RESEARCH ARTICLE

The effects of water deficit on the expression of monoterpene synthases and essential oils composition in *Salvia* ecotypes

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Abstract The medicinal sage plant (*Salvia* spp.), belonging to Lamiaceae family, is one of the most important medicinal and aromatic plants. The members of this genus are globally known due to its antimicrobial, antioxidant, astringent, spasmolytic, antihidrotic and specific sensorial properties. In this study, we investigated the potential impact of water deficit on transcript abundance, and essential oil composition of five major metabolites, i.e. 1–8 cineole, α - β -thujone, camphor, and borneol in three genotypes of *Salvia* spp. Results showed that relative expression of three genes and their corresponding metabolites increased together at three stages under drought condition, but the *CS* gene transcript decreased independently from 1,8-cineole in garden sage. Furthermore, borneol changed differently compared to the *BS* gene expression in control and drought treatment plants of *S. reuterana* (Yasuj). The competitive synthesis of β -thujone, and α -thujone by *SS* gene were demonstrated in *S. officinalis* and Yasuj ecotype of *S. reuterana*; whereas, no change was observed for Urmia ecotype of *S. reuterana*. There was no precursor shortage to synthesis of borneol and camphor in garden sage; however increasing the *BS* led to high production of borneol and low camphor in *S. reuterana* under drought stress. As a mechanism, secondary metabolites enable the plants to cope with

unfavorable conditions, but genetic differences might affect the quantity and quality of these compounds.

Keywords *Salvia* · Sage · α - β -thujone · Monoterpene · GC/MS · Drought stress

Introduction

Garden sage, *Salvia officinalis* L., is one of the broad-appealing herbal plants due to the presence of a vast amount of aromatic oils as well as compounds with valuable medicinal properties. This plant belongs to the genus *Salvia* and Lamiaceae family, one of the largest genus comprising about 900 species. Although garden sage is currently naturalized all around the world, this plant is native to the Middle East and Mediterranean regions (Czygan 2004; Ghorbani and Esmaeilzadeh 2017; Miura et al. 2001). Throughout history, this plant has been widely used in food, medicine, and beverage preparations because of its tasty and spicy flavor. Moreover, it has been considered as a preservative in the food industry thanks to its strong antimicrobial activity (Behradmanesh et al. 2013). In the current era, concerning advanced medicinal and biological studies, the species belonging to *Salvia* have considerably drawn scientist's attention owing to highly valuable compounds. Specifically, essential oils, which contain more than 100 active compounds, have shown important bioactivities, such as anticancer, anti-inflammatory, choleric, antimutagenic, antioxidant, and antimicrobial activities (Ghorbani and Esmaeilzadeh 2017). These metabolites are mainly divided into groups of monoterpenes, sesquiterpenes, diterpenes, and triterpenes (Badiie et al. 2012).

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Biosynthesis of terpenoids is well-developed in many plants; however, the differential expression of these genes in some medicinal plants has not been understood yet. It is generally accepted that the immediate building blocks of all terpenoids are prenyl diphosphates, that is, farnesyl pyrophosphate (FPP), geranylgeranyl pyrophosphate (GGPP), and diphosphate (GPP) (McGarvey and Croteau 1995). Besides, these compounds are mostly formed in plastids via the 1-deoxy-d-xylulose-5-phosphate pathway (Lichtenthaler 1999). Three monoterpene synthases are involved in the first steps of monoterpene formation. The (+)-sabinene synthase (SS) is the first enzyme in the production of two major monoterpenes, α - and β -thujone. The 1,8-cineole synthase (CS) catalyzes the one-step conversion of GPP to 1,8-cineole (Croteau and Karp 1977). The third important enzyme is (+)-bornyl diphosphate synthase (BS), which produces bornyl diphosphate (Croteau and Karp 1979). The bornyl diphosphate subsequently hydrolyzed to borneol and then oxidized to camphor (Corsi and Bottega 1999; Venkatachalam et al. 1984). The regulation of these genes in *S. officinalis* is not completely known (Fig. 1).

It has been well established that plants subjected to unfavorable conditions, such as water shortage produce increased concentrations of specific metabolites compared to those growing in normal conditions. When a plant is exposed to the drought stress, its stomata close, leading to a reduction of the uptake of CO_2 . Consequently, the reduction equivalents consumed in the Calvin cycle significantly decrease and result in a tremendous oversupply of $\text{NADPH} + \text{H}^+$. As a result, secondary metabolic processes are pushed to the formation of highly reduced metabolites, such as isoprenoids, alkaloids, or phenols (Selmar and Kleinwächter 2013a). Furthermore, stress-

related enhancement of secondary metabolites might be due to increased activity of metabolic enzymes, or co-regulation of these genes in response to the applied stresses (Abedini and Monfared 2018). The main objective of this research was to study transcript abundance of three important monoterpene synthases, i.e. SS, CS, and BS, in three genotypes of *Salvia* spp. growing under drought conditions. The metabolite content of the drought treated and well-irrigated samples were measured using GC/MS. Specifically, the relatedness of five major compounds, i.e. 1,8-cineole, β -thujone, α -thujone, camphor, and borneol, with the expression of each gene was addressed.

Materials and methods

Plant materials and drought treatments

The seeds of garden sage (*S. officinalis* L.) were provided by a commercial nursery, and two ecotypes of *S. reuterana* Boiss. were obtained from Research Institute of Forest and Rangelands (RIFR), Iran, which had been collected from Yasuj and Urmia. The seeds were planted in rectangular pots containing coco peat and perlite (2:1) and placed in greenhouse with 16/8 h (light/dark). At four-leaf stage, the seedlings were transplanted into plastic pots (30 × 35 cm) containing 2:1:1 mixtures of soil, farmyard manure and coco peat. In this study the *Salvia* plants were biennial and perennial. Therefore, the required chilling (low temperature at 4 °C) for flowering was performed by transferring the one-year pot plants to growth chamber with 4 °C, 80% RH, 1000 lx light and 10/14 h (light/dark) photoperiod condition for three months. The drought treatment was carried out at four-leaf stage of seedling growth. To this

Fig. 1 Biosynthetic pathway of 1,8-cineole, sabinene and bornyl diphosphate in *Salvia officinalis* L. Three distinct monoterpene synthases, i.e. 1,8-cineole synthase (CS), (+)-sabinene synthase (SS), and (+)-bornyl diphosphate synthase (BS) are responsible for production of 1,8-cineole, sabinene and bornyl diphosphate, respectively

end, half of the studied plants were well-irrigated and considered as control plants; whereas, the other plants were imposed to water deficiency ($30 \pm 5\%$ FC) for 20 days. Aerial parts of the plants were collected from cultivated plants at three growth phase, namely vegetative, pre-flowering, and flowering stage, and stored at -80°C until further processing. The leaves were lyophilized before the SPME GC–MS analysis.

Extraction of essential oil

Essential oil extraction and analysis were carried out based on the SPME (solidphase micro-extraction) method (Cozzolino et al. 2016). Briefly, the optimization of SPME extraction and desorption conditions were performed by analyzing dried leaves of *S. officinalis* L., used as the matrix. The nature of the adsorptive phase, the sampling temperature, and the extraction time required to achieve equilibration between the analytes and the fiber were investigated by applying a univariate experimental design. Four SPME fibers (Supelco) with different stationary phases and film thicknesses, including PDMS (100 μm), DVB/CAR/PDMS (50/30 μm), CAR/PDMS (75 μm), and PDMS/DVB (65 μm), were tested.

The following sample preparation procedure was adopted: 15 mg of lyophilized dried sage leaves were mixed into a 20 ml screw-on cap HS vial (Supelco, Bellefonte, PA, USA) with 5 ml of 5% Ethanol and 0.5 mg of Na_2SO_4 . After stirring, vials were sealed with a Teflon (PTFE) septum and an aluminum cap (Chromacol, Hertfordshire, UK) for the production of headspace and the successive analysis. The sample vial was put into a dry block-heater at 40°C for 20 min to equilibrate the system. The extraction and injection processes were automatically performed using an autosampler MPS 2 (Gerstel, Mülheim, Germany). Then, the fiber was automatically inserted into the vial's septum for 10 min, to allow the volatile compounds to be absorbed onto the SPME fiber surface. Each SPME fiber was conditioned before its first use, as recommended by the manufacturer.

Gas Chromatography–Mass Spectrometry (GC–MS) analysis

In order to desorb the volatile metabolites, the SPME fiber was introduced into the injector port of the gas chromatograph device, model GC 7890A, Agilent (Agilent Technologies, Santa Clara, CA, USA) coupled with a mass spectrometer 5975 C (Agilent) wherein the metabolites were thermally desorbed and transferred directly to a capillary column HP-Innowax (30 m $\times$ 0.25 mm $\times$ 0.5 μm Agilent J&W).

The oven temperature program was initially set at 40°C for 1 min, ramped to 200°C at 4°C min^{-1} , increased to 240°C at $10^\circ\text{C min}^{-1}$ and held for 5 min. The temperature of the ion source and the quadrupole were kept at 230°C and 150°C , respectively; helium was used as carrier gas with a flow of 1.5 ml min^{-1} ; injector temperature was held at 240°C , and the pulsed splitless mode was used for the analysis. The fiber was maintained in the injector for 10 min. Mass Spectra were acquired at an ionization energy of 70 eV, and volatile components were detected by mass selective detector. The detector operated in a mass range between 30 and 300 m/z with a scan rate of 2.7 scans s^{-1} . Each sample was analyzed in three replications, and blanks were run between sets of three analyses. A DVB/CAR/PDMS fiber was used to select the extraction temperature and time. Extraction was performed at temperatures of 25°C , 40°C and 60°C for 10, 20, 30 and 45 min. The sum of the peak areas obtained under each parameter, the number of extracted metabolites and the reproducibility were used to evaluate the optimal SPME conditions. The relative peak areas (RPAs) of individual metabolites are related to total peak area. Based on the peak resolution, their areas were calculated from the total ion current.

RNA extraction, cDNA synthesis and qRT-PCR assay

In order to assess the gene expression, total RNA from fresh leaves of each sample was extracted using BIOZOL Total RNA Extraction Reagent (Bioer Technology Company Limited, Binjiang, China), according to the manufacturer's instructions. Then, the quality and quantity of the extracted RNA were assessed via gel electrophoresis and Nanodrop spectrophotometer (BioTek-Epoch, Winooski, VT, USA), respectively. Afterward, the samples were treated with DNase I (fermentase) to remove any genomic DNA contamination. Treated samples were standardized to contain an equal amount of RNA (2 μg) and subjected to reverse transcriptase to the synthesis of first-strand cDNA. The specific primers for the surveyed genes in sage were used as published by Grausgruber-Gröger and co-workers (Table 1). In the current study, we evaluated the mRNA level of cineole synthase (CS), bornyldiphosphate synthase (BS), and sabinene synthase (SS). Then, exclusivity performance of each primer pairs was verified using PCR assay, and the specific length of the amplicons was further confirmed by gel electrophoresis.

The quantitative real-time PCR was performed using CorbettRotor Gene 3000 system in Slarno Lab, Italy, with the fluorescent dye SYBR[®]Green Master Mix. The qRT-PCR was run at 95°C for 180 s, 40 cycles at 95°C (30 sec), 61°C (60 s), 72°C (45 sec). After completion of each run, the dissociation curves were obtained by

Table 1 The primers of Bornyl diphosphate synthase (*BS*), 1,8-Cineole synthase (*CS*), Sabinene synthase (*SS*) and actin genes used for qRT-PCR assay

Primer	Sequence (5' to 3')	Accession No.
<i>BS</i> -Forward	TATGCGAGAAGGCCAGACATG	AF051900
<i>BS</i> -Reverse	AGCCTATCCCTCACAAATGGG	
<i>CS</i> -Forward	CGACATGAATCCGACTGTGCTAGAAC	AF051899
<i>CS</i> -Reverse	GGAAGCTCGTGGACAAGACC	
<i>SS</i> -Forward	AGGAACCACCGATCCAGACA	AF051901
<i>SS</i> -Reverse	GGTGCCCTGAGGTCTCTGTT	
Actin-Forward	AGGAACCACCGATCCAGACA	
Actin-Reverse	GGTGCCCTGAGGTCTCTGTT	

ramping up the temperature from 65 °C to 95 °C (0.5 °C/s). In this study, we used three technical replicates for each sample. The data were analyzed with Rotor Gene software (Version 6.1.81). Moreover, to quantify cycle thresholds (C_t) for each gene, we used the Livak method ($2^{-\Delta\Delta C_t}$), with the actin gene as an internal reference. Excel (Microsoft 2010) was utilized to calculate relative gene expression.

Statistical analysis

The experiment was conducted in a factorial design (two levels of water regimes $\times$ three genotypes) with three growth stages (vegetative, pre-flowering, and flowering). The main and interaction effects of the experimental factors were analyzed using ANOVA, and the mean comparison was calculated via Duncan multiple range test (MRT) with SAS.9.3.

Results

Phytochemical analysis

All volatile metabolites from *S. officinalis* and two native ecotypes of *S. reuterana*, i.e. Yasuj and Urmia, were measured (Supplementary Table 1). The garden sage represented 48 and 68 volatile compounds in the first phase of growth, 76, and 71 in the pre-flowering stage, and 74 and 65 in flowering time in the stressed and unstressed plant samples, respectively. Briefly, according to the statistical analyses, five major volatile compounds, including 1,8-cineole, β -thujone, α -thujone, camphor and borneol, showed different amounts at the three growth stages in control and drought treated plants (DTPs). Under drought stress, 1,8-cineole (14.03%) and camphor (5.74%) were the highest at flowering; on the other side, β -thujone (48.68%), borneol (0.33%) and α -thujone (4.66%) were the maximum at vegetative stage in garden sage (Fig. 2). The 3-way ANOVA results also showed a significant difference ($p < 0.01$) in 1,8-Cineole, α -thujone and β -thujone

metabolites (Table 2). Of this, 1,8-Cineole showed the highest concentration in the interaction of garden sage $\times$ drought and during all three stages. On the other hand, the maximum content of α -thujone and β -thujone was detected in the interaction of garden sage $\times$ drought $\times$ vegetative stage (Table 2). Overall, the imposition of differential water regimes caused these volatile compounds in the vegetative stage to increase. The interaction between genotypes and stages was statistically significant ($p < 0.01$) in all of the five compounds. Moreover, the two-way interaction effect between species and water regime was significant ($p < 0.01$) in two 1,8-cineole, α thujone; while camphor and borneol showed a significant difference at $p < 0.05$.

In this study, we also measured the metabolic profile of two ecotypes of *S. reuterana*, i.e. Yasuj and Urmia. These ecotypes grow as wild plants in two geographically different places in Iran. Thus, despite their genetic similarity, we assumed that the location can have an impact on their phytochemical profiles. Based on the GC–MS results (Figs. 2, 3, 4), the concentration of 1,8-cineole in control plants significantly increased as the plant develops from vegetative (2.40%) to the flowering stage (7.49%) (Fig. 2). In contrast, Yasuj DTPs showed a reverse pattern of this metabolite with lower content pre-flowering stage (2.45%) compared to vegetative time (3.78%). Interestingly, drought determined a decrease of 1,8-cineole at the flowering stage in Yasuj DTPs (0.78%) as compared to the vegetative growth (3.78%). In contrast, β -thujone (48.68%), α -thujone (4.66%) and borneol (0.33%) were in maximum content at vegetative stage of DTPs, whereas there was no significant difference between the three stages in control plants (Figs. 3, 4). Even though camphor content was higher at flowering stage (5.74%) in DTPs, no significant difference between the vegetative and pre-flowering stages was observed. However, lack of water led to accumulation of several compounds such as 3-pentanol 2-methyl, 2-pentanol and methyl anisole in DTPs. The same observations were not recorded in unstressed samples. Moreover, the amount of sabinene (5.64%) and thymol (2.08%) at vegetative, and β -cubebene (32.20%),

Fig. 2 The transcript abundance of *CS* gene and 1,8-cineole content in drought stressed and control Garden sage (*S. officinalis*) and two ecotypes of *S. reuterana* (Yasuj and Urmia). *CS*: 1,8-cineole synthase, V: vegetative stage, PF: pre-flowering stage, F: flowering stage

Table 2 Analysis of variance (ANOVA) for genotype, water regime and stage containing one-way, two-way and three-way ANOVA

Source of variation	df	1,8-Cineole	α -thujone	β -thujone	Camphor	borneol
Genotype	2	477.31**	63.56**	6665.76**	90.71**	0.155**
Water regime	1	0.11 ^{ns}	1.01**	0.02 ^{ns}	0.01 ^{ns}	0.002 ^{ns}
Genotype $\times$ Water regime	2	56.68**	0.52**	1.95 ^{ns}	0.27*	0.009*
Stage	2	10.51**	0.01 ^{ns}	186.17**	4.36**	0.017 ^{ns}
Genotype $\times$ Stage	4	5.25**	1.05**	228.12**	3.53**	0.004**
Water regime $\times$ Stage	2	14.28**	1.69**	16.39**	0.19 ^{ns}	0.000 ^{ns}
Genotype $\times$ Water regime $\times$ Stage	4	7.40**	1.53**	14.66**	0.26*	0.000 ^{ns}
Error		0.49	0.03	0.80	0.08	0.002

ns, * and ** represent non-significant and significant at the 0.05 and 0.01 probability levels, respectively

Fig. 3 The transcript abundance of *SS* gene and the corresponding metabolites, α - and β -thujone, content in drought treated and control Garden sage (*S. officinalis*) and two ecotypes of *S. reuterana* (Yasuj

and Urmia). *SS*: sabinene synthase, V: vegetative stage, PF: pre-flowering stage, F: flowering stage

Fig. 4 The transcript abundance of *BS* gene and the corresponding metabolites, camphor and borneol, content in drought stressed and control Garden sage (*S. officinalis*) and two ecotypes of *S. reuterana*

(Yasuj and Urmia). *BS*: bornyl diphosphate synthase, V: vegetative stage, PF: pre-flowering stage, F: flowering stage

bicyclo germanacrene (32%), δ/β -Cadinene (6.39%) at flowering stage were highest in normal samples and DTPs, respectively (Supplementary Table 1). The application of drought stress also changed the profile of volatile compounds at flowering time. Comparison between well-irrigated plants and stress treated plants showed 52 and 66 compounds, respectively, indicating the considerable effects determined by water shortage in this stage. β -cubebene, 3-Octanol, epi-bicyclo sesquiphellandrene, calarene, Germacrene D are some examples having functional roles in response to stresses that were detected in drought treated plants only (Supplementary Table 1).

Similarly, lack of water caused a higher metabolite production than control samples in Urmia. β -Elemene, β -cubebene, cedren-9-Ene, alloaromadendrene, selina 3,7 (II) diene, cis α -bisabolene, and β -selinene were some of the identified metabolites produced at DTPs in the vegetative stage of this ecotype (Supplementary Table 1). The results also indicated that the production of 1,8-cineole rose considerably in both DTPs and controls when plant growth switched from vegetative to the flowering stage. Besides, there are slight differences in production of camphor, α and β -thujones, borneol between control and DTP samples (Figs. 3, 4).

Gene expression analysis

The *CS* gene converting geranyl-diphosphate to 1,8-cineole, showed a different expression patterns in each genotype. In garden sage, water shortage strongly changed the expression of *CS* (Fig. 2). The expression rate of this gene at the flowering stage was higher than at the vegetative stage. On the other hand, the expression of *CS* in well-irrigated samples significantly declined as compared to drought stress samples in Yasuj ecotype of *S. reuterana*. At vegetative stage of control samples, the transcript abundance of this gene was considerably higher than at flowering stage; whereas, a reverse pattern is observed in drought treated samples. Similarly, in Urmia ecotype, the mRNA level of *CS* in control samples was significantly higher than DTPs. In contrast to drought treated samples, expression of this gene in control samples increased as the plant passed from vegetative to flowering stage (Fig. 2). These results illustrate the similar expression of this gene in two ecotypes of *S. reuterana*. However, the mRNA level of this gene in all ecotypes exposed to drought stress is higher than in unstressed plants.

The *SS* gene is involved in the second branch point leading to conversion of geranyl-diphosphate to sabinene. The transcript abundance of this gene in sage cultivar showed no significant difference between control samples and drought treated samples (Fig. 3). Nonetheless, expression rate of *SS* at vegetative growth stage was higher

than at flowering time. In Yasuj ecotype, however, expression of the *SS* was lower at vegetative stage, then considerably increased in the second growth stage (pre-flowering), and eventually, declined again at flowering stage. The expression of this gene was lower at vegetative stage than at flowering stage in both experimental conditions. Subjecting the Urmia ecotype to stress resulted in higher transcription of *SS* gene at flowering stage (Fig. 3). Accordingly, the results indicate a different response of *SS* gene in all genotypes, although generally the expression of *SS* down-regulated in drought treated plants compared to unstressed plants, probably because of the metabolic shut down caused by the extreme water shortage. The corresponding enzyme, to catalyze geranyl-diphosphate to bornyl diphosphate, is *BS*. Regarding the qRT-PCR results, the relative expression of this gene in DTPs was higher than in control samples, although this change was not remarkable (Fig. 4).

Discussion

Salvia species has been one of the broad-appealing medicinal plants since long time. The family of this genus is widely distributed all around the world (Ghorbani and Esmailizadeh 2017). It is widely accepted that variations in plant environment can lead to changes in its metabolism. Therefore, this study was aimed to investigate the potential impact of drought stress on sage metabolite biosynthesis. To this end, we measured the metabolite content of three genotypes exploring possible changes in phytochemical patterns, when subjected to drought stress. Furthermore, the expression analysis of three most abundant and important monoterpene synthase genes, that is cineole synthase (*CS*), leading directly to 1,8-cineole formation, bornyl diphosphate synthase (*BS*), generating the precursor of camphor, and sabinene synthase (*SS*) responsible for the first step in the production of α - and β -thujone, was investigated (Selmar and Kleinwächter 2013b; Wise et al. 1998).

Generally, the concentration and composition of volatile compounds in *Salvia* genotypes mainly depend on genetic, seasonal, climatic, and environmental factors (Nowak et al. 2010). For instance, it has been suggested that younger leaves contain more essential oils and volatile compounds compared to aged leaves (Gershenzon et al. 2000). Additionally, experiencing stress conditions results in changes in the metabolic process. In medicinal plants, several studies have indicated the potential influence of water shortage on the accumulation of secondary metabolites, some of which have pharmacological or industrial values (Fatima et al. 1999; Khalid 2006; Misra and Srivastava 2000; Simon et al. 1992). Nonetheless, it is worthwhile to

note that, whenever a plant is exposed to drought stress, its growth rate or final biomass production decreases dramatically compared to that of cultivating in well-irrigated land. As a result, higher concentrations of several compounds may reflect the reduction of biomass (Paulsen and Selmar 2016). In sage, moderate and severe drought boosted the production of essential oils and characterizing compounds like camphor and cineole although it determined lower α - β -thujone accumulation (Bettaieb et al. 2009; Hendawy and Khalid 2005). Phytochemical analysis of volatile compounds in three genotypes of sage demonstrated different responses to the applied stress. However, as we anticipated, two ecotypes belonging to *S. reuterana* (i.e. Yasuj and Urmia) mainly showed a similar pattern, while differed from the garden sage (*S. officinalis*). In garden sage, the amount of 1,8-cineole, camphor, and borneol were higher than control samples. The two and three way interaction effect was also significant in 1,8-cineole, α thujone and β -thujone metabolites with maximum concentration of metabolites were observed in interaction of garden sage $\times$ drought during all three stages. In contrast, in water-stressed samples, the amount of α - β -thujone decreased gradually. It is also interesting to mention that the concentration of α -thujone in common sage is one of the characterizing factors in sage essence quality; lower the amount of α -thujone, better will be the quality of the extract (Corell et al. 2007). As a consequence, subjecting the plants to water shortage can lead to better essence quality, which is desirable from a commercial and pharmacological perspective. Previously, Nowak et al. (2010) reported that the application of drought stress determined a higher production of monoterpenes in common sage, supporting our results. So far, two hypotheses accounting for this fact have been offered: (i) increased production due to the strong enhancement in over-reduction via promoting reactions which consume $\text{NADPH} + \text{H}^+$, as elaborated by Selmar and Kleinwächter (2013b); (ii) increased activity of enzymes corresponding in these reactions (Selmar and Kleinwächter 2013a). Analysis of the expression rate of three genes involved in these reactions may clarify the mechanisms underlying the aforementioned higher production of monoterpenes.

As mentioned in the introduction, three genes, *CS*, *BS*, and *SS* are coding key enzymes involved in the biosynthesis of three major monoterpenes (Croteau and Karp 1977; Croteau and Karp 1979; Nowak et al. 2010). Imposition of drought stress up-regulated the expression of 1,8-cineole synthase and bornyl diphosphate synthase genes at three growth stages. As far as metabolomics data are concerned, subjecting plants to water shortage resulted in higher production of cineole and camphor, accounting for the potential role of the corresponding genes. Accordingly, the results indicate that drought stress enhances the

expression of *CS* and *BS*; consequently, higher expression of these genes increases the catalysis of reactions leading to production of cineole and camphor. On the other hand, the mRNA level of the *SS* gene in drought treated plants significantly decreased, probably due to the metabolic downregulation caused by the severe water shortage. Application of exogenous stresses/elicitors, either biotic or abiotic, has uncovered interesting regulatory mechanisms inside the plants. Schmiderer et al. (2010) reported that the treatment of *S. officinalis* with gibberellic acid (GA) led to higher expression of monoterpene synthases, as well as increasing 1,8-cineole and camphor content; conversely blocking the biosynthesis of GA decreased the transcription of these genes, resulting in higher accumulation of α and β -thujone. The results also showed that water shortage in *S. officinalis* had a positive impact on the accumulation of monoterpenes; whereas, negative influence on the formation of α and β -thujone. Considering all evidence, we can firmly conclude that apart from the ecological functions of monoterpenes, these compounds are also related to the dissipation of strong oversupply of energy produced in the plant, particularly in leaves, under water stress. Moreover, the expression of the various monoterpene synthases is regulated differentially in the plant. It should also be mentioned that all three enzymes originate from a common substrate; hence, competing with each other. As a result, drought stress not only decreases the activity of *SS* gene but also provides further substrates for the two other enzymes, thereby lowering the competition among these enzymes and *SS*. This phenomenon can be due to the presence of regulatory elements within the promoter region on the genes (Abedini et al. 2018), which requires further investigations to be more clearly demonstrated.

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