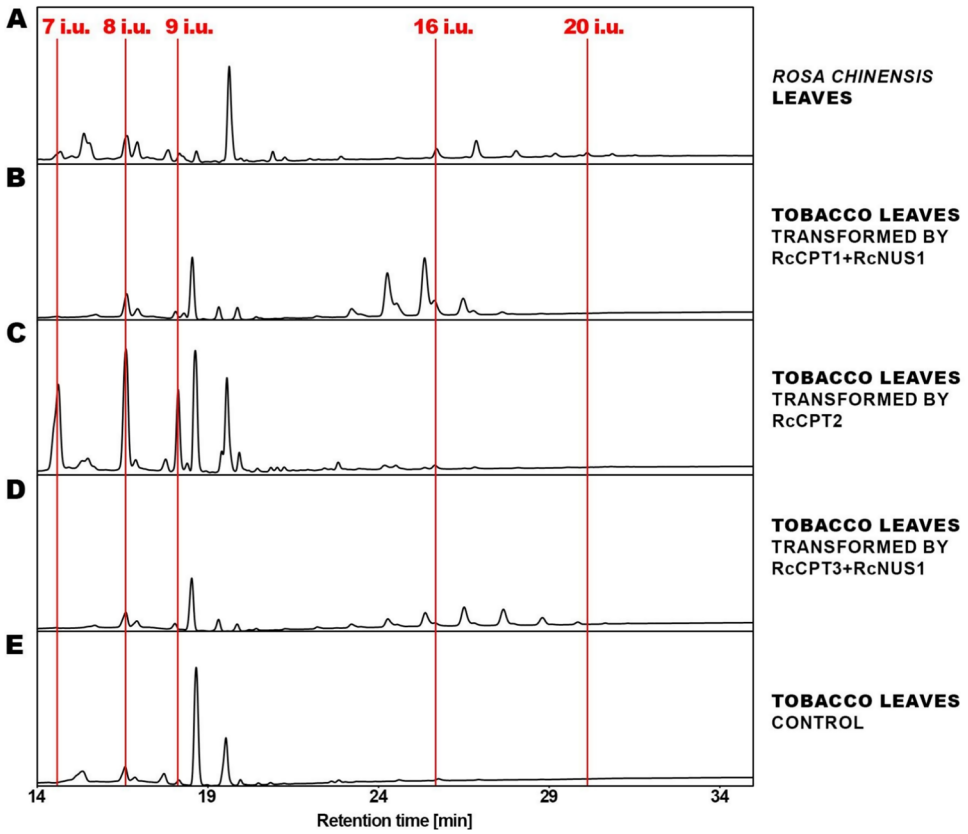


Fig. 7 Polyisoprenoids extracted from the *R. chinensis* leaves and tobacco leaves transiently transformed by *R. chinensis* CPTs and NUS1. HPLC/UV method for shorter compounds identification was used. Representative HPLC/UV chromatograms are presented and the main polyisoprenoids are indicated

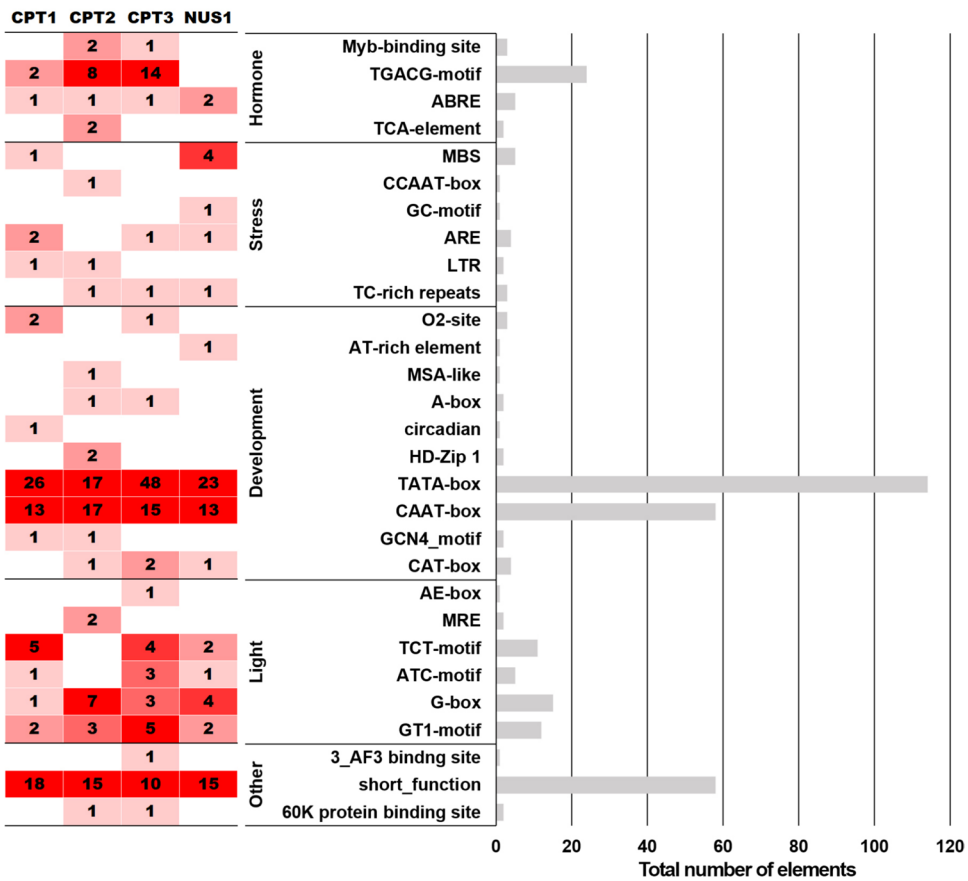


Fig. 8 Various *cis*-acting elements in the promoter (2.0 kb upstream of the translation initiation sites) of RcCPTs genes. The statistics of total number of RcCPTs contain various *cis*-acting elements

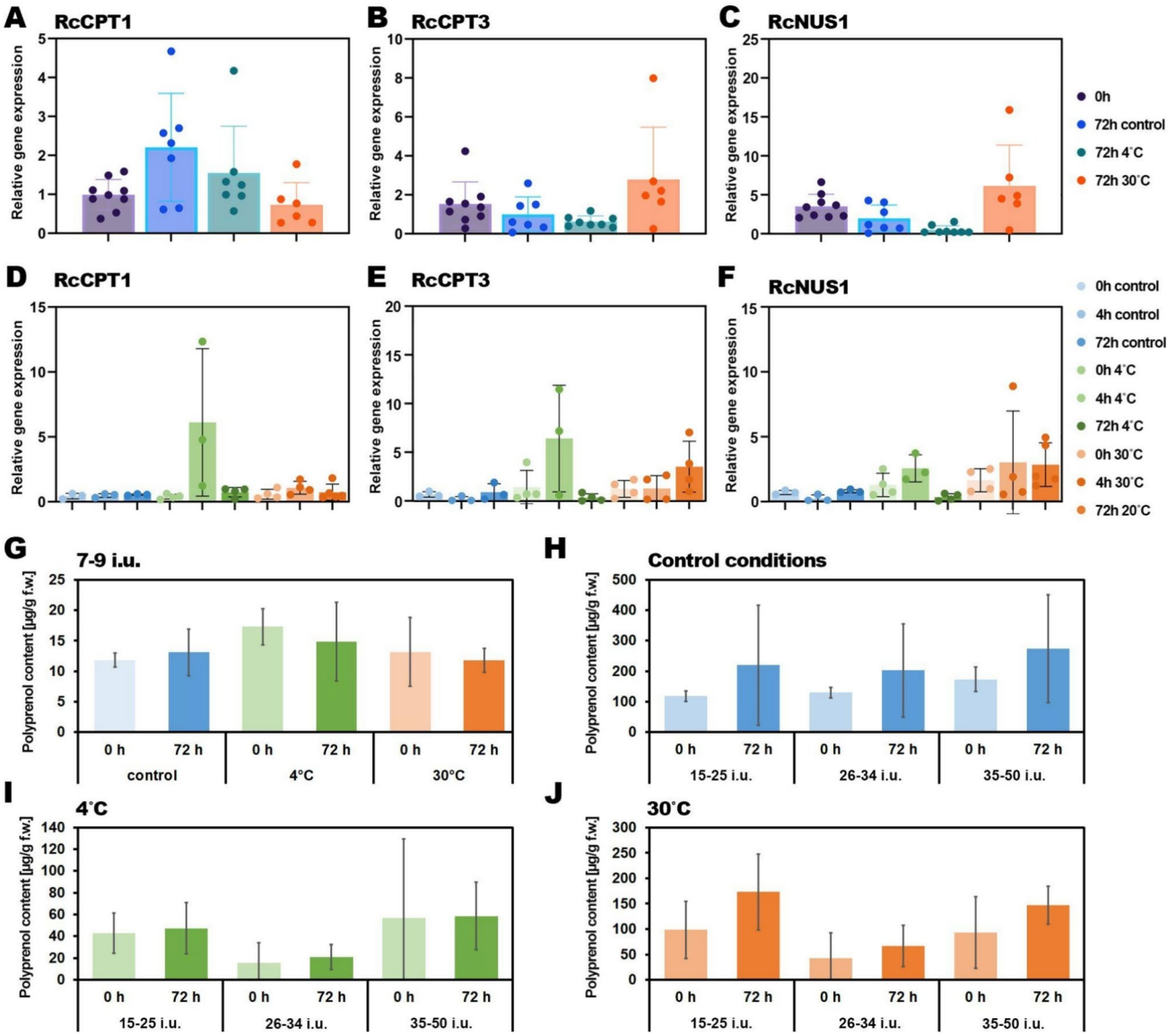


Fig. 9 Gene expression and polyisoprenoid content in *R. chinensis* leaves. Upper panel: *RcCPTs* and *RcNUS1* expression in *R. chinensis* leaves, from plants grown under control conditions and under 4 °C and 30 °C stress conditions. Panel **A**–**C** shows the results for small plants and panel **D**–**F** for large plants. Lower panel: polyprenols content in *R. chinensis* leaves from older plants, **G** polyprenols 7–9 i.u.; **H** longer polyprenols from plants grown under control conditions and **I** under 4 °C and **J** 30 °C stress conditions. All values are presented as mean ± SD ($n \geq 3$)

of cold exposure (Fig. 9d–f). After 72 h, a decrease relative to the 4-h time point was detected for all three genes (Fig. 9d–f). For *CPT3* and *NUS1*, individual plants showing elevated expression after both 4 and 72 h of high-temperature treatment were also observed, although variability among biological replicates was relatively high (Fig. 9e, f).

The expression of *RcCPTs*/*CPTB* in *R. chinensis* roots was also examined. No expression was detected for *CPT3* and *NUS1* in roots. The *CPT1* gene was expressed, but its level remained unchanged under temperature treatments (data not shown).

Reliable data for *CPT2* expression could not be obtained due to difficulties in designing specific primers for this gene.

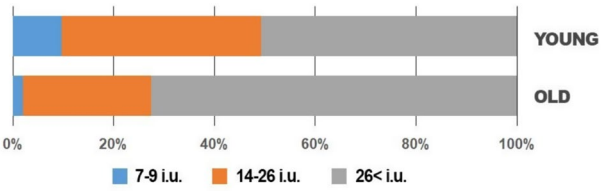
Polyisoprenoid content in leaves of *R. chinensis* in response to abiotic factors

Given previous reports indicating that the level of plastidial polyisoprenoids is heat stress-dependent and that polyisoprenoids may modulate membrane fluidity in response to stress (Akhtar et al. 2017, Van Gelder et al. 2018; Buszewicz et al. 2021), the content of polyisoprenoids

in older *R. chinensis* plants was assessed in response to temperature treatment. The results obtained were categorized into four groups based on the length of the analyzed polyisoprenoid chains. No significant differences were observed in the content of polyisoprenoids within the 7–9 i.u. family between the "zero time" samples and those collected after 72 h, both under control conditions and temperature treatments (Fig. 9g). Similarly, no differences were found for the 15–25 i.u., 26–34 i.u., and 35–50 i.u. families (Fig. 9h–j). However, for the mentioned medium-length and very long polyisoprenoid families, considerable differences in content were observed between individual plants growing under the same conditions.

Considering that promoter analysis revealed that CPTs responsible for the elongation of polyisoprenoid chains may be regulated by changes in plant exposure to light, it was decided to further investigate whether such changes would affect the polyisoprenoid content in rose leaves. Leaves from an English rose "Abraham Darby" shrub growing under natural conditions were used for the study. To minimize changes in the environment impacting individual plants, which may in turn influence the levels of polyisoprenoids, all leaves used in the experiment were obtained from the same shrub. No significant changes in the polyisoprenoid content were observed between leaves grown under shading and those exposed to sunlight (Suppl. Fig. 2). However, similar to the results obtained in the temperature treatment experiment, it was noted that the content of short-chain (7–9 i.u.) polyisoprenoids showed minimal variation between individual leaves from the same conditions, while the content of very long (> 26 i.u.) polyisoprenoids exhibited considerable variation among leaves from the same treatment (Fig. 9g–j).

To investigate the potential causes of the substantial variation in the content of very long polyisoprenoids, the polyisoprenoid content in young versus old rose leaves was examined. The content of polyisoprenoid families of various chain lengths was compared in leaves that were approximately 2 weeks old, those that appeared at the beginning of the spring season, and old leaves that had been present on the shrub since the previous season. Both young and old leaves were collected from the same English rose "Abraham Darby" shrub. In young leaves, the ratio between the contents of polyisoprenoids of different chain lengths was 1:4:5 (shortest 7–9 i.u.: medium 15–26 i.u.: longest > 26 i.u.) (Fig. 10). In old leaves, the ratio was 1:13:37 (shortest:medium:longest) (Fig. 10). Over the course of the leaf's life, the content of 7–9 i.u. polyprenols increased by 6.5-fold, medium-length 15–26 i.u. polyprenols by approx. 20-fold, and the longest > 26 i.u. polyprenols by approx. 44-fold. This indicates that the content of > 26 i.u. long polyisoprenoids in rose leaves is most strongly dependent on leaf age.



Content [µg/g f.w.]	Old leaves	Young leaves	Fold change
7-9 i.u.	35.42	5.44	6.5x
14-26 i.u.	439.27	22.26	19.73x
26< i.u.	1252.12	28.60	43.78x
Total	1726.82	56.30	30.67x

Fig. 10 Distribution of total polyisoprenoids content in young (about 2 weeks old) versus old (about 12 months old) leaves of English shrub rose "Abraham Darby" which grew in a natural environment

Discussion

Despite extensive research on terpenoids in roses, the polyisoprenoid fraction remains poorly characterized in terms of biosynthesis, subcellular localization, and biological function. Our study provides new insight into both the diversity of polyisoprenoid families and the *cis*-prenyltransferases responsible for their synthesis in roses.

Polyisoprenoids of 7–9 i.u. and the potential role of RcCPT2

The shortest detected compounds in rose, exclusively in leaves, corresponding to the 7–9 i.u. family (Fig. 3a), are comparable in length to polyprenols 9–11 i.u found in the leaves of *A. thaliana*, where they are known to accumulate in chloroplast membranes. Such short-chain polyprenols have been proposed to modulate membrane fluidity and stability, particularly under changing environmental conditions (Akhtar et al. 2017; Van Gelder et al. 2018; Buszewicz et al. 2021). It is therefore plausible that the 7–9 i.u. polyisoprenoids detected in rose leaves may play a similar structural role in the thylakoid membranes.

In this study, a chloroplast-localized *cis*-prenyltransferase, RcCPT2, was identified as the enzyme responsible for the formation of this 7–9 i.u. family in *R. chinensis* (Figs. 6, 7c). RcCPT2 is a homomeric CPT containing an RXG motif, expressed exclusively in leaves, which corresponds well with the tissue distribution of the 7–9 i.u. polyisoprenoids. Phylogenetic analysis revealed that RcCPT2 is homologous to other chloroplast CPTs, such as AtCPT7 from *A. thaliana*, SlCPT4 and SlCPT5 from *S. lycopersicum*, TkCPT5 and TkCPT6 from *T. koksaghyz*, and LsCPT2 from *L. sativa* (Fig. 4a). These enzymes are known to produce polyprenols of similar lengths—typically Pren-9 to Pren-11 (Akhtar et al. 2012, 2017; Müller et al. 2025).

Polyisoprenoids of 15–26 i.u. and the potential role of RcCPT1

A distinct family of polyisoprenoids with chain lengths of 15–26 i.u. was detected in all analyzed organs of roses (Figs. 2, 3). Within this group, both polyprenols and dolichols were identified (Fig. 2b). Similar-length dolichols (13–16 i.u.) have been reported in *A. thaliana*, where they function as glycosyl carrier lipids in N-glycosylation within the endoplasmic reticulum (Lucas and Waechter 1976; Gawarecka et al. 2021). The detection of 15–26 i.u. polyisoprenoids in roses suggests an analogous role in glycoprotein biosynthesis.

The biosynthesis of this family in *R. chinensis* appears to be mediated by the heteromeric *cis*-prenyltransferase RcCPT1. RcCPT1 is localized in the endoplasmic reticulum and, when expressed alone in *N. benthamiana* leaves, does not produce additional polyisoprenoid products (Fig. 7, Suppl. Fig. 2b). However, co-expression with the CPT-binding partner *RcNUS1* results in the formation of polyisoprenoids of 13–18 i.u. (Fig. 7b), confirming that RcCPT1 functions as a heteromeric enzyme complex. RcCPT1 is expressed both in leaves and roots, which corresponds with the detection of 15–21 i.u. polyisoprenoids as the sole family present in root tissues. These findings indicate that RcCPT1, together with RcNUS1, likely catalyzes the synthesis of medium-length polyisoprenoids.

Polyisoprenoids > 26 i.u. and the potential role of RcCPT3

In roses, the longest polyisoprenoids detected ranged from 27 to 50 i.u., predominantly in leaves and young shoots (Fig. 2a). These molecules are longer than the polyisoprenoids typically associated with membranes yet much shorter than those forming natural rubber. Earlier studies on *Rosaceae* leaves reported polyisoprenoids up to 35 i.u. (Swiezewska et al. 1992, 1994); however, our data extend this range to 50 i.u. Such "intermediate-length" polyisoprenoids have not been functionally characterized, but their organ-specific distribution suggests specialized roles in leaf or shoot physiology.

The synthesis of these > 26 i.u. polyisoprenoids in *R. chinensis* is likely associated with RcCPT3, a heteromeric *cis*-prenyltransferase also localized in the endoplasmic reticulum (Fig. 6). RcCPT3 alone does not produce detectable products when transiently expressed in *N. benthamiana*, but co-expression with *RcNUS1* results in the formation of polyisoprenoids of 14–21 i.u. (Fig. 7d, Suppl. Fig. 2c), suggesting that the enzyme complex is catalytically active yet may require additional, as-yet-unidentified cofactors to achieve the synthesis of chains longer than 26 i.u.. *RcCPT3* expression is confined to leaves, consistent with the occurrence of very long polyisoprenoids in this organ.

Interestingly, in the leaves of *R. chinensis*, we observed that polyisoprenoids longer than 26 i.u. form two distinct families (Fig. 3b). This phenomenon was not observed in English roses, where only a single family of long-chain polyisoprenoids is present (Fig. 2a). Until now, it has been generally assumed that each *cis*-prenyltransferase is responsible for the synthesis of a single polyisoprenoid family or, one component. In *R. chinensis*, however, we report a situation in which the number of polyisoprenoid families exceeds the number of identified CPTs—a scenario that, to our knowledge, has not been described in any other organism before. This observation raises the question of what additional mechanisms might be involved in determining or extending polyisoprenoid chain length?

Previous studies have demonstrated that polyisoprenoid chain length may depend not only on the CPT enzyme itself but also on accessory proteins, the nature of the allylic diphosphate primer, and substrate availability (Takahashi and Koyama 2006; Qu et al. 2015; Lakusta et al. 2019). The allylic diphosphate primer (e.g., dimethylallyl diphosphate, geranyl diphosphate, farnesyl diphosphate, or geranylgeranyl diphosphate) that initiates the polymerization reaction plays a role in the ultimate chain length (Takahashi and Koyama 2006; Akhtar et al. 2012; Müller et al. 2025). Different CPTs exhibit distinct substrate preferences, influencing the resulting product profile. For example, some CPTs prefer farnesyl diphosphate (C15) as a primer, while others prefer dimethylallyl diphosphate (C5) (Kutsukawa et al. 2022). Interestingly, in some experimental settings, an excess of substrates (IPP or FPP) has been observed to lead to the production of shorter polyisoprenoid chains (Kharel and Koyama 2002). Further investigation will be necessary to elucidate the exact mechanism underlying the formation of two distinct polyisoprenoid families in *R. chinensis*, most likely mediated by RcCPT3. Nonetheless, this discovery broadens the conceptual framework of CPTs activity and challenges the long-standing assumption of a one-enzyme–one-family relationship.

Function of polyisoprenoids in roses

Polyisoprenoids are best known and most thoroughly studied for their essential role as lipid carriers in protein glycosylation, a fundamental process conserved across all domains of life. However, this canonical function is universal and not the focus of the present study. Instead, we are interested in the presence of unusually long-chain (> 26 i.u.) polyisoprenoids in roses, which suggests additional, potentially unique biological roles beyond glycosylation.

Roses are perennial plants exposed repeatedly to fluctuating and often unfavorable temperature conditions throughout

their lifespan. It is plausible that these > 26 i.u. long-chain polyisoprenoids contribute to the plant's ability to withstand temperature treatment, possibly by modulating membrane properties.

Various studies have implicated polyisoprenoids in plant responses to temperature stress, although the effects appear species—and context-dependent. In *A. thaliana*, heat stress (38 °C) upregulates the *cis*-prenyltransferase *CPT7* via Heat Shock Transcription Factors (HSFA1), increasing polyprenol content (Pren-9 to Pren-11) in leaves (Buszewicz et al. 2021). Conversely, in cycads such as *Cycas multipinnata* and *Cycas panzhihuaensis*, heat stress did not alter polyprenol levels, and cold stress responses varied between species (Zhu et al. 2022; Zheng et al. 2021). In *C. geoides*, lower temperatures induce dolichol accumulation in root cultures (Skorupińska-Tudek et al. 2007).

Our data show that in *R. chinensis*, neither low (4 °C) nor elevated (30 °C) temperatures led to changes in polyisoprenoid content or *RcCPT*s and *RcNUS1* gene expression in leaves (Fig. 9a–f). Trends suggesting mild downregulation of *RcCPT3* and *RcNUS1* under cold treatment, and slight upregulation under 30 °C, were overshadowed by high variability among individual plants, indicating that other intrinsic or environmental factors may predominate in regulating polyisoprenoid biosynthesis in roses.

Light influences isoprenoid metabolism through activation of the MEP pathway, promoting polyprenol and pigment accumulation while repressing dolichol and phytosterol biosynthesis (Lipko et al. 2023). Furthermore, light-generated reactive oxygen species (ROS), especially singlet oxygen, have been reported to increase polyisoprenoid levels in plants (Komaszylo Née Siedlecka et al. 2016). In our experiments, reducing sunlight exposure did not alter polyisoprenoid levels in rose's leaves. Yet, similar to temperature treatment results, > 26 i.u. long polyisoprenoids showed high variability among leaves from identical light conditions, suggesting that factors other than light intensity critical determinants of polyisoprenoid accumulation.

Building on this, a particularly noteworthy finding relates to the age-dependent accumulation patterns of polyisoprenoids in rose leaves. It is not only the overall increase in their content that is striking, but also the markedly different rates at which polyisoprenoids of various chain lengths accumulate. While total polyisoprenoid levels increased more than 30-fold from young (2 weeks) to old (12 months) leaves, very long polyisoprenoids (> 26 i.u.) rose almost 44-fold, in contrast to shorter chains (7–9 i.u.), which increased approximately 6.5-fold (Fig. 10).

Age-related accumulation is well-known observations in other organisms (Swiezewska et al. 1994), though the mechanisms governing polyisoprenoid recycling or degradation remain unknown. No enzymes responsible for their breakdown have been identified. This suggests that polyisoprenoids accumulate throughout the organism's life, potentially reflecting their involvement in long-term physiological processes. The differential accumulation of polyisoprenoids of varying chain lengths raises intriguing questions about their distinct biological roles. The markedly higher increase of very long polyisoprenoids may relate to specialized functions in mature leaves, such as enhanced protection against oxidative stress or membrane stabilization, which could be particularly important in perennial plants like roses facing recurring environmental challenges.

To fully elucidate how polyisoprenoids contribute to stress adaptation and leaf physiology, detailed knowledge of their subcellular localization is essential. Short-chain polyisoprenoids are known to integrate into membranes, modulating their physical and chemical properties. In contrast, natural rubber-length polyisoprenoids are sequestered in vesicles enveloped by phospholipid monolayers (Hagel et al. 2008). The chain-length threshold dictating the shift from membrane association to vesicular compartmentalization remains unknown.

Moreover, it is unclear whether > 26 i.u. long polyisoprenoids in rose leaves are transported intra- or extracellularly and whether they might function at the plant organ surface. Clarifying these aspects will be crucial to understanding the biological relevance and potential adaptive advantages conferred by these unique polyisoprenoids in roses.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00425-026-04924-0.

Acknowledgements The authors wish to express their sincere gratitude to prof. Ewa Swiezewska for insightful comments during the research process and for valuable assistance with the preparation of the manuscript.

Author contributions A.W., as the principal investigator of the research project that partially funded this study, was responsible for the main research concept, performed wet-lab experiments, and drafted the manuscript. K.S. carried out the bioinformatics analyses. B.B. cultivated *R. chinensis* plants. K. S.-T. performed an analysis of polyisoprenoids in the roots of *R. chinensis*. A.J. performed the LC–MS analysis. L.S. was the principal investigator of another research project that also partially funded this study.

Funding This work was supported by the Institute of Biochemistry and Biophysics Polish Academy of Sciences Midigrant FBW-MG-01/2024 and by the National Science Centre of Poland by Grant 2019/35/B/NZ1/03794.

Data availability The experimental data that support the findings of this study are available in the Supplemental Materials.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Akhtar TA, Matsuba Y, Schauvinhold I, Yu G, Lees HA, Klein SE, Pichersky E (2012) The tomato *cis*-prenyltransferase gene family. Plant J 73:640–652. https://doi.org/10.1111/tpj.12063

Akhtar TA, Surowiecki P, Siekierska H, Kania M, Van Gelder K, Rea KA, Virta LKA, Vatta M, Gawarecka K, Wojcik J, Danikiewicz W, Buszewicz D, Swiezewska E, Surmacz L (2017) Polyprenols are synthesized by a plastidial *cis*-prenyltransferase and influence photosynthetic performance. Plant Cell 29:1709–1725. https://doi.org/10.1105/tpc.16.00796

Baczewska AH, Dmuchowski W, Jozwiak A, Gozdowski D, Brągoszewska P, Dąbrowski P, Swiezewska E (2014) Effect of salt stress on prenol lipids in the leaves of *Tilia* 'Euchlora.' Dendrobiology 72:177–186. https://doi.org/10.12657/denbio.072.015

Baczewska-Dąbrowska AH, Dmuchowski W, Gozdowski D, Gworek B, Jozwiak A, Swiezewska E, Dąbrowski P, Suwara I (2021) The importance of prenol lipids in mitigating salt stress in the leaves of *Tilia* × *euchlora* trees. Trees 36:393–404. https://doi.org/10.1007/s00468-021-02214-8

Bajda A, Konopka-Postupolska D, Krzymowska M, Hennig J, Skorupinska-Tudek K, Surmacz L, Wójcik J, Matysiak Z, Chojnacki T, Skorzynska-Polit E, Drazkiewicz M, Patrzylas P, Tomaszewska M, Kania M, Swist M, Danikiewicz W, Piotrowska W, Swiezewska E (2009) Role of polyisoprenoids in tobacco resistance against biotic stresses. Physiol Plant 135(4):351–364. https://doi.org/10.1111/j.1399-3054.2009.01204.x

Bianchini G, Sánchez-Baracaldo P (2024) Flexible, modular software to visualise and manipulate phylogenetic trees. Ecol Evol 14:2. https://doi.org/10.1002/ece3.10873

Breitling J, Aebi M (2013) N-linked protein glycosylation in the endoplasmic reticulum. Cold Spring Harb Perspect Biol 5(8):a013359. https://doi.org/10.1101/cshperspect.a013359

Buszewicz D, Kowalewska Ł, Mazur R, Zajbt-Łuczniewska M, Surmacz L, Sosnowska K, Welc R, Jemioła-Rzemińska M, Link-Lenczowski P, Onysk A et al (2021) HSFA1 proteins mediate heat-induced accumulation of CPT7-derived polyprenols affecting thylakoid organization. https://doi.org/10.1101/2021.12.22.473876

Ciepichal E, Jemiola-Rzeminska M, Hertel J, Swiezewska E, Strzalka K (2011) Configuration of polyisoprenoids affects the permeability and thermotropic properties of phospholipid/polyisoprenoid model membranes. Chem Phys Lipids 164:300–306. https://doi.org/10.1016/j.chemphyslip.2011.03.004

Conart C, Bomzan DP, Huang X-Q, Bassard J-E, Paramita SN, Saint-Marcoux D, Rius-Bony A, Hivert G, Anchisi A, Schaller H et al (2023) A cytosolic bifunctional geranyl/farnesyl diphosphate synthase provides MVA-derived GPP for geraniol biosynthesis in rose flowers. Proc Natl Acad Sci U S A. https://doi.org/10.1073/pnas.2221440120

Dmuchowski W, Brągoszewska P, Gozdowski D, Baczewska-Dąbrowska AH, Chojnacki T, Jozwiak A, Swiezewska E, Suwara I, Gworek B (2020) Strategies of urban trees for mitigating salt stress: a case study of eight plant species. Trees 36:899–914. https://doi.org/10.1007/s00468-020-02044-0

Gawarecka K, Siwinska J, Poznanski J, Onysk A, Surowiecki P, Sztompka K, Surmacz L, Ahn JH, Korte A, Swiezewska E, Ihnatowicz A (2021) *cis*-Prenyltransferase 3 and α/β-hydrolase are new determinants of dolichol accumulation in Arabidopsis. Plant Cell Environ 45:479–495. https://doi.org/10.1111/pce.14223

Grabińska KA, Park EJ, Sessa WC (2016) *cis*-Prenyltransferase: new insights into protein glycosylation, rubber synthesis, and human diseases. J Biol Chem 291:18582–18590. https://doi.org/10.1074/jbc.R116.739490

Grabińska KA, Edani BH, Park EJ, Kraehling JR, Sessa WC (2017) A conserved C-terminal RXG motif in the NgBR subunit of *cis*-prenyltransferase is critical for prenyltransferase activity. J Biol Chem 292:17351–17361. https://doi.org/10.1074/jbc.M117.806034

Hagel J, Yeung E, Facchini P (2008) Got milk? The secret life of laticifers. Trends Plant Sci 13:631–639. https://doi.org/10.1016/j.tplants.2008.09.005

Hartley MD, Imperiali B (2012) At the membrane frontier: a prospectus on the remarkable evolutionary conservation of polyprenols and polyprenyl-phosphates. Arch Biochem Biophys 517:83–97. https://doi.org/10.1016/j.abb.2011.10.018

Hibrand Saint-Oyant L, Ruttink T, Hamama L, Kirov I, Lakhwani D, Zhou NN, Bourke PM, Daccord N, Leus L, Schulz D, de Van Geest H, Hesselink T, Van Laere K, Debray K, Balzergue S, Thouroude T, Chastellier A, Jeauffre J, Voisine L, Gaillard S, Borm TJA, Arens P, Voorrips RE, Maliepaard C, Neu E, Linde M, Le Paslier MC, Bérard A, Bounon R, Clotault J, Choisne N, Quesneville H, Kawamura K, Aubourg S, Sakr S, Smulders MJM, Schijlen E, Bucher E, Debener T, De Riek J, Foucher F (2018) A high-quality genome sequence of *Rosa chinensis* to elucidate ornamental traits. Nat Plants 4:473–484. https://doi.org/10.1038/s41477-018-0166-1

Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2017) UFBoot2: improving the ultrafast bootstrap approximation. Mol Biol Evol 35:518–522. https://doi.org/10.1093/molbev/msx281

Hou Y, Fan C, Sun J, Chang Y, Lu J, Sun J, Wang C, Liu J (2022) Genome-wide identification, evolution, and expression analysis of the TCP gene family in Rose (*Rosa chinensis* Jacq.). Horticulturae 8:961. https://doi.org/10.3390/horticulturae8100961

Janas T, Walińska K, Chojnacki T, Świeżewska E, Janas T (2000) Modulation of properties of phospholipid membranes by the long-chain polyprenol (C160). Chem Phys Lipids 106:31–40. https://doi.org/10.1016/S0009-3084(00)00129-8

Jozwiak A, Ples M, Skorupinska-Tudek K, Kania M, Dydak M, Danikiewicz W, Swiezewska E (2013) Sugar availability modulates polyisoprenoid and phytosterol profiles in *Arabidopsis thaliana* hairy root culture. Biochim Biophys Acta (BBA) Mol Cell Biol Lipids 1831:438–447. https://doi.org/10.1016/j.bbalip.2012.11.006

Jozwiak A, Lipko A, Kania M, Danikiewicz W, Surmacz L, Witek A, Wojcik J, Zdanowski K, Pączkowski C, Chojnacki T, Poznanski J, Swiezewska E (2017) Modeling of dolichol mass spectra isotopic envelopes as a tool to monitor isoprenoid biosynthesis. Plant Physiol 174:857–874. https://doi.org/10.1104/pp.17.00036

Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haeseler A, Jermiin LS (2017) ModelFinder: fast model selection for accurate

phylogenetic estimates. Nat Methods 14:587–589. https://doi.org/10.1038/nmeth.4285
Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010
Kharel Y, Koyama T (2002) Molecular analysis of *cis*-prenyl chain elongating enzymes. Nat Prod Rep 20:111–118. https://doi.org/10.1039/b108934j
Kharel Y, Takahashi S, Yamashita S, Koyama T (2006) Manipulation of prenyl chain length determination mechanism of *cis*-prenyltransferases. FEBS J 273:647–657. https://doi.org/10.1111/j.1742-4658.2005.05097.x
Komaszylo Née Siedlecka J, Kania M, Masnyk M, Cmoch P, Lozinska I, Czarnocki Z, Skorupinska-Tudek K, Danikiewicz W, Swiezewska E (2016) Isoprenoid alcohols are susceptible to oxidation with singlet oxygen and hydroxyl radicals. Lipids 51(2):229–244. https://doi.org/10.1007/s11745-015-4104-y
Kong Y, Wang H, Lang L, Dou X, Bai J (2022) Genome-wide analysis of terpene synthase gene family in *Rosa chinensis*. https://doi.org/10.21203/rs.3.rs-1505144/v1
Kutsukawa R, Imaizumi R, Suenaga-Hiromori M, Takeshita K, Sakai N, Misawa S, Yamamoto M, Yamaguchi H, Miyagi-Inoue Y, Waki T, Kataoka K, Nakayama T, Yamashita S, Takahashi S (2022) Structure-based engineering of a short-chain *cis*-prenyltransferase to biosynthesize nonnatural all-*cis*-polyisoprenoids: molecular mechanisms for primer substrate recognition and ultimate product chain-length determination. FEBS J 289:4602–4621. https://doi.org/10.1111/febs.16392
Lakusta AM, Kwon M, Kwon E-JG, Stonebloom S, Scheller HV, Ro D-K (2019) Molecular studies of the protein complexes involving *cis*-prenyltransferase in Guayule (*Parthenium argentatum*), an alternative rubber-producing plant. Front Plant Sci 10:165. https://doi.org/10.3389/fpls.2019.00165
Lescot M (2002) PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. Nucleic Acids Res 30:325–327. https://doi.org/10.1093/nar/30.1.325
Li J, Zheng G, Li S, Tang Z, Sun J, Xin X (2020) Characterization of *Rosa chinensis* CBF genes and the function of RcCBF6 in cold tolerance. J Plant Biol 63:267–278. https://doi.org/10.1007/s12374-020-09248-4
Li H, Li Y, Yan H, Bao T, Shan X, Caissard J-C, Zhang L, Fang H, Bai X, Zhang J et al (2024) The complexity of volatile terpene biosynthesis in roses: particular insights into β-citronellol production. Plant Physiol 196:1908–1922. https://doi.org/10.1093/plphys/kiae444
Lipko A, Pączkowski C, Perez-Fons L, Fraser PD, Kania M, Hoffman-Sommer M, Danikiewicz W, Rohmer M, Poznanski J, Swiezewska E (2023) Divergent contribution of the MVA and MEP pathways to the formation of polyprenols and dolichols in Arabidopsis. Biochem J 480(8):495–520. https://doi.org/10.1042/BCJ20220578
Lucas JJ, Waechter CJ (1976) Polyisoprenoid glycolipids involved in glycoprotein biosynthesis. Mol Cell Biochem 11:67–78. https://doi.org/10.1007/BF01792788
Magnard J-L, Roccia A, Caissard J-C, Vergne P, Sun P, Hecquet R, Dubois A, Hibrand-Saint Oyant L, Jullien F, Nicolè F et al (2015) Biosynthesis of monoterpene scent compounds in roses. Science 349:81–83. https://doi.org/10.1126/science.aab0696
Manat G, Roure S, Auger R, Bouhss A, Barreteau H, Mengin-Lecreulx D, Touzé T (2014) Deciphering the metabolism of undecaprenyl-phosphate: the bacterial cell-wall unit carrier at the membrane frontier. Microb Drug Resist 20:199–214. https://doi.org/10.1089/mdr.2014.0035
Milewska-Hendel A, Baczewska AH, Sala K, Dmuchowski W, Brągoszewska P, Gozdowski D, Jozwiak A, Chojnacki T, Swiezewska E, Kurczynska E (2017) Quantitative and qualitative characteristics of cell wall components and prenyl lipids in the leaves of *Tilia* × *euchlora* trees growing under salt stress. PLoS ONE 12:e0172682. https://doi.org/10.1371/journal.pone.0172682
Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, Lanfear R (2020) IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol 37:1530–1534. https://doi.org/10.1093/molbev/msaa015
Młodzińska-Michta E, Swiezewska E, Hoffman-Sommer M, Piłka N, Radkiewicz M, Jarzembowski P (2023) Adaptation of the maize seedling seminal roots to srought: essential role of plasma membrane H+-ATPases activity. Acta Soc Bot Pol 92:1–15. https://doi.org/10.5586/asbp/177274
Mooibroek H, Cornish K (2000) Alternative sources of natural rubber. Appl Microbiol Biotechnol 53:355–365. https://doi.org/10.1007/s002530051627
Müller B, Niephaus E, Eisenreich W, Bröker JN, Twyman RM, Prüfer D, Schulze Gronover C (2025) The *cis*-prenyltransferase protein family in *Taraxacum koksaghyz*. Plant J. https://doi.org/10.1111/tpj.17233
Nakagawa T, Kurose T, Hino T, Tanaka K, Kawamukai M, Niwa Y, Toyooka K, Matsuoka K, Jinbo T, Kimura T (2007) Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. J Biosci Bioeng 104:34–41. https://doi.org/10.1263/jbb.104.34
Nelson BK, Cai X, Nebenführ A (2007) A multicolored set of in vivo organelle markers for co-localization studies in Arabidopsis and other plants. Plant J 51:1126–1136. https://doi.org/10.1111/j.1365-313X.2007.03212.x
Noh Y-M, Ait Hida A, Raymond O, Comte G, Bendahmane M (2023) The scent of roses, a bouquet of fragrance diversity. J Exp Bot 75:1252–1264. https://doi.org/10.1093/jxb/erad470
Qu Y, Chakrabarty R, Tran HT, Kwon E-JG, Kwon M, Nguyen T-D, Ro D-K (2015) A lettuce (*Lactuca sativa*) homolog of human Nogo-B receptor interacts with *cis*-prenyltransferase and is necessary for natural rubber biosynthesis. J Biol Chem 290:1898–1914. https://doi.org/10.1074/jbc.M114.616920
Raymond O, Gouzy J, Just J, Badouin H, Verdenaud M, Lemainque A, Vergne P, Moja S, Choisne N, Pont C et al (2018) The Rosa genome provides new insights into the domestication of modern roses. Nat Genet 50:772–777. https://doi.org/10.1038/s41588-018-0110-3
Rush JS, Gao N, Lehrman MA, Waechter CJ (2008) Recycling of dolichyl monophosphate to the cytoplasmic leaflet of the endoplasmic reticulum after the cleavage of dolichyl pyrophosphate on the lumenal monolayer. J Biol Chem 283:4087–4093. https://doi.org/10.1074/jbc.M707067200
Skorupińska-Tudek K, Sternik J, Bajda A, Klobus G, Swiezewska E (2007) Changes in polyisoprenoid alcohols accumulation upon abiotic stress in plants. https://science24.com/paper/10759#gsc.tab=0
Soundararajan P, Won SY, Kim JS (2019) Insight on Rosaceae family with genome sequencing and functional genomics perspective. BioMed Res Int 2019:1–12. https://doi.org/10.1155/2019/7519687
Stanke M, Diekhans M, Baertsch R, Haussler D (2008) Using native and syntenically mapped cDNA alignments to improve de novo gene finding. Bioinformatics 24:637–644. https://doi.org/10.1093/bioinformatics/btn013
Surmacz L, Swiezewska E (2011) Polyisoprenoids—secondary metabolites or physiologically important superlipids? Biochem Biophys Res Commun 407:627–632. https://doi.org/10.1016/j.bbrc.2011.03.059
Surmacz L, Swiezewska E (2013) What we do and do not know about the cellular functions of polyisoprenoids. Isoprenoid

Synth Plants Microorg. https://doi.org/10.1007/978-1-4614-4063-5_21

Surmacz L, Plochocka D, Kania M, Danikiewicz W, Swiezewska E (2014) *cis*-Prenyltransferase AtCPT6 produces a family of very short-chain polyisoprenoids in planta. Biochim Biophys Acta (BBA) Mol Cell Biol Lipids 1841:240–250. https://doi.org/10.1016/j.bbalip.2013.11.011

Swiezewska E, Danikiewicz W (2005) Polyisoprenoids: structure, biosynthesis and function. Prog Lipid Res 44:235–258. https://doi.org/10.1016/j.plipres.2005.05.002

Swiezewska E, Chojnacki T, Jankowski J, Singh AK, Olsson J (1992) The occurrence of long-chain polyprenols in leaves of plants of Rosaceae family and their isolation by time-extended liquid chromatography. Biochem Cell Biol 70:448–454. https://doi.org/10.1139/o92-068

Swiezewska E, Sasak W, Mankowski T, Jankowski W, Vogtman T, Krajewska I, Hertel J, Skoczylas E, Chojnacki T (1994) The search for plant polyprenols. Acta Biochim Pol 41:221–260. https://doi.org/10.18388/abp.1994_4713

Takahashi S, Koyama T (2006) Structure and function of *cis*-prenyl chain elongating enzymes. Chem Rec 6:194–205. https://doi.org/10.1002/tcr.20083

Tello-Ruiz MK, Jaiswal P, Ware D (2022) Gramene: a resource for comparative analysis of plants genomes and pathways. Methods Mol Biol. https://doi.org/10.1007/978-1-0716-2067-0_5

Van Gelder K, Rea KA, Virta LKA, Whitnell KL, Osborn M, Vatta M, Khozin A, Skorupinska-Tudek K, Surmacz L, Akhtar TA (2018) Medium-chain polyprenols influence chloroplast membrane dynamics in *Solanum lycopersicum*. Plant Cell Physiol 59:2350–2365. https://doi.org/10.1093/pcp/pcy157

Weremczuk A, Swiezewska E (2025) Plant polyisoprenoids: biosynthesis and functional implications during stress responses. Crit Rev Plant Sci 44(6):449–470. https://doi.org/10.1080/07352689.2025.2593213

Zhang Y, Wu Z, Feng M, Chen J, Qin M, Wang W, Bao Y, Xu Q, Ye Y, Ma C, Jiang C-Z, Gan S-S, Zhou H, Cai Y, Hong B, Gao J, Ma N (2021) The circadian-controlled PIF8–BBX28 module regulates petal senescence in rose flowers by governing mitochondrial ROS homeostasis at night. Plant Cell 33:2716–2735. https://doi.org/10.1093/plcell/koab152

Zheng Y, Yang Y, Wang M, Hu S, Wu J, Yu Z (2021) Differences in lipid homeostasis and membrane lipid unsaturation confer differential tolerance to low temperatures in two *Cycas* species. BMC Plant Biol 21:377. https://doi.org/10.1186/s12870-021-03158-4

Zhu H, Wu Y, Zheng Y (2022) Effects of heat shock on photosynthesis-related characteristics and lipid profile of *Cycas multipinnata* and *C. panzhihuaensis*. BMC Plant Biol 22:442. https://doi.org/10.1186/s12870-022-03825-0