# Drought stress adaptation modulates plant secondary metabolite production in *Salvia dolomitica* Codd.

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**Abstract**

Sages are important medicinal and aromatic plants. While *Salvia officinalis* and *S. miltiorrhiza* have been widely studied, poor information are available on *S. dolomitica* which is recently addressing attention for its antiplasmodial and anti-inflammatory properties. The current study investigated the performances and the metabolic profile of this minor species in response to drought stress. Plants were exposed to three treatments: control, moderate drought stress, or severe drought stress. Changes in growth and ecophysiological traits, in active and volatile compounds, and essential oils production were determined. As the terpenoids are the most representative class of secondary metabolites, the gene expression of key enzymes of terpenoid biosynthesis has been also investigated. Moderate drought induced a drop of leaf water potential, growth, and stomatal conductance, while an increase of deyhdrin expression level. However, serious stress symptoms such as decrease of net photosynthesis and transpiration rate and increase of endogenous abscisic acid were observed only in severe drought stressed plants. Both drought stress conditions lead to modulate the expression of some genes involved in BVOCs and EOs biosynthesis and the metabolic profile. In particular, drought induced an increase of sesquiterpenes, a class of terpenoids with high importance in food, cosmetic and pharmaceutical industry. Thus, we can speculate that moderate drought stress, in addition to allowing water savings during cultivation, can lead to an improvement in the production of secondary metabolites in *S. dolomitica*.

**Keywords:** ABA; farnesyl diphosphate synthase; sage; sesquiterpenes; volatilome; water deprivation; medicinal and aromatic plants; metabolome

## 1. Introduction

Drought, in combination with high levels of irradiance and increasing temperature, is considered the most severe abiotic stress that inhibits plant survival (Morales et al., 2013; Nogués et al., 2015). Water shortage induced a series of changes at morphological, physiological, biochemical and molecular level that adversely affect plant growth, health, and productivity (Mitchell et al., 2013; Caser et al., 2016, 2017 and 2018).

The quality of medicinal and aromatic plants (MAPs) relies on the composition and concentration of plant secondary metabolites (PSMs), which are definitely influenced by environmental conditions such as drought (Kleinwächter and Selmar, 2015; Mandoulakani et al., 2017). This effect occurs to all major classes of PSMs and depends on plant species and cultivation practices (Kleinwächter et al., 2015).

Among PSMs, terpenoids represent the most diverse and largest class of compounds produced by plants (Tholl, 2015). Mono- and sesquiterpenes are the main constituents of biogenic volatile organic compounds (BVOCs) and essential oils (EOs), providing typical aroma and biological properties (Raut and Karuppayil, 2014; Loreto et al., 2014; Nogués et al., 2015; Caser et al., 2016; Moradi et al., 2017; Radwan et al., 2017).

Recently, a model was described to explain how drought affects PSMs production (Selmar and Kleinwächter, 2013). Authors reported that, during water-shortage conditions, the stomata are closed to minimize transpiration and impairs influx of carbon dioxide ($CO_2$) into the leaves. Consequently, the lower content of $CO_2$ molecules is fixed via the Calvin Cycle and the fewer reduced reduction equivalents (e.g. NADPH + $H^+$) are consumed and re-oxidized. Thus, large amounts of NADPH + $H^+$ accumulate, generating an over-reduced state. In this condition, plants promote all reactions to consume NADPH + $H^+$, including terpenoids and phenols biosynthesis. Generally, PSMs changes induced by drought improve the product quality of many MAPs (Mandoulakani et al., 2017; Caser et al., 2018; Kleinwächter and Selmar, 2014). However, this model has not been effectively addressed so far. Recently, in *Salvia officinalis*, Radwan et al.

(2017) verified that the increase of monoterpenes biosynthesis was due not only to a passive shift caused by the stress related over-reduced status, but also to an active biosynthesis of plant growth regulators, changes of biochemical pathway and up-regulation of the main genes involved in terpenoids. In *S. miltiorrhiza*, Ma et al. (2012) isolated and studied the expression of several genes, coding different enzymes involved in both the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway and the mevalonate (MVA) pathway leading to terpenoid biosynthesis. These enzymes, starting from the universal isoprene precursor isopentenyl diphosphate and its isomer lead to the formation of diverse terpenoids such as mono (C10)- and sesqui (C15)-terpenoids, carotenoids and chlorophylls, and bicyclic diterpenoids activated during biotic and abiotic stress responses (Prisic et al., 2004; Wenping et al., 2011).

This study aimed to contribute to unravel the mechanisms underlying the answers to drought stress in *Salvia dolomitica* Codd, addressing particular attention to the impact on PSMs.

## 2. Materials and methods

### *2.1. Plant material and experimental conditions*

A total of 120 clonally propagated plants of *Salvia dolomitica* Codd. were transplanted in plastic pots (9 cm in diameter) containing peat (Silver Torf, Agrochimica, Bolzano, Italy) and Agriperlite® (70:30). A slow-release fertilizer (Osmocote 15:11:13; Scotts Europe, The Netherland) was used. Cultivation lasted for a total of 34 days and was performed in a climate chamber with semi-controlled growth conditions (25 °C, 60% air humidity, 300 μmol $m^{-2}$ $s^{-1}$ and 16/8 h photoperiod), located at the University of Torino (Italy, 45°06’23.21’’N Lat, 7°57’82.83’’E Long). A complete randomized design with three levels of irrigation was applied. The levels of irrigation were: 100% container capacity (CC) as well-watered (WW, control), 50% CC as moderate drought stress (MDS) or 0% CC as severe drought stress (SDS) treatments. For each irrigation regime, a total of 40 plants (four replications with 10 plants each) were treated. All the water amounts were kept constant throughout the experiment by gravimetric determinations as reported by Caser et al.

(2016). Morphological, physiological and biochemical parameters were monitored after 0, 4, 7, 11, 14, 18, 21, 25, 28, 32 and 34 days of cultivation to describe the plant responses to drought during time.

*2.2. Morphological parameters*

Plant growth (Growth Index, G.I.) was monitored by estimating the occupied volume of each plant through the measurement of the height, the broadest diameter and the perpendicular diameter (Demasi et al., 2017). At the end of the experiment (day 34), roots and aerial parts of ten plants per irrigation level were weighted to record fresh biomass. Later, they were oven-dried at 45 °C for one week and dry biomass was measured. Root to aerial (R:A) dry weight ratio was calculated.

*2.3. Photosynthetic pigments*

The relative quantity of chlorophyll was measured on six leaves per plant, randomly selected, for a total of six plants per irrigation level, by using the Chlorophyll Meter SPAD-502 (Konica Minolta Sensing Inc., Osaka, Japan).

Chlorophyll and carotenoids were extracted from 50 mg of fresh fully formed leaves from six plants per irrigation level. After an over-night extraction in 5 ml of methanol at 4 °C in the dark, pigments were spectrophotometrically determined at 665, 652, and 470 nm using a Ultrospec 2100 pro (Amersham Biosciences, UK) as described by Caser et al. (2013). The data were reported in mg $g^{-1}$ leaf fresh weight (FW).

*2.4. Phenols, flavonoids and antioxidant activity*

One-hundred mg of fresh leaves per irrigation level were powdered and homogenized with 1 ml of aqueous solution at 70% (v/v) methanol. After 30 minutes on ice, the extracts were centrifuged at 10,000 rpm for 10 minutes at 25 °C to recover the supernatant for the following determination of phenol and flavonoid content, and the antioxidant activity.

The total phenols were determined colorimetrically using Folin-Ciocalteau's reagents, as described by Singleton and Rossi (1965) and indicated as mg gallic acid equivalent (GAE) $g^{-1}$FW. Total flavonoid content was also determined spectrophotometrically using the colorimetric method of Kim et al. (2003), based on the formation of a complex flavonoid-aluminium and indicated as mg $g^{-1}$FW. The antioxidant activity was determined by using the ferric reducing antioxidant power (FRAP) method with minor modification (Szôllôsi and Szôllôsi Varga, 2002) and indicated as µmol $Fe^{2+}mg^{-1}$. The working solution always freshly prepared contained 7.5 mM acetate buffer, pH 3.6, 0.1 mM tripyridyltriazine (TPTZ), and 0.05 mM $FeCl_3$ 6H2O. At low pH, when the tripyridyltriazine ($Fe^{3+}$-TPTZ) complex is reduced to the ferrous form ($Fe^{2+}$), an intensive blue color of ($Fe^{2+}$-TPTZ) can be monitored spectrophotometrically at 593 nm.

Samples were measured in three replicates. At the end of the experiment (day 34), the total amount of total phenols, flavonoids and antioxidant activity per plant (mg plant $FW^{-1}$) was estimated on the basis of the aerial fresh biomass.

*2.5. Ecophysiological evaluation*

The method of Scholander et al. (1965) was used to estimate the midday leaf water potential (MLWP; MPa) in three mature and fully expanded leaves of six plants per irrigation level with a pressure bomb (Soil Moisture Equipment, Santa Barbara, CA, USA). Moreover, the internal $CO_2$ concentration (*C*i; µmol $mol^{-1}$), the transpiration rate (*E*; mmol $m^{-2}$ $s^{-1}$), the stomatal conductance ($g_s$; mmol $m^{-2}$ $s^{-1}$), and the net photosynthetic rate (*A*; µmol $m^{-2}$ $s^{-1}$) were measured with a portable infrared gas analyser ADC-LCPro+ (The Analytical Development Company Ltd., Hoddesdon, UK). These parameters were monitored in healthy and fully expanded leaves of six plants per irrigation level between 10:00 and 12:00 a.m. when the vapour pressure deficit (VPD) was constantly around 2.4 kPa (± 0.06 std err) with air temperature of 26.6 ± 0.11 °C.

*2.6. Endogenous abscisic acid determination*

153 The concentration of endogenous abscisic acid (ABA) was quantified every week in mature
154 leaves of six plants per irrigation level through an High Performance Liquid Chromatography
155 (HPLC), based on Solid Phase Extraction (SPE) (Bosco et al., 2013; Demasi et al., 2017). Leaves
156 were grounded in liquid nitrogen and 0.5 g of each sample was suspended in 4 ml of the extraction
157 solution (65% pure methanol, 25% ultrapure water, 10% aqueous hydrogen chloride 1 M) for 2 h at
158 4 °C, in the dark. Samples were then filtered and the eluates were added to a SPE cartridge
159 (Supelclean SPE LC-NH2, Supelco Analytical, USA). ABA was eluted with 5% of phosphoric acid
160 ($H_3PO_4$) in methanol. The procedure was carried out under artificial light with amber glassware,
161 preventing degradation. The chromatographic analysis of the eluate was performed with HPLC
162 1200 Series (Agilent Technologies, Böblingen, Germany) and the signal was monitored at 265 nm
163 with a diode array detector. The content (pmol $mg^{-1}$ FW) was calculated based on a calibration
164 curve constructed from the matrix-matched calibration standards.
165
166 *2.7. Analysis of biogenic volatile organic compounds*
167 The BVOCs evaluation was conducted on 3 grams of shoots by using a Supelco Solid Phase
168 Micro Extraction (SPME) (Supelco, Bellefonte, PA, USA) with polydimethylsiloxane (PDMS, 100
169 µm) at day 14. Each sample was introduced into a 100 ml glass conical flask and equilibrated for 30
170 min at 25°C. After the equilibration time, the fiber was exposed to the headspace for 15 min at
171 room temperature; once sampling was finished the fiber was withdrawn into the needle and
172 transferred to the injection port of the Gas Chromatography–Electron Impact Mass Spectrometry
173 (GC-EIMS) system where the fiber was desorbed. GC-EIMS analysis was performed with a Varian
174 CP 3800 gas chromatograph (Varian, Inc., Palo Alto, CA) equipped with a DB-5 capillary column
175 (30 m × 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector
176 chromatograph (Varian, Inc., Palo Alto, CA). Analytical conditions were: injector and transfer line
177 temperature at 250 °C and 240 °C, respectively; oven temperature programmed from 60 °C to
178 240 °C at 3 °C $min^{-1}$; helium as carrier gas set at 1 mL $min^{-1}$; and the injection in splitless mode.

Identification of the constituents was conducted by the comparison of their retention times with those of authentic samples, and on computer matching against commercial (Adams, 1995) and home-made library mass spectra built from pure substances and MS literature data (Davies, 1990).

The relative proportions of the volatile constituents were expressed in percentages obtained by peak-area normalisation, and all relative response factors were taken as one.

*2.8. Essential oil isolation*

Twenty grams of dried leaves were hydrodistilled using a Clevenger-type apparatus (2 h). The yields of distillation were not determined due to the low amount of the starting plant material. The EOs obtained were solubilized in *n*-hexane, dried over anhydrous sodium sulphate and filtered, then stored in a vial at 4 °C in the dark until use. GC-EIMS was used to analyze all the EO obtained (injection of 0.2 μL) as reported by Caser et al. (2016).

*2.9. RNA isolation and real time RT-PCR analysis*

Leaves collected from six plants at the end of the experiment (day 34) were pooled to form three biological replicates (two plants for each biological replicate). Total RNA was extracted using the Spectrum™ Plant Total RNA extraction kit (Sigma Aldrich) starting from 80 mg of material, and RNA quantity were checked using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific). RNA was then treated with DNase I (Invitrogen, Thermo Fisher Scientific) in accordance with the manufacturer's instructions. For each biological replicate, first-strand cDNA was synthesized starting from 500 ng of total RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Thermo Fisher Scientific) according to the manufacturer's instructions. Since the absence of *S. dolomitica* reference genome, gene-specific primers (Table 1) were selected on the base of the closest phylogenetically species, *S. miltiorrhiza* and designed using Primer Express® software (v3.0, Applied Biosystems, Thermo Fisher Scientific). Reactions were carried out using Power SYBR® Green PCR Master Mix (Applied Biosystems, Thermo Fisher

205 Scientific) as reported in Chitarra et al. (2017). Three technical replicates were run for each
206 biological replicate, and the expression of target genes was quantified after normalization to
207 glyceraldehyde-3-phosphate dehydrogenase (*GAPDH)* housekeeping gene. The results were
208 calculated as expression ratios (Relative Quantity, RQ) to control (WW).
209
210 *2.10. Statistical methods*
211 Data were firstly tested for the variance homogeneity. All the measured and the derived data
212 were then subjected to post-hoc test using Ryan-Einot-Gabriel-Welsch-F test (REGW-F) and
213 Tukey's test for gene expression analyses. The critical value for statistical significance was $P <$
214 0.05. All computations were conducted with SPSS statistical package (version 21.0; SPSS Inc.,
215 Chicago, Illinois).
216
217 **3. Results and discussion**
218 *3.1. Plant growth*
219 Moderate drought stress (MDS) and severe drought stress (SDS) reduced growth starting
220 from day 21 onward when compared to well-watered (WW) (G.I. Table 2). MDS and SDS also
221 drastically reduced the total (-74% and -83%, respectively), aerial (-80% and -82%, respectively)
222 and root dry biomass (-60% and -85%, respectively) compared to control(Table 3).
223 Dehydration often reduces the overall plant growth (Caser et al., 2012, 2016, 2017 and
224 2018; Soni and Abdin, 2017), attributable to the considerable reduction of photosynthesis, cell
225 turgidity, cell growth, and to the increasing evapotranspiration (Rahimi et al., 2017). This was seen
226 in many MAPs, such as *Mentha pulegium* (Hassanpour et al., 2014), *M. piperita* (Rahimi et al.,
227 2017), *M. spicata* (Delfine et al., 2005) and *Rosmarinus officinalis* (Delfine et al., 2005). Within the
228 genus *Salvia*, different morphological responses to drought are reported in literature. No differences
229 in biomass production was observed in drought stressed *S. officinalis* plants (Radwan et al., 2017)

while, a reduction was seen in other *Salvia* species as *S. splendens* (Burnett et al., 2005), *S. miltiorrhiza* (Liu et al., 2011) and in a previous study on *S. dolomitica* (Caser et al., 2012).

*S. dolomitica* significantly increased the root to aerial ratio when grown in MDS in comparison to WW (+100%) and SDS treatments (+244%). Roots are the only source to acquire water from soil, therefore the root density and size are key responses of plants to drought stress. Mediterranean plant species typically have higher R:A ratios than plants from more mesic biomes (Valliere and Allen, 2016), possibly as a result of the adaptation to seasonal drought. Besides, Mahajan and Tuteja (2005) reviewed that leaves are generally more sensitive to stresses than roots. This often results in an increase in R:A when water is limited, as also seen in *S. sinaloensis*, *Allium cepa* and *Artemisia californica* (Farooq et al., 2009; Valliere and Allen, 2016; Caser et al., 2018). However, when drought conditions are too severe, there is also a dramatic reduction of roots, as proved in *Helichrysum petiolare* (Caser et al., 2016).

### *3.2. Photosynthetic pigments*

A decrease in chlorophyll and carotenoid content in plants subjected to drought stress is commonly known in several species, including MAPs (Caser et al., 2016). In *S. dolomitica*, only SDS significantly reduced the content of both pigments compared to WW and MDS starting from day 21 (total chlorophylls: 1.46, 1.45 and 1.19 mg $g^{-1}$; carotenoids: 2.93, 2.75 and 1.54 mg $g^{-1}$ in WW, MDS and SDS, respectively) up to their senescence (Table 4), combined to a growth reduction at the same time points, as previously seen in *S. sinaloensis* (Caser et al., 2018). Differently, in *S. officinalis* a strong reduction in chlorophyll content was observed also in plants treated with MDS (-78.5%) (Bettaieb et al., 2011).

According to Flexas and Medrano (2002), green leaf colour in $C_3$ plants can be reduced by increasing drought stress. However, in the present study no significant differences on SPAD values among treatments were noted (Table 4), as previously found in *S. dolomitica* and *S. sinaloensis* by Caser et al. (2012 and 2018).

256

257 *3.3. Phenols, flavonoids and antioxidant activity*

258 Drought induces oxidative stress in plants, in which reactive oxygen species (ROS) are
259 commonly produced (Munné-Bosch and Peñuelas, 2003). Polyphenols and flavonoids are among
260 the most adaptable natural compounds, helping plants to scavenge ROS (di Fernando et al., 2014).
261 An increase of phenolic compounds biosynthesis was seen in drought-stressed plants of *Labisia*
262 *pumila* (Jaafar et al., 2012), *Salvia officinalis* (Radwan et al., 2017) and *S. sinaloensis* (Caser et al.,
263 2018).

264 In the present study, the rate of total phenols, flavonoids and antioxidant activity in treated
265 plants was evaluated during the entire experiment (Table 5). *S. dolomitica* plants subjected to SDS
266 conditions showed a strong decrease in the content of total phenols, flavonoids and antioxidant
267 activity starting from day 4 till day 11. Later, no differences occurred among treatments in total
268 phenols and flavonoids till the end of the experiment. No differences among WW and MDS
269 occurred in phenols and flavonoids content, with the exception for day 32 (29.0 and 12.7 mg GAE
270 $g^{-1}$ of phenols and 8.3 and 5.7 mg $g^{-1}$ of flavonoids in WW and MDS, respectively). Regarding
271 antioxidant activity, at day 25, SDS induced a sharply significant increase compared to other
272 treatments (98.7, 101.5 and 153.1 μmol $Fe^{2+}g^{-1}$ in WW, MDS and SDS, respectively). This time
273 point coincides with the complete senescence. At the end of the experiment (day 25 for SDS and
274 day 34 for WW and MDS), the total amount of total phenols, flavonoids and antioxidant activity per
275 plant was estimated on the basis of the plant fresh biomass (Table 5). Here, results highlighted that
276 all the parameters were significantly reduced by MDS and SDS compared to WW (305.2, 53.2 and
277 20.5 mg $GAEg^{-1}$ of phenols, 105.7, 17.1 and 5.3 mg $g^{-1}$ of flavonoids and 1815.8, 337.5 and 134.7
278 μmol $Fe^{2+}g^{-1}$ of antioxidant activity, respectively).

279 Considering that drought-tolerant species are known to increase the accumulation of
280 antioxidants, which help to protect plant cells from ROS (Moradi et al., 2017), these results point at

281 *S. dolomitica* as a drought-sensitive species. A low amount of these metabolites produced under
282 drought stress conditions was also seen in *S. miltiorrhiza* (Liu et al., 2011).
283
284 *3.4. Ecophysiological traits*
285 The decline in the levels of pigments (chlorophyll and carotenoids) under drought stress
286 conditions, in parallel to the increased level of stress, is in tune with depressed physiological needs
287 of photosynthetic activity in favour of lower water loss and assuming lower growth. As expected, in
288 *S. dolomitica* water shortage affected midday leaf water potential (MLWP), internal $CO_2$
289 concentration (*C*i), transpiration rate (*E*), stomatal conductance ($g_s$) and net photosynthetic rate (*A*)
290 (Figure 1).
291 The MLWP in the WW plants kept constant during the entire experiment (-0.34 MPa) (Fig.
292 1A). In MDS plants, MLWP was significantly lower at the days 18, 28, 32 and 34 (-0.40, -0.52, -
293 0.50 and -0.46 MPa, respectively) compared to controls. Severe drought stress significantly
294 constantly reduced MLWP from day 7 (-0.53 MPa) until day 25 (-1.00 MPa), when the complete
295 leaf withering occurred. Within the genus *Salvia*, similar results were found also in *S. splendens*
296 'Bonfire' and *S. sinaloensis* whose leaves reached a LWP of -1.40 and -1.10 MPa under similar
297 severe drought conditions, respectively (Eakes et al., 1991; Caser et al., 2018). Even more, *S.*
298 *officinalis* and *S. mellifera* plants under the same stress conditions showed much lower LWP (-4.8
299 and -8.0 MPa, respectively) (Hargrave et al., 1994; Bettaieb et al., 2011).
300 No differences in *C*i were observed between WW and MDS plants, ranging between 255.0
301 and 483.4 μmol $mol^{-1}$ during the experiment (Fig. 1B), while a significant increase in SDS plants
302 was observed starting from day 14 (423.0 μmol $mol^{-1}$) to day 25 (493.0 μmol $mol^{-1}$). Similarly, also
303 in *E* no differences between WW and MDS plants were highlighted (Fig. 1C), whereas, a significant
304 decrease occurred in SDS plants in the days 4, 7, 11, 14 and 25, compared to the other treatments.
305 Regarding stomatal conductance ($g_s$) (Fig. 1D), differences between WW and MDS plants occurred
306 at days 7, 21, 25, 28 and 34. SDS plants showed a significant and constant decrease starting from

307 day 7 (0.10 mmol $m^{-2}$ $s^{-1}$) until the complete senescence (0.01 mmol $m^{-2}$ $s^{-1}$). Net photosynthetic
308 rate (*A*) (Fig. 1E) followed a similar trend in SDS treatment starting from day 14 (1.78 μmol $mol^{-2}$ $s^{-1}$).
309 Differences between WW and MDS appeared only at day 25 (15.32 and 5.16 μmol $mol^{-2}$ $s^{-1}$,
310 respectively). The decrease of photosynthetic activity under drought stress may be due to stomatal
311 or non-stomatal mechanisms (Caser et al., 2018). In drought tolerant species, the reduction of
312 photosynthesis is due to stomatal closure and limitation of water losses. In drought sensitive plants,
313 the reduction of net photosynthesis is mainly due to water shortage, inducing severe damages in
314 plants. Here, SDS strongly reduced the assimilation processes, with a significant decrease of $g_s$ and
315 saving internal $CO_2$, suggesting an efficient adaptive stomatal modulation.
316
317 *3.5. Endogenous ABA content*
318 Abscisic acid (ABA) is known to be synthesized under different stress conditions (Demasi et
319 al., 2017) either at the root or leaf level. ABA has an inhibitory effect on cell growth and it leads to
320 the depolarization of guard cell membranes triggering osmotic ion efflux and the loss of guard cell
321 turgor (McAdam and Brodribb, 2016).
322 Endogenous ABA content in the leaves of *S. dolomitica* under WW and MDS plants did
323 not differ during the entire experiment with a mean value equal to 0.16 pmol $mg^{-1}$ (Fig. 1F). On the
324 contrary, SDS induced a strong significant increase in the hormone concentration already at day 7
325 (~14 fold more than WW and MDS) until the complete plant senescence (~39 fold more than WW
326 and MDS). Endogenous ABA plays an important role in drought adaptation and, in *S. dolomitica*
327 rapidly increased under severe water shortage conditions, enhancing drought tolerance as was
328 observed in leaves of *Cichorium intybus* treated with similar severe drought conditions
329 (Ghanaatiyan and Sadeghi, 2017).
330 Within the genus *Salvia* only few studies reported the content of ABA in response to not
331 optimal growing conditions. Kondrat'eva et al. (2008) found an increase of ABA in *S. sclarea* under

cold stress (ranged between 5.1 and 7.1 pmol $mg^{-1}$). While, Asensi-Fabado et al. (2013) in *S. officinalis* under heat stress (ranged between 3.0 and 6.0 pmol $mg^{-1}$).

*3.6. Biogenic volatile organic compounds production*

Intensity and profile of BVOCs emitted by plants is dependent on the genetic variability and plasticity of phenotypes (Dicke and Loreto, 2010). Their emission can vary drastically depending on the species, organ, developmental stage and environmental conditions (Holopainen amd Gershenzon, 2010). Several authors highlighted that any stress condition could potentially alter the rate and composition of BVOCs (Niinemets et al., 2013). As reported by Loreto et al. (2014) under stress conditions, the investment of carbon into foliar BVOC increases, resulting in considerably larger quantities being released into the atmosphere. In fact, abiotic and biotic stresses can enhance their emission to communicate with other organisms (Loreto and Schnitzler, 2010).

The total emitted and identified BVOCs from the analyzed shoots of *S. dolomitica* are reported in Table 6. Overall, a number of 36, 33 and 37 compounds were recognized in WW, MDS and SDS plants, accounting for 94.43%, 81.38% and 98.18% of the total compositions, respectively. Figure 2A shows how the main volatile fractions changed in *S. dolomitica* plants subjected to different drought treatments. Well watered plants were characterized mainly by monoterpene hydrocarbons (mh); this volatile fraction strongly decreased due to increasing stress conditions (57.71%, 30.97% and 29.41% in WW, MDS and SDS, respectively). Conversely, under MDS and SDS conditions, an increase in sesquiterpene hydrocarbons (sh) was highlighted (34.09%, 47.19% and 66.32% in WW, MDS and SDS, respectively). Drought conditions softly affected also the production of the other reported volatile molecule class, the oxygenated monoterpene (om) (1.72%, 2.92% and 2.19% in WW, MDS and SDS, respectively).

Several recent reviews addressed the roles of BVOCs in enhancing the tolerance of plants to various general abiotic stressors (Possel and Loreto, 2013). However, the literature concerning BVOC emission in relation to water availability is ambiguous. *S. dolomitica* as other Labiatae

358 species accumulate terpenes in specialized structures (i.e. glandular hairs) (Bassolino et al., 2015)
359 and their terpene emission is considered to be the consequence of terpene volatilization from these
360 structures, which is generally temperature dependent (Llusia and Peñuelas, 2000). In the present
361 study, plants under severe stress conditions showed an increase in the total amount of analysed
362 components and a strong decrease of hydrogenated monoterpenens in concomitance with a sharp
363 increase of hydrogenated sesquiterpenes. Llusià and Peñuelas (1998) reported that a reduction of
364 monoterpene emission under severe drought conditions could be expected due to stomata closure.
365 However, sesquiterpenes are not generally emitted in large amounts constitutively (Possel and
366 Loreto, 2013) but they can be enhanced by biotic and abiotic stresses as indirect defence
367 mechanism.

368 All the investigated headspaces showed different array of the main constituents. The
369 chemical profile in WW plants was characterized by Limonene > Δ-3-carene > Germacrene D > β-
370 Caryophyllene > (E)-β-ocimene, in MDS plants, by Germacrene D > Limonene > β-Caryophyllene
371 > α-guaiene > Δ-3-carene, and in SDS plants by Germacrene D > Limonene > Bicyclogermacrene >
372 β-Caryophyllene > α-guaiene. Among the cited constituents, a very sharp increase (~+260%) was
373 observed for the sesquiterpene hydrocarbons Germacrene D (from 8.57% to 22.35% and 22.16% in
374 WW and MDS and SDS, respectively). On the opposite, the monoterpene hydrocarbons (E)-β-
375 ocimene reduced of ~60% when plants were subjected to MDS and SDS. Arey et al. (1995)
376 suggested that the sesquiterpenes emission in *S. mellifera*, mainly composed by β-caryophyllene
377 and Germacrene D, is not dependent to seasons but that any disturbances to the plants may exert
378 influence on the total observed emission variability. Very few studies reported the impact of
379 drought on volatile sesquiterpenes emissions in MAPs with misaligned results. Ormeño et al. (2007)
380 observed a reduction in sesquiterpenes (allo-aromadendrene, α-zingiberene and α-cadinene) in
381 drought stressed *Rosmarinus officinalis* plants, while an increase of Germacrene D was evidenced
382 in *Thymus vulgaris* and *T. serpyllum* (Moradi et al., 2017).
383

384 *3.7. Essential oils*

385 Essential oil synthesis in plants is influenced by several factors such as light, seasonal
386 variation, climate change, plant growth regulators, and environmental stresses such as drought
387 (Mandoulakani et al., 2017).

388 A total of 82 constituents were detected in the investigated EOs, of which 42, 46 and 52 in
389 WW, MDS and SDS plants, respectively (Table 7). Drought stress conditions induced only a slight
390 decrease in the total amount of the identified constituents (97.3, 95.5 and 95.9% at WW, MDS and
391 SDS plants, respectively), but affected the main chemical classes, particularly the sesquiterpenes
392 (Fig. 2B). Only the oxygenated monoterpens were reduced under stress conditions (7.5%, 1.1% and
393 1.5%, in WW, MDS and SDS, respectively), while MDS and SDS reduced the total amount of the
394 sesquiterpene hydrocarbons (53.4%, 32.9% and 33.2% in WW, MDS and SDS, respectively) and
395 increased the oxygenated sesquiterpenes (26.0%, 53.2% and 53.1% in WW, MDS and SDS,
396 respectively).

397 The main constituent in WW EOs was the sesquiterpene hydrocarbon β-Caryophyllene. This
398 constituent strongly decreased with drought (21.2%, 0.6% and 0.6% in WW, MDS and SDS,
399 respectively). In stressed plants the main constituent was instead the oxygenated sesquiterpene
400 Longipinalol, which deeply increased under drought conditions (0.8%, 41.9% and 41.5% in WW,
401 MDS and SD, respectively). Specifically, the chemical profile of WW plants was composed by β-
402 Caryophyllene > δ-cadinene > 1H-cyclopropanaphtalene > α-eudesmol > epi-α-cadinol, while for
403 MDS and SDS by Longipinalol > Trans-β-guaiene > β-pinene > α-humulene > δ-cadinene.

404 Within the genus *Salvia*, drought stress resulted to slightly increase the total amount of EO
405 constituents in *S. officinalis* (i.e. camphor, α-thujone and 1.8-cineole) (Bettaieb et al., 2009) and *S.*
406 *sinaloensis* (i.e. camphor) (Caser et al., 2018). *S. dolomitica* EOs were previously evaluated by
407 Kamatou et al. (2007) in South Africa wild plants and by Bassolino et al. (2015) in potted cultivated
408 plants. Quite surprisingly, these profiles differ greatly. Wild plants contained mainly oxygenated
409 monoterpenes (71.8%), while cultivated plants mainly hydrocarbons (71.5%) and oxygenated

410 sesquiterpenes (13.6%), with β-caryophyllene as main constituent. In our study, WW plants
411 presented a profile similar to what found by Bassolino et al. (2015). These variations in the EO
412 compositions might have arisen from several factors (climatical, seasonal, geographical, geological,
413 extraction method), as mentioned Gonzáles-Coloma et al. (2011) in other Labiatae species.

414 Sesquiterpenes represent an extremely diverse, heterogeneous and large group of natural
415 compounds. Since these compounds play essential roles in plant defense response, their
416 accumulation under abiotic stress is consistent with the carbon balance theory, which states that the
417 investment in plant defense increases in response to a growth limitation. As an example, high
418 amount of sesquiterpenes were observed in *Inula montana* plants subjected to different abiotic
419 stresses (i.e. altitude, drought and soil composition) (Roux et al., 2017). In this work, plants
420 subjected to drought showed a reduction of hydrocarbons sesquiterpenes and an increase in
421 oxygenated sesquiterpenes (Fig. 3). These dynamics were already supposed to be a defence
422 mechanism of the plants against hostile environment such as intense light conditions or water
423 shortage.

424

425 *3.8. Genes involved in terpenoid biosynthesis*

426 Plants are compatible to biotic and abiotic stresses by modulating the expression of genes
427 which are responsible in both primary and secondary metabolism (Dolzhenko et al., 2010).
428 Deyhdrin is one of the most important genes expressed in plants during water deficit condition and
429 its ABA binding-site is often used as target to attest the drought stress (George et al., 2017). These
430 proteins of LEA family help to maintain large amounts of water inside the plant cell during water
431 stress, thereby protecting the plants proteins and biomembranes (Battaglia et al., 2008).

432 Here, the expression profile of the dehydrin gene (*DH*) increased concurrently with the
433 strength of the water stress (c.a. 1.5 and 3 fold in MDS and SDS compared to WW, respectively)
434 (Fig. 3 in the box), confirming how plants perceived the drought stress and activated molecular
435 responses. A similar trend was seen for the following genes that code for enzymes involved in the

terpenoid biosynthesis: geranyl diphosphate synthases (GPPS), farnesyl diphosphate synthase (FPPS), geranylgeranyl diphosphate synthase (GGPPS) and copalyl diphosphate synthases (CPS). As reported in *S. miltiorrhiza* (Wenping et al., 2011), GPPS catalyses the condensation of two units of isopentenyl pyrophosphate (IPP) and one unit of dimethyl allyl pyrophospahate (DMAPP) to form geranyl diphosphate (GPP), precursor of almost all the monoterpens, while FPPS catalysed the formation of farnesyl diphosphate (FPP), precursor of almost all sesquiterpenes. Lastly, GGPPS catalysed the formation of geranyl geranyl diphosphate (GGPP), precursor of triterpenes (C20), carotenoids and chlorophylls, and CPS catalyses the cyclization reaction that converts GGPP to form copalyl diphosphate (CPP).

In this study, all the genes were up regulated in stressed plants. This was particularly evident for FPPS and CPS2 genes (c.a. 18 and 8 fold, respectively) (Fig. 3C and F). Comparing the two drought stresses, except for GPPS2D and CPS3 (Fig. 3B and G), the highest levels of expression were found in MDS plants. This highlight that the MDS treatment induced with greater efficiency the transcriptional upregulation of different enzymes involved in the terpenoid biosynthesis and consequently in BVOCs and EOs production.

Within the Labiatae family, CPS genes were isolated in *Salvia fruticosa*, *S. miltiorrhiza* and *R. officinalis* (Božićet al., 2015). Wenping et al. (2011) and Ma et al. (2012) highlighted that these genes have diverse expression patterns that are tightly controlled at different developmental stages (seed germination, seedling growth, vegetative stage and reproductive stage). As confirmed by our study, they play important roles also in interactions with environmental factors by inducing the biosynthesis of PSMs, as well as the other studied genes.

## 4. Conclusion

In summary, this study represents an integrated approach, combining metabolomics and physiological studies that allowed us to get new insights on the impact of drought in mechanisms and processes involved in MAPs, and particularly in *S. dolomitica* adaptation.

Moderate drought stressed plants perceived the water stress, as testified by the drop of leaf water potential, the reduced growth index, the constant decrease of stomatal conductance and the increased deyhdrin expression level. However, only severe drought stressed plants showed serious stress symptoms such as significant decrease of net photosynthesis, increase of endogenous ABA, and decrease of transpiration rate. Thus, these traits revealed that the optimum irrigation conditions for the growth and health of *S. dolomitica* plants was of circa 50% of control irrigation, since did not affect its quality, suggesting the possibility to ameliorate the water-management practices in MAPs sector.

Plant secondary metabolites are a fascinating class of phytochemicals exhibiting immense chemical diversity. MAPs are commonly known to produce a wide range of these molecules with different industrial purposes. Here, both drought stress conditions (mainly MDS) lead to modulate the expression of some genes involved in BVOCs and EOs biosynthesis, mainly sesquiterpenes, a class of terpenoids with high importance in food, cosmetic and pharmaceutical industry. Since previous studies indicated that the EOs of *S. dolomitica* exhibited antiplasmodial and anti-inflammatory activities (Fisher et al., 2005; Kamatou et al., 2007a,b, 2008 and 2010), we can speculate that moderate drought stress can be beneficial for the PSMs production in *S. dolomitica*.

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487 **References**

488 Adams, R.P., 1995. Identification of essential oil components by gas chromatography-mass
489 spectroscopy. Carol Stream, IL: Allured.

490 Arey, J., Crowley, D. E., Crowley, M., Resketo, M., Lester, J., 1995. Hydrocarbon emissions from
491 natural vegetation in California's south coast air basin. Atmosph. Environ. 29, 2977-2988.

492 Asensi-Fabado, M. A., Olivan, A., Munné-Bosch, S., 2013. A comparative study of the hormonal
493 response to high temperatures and stress reiteration in three Labiatae species. Environ. Exp.
494 Bot. 94, 57-65.

495 Bassolino, L., Giacomelli, E., Giovanelli, S., Pistelli, L., Cassetti, A., Damonte, G., Bisio, A.,
496 Ruffoni, B., 2015. Tissue culture and aromatic profile in *Salvia dolomitica* Codd. Plant Cell
497 Tiss Organ 121, 83-95.

498 Battaglia, M., Olvera-Carrillo, Y., Garciarrubio, A., Campos, F., Covarrubias, A.A., 2008. The
499 enigmatic LEA proteins and other hydrophilins. Plant Physiol. 148, 6-24.

500 Bettaieb, I., Zakhama, N., Aidi Wannes, W., Kchouk, M.E., Marzouk, B., 2009. Water deficit
501 effects on *Salvia officinalis* fatty acids and essential oils composition. Sci. Hortic. 120, 271–
502 275.

503 Bettaieb, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F., Marzouk, B., 2011. Drought effects on
504 polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. Acta
505 Physiol. Plant. 475, 1103-1111.

506 Bosco, R., Caser, M., Vanara, F., Scariot, V., 2013. Development of a rapid LC-DAD/FLD method
507 for the simultaneous determination of auxins and abscisic acid in plant extracts. J. Agric.
508 Food Chem. 61, 10940–10947.

509 Božić, D., Papaefthimiou, D., Brückner, K., de Vos, R.C.H., Tsoleridis, C.A., Katsarou, D.,
510 Papanikolaou, A., Pateraki, I., Chatzopoulou, F.M., Dimitriadou, E., Kostas, S., Manzano, D.,
511 Scheler, U., Ferrer, A., Tissier, A., Makris, A.M., Kampranis, S.C., Kanellis, A.K., 2015.
512 Towards elucidating carnosic avid biosynthesis in Lamiaceae: functional characterization of

513 the three first steps of the pathway in *Salvia fruticosa* and *Rosmarinus officinalis*. PLoS ONE
514 10: doi:10.1371/journal.pone.0124106.
515 Burnett, S.E., Pennisi, S.V., Thomas, P.A., van Iersel, M.W., 2005. Controlled drought affects
516 morphology and anatomy of *Salvia splendens*. J. Amer. Soc. Hortic. Sci. 130, 775-781.
517 Caser, M., Ruffoni, B., Scariot, V., 2012. Screening for drought tolerance in *Salvia* spp. and
518 *Helichrysum petiolare*: a way to select low maintenance ornamental plants. Acta Hortic. 953,
519 239-246.
520 Caser, M., Scariot, V., Gaino, W., Larcher, F., Devecchi, M., 2013. The effects of sodium chloride
521 on the aesthetic value of *Buxus* spp. Eur. J. Hortic. Sci. 78, 153-159.
522 Caser, M., D'Angiolillo, F., Chitarra, W., Lovisolo, C., Ruffoni, B., Pistelli, Lu., Pistelli, La.,
523 Scariot, V., 2016. Water deficit trigger changes in valuable physiological and phytochemical
524 parameters in *Helichrysum petiolare* Hilliard & B.L. Burtt. Ind. Crops Prod. 83, 680-692.
525 Caser, M., Lovisolo, C., Scariot, V., 2017. The influence of water stress on growth, ecophysiology
526 and ornamental quality of potted *Primula vulgaris* 'Heidy' plants. New insights to increase
527 water use efficiency in plant production. Plant Growth Regul. 83, 361-373.
528 Caser, M., D'Angiolillo, F., Chitarra, W., Lovisolo, C., Ruffoni, B., Pistelli, Lu., Pistelli, La.,
529 Scariot, V., 2018. Ecophysiological and phytochemical responses of *Salvia sinaloensis* Fern.
530 to drought stress. Plant Growth Regul. 84, 383-394.
531 Chitarra, W., Perrone, I., Avanzato, C. G., Minio, A., Boccacci, P., Santini, D., Gilardi, G.,
532 Siciliano, I., Gullino, M.L., Delledonne, M., Mannini, F., Gambino, G., 2017. Grapevine
533 grafting: Scion transcript profiling and defense-related metabolites induced by rootstocks.
534 Front. Plant Sci. 8, 654 doi:10.3389/fpls.2017.00654.
535 Davies, N.W., 1990. Gas chromatographic retention indexes of monoterpenes and sesquiterpenes on
536 methyl silicone and carbowx20M phases. J. Chrom. 503, 1–24.

537 Delfine, S., Loreto, F., Pinelli, P. Tognetti, R., Alvino, A., 2005. Isoprenoids content and
538 photosynthetic limitations in rosemary and spearmint plants under water stress. Agric.
539 Ecosyst. Env. 106, 243-252.
540 Demasi, S., Caser, M., Handa, T., Kobayashi, N., De Pascale, S., Scariot, V., 2017. Adaptation to
541 iron deficiency and high pH in evergreen azaleas (*Rhododendron* spp.): potential resources for
542 breeding. Euphytica 213, 148.
543 di Ferdinando, M., Brunetti, C., Agati, G., Tattini, M., 2014. Multiple functions of polyphenols in
544 plants inhabiting unfavorable Mediterranean areas. Environ. Exp. Bot. 103, 107-116.
545 Dicke, M., Loreto, F., 2010. Induced plant volatiles: from genes to climate change. Trends Plant
546 Sci. 15, 115-117.
547 Dolzhenko, Y., Bertea, C.M., Occhipinti, A., Bossi, A., Maffei, M.E.J., 2010. UV-B modulates the
548 interplay between teerpenoids and flavonoids in peppermint (*Mentha* x *piperita* L.).
549 Photochem. Photobiol. B 100, 67-75.
550 Eakes, D.J., Wright, R.D., Seiler, J.R., 1991. Moisture stress conditioning effects on *Salvia*
551 *splendens* 'Bonfire'. J. Amer. Soc. Hort. Sci. 116, 716-719.
552 Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., 2009. Plant drought stress:
553 effects, mechanisms and management. In: Lichtfouse E., Navarrete M., Debaeke P.,
554 Véronique S., Alberola C. (eds) Sustainable Agriculture. Springer, Dordrecht.
555 Fisher, V.L., 2005. Indigenous *Salvia* species—an investigation of the antimicrobial activity, anti-
556 oxidant activity and chemical composition of leaf extracts. M.Sc. Dissertation. University of
557 the Witwatersrand, Johannesburg, South Africa.
558 Flexas, J., Medrano, H., 2002. Droguth-inhibition of photosynthesis in C3 plants: stomatal and non-
559 stomatal limitations revisited. Ann. Bot. 89, 183-189.
560 George, K.J., Malik, N., Kumar, I.P.V., Krishnamurthy, K.S., 2017. Gene expression analysis in
561 drought tolerant and susceptible black pepper (*Piper nigrum* L.) in response to water deficit
562 stress. Acta Physiol. Plant. 39, 104.

563 Ghanaatiyan, K., Sadeghi, H., 2017. Differential responses of chicory ecotypes exposed to drought
564 stress in relation to enzymatic and non-enzymatic antioxidants as well as ABA concentration.
565 J. Hortic. Sci. Biotech. 92, 404-410.
566 Gonzalez-Coloma, A., Delgado, F., Rodilla, J. M., Silva, L., Sanz, J., Burillo, J., 2011. Chemical
567 and biological profiles of *Lavandula luisieri* essential oils from western Iberia Peninsula
568 populations. Biochem. Syst. Ecol. 39, 1-8.
569 Hargrave, K.R., Kolb, K.J., Ewers, F.W., Davis, S.D., 1994. Conduit diameter and drought-induced
570 embolism in *Salvia mellifera* Greene (Labiatae). New Phytol. 126, 695-705.
571 Hassanpour, H., Khavari-Nejad, R.A., Niknam, V., Razavi, K., Najafi, F., 2014. Effect of
572 penconazole and drought stress on the essential oil composition and gene expression of
573 *Mentha pulegium* L. (Lamiaceae) at flowering stage. Acta Physiol. Plant. 36, 1167-1175.
574 Holopainen, J.K., Gershenzon, J., 2010. Multiple stress factors and the emission of plant VOCs.
575 Trends Plant Sci. 15**,** 176-184.
576 Jaafar, H.Z.E., Ibrahim, M.H., Fakri, N.F.M., 2012. Impact of soil field water capacity in secondary
577 metabolites, phenylalanine ammonia-lyase (PAL), maliondialdehyde (MDA) and
578 photosynthetic responses of Malaysian kacip Fatimah (*Labisia pumila* Benth). Molecules 17,
579 7305-7322.
580 Kamatou, G.P.P., Van Vuuren, S.F., Van Heerden, F.R., Seaman, T., Viljoen, A.M., 2007a.
581 Antibacterial and antimycobacterial activities of South African *Salvia* species and isolated
582 compounds from *S. chamelaegnea*. South Afr. J. Bot. 73, 552-557.
583 Kamatou, G.P.P., Viljoen, A.M., Figueiredo, A.C., Tilney, P.M., Van Zyl, R.L., Van Vuuren, S.F.,
584 Pedro, L.G., Barroso, J.G., (2007b). Glandular trichomes, essential oil composition and
585 biological activities of *Salvia albicaulis* Benth and *S. dolomitica* Codd, two species from the
586 Cape region of South Africa. South Afric. J. Bot. 73, 102-108.
587 Kamatou, G.P.P., Makunga, N.P., Ramogola, W.P.N., Viljoen, A.M., 2008. South African *Salvia*
588 species: a review og biological activities and phytochemistry. J. Ethnopharm. 119, 664-672.

589 Kamatou G.P.P., Viljoen, A.M., Steenkamp, P., 2010. Antioxidant, antiinflammatory activities and
590 HPLC analysis of South African *Salvia* species. Food Chem. 119, 684-688.
591 Kim, D.O., Jeong, S.W., Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemicals from
592 various cultivars of plums. Food Chem. 81, 321-326.
593 Kleinwächter, M., Selmar, D., 2014. Influencing the Product Quality by Applying Drought Stress
594 During the Cultivation of Medicinal Plants. In: Ahmad P., Wani M. (eds) Physiological
595 Mechanisms and Adaptation Strategies in Plants Under Changing Environment. Springer,
596 New York, NY.
597 Kleinwächter, M., Selmar, D., 2015. New insights explain that drought stress enhances the qualityt
598 of spice and medicinal plants: potential applications. Agron. Sust. Develop. 35, 121-131.
599 Kleinwächter, M., Paulsen, J., Bloem, E., Schnug, E., Selmar, D., 2015. Moderate drought and
600 signal transducer induced biosynthesis of relevant secondary metabolite in thyme (*Thymus*
601 *vulgaris*), greater celandine (*Chelidonium majus*) and parsley (*Petroselinum crispum*). Ind.
602 Crops Prod. 64, 158-166.
603 Kondrat'eva, V.V., Voronkova, T.V., Shelepova, O.V., Olekhnovich, L.S., 2008. Physiological and
604 biochemical aspects of clary (*Salvia sclarea* L.) overwintering in central Russia. Biol. Bull.
605 35, 255-261.
606 Liu, H., Wang, X., Wang, D., Zou, Z., Liang, Z., 2011. Effect of drought stress on growth and
607 accumulation of active constituents in *Salvia miltiorrhiza* Bunge. Ind. Crops Prod. 33, 84-88.
608 Llusia, J., Peñuelas, J., 1998. Changes in terpene content and emission in potted Mediterranean
609 woody plants under severe drought. Can. J. Bot. 76, 1366-1373.
610 Llusia, J., Peñuelas, J., 2000. Seasonal patterns of terpene content and emission from seven
611 Mediterranean woody species in field conditions. Am. J. Bot. 87, 133-140.
612 Loreto, F., Schnitzler, J. P., 2010. Abiotic stresses and induced BVOCs. Trends Plant Sci. 15, 154-
613 166.

614 Loreto, F., Dicke, M., Schnitzler, J.P., Turlings, T.C.J., 2014. Plant volatiles and the environment.
615 Plant Cell Environ. 37, 1905-1908.
616 Ma, Y., Yuan, L., Wu, B., Li, X., Chen, S., Lu, S., 2012. Genome-wide identification and
617 characterization of novel genes involved in terpenoid biosynthesis in *Salvia miltiorrhiza*. J.
618 Exp. Bot. 63, 2809-2823.
619 Mahajan, S., Tuteja, N., 2005. Cold, salinity and drought stresses: an overview. Arch. Biochem.
620 Biophys. 444, 139-158.
621 Mandoulakani, B.A., Eyvazpour, E., Ghadimzadeh, M., 2017. The effect of drought stress on the
622 expression of key genes involved in the biosynthesis of phenylpropanoids and essential oil
623 components in basil (*Ocimum basilicum* L.). Phytochemistry 139, 1-7.
624 McAdam, S.A.M., Brodribb, T.J., 2016. Linking turgor with ABA biosynthesis: implications for
625 stomatal responses to vapour pressure deficit across land plants. Plant Physiol. DOI:
626 https://doi.org/10.1104/pp.16.00380.
627 Mitchell, P.J., O'Grady, A.P., Tissue, D.T., White, D.A., Ottenschlaeger, M.L., Pinkard, E.A.,
628 2013. Drought response strategies derfine the relative cnotributions of hydraulic dysfunction
629 and carbohydrate depletion during tree mortality. New Phytol. 197, 862-872.
630 Moradi, P., Ford-Lloyd, B., Pritchard, J., 2017. Metabolomic approach reveals the biochemical
631 mechanisms underlying drought stress tolerance in thyme. Anal. Biochem. 527, 49-62.
632 Morales, C.G., Pino, M.T., del Pozo, A., 2013. Phenological and physiological responses to drought
633 stress and subsequent rehydration cycles in two raspberry cultivars. Sci. Hortic. 162, 234-241.
634 Munné-Bosch, S., Peñuelas, J., 2003. Photo- and antioxidative protection, and a role for salicyclic
635 acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. Planta 217,
636 758-766.
637 Niinemets, Ü., Kännaste, A., Copolovici, L., 2013. Quantitative patterns between plant volatile
638 emissions induced by biotic stresses and the degree of damage. Front. Plant Sci. 4, 262.

639 Nogués, I., Muzzini, V., Loreto, F., Bustamante, M.A., 2015. Drought and soil amendment effects
640 on monoterpene emission in rosemary plants. Sci. Total Environ. 538, 768-778.
641 Ormeño, E., Mévy, J.P., Vila, B., Bousquet Mélou, A., Greff, S., Bonin, G., Fernandez, C., 2007.
642 Water deficit stress induces different monoterpene and sesquiterpene emission changes in
643 Mediterranean species. Relationship between terpene emissions and plant water potential.
644 Chemosphere 67, 276-636.
645 Possell, M., Loreto, F., 2013. The role of volatile organic compounds in plant resistance to abiotic
646 stresses: responses and mechanisms. In: Biology, Controls and Models of Tree Volatile
647 Organic Compound Emissions (Eds Ü. Niinemets, RK Monson), pp. 209–235. Springer,
648 Berlin, Germany.
649 Prisic, S., Xu, M., Wilderman, P.R., Peters, R.J., 2004. Rice contains two disparate *ent*-copalyl
650 diphosphate synthases with distinct metabolic functions. Plant Physiol. 136, 4228-4236.
651 Radwan, A., Kleinwächter, M., Selmar, D., 2017. Impact of drought stress on specialised
652 metabolism: biosynthesis and the expression of monoterpene synthases in sage (*Salvia*
653 *officinalis*). Phytochemistry 141, 20-26.
654 Rahimi, Y., Taleei, A., Ranjbar, M., 2017. Changes in the expression of key genes involved in the
655 biosynthesis of menthol and menthofuran in *Mentha piperita* L. under drought stress. Acta
656 Physiol. Plant. 39, 203.
657 Raut, J.S., Karuppayil, S.M., 2014. A status review on the medicinal properties of essential oils.
658 Ind. Crops Prod. 62, 250-264.
659 Roux, D., Alnaser, O., Garayev, E., Baghdikian, B., Elias, R., Chiffolleau, P., Ollivier, E., Laurent,
660 S., El Maataoui, M., Sallanon, H., 2017. Ecophysiological and phytochemical characterization
661 of wild populations of *Inula montana* L. (Asteraceae) in Southeastern France. Flora 236, 67-
662 75.

663 Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemmingsen, E.A., 1965. Sap pressure in
664 vascular plants. Negative hydrostatic pressure can be measured in plants. Science 148, 339–
665 346.
666 Selmar, D., Kleinwachter, M., 2013. Influencing the product quality by deliberately applying
667 drought stress during the cultivation of medicinal plants. Ind. Crops Prod. 42, 558-666.
668 Singleton, V.L., Rossi, J.A.J., 1965. Colorimetry of total phenolics with phosphomolybdic–
669 phosphotungstic acid reagent. Am. J. Enol. Viticol. 16, 144–158.
670 Soni, P. Abdin, M.Z., 2017. Water deficit-induced oxidative stress affects artemisinin content and
671 expression of proline metabolic genes in *Artemisia annua* L. FEBS OpenBio **7**, 367-381.
672 Szôllôsi, R., Szôllôsi Varga, I., 2002. Total antioxidant power in some species of Labiatae
673 (adaptation of FRAP method). Acta Biol. Szegediensis 46, 125-127.
674 Tholl, D., 2015. Biosynthesis and Biological Functions of Terpenoids in Plants. In: Schrader J.,
675 Bohlmann J. (eds) Biotechnology of Isoprenoids. Advances in Biochemical
676 Engineering/Biotechnology, vol 148. Springer, Cham.
677 Valliere, J.M., Allen, E.B., 2016. Interactive effects of nitrogen deposition and drought-stress on
678 plant-soil feedbacks of *Artemisia californica* seedlings. Plant Soil 403, 277-290.
679 Wenping, H., Yuan, Z., Jie, S., Lijun, Z., Zhezhi, W., 2011. *De novo* transcriptome sequencing in
680 *Salvia miltiorrhiza* to identify genes involved in the biosynthesis of active ingredients.
681 Genomics 98, 272-279.
682

683 **Conflict of Interest Statement:** The authors declare that they have no conflict of interest.

684

685 **TABLES**

686 **Table 1.** Oligonucleotides used in this study in qRT PCR experiments.

| Name | Putative Gene Description | Primer | Primer sequences 5'-3' | References |
|---|---|---|---|---|
| ***DH*** | Dehydrin | Forward | GAGGTAGAGGGGGAAAATGG | This study |
| | | Reverse | CCGATGTGTCTACGCATTTC | |
| ***GPPSB*** | Geranyl diphosphate synthase | Forward | GGCGTATGGGTTACACAAGC | This study |
| | | Reverse | GCACCAAGGCTAGAGAGCTG | |
| ***GPPS2D*** | Geranyl diphosphate synthase | Forward | GCTGTCCCCCAAGTTTGAT | This study |
| | | Reverse | CTCTCCATCACGCGAAGC | |
| ***FPPS*** | Farnesyl diphosphate synthase | Forward | GCGGGTGAGGACCTGGAGAAACAT | Ma et al. 2012 |
| | | Reverse | CAGGGCCTTTACAACCAGCCAAGAA | |
| ***GGPPS2*** | Geranylgeranyl diphosphate synthase | Forward | CCAGATTGTGGACTTGTCGAGCGA | Ma et al. 2012 |
| | | Reverse | CAACACACCTGGCGTACTTCCTCAA | |
| ***CPS1*** | Copalyl diphosphate synthase | Forward | CCACATCGCCTTCAGGGAAGAAAT | Ma et al. 2012 |
| | | Reverse | TTTATGCTCGATTTCGCTGCGATCT | |
| ***CPS2*** | Copalyl diphosphate synthase | Forward | GGTCTCATCGCCTTCAACGAAGAT | Ma et al. 2012 |
| | | Reverse | TCCTTATCCTTTATGCTCCCATCCA | |
| ***CPS3*** | Copalyl diphosphate synthase | Forward | GGAGATGCCAATTCGAACATCAGA | Ma et al. 2012 |
| | | Reverse | TCAAATATAGTTGCGGCGGCCAAA | |
| ***CPS4*** | Copalyl diphosphate synthase | Forward | CGGCTGCCTTGGGCTACAACAATA | Ma et al. 2012 |
| | | Reverse | TCCCTGGTGACCTCCTCCTTCCCA | |
| ***GAPDH*** | Glyceraldehyde-3-phosphate dehydrogenase | Forward | ACCCTCACGGGGAAGACCATC | This study |
| | | Reverse | ACCACGGAGACGGAGGACAAG | |

687

688 **Table 2.** Average values of growth index (G.I., $cm^3$) during the experiment. *S. dolomitica* plants
689 were well watered (100% container capacity, 100% CC, WW) or subjected to moderate drought
690 stress (50% CC, MDS) or severe drought stress (0% CC, SDS).

| **G.I. ($cm^3$)** | Days | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | 0 | 4 | 7 | 11 | 14 | 18 | 21 | 25 | 28 | 32 | 34 |
| WW | 8134 | 3186 | 3525 | 7114 | 6464 | 4788 | 8355a | 11921a | 12059 | 12156 | 12456 |
| MDS | 8134 | 5726 | 5837 | 4641 | 4561 | 4779 | 4058b | 3336b | 3451 | 3587 | 4002 |
| SDS | 8143 | 2951 | 3011 | 3611 | 2874 | 2786 | 1534c | 778c | - | - | - |
| *P* | ns | ns | ns | ns | ns | ns | ** | * | ** | ** | ** |

691 Means followed by the same letter do not differ significantly, according to REGW-F test (NS = non significant;
692 *P<0.05; ** P<0.001).

693

694 **Table 3.** Total, aerial and root dry mass accumulation (g DW $plant^{-1}$) and root:aerial (R:A) ratio of
695 treated *S. dolomitica* plants at the end of the experiment. Plants were well watered (100% container
696 capacity, 100% CC, WW) or subjected to moderate drought stress (50% CC, MDS) or severe
697 drought stress (0% CC, SDS). In brackets are reported the percentage variations referred to controls.

| Treatments | Dry mass accumulation (gDW $plant^{-1}$) | | | R:A ratio |
|---|---|---|---|---|
| | Total | Aerial part | Root | |
| WW | 6.24 a (100%) | 4.30 a (100%) | 1.94 a (100%) | 0.45 b (100%) |
| MDS | 1.64 b (26%) | 0.86 b (20%) | 0.78 b (40%) | 0.91 a (200%) |
| SDS | 1.06 b (17%) | 0.77 b (18%) | 0.29 c (15%) | 0.38 c (82%) |
| *P* | ** | ** | ** | * |

698 Means followed by the same letter do not differ significantly, according to REGW-F test (NS = non significant;
699 *P<0.05; ** P<0.001).

700

701 **Table 4.** SPAD values, chlorophyll (a + b) and total carotenoid (Car) measured on *S. dolomitica*
702 plants treated with three irrigation regimes: well watered (100% container capacity, 100% CC,
703 WW), moderate drought stress (50% CC, MDS), or severe drought stress (0% CC, SDS).

| **SPAD** | Days | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | 0 | 4 | 7 | 11 | 14 | 18 | 21 | 25 | 28 | 32 | 34 |
| WW | 28.1 | 34.2 | 36.4 | 36.8 | 35.9 | 34.4 | 38.0 | 38.4 | 38.1 | 38.7 | 38.8 |
| MDS | 28.1 | 32.1 | 32.7 | 35.3 | 35.0 | 32.1 | 33.8 | 33.9 | 34.1 | 34.2 | 34.1 |
| SDS | 28.1 | 28.3 | 29.3 | 32.3 | 33.2 | 33.0 | 31.5 | 30.0 | - | - | - |
| *P* | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| **Chl (a+b)** (mg $g^{-1}$ FW) Treatments | | | | | | | | | | | |
| WW | 1.42 | 1.46 | 1.42 | 1.46 | 1.48 | 1.49 | 1.46 a | 1.45 a | 1.48 | 1.49 | 1.45 |
| MDS | 1.42 | 1.41 | 1.43 | 1.31 | 1.39 | 1.48 | 1.45 a | 1.46 a | 1.47 | 1.45 | 1.43 |
| SDS | 1.42 | 1.43 | 1.39 | 1.48 | 1.27 | 1.29 | 1.19 b | 1.11 b | - | - | - |
| *P* | ns | ns | ns | ns | ns | ns | ** | ** | ns | ns | ns |
| **Carotenoids** (mg $g^{-1}$ FW)) Treatments | | | | | | | | | | | |
| WW | 2.18 | 2.24 | 2.04 | 2.84 | 2.65 | 2.45 | 2.93 a | 2.46 a | 2.58 | 2.78 | 2.94 |
| MDS | 2.18 | 1.85 | 2.16 | 2.02 | 2.05 | 2.35 | 2.75 a | 2.45 a | 2.74 | 2.95 | 2.87 |
| SDS | 2.18 | 2.20 | 2.01 | 2.23 | 2.32 | 1.96 | 1.54 b | 1.07 b | - | - | - |
| *P* | ns | ns | ns | ns | ns | ns | * | ** | ns | ns | ns |

Means followed by the same letter do not differ significantly, according to REGW-F test (NS = non significant; *P<0.05; ** P<0.001).

**Table 5**. The rate during the experiment and the total amount (mg plant$^{-1}$ FW) of leaf phenols, flavonoids, and antioxidant activity of treated *S. dolomitica* plants. Plants were well watered (100% container capacity, 100% CC, WW) or subjected to moderate drought stress (50% CC, MDS) or severe drought stress (0% CC, SDS). In brackets are reported the percentage variations referred to controls.

| **Phenols** (mg GAE g$^{-1}$) Treatments | Days 0 | 4 | 7 | 11 | 14 | 18 | 21 | 25 | 28 | 32 | 34 | Total amount |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| WW | 58.3 | 38.7a | 27.5a | 34.8a | 31.8 | 21.7 | 21.5 | 21.0 | 18.0 | 29.0 | 21.8 | 305.2a (100%) |
| MDS | 58.3 | 39.6a | 29.1a | 31.6a | 38.7 | 22.5 | 22.0 | 20.0 | 18.5 | 12.7 | 18.6 | 53.2b (17%) |
| SDS | 58.3 | 13.8b | 11.2b | 9.9b | 29.1 | 17.3 | 23.3 | 21.3 | - | - | - | 20.5c (7%) |
| *P* | ns | * | ** | ** | ns | ns | ns | ns | ns | ** | ns | ** |
| **Flavonoids** (mg g$^{-1}$) Treatments | | | | | | | | | | | | |
| WW | 22.1 | 11.3a | 7.3a | 10.5a | 11.3 | 6.8 | 7.0 | 6.3 | 5.3 | 8.3 | 7.3 | 105.7a (100%) |
| MDS | 22.1 | 10.8a | 7.0a | 7.9ab | 8.2 | 7.1 | 6.5 | 6.1 | 5.6 | 5.7 | 6.0 | 17.1b (16%) |
| SDS | 22.1 | 4.2b | 3.8b | 5. b | 7.9 | 5.3 | 7.1 | 6.0 | - | - | - | 5.3c (5%) |
| *P* | ns | * | ** | ** | ns | ns | ns | ns | ns | * | ns | ** |
| **FRAP** (µmol $Fe^{2+}$ g$^{-1}$) Treatments | | | | | | | | | | | | |
| WW | 380.3 | 193.5a | 130.3a | 168.5a | 148.3 | 115.3 | 120.4 | 98.7b | 99.1 | 163.4 | 125.4 | 1815.8a (100%) |
| MDS | 380.3 | 190.6a | 135.1a | 143.4a | 154.3 | 121.5 | 108.3 | 101.5b | 102.3 | 105.0 | 118.0 | 337.5b (18%) |
| SDS | 380.3 | 83.1b | 64.5b | 80.5b | 150.2 | 98.4 | 116.1 | 153.1a | - | - | - | 134.7c (7%) |
| *P* | ns | ** | ** | ** | ns | ns | ns | ** | ns | * | ns | ** |

Means followed by the same letter do not differ significantly, according to REGW-F test (NS = non significant; *P<0.05; ** P<0.001).

**Table 6.** Chemical composition (%) of volatiles emitted from *Sa. dolomitica* plants after well watered irrigation (WW), moderate drought stress (MDS) or severe drought stress (SDS). All

constituents are ordered on the basis of their linear retention index (LRI). In bold are indicated the most relevant constituents.

| Category* | Constituents (%) | l*RI* | WW | MDS | SDS |
|---|---|---|---|---|---|
| mh | α-thujene | 939 | 0.33 | 0.11 | 0.35 |
| mh | α-pinene | 953 | 5.75 | 2.97 | 2.76 |
| mh | camphene | 980 | 4.05 | 2.04 | 1.88 |
| mh | β-pinene | 991 | 3.35 | 1.85 | 1.64 |
| mh | myrcene | 1031 | 5.09 | 2.01 | 1.76 |
| mh | α-phellandrene | 1040 | 0.57 | 0.18 | 0.27 |
| **mh** | **Δ-3-carene** | **1050** | **9.14** | **4.16** | **4.53** |
| **mh** | **limonene** | **1088** | **19.80** | **13.51** | **12.24** |
| **mh** | **(E)-β-ocimene** | **1097** | **7.39** | **3.19** | **2.99** |
| mh | (Z)-β-ocimene | 1098 | 0.64 | 0.23 | 0.27 |
| mh | γ-terpinene | 1110 | 0.85 | 0.38 | 0.43 |
| mo | *cis*-sabinene hydrate | 1125 | 0.08 | 0.21 | 0.17 |
| mh | terpinolene | 1143 | 0.75 | 0.32 | 0.28 |
| mo | *trans*-sabinene hydrate | 1165 | 0.18 | 0.21 | 0.24 |
| mh | *allo*-ocimene | 1189 | 0.91 | 0.29 | 0.26 |
| mo | isoborneol | 1204 | 0.10 | 0.12 | 0.00 |
| mo | borneol | 1285 | 1.36 | 2.39 | 1.79 |
| sh | Δ-elemene | 1339 | 0.49 | 0.98 | 1.12 |
| sh | α-cubebene | 1376 | 0.37 | 0.40 | 0.57 |
| sh | isoledene | 1380 | 0.52 | 0.53 | 0.68 |
| sh | α-copaene | 1391 | 1.93 | 2.23 | 2.63 |
| sh | β-bourbonene | 1398 | 0.22 | - | 1.89 |
| sh | β-cubebene | 1418 | 0.21 | 0.47 | 0.40 |
| sh | β-elemene | 1429 | 0.11 | 0.31 | 0.37 |
| sh | α-gurjunene | 1432 | 0.72 | 0.79 | 0.89 |
| **sh** | **β-caryophyllene** | **1439** | **7.86** | **9.47** | **9.09** |
| sh | β-copaene | 1454 | 0.92 | 1.29 | 2.00 |
| sh | β-gurjunene | 1458 | 0.38 | 0.46 | 0.65 |
| **sh** | **α-guaiene** | **1476** | **3.68** | **4.51** | **5.21** |
| sh | aromadendrene | 1477 | 0.40 | 0.58 | 0.77 |
| sh | αβ-humulene | 1480 | 0.75 | 1.16 | 1.15 |
| sh | allo-aromadendrene | 1485 | 0.46 | 0.72 | 0.94 |
| sh | γ-muurolene | 1494 | 0.89 | 0.96 | 1.02 |
| **sh** | **germacrene D** | **1503** | **8.57** | **22.35** | **22.16** |
| **sh** | **bicyclogermacrene** | **1517** | **4.21** | **-** | **9.27** |
| sh | γ-cadinene | 1524 | 1.40 | - | 1.84 |
| sh | δ-cadinene | 1581 | - | - | 3.68 |
| | **Total** | | **94.43** | **81.38** | **98.18** |
| | **Monoterpene Hydrocarbons (mh %)** | | **57.71** | **30.97** | **29.41** |
| | **Oxygenated Monoterpene (om %)** | | **1.72** | **2.92** | **2.19** |
| | **Sesquiterpene Hydrocarbons (sh %)** | | **34.09** | **47.19** | **66.32** |

*****All the constituents identified belong to monoterpene hydrocarbons (mh), oxygenated monoterpene (om) and sesquiterpene hydrocarbons (sh).

**Table 7.** Chemical composition (%) of essential oils extracted from *Salvia dolomitica* plants after well watered irrigation (WW), moderate drought stress (MDS) or severe drought stress (SDS). All

constituents are ordered on the basis of their linear retention index (LRI). In bold are indicated the most relevant constituents.

| Category* | Constituents (%) | l*RI* | WW | MDS | SDS |
|---|---|---|---|---|---|
| mh | α-pinene | 319 | 1.4 | 1.0 | 1.0 |
| mh | camphene | 340 | 0.6 | | |
| **mh** | **β-pinene** | **386** | **0.2** | **6.1** | **6.0** |
| mh | myrcene | 408 | 0.5 | 0.2 | 0.2 |
| mh | α-phellandrene | 435 | 0.2 | | |
| mh | Δ-3-carene | 444 | 1.5 | | |
| mh | α-terpinene | 462 | 0.2 | | |
| mh | p-cymene | 471 | 0.3 | | |
| mh | limonene | 481 | 2.0 | 0.3 | 0.3 |
| om | 1,8-cineolo | 485 | 3.4 | 0.3 | 0.3 |
| om | (Z)-β-ocimene | 498 | 0.6 | 0.2 | 0.2 |
| mh | γ-terpinene | 545 | 0.3 | | 0.1 |
| om | terpinolene | 612 | 0.1 | | |
| om | trans-pinocarveol | 724 | 0.1 | | |
| om | pinocarvone | 781 | | | 0.1 |
| ac-10 | borneol | 789 | 2.4 | 0.4 | 0.4 |
| nt | 4-terpineol | 820 | 0.3 | 0.1 | 0.1 |
| om | myrtenal | 864 | | 0.5 | 0.5 |
| om | safranal | 950 | | | 0.2 |
| om | N-decanal | 1084 | | | 0.1 |
| sh | lavandulyl acetate | 1111 | | 1.4 | 1.3 |
| sh | trans-pinocarvyl acetate | 1135 | | 0.2 | 0.2 |
| sh | myrtenyl acetate | 1195 | | 0.8 | 0.8 |
| om | α-cubebene | 1267 | 0.3 | | |
| sh | isoledene | 1308 | 0.4 | | |
| sh | α-copaene | 1334 | 2.3 | 0.5 | 0.5 |
| sh | trans-myrtanol acetate | 1347 | | 0.2 | 0.2 |
| om | sativene | 1364 | 0.1 | | |
| sh | α-gurjunene | 1421 | 1.0 | 0.2 | 0.2 |
| **sh** | **β-caryophyllene** | **1442** | **21.2** | **0.6** | **0.6** |
| sh | lavandulyl isobutirate | 1452 | 0.9 | 0.2 | 0.2 |
| sh | β-copaene | 1464 | 0.3 | | |
| sh | β-gurjunene | 1475 | 0.9 | | |
| **sh** | **1H-cyclopropanaphtalene** | **1486** | **6.9** | | |
| sh | α-guaiene | 1491 | 0.8 | 0.2 | 0.2 |
| sh | aromadendrene | 1491 | 0.1 | | |
| **sh** | **α-humulene** | **1527** | **2.2** | **3.8** | **3.8** |
| sh | alloaromadendrene | 1546 | 0.9 | 0.5 | 0.5 |
| sh | trans-cadina 1(6).4-diene | 1567 | 0.7 | | |
| sh | γ-muurolene | 1586 | 0.8 | 0.6 | 0.6 |
| sh | β-selinene | 1608 | 0.2 | 0.7 | 0.7 |
| sh | cis-β-guaiene | 1621 | 0.5 | | |
| sh | valencene | 1624 | 0.3 | | |
| om | viridiflorene | 1628 | 3.0 | 0.2 | 0.2 |
| **sh** | **trans-β-guaiene** | **1646** | **1.9** | **18.6** | **18.5** |
| sh | α-bulnesene | 1658 | 0.1 | | 0.1 |
| sh | geranyl isobutyrate | 1678 | | 1.4 | 1.7 |
| sh | trans-γ-cadinene | 1676 | 3.6 | | |
| **sh** | **δ-cadinene** | **1700** | **7.1** | **3.1** | **3.2** |
| os | trans-cadina-1(2).4-diene | 1718 | 0.7 | | |

| | | | | | |
|---|---|---|---|---|---|
| sh | α-cadinene | 1733 | 0.3 | | |
| os | α-calacorene | 1744 | | 1.3 | 1.3 |
| os | elemol | 1759 | 0.2 | | |
| os | germacrene D | 1786 | 0.5 | | |
| **os** | **longipinalol** | **1801** | **0.8** | **41.9** | **41.5** |
| os | caryophyllene alcohol | 1806 | 0.1 | | |
| os | spathunelol | 1825 | 0.4 | 1.5 | 1.5 |
| os | caryophyllene oxide | 1837 | 3.8 | 0.2 | 0.2 |
| os | 5-epi-7-epi-α-eudesmol | 1894 | 1.6 | 0.3 | 0.3 |
| os | humulene oxide | 1897 | 0.3 | 1.0 | 1.0 |
| os | 1.10-di-epi-cubenol | 1915 | 0.5 | 0.9 | 1.0 |
| os | 1-epi-cubenol | 1944 | 1.3 | 0.7 | 0.8 |
| os | γ-eudesmol | 1951 | 0.9 | | 0.1 |
| os | caryophylla-4(14).8(15)-dien-5-ol | 1962 | 0.6 | | |
| **os** | **epi-α-cadinol** | **1973** | **4.3** | **0.9** | **0.9** |
| os | α-muurolol | 1984 | 0.1 | 0.4 | 0.4 |
| os | β-eudesmol | 1993 | 1.4 | | |
| **os** | **α-eudesmol** | **2000** | **4.4** | **2.2** | **2.3** |
| os | 14-hydroxy-9-epi-(E)-caryophyllene | 2028 | 2.3 | | |
| os | bulnesol | 2033 | 0.1 | 0.1 | 0.1 |
| os | α-cadinol | 2003 | | 0.3 | 0.3 |
| os | valeranone | 2047 | 0.9 | 0.4 | 0.4 |
| os | cadalene | 2050 | | 0.2 | 0.2 |
| os | khusinol | 2051 | 0.1 | 0.2 | 0.2 |
| os | α-bisabolol | 2072 | | 0.2 | 0.2 |
| os | eudesma-4(15).7-dien-1-β-ol | 2076 | 0.3 | | |
| os | acorenone | 2078 | 0.1 | | |
| nt | trans-α-bergamotol | 2097 | 0.2 | | |
| os | γ-atlantone | 2116 | | 0.3 | 0.3 |
| os | oplopanone | 2153 | 0.1 | | |
| nt | hexadecanal | 2239 | 0.2 | 0.1 | 0.1 |
| os | lanceol acetate (z) | 2455 | | 0.2 | 0.2 |
| | **Total** | | **97.3** | **95.5** | **95.9** |
| | **Monoterpene Hydrocarbons (mh %)** | | **7.2** | **7.6** | **7.5** |
| | **Oxygenated Monoterpene (om %)** | | **7.5** | **1.1** | **1.5** |
| | **Sesquiterpene Hydrocarbons (sh %)** | | **53.4** | **32.9** | **33.2** |
| | **Oxygenated Sesquiterpenes (os %)** | | **26.0** | **53.2** | **53.1** |
| | **Non terpenoid (nt %)** | | **0.6** | **0.3** | **0.3** |
| | **Apocarotenoids (ac-10 %)** | | **2.4** | **0.4** | **0.4** |

*All the constituents belong to non terpene derivates (nt), monoterpene hydrocarbons (mh), oxygenated monoterpene (om), sesquiterpene hydrocarbons (sh), oxygenated sesquiterpene (os), and apocarotenoids (ac-10).

728

729 **Figure captions**

730

731 **Figure 1.** Midday leaf water potential (MLWP - A), gas exchange (internal $CO_2$ concentration, C*i* -
732 B; transpiration rate, *E* - C; stomatal conductance, *gs* - D; net photosynthetic rate, *A* - E) and
733 internal abscisic acid content (ABA - F) dynamics measured on *S. dolomitica* plants treated with
734 well watered irrigation (WW), moderate drought stress (MDS), or severe drought stress (SDS).
735 Mean values showing the same letter are not statistically different at P≤0.05 according to the
736 REGW-F post-hoc test. The statistical relevance of 'Between-Subjects Effects' tests (ns=non
737 significant, *=P<0.05, ** P<0.001 ) was evaluated.

738

739 **Figure 2.** Radar charts showing changes in terpenoids content of biogenic volatile organic
740 compounds (BVOCs - A) and essential oils (EOs - B) of *S. dolomitica* plants in responses to well
741 watered irrigation (WW, black line), moderate drought stress (MDS, dark grey line) or severe
742 drought stress (SDS, light grey line). All the constituents belong to non terpene derivates (nt),
743 monoterpene hydrocarbons (mh), oxygenated monoterpene (om), sesquiterpene hydrocarbons (sh),
744 oxygenated sesquiterpene (os), and apocarotenoids (ac-10).

745

746 **Figure 3.** Flowchart for assembling isoprenoid building blocks to produce terpenes and relative
747 transcriptional modulation of genes involved in *S. dolomitica* terpenoid biosynthesis. Relative gene
748 expression levels obtained by RT-qPCR analysis of the *DH* (in the box): dehydrin; *GPPSB* (A) *and*
749 *GPPS2D* (B)*:* geranyl diphosphate synthases; *FPPS* (C): farnesyl diphosphate synthase; *GGPPS2*
750 (D)*:* geranylgeranyl diphosphate synthase; *CPS1* (E), *CPS2* (F), *CPS3* (G) and *CPS4* (H)*:* copalyl
751 diphosphate synthases. In the box relative transcriptional modulation. Genes were tested on plants
752 subjected to moderate water stress (MDS), severe water stress (SDS) or well-watered (WW)
753 treatments. Mean values showing the same letter are not statistically different at P≤0.05 according

754 to the Tukey's post-hoc test. Bars represent the standard deviation of the mean (n=3). MVA,
755 Mevalonate pathway; MEP, Methylerythritol phosphate patway; IPP, isopenthenyl diphosphate;
756 DMAPP, dimethylallyl diphosphate.