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Advances in the study of key genes and transcription factors regulating the mevalonate synthesis pathway in Edible and medicinal plants

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Abstract

Terpenoids are one of the secondary metabolites of plants and are the most widely distributed in nature with a diverse skeleton and significant biological activity, among which sesquiterpenoids are the most abundant. Sesquiterpenoids and triterpenoids are mainly synthesized by the mevalonate pathway, and the processes involve a variety of key enzymes and are regulated by transcription factors. They play important roles in plant growth and development, environmental adaptation, and resistance to pests and diseases. It is important to understand the gene functions of key enzymes to increase the production of sesquiterpenoids and triterpenoids to meet the market demand and to study the mevalonate biosynthesis pathway in depth. In this paper, the progress of gene cloning and transcription factor regulation of sesquiterpenes, triterpenoids and key enzymes of mevalonate synthesis pathway will be briefly reviewed.

Keywords: sesquiterpenoids and triterpenoids; mevalonate pathway; critical gene cloning; transcription factors.

Practical Application: Grasping the gene function of key enzymes is of great significance for increasing the production of sesquiterpenoids and triterpenoids, meeting the market demand and deeply studying the biosynthetic pathway of mevalonate.

1 Introduction

Sesquiterpenoids and triterpenoids are mainly synthesized by the mevalonate pathway (MVA) (Niu et al., 2021) and are used in various industries such as clinical, cosmetic, and food applications. However, the content of natural plant terpenoids is relatively low, industrial production is uneconomical, the cost is high, and the utilization rate is low, so it is difficult to meet the market demand. Various compounds with novel structures have been found and shown a variety of biological activities (Socorro et al., 2021). With the wide application of various biotechnological methods and synthetic biology strategies, the key enzymes for the synthesis of sesquiterpenes and triterpenoids have become a new research hotspot, attracting the attention of more and more scholars. Thus, a variety of key genes of MVA biosynthesis pathway have been successfully cloned and expressed, providing new ideas for metabolic pathways of sesquiterpenoids and triterpenoids for application in plant engineering. Plant metabolites are not only regulated by environmental factors, but some endogenous factors such as transcription factors are also influencing the levels of secondary metabolites (Thakur & Vasudev, 2022). The MVA synthesis pathway is regulated by a variety of key enzymes and transcription factors. They play a crucial role in the interaction between plants (Huang et al., 2019) and the interaction with the ecological environment (Campbell et al., 2019; Warsi et al., 2023). This paper reviews the research on key enzymes and transcription factors involved in regulating plant MVA synthesis pathway. The brief description is as follows.

2 Sesquiterpenoids and triterpenoids

The sesquiterpenoids are the largest secondary metabolite group of plants and are the most abundant class of terpenoids. They are polymerized from three isoprene structural units, and their general structural formula is $(C_5H_8)_3$. The sesquiterpenoids have a large number of species and complex structures, widely distributed in plants and microorganisms, with more than 200 structural skeletons (Chen et al., 2020). According to its structural carbon ring number, it can be divided into acyclic, monocyclic, bicyclic, tricyclic and tetracyclic sesquiterpenes. According to the number of carbon atoms constituting the ring, it can be divided into five-membered rings, six-membered rings, seven-membered rings, up to twelve-membered rings. It can also be divided into sesquiterpene alcohols, sesquiterpene aldehydes, sesquiterpene ketones, and sesquiterpene lactones according to the oxygen-containing functional groups. The sesquiterpenoids are also important constituents of plant volatile oils with a variety of pharmacological activities such as anti-cancer (Abu-Izneid et al., 2020), anti-oxidation (Zhang et al., 2021), anti-inflammatory (Tungcharoen et al., 2018; Lima et al., 2021), and anti-microbial (Perveen et al., 2020).

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The glycosides of triterpenoids act as a surfactant due to their lipophilic nature and are also known as triterpene saponins. Triterpenoid saponins are composed of triterpene sapogenin and sugars. The majority of triterpenes are tetracyclic triterpenes and pentacyclic triterpenes. Triterpenoids and their glycosides are widely found in nature and have various pharmacological activities such as regulation of lipid metabolism (Dembitsky, 2021), anti-cancer (Ren & Kinghorn, 2019), and protecting liver. Triterpenoids have become one of the most important natural products of liver protecting agents (Xu et al., 2018).

3 MVA biosynthetic pathway

The mevalonate pathway (MVA) synthesis pathway is mainly found in the cytoplasm, endoplasmic reticulum and peroxisomes (Jin et al., 2020). The MVA pathway begins with the condensation of two molecules of acetyl coenzyme A by acetyl-CoA C-acetyl transferase (AACT) to form acetyl acetyl coenzyme A, followed by hydroxymethylglutaryl coenzyme A synthase (HMG-CoA synthase, HMGS) catalyzing the aldol condensation reaction of acetyl acetyl coenzyme A combined with a third molecule by three molecules of acetyl coenzyme A to produce 3-hydroxy-3-methylglutaryl monoacyl coenzyme A (HMG-CoA). 3-Hydroxy-3-methyl glutaryl coenzyme A reductase (HMGR) is the first rate limiting enzyme in the mevalonate pathway, which catalyzes the irreversible production of mevalonate acid (MVA) from HMG CoA, and participates in the first rate limiting step in the mevalonate pathway. Mevalonate kinase (MVK) is one of the key rate-limiting enzymes of the MVA pathway. As the first ATP-dependent enzyme in the enzymatic reaction of the MVA pathway, it is responsible for transferring the phosphate group from triphosphate adenosine to the fifth hydroxyl group of MVA to form mevalonate-5-phosphate and release ADP. After three steps of enzymatic reaction, it finally generates isopentenyl pyrophosphate (IPP) (Li et al., 2017; Tholl, 2015). Phosphomevalonate kinase (PMK) belongs to the GHMP kinase superfamily. It is the second of three consecutive ATP-dependent rate-limiting reactions in the MVA pathway, whose role is to transfer the γ-phosphate group from ATP to mevalonate-5-phosphate (MVAP) to generate mevalonate-5-diphosphate (MVAPP). IPP is the basic skeleton of isoprenoid biosynthesis, from which all sesquiterpenes, sterols and other compounds have evolved (Bergman et al., 2019). IPP is produced by Diphosphomevalonate decarboxylase (MVD) catalyzing the decarbonylation of 6-carbon mevalonate pyrophosphate. In MVA pathway, only IPP is formed, while the MEP pathway produces IPP and dimethylallyl pyrophosphate (DMAPP) (Rohdich et al., 2003) in a ratio of 6:1, and they are isomers of each other (Wang & Oldfield, 2014). To maintain the equilibrium of both IPP and DMAPP, isopentenyl-diphosphate isomerase (Isopentenyl-diphosphate Δ3-Δ2-isomerase, IDI) partially converts IPP to DMAPP (Phillips et al., 2008). IPP and DMAPP are both used as synthetic intermediates. Due to the different subcellular localization of short chain isopentenyl pyrophosphate transferase, the formation of isopentenyl pyrophosphate intermediates has interval specificity. In the cytoplasm, farnesyl diphosphate synthase (FPPS) catalyzes the synthesis of the sesquiterpene precursor farnesyl diphosphate (FPP) from two molecules of IPP and one molecule of DMAPP. In plastids, one molecule of DMAPP and one or three molecules of IPP are catalyzed by geranyl diphosphate synthase (GPPS) and geranylgeranyl diphosphate synthase (GGPPS) to form geranyl pyrophosphate (GPP) and geranylgeranyl diphosphate (GGPP) (Abbas et al., 2017). Then squalene synthase (SQS) catalyzes the internal cyclization of two molecules of FPP to produce one molecule of squalene (SQ). Therefore, SQS is considered to be the key enzyme in the biosynthesis of the carbon ring skeleton in the middle of triterpene saponins. The synthesis pathway is shown in the Figure 1.

4 Gene cloning of MVA key enzymes

4.1 AACT clone

Sesquiterpene is an important component of volatile oil in *Santalum album* L.. Niu et al successfully cloned an AACT gene from Indian sandalwood for the first time by cDNA end amplification (RACE) technique (Niu et al., 2021). Its cDNA was 1538 bp in full length and contained a 1248 bp open reading frame (ORF) encoding 415 putative amino acid residues, and the differential expression levels of SaAACT gene in different tissues after MeJA treatment were detected by RT-PCR. The results showed that SaAACT was expressed at higher levels in the roots, followed by mature leaves > heartwood > young leaves. Overexpression of the AACT gene increased sesquiterpene content, which was positively correlated with its expression. It indicates that MeJA may be an effective method to induce high yield of sesquiterpenoids.

The WRKY transcription factor family is one of the largest groups of transcription factors in plants and plays an important role in plant growth and development as well as in biotic and abiotic stress responses (Zhao et al., 2019). It has been found

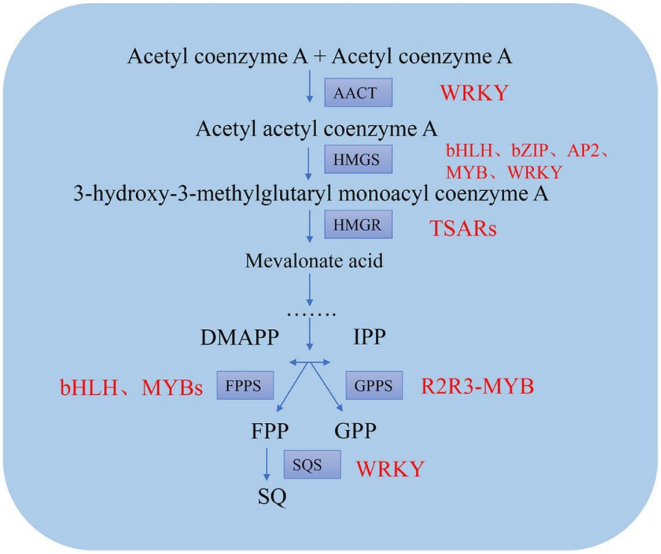

Figure 1. MVA synthesis pathway. Solid boxes indicate key enzymes and red indicates transcription factors. AACT: acetyl-CoA C-acetyl transferase; HMGS:3-hydroxy-3-methy-lglutatryl-CoA synathase; HMGR:3-hydroxy-3-methy-lglutaryl coenzyme A reductase; FPPS: farnesyl diphosphate synthase; GPPS: geranyl diphosphate synthase; SQS: squalene synthase.

that WRKY positively regulates ginsenoside biosynthesis by binding to the W-box element of the gene promoter (Di et al., 2021). Su et al identified W-box elements from the PtAACT gene promoter of *Psammosilene tunicoides* W.C.Wu & C.Y.Wu by full-length transcriptome data. Interestingly, PtWRKY70 was up-regulated with PtAACT after SA treatment and also, increased the biosynthesis of triterpenoid saponins in *Psammosilene tunicoides* W.C.Wu & C.Y.Wu (Su et al., 2021). In summary, it is suggested that there may be an interaction between the WRKY transcription factor family and the AACT key enzymes in the biosynthetic pathway of triterpenoid saponins.

4.2 HMGS clone

Ginkgolide is an important active ingredient in *Ginkgo biloba* L., consisting of sesquiterpene lactones and diterpene lactones. Meng et al obtained the inferred HMGS gene from *Ginkgo biloba* for the first time by PCR and RACE methods. the full-length cDNA sequence of GbHMGS is 1945 bp, contains a 1422 bp ORF, and encodes a 474 amino acid protein (Meng et al., 2017). It is highly homologous with *Taxus chinensis* (Pilg.) Rehder, *Pinus sylvestris* L. and *Camptotheca acuminata* Decne.. A comparative model of the 3D structure of GbHMGS was generated using SWISS-MODEL. The polypeptide chain of GbHMGS contains three structural domains, namely the N-terminal, the catalytic region and the C-terminal. The N-terminus contains a conserved signal peptide sequence and the C-terminus contains an important catalytic cysteine residue. The predicted 3D model of GbHMGS consists of two structural regions, called the lower and upper regions. In addition, the interface between the upper and lower regions defines the acetylacetyl coenzyme a binding site, and all conserved motifs and active sites are located in the five-layer core structure of the upper region. It is important for the elucidation of sesquiterpene lactones biosynthesis.

Characterization of key enzymes and transcription factors regulating triterpene saponin biosynthesis in *Camellia sinensis* (L.) Kuntze revealed that HMGS genes were highly expressed in flowers and seeds of tea tree, producing more saponins. Various transcription factors such as bHLH, bZIP, AP2, MYB, and WRKY are highly co-expressed with CsHMGS, and these transcription factors may regulate triterpene saponin biosynthesis through transcriptional regulation of genes such as CsHMGS (Chen et al., 2022).

4.3 HMGR clone

Panax ginseng C.A.Mey. is a common herbal medicine containing triterpenes and their glycosides, and HMGR is the first rate-limiting enzyme of the MVA pathway. Luo et al cloned the open reading frame of PgHMGR from *Panax ginseng* C.A.Mey.. cDNA sequence was 1770 bp in length and encoded 589 amino acids (Luo et al., 2013). Bioinformatics analysis showed that the protein encoded by PgHMGR2 contains two transmembrane regions and an HMG-CoA-reductase structural domain without a signal peptide. Based on real-time fluorescence quantitative PCR analysis, the expression level of PgHMGR was highest in flowers, followed by leaves and roots, and the lowest was in stems. Some studies have also shown the presence of the ABRE factor of the TACGTG sequence in the PqHMGR promoter, which regulates ginsenoside levels by responding to abscisic acid (ABA) (Kochan et al., 2019). Yu et al. were the first to clone the PgHMGR gene, which has a conserved structural domain of HMG-CoA_ reductase_ classI (HMG-CoA reductase), from the roots of *Platycodon grandiflorus* (Jacq.) A.DC., and successfully induced a recombinant protein. The medicinal value of *Platycodon grandiflorus* (Jacq.) A.DC. is derived from the triterpenoid saponins of *Platycodon grandiflorus* (Jacq.) A.DC.. Laying the foundation for further research on the triterpene biosynthesis pathway of *Platycodon grandiflorus* (Jacq.) A.DC. (Yu et al., 2022). In summary, we suggest that HMGR may be involved in the biosynthesis of sesquiterpenes and triterpenes.

The transcription factors TSAR1 and TSAR2 of the basic helix-loop-helix family were found to mediate the biosynthesis of triterpene saponins in *Medicago truncatula* Gaertn.. TSAR1 and TSAR2 are mediated through direct binding of TSARs to the HMGR1 promoter N-box, which synergistically regulates and activates them in trans with genes encoding HMGR1 and E3 ubiquitin ligases that control HMGR1 levels, respectively. Importantly, overexpression of TSAR1 or TSAR2 in *Medicago truncatula* Gaertn. hairy roots resulted in elevated transcript levels of known triterpene saponin biosynthesis genes and strongly increased the accumulation of triterpene saponins. The above proves that transcription factors have an important role in *Medicago truncatula* Gaertn. clover triterpene metabolism (Mertens et al., 2016).

5 Cloning of other enzymes

5.1 FPPS clone

Farnesyl pyrophosphate synthase (FPPS) plays an important role in the MVA synthesis pathway in plants. Artemisinin synthesized from *Artemisia annua* L. is a potent antimalarial sesquiterpene lactone and is a potent drug for the treatment of cerebral malaria and anti-chloroquine falciparum malaria (Zhang et al., 2019; Oliveira et al., 2022). FPPS is a key enzyme in the artemisinin biosynthesis pathway. To increase the production, Elfahmi et al enhanced its production by semi-synthetic production in Saccharomyces cerevisiae using pBEVY vector transformed into Saccharomyces cerevisiae and by PCR, restriction and sequencing analysis, it can be concluded that FPPS gene was successfully constructed, transformed and expressed in Saccharomyces cerevisiae (Elfahmi et al., 2021). The direct cloning method using yeast is simpler, more cost effective and less time consuming.

Patchouli oil, the main medicinal ingredient of *Pogostemon cablin* (Blanco) Benth., is an important natural spice. To clarify the function and molecular regulation mechanism of FPPS gene in *Pogostemon cablin* (Blanco) Benth., the full-length cDNA of PcFPPS was cloned and characterized. The results showed that PcFPPS had the highest expression in flowers, subcellular localization analysis indicated a role in the cytoplasm, and overexpression increased terpene biosynthesis. Yeast hybridization experiments showed that the transcription factor PcWRKY44 can bind to the promoter of PcFPPS and promote the biosynthesis of patchouli alcohol in *Pogostemon cablin* (Blanco) Benth.. All these results suggest that PcFPPS plays an important role in *Pogostemon*

cablin (Blanco) Benth. terpene biosynthesis and is regulated by PcWRKY44 (Wang et al., 2022).

Some researchers have found that transcription factors in the bHLH family can regulate the biosynthesis of different secondary metabolites in various plant species and have been shown to play a regulatory role in the biosynthesis of plant triterpenoids (Zhou & Memelink, 2016). Yin et al functionally characterized BpMYB21 and BpMYB61 in response to MeJA and SA in the synthesis of *Betula pendula* Roth triterpenoids (Yin et al., 2020). The results showed that the transcription factors BpMYB21 and BpMYB61 belonged to R2R3 IFs and both had active promoters, but showed positive and negative regulation in response to methyl jasmonate (MeJA) and salicylic acid (SA) in MeJA and SA responses, respectively. The key triterpenoid synthesis gene FPPS was up-regulated in BpMYB21 and BpMYB61 transgenic birch. Therefore, it is possible that the transcription factors promote the production of *Betula pendula* Roth triterpenoids.

5.2 GPPS clone

The main biologically active substances in *Tripterygium wilfordii* Hook.f. are sesquiterpene alkaloids and triterpenoids (Guo et al., 2019). Tu et al designed specific primers based on their suspension cell transcriptome data and cloned the full-length cDNA of the TwGPPSl and TwGPPS2 genes of *Tripterygium wilfordii* Hook.f. using rapid amplification of cDNA ends, and obtained for the first time the complete open reading frame length of TwGPPSl nucleotide of 1278 bp, encoding a 425 amino acid protein with a predicted protein isoelectric point of 6.68. The molecular mass was 47.189 kDa (Tu et al., 2017). They further constructed the prokaryotic recombinant expression vectors pET-32a-TwGPPSl and pET-32a-TwGPPS2 and successfully induced the recombinant proteins, which laid the foundation for in-depth study on the function of this key enzyme gene and the biosynthetic pathway of *Tripterygium wilfordii* Hook.f..

R2R3-MYB represents one of the largest plant TF families and can act as a transcriptional activator or repressor of genes. The finding that R2R3-MYB represses the expression of MsGPPS genes in *Mentha* L. extends the knowledge of R2R3-MYB-mediated regulation of plant secondary metabolism (Reddy et al., 2017).

5.3 SQS clone

Crataegus pinnatifida Bunge contains chemical components such as triterpenes and sesquiterpenes, which have the ability to promote apoptosis in cancer cells (Dong et al., 2021). To understand the structural characteristics of triterpenoid squalene synthase genes in *Crataegus pinnatifida* Bunge, Shan et al cloned two squalene synthase genes, CpSQS1 and CpSQS2, from *Crataegus pinnatifida* Bunge fruits for the first time by RT-PCR (Shan et al., 2020). The ORF lengths of CpSQS1 and CpSQS2 are 1239 bp and 1233 bp, respectively. Online tools predict that CpSQS1 and CpSQS2 are stable acidic proteins. The secondary structure is mainly composed of α-helix structure. Structure-function domain analysis showed that amino acids 35-367 of CpSQS1 and CpSQS2 cDNAs contain a conserved trans-isoprene pyrophosphate synthase structural domain. Transmembrane structural domain analysis predicted that two transmembrane structural domains were found in CpSQS1 and CpSQS2. It laid the foundation for further study of the *Crataegus pinnatifida* Bunge triterpene metabolic pathway.

Celastrol is the most representative triterpene of *Tripterygium wilfordii* Hook.f.. Liu used the information from the Raijin transcriptome database and designed primers using Primer 5.0 to clone a squalene synthase TwSQS with an ORF of 1242 bp, encoding 413 amino acids (Liu, 2018). The TwSQS-encoded protein has a transmembrane helix and signal peptide analysis showed no signal peptide, presumably all are non-secretory proteins. This study also proposes to silence key genes of the triterpene synthesis pathway by RNA interference (RNAi) technology using reverse genetics to interfere with genes such as SQS, a key enzyme in the triterpene synthesis pathway of Ragweed. FPP is a common precursor for sesquiterpenes and triterpenes, resulting in more precursors for the sesquiterpene synthesis pathway FPP, thus increasing the accumulation of sesquiterpene alkaloids. Regulation of SQS genes can regulate the accumulation of secondary metabolites in plants.

Camellia oil, the main product of *Camellia oleifera* C.Abel, is a high-quality vegetable cooking oil. And studies have shown a significant positive correlation between the CoSQS gene and squalene content of oil tea seed kernels (Zhou et al., 2013). To investigate the biological function of the *Camellia oleifera* C.Abel squalene synthase promoter pCoSQS and the relationship with the transcription factor CoWRKY, a yeast monohybrid approach was used for validation. The results indicate that pCoSQS can exercise promoter functions and provide preliminary evidence for a possible reciprocal relationship between the CoWRKY1 transcription factor and pCoSQS (Yang, 2021).

6 Summary

With the rapid development of the economy and the improvement of human health concept, many plants that combine edible, medicinal and ornamental uses have attracted much attention and the development fever continues to rise. It is reported that many plants with the same origin of medicine and food have biological activities. According to the literature and the latest version of the 2020 Directory of Medicinal and Food Sources, medicinal and food plants include *Mentha* L., *Camellia oleifera* C.Abel, *Panax ginseng* C.A.Mey. and *Santalum album* L. mentioned in this article, and health foods include *Ginkgo biloba* L. and *Medicago truncatula* Gaertn.. *Mentha* L. is not only used as a cosmetic additive to whiten and remove blemishes, but it can also be used for food stagnation disorders. *Camellia oleifera* C.Abel can be used for both weight loss people and for activating blood circulation and eliminating food. *Panax ginseng* C.A.Mey. contains dietary fiber and trace elements to improve the structure of intestinal flora(Mu et al., 2022). The heartwood of *Santalum album* L. is the medicinal part in the 2020 edition of the Chinese Pharmacopoeia, and the leaves and bark are recorded as edible parts in the *Compendium of Materia Medica*(Jia et al., 2021).

Through corresponding technical means, more and more key enzymes of biosynthetic processes as well as transcription factor functions have been characterized, providing new importance

to elucidate the regulatory role of transcription factors on key enzymes. The targeted synthesis of terpenoids is achieved by genetic engineering to improve plant cells, tissues, plants, etc. for mass production of secondary metabolites, or by constructing promoters for specific expression of key enzymes for efficient production of target products. Taken together, most of the genes on the MVA synthesis pathway are associated with the active ingredients of sesquiterpenes and triterpenoids.

The clarification of the functions of each gene can help to provide a reference for plant evolutionary classification, and the analysis of the upstream promoter structure can help to select the relevant trait-related breeding (Braga & Pavan, 2021), which is conducive to fully exploit the huge resource of medicinal food homologs and enhance its exploitation. It provides insights and strategies for the application of key enzymes of the MVA synthesis pathway to metabolic processes.

Conflicts of interest

There are no conflicts to declare.

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References

Abbas, F., Ke, Y., Yu, R., Yue, Y., Amanullah, S., Jahangir, M. M., & Fan, Y. H. (2017). Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta*, 246(5), 803-816. http://dx.doi.org/10.1007/s00425-017-2749-x. PMid:28803364.

Abu-Izneid, T., Rauf, A., Shariati, M. A., Khalil, A. A., Imran, M., Rebezov, M., Uddin, M. S., Mahomoodally, M. F., & Rengasamy, K. R. R. (2020). Sesquiterpenes and their derivatives-natural anticancer compounds: an update. *Pharmacological Research*, 161, 105165. http://dx.doi.org/10.1016/j.phrs.2020.105165. PMid:32835868.

Bergman, M. E., Davis, B., & Phillips, M. A. (2019). Medically useful plant terpenoids: biosynthesis, occurrence, and mechanism of action. *Molecules (Basel, Switzerland)*, 24(21), 3961. http://dx.doi.org/10.3390/molecules24213961. PMid:31683764.

Braga, R., & Pavan, M. A. (2021). Pepper resistance to PepYMV and PepYMV-Lins and development of resistant hybrids. *Food Science and Technology (Campinas)*, 47(1), 21-26.

Campbell, D. R., Sosenski, P., & Raguso, R. A. (2019). Phenotypic plasticity of floral volatiles in response to increasing drought stress. *Annals of Botany*, 123(4), 601-610. http://dx.doi.org/10.1093/aob/mcy193. PMid:30364929.

Chen, C., Zhu, H. Q., Kang, J. X., Warusawitharana, H. K., Chen, S., Wang, K. X., Yu, F., Wu, Y. Y., He, P., Tu, Y. Y., & Li, B. (2022). Comparative transcriptome and phytochemical analysis provides insight into triterpene saponin biosynthesis in seeds and flowers of the tea plant (*Camellia sinensis*). *Metabolites*, 12(3), 204. http://dx.doi.org/10.3390/metabo12030204. PMid:35323647.

Chen, D. L., Li, X., & Zhou, X. J. (2020). Research progress on sesquiterpenes and its pharmacological activities in genus Carpesium. *Zhongguo Zhongyao Zazhi*, 45(1), 37-51. PMid:32237409.

Dembitsky, V. M. (2021). In silico prediction of steroids and triterpenoids as potential regulators of lipid metabolism. *Marine Drugs*, 19(11), 650. http://dx.doi.org/10.3390/md19110650. PMid:34822521.

Di, P., Wang, P., Yan, M., Han, P., Huang, X. Y., Yin, L., Yan, Y., Xu, Y. H., & Wang, Y. P. (2021). Genome-wide characterization and analysis of WRKY transcription factors in *Panax ginseng*. *BMC Genomics*, 22(1), 834. http://dx.doi.org/10.1186/s12864-021-08145-5. PMid:34794386.

Dong, J. Q., Chen, J. P., & Gong, S. X. (2021). Research progress on chemical constituents and pharmacological effects of Crataegi Fructus and predictive analysis on Q-Marker. *Chinese Traditional and Herbal Drugs*, 52(09), 2801-2818.

Elfahmi, Hapsari, R. A., Chrysanthy, T., Synthiarini, V., Masduki, F. F., Setiawan, A., & Muranaka, T. (2021). Expression of two key enzymes of artemisinin biosynthesis FPS and ADS genes in *Saccharomyces cerevisiae*. *Advanced Pharmaceutical Bulletin*, 11(1), 181-187. http://dx.doi.org/10.34172/apb.2021.019. PMid:33747865.

Guo, S. Y., Yan, Q., & Li, J. (2019). Cloning and bioinformatic analysis of DXR and HMGR genes from *Tripterygium regelii*. *Zhongguo Xiandai Zhongyao*, 21(11), 1482-1488.

Huang, W., Gfeller, V., & Erb, M. (2019). Root volatiles in plant-plant interactions II: Root volatiles alter root chemistry and plant-herbivore interactions of neighbouring plants. *Plant, Cell & Environment*, 42(6), 1964-1973. http://dx.doi.org/10.1111/pce.13534. PMid:30754075.

Jia, C. L., Wang, J. Y., Zhao, K. J., & Peng, H. S. (2021). Records of Medicinaland Edible Plants in the Wood Section of *Compendium of Materia Medica* and Relevation. *Zhongguo Xiandai Zhongyao*, 23(6), 1094-1102.

Jin, X., Baysal, C., Gao, L., Medina, V., Drapal, M., Ni, X., Sheng, Y., Shi, L., Capell, T., Fraser, P. D., Christou, P., & Zhu, C. (2020). The subcellular localization of two isopentenyl diphosphate isomerases in rice suggests a role for the endoplasmic reticulum in isoprenoid biosynthesis. *Plant Cell Reports*, 39(1), 119-133. http://dx.doi.org/10.1007/s00299-019-02479-x. PMid:31679061.

Kochan, E., Balcerczak, E., Szymczyk, P., Sienkiewicz, M., Zielińska-Bliźniewska, H., & Szymańska, G. (2019). Abscisic acid regulates the 3-Hydroxy-3-methylglutaryl CoA reductase gene promoter and ginsenoside production in *Panax quinquefolium* hairy root cultures. *International Journal of Molecular Sciences*, 20(6), 1310. http://dx.doi.org/10.3390/ijms20061310. PMid:30875925.

Li, Q., Ding, G., Li, B., & Guo, S. X. (2017). Transcriptome analysis of genes involved in dendrobine biosynthesis in *Dendrobium nobile* Lindl. infected with Mycorrhizal fungus MF23 (Mycena sp.). *Scientific Reports*, 7(1), 316. http://dx.doi.org/10.1038/s41598-017-00445-9. PMid:28331229.

Lima, T. C., Souza, R. J., Moraes, M. H., Matos, S. S., Almeida, F. H., Steindel, M., & Biavatti, M. W. (2021). Isolation and characterization of sesquiterpene lactones from Calea uniflora Less. and their leishmanicidal and trypanocidal activities. *Food Science and Technology (Campinas)*, 44(6), 696-699.

Liu, Y. (2018). *Cloning and expression analysis of key enzyme genes of triterpenoid biosynthesis pathway in Tripterygium wilfordii Hook.f.* China: Northwest A&F University.

Luo, H. M., Song, J. Y., Li, X. Y., Sun, C., Li, C. F., Luo, X., Li, Y., & Chen, S. L. (2013). Cloning and expression analysis of a key device of HMGR gene involved in ginsenoside biosynthesis of *Panax ginseng* via synthetic biology approach. *Yao Xue Xue Bao*, 48(2), 219-227. PMid:23672018.

Meng, X., Song, Q. L., Ye, J. B., Wang, L. L., & Xu, F. (2017). Characterization, function, and transcriptional profiling analysis of 3-Hydroxy-3-methylglutaryl-CoA Synthase Gene (GbHMGS1) towards stresses and exogenous hormone treatments in *Ginkgo biloba*. *Molecules (Basel, Switzerland)*, 22(10), 1706. http://dx.doi.org/10.3390/molecules22101706. PMid:29023415.

Mertens, J., Pollier, J., Vanden Bossche, R., Lopez-Vidriero, I., Franco-Zorrilla, J. M., & Goossens, A. (2016). The bHLH transcription factors TSAR1 and TSAR2 regulate triterpene saponin biosynthesis in *Medicago truncatula*. *Plant Physiology*, 170(1), 194-210. http://dx.doi.org/10.1104/pp.15.01645. PMid:26589673.

Mu, R., Xia, Y. S., Zhang, Y. T., Bo, P. P., Sun, Y. S., Wang, Z. T., & Hua, M. (2022). Research Progress on the Chemical Composition and Intestinal Flora Regulation of Dietary Fiber from the Edible and Medicinal Plants. *Science and Technology of Food Industry*, 43(18), 493-500.

Niu, M., Yan, H., Xiong, Y., Zhang, Y., Zhang, X., Li, Y., da Silva, J. A. T., & Ma, G. (2021). Cloning, characterization, and functional analysis of acetyl-CoA C-acetyltransferase and 3-hydroxy-3-methylglutaryl-CoA synthase genes in *Santalum album*. *Scientific Reports*, 11(1), 1082. http://dx.doi.org/10.1038/s41598-020-80268-3. PMid:33441887.

Oliveira, R. G., Cruz, L. R., & Dias, L. C. (2022). Artemisinina e derivados: descoberta, estratégias sintéticas e obtenção industrial. *Food Science and Technology (Campinas)*, 45(7), 831-846. https://doi.org/10.21577/0100-4042.20170888.

Perveen, S., Alqahtani, J., Orfali, R., Aati, H. Y., Al-Taweel, A. M., Ibrahim, T. A., Khan, A., Yusufoglu, H. S., Abdel-Kader, M. S., & Taglialatela-Scafati, O. (2020). Antibacterial and Antifungal Sesquiterpenoids from Aerial Parts of *Anvillea garcinii*. *Molecules (Basel, Switzerland)*, 25(7), 1730. http://dx.doi.org/10.3390/molecules25071730. PMid:32283756.

Phillips, M. A., D'auria, J. C., Gershenzon, J., & Pichersky, E. (2008). The Arabidopsis thaliana type I Isopentenyl Diphosphate Isomerases are targeted to multiple subcellular compartments and have overlapping functions in isoprenoid biosynthesis. *The Plant Cell*, 20(3), 677-696. http://dx.doi.org/10.1105/tpc.107.053926. PMid:18319397.

Reddy, V. A., Wang, Q., Dhar, N., Kumar, N., Venkatesh, P. N., Rajan, C., Panicker, D., Sridhar, V., Mao, H. Z., & Sarojam, R. (2017). Spearmint R2R3-MYB transcription factor MsMYB negatively regulates monoterpene production and suppresses the expression of geranyl diphosphate synthase large subunit (MsGPPS.LSU). *Plant Biotechnology Journal*, 15(9), 1105-1119. http://dx.doi.org/10.1111/pbi.12701. PMid:28160379.

Ren, Y., & Kinghorn, A. D. (2019). Natural Product Triterpenoids and Their Semi-Synthetic Derivatives with Potential Anticancer Activity. *Planta Medica*, 85(11-12), 802-814. http://dx.doi.org/10.1055/a-0832-2383. PMid:30658371.

Rohdich, F., Zepeck, F., Adam, P., Hecht, S., Kaiser, J., Laupitz, R., Gräwert, T., Amslinger, S., Eisenreich, W., Bacher, A., & Arigoni, D. (2003). The deoxyxylulose phosphate pathway of isoprenoid biosynthesis: studies on the mechanisms of the reactions catalyzed by IspG and IspH protein. *Proceedings of the National Academy of Sciences of the United States of America*, 100(4), 1586-1591. http://dx.doi.org/10.1073/pnas.0337742100. PMid:12571359.

Shan, T. Y., Yu, D. Q., Han, X. J., Xu, R., Zha, L. P., & Yuan, Y. (2020). Cloning and prokaryotic expression analysis of squalene synthase CpSQS1 and CpSQS2 from *Crataegus pinnatifida*. *Zhongguo Zhongyao Zazhi*, 45(6), 1334-1341. PMid:32281345.

Socorro, F. M., Flavio, H. O., Oscar, C. H., Mauricio, L. R., Carolina, B. S., Hugo, A., & Jose Armando, L. G. (2021). Chitosan-induced production of secondary metabolites in plant extracts of *Piper auritum*, and the in vitro fungicidal activity against *Fusarium oxysporum* f. sp. vanilla. *Food Science and Technology (Campinas)*, 39(1), 198-206.

Su, L., Li, S., Qiu, H., Wang, H., Wang, C., He, C., Xu, M., & Zhang, Z. (2021). Full-length transcriptome analyses of genes involved in triterpenoid saponin biosynthesis of *Psammosilene tunicoides* hairy root cultures with exogenous salicylic acid. *Frontiers in Genetics*, 12, 657060. http://dx.doi.org/10.3389/fgene.2021.657060. PMid:33854529.

Thakur, S., & Vasudev, P. G. (2022). MYB transcription factors and their role inMedicinal plants. *Molecular Biology Reports*, 49(11), 10995-11008. http://dx.doi.org/10.1007/s11033-022-07825-z. PMid:36074230.

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants. *Advances in Biochemical Engineering/Biotechnology*, 148, 63-106. http://dx.doi.org/10.1007/10_2014_295. PMid:25583224.

Tu, L. C., Zhang, Y. F., Su, P., Hu, T. Y., Tong, Y. R., Guan, H. Y., Zhao, Y. J., Zhang, X. N., Yuan, Y., Gao, W., & Huang, L. Q. (2017). [Cloning and protein expression analysis of geranyl diphosphate synthase genes in Tripterygium wilfordii]. *Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica*, 42(2), 220-225. PMid:28948723.

Tungcharoen, P., Wattanapiromsakul, C., Tansakul, P., Nakamura, S., Matsuda, H., & Tewtrakul, S. (2018). Antiinflammation constituents from *Curcuma zedoaroides*. *Phytotherapy Research*, 32(11), 2312-2320. http://dx.doi.org/10.1002/ptr.6173. PMid:30109745.

Wang, W., & Oldfield, E. (2014). Bioorganometallic chemistry with IspG and IspH: structure, function, and inhibition of the [Fe(4)S(4)] proteins involved in isoprenoid biosynthesis. *Angewandte Chemie International Edition*, 53(17), 4294-4310. http://dx.doi.org/10.1002/anie.201306712. PMid:24481599.

Wang, X. B., Tang, Y., Huang, H. L., Wu, D., Chen, X. Z., Li, J. R., Zheng, H., Zhan, R. T., & Chen, L. K. (2022). Functional analysis of *Pogostemon cablin* farnesyl pyrophosphate synthase gene and its binding transcription factor PcWRKY44 in regulating biosynthesis of patchouli alcohol. *Frontiers in Plant Science*, 13, 946629. http://dx.doi.org/10.3389/fpls.2022.946629. PMid:36092423.

Warsi, M. K., Howladar, S. M., & Alsharif, M. A. (2023). Regulon: an overview of plant abiotic stress transcriptional regulatory system and role in transgenic plants. *Food Science and Technology (Campinas)*, 83, e245379. PMid:34495147.

Xu, G. B., Xiao, Y. H., Zhang, Q. Y., Zhou, M., & Liao, S. G. (2018). Hepatoprotective natural triterpenoids. *European Journal of Medicinal Chemistry*, 145, 691-716. http://dx.doi.org/10.1016/j.ejmech.2018.01.011. PMid:29353722.

Yang, R. (2021). *Preliminary study on CoSQS promoter and its interaction with CoWRKY transcription factors in Camellia oleifera Abel*. China: Central South University of Forestry and Technology.

Yin, J., Sun, L., Li, Y., Xiao, J. L., Wang, S. Y., Yang, J., Qu, Z. Y., & Zhan, Y. G. (2020). Functional identification of BpMYB21 and BpMYB61 transcription factors responding to MeJA and SA in *birch triterpenoid* synthesis. *BMC Plant Biology*, 20(1), 374. http://dx.doi.org/10.1186/s12870-020-02521-1. PMid:32787836.

Yu, H. W., Liu, M. L., Li, J., Peng, H. S., Han, B. X., Cha, L. P., & Gui, S. Y. (2022). Cloning and prokaryotic expression analysis of *Platycodon grandiflorus* (Jacq.) A.DC. PgHMGS and PgHMGR genes. *Zhongchengyao*, 44(9), 3062-3068.

Zhang, C., Zhu, Y., Yin, X. P., Wei, Q. W., Zhang, N. N., Li, C. X., Xie, T., & Chen, R. (2019). Advances in synthesis of artemisinin based on plant genetic engineering. *Zhongguo Zhongyao Zazhi*, 44(19), 4285-4292. PMid:31872711.

Zhang, J., Shi, L. Y., Ding, L. P., Liang, H., Tu, P. F., & Zhang, Q. Y. (2021). Antioxidant terpenoids from the red alga *Laurencia tristicha*.

Natural Product Research, 35(23), 5048-5054. http://dx.doi.org/10.1080/14786419.2020.1774762. PMid:32510234.

Zhao, K. Z., Zhang, D. W., Lv, K., Zhang, X. M., Cheng, Z. H., Li, R. H., Zhou, B., & Jiang, T. B. (2019). Functional characterization of poplar WRKY75 in salt and osmotic tolerance. *Plant Science*, 289, 110259. http://dx.doi.org/10.1016/j.plantsci.2019.110259. PMid:31623781.

Zhou, C. F., Yao, X. H., & Lin, P. (2013). Squalene synthase gene cloning and analysis of Camellia oleifera based on RNA-Seq. *Guangxi Zhi Wu*, (6), 852-858.

Zhou, M., & Memelink, J. (2016). Jasmonate-responsive transcription factors regulating plant secondary metabolism. *Biotechnology Advances*, 34(4), 441-449. http://dx.doi.org/10.1016/j.biotechadv.2016.02.004. PMid:26876016.