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REVIEW ARTICLE

# An investigation of the *HMGR* gene and *IPI* gene expression in black caraway (*Bunium persicum*)

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## Abstract

Black caraway is of great importance for its terpene compounds. Many genes are involved in the biosynthesis of secondary metabolites in medicinal plants. For this study, black caraway seeds were collected from five different regions, i.e. [Isfahan; Kerman (Khabr); Semnan; Kerman (Sirch); and Hormozgan]. The black caraway seed oil was extracted and analyzed by means of the gas chromatography method. There was a negatively significant correlation ($p \leq 0.05$) observed between cuminaldehyde and gammaterpinene compounds. 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGR*) and isopentenyl pyrophosphate isomerase (*IPI*) play an important role in the biosynthesis of secondary metabolites. Appropriate primers were designed for these genes based on the conserved regions in other plants. Amplified fragments were then sequenced. Blastn results indicated the similarity of the high RNA sequences between new sequences and other *HMGR* and *IPI* gene sequences in GenBank, and it also identified the *HMGR* and *IPI* gene sequences of *B. persicum*. A fragment of the *HMGR* gene with KJ143741 number was recorded in the gene bank. Quantitative PCR showed that the relative expression of two genes in different growth stages of *B. persicum* was significantly different between the germination stage and the multi-leaf stage, and also between the germination stage and the flowering stage ($p < 0.05$); however, there was no significant difference observed between the flowering stage and the multi-leaf stage. The results indicated that the expression of *HMGR* increased from the germination stage to the adult plant, and then it got stable until the flowering stage; in the same vein, the expression of *IPI* increased continuously from the germination stage to the flowering stage. The expression of *HMGR* and *IPI* genes occurred differently at the germination stage of five ecotypes. The Hormozgan ecotype showed the least expression rate.

**Keywords** Black caraway · *Bunium persicum* · Secondary metabolites · *HMGR* · *IPI*

## Introduction

Being considered as a non-crop and non-domestic plant, black caraway or mountainous Black Zira (*Bunium persicum*) is one of the economical and important medicinal plants in Iran and across the world (Sofi et al. 2009; Jalilzadeh-Amin et al. 2011). This plant belongs to the Apiaceae (Umbelliferae) family and naturally grows in temperate and arid regions of Iran and some other countries (Jalilzadeh-Amin et al. 2011). Terpene compounds are the major active ingredients contained in *B. persicum*. These compounds have a large amount of γ-terpinene (Oroojalian et al. 2010) that is a monoterpene. In another study, the antioxidant properties of *B. persicum* and thyme were examined and they were attributed to the compounds in the essential oils of these plants that contained terpene, especially γ-terpinene (Shahsavari et al. 2008). The results of the data analysis

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using geNorm and Bestkeeper-1 software tools showed that the elongation factor 1-alfa (*EF1A*) gene is expressed more stable than the two other genes and that the standard error (SE) was higher in β-actin (*ACT*) and Ubiquitin (*UBC*) genes; it was also demonstrated that they are expressed less than *EF1A*. Considering the studies done on the stability, and uniformity and stability of the *EF1A* gene expression in different ecotypes of *B. persicum*, it is recommended that the data of the target gene expression be normalized (Darvishi Zeidabadi et al. 2015).

Isoprenoids are generally made through one of the two pathways, i.e., either the mevalonate pathway is used by the cells of eukaryotes and some prokaryotes, or the alternate path of 2-C-methyl-D-erythritol 4-phosphate (MEP) that is used by most prokaryotes (Campos et al. 2001; Goldstein and Brown 1990; Hecht et al. 2001; McAteer et al. 2001). The enzyme of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR, EC 1.1.1.34) catalyzes the NADPH-dependent reduction of HMG-CoA to mevalonate, being considered as the first committed step in the isoprenoid pathway to produce the largest group of contemporary natural products (Wu et al. 2012) (Fig. 1).

Terpenoids are derived from the repeated condensation of isoprenoids among which mevalonic acid acts as a precursor. Within plant cells, the concentration of mevalonic acid (MVA) is strictly controlled by the *HMGR* activity. *HMGR* is one of the most heavily regulated enzymes ever identified (Goldstein and Brown 1990).

Enzyme *HMGR* converts 3-hydroxy-3-methylglutaryl coenzyme A to mevalonic acid and is a significant key regulator and controller of isoprene metabolism in mammals and fungi (Chappell et al. 1995).

Studies indicate that *HMGR* is an important control point for the mevalonate pathway in plants (Stermer et al. 1994). Since *HMGR* is a key enzyme in the pathway that leads to compounds with diverse and important functions in plants, it is not surprising that the *HMGR's* activity in plants is controlled by a variety of developmental and environmental signals. The higher levels of the *HMGR's* activity are usually associated with the rapidly growing parts of the plant, including apical buds and roots, with a much reduced activity observed in mature tissues (Brooker and Russell 1975; Bach et al. 1980). As an instance, the relative activity of microsomal *HMGR* observed in the mature leaves of pea

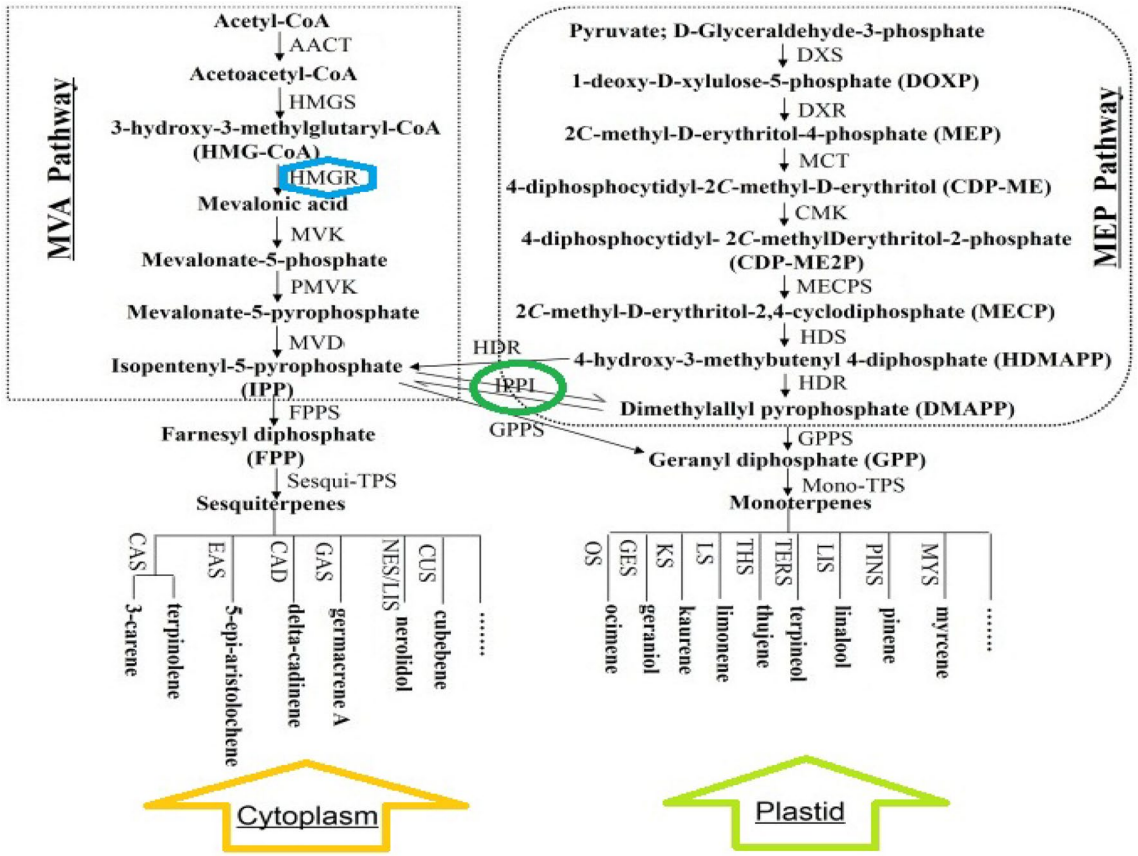


**Fig. 1** Biosynthetic pathway of monoterpenes and sesqui terpenes

seedlings was shown to be only 7% of the activity observed in the apical buds (Brooker and Russell 1975).

The *HMGR's* activity is controlled not only at the mRNA level, but also post-translationally. Some studies have suggested that the *HMGR's* activity in plants is regulated by reversible phosphorylation, as it is done in animals (Russell et al. 1985; Sipat 1982). *IPI* catalyzes the interconversion of isopentenyl diphosphate (IDP) and dimethylallyl diphosphate (DMAPP). It has been reported that the *S. cerevisiae IPI* gene (*IDI*) is an essential and single-copy gene, and that the disruption of the *IDI* gene results in a lethal phenotype (Mayer et al. 1992). Based on the relevant knowledge, the conversion of IPP to DMAPP by *IPI* in plants is thought to be necessary for isoprenoid biosynthesis through the cytosolic MVA pathway (Heintz et al. 1972; Nes and Venkatramesh 1999; Lange and Ghassemian 2003). IPP and DMAPP are synthesized in plants via the cytosolic mevalonate (MVA) pathway and the plastidic methylerythritol phosphate (MEP) pathway, respectively (Okada et al. 2008). IPP is consequently condensed to DMAPP to yield the short-chain isoprenoid precursors geranyl diphosphate, farnesyl diphosphate (FPP), and geranylgeranyl diphosphate (GGPP) that are further metabolized to monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20). IPP isomerase (IPI; EC 5.3.3.2) catalyzes the interconversion of IPP to DMAPP, being regarded an essential starter moiety for the condensation reactions (Xuan et al. 1994).

The transcriptional co-regulation of the genes involved in secondary metabolite pathways is an important mechanism to regulate such pathways in general. To understand the correlation between the expression pattern of various genes and the content of terpenoid metabolites involved in artemisinin biosynthesis, the qPCR method was used (Yang et al. 2015).

In this study, two genes (*HMGR* and *IPI*) of Black Zira and their relative expressions in various stages of the plant growth were identified, and they were also analyzed in the 5-ecotype germination stage. The GC–MS analysis was used to assess the ingredients of the seeds of five Black Zira ecotypes.

## Materials and methods

### Plant material

Five *B. persicum* ecotypes [Isfahan, Keman (khabr), Semnan, Kerman (sirch) and Hormozgan] were used in the experiments. The seeds were surface-sterilized and germinated synchronously based on the protocol developed by Sharifi and Pouresmael (2006). The seeds were sterilized with 0.2% (v/v) sodium hypochlorite for 20 min, followed by 70% (v/v) ethanol for 1 min. Having been washed, the seeds were treated with hormones (gibberellic 100 µm acid and thidiazuron 6 µm) for 24 h, and they were then washed with the sterile water. The seeds were transferred into a sterile Petri dish and incubated in the dark at 4 °C for an additional 3 weeks. The seeds subsequently started to be germinated. The seeds of all the ecotypes were grown in a greenhouse. Following 15 days, a sample of seedlings was kept at − 80 °C for a further analysis. Other samples of the multi-leaf stage and the flowering stage were taken and kept for further tests at − 80 °C.

### GC–MS analysis

The ground dry seeds (10 g) of each of the five ecotypes (Isfahan, Keman-khabr, Semnan, Kerman-sirch and Hormozgan) were extracted using 120 ml distilled water for approximately 3 h in the Clevenger instrument. The essential oil of each of the ecotype seeds was analyzed using capillary GC on a Thermo-UFM gas chromatograph (Thermo, Inc.) equipped with an HP-5 ms column with 5% phenyl methyl silo hexane capillary (0.4 µm film thicknesses, 10 m × 0.1 mm i.d.) and an Chrom-Card A/T Network Mass Selective Detector. The oven temperature was programmed for 1 min at 60 °C and 60–280 °C, and 80 °C per minute, and a final time of 4 min. Helium was used as the carrier gas. The sample size was 1 µl in the splitless mode. The terpenoids were identified by comparing the mass spectra and the retention indices with the wiley-5 library.

### Polymerase chain reaction and *gene detection*

*HMGR* and *IPI* sequences were searched through the GenBank. The sequences of *HMGR* and *IPI* in the other family, especially Apiaceae, were analyzed by MegAlign 5.00 software (© 1993–2001 DNASTAR Inc.) and were simulated using the Clustal W method. The conserved areas for *HMGR* and *IPI* genes were identified, and appropriate primers were designed for those areas. The required features of the primers were examined by OligoAnalyser software. The primers were then designed in the conserved regions of the genes (Table 1).

For RNA isolation, 50–100 mg of the plant tissue was ground and homogenized with the lysis buffer of the Ribospin plant Cat. no: 307–150 Kit, according to the manufacturer's instructions (GeneAll, Korea). The RNA was treated with *DNase I* (Fermentas) to remove the remaining genomic DNA. The RNA quality was examined using agarose gel 1/5%, and its quantity was measured using Nanodrop (Epoch™ Multi-Volume Spectrophotometer). RNA (1 µg) was reverse transcribed using RevertAid™H Minus-MuLV reverse transcriptase (Fermentas) and 0.1 µg oligo (dT) 18 primer in accordance with the manufacturer's instructions. Complementary DNA (cDNA) was performed

**Table 1** Primers used in qPCR, RACE PCR, and gene cloning

| Primer no. | Primer name | Use | Primer sequence |
|---|---|---|---|
| 1 | $IPI1_{(forward)}$ | qPCR | 5′-GAT GTG AAA GTA AAT CCC AAC CCT G-3′ |
| 2 | $IPI1_{(reverse)}$ | qPCR | 5′-GAA TCG TTT CCA TAT CAG CAA CTT CC-3′ |
| 3 | $HMGR1_{(forward)}$ | qPCR | 5′-GAT GCD ATG GGA ATG AAC ATG GT-3′ |
| 4 | $HMGR1_{(reverse)}$ | qPCR | 5′-GCA CAG TGG TTT TCA AYA CCT TCT TCA C-3′ |
| 10 | $EF1A_{(forward)}$ | qPCR | 5′-CTG GTG GTT TTG AAG CTG GT-3′ |
| 11 | $EF1A_{(reverse)}$ | qPCR | 5′-TGT TGT CAC CCT CGA ATC CA-3′ |

by the RevertAid™ First strand cDNA synthesis kit from fermentase.

Tissues from different growth stages were collected and used for the amplification and analysis of the expression levels of *IPI* and *HMGR* genes. For each sample, the reaction was set up with the 10 µl Ampliqon PCR Master Mix, 1 µl forward (10 pmol) primer, 1 µl reverse (10 pmol) primer, and 7 µl $dH_2O$ and 1 µl (5 ng) cDNA as the template. The PCR program was used within the temperature range (gradient) for checking the optimal temperature of the primers. The cycling program for the PCR included 94 °C 4 min, 1 cycle, and 94 °C 30 s, 57 °C 45 s, 72 °C 1 min, 35 cycles; 72 °C 10 min, for *HMGR* and *IPI*. The quality of the PCR product was examined using agarose gel 1%. The target gene fragments were amplified by specific primers, and after observing the electrophoresis pattern and ensuring the reproduction quality, they were submitted for purifying and sequencing the PCR product. After getting sequenced, the fragments were aligned and assembled. The sequencing results were analyzed by SeqMan and BLAST software.

### Gene expression

Tissues of different ecotypes from the germination stage were collected to analyze the correlation between the expression levels of the genes encoding the biosynthetic material and the secondary metabolites content. RNA was extracted using the Ribospin plant Cat. no: 307–150 Kit, according to the manufacturer's instructions (GeneAll, Korea). The single-stranded cDNA was synthesized by RevertAid™ H Minus-Mulv reverse transcriptase (Fermentas), using 0.1 µg Oligo $(dT)_{18}$-primer. ABio-Rad iCycler Real-time PCR system (MiniOpticon) was used to perform the amplification task. For each sample, the reaction was set up with 1 µl EvaGreen® qPCR Real Time Master Mix with ROX (Solis BioDyne), 1 µl forward primer, 1 µl reverse primer, and 7 µl $dH_2O$ and 1 µl cDNA as the template. The thermal cycling program included 95 °C 15 min, 1 cycle; 95 °C 15 s, 57 °C 60 s, 72 °C 30 s, 40 cycle; 95 °C 15 s, 60 °C 1 min, 95 °C 15 s, 1 cycle. The primers for each gene are listed in Table 1.

The relative fold changes in the gene expression were measured based on the comparative method (Livak and Schmittgen 2001; Sehringer et al. 2005; Cikos et al. 2007) with three replications. In this method, the levels of the target gene amplification in an experimental sample are compared with the levels of the target gene amplification in another sample or standard, both of which being first normalized to the amplification levels of a normalizing gene. For this study, the elongation factor 1-alfa (*EF1A*) gene was used as the normalizing factor. Before examining *HMGR* and *IPI*, the relative expression efficiency of each specific gene and the housekeeping gene was measured, and then the relative expression levels were analyzed using the REST 2009 software V. 2.0.13 (Qiagen, Hilden, Germany) (Pfaffl et al. 2002).

## Results and discussion

### GC–MS analysis

The analysis results of the essential oil from five ecotype seeds, making use of GC–MS, showed component differences in various ecotypes. Four components showed to have a higher content rate from among all other components (i.e., P-cymene, γ-terpinene, cuminaldehyde, and γ-terpinene-7al). Cuminaldehyde and γ-terpinene-7al had higher quantities in comparison with the three other components (Figs. 2, 3, 4).

A DNA fragment encoding the HMG-CoA reductase was obtained by PCR from $HMGR1_{(forward)}$ (5′-GAT GCD ATG GGA ATG AAC ATG GT-3′) and $HMGR1_{(reverse)}$ (5′-GCA CAG TGG TTT TCA AYA CCT TCT TCA C-3′) as primers; also for IPI, $IPI1_{(forward)}$ was (5′-GAT GTG AAA GTA AAT CCC AAC CCT G-3′) and $IPI1_{(reverse)}$ was (5′-GAA TCG TTT CCA TAT CAG CAA CTT CC-3′).

The sequencing results showed that 217 bases had been sequenced from *HMGR* and 214 bp from *IPI* genes (Fig. 5).

The nucleotide sequences of both genes were analyzed using BLASTN software. The *HMGR* nucleotide sequence showed 85% similarity with *Artemisia annua HMGR*, 82% similarity with *Tanacetum parthenium HMGR*, and some similarities with a few other nucleotide fragments of different plants in GenBank. The *IPI* nucleotide sequence showed over 93% similarity with *Dacus carruta IPI*

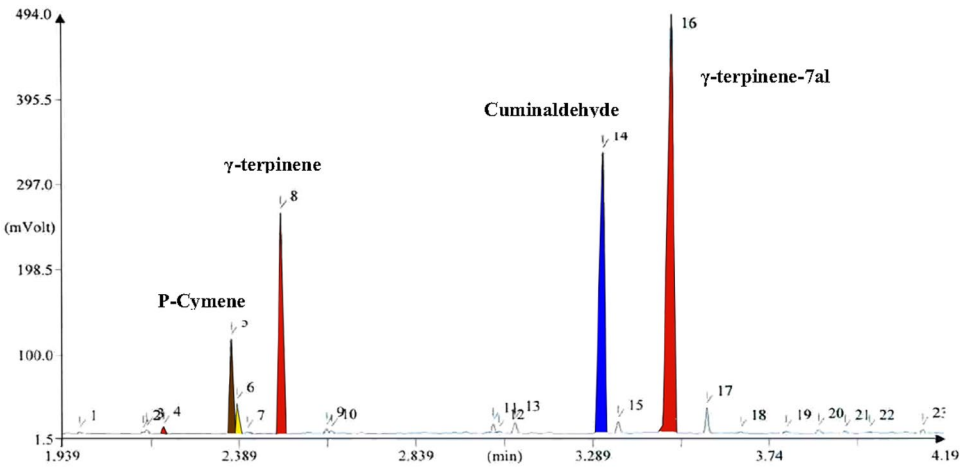


**Fig. 2** GC–MS chromatogram of the essence analysis for Kerman (sirch) ecotype of *B. persicum*

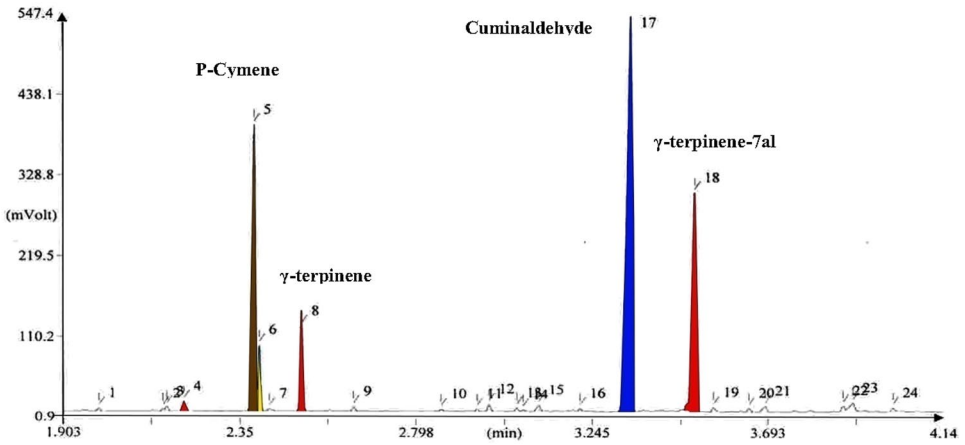


**Fig. 3** GC–MS chromatogram of the essence analysis for Hormozgan ecotype of *B. persicum*

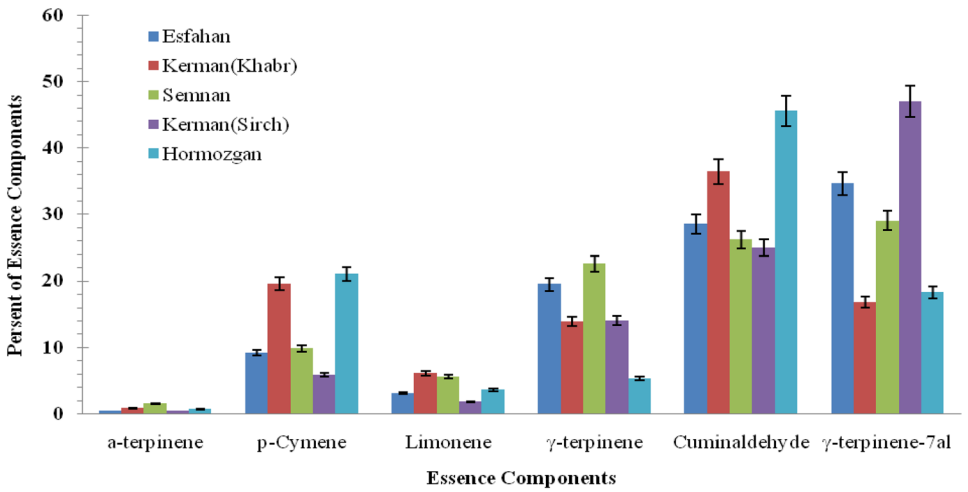


**Fig. 4** Different essence components of five ecotype seeds of *B. persicum*

(JX100860), 83% similarity with *Medicago truncatula IPI* (XM-003624130.1), and some similarities with a few other nucleotide fragments of different plants in GenBank. The partial gene fragment of *HMGR* was registered for the first time for *B. persicum* under number KJ143741 in GenBank (NCBI).

**Fig. 5** Partial sequences from A-*HMGR* and B- *IPI*

A- *HMGR*
5′-ATG CGA TGG GAA TGA ACA TGG TCG CTT GGA TTG GCA GCT GTC TGC GAT TTT CTT CAT GTG ATT TCC CCG ATA TGG ATG TGA TCG GCA TAT CTG GTA ACT TTT GTT CTG ATA AGA AAC CTG CTG CTG TCA ATT GGA TTG AGG GGC GTG GAA AAT CTG TTG TCT GTG AGG CAA CCA TAA CTG AGG ATG TTG TGA AGA AGG TAT TGA AAA CCA CTG TGC A-3′ 217bp

B- *IPI*
5′_GATGTGAAAGTAAATCCCAACCCTGAGGGCTGCTTGACTTATATATGTAGAACCA AGAACAGCTGAGAGAATTGCTGAGAAAAGCCGATGCTGGTGAGGAAGGCTTGAAAC TATCCCCTTGGTTCAGACTAATTGTTGACAATTTCTTGTTCAAATTGTGGGACCATGC TCAGAAAGGAACCCTCGAGGAAGTTGCTGATTCGGAAACGATTCA-3′- 214bp

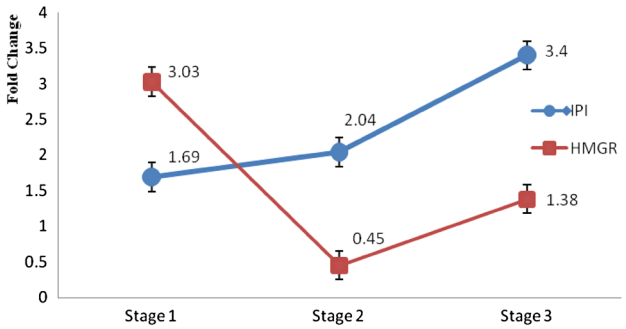


**Fig. 6** Relative expression (fold change) of *HMGR* and *IPI* genes in the three stages of the *B. persicum* growth in Kerman (sirch) ecotype: stage 1—germination, stage 2—multi-leaf, and stage 3—flowering

### Gene expression analysis

The sensitive quantitative Real Time PCR was performed using the total RNA isolated from various tissues of *B. persicum. EF1A* was used as the housekeeping gene. This gene should be highly, stably, and constitutively expressed in all conditions and tissues to be analyzed (Deprez et al. 2002; Thellin et al. 1999; Schmittgen and Zakrajsek 2000; Brunner et al. 2004); besides, *EF1A* is expressed in a higher and more uniformed form, compared with other genes in *B. persicum* (Darvishi Zeidabadi et al. 2015).

However, the gene is expressed highly in the leaves and abundantly in the flowers and roots. The efficiency of the three gene primers was calculated using the REST-2009 software. The rates were 0.98, 0.91, and 0.95 for *EF1A*, *HMGR*, and *IPI*, respectively. The expression of *HMGR* and *IPI* could be detected in all tissues from the germination stage to the flowering stage.

The results of the relative expression analysis of *HMGR* and *IPI* genes showed a significant difference between different stages. The *HMGR* gene showed a significant expression difference between the germination stage and the multi-leaf stage (3.03-fold), and also between the germination stage and the flowering stage (1.38-fold) ($p < 0.05$); however, there was no significant difference between the expression in the flowering stage and the multi-leaf stage (Fig. 6).

On the other hand, the results showed that the expression of *HMGR* increases from the germination stage to the mature-plant stage, and it then becomes stable until the flowering stage. The *IPI* gene showed no significantly different expression between the multi-leaf stage and the germination stage, but there was a significant difference between the flowering stage and the multi-leaf stage (2.04-fold), and also between the flowering stage and the germination stage (3.4-fold). The higher levels of the *HMGR* activity are usually associated with the rapid growth of the plant parts, including apical buds and roots, with a much reduced activity observed in mature tissues (Brooker and Russell 1975; Bach et al. 1980). *HMGR* also exhibited a lower expression rate in old leaves in comparison with other tissues in *A. annua* L. (Olofsson et al. 2011). The overexpression of both *HMGR* and *ads* genes in *A. annua* L. plants results not only in an increase in the artemisinin content, but it also enhances the synthesis of other isoprenoids, including the essential oils (Alam et al. 2014). The stability of the *HMGR* expression at the multi-leaf stage to the flowering stage might have happened since these stages are very important and plants need a highly strong defense system. The plastidic pathway was predicted to play an important role in the shift from the vegetative growth stage to the reproductive growth stage (Vail 2008).

*HMGR* expression is highly upregulated in the plants that shift into the reproductive growth stage (Vail 2008). However, *HMGR* is known to be regulated post-transcriptionally (Hey et al. 2006). During the flowering stage, the plant is in a vital and vulnerable phase; therefore, the need for the defensive compounds is by far higher (Vail 2008). Isoprenoids produced in the plastid are important for floral pigmentation (carotenoids) and fragrances (monoterpenes) (Vail 2008). Furthermore, Towler and Weathers (2007) demonstrated that the inhibition of the MEP pathway reduced the artemisinin production significantly.

However, the results showed that the mRNA levels of key isoprenoid biosynthetic genes in the plastid remained fixed in flower-budding plants compared to vegetative plants, and that they were also downregulated during the full flowering stage. Photosynthesis in the shoot is likely to assume

a weaker role in the plant shifts to the reproductive phase. Cytosolic IPP is also likely to provide a crosstalk source of isoprene (IPP) biosynthesis in the plastid, since the cytosolic pool of IPP is probably very large due to the high increases in *HMGR* transcripts.

MEP pathway genes are known to be regulated post-transcriptionally (Sauret-Gueto et al. 2006); therefore, it is possible that although the levels of transcripts are unchanged as the plant shifts to the reproductive stage and the flowering stage, the post-translational regulation may occur.

Page et al. (2004) realized that *Nicotiana benthamiana* leaves where the *IPI* expression was downregulated by the tobacco rattle virus-mediated gene silencing exhibited a mottled whitish pale green phenotype and an 80% reduction in the level of chlorophyll compared with control leaves. They concluded that although not absolutely required, *IPI* plays a significant role in plastidic isoprenoid biosynthesis in higher plants (plants of relatively complex or advanced characteristics, especially vascular plants, including flowering plants).

The *atipi1atipi2* double mutant conditionally showed a 20% decrease in chlorophyll and carotenoids compared with control plants under the continuous light. Thus, a pale green phenotype seems to be a common feature of plants defective in the *IPI* activity, although the extent of variegation varies significantly among plant species. However, the appearance of a pale green phenotype was greatly absent from the Arabidopsis *ipi* double mutant under LD (long day) conditions, indicating that the *IPI* activity is required for the production of photosynthetic pigments using the MEP pathway under conditions necessitating an increased isoprenoid production rate (Okada et al. 2008). In contrast to the *atipi1atipi2* double mutant, neither the *atipi1* single mutant nor the *atipi2* single mutant demonstrates any visible phenotype. This observation was interesting given that *AtIPI1* was localized in the cytoplasm, whereas *AtIPI2* was targeted at mitochondria (Okada et al. 2008). The observations also indicated that cytoplasmic *AtIPI1* was sufficient for the synthesis of isoprenoids in mitochondria, and that IPP, DMAPP and other prenyl diphosphates such as FPP were capable of moving between the mitochondria and the cytosol (Okada et al. 2008).

The analysis of the expression of *HMGR* and *IPI* for five ecotypes of *B. persicum* in the germination stage demonstrated a significant difference among ecotypes. These results show a variation among ecotypes concerning the expression factor. The Hormozgan ecotype was observed to have less expression than other ecotypes, so it was measured as the expression for other ecotypes (Fig. 7). The results showed that the Semnan ecotype had the highest expression of *IPI*.

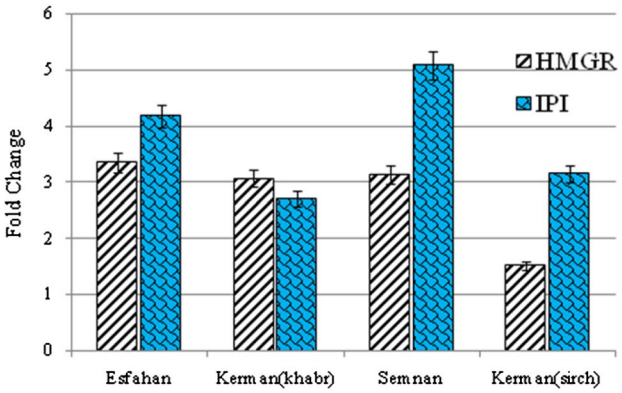


**Fig. 7** Relative expression (fold change) of *HMGR* and *IPI* genes in four different ecotypes to Hormozgan ecotype of *B. persicum*

There was a negatively significant correlation ($p \leq 0.05$) between cuminaldehyde and γ-terpinene-7al that shows a variation among the five ecotypes (Fig. 8a). There was a positive correlation between the *HMGR* expression and the *IPI* expression (Fig. 8b). The expressions of two genes in different ecotypes were correlated; hence, it can be concluded that the *IPI* expression has also been highly similar to the high expression of *HMGR*. The overexpression of *HMGR*, considered as a rate-limiting enzyme, upgrades the flux rate of the MVA pathway and leads to an increase in the terpenoid biosynthesis and *IPI* (isopentenyl diphosphate isomerase) (Qaderi et al. 2014). Cytosolic *IPI* increases the pool of the precursor DMAPP in plastid. The accumulation of DMAPP in plastid (the first precursor in the production of diterpenoids) leads to an increase in the amount of diterpenoids (Roberts 2007).

The correlation of *HMGR* and *IPI* CT with cuminaldehyde and γ-terpinene-7al was not significant. This indicates that the production of secondary metabolites such as γ-terpinene and cuminaldehyde has undergone the post-transcriptional regulation (Table 2). Although the levels of *HMGR* transcripts have remarkably increased, a consequent increase in the enzyme activity and the carbon flux may not necessarily follow the increased transcript levels (Re et al. 1995).

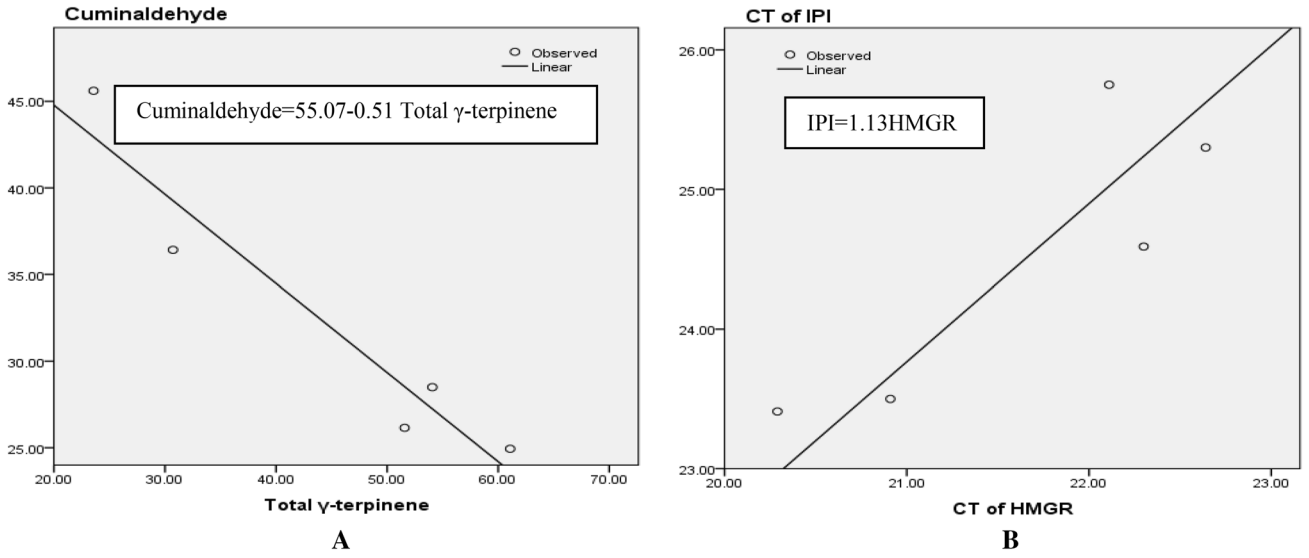


**Fig. 8** The curve of regression between: **a** total γ-terpinene and cuminaldehyde, and **b** CT of HMGR and CT of IPI

**Table 2** Pearson correlation between important essence components and specific genes CT

| | Cuminaldehyde | γ-Terpinene | IPIct | HMGRct |
|---|---|---|---|---|
| Cuminaldehyde | 1 | | | |
| γ-Terpinene | −0.833* | 1 | | |
| IPIct | 0.305 | −0.361 | 1 | |
| HMGRct | −0.259 | 0.079 | 0.611 | 1 |

*Correlation is significant at the 0.05 level

## Compliance with ethical standards

**Conflict of interest** We declare that the authors of this paper have no conflicting interests.

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