The genome of the camphor tree and the genetic and climatic relevance of the top-geoherbalism in this medicinal plant

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Abstract

Camphor tree (*Cinnamomum camphora* (L.) J. Presl), a species in the magnoliid family Lauraceae, is known for its rich volatile oils and is used as a medical cardiotonic and as a scent in many perfumed hygiene products. Here, we present a high-quality chromosome-scale genome of *C. camphora* with a scaffold N50 of 64.34 Mb and an assembled genome size of 755.41 Mb. Phylogenetic inference revealed that the magnoliids are a sister group to the clade of eudicots and monocots. Comparative genomic analyses identified two rounds of ancient whole-genome duplication (WGD). Tandem duplicated genes exhibited a higher evolutionary rate, a more recent evolutionary history and a more clustered distribution on chromosomes, contributing to the production of secondary metabolites, especially monoterpenes and sesquiterpenes, which are the principal essential oil components. Three-dimensional analyses of the volatile metabolites, gene expression and climate data of samples with the same genotype grown in different locations showed that low temperature and low precipitation during the cold season modulate the expression of genes in the terpenoid biosynthesis pathways, especially *TPS* genes, which facilitates the accumulation of volatile compounds. Our study lays a theoretical foundation for policy-making regarding the agroforestry applications of camphor tree.

Keywords: *Cinnamomum camphora*, genome, top-geoherbalism, tandem duplication, climatic factors

Introduction

Top-geoherbalism, also known as “Daodi” in China and “Provenance” or “Terroir” in Europe, refers to traditional herbs grown in certain native ranges with better quality and efficacy than those grown elsewhere, in which the relevant characteristics are selected and shaped by thousands of years of the clinical application of traditional medicine (Brinckmann, 2015). The concept of top-geoherbalism is documented in the most ancient and classic Chinese Materia Medica (Divine Husbandman’s Classic of Materia Medica; *Shen Nong Ben Cao Jing*) from approximately 221 B.C. to 220 A.D. (Zhao et al., 2012), which reported that the origin and growing conditions of most herbs were linked to their quality. Historical literature documented cases where the misuse of traditional Chinese medicine (TCM) in prescriptions led to a reduction or absence of therapeutic effects. For example, the application of dried tender shoots of *Cinnamomum cassia* Presl (a component of *Guizhi soup* recorded in *Prescriptions for Emergencies*) from non-top-geoherb regions led to a deficiency in the treatment of fever, while the replacement of this TCM with materials from top-geoherb regions results in proper fever treatment. Currently, TCM from top-geoherb regions accounts for 80% of the market occupancy and economic profits of all TCMs (Huang, 2012), and the significance of top-geoherbs has been revived by the increasing trend of the protection of botanicals with “geographical indication” (GI) (Brinckmann, 2015). Thus, a deep understanding of the pattern and mechanism of top-geoherbalism will strongly guide producers and consumers of TCM and improve the standardization and internalization of the TCM market, especially in the economic context of the Belt and Road Initiative (Hinsley et al., 2020).

A substantial basis of the top-geoherbalism of TCMs is secondary metabolites that play principal roles in the therapeutic effects of herbs. The content of secondary metabolites is a continuous quantitative trait that is determined by three factors: genotype, environment and the interaction between the genotype and environment. Li et al. (2020) showed that cultivars of opium poppy with similar copy number variations in benzyloisoquinoline alkaloid biosynthetic genes were likely to exhibit similar alkaloid contents. To identify the ecological and climatic factors driving the development top-geoherbs, the appropriate strategy is to grow herbs with the same genotype (eliminating the effect of genetic variation) in different environmental settings and study how environmental factors are correlated with secondary metabolites by altering gene expression. However, most published studies confound the effects of genetic variation and environmental factors. For example, Tan et al. (2015) identified metabolite markers for distinguishing *Radix Angelica sinensis* from top-geoherb regions and non-top-geoherb regions by analysing the volatile metabolites of processed herbal medicine from drug stores in different regions. The roots of *Paeonia veitchii* showed higher bioactivities when grown at lower average annual temperatures and high elevations based on the analyses of environmental factors and phytochemicals of different samples from seven populations (Yuan et al., 2020). Liu et al. (2020) revealed a correlation between iridoid accumulation and increased temperature by examining 441 individuals from 45 different origins. The confounding

factor of genetic variation led to the misinterpretation of how environmental factors alone affect secondary metabolites. In addition, top-geoherb TCMs have been annotated with their cultural properties, including their cultivation, harvesting and postharvest processing (Huang, 2012), which is outside of the scope of the current study.

Cinnamomum camphora (L.) J. Presl (Figure 1a), also known as camphor tree is native to China, India, Mongolia and Japan (Chen et al., 2017; Yoshida et al., 1969) and was later introduced to Europe and the southern United States (Bottoni et al., 2021; Hamidpour et al., 2012; Ravindran et al., 2004). Camphor trees are divided into five chemotypes according to their dominant volatile oil components as linalool, camphor, eucalyptol, borneol and isonerolidol types (Luo et al., 2021). Their leaves are especially rich in volatile oil components, including monoterpenes, sesquiterpenes and diterpenes (Hou et al., 2020; Tian et al., 2021). Camphor tree has high medicinal, ornamental, ecological and economic value (Chen, 2020; Liu et al., 2019; Zhang et al., 2020). As a TCM, *C. camphora* can be used to treat rheumatism and arthralgia, sores and swelling, skin itching, poisonous insect bites, etc. Among the many chemical components of the species, camphor is used as a cardiogenic, deodorant and stabilizer in medicine, daily-use chemical production and industry, and linalool is most frequently used as a scent in 60% to 80% of perfumed hygiene products and cleaning agents (Consultation, 2021; Eggersdorfer, 2000; Letizia et al., 2003).

C. camphora belongs to Lauraceae within the magnoliid group comprising four orders (Laurales, Magnoliales, Canellales and Piperales). Magnoliids are the third largest group of angiosperms, including approximately 9,000-10,000 species (Massoni et al., 2015; Palmer et al., 2004). From an evolutionary point of view, the mysterious phylogenetic position of magnoliids within angiosperms has been debated for decades. Recently, the debate on the phylogenetic position of magnoliids has focused on three main topologies (Endress and Doyle, 2009; Moore et al., 2007; Qiu et al., 2010; Zeng et al., 2014) positioning magnoliids as (a) a sister group to eudicots (Chaw et al., 2019; Lv et al., 2020; Shang et al., 2020); (b) a sister group to monocots (Qin et al., 2021); or (c) a sister group to the clade of monocots and eudicots (Chen et al., 2019; Chen et al., 2020; Chen et al., 2020; Hu et al., 2019; Rendón-Anaya et al., 2019). Furthermore, the different whole-genome duplication (WGD) events that have occurred in specific lineages of magnoliids and the divergences time between different magnoliid plants remain unclear (Chaw et al., 2019; Chen et al., 2019; Chen et al., 2020; Chen et al., 2020; Hu et al., 2019; Lv et al., 2020; Qin et al., 2021; Rendón-Anaya et al., 2019; Shang et al., 2020).

Despite the economic and evolutionary value of *C. camphora*, the lack of a high-quality genome for the species has greatly restricted the progress of genetic research and the identification of the biosynthetic genes underlying the production of essential volatile compounds with medicinal effects (Chaw et al., 2019; Chen et al., 2019; Chen et al., 2020; Chen et al., 2020; Hu et al., 2019; Lv et al., 2020; Rendón-Anaya et al., 2019; Shang et al., 2020). In our study, we *de novo* assembled the chromosome-scale genome of *C. camphora*, explored the genomic characteristics of *C. camphora* and investigated the genetic and climatic factors underlying the

132 top-geoherbalism of this well-known TCM.
133

134
135 **Figure 1** The camphor tree and landscape of its genome. (a) Images of a camphor tree and its multiple
136 tissues. (i) An ancient camphor tree, (ii) tissue-cultured seedlings, (iii) seedlings, (iv) young leaves, (v)
137 flowers and (vi) fruits. (b) Circos plot of the *C. camphora* genome assembly. Circles from outside to inside:
138 (i) chromosomes, (ii) Gypsy LTR density, (iii) Copia LTR density, (iii) total LTR density, (iv) gene density
139 and (v) GC content. These density metrics were calculated with 1 Mb nonoverlapped sliding windows.
140 The syntenic genomic blocks (>300 kb) are illustrated with orange lines.

141 142 Results

143 *C. camphora* genome assembly and annotation

144 A haplotype-resolved genome assembly was obtained by using Hifiasm (Cheng et al.,
145 2021) integrating HiFi long reads (24.95 Gb; ~32.83X based on a previous flow
146 cytometry-based estimated genome size of 760 Mb (Wu, 2014)) and Hi-C short reads
147 (63.78 Gb; ~83.92X) with the default parameters. The two contig-level haplotype
148 genomes showed total sizes of 768.97 Mb and 752.05 Mb, respectively, after
149 removing redundant sequences with Purge_haplotigs (Roach et al., 2018). Compared
150 with the earlier published *C. kanehirae* genome (Chaw et al., 2019), the contig N50
151 was 2.01 Mb for haplotype A and 2.25 Mb for haplotype B; these values are ~2.2- and
152 ~2.5-fold higher, respectively, than the values of the previously published *C.*
153 *kanehirae* genome (Chaw et al., 2019), respectively (Table 1). Benchmarking
154 Universal Single-Copy Orthologue (BUSCO) analyses showed that the contig sets of
155 haplotype A and haplotype B contained 94.1% and 96.0% complete sets of the core
156 orthologous genes of viridiplantae, respectively (Table S2). Subsequently, the two
157 nonredundant contig sets were anchored to 12 chromosomes based on Hi-C contacts
158 (Figure S1). Overall, the assembled genome size was consistent with previous
159 reports (Table 1; Table S3). As the chromosome-level haplotype A genome was much
160 closer to the estimated genome size than the haplotype B genome, it was used for
161 subsequent analyses.

162 The final assembled *C. camphora* genome consisted of 12 chromosomes, 1,025
163 scaffolds and 1,686 contigs, with a scaffold N50 of 64.34 Mb (Table 1; Figure 1b). The
164 number of assembled chromosomes was consistent with previous cytological
165 observations (<http://ccdb.tau.ac.il>). The length of the chromosomes ranged from
166 84.54 Mb (Chr1) to 36.36 Mb (Chr12) (Table S5). The high fidelity of the assembly
167 was corroborated by multiple lines of evidence. First, the mapping rates of RNA-Seq
168 paired-end reads against the assembled genome were high (93%-95%). Second, the
169 high completeness of this assembly was supported by a 96.2% BUSCO value (Table
170 1; Table S4), suggesting high completeness at the gene level, which was much better
171 than the completeness of the *C. kanehirae* genome assembled via PacBio CLR
172 sequencing. Third, the long terminal repeat (LTR) assembly index (LAI) score (Ou et
173 al., 2018) was 13.82, indicating the “reference” level of the genome and reflecting
174 completeness at the transposable element (TE) level.

175 By combining *ab initio* prediction, orthologous protein and transcriptomic data, we
176 annotated 24,883 protein-coding genes using the MAKER pipeline (Cantarel et al.,
177 2008) (Table 1). The average lengths of the gene regions, coding sequences (CDSs),
178 exon sequences, and intron sequences were 9,295.00, 1,189.59, 309.29, and
179 1,548.48 bp, respectively (Table 1). Among the predicted protein-coding genes,
180 97.06% could be annotated in at least one of the following protein-related databases:
181 NCBI nonredundant protein (NR) (97.04%), Swiss-Prot (80.87%), Pfam (72.12%), GO
182 (46.59%) or KEGG (45.08%) (Table 1).

183 A total of 361.37 Mb of TEs were identified (Table S6). Long-terminal repeat
184 retrotransposons (LTR-RTs) were the most abundant type of repetitive sequence,
185 accounting for 46.86% of the whole genome, similar to the proportion of LTRs found in
186 *C. kanehirae* (Table 1). A total of 3,731 intact LTR-RTs were identified, and the
187 frequency distribution of insertion times showed a burst of LTR-RTs 1-2 million years
188 ago (Mya) (Figure S2), consistent with previous reports in magnoliids. Gypsy
189 elements were the largest LTR-RT superfamily in *C. camphora*, constituting 15.42% of
190 the genome. The second largest superfamily was Copia, accounting for 7.69% of the
191 genome. Other unclassified LTR-RTs encompassed 4.83% of the genome. Among
192 DNA retrotransposons, terminal inverted repeat sequences (TIRs) and non-TIRs
193 comprised 14.05% and 5.87% of the genome, respectively.

194

Table 1 The statistics for genome assemblies of *Cinnamomum*

	<i>C. camphora</i>	<i>C. kanehirae</i>
Gene assembly		
Genome size (Mb)	755.41	730.43
Scaffold number (n)	1,037	2,153
Scaffold N50 (Mb)	64.34	50.35
Scaffold L51 (n)	5	6
Chromosome-scale scaffolds (Mb)	697.81 (92.38%)	672.85 (92.12%)
Contig number (n)	1,686	-
Contig N50 (Mb)	2.01	0.90
Contig L51 (n)	108	-
GC content of the genome (%)	39.49	38.22
Complete BUSCOs (%)	96.20	88.50
TE annotation		
TE content (%)	47.86	47.87
LTR content (%)	27.94	25.53
Copia content (%)	7.69	-
Gypsy content (%)	15.42	-
LAI	13.82	-
Gene annotation		
Number of predicted genes (n)	24,883	27,899
Average gene length (bp)	9,295.00	7,591.84
Average CDS length (bp)	1,189.59	1,310.51
Average exon length (bp)	309.29	241.55
Average exon number per transcript (n)	4.80	5.40
Average intron length (Mb)	1,548.48	1,425.80
Average intron number per transcript (n)	4.40	4.06
Gene function annotation		
Nr	24,147 (97.04%)	-
Swissprot	20,122 (80.87%)	-
Pfam	17,945 (72.12%)	-
GO	11,593 (46.59%)	-
KEGG	11,218 (45.08%)	-
Total	24,152 (97.06%)	-

The assembly for *C. camphora* was compared with *C. kanehirae*. Dash (-) indicates data were not shown in the original research.

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196

197 **Phylogenetic and WGD analyses**

198 The concatenate phylogenetic tree constructed with 172 single-copy orthologous
199 genes among 16 species showed that *C. camphora* was clustered with two other
200 Lauraceae species and that the formed clade was then clustered with other
201 magnoliids (Figure 2; Figure S3a). More importantly, the magnoliids were sister to the
202 combined clade of eudicots and monocots rather than to either monocots or eudicots.
203 In addition, the coalescence-based phylogenetic tree showed the same topology for

204 magnoliids as the concatenation tree (Figure S3b). This phylogenetic topology is
 205 consistent with some previous studies (Chen et al., 2019; Chen et al., 2020; Chen et
 206 al., 2020; Hu et al., 2019; Rendón-Anaya et al., 2019) but contrasts with other reports
 207 (Chaw et al., 2019; Lv et al., 2020; Qin et al., 2021; Shang et al., 2020). The
 208 divergence time between magnoliids and the clade of monocots and eudicots was
 209 inferred to be 147.47-170.11 Mya (Figure 2) in MCMCTree with fossil calibration
 210 (Yang et al., 2007), coinciding with the estimates in other studies (Chen et al., 2019;
 211 Chen et al., 2020; Chen et al., 2020; Hu et al., 2019; Rendón-Anaya et al., 2019). In
 212 the clade of magnoliids, the divergence time between Laurales and Magnoliales was
 213 approximately 138.96 Mya, after which the divergence of Lauraceae and
 214 Calycanthaceae occurred approximately 114.26 Mya, and finally, *C. camphora*
 215 diverged from the most recent common ancestor of *C. camphora* and *C. kanehirae*
 216 approximately 28.03 Mya.
 217

218
 219 **Figure 2** Phylogenetic analyses. The phylogenetic tree was constructed based on 172 single-copy
 220 orthologous genes of 16 species using two ANA-grade species as outgroups; node age and 95%
 221 confidence intervals are labeled. Pie charts show the proportions of gene families that underwent
 222 expansion or contraction. Predicted whole-genome duplication (WGD) events were only indicated for
 223 Laurales and Magnoliales.
 224

225 The intragenomic collinearity and syntenic depth ratio of *C. camphora* showed clear
 226 syntenic evidence of ancient WGD events (Figure 1b; Figure S4). Only the paralogous
 227 gene set derived from duplicates produced by WGD was used for the analyses of
 228 synonymous substitutions per site (*Ks*) (see “Methods” section). Two ancient WGD
 229 events shared within the Laurales lineage (*C. camphora*, *C. kanehirae* and *Persea*
 230 *americana*) occurred approximately 85.66 Mya and 137.90 Mya, represented by two
 231 signature peaks with *Ks* values of approximately 0.52 and 0.83 (Figure 3a). The
 232 recent *Ks* peak (85.66 Mya) occurred much earlier than the divergence time (42.27
 233 Mya) between *P. americana* and the common ancestor of *Cinnamomum*, indicating
 234 that this round of WGD was shared between *Cinnamomum* and *Persea* (Figure 2;

235 Figure 3a). Additionally, the *Ks* peak (137.90 Mya) occurred earlier than the
236 divergence (114.26 Mya) between Lauraceae species and *Chimonanthus salicifolius*,
237 implying that this round of WGD was shared between Lauraceae and Calycanthaceae
238 (Figure 2; Figure 3a). We also detected a WGD event in *Liriodendron chinense* that
239 occurred approximately 116.67 Mya, corresponding to a *Ks* peak of 0.70 (Figure 3a),
240 which is consistent with a previous study (Chen et al., 2019).

241 To confirm that *C. camphora* underwent two rounds of ancient WGD, we performed
242 intergenomic synteny analyses between the genomes of *C. camphora* and *L.*
243 *chinense* together with *Vitis vinifera*. A “4:2” syntenic relationship was detected
244 between *C. camphora* and *L. chinense* (Figure S5a). Given the hexaploidy event
245 (γ -WGT) shared among core eudicots, including *V. vinifera* (French-Italian Public
246 Consortium for Grapevine Genome Characterization, 2007), an overall “4:3” syntenic
247 relationship was observed between *C. camphora* and *V. vinifera* (Figure S5b).
248 Furthermore, we discovered a “2:3” syntenic relationship between *L. chinense* and *V.*
249 *vinifera* (Figure S5c). Thus, the syntenic relationships among the *L. chinense*:*V.*
250 *vinifera*:*C. camphora* genomes showed a 2:3:4 ratio (Figure 3b). Our results echoed
251 previous studies (Chaw et al., 2019; Chen et al., 2019), indicating two rounds of
252 ancient WGD in Lauraceae.

253 To determine the functional roles of the genes retained after WGD events, GO and
254 KEGG enrichment analyses were performed. The results showed that the genes
255 retained after WGD were enriched in basic physiological activities, processes and
256 pathways, such as structural constituents of ribosomes, NADPH dehydrogenase
257 activity, photosynthesis and plant hormone signal transduction, suggesting that two
258 rounds of WGD events enhanced the adaptability of *C. camphora* to changing
259 environments by improving basic physiological activities and primary metabolism
260 (Figure S6; Figure S7; Table S7; Table S12).

261

Figure 3 Gene duplication and evolution. (a) Ks distribution of paralogues in magnoliid species (*C. camphora*, *C. kanehirae*, *P. americana* and *L. chinense*) and orthologues between these magnoliids and *V. vinifera*. (b) Synteny blocks among *C. camphora*, *L. chinense* and *V. vinifera*. (c) Ka/Ks ratio distributions of gene pairs derived from different types of duplication. WGD, whole-genome duplication; TD, tandem duplication; PD, proximal duplication; TRD, transposed duplication; DSD, dispersed duplication. (d) Ks ratio distributions of gene pairs derived from different types of duplication. (e) GO enrichment analyses of genes from different types of duplication. The enriched GO terms with adjusted *P*

values < 0.01 are presented. The colours of the bubbles indicate the statistical significance of the enriched GO terms. The sizes of the bubbles indicate the number of genes associated with one GO term. (f) KEGG enrichment analyses of genes resulting from different types of duplication. Enriched KEGG pathways with adjusted *P* values < 0.01 are presented. The colours of the bubbles represent the statistical significance of enriched KEGG pathways. The sizes of the bubbles indicate the number of genes associated with one KEGG pathway.

Tandem and proximal duplications contribute to terpene synthesis in *C. camphora*

A total of 18,938 duplicated genes were identified, including 7,043 WGD genes (37.19%), 1,597 tandem duplication (TD) genes (8.43%), 1,079 proximal duplication (PD) genes (5.70%), 5,127 dispersed duplication (DSD) genes (27.07%) and 3,779 transposed duplication (TRD) genes (19.95%) (Figure S8). The *Ks* and *Ka/Ks* values among different types of duplicated genes were calculated (Figure 3c; Figure 3d). The *Ks* values of TD and PD genes were much smaller than those of other types of duplications (Figure 3d), suggesting that TD and PD genes were formed recently. Additionally, the higher *Ka/Ks* ratios of TD and PD genes (Figure 3c) implies that they were subject to more rapid sequence divergence.

We also performed GO and KEGG enrichment analyses of the gene sets associated with the five duplication types (Figure S6; Figure S7; Table S7-S16). The GO results showed that the biological process (BP) and molecular function (MF) categories of secondary metabolite, monoterpene, sesquiterpene and terpene biosynthesis and metabolism were significantly enriched in the TD and PD gene sets, but no enrichment of these GO terms was found in the WGD, DSD and TRD gene sets (Figure 3e). Regarding the KEGG results, the TD and PD gene sets were significantly enriched in the terpenoid backbone, monoterpene, sesquiterpene and phenylpropanoid biosynthesis pathways. These secondary metabolite biosynthesis pathways were not enriched in the other three duplication gene sets (Figure 3f). In addition, KEGG pathways related to responses to environmental stimuli, such as the MAPK signalling pathway, steroid hormone biosynthesis and plant-pathogen interaction, were also significantly enriched in the TD and PD gene sets. All of these results indicated that the rapidly evolving TD and PD genes played essential roles in the synthesis of secondary metabolites, especially monoterpenes and sesquiterpenes, and the response to environmental stimuli in *C. camphora*.

Metabolic reflection of top-geoherbalism

To evaluate how environmental factors affect metabolite accumulation, we grew tissue-cultured seedlings from a single mother plant in four major production locations, including Qinzhou, Nanning, Baise and Liuzhou, beginning in May 2018. Mature leaves were harvested from the four locations in November 2020 for RNA-Seq and metabolite assessment. Thus, we were able to compare the effects of environmental conditions on metabolite accumulation based on the fixed genotype.

Volatile metabolites are a substantial basis of the top-geoherbalism of camphor tree. Based on targeted gas chromatography-mass spectrometry (GC-MS) analyses, we

314 identified 153 nonredundant volatile metabolites (Supplemental Table 17). The 153
315 volatile metabolites included 82 terpenes (53.60%), 14 esters (9.15%), 13 ketones
316 (8.50%), 12 heterocyclic compounds (7.84%), 10 alcohols (6.54%), 9 aromatics
317 (5.88%) and 13 additional metabolites (8.49%) that did not belong to these six main
318 types (Figure 4a). For quality control, we performed hierarchical clustering and
319 principal component analyses (PCA) of metabolic abundances in these 24 samples
320 (Figure S9). The first replicate in Baise ("Baise_1") was identified as an outlier and
321 was removed from further analyses.

322 The volatile metabolites of the samples from the four locations exhibited distinct
323 clustering in the PCA plot (Figure 4c). Notably, the metabolites of samples from Baise
324 and Liuzhou were clearly separated from those of Qinzhou and Nanning according to
325 principal component 1 (PC1), which accounted for 43.2% of the total variance. The
326 heatmap based on the relative abundances of volatile metabolites showed that
327 samples from Baise and Liuzhou exhibited higher abundances than those from
328 Qinzhou and Nanning, especially with regard to terpenes (Figure 4b; Figure S10).

329 Linalool, borneol, camphor, 1,8-cineole and isonerolidol are the five main
330 phytochemicals determining the medicinal value and top-geoherbalism of *C.*
331 *camphora*. A detailed comparison of the five volatile terpenoids implied that samples
332 from Liuzhou showed the highest total abundance, followed by the samples from
333 Baise, while the abundance was lowest in Qinzhou (Figure 4d). The abundance
334 profiling of camphor and borneol in the four locations echoed the trends of the total
335 abundance of the five volatile terpenoids. The abundance of linalool and isonerolidol
336 peaked in Baise and Liuzhou, respectively, while that of 1,8-cineole peaked in
337 Nanning. Taken together, these results suggested that Baise and Liuzhou are
338 top-geoherb regions of the camphor tree in Guangxi Province.

339

Figure 4 Abundance patterns of volatile metabolites in *C. camphora* planted in different locations. (a) Pie charts show the proportions of different types of metabolites identified in the current study. (b) Hierarchical clustering heatmap of metabolic abundance profiles in the four planting locations, including Qinzhou, Nanning, Baise and Liuzhou, indicated on the x axis. Metabolic abundance was averaged and z-score transformed. The rows are clustered by the types of metabolites. (c) Principal component analyses (PCA) of metabolites of *C. camphora* planted in the four locations. The circles represent the 95% confidence intervals. (d) The relative abundances of linalool, borneol, camphor, 1,8-cineole and isomerolidol in the four planting locations.

Modulation of gene expression related to top-geoherbalism

TPSs are the rate-limiting enzymes in the production of terpenoids (Chen et al., 2011; Tholl, 2015), including monoterpenes, sesquiterpenes and diterpenoids (see “Methods” section). The identified *TPS* genes of *C. camphora*, *C. kanehirae*, *P. americana*, *L. chinense* and *Arabidopsis thaliana* were clustered into six clades in the phylogenetic tree, corresponding to the *TPS-a*, *TPS-b*, *TPS-c*, *TPS-e*, *TPS-f* and *TPS-g* subfamilies (Chen et al., 2011), among which *TPS-b* and *TPS-g* encode the

enzymes catalysing the production of 10-carbon monoterpenoids from geranyl diphosphate (GPP), and *TPS-a* genes are responsible for catalysing the production of 15-carbon sesquiterpenoids from farnesyl diphosphate (FPP) (Figure 5a,b). The copy number variation of the *TPS* genes showed that *TPS-b* was greatly expanded in *C. kanehirae* and *C. camphora* (Figure 5a,b), which resulted in the high abundance of monoterpenoids in *Cinnamomum* (Chen et al., 2020; Chen et al., 2020). Specifically, 44 *TPS-b* genes were identified in *C. camphora*, accounting for 61% of all its *TPS* genes, consistent with the percentage observed in *C. kanehirae* (63%) and much higher than those in *L. chinense* (33%), *A. thaliana* (18%) and *P. americana* (12%). We also observed more copies of the *TPS-g* subfamily in *C. camphora* than in *A. thaliana* and *P. americana*. However, no expansion of *TPS-a* genes was detected in *Cinnamomum*.

Next, we examined how the *TPS* genes were distributed across the genome. Chromosome 9 and chromosome 7 harboured the most *TPS* genes (27 and 25, respectively), followed by chromosome 3 (13 *TPS* genes) (Figure 5c; Table S18). Interestingly, genes from the seven subfamilies were observed as tandem duplicates. Two large *TPS-b* gene clusters were identified on chromosome 9 (21 genes; ca. 38.78-40.12 Mb) and chromosome 7 (10 genes; ca. 12.70-16.03 Mb), respectively. In addition, two large *TPS-a* gene clusters were detected on chromosome 3 (10 genes; ca. 31.90-32.52 Mb) and chromosome 7 (6 genes; ca. 19.49-19.90 Mb), respectively. The remaining smaller *TPS* gene clusters were scattered throughout the genome of *C. camphora*.

Notably, the *TPS* genes exhibited a strong tissue-specific expression pattern (Figure 5d; Table S19), especially *TPS-a*, *TPS-b* and *TPS-g*. We downloaded previously published RNA-Seq data (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA747104>) from three tissues (leaf, stem and root) of *C. camphora* to determine the gene expression profile of *TPS* genes. As leaves are the major tissue used in medicine, we focused on the *TPS* genes with high expression levels in leaves. Six *TPS-a* genes and twelve *TPS-b* genes showed higher expression in leaves than in stems and roots. All of these results indicated that the expansion of the *TPS-b* subfamily and the tandem duplication of *TPS-b* genes probably contribute to monoterpenoid biosynthesis in *C. camphora*.

389

Figure 5 Genes involved in the biosynthesis of volatile terpenoids. (a) Phylogenetic analyses of *TPS* genes in *C. camphora*. The phylogenetic tree was constructed based on *TPS* gene sequences from four magnoliid genomes (*C. camphora*, *C. kanehirae*, *P. americana* and *L. chinense*) and *A. thaliana*. (b) Copy numbers of *TPS* genes in the genomes of four magnoliids and *A. thaliana*. (c) Distribution of the *TPS* genes on seven chromosomes of *C. camphora*. (d) Tissue-specific expressions of *TPS-a*, *TPS-b* and *TPS-g* subfamilies.

The biosynthetic pathways of terpenoids are derived from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) produced via the methylerythritol phosphate (MEP) and mevalonate (MVA) pathways, respectively (Li et al., 2019; Zhou and Pichersky, 2020). Comparative transcriptome analyses of samples from the four locations were performed to examine the expression patterns of genes involved in the MEP and MVA pathways. We combined four differentially expressed gene (DEG) sets identified in Liuzhou vs. Qinzhou, Liuzhou vs. Nanning, Baise vs. Qinzhou and Baise vs. Nanning (Figure S11). We detected 22 and 23 DEGs in the MEP and MVA pathways, respectively (Figure 6; Table S20). Generally, the DEGs involved in all the steps of the MEP and MVA pathways showed higher expression in Baise and Liuzhou than in Qinzhou and Nanning, except for the *2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase (MDS)* gene, which was absent in the combined DEG set. The same expression pattern was observed for the

genes related to downstream steps, including *isopentenyl diphosphate isomerase* (*IDI*), *geranyl diphosphate synthetase* (*GPPS*), *farnesyl diphosphate synthetase* (*FPPS*), *TPS-a*, *TPS-b* and *TPS-g*. The modulation of gene expression in the terpenoid biosynthesis pathway echoed the higher accumulation of monoterpenoids and sesquiterpenoids observed in Baise and Liuzhou than in Qinzhou and Nanning.

Figure 6 Biosynthetic pathways of monoterpenoids and sesquiterpenoids. Relative expression profiling of genes involved in volatile terpenoid biosynthesis among the four planting locations (Qinzhou, Nanning, Baise and Liuzhou). Gene expression was extracted from the combined differentially expressed gene (DEG) set (Liuzhou vs. Qinzhou, Liuzhou vs. Nanning, Baise vs. Qinzhou and Baise vs. Nanning). MEP, mevalonate pathway; MEP, methylerythritol phosphate pathway; ACAT, acyl-coenzyme A-cholesterol acyl-transferase; HMGS, hydroxymethylglutaryl coenzyme A synthase; HMGR, hydroxymethylglutaryl coenzyme A reductase; MVK, mevalonate kinase; PMK, phospho-mevalonate kinase; MVD, mevalonate diphosphate decarboxylase; DXS, 1-deoxy-D-xylulose 5-phosphate synthase; DXR, 1-deoxy-D-xylulose 5-phosphate reductoisomerase; MCT, 2-C-methyl-D-erythritol-4-phosphate cytidyltransferase; CMK, 4-(cytidine-5-diphospho)-2-C-methyl-D-erythritol kinase; MDS, 2-C-methyl-D-erythritol-2,4-cyclodiphosphate synthase; HDS, (E)-4-hydroxy-3-methyl-but-2-enyl-pyrophosphate synthase; HDR, (E)-4-hydroxy-3-methyl-but-2-enyl-pyrophosphate reductase.

Climatic factors underlying the top-geoherbalism of *C. camphora*

To determine what climatic factors caused the differences in volatile metabolites among the *C. camphora* plants of the same genotype grown in different locations (Figure 7a), we downloaded the climate data of Qinzhou, Nanning, Baise and Liuzhou from three years (2018-2020) from the National Meteorological Information Centre (<http://data.cma.cn/en>). The 17 climatic factors could be classified into

438 temperature-related, wind-related, pressure-related, precipitation-related,
439 humidity-related and sunshine-related factors. (Table S21; Table S22).

440 PCAs of the climatic factors showed that PC1 (accounting for 53.2% of the total
441 variance) separated Liuzhou from Qinzhou, Nanning and Baise, while PC2
442 (accounting for 25.8% of the total variance) split Baise from Qinzhou, Nanning and
443 Liuzhou (Figure 7b). To examine the contributions of climatic factors to PC1 and PC2,
444 we loaded them in the PCA plot (Figure 7c). Temperature-related factors were the
445 main variables contributing to PC1, including mean minimum temperature (16.88%),
446 mean temperature (16.32%) and mean maximum temperature (14.50%), and
447 precipitation-related factors mainly contributed to PC2, including daily precipitation
448 (27.29%) and maximum daily precipitation (19.75%) (Figure 7d).

449 To examine the detailed differences in temperature- and precipitation-related
450 factors in these four locations, we plotted the monthly observations of mean minimum
451 temperature, mean temperature, mean maximum temperature, daily precipitation and
452 maximum daily precipitation (main contributors to PC1 and PC2) from 2018 to 2020
453 (Figure 7e, f; Figure S12; Table S22). The mean temperature of Liuzhou was much
454 lower than those of the other locations, especially in the two colder quarters of the
455 year. Additionally, daily precipitation was lower in Baise than in the other locations,
456 especially in early spring. All of these results suggested that relatively low
457 temperature and precipitation during the cold season imposes some degree of stress
458 on the plants and thus stimulated the production of the desired terpenoids in *C.*
459 *camphora*.

460

Figure 7 Analyses of climatic factors in different planting locations. (a) Geographical distribution of *C. camphora* planting locations, including Qinzhou, Nanning, Baise and Liuzhou. (b) Principal component analyses (PCA) of seventeen climatic factors in the four planting locations in 2018, 2019 and 2020. (c) The loadings of climatic factors in the PCA plot. The colours of the arrows represent the percentages of the contributions of climatic factors to the PCs. (d) Histograms of the percentages of the contributions of different climatic factors to PC1 and PC2. The red dashed lines indicate the average contributions of different climatic factors. Only the top eight climatic factors are shown. (e) Monthly observations of the mean temperature in the four planting locations. Single and double asterisks indicate statistically significance levels of $P < 0.05$ and $P < 0.01$, respectively, between Liuzhou and Qinzhou/Nanning (paired-sample Student's *t* test). (f) Monthly observations of daily precipitation in the four planting locations. A single asterisk indicates the statistically significance levels of $P < 0.05$ between Baise and Qinzhou/Nanning (paired-sample Student's *t* test).

Discussion

Here, by decoding the genome of the camphor tree, we revealed that magnoliids are a sister group to the clade of eudicots and monocots. The two rounds of WGD identified

478 in *C. camphora* were dated to ca. 85.66 Mya and 137.90 Mya, respectively. The
479 former was shared with Lauraceae species, and the latter occurred before the
480 divergence of Lauraceae and Calycanthaceae. We found that rapidly evolving TD
481 genes play key roles in the synthesis of secondary metabolites, especially for
482 monoterpenes and sesquiterpenes. By analysing volatile metabolites in leaves
483 sampled from plants of the same genotype grown in four different locations, we found
484 higher accumulation of the key volatile metabolites in regions with lower temperature
485 and precipitation in the cold season, which was attributed to the differential expression
486 of genes related to the MVA and MEP pathways as well as their downstream steps in
487 terpene biosynthesis. Our study confirmed that abiotic stress contributes to the
488 development of top-geoherbs, laying a theoretical foundation for policy making on the
489 agroforestry applications of camphor trees.

490 In addition to releasing the first chromosome-level genome of this significant
491 economic tree species, our study is innovative in two regards. First, it is among the
492 very few innovative three-dimensional evaluations of the top-geoherbalism of a TCM
493 species, integrating phytochemical, genetic and climatic analyses of camphor tree
494 germplasm (Li et al., 2017). Second, our study is the first to fix the genotypic variation
495 of the germplasm so that it is feasible to investigate how climatic factors alone affect
496 the accumulation of metabolites (Li et al., 2020; Mandim et al., 2021). By integrating
497 these two novel approaches, our study provides an example of how to
498 comprehensively scrutinize the genetic and climatic factors affecting the composition
499 of secondary metabolites.

500 The dominant climatic factors affecting the secondary metabolites of the camphor
501 tree are not always the key factors affecting in other traditional herbs. For example,
502 humidity and sunshine time are the chief limiting factors in the production of artemisinin
503 in *Artemisia annua* L. (Li et al., 2017). Additionally, lower temperature in the coldest
504 quarter imposes stress on camphor tree and increases the production of desired
505 volatile compounds. In other cases, a higher temperature imposes stress and
506 enhances the production of desired metabolites (Liu et al., 2020). The limiting
507 ecological and climatic factors depended on the native ranges of the herbs and climatic
508 conditions during their growing season (Körner, 2021). A thorough investigation of
509 each specific case would be beneficial for decision making.

510 Some caveats need to be considered when interpreting our results. First, broader
511 planting of the same genotype (i.e., covering all provinces in South China and even
512 some Southeast Asian countries) would provide a thorough understanding of the
513 optimal climatic variables resulting in the greatest consistency and efficacy of the
514 medicinal components of camphor tree (Sharma et al., 2020). Second, the further
515 experimental verification of the effect of temperature on metabolite contents and the
516 functional validation of selected candidate genes (such as *TPS-b*, *TPS-g*, of *TPS-a*) of
517 the biosynthetic pathway under controlled laboratory conditions would enhance our
518 conclusions (Lau and Sattely, 2015; Nett et al., 2020). Third, soil characteristics
519 (including nutritional status, humidity and rhizosphere microorganisms, etc.) are
520 crucial for the accumulation of secondary metabolites (Ciancio et al., 2019; de Vries et

al., 2020). However, owing to the absence of appropriate data for assessing these characteristics, we overlooked ecological factors such as soil conditions. Further studies addressing the abovementioned limitations would provide an in-depth framework for understanding the genetic and environmental factors related to top-geoherbalism.

Taken together, our results lay a theoretical foundation for the optimal production of this economically significant tree species. The distributional range of camphor tree largely overlaps with the land and maritime portions of the silk road of the Belt and Road Initiative (Hinsley et al., 2020); thus, products obtained from camphor tree, such as TCMs or essential oil, show a high chance of being exported to connected countries in the Middle West, Western Europe and even North Africa. As tissue culture methods for camphor trees are well established and the growth rate of the trees is relatively fast (Shi et al., 2010), the commercial cultivation of trees *ex situ* is probably a sustainable and economic method in addition to importation (Brinckmann, 2015). Thus, our research will provide guidance for policy making, genotype selection and the optimization of climatic conditions for growth by domestic and international government stakeholders, farmers, merchandisers and consumers.

Methods

Plant materials

The sequences utilized for *de novo* genome assembly of the genome were obtained from the fresh leaves of a single camphor tree (*C. camphora* var. *linaloolifera* Fujita; NO.95), grown at Guangxi Forestry Research Institute.

Tissue-cultured seedlings were planted in four locations in Guangxi Province in China in May 2018, including Qinzhou (21°58'52"N, 108°39'14"E), Nanning (22°49'00"N, 108°19'39"E), Baise (23°54'09"N, 106°37'06"E) and Liuzhou (24°19'35.0"N, 109°25'41"E). Fresh leaves were collected from the four locations for RNA-Seq analyses and volatile compound quantification in November 2020.

DNA sequencing

High-molecular-weight (HMW) genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, USA), and 50 µg of HMW genomic DNA was prepared to generate SMRTbellTM libraries with a 20 kb insert size. Subsequently, PacBio circular consensus sequencing (CCS) data were produced on the PacBio Sequel II platform. Hi-C libraries were constructed from the tender leaves of *C. camphora* with fragments labelled with biotin and sequenced on the Illumina NovaSeq 6000 platform.

RNA sequencing

Total RNA was extracted from each sample by using an RNAprep Pure Plant kit (TIANGEN, Beijing, China) according to the user manual. cDNA was synthesized from 20 µg of total RNA using Rever Tra Ace (TOYOBO, Osaka, Japan) with oligo (dT) primers following the user manual. High-throughput sequencing was then performed on the Illumina NovaSeq 6000 platform.

De novo genome assembly and quality assessment

The *C. camphora* genome was assembled by integrating the sequencing data obtained with PacBio CCS technology and the Hi-C method using Hifiasm (Cheng et al., 2021) with the default parameters. We assembled two contig-level genomes, including a monoploid genome (haplotype A) and an allele-defined genome (haplotype B). Before Hi-C scaffolding, we filtered the redundant contigs from the contig-level genomes by using Purge_haplotigs (Roach et al., 2018). The Hi-C reads were assessed with the HiC-Pro program (Servant et al., 2015) and uniquely mapped to the contig assemblies. Juicer tools (Durand et al., 2016) and 3D-DNA pipelines (Dudchenko et al., 2017) were used to perform chromosome scaffolding. The BUSCO (Seppey et al., 2019) method was used to evaluate the completeness of the chromosome-level genomes.

Repetitive sequences and gene annotation

We used the EDTA pipeline (Ou et al., 2019) to annotate transposable elements in the *C. camphora* genome, including LTR, TIR and nonTIR elements. LAI assessment and LTR insertion time estimation were performed by LTR_retriever (Ou and Jiang, 2018) with the synonymous substitution rate set as 3.02e-9 (Cui et al., 2006). TRF software (Benson, 1999) with modified parameters ("trf 1 1 2 80 5 200 2000") was used to identify tandem repeats.

For gene model annotation, we trained *ab initio* gene predictors, including AUGUSTUS (Stanke and Morgenstern, 2005) and SNAP (Korf, 2004), on the repeat-masked genome using a combination of protein and transcript data. We used the annotated proteome data of *A. thaliana*, *L. chinense* (Chen et al., 2019), *C. kanehirae* (Chaw et al., 2019) and *P. americana* (Rendón-Anaya et al., 2019) and the Swiss-Prot database as the protein data. We employed RNA-Seq data from the four locations as the transcript data. To train AUGUSTUS, BRAKER2 (Bruna et al., 2021) was applied with the transcript data from aligned RNA-Seq bam files produced by Hisat2 (Kim et al., 2015). SNAP was trained under MAKER (Cantarel et al., 2008) with two iterations. Transcript data were supplied in the form of a *de novo* assembled transcriptome generated in Trinity (Haas et al., 2013) and a reference-based assembly generated by StringTie (Pertea et al., 2016). After training, the AUGUSTUS and SNAP results were fed into MAKER again along with all other data to produced synthesized gene models.

Functional annotations of the protein-coding sequences were obtained via BLASTP ("e-value 1e-10") searches against entries in both the NR and Swiss-Prot databases. The prediction of gene sequence motifs and structural domains was performed using InterProScan (Jones et al., 2014). The annotations of the GO terms and KEGG pathways of the genes were obtained from eggNOG-mapper (Huerta-Cepas et al., 2017).

Phylogenetic analyses and estimation of divergent times

A total of 16 plant species, including five magnoliids (*C. camphora*, *C. kanehirae*, *P. americana*, *Ch. salicifolius* and *L. chinense*), four monocots (*Zea mays*, *Oryza sativa*,

609 *Ananas comosus* and *Musa acuminata*), five eudicots (*A. thaliana*, *Populus*
610 *trichocarpa*, *Medicago truncatula*, *Solanum lycopersicum* and *V. vinifera*) and two
611 ANA-grade species (*Nymphaea colorata* and *Amborella trichopoda*) were used to
612 infer the phylogenetic tree. All genomes except for that of *Ch. salicifolius*
613 (http://xhhuanglab.cn/data/Chimonanthus_salicifolius.html) were downloaded from
614 JGI (<ftp://ftp.jgi-psf.org/pub/comp/gen/phytozome/v12.0/>) and Ensembl Plants
615 (<http://plants.ensembl.org/info/data/ftp/index.html>). Paralogues and orthologues were
616 identified among the 16 species by using the OrthoFinder pipeline (Emms and Kelly,
617 2019) with the default parameters, and the protein sequences of the 172 identified
618 single-copy orthologous genes were used for the construction of the phylogenetic tree.
619 The concatenated amino acid sequences were aligned with MAFFT (Katoh and
620 Standley, 2013) and trimmed with trimAl (Capella-Gutiérrez et al., 2015). A maximum
621 likelihood (ML) phylogenetic tree was constructed using IQ-TREE (Nguyen et al.,
622 2015) with ultrafast bootstrapping (-bb 1000), using *N. colorata* and *A. trichopoda* as
623 outgroups. The best-fitting substitution models were selected by ModelFinder
624 (Kalyaanamoorthy et al., 2017). In addition, ASTRAL-III v5.7.3 (Zhang et al., 2018)
625 was applied to infer the coalescence-based species tree with 172 gene trees. The
626 species tree was then used as an input to estimate the divergence time in the
627 MCMCTree program in the PAML package (Yang et al., 2007). The calibration time
628 was obtained from TimeTree (Kumar et al., 2017): 107-135 Mya for the divergence of
629 *V. vinifera* and *A. thaliana*, 42-52 Mya for *Z. mays* and *O. sativa*, 97-116 Mya for *O.*
630 *sativa* and *M. acuminata* and 173-199 Mya for *A. trichopoda* and *A. thaliana*. The
631 expansion and contraction of gene families were inferred with CAFÉ (De Bie et al.,
632 2006) based on the chronogram of the 16 species

633

634 **Genome duplication and syntenic analyses**

635 To identify the pattern of genome-wide duplications in *C. camphora*, we divided
636 duplicated genes into five categories, WGD, TD, PD (duplicated genes separated by
637 less than 10 genes on the same chromosome), TRD, and DSD (the remaining
638 duplicates other than the four specified types) gene, using DupGen_Finder (Qiao et
639 al., 2019) with the default parameters. The *Ka*, *Ks*, and *Ka/Ks* values were estimated
640 for duplicated gene pairs based on the YN model in *KaKs_Calculator2* (Wang et al.,
641 2010), followed by the conversion of amino acid alignments into the corresponding
642 codon alignments with PAL2NAL (Suyama et al., 2006). The genes in the five
643 duplicate categories were further subjected to GO and KEGG analyses with the R
644 package clusterProfiler (Yu et al., 2012). The enriched items were selected according
645 to an FDR criterion of 0.05. The dating of ancient WGDs and orthologue divergence
646 were estimated based on the formula $T = Ks/2r$, where *Ks* refers to the synonymous
647 substitutions per site, and *r* (3.02e-9) is the synonymous substitution rate for
648 magnoliids (Cui et al., 2006).

649 Genomic synteny blocks to be employed for intra- and interspecies comparisons
650 among magnoliids were identified with MCscan software (Tang et al., 2008). We
651 performed all-against-all LAST analyses (Kielbasa et al., 2011) and chained the LAST
652 hits according to a distance cut-off of 10 genes, requiring at least five gene pairs per

syntenic block. The syntenic “depth” function implemented in MCScan was applied to estimate the duplication histories of the respective genomes. Genomic synteny was visualized with the Python version of MCScan (Tang et al., 2008), the R package Rldeogram (Hao et al., 2020) and Circos (Krzywinski et al., 2009).

Gene expression profiling

The raw RNA-Seq data were filtered by using FASTP (Chen et al., 2018). The clean data were aligned to our assembled *C. camphora* genome with Hisat2 (Kim et al., 2015), and the quantification of gene expression was calculated with StringTie (Pertea et al., 2015). The Python script preDE.py built into StringTie was used to convert the quantification results into a count matrix. DEGs were detected with the DESeq2 R package (Love et al., 2014) with an FDR < 0.05.

TPS gene family

TPS genes are classified into seven subfamilies, including *TPS-a*, *TPS-b*, *TPS-c*, *TPS-d*, *TPS-e*, *TPS-f* and *TPS-g* (Chen et al., 2011). The *TPS* genes included in the *TPS-a* subfamily are responsible for forming 15-carbon sesquiterpenoids. The *TPS-b* and *TPS-g* superfamilies encode the enzymes producing 10-carbon monoterpenoids. The *TPS-c*, *TPS-e* and *TPS-f* subfamilies encode diterpene synthases, which catalyse the formation of 20-carbon isoprenoids. The *TPS-d* subfamily is gymnosperm specific and encodes enzymes involved in the production of 20-carbon isoprenoids (Martin et al., 2004). The *TPS* genes were predicted based on both their conserved domains (PF01397 and PF03936) and BLAST analyses. Conserved domains were used as search queries against the predicted proteome using hmmsearch in HMMER (<https://www.ebi.ac.uk/Tools/hmmer/>). *TPS* protein sequences from *A. thaliana* and *C. kanehirae* were used as queries to identify the *TPS* genes of *C. camphora*, *P. americana*, *L. chinense* and *V. vinifera*. The protein sequence hits of *TPS* genes were aligned with MAFFT (Katoh and Standley, 2013) and trimmed with trimAl (Capella-Gutierrez et al., 2009). The *TPS* gene tree was constructed using RAxML (Stamatakis, 2014) with 1,000 bootstrap replicates. The *TPS-c* subfamily was designated as the outgroup. The distribution of *TPS* genes on the chromosomes was visualized in TBtools (Chen et al., 2020).

Identification of genes involved in the MVA and MEP pathways

The MVA pathway involves six gene families, including the *acyl-coenzyme A-cholesterol acyl-transferase* (ACAT), *hydroxymethylglutaryl coenzyme A synthase* (HMGS), *hydroxymethylglutaryl coenzyme A reductase* (HMGR), *mevalonate kinase* (MVK), *phospho-mevalonate kinase* (PMK), and *mevalonate diphosphate decarboxylase* (MVD) genes. Seven gene families are involved in the MEP pathway, including the *1-deoxy-D-xylulose 5-phosphate synthase* (DXS), *1-deoxy-D-xylulose 5-phosphate reductoisomerase* (DXR), *2-C-methyl-D-erythritol-4-phosphate cytidyltransferase* (MCT), *4-(cytidine-5-diphospho)-2-C-methyl-D-erythritol kinase* (CMK), *MDS*, *(E)-4-hydroxy-3-methyl-but-2-enyl-pyrophosphate synthase* (HDS) and *(E)-4-hydroxy-3-methyl-but-2-enyl-pyrophosphate reductase* (HDR) genes. The

genes in both the MVA and MEP pathways are well documented in the model plants. To identify candidate genes related to the two pathways in the *C. camphora* genome, we collected the protein sequences of genes in the MVA and MEP pathways identified in *A. thaliana*. Using each *A. thaliana* gene as a query sequence, BLASTP (“-evaluate 1e-10”) analyses was performed to identify orthologous genes in *C. camphora*.

Determination of volatile metabolites

Six biological replicates were sampled in each location. The samples were ground into powder in liquid nitrogen. Two grams of the powder was transferred to a 20 ml headspace vial. The vials were sealed using crimp-top caps with TFE-silicone headspace septa. In solid-phase microextraction (SPME) analyses, each vial was placed at 60°C for 12 min, and a 65 µm divinylbenzene/carboxene/polydimethylsiloxane fibre (Supelco, Bellefonte, PA, USA) was then exposed to the headspace of the sample for 30 min at 60°C. Volatile metabolites were detected by MetWare (<http://www.metware.cn/>) based on the Agilent 7890B-7000D platform. The desorption of the volatile metabolites from the fibre coating was carried out in the injection port of the GC apparatus at 250°C for 10 min in spitless mode. The identification and quantification of volatile metabolites were carried out with a 30 m x 0.25 mm x 1.0 µm DB-5MS (5% phenyl-polymethylsiloxane) capillary column. Helium was used as the carrier gas at a linear velocity of 1.0 ml/min. The oven temperature was programmed to increase from 40°C (6 min) at 5°C/min until it reached 280°C, where it was held for 5 min. Mass spectra were recorded in electron impact (EI) ionization mode at 70 eV. The quadrupole mass detector, ion source and transfer line temperatures were set at 150, 230 and 280°C, respectively. Volatile compounds were identified by comparing the mass spectra with the data system library (MWGC) and linear retention index.

Climatic factor collection and analyses

Data on 17 climatic factors from Qinzhou, Nanning, Baise and Liuzhou from 2018 to 2020 were downloaded from the National Meteorological Information Centre (<http://data.cma.cn/en>). The analyses of the climatic factors were performed in the R package FactoMineR (Lê et al., 2008).

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743

Conflict of interest

745 No conflict of interest was declared.

746

Author contributions

748 L.W., C.L. K.X.L. and R.H.J. conceived and designed the study; R.H.J., X.L.C., C.S.Z.,
749 P.W. and K.X.L. prepared the materials; C.L., X.L.C., X.Z.L., D.P. and X.X.H.
750 performed data analyses; R.H.J., X.L.C., D.E.H, C.L. and L.W. wrote the manuscript;
751 all authors read and approved the final draft.

752

Data availability

754 All the raw sequence reads of *C. camphora* have been deposited in NCBI under the
755 BioProject accession number PRJNA761572. The *C. camphora* genome assembly
756 and annotations have been deposited on the cyVerse platform
757 (https://data.cyverse.org/dav-anon/iplant/home/licheng_caas/C.camphora_genome).

758

Reference

- 760 Brinckmann, J.A. (2015) Geographical indications for medicinal plants: Globalization,
761 climate change, quality and market implications for geo-authentic botanicals.
762 *World J. Trad. Chinese Med.* **1**,16-23.
- 763 Benson, G. (1999) Tandem repeats finder: a program to analyze DNA sequences.
764 *Nucleic Acids Res.* **27**,573-580.
- 765 Bottoni, M., Milani, F., Mozzo, M., Radice Kolloffel, D.A., Papini, A., Fratini, F., Maggi,
766 F. and Santagostini, L. (2021) Sub-tissue localization of phytochemicals in
767 *Cinnamomum camphora* (L.) J. Presl. growing in Northern Italy. *Plants* **10**,1008.
- 768 Bruna, T., Hoff, K.J., Lomsadze, A., Stanke, M. and Borodovsky, M. (2021) BRAKER2:
769 automatic eukaryotic genome annotation with GeneMark-EP+ and AUGUSTUS
770 supported by a protein database. *NAR Genom. Bioinform.* **3**,a108.
- 771 Cantarel, B.L., Korf, I., Robb, S.M., Parra, G., Ross, E., Moore, B., Holt, C., Sanchez,
772 A.A. and Yandell, M. (2008) MAKER: an easy-to-use annotation pipeline
773 designed for emerging model organism genomes. *Genome Res.* **18**,188-196.
- 774 Capella-Gutiérrez, S., Silla-Martinez, J.M. and Gabaldón, T. (2009) TrimAl: a tool for
775 automated alignment trimming in large-scale phylogenetic analyses.
776 *Bioinformatics* **25**,1972-1973.
- 777 Chaw, S., Liu, Y., Wu, Y., Wang, H., Lin, C.I., Wu, C., Ke, H., Chang, L., Hsu, C.,
778 Yang, H., Sudianto, E., Hsu, M., Wu, K., Wang, L., Leebens-Mack, J.H. and Tsai,
779 I.J. (2019) Stout camphor tree genome fills gaps in understanding of flowering
780 plant genome evolution. *Nat. Plants* **5**,63-73.
- 781 Chen, C., Chen, H., Zhang, Y., Thomas, H.R., Frank, M.H., He, Y. and Xia, R. (2020)
782 TBtools: An Integrative Toolkit developed for interactive analyses of big biological
783 data. *Mol. Plant* **13**,1194-1202.
- 784 Chen, C., Zheng, Y., Liu, S., Zhong, Y., Wu, Y., Li, J., Xu, L. and Xu, M. (2017) The

complete chloroplast genome of *Cinnamomum camphora* and its comparison with related Lauraceae species. *PeerJ* **5**,e3820.

Chen, C., Zhong, Y., Yu, F. (2020) Deep sequencing identifies miRNAs and their target genes involved in the biosynthesis of terpenoids in *Cinnamomum camphora*. *Ind. Crop Prod.* **145**,111853.

Chen, F., Tholl, D., Bohlmann, J. and Pichersky, E. (2011) The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *Plant J.* **66**,212-229.

Chen, J., Hao, Z., Guang, X., Zhao, C., Wang, P., Xue, L., Zhu, Q., Yang, L., Sheng, Y., Zhou, Y., Xu, H., Xie, H., Long, X., Zhang, J., Wang, Z., Shi, M., Lu, Y., Liu, S., Guan, L., Zhu, Q., Yang, L., Ge, S., Cheng, T., Laux, T., Gao, Q., Peng, Y., Liu, N., Yang, S. and Shi, J. (2019) *Liriodendron* genome sheds light on angiosperm phylogeny and species-pair differentiation. *Nat. Plants* **5**,18-25.

Chen, S., Sun, W., Xiong, Y., Jiang, Y., Liu, X., Liao, X., Zhang, D., Jiang, S., Li, Y., Liu, B., Ma, L., Yu, X., He, L., Liu, B., Feng, J., Feng, L., Wang, Z., Zou, S., Lan, S. and Liu, Z. (2020) The *Phoebe* genome sheds light on the evolution of magnoliids. *Hortic. Res.* **7**,146.

Chen, S., Zhou, Y., Chen, Y. and Gu, J. (2018) Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*, **34**, i884-i890.

Chen, Y., Li, Z., Zhao, Y., Gao, M., Wang, J., Liu, K., Wang, X., Wu, L., Jiao, Y., Xu, Z., He, W., Zhang, Q., Liang, C., Hsiao, Y., Zhang, D., Lan, S., Huang, L., Xu, W., Tsai, W., Liu, Z., Van de Peer, Y. and Wang, Y. (2020) The *Litsea* genome and the evolution of the laurel family. *Nat. Commun.* **11**,1675.

Cheng, H., Concepcion, G.T., Feng, X., Zhang, H. and Li, H. (2021) Haplotype-resolved *de novo* assembly using phased assembly graphs with hifiasm. *Nat. Methods* **18**,170-175.

Ciancio, A., Pieterse, C.M.J. and Mercado-Blanco, J. Editorial: Harnessing useful rhizosphere microorganisms for pathogen and pest biocontrol - second edition. *Front. Microbiol.* **10**,1935.

Consultation Shenzhen Limu Information (2021) China's linalool industry market development scale and forecast in 2021.

Cui, L., Wall, P.K., Leebens-Mack, J.H., Lindsay, B.G., Soltis, D.E., Doyle, J.J., Soltis, P.S., Carlson, J.E., Arumuganathan, K., Barakat, A., Albert, V.A., Ma, H. and DePamphilis, C.W. (2006) Widespread genome duplications throughout the history of flowering plants. *Genome Res.* **16**,738-749.

De Bie, T., Cristianini, N., Demuth, J.P. and Hahn, M.W. (2006) CAFÉ: a computational tool for the study of gene family evolution. *Bioinformatics* **22**, 1269-1271.

de Vries, F.T., Griffiths, R.I., Knight, C.G., Nicolitch, O. and Williams, A. (2020) Harnessing rhizosphere microbiomes for drought-resilient crop production. *Science* **368**,270-274.

Dudchenko, O., Batra, S.S., Omer, A.D., Nyquist, S.K., Hoeger, M., Durand, N.C., Shamim, M.S., Machol, I., Lander, E.S., Aiden, A.P. and Aiden, E.L. (2017) *De novo* assembly of the *Aedes aegypti* genome using Hi-C yields

829 chromosome-length scaffolds. *Science* **356**,92-95.

830 Durand, N.C., Shamim, M.S., Machol, I., Rao, S.S., Huntley, M.H., Lander, E.S. and
831 Aiden, E.L. (2016) Juicer provides a one-click system for analyzing
832 loop-resolution Hi-C experiments. *Cell Syst.* **3**,95-98.

833 Eggersdorfer, M. (2000) *Terpenes*". *Ullmann's Encyclopedia of Industrial Chemistry*.

834 Emms, D.M. and Kelly, S. (2019) OrthoFinder: phylogenetic orthology inference for
835 comparative genomics. *Genome Biol.* **20**,238.

836 Endress, P.K. and Doyle, J.A. (2009) Reconstructing the ancestral angiosperm flower
837 and its initial specializations. *Am. J. Bot.* **96**,22-66.

838 Haas, B.J., Papanicolaou, A., Yassour, M., Grabherr, M., Blood, P.D., Bowden, J.,
839 Couger, M.B., Eccles, D., Li, B., Lieber, M., MacManes, M.D., Ott, M., Orvis, J.,
840 Pochet, N., Strozzi, F., Weeks, N., Westerman, R., William, T., Dewey, C.N.,
841 Henschel, R., LeDuc, R.D., Friedman, N. and Regev, A. (2013) *De novo*
842 transcript sequence reconstruction from RNA-seq using the Trinity platform for
843 reference generation and analyses. *Nat. Protoc.* **8**,1494-1512.

844 Hamidpour, R., Hamidpour, S., Hamidpour, M. and Shahlari, M. (2012) Camphor
845 (*Cinnamomum camphora*), a traditional remedy with the history of treating
846 several diseases. *Int. J. Case Rep. Images* **4**,86.

847 Hao, Z., Lv, D., Ge, Y., Shi, J., Weijers, D., Yu, G., Chen, J. (2020) Rldeogram:
848 drawing SVG graphics to visualize and map genome-wide data on the idiograms.
849 *PeerJ Comput. Sci.* **6**, e251.

850 Hinsley, A., Milner-Gulland, E.J., Cooney, R., Timoshyna, A., Ruan, X.D. and Lee, T.M.
851 (2020) Building sustainability into the Belt and Road Initiative's Traditional
852 Chinese Medicine trade. *Nat. Sustain.* **3**,96-100.

853 Hou, J., Zhang, J., Zhang, B., Jin, X., Zhang, H. and Jin, Z. (2020) Transcriptional
854 analyses of metabolic pathways and regulatory mechanisms of essential oil
855 biosynthesis in the leaves of *Cinnamomum camphora* (L.) Presl. *Front. Genet.*
856 **11**,598714.

857 Hu, L., Xu, Z., Wang, M., Fan, R., Yuan, D., Wu, B., Wu, H., Qin, X., Yan, L., Tan, L.,
858 Sim, S., Li, W., Saski, C.A., Daniell, H., Wendel, J.F., Lindsey, K., Zhang, X., Hao,
859 C. and Jin, S. (2019) The chromosome-scale reference genome of black pepper
860 provides insight into piperine biosynthesis. *Nat. Commun.* **10**,4702.

861 Huang, L. (2012) *Molecular Pharmacognosy*. Shanghai Scientific and Technical
862 Publishers.

863 Huerta-Cepas, J., Forslund, K., Coelho, L.P., Szklarczyk, D., Jensen, L.J., von Mering,
864 C. and Bork, P. (2017) Fast genome-wide functional annotation through orthology
865 assignment by eggNOG-Mapper. *Mol. Biol. Evol.* **34**,2115-2122.

866 Jones, P., Binns, D., Chang, H.Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H.,
867 Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A.F., Sangrador-Vegas, A.,
868 Scheremetjew, M., Yong, S.Y., Lopez, R. and Hunter, S. (2014) InterProScan 5:
869 genome-scale protein function classification. *Bioinformatics* **30**,1236-1240.

870 Kalyanamoorthy, S., Minh, B.Q., Wong, T.K.F., von Haeseler, A. and Jermin, L.S.
871 (2017) ModelFinder: fast model selection for accurate phylogenetic estimates.
872 *Nat. Methods* **14**, 587-589.

873 Katoh, K. and Standley, D.M. (2013) MAFFT multiple sequence alignment software
874 version 7: improvements in performance and usability. *Mol. Biol. Evol.*
875 **30**,772-780.

876 Kielbasa, S.M., Wan, R., Sato, K., Horton, P. and Frith, M.C. (2011) Adaptive seeds
877 tame genomic sequence comparison. *Genome Res.* **21**, 487-493.

878 Kim, D., Langmead, B. and Salzberg, S.L. (2015) HISAT: a fast spliced aligner with
879 low memory requirements. *Nat. Methods* **12**,357-360.

880 Körner C. (2021) The cold range limit of trees. *Trends Ecol. Evol.* **13**,S0169-S5347.

881 Korf, I. (2004) Gene finding in novel genomes. *BMC Bioinformatics* **5**,59.

882 Krzywinski, M., Schein, J., Birol, I., Connors, J., Gascoyne, R., Horsman, D., Jones,
883 S.J. and Marra, M.A. (2009) Circos: an information aesthetic for comparative
884 genomics. *Genome Res.* **19**, 1639-1645.

885 Lau, W. and Sattely, E.S. (2015) Six enzymes from mayapple that complete the
886 biosynthetic pathway to the etoposide aglycone. *Science* **11**,1224-1228.

887 Lê S, Josse J and Husson F (2008) FactoMineR: An R package for multivariate
888 analyses. *J. Stat. Softw.* **25**,1-18.

889 Letizia, C.S., Cocchiara, J., Lalko, J. and Api, A.M. (2003) Fragrance material review
890 on linalool. *Food Chem. Toxicol.* **41**,943-964.

891 Li, C., He, Q., Zhang, F., Yu, J., Li, C., Zhao, T., Zhang, Y., Xie, Q., Su, B., Mei, L.,
892 Zhu, S. and Chen, J. (2019) Melatonin enhances cotton immunity to *Verticillium*
893 wilt via manipulating lignin and gossypol biosynthesis. *Plant J.* **100**,784-800.

894 Li, L., Josef, B.A., Liu, B., Zheng, S., Huang, L. and Chen, S. (2017)
895 Three-Dimensional Evaluation on Ecotypic Diversity of traditional Chinese
896 medicine: A case study of *Artemisia annua* L. *Front. Plant Sci.* **8**.

897 Li, Q., Ramasamy, S., Singh, P., Hagel, J.M., Dunemann, S.M., Chen, X., Chen, R.,
898 Yu, L., Tucker, J.E., Facchini, P.J. and Yeaman, S. (2020) Gene clustering and
899 copy number variation in alkaloid metabolic pathways of opium poppy. *Nat.*
900 *Commun.* **11**,1190.

901 Li, Y., Kong, D., Fu, Y., Sussman, M.R. and Wu, H. (2020) The effect of
902 developmental and environmental factors on secondary metabolites in medicinal
903 plants. *Plant Physiol. Biochem.* **148**,80-89.

904 Liu, L., Zuo, Z., Xu, F. and Wang, Y. (2020) Study on quality response to
905 environmental factors and geographical traceability of wild gentiana rigescens
906 Franch. *Front. Plant Sci.* **11**,1128.

907 Liu, X., Meng, Y., Zhang, Z., Wang, Y., Geng, X., Li, M., Li, Z. and Zhang, D. (2019)
908 Functional nano-catalyzed pyrolyzates from branch of *Cinnamomum camphora*.
909 *Saudi J. Biol. Sci.* **26**,1227-1246.

910 Love, M.I., Huber, W. and Anders, S. (2014) Moderated estimation of fold change and
911 dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550.

912 Luo, Q., Xu, C., Zheng, T., Ma, Y., Li, Y. and Zuo, Z. (2021) Leaf morphological and
913 photosynthetic differences among four chemotypes of *Cinnamomum camphora*
914 in different seasons. *Ind. Crop Prod.* **169**,113651.

915 Lv, Q., Qiu, J., Liu, J., Li, Z., Zhang, W., Wang, Q., Fang, J., Pan, J., Chen, Z., Cheng,
916 W., Barker, M.S., Huang, X., Wei, X. and Cheng, K. (2020) The *Chimonanthus*

- 917 *salicifolius* genome provides insight into magnoliid evolution and flavonoid
918 biosynthesis. *Plant J.* **103**,1910-1923.
- 919 Mandim, F., Petropoulos, S.A., Dias, M.I., Pinela, J., Kostic, M., Soković, M.,
920 Santos-Buelga, C., Ferreira, I. and Barros, L. (2021) Seasonal variation in
921 bioactive properties and phenolic composition of cardoon (*Cynara cardunculus*
922 var. *altilis*) bracts. *Food Chem.* **336**,127744.
- 923 Massoni J, Couvreur TL and Sauquet H (2015) Five major shifts of diversification
924 through the long evolutionary history of Magnoliidae (angiosperms). *BMC Evol.*
925 *Biol.* **15**.
- 926 Moore, M.J., Bell, C.D., Soltis, P.S, and Soltis, D.E. (2007) Using plastid
927 genome-scale data to resolve enigmatic relationships among basal angiosperms.
928 *PNAS* **104**,19363-19368.
- 929 Nett, R.S., Lau, W. and Sattely, E.S. (2020) Discovery and engineering of colchicine
930 alkaloid biosynthesis. *Nature* **584**,48-153.
- 931 Nguyen, L.T., Schmidt, H.A., von Haeseler, A. and Minh, B.Q. (2015) IQ-TREE: a fast
932 and effective stochastic algorithm for estimating maximum-likelihood phylogenies.
933 *Mol. Biol. Evol.* **32**, 268-274.
- 934 Ou, S. and Jiang, N. (2018) LTR_retriever: A highly accurate and sensitive program
935 for identification of long terminal repeat retrotransposons. *Plant Physiol.*
936 **176**,1410-1422.
- 937 Ou, S., Su, W., Liao, Y., Chougule, K., Agda, J., Hellinga, A.J., Lugo, C., Elliott, T.A.,
938 Ware, D., Peterson, T., Jiang, N., Hirsch, C.N. and Hufford, M.B. (2019)
939 Benchmarking transposable element annotation methods for creation of a
940 streamlined, comprehensive pipeline. *Genome Biol.* **20**,275.
- 941 Palmer, J.D., Soltis, D.E. and Chase, M.W. (2004) The plant tree of life: an overview
942 and some points of view. *Am. J. Bot.* **91**,1437-1445.
- 943 Perte, M., Kim, D., Perte, G.M., Leek, J.T. and Salzberg, S.L. (2016)
944 Transcript-level expression analyses of RNA-seq experiments with HISAT,
945 StringTie and Ballgown. *Nat. Protoc.* **11**,1650-1667.
- 946 Qiao, X., Li, Q., Yin, H., Qi, K., Wang, R., Zhang S. and Paterson, A. (2019) Gene
947 duplication and evolution in recurring polyploidization-diploidization cycles in
948 plants. *Genome Biol.* **20**, 38.
- 949 Qin, L., Hu, Y., Wang, J., Wang, X., Zhao, R., Shan, H., Li, K., Xu, P., Wu, H., Yan, X.,
950 Liu, L., Yi, X., Wanke, S., Bowers, J.E., Leebens-Mack, J.H., DePamphilis, C.W.,
951 Soltis, P.S., Soltis, D.E., Kong, H. and Jiao, Y. (2021) Insights into angiosperm
952 evolution, floral development and chemical biosynthesis from the *Aristolochia*
953 *fimbriata* genome. *Nat. Plants* <https://doi.org/10.1038/s41477-021-00990-2>
- 954 Qiu, Y., Li, L., Wang, B., Xue, J., Hendry, T.A., Li, R., Brown, J.W., Liu, Y., Hudson,
955 G.T. and Chen, Z. (2010) Angiosperm phylogeny inferred from sequences of four
956 mitochondrial genes. *J. Syst. Evol.* **48**,391-425.
- 957 Ravindran, P.N., Nirmal Babu, K. and Shylaja, M. (2004) *Cinnamomum* and *Cassia*, the
958 *Genus Cinnamomum*. USA:CRC Press: Boca Raton.
- 959 Rendón-Anaya, M., Ibarra-Laclette, E., Mendez-Bravo, A., Lan, T., Zheng, C.,
960 Carretero-Paulet, L., Perez-Torres, C.A., Chacon-Lopez, A., Hernandez-Guzman,

961 G., Chang, T.H., Farr, K.M., Barbazuk, W.B., Chamala, S., Mutwil, M., Shivhare,
962 D., Alvarez-Ponce, D., Mitter, N., Hayward, A., Fletcher, S., Rozas, J., Sanchez,
963 G.A., Kuhn, D., Barrientos-Priego, A.F., Salojarvi, J., Librado, P., Sankoff, D.,
964 Herrera-Estrella, A., Albert, V.A. and Herrera-Estrella, L. (2019) The avocado
965 genome informs deep angiosperm phylogeny, highlights introgressive
966 hybridization, and reveals pathogen-influenced gene space adaptation. *PNAS*
967 **116**,17081-17089.

968 Roach, M.J., Schmidt, S.A. and Borneman, A.R. (2018) Purge Haplotigs: allelic contig
969 reassignment for third-gen diploid genome assemblies. *BMC Bioinformatics*
970 **19**,460.

971 Seppey, M., Manni, M. and Zdobnov, E.M. (2019) BUSCO: Assessing Genome
972 Assembly and Annotation Completeness. *Methods Mol. Biol.* **1962**,227-245.

973 Servant, N., Varoquaux, N., Lajoie, B.R., Viara, E., Chen, C.J., Vert, J.P., Heard, E.,
974 Dekker, J. and Barillot, E. (2015) HiC-Pro: an optimized and flexible pipeline for
975 Hi-C data processing. *Genome Biol.* **16**,259.

976 Shang, J., Tian, J., Cheng, H., Yan, Q., Li, L., Jamal, A., Xu, Z., Xiang, L., Saski, C.A.,
977 Jin, S., Zhao, K., Liu, X. and Chen, L. (2020) The chromosome-level wintersweet
978 (*Chimonanthus praecox*) genome provides insights into floral scent biosynthesis
979 and flowering in winter. *Genome Biol.* **21**,200.

980 Sharma, S., Walia, S., Rathore, S., Kumar, P. and Kumar, R. (2020) Combined effect
981 of elevated CO₂ and temperature on growth, biomass and secondary metabolite
982 of *Hypericum perforatum* L. in a western Himalayan region. *J. Appl. Res. Med.*
983 *Aroma.* **16**,2214-7861.

984 Shi, X., Dai, X., Liu, G., Zhang, J., Ning, G. and Bao, M. (2010). Cyclic secondary
985 somatic embryogenesis and efficient plant regeneration in camphor tree
986 (*Cinnamomum camphora* L.). *In Vitro Cell Dev. Pl.* **46**,117-125.

987 Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analyses and
988 post-analyses of large phylogenies. *Bioinformatics* **30**,1312-1313.

989 Stanke, M. and Morgenstern, B. (2005) AUGUSTUS: a web server for gene prediction
990 in eukaryotes that allows user-defined constraints. *Nucleic Acids Res.*
991 **33**,W465-W467.

992 Suyama, M., Torrents, D. and Bork, P. (2006) PAL2NAL: robust conversion of protein
993 sequence alignments into the corresponding codon alignments. *Nucleic Acids*
994 *Res.* **34**, W609-W612.

995 Tan, H., Hu, D., Song, J., Xu, Y., Cai, S., Chen, Q., Meng, Q., Li, S., Chen, S., Mao, Q.
996 and Xu, H. (2015) Distinguishing *Radix Angelica sinensis* from different regions
997 by HS-SFME/GC-MS. *Food Chem.* **186**,200-206.

998 Tang, H., Bowers, J.E., Wang, X., Ming, R., Alam, M. and Paterson, A.H. (2008)
999 Synteny and collinearity in plant genomes. *Science* **320**, 486-488.

1000 Tholl, D. (2015) Biosynthesis and biological functions of terpenoids in plants. *Adv*
1001 *Biochem. Eng. Biotechnol.* **148**,63-106.

1002 Tian, Z., Luo, Q. and Zuo, Z. (2021) Seasonal emission of monoterpenes from four
1003 chemotypes of *Cinnamomum camphora*. *Ind. Crop Prod.* **163**,113327.

1004 Wang, D., Zhang, Y., Zhang, Z., Zhu, J. and Yu, J. (2010) KaKs_Calculator 2.0: a

1005 toolkit incorporating gamma-series methods and sliding window strategies.
1006 *Genomics Proteom. Bioinform.* **8**, 77-80.
1007 Wu, Y., Xiao, F., Xu, H., Zhang, T. and Jiang, X. (2014) Genome Survey in
1008 *Cinnamomum camphora* L. Presl. *J. Plant. Genet. Resour.* **15**,149-152.
1009 Yang, Z. (2007) PAML 4, phylogenetic analyses by maximum likelihood. *Mol. Biol.*
1010 *Evol.* **24**,1586-1591.
1011 Yoshida, T., Muraki, S., Kawamura, H. and Komatsu, A. (1969) Minor constituents of
1012 Japanese ho-leaf oil the structures of (+)-tagetanol and (-)-trans-Hotrienol. *Agric.*
1013 *Biol. Chem.* **3**,343-352.
1014 Yu, G., Wang, L.G., Han, Y., and He, Q.Y. (2012) clusterProfiler: an R package for
1015 comparing biological themes among gene clusters. *OMICS* **16**, 284-287.
1016 Yuan, M., Yan, Z., Sun, D., Luo, X., Xie, L., Li, M., Wang, S., Shi, Q. and Zhang, Y.
1017 (2020) New insights into the impact of ecological factor on bioactivities and
1018 phytochemical composition of *Paeonia veitchii*. *Chem. Biodivers* **17**.
1019 Zeng, L., Zhang, Q., Sun, R., Kong, H., Zhang, N. and Ma, H. (2014) Resolution of
1020 deep angiosperm phylogeny using conserved nuclear genes and estimates of
1021 early divergence times. *Nat. Commun.* **5**,4956.
1022 Zhang, C., Rabiee, M., Sayyari, E. and Mirarab, S. (2018) ASTRAL-III: polynomial
1023 time species tree reconstruction from partially resolved gene trees. *BMC*
1024 *Bioinformatics* **19**,153.
1025 Zhang, Z., Wu, X., Lai, Y., Li, X., Zhang, D. and Chen, Y. (2020) Efficient extraction of
1026 bioenergy from *Cinnamomum camphora* leaves. *Front. Energy Res.* **8**.
1027 Zhao, Z., Guo, P. and Brand, E. (2012) The formation of daodi medicinal materials. *J.*
1028 *Ethnopharmacol.* **140**,476-481.
1029 Zhou, F. and Pichersky, E. (2020) More is better: the diversity of terpene metabolism
1030 in plants. *Curr. Opin. Plant Biol.* **55**,1-10.
1031
1032