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Relationship between gene expressions and related terpenes at three developmental stages in *Artemisia annua*

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Abstract

Importance of terpenes in different aspects of human's life, makes it necessary to determine the relationship between them and their genes just in order to fathom such relationship for further exploitation of their production process, either increasing the volume of synthesis or changing the mixture of terpene content, for exponentially demanding for these invaluable compounds. To do so, In this study some genes in 2-c-methyl-d-erythritol 4- phosphate and Mevalonate pathways namely *Farnesyl diphosphate synthase, Hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase* and *Isiopentenyl diphosphate isomeras* were evaluated in three of *Artemisia annua* tissues including leaf, bloom and flower. Real-time PCR results of those genes compared with the information taken from GC-Mass outputs of terpenes, yields the final result of this research. Results showed, relationship between genes and terpenes was not similar in tissues. In certain tissues, these genes play a limiting role, while in others, some other downstream genes were possibly more important, still the effect of post-transcriptional modification cannot be ignored. In the end, although those investigated genes in this research are somehow crucial in terpene production, in order to make a more obvious picture of terpene synthesis, evaluation of more immediate genes to terpenes, particularly terpene synthases, and proteomics data of these genes needed to make the relationship between RNA transcrips and metabolites more reliable.

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Introduction

Secondary metabolites in plant are categorized in three groups among which terpenes are the largest and have the most diversity in structure (more than 50000 known types) (Hsieh and Goodman, 2005; Keeling and Bohlmann, 2006; Zwenger and Basu, 2008). Most of volatile terpenoids include isoprene, monoterpene and sesquiterpene comprise the biggest volatile compounds in plants (Nagegowda, 2010). Information concerning synthesis and chemistry of terpenes and isoterpenes accelerates steps towards perception of plant metabolic and biochemistry processes (Zwenger and Basu, 2008). Furthermore, understanding the function of involved genes in terpenes production could help to decipher either new compounds or new pathways (Zwenger and Basu, 2008). Due to terpenes importance in plant development and their potential in view of pathway engineering, identification and characterization of genes responsible for terpene production in many species have been carried out (Ma *et al*., 2012).

Terpenes have a vast range of duties: in cell membrane structure(sterol), reduction- oxidation (Redox) reactions, light harvest (chlorophyll, phytol) and protection (carotenoid), growth and development regulation (gibberellin), protein alteration (ubiquinone), hormonal function (steroid hormone) and etc. (Hsieh and Goodman, 2005; Wang *et al*., 2008; Nagegowda, 2010; Olofsson *et al*., 2011). Moreover, Frequency and diversity of terpene compounds cause widespread ecosystem effects in nature (Zwenger and Basu, 2008). Terpenes cause high-temperature tolerance, protection against pest, and attraction of pollinators and seed diffusers in plants (Cheng *et al*., 2007; Han *et al*., 2008; Nagegowda, 2010). Terpenes are also being exploited in medicine, pesticide, biofuel, food and cosmetic industries (Zwenger and Basu, 2008; Ma *et al*., 2012).

In general, Terpene biosynthesis is divided into three phases. In the first phase, two important precursors, isopentenyl diphosphate/ pyrophosphate (IDP / IPP) and dimethylallyl diphosphate/ pyrophosphate (DMADP / DMAPP) that are active basic building units, are produced via two pathways, Mevalonate (MVA) and 2-c-methyl-d-erythritol 4- phosphate (MEP). MVA occurs in cytosol, peroxisome and endoplasmic reticulum while MEP occurs in plastid. The former pathway is more general and is found in most organisms but the later one is more specific and exists in eubacteria, algae and plants (Nagegowda. 2010; Ma *et al*., 2012). In MVA, acetyl coenzyme A is converted to IDP which would be affected by another enzyme that changes it to its isomer DMADP. In MEP, IDP and DMADP are produced from pyruvate and glyceraldehyde 3-phosphate through another series of reactions (Cheng *et al*., 2007; Olofsson *et al*., 2011; Ma *et al*., 2012). In general, MVA products are precursors for sesquiterpenes, triterpenes and sterol (Wen and Yu, 2011), whereas MEP products act as precursors for monoterpenes, diterpenes and carotenoid.

In the second phase some materials consist of geranyl diphosphate(GDP), farnesyl diphosphate (FDP) and geranylgeranyl diphosphate (GGDP), are produced ,using IDP and DMADP, by some enzymes called prenyl transferase (PTs) [or isoprenyl diphosphate synthase (IDSs)]. These enzymes consist of three families: short, medium and large chain. Enzymes including Geranyl diphosphate synthase (GDS), FDS and Geranylgeranyl diphosphate synthase (GGDS) are grouped as short chain family (Ma *et al*., 2012). FDS binds two IDPs to one DMADP in direction of head to tail and forms a C15 material called farnesyl diphosphate (FDP) that acts as sesquiterpenes precursor. GDS binds one unit of IDP to one unit of DMADP and forms one C10 material called geranyl diphosphate (GDP) that is monoterpenes precursor. GGDS binds three units of IDP to one DMADP and forms one C20 material called Geranylgeranyl diphosphate (GGDP) that is diterpenes precursor (Cheng *et al*., 2007).

In the third phase, terpene synthase/cyclase (*TPSs*) occurs that convert the second phase products to the final material terpenes. These enzymes are divided into seven subfamilies including TPSa, TPSb,..and TPSg. All of them use DMADP, FDP, GDP and GGDP as precursor (Keeling and Bohlmann, 2006). Beside

these enzymes, there are some other enzymes that are active at the end phase of terpene production and are involved in altering terpenes' basic skeleton (Ma *et al*., 2012).

In this study relative expression of three important genes: *Isiopentenyl diphosphate isomeras (IDI)*, *Farnesyl diphosphate synthase (FDS)* and *Hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase (HDR)* genes from two pathways (MEP and MVA) along with some of their related products namely monoterpenes and sesquiterpenes, were evaluated in three tissues including leaf, bloom and flower of *A. annua*.

Materials and methods

Plant material and growth conditions

Seeds of *A. annua,* obtained from the Iranian Biological Resource Center (IBRC), were grown in regulated condition in greenhouse. The specimen was preserved in the Herbarium (voucher no. 1000060) of IBRC for reference. Seeds were first treated with sodium hypochlorite 20% and then washed with distilled water for three times. Treated seeds were located in petri dishes for 14 days in condition of 3000 L light and periodic regime of 8 hours darkness and 16 hours brightness. Upon germination, seeds were transferred to small plastic pots, which were sealed with clear plastic covers. They were then put in growth chamber for 21 days in the following condition: 25 ^{o}C temperature, 55% humid, 5000 L light, regime of 8 to 16 hours darkness and brightness, respectively. After then, they were transplanted and kept in large pots up to flowering under 25 ^{o}C temperature, 55% humid, 7000 L light and regime of 8 to 16 darkness and brightness.

GC-Mass analysis

Explants taken from leaf, bloom and flower of *A. annua* at the flowering time were put in paper bags and dried by placing them in shade. In order to extract essential oil, explants were crushed and poured in glass vials along with four metal marbles. Some amount of hexane were then added and placed on vortex. The samples were left in room temperature for four hours, then were filtered, and were injected into GC-MS device (Munoz-Bertomeu *et al*., 2006). To analyze the essential oil, Agilent Technologies 7890 A with one MSD (5975), HP-5MS column with 30 meters long and 0.25 millimeters diameter and layer thickness of 0.25 millimeters was used. Injection was accomplished in split mood and at 250 ^{o}C temperature. The oven temperature regime was 60 ^{o}C for three minutes and then it was raised to 150 ^{o}C by rate of three degrees centigrade per minute. This condition was maintained for one minute and then raised to 260 ^{o}C with the rate of the previous stage and then maintained ten minutes. Helium with flow speed of one mm per minute and average speed of one cm per second was used as the carrier gas. The ionizing source temperature was 230 ^{o}C. Constituents of the oil were identified by comparison of their retention indices relative to a homologous n-alkane (C8–C28) series and matching their mass spectra with those of reference compounds in Wiley and NIST libraries.

RNA Isolation and Real-Time RT-PCR

RNA of samples from leaves, bloom and flower of adult plants for three replications were extracted using RNasy Plant Mini Kit, a product of Qiagene co., according to the corporation's instructions. cDNA was synthesized using a Fermentas's product instructions RevertAidTM First Strand cDNA Synthesis K1622, according to the company's protocol. Then, synthesized cDNA was transferred to -20 ^{o}C to store.

FDS, *IDI* and *HDR* primers were designed using PRIMER3 software and were tested by PRIMER BLAST software. In order to measure gene expression quantity, Real Time PCR device from BIORAD was used. *18s rRNA* was selected as reference gene and its primers were selected according to Zeng *et al*., (2008). The primer sequences and their amplification length are shown in table 1.

Table 1. Primer sequences of studied genes and their amplification length.

Gene	Accession number	Primers (F: forward, R:reverse)	amplification length
FDS	U36376.1	F 5'-CTGCCCTTGGTTGGTGTATT-3'	169
		R 5'-ATTCTCGGGACATGGTTACG-3'	
IDI	DQ666334.1	F 5'-GGGCGAACATGAACTTGATT-3'	154
		R 5'-CAGCTTGAGACCCTCCTCAC-3'	
HDR	GQ119345.1	F 5'-AATTCTCCATGGCGTCTTTG-3'	170
		R 5'-ATTATGCCTGGACACCTTCG-3'	

Data analysis

Data from Real Time PCR were analyzed using "Rest ©2001 and 2002 Michael W. Pfaffl and Graham W. Horgan" software. The results were then interpreted regarding to GC-MS data. The leaf was used as reference tissue in REST software. Graphs were drawn for bloom and flower in comparison with leaf (table 3). For monoterpenes both *HDR* and *IDI* and for sesquiterpenes, *FDS* and *IDI* were compared.

Results and discussion

Gene expression

According to real time PCR results (table 3), *IDI* expression level in bloom of *A.annua* was more than that in leaf and flower. Gene expression in flower was less than that in leaf. With regard to *IDI* function that acts as regulating factor in the pathways of different tissues, this regulation can result in production of diverse terpene compounds. The amount of terpene production depends on the kind of tissue, developmental stages and environment. In other words, *IDI* produces different terpene compounds depending on the way it is regulated in different tissues. One study on *Curcuma wenyujin* showed that *IDI* had more expression level in roots compared to stems and leaves (Gao *et al.*, 2012).

HDR expression in bloom and flower of *A.annua* was down-regulated compared to leaf (table 3). *HDR* expression, however, in flower was more than that in bloom. This gene generates basic terpenes and is very important as rate-limiting enzyme. High expression level of it in leaf might imply that more precursors are required in this tissue compared to others. It is obvious that compounds resulted from terpenes like chlorophyll and gibberellin that are needed in leaf and therefore may cause gene activity be high in that tissue. Although Studies on *Camptotheca acuminate* and *Arabidopsis thaliana* showed a high expression level of *HDR* gene in flower (Wang *et al.*, 2008), Another study, showed that this gene is expressed in higher level in old leaves than other tissues of *A. annua* (by 10 to 30 folds) (Olofsson *et al.*, 2011). The gene level in bloom was less than that in leaf, indicating that less terpene-related material is required compared to leaf. Gene expression level in flower was more than that in bloom but this level was less than that in leaf indicating that more precursors are required in flower than that in bloom but in leaf was more than both. Another research on *Oncidium orchard* showed that the expression level of *HDR* in semi-open flowers was more than that in fully-open flowers (Huang *et al.*, 2009).

The expression level of *FDS* in flower was more than that in both leaf and bloom. In contrast to *IDI* and *HDR, FDS* had a more specific role in producing terpene compounds. FDS uses IDI and DMADP to build intermediate precursor (FDP) for certain groups of terpenes. In short, different requirement of the gene products by different plants and tissues causes different regulations of the gene. In one study on *Euphorbia pekinensis Rupr* the highest expression level of *FDS* was observed in root whereas low expression was found in leaf and stem (Cao X *et al.*, 2012). Our result indicated that expression level of this gene in leaf was more than that in bloom and it means that products of this gene are more needed in leaf than in bloom. High expression level of *FDS* was also observed in flower and young leaf of *Withania somnifera*. L. (Gupta *et al.*, 2011).

GC-Mass results

Terpenes were detected in the three tissues in different concentrations. However, some monoterpene and sesquiterpenes were produced more in bloom in comparison with leaf (table 2).1,8-cineole and α-pinene in bloom were more than that in leaf and it was less in flower than that in leaf. Study on shoot of *Menta piperita L.* showed more 1,8-cineole than some other monoterpene including α-pinene, b-pinene and sabinene (Gershenzon *et al*., 2000). In addition, a study on *Pinus elliottii* showed more production of α-pinene than b-pinene in foliage (Tingey *et al*., 1980). In our study, artemisia alcohol was produced more in flower than that in bloom and more in bloom than that in leaf. Atemisia ketone imitates artemisia alcohol pattern but the amount in flower was far more than that in other tissues. In one study on Chinese cultivar of *A annua*, artemisia ketone was produced more than artemisia alcohol and camphor. In Vietnamese cultivar of that species, camphor was produced more than 1,8-cineole (Woerdenbag *et al*., 2006).

The pattern of production of camphene, camphor, myrtenal, terpineol-4 and p-cymene was similar and they were produced less in bloom and flower compared to leaf. Copaene in bloom and flower of *A. annua* was more than that in leaf, but β-selinene and arteannuin B were produced more in leaf than that in bloom, and in bloom were more than that in flower (table 2).

Table 2. Monoterpene and sesquiterpenes measured in leaf, bloom and flower of *A.annua* by GC-MS.

Terpenes		RI*	Leaf	Bloom	Flower
Monoterpene	a-pinene	436	1.28972	4.145328	1.147144
	camphene	945	5.44737	1.022787	2.958424
	sabinene	971	0.05091	1.441962	0.67923
	b-pinene	975	0.6788	1.106622	0.649042
	yomogi alcohol	995	0.20364	0.100602	0.558478
	p-cymene	1025	0.79759	0.352107	0.286786
	1,8-Cineole	1032	9.01107	12.39554	7.154556
	artemisia ketone	1058	9.68987	10.68058	36.52748
	artemesia alcohol	1073	0.28849	0.570078	2.218818
	trans-Pinocarveol	1141	0.40728	1.710234	0.15094
	camphor	1147	25.30227	14.00259	15.56191
	borneol	1169	0.39031	0.268272	0.090564
	terpinen-4-ol	1179	0.23758	0.134136	0.105658
	a-Terpineol	1191	0.11879	0.905418	0.407538
	myrtenal	1193	0.20364	0.16767	0.166034
sesquiterpene	copaene	1336	0.1697	1.00602	0.467914
	β-Selinene	1476	9.24865	8.836209	2.445228
	arteannuin B	1969	21.92524	7.04214	4.588576

*Retention index relative to n-alkanes on HP-5MS column.

Relationship between gene expression and GC-Mass results

IDI expression in bloom was more than that in leaf and as a result some monoterpenes were produced more in bloom than that in leaf. These monoterpenes consist of α-pinene, sabinene, b-pinene, 1,8-cineole, artemisia alcohol, artemisia ketone, trans-pinocarveol and a-terpineol (table 3). According to MEP pathway (Fig. 1), it cannot be stated that the production of these compounds have a direct relationship with *IDI,* but, to some extent, this gene is important in their productions.

HDR expression in bloom was less than that in leaf and some terpenes including camphene, borneol, camphor, myrtenal, p-cymene, terpineol-4 and yomogi alcohol were less in bloom than that in leaf as well. This result can be related to *HDR* low expression. Considering *HDR* (as an enzyme that provides precursors to feed MEP downstream enzymes) down regulation in flower and bloom compared with that in leaf and also *IDI* low expression in flower compared with leaf, there were some components that were produced more in flower and bloom than that in leaf. These results may mean that activity of some genes like Terpene synthase/cyclase (*TPSs*) may cause precursors to produce different terpenes from the pool of isoprene precursor with respect to their requirements in different tissues and in different developmental stages or physiological situations.

FDS expression in bloom was less than that in leaf while *IDI* expression in bloom was more than that in leaf (table 3) which resulted in less sesquiterpenes production in bloom compared with leaf (table 3). This result seems to be logical since *FDS* is located in a lower position than *IDI* in MVA pathway and the relationship between sesquiterpenes production and *FDS* is more powerful than that with *IDI*. Albeit in one study on *A. annua* no direct relationship between *FDS* and artemisinine (a kind of sesquiterpenes) content was reported (Zare mehrjerdi *et al.*, 2013). Considering more *FDS* expression in flower compared with that in leaf, some sesquiterpene contents were less in flower than that in leaf. These results can clearly highlight *TPSs* function in specifying *FDS* products to produce different terpenes (table 3). Another study on three varieties of *Ocimum basilicum* showed low relationship between transcription and enzyme activity level of *FDS*, *GDS* (a gene that provides precursors for monoterpenes) and *TPSs*; however, Total terpene content showed a direct relationship with *TPSs* but not with *FDS* and *GDS* (Iijima *et al.*, 2004).

Table 3. Comparison between terpenes and related genes: Production content and gene expression are shown by plus and minus marks, minus means that the production content was less than that in leaf and plus means that the production was more than that in leaf.

Terpenes		Bloom	Flower
Terpenes	1,8-Cineole	+	-
	a-pinene	+	-
	arteannuin B	-	-
	artemesia alcohol	+	+
	artemisia ketone	+	+
	a-Terpineol	+	+
	borneol	-	-
	b-pinene	+	-
	camphene	-	-
	camphor	-	-
	copaene	+	+
	myrtenal	-	-
	p-cymene	-	-
	sabinene	+	+
	terpineol-4	-	-
	trans-Pinocarveol	+	-
	yomogi alcohol	-	+
	β-Selinene	-	-
Genes	*FDS*	-	+
	HDR	-	-
	IDI	+	-

Table 4. Probable limiting genes for mentioned monoterpenoids in different tissues of *A. annua*. (Yes= limiting factor no= non-limiting factor N/A= the relationship is complicated). As an example: about camphor, in leaf, *IDI* has a complicated relationship with the compound but it seems that *HDR* has a positive relationship or having limiting effect on its production. On the other hand, in bloom, *IDI* has no limiting effect on the production of the compound.

Tissue	Leaf		bloom		flower	
compounds	*IDI*	*HDR*	*IDI*	*HDR*	*IDI*	*HDR*
1,8-Cineole	yes	N/A	yes	no	yes	N/A
artemesia alcohol	N/A	no	N/A	N/A	no	N/A
artemisia ketone	N/A	no	N/A	N/A	no	N/A
a-Terpineol	N/A	no	yes	no	N/A	no
borneol	N/A	yes	N/A	N/A	yes	N/A
b-pinene	yes	N/A	yes	no	yes	N/A
camphene	N/A	yes	no	yes	N/A	yes
camphor	N/A	yes	no	yes	N/A	yes
myrtenal	N/A	yes	N/A	N/A	yes	N/A

Tissue	Leaf		bloom		flower	
p-cymene	N/A	yes	N/A	N/A	yes	N/A
sabinene	N/A	no	yes	no	N/A	no
terpineol-4	N/A	yes	N/A	N/A	yes	N/A
trans-Pinocarveol	yes	N/A	yes	no	yes	N/A
α-pinene	yes	N/A	yes	no	yes	N/A
yomogi alcohol	no	N/A	no	yes	no	N/A

In that study it was stated that when flux of reaction increases in terpene pathways, *FDS* and *GDS* possibly exert some regulation on precursors which are flowing to monoterpenes and sesquiterpenes synthase enzymes, but they don't have much effect on terpene production. This explanation is mentioned to be true providing that monoterpene and sesquiterpene pathways are not completely independent as they are not (Iijima *et al*., 2004).

Table 5. Probable limiting genes for mentioned sesquiterpenes in different tissues of *A. annua*.

Tissue	leaf		bloom		flower	
compounds	*IDI*	*FDS*	*IDI*	*FDS*	*IDI*	*FDS*
copaene	N/A	N/A	yes	no	N/A	N/A
β-Selinene	N/A	N/A	N/A	N/A	yes	no
arteannuin B	N/A	N/A	N/A	N/A	yes	no

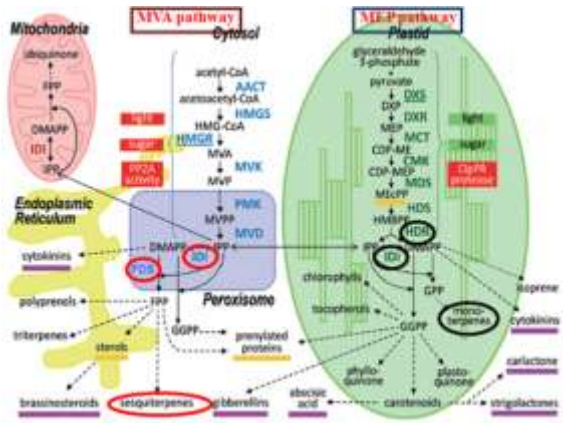

Fig.1. MEP and MVA pathways and genes which are evaluated in this study. MEP is located in the plastid whereas MVA is located in cytoplasm and peroxisome. Red circled genes and their related product sesquiterpenes are shown on the left whereas black circled genes and their corresponding product monoterpenes are shown on the right (the diagram extracted from Pulido *et al*., 2012).

Conclusion

On the whole, In order to get insight into the relationship between genes and their products in *A. annua,* some monoterpene and sesquiterpene and *FDS*, *HDR* and *IDI* expression levels in bloom and flower were compared with these amounts in leaf. This is true that these genes are very important in producing mentioned compounds, but considering the fact that there are some other enzymes (*TPSs* and *GDS*) between evaluated enzymes and compounds, it is difficult to attribute genes, up or down regulation to high or low production of compounds (Fig. 1). Moreover, some genes of terpene pathway are regulated post-transcriptionally and therefore, down or up regulation of expression of studied genes could not absolutely be annotated to the low or high production of terpenes. Yet, by comparison between gene expression and the level of terpene production, at least for some terpenes and corresponding genes one can see a relationship. Probable role of such evaluated genes in the synthesis of corresponding terpenes can just be tabulated in tables 4 and 5. It can be seen in these two tables that for most evaluated compounds the relationship between their genes is complicated and not direct and straightforward.

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References

Cao X, Yin T, Miao Q, Li C, Ju X, Sun Y, Jiang J. 2012. Molecular characterization and expression analysis of a gene encoding for farnesyl diphosphate synthase from *Euphorbia pekinensis Rupr*. Molecular Biology Reports **39**, 1487-92.

Cheng AX, Lou YG, Mao YB, Lu S, Wang LJ, Chen X. 2007. Plant terpenoids: Biosynthesis and ecological functions. Journal of Integrated Plant Biology **49**, 179–186.

Gao Y, Wu Y, Xu Y, Chen Z, Li H, Yin X, Zeng Zh, Chen R. 2012. Molecular cloning, characterization and functional analysis of a new isopentenyl diphosphate isomerase gene (IPI) from *Curcuma wenyujin*. Journal of Medicinal Plants Research **6**, 3148-3155.

Gershenzon J, McConkey ME, Croteau RB. 2000. Regulation of monoterpene accumulation in leaves of peppermint. Plant Physiology **122**, 205-213.

Gupta P, Akhtar N, Tewari Sh, Sangwan R, Trivedi P. 2011. Differential expression of farnesyl diphosphate synthase gene from *Withania somnifera* in different chemotypes and in response to elicitors. Plant Growth Regulation **65**, 93-100.

Han Min Ye J, Qiao X, Xu M, Wang B, Guo DA. 2008. Characterization of phenolic compounds in the Chinese herbal drug *Artemisia annua* by liquid chromatography coupled to electrospray ionization mass spectrometry. Journal of Pharmaceutical and Biomedical Analysis **47**, 516–525.

Hsieh MH, Goodman HM. 2005. The Arabidopsis IspH Homolog is Involved in the Plastid Nonmevalonate Pathway of Isoprenoid Biosynthesis. Plant Physiology **138**, 641-653.

Huang JZ, Cheng TC, Wen PJ, Hsieh MH, Chen FC. 2009. Molecular characterization of the *Oncidium orchid HDR* gene encoding 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase, the last step of the methylerythritol phosphate pathway. Plant Cell Reports **28**, 1475–1486.

Iijima Y, Davidovich-Rikanati R, Fridman E, Gang DR, Bar E, Lewinsohn E, Pichersky. 2004. The biochemical and molecular basis for the divergent patterns in the biosynthesis of terpenes and phenylpropenes in the peltate glands of three cultivars of basil. Plant Physiology **136**, 3724–3736.

Keeling CI, Bohlmann J. 2006. Genes, enzymes and chemicals of terpenoid diversity in the constitutive and induced defense of conifers against insects and pathogens. New Phytologist Journal **138**, 641–653.

Ma Y, Yuan L, Wu B, Li X, Chen S, Lu S. 2012. Genome-wide identification and characterization of novel genes involved in terpenoid biosynthesis in *Salvia miltiorrhiza*. Journal of Experimental Botany **63**, 2809–2823.

Munoz-Bertomeu J, Arrillaga I, Ros R, Segura J. 2006. Up-regulation of 1-deoxy-Dxylulose-5-phosphatesynthase enhances production of essential oils in transgenic spike lavender. Plant Physiology **142**, 890-900.

Nagegowda DA. 2010. Plant volatile terpenoid metabolism: Biosynthetic genes, transcriptional regulation and subcellular compartmentation. FEBS Letters **584**, 2965–2973.

Olofsson L, Engström A, Lundgren A, Brodelius PE. 2011. Relative expression of genes of terpene metabolism in different tissues of *Artemisia annua L.* BMC Plant Biology **11**, 45.

Tingey DT, Manning M, Grothaus LC, Burns WF. 1980. Influence of light and temperature on monoterpene emission rates from Slash Pine. Plant Physiology **65**, 797-801.

Wang Q, Pi Y, Hou R, Jiang K, Huang Z, Hsieh M, Sun X. 2008. Molecular cloning and characterization of 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase (*CaHDR*) from *Camptotheca acuminata* and its functional identification in *Escherichia coli*. Biochemistry and molecular Biology reports **29**, 112-118.

Wen W, Yu R. 2011. Artemisinin biosynthesis and its regulatory enzymes: Progress and perspective. Pharmacognosy Reviews **5**, 189 194.

Woerdenbag H, Bos R, Salomons M C, Hendriks H, Pras N, Malingré TH. 1993. Volatile

constituents of *Artemisia annua* L. (Asteraceae). Flavour and Fragrance Journal **,** 131–137.

Zare Mehrjerdi M, Bihamta MR, Omidi M, Naghavi MR, Soltanloo H, Ranjbar M. 2013. Effects of exogenous methyl jasmonate and 2-isopentenyladenine on artemisinin production and gene expression in *Artemisia annua*. Turkish Journal of Botany **37**, 499-505.

Zeng Q, Zhao C, Yin L, Yang R, Zeng X, Huang Y, Feng L, Yang X. 2008. Cloning of artemisinin biosynthetic cDNAs and novel ESTs and quantification of low temperature-induced gene overexpression. Science in China. Series C, Life Sciences/ Chinese Academy of sciences **51(3)**, 232-44.

Zwenger S, Basu C. 2008. Plant terpenoids: applications and future potentials. Biotechnology and Molecular Biology Reviews **3**, 001-007.