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

3

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27 **Abstract**

28 Sage is an important medicinal and aromatic plant. While *Salvia officinalis* and *S. miltiorrhiza* have  
29 been widely studied, little information regarding *S. dolomitica* exists, although it has recently  
30 attracted attention due to its anti-plasmodial and anti-inflammatory properties. This study  
31 investigated the performance and metabolic profile of this species in response to two drought  
32 treatments (moderate or severe) relative to well-watered control plants. Changes in growth and  
33 ecophysiological traits, as well as in bioactive and volatile compounds and essential oil production  
34 were determined. Given that terpenoids are the most representative class of secondary metabolites,  
35 the gene expression of key enzymes of terpenoid biosynthesis was also investigated. Moderate  
36 drought stimulated a decline in leaf water potential, growth and stomatal conductance, as well as an  
37 increase in dehydrin expression. Serious stress symptoms occurred only in severe drought-stressed  
38 plants, where a decline in net photosynthesis and transpiration and an increase in endogenous  
39 abscisic acid was observed. Both drought stress conditions led to modulate the expression of some  
40 genes involved in biogenic volatile organic compound and essential oil biosynthesis and metabolic  
41 profile. In particular, drought induced an increase in sesquiterpene production, a class of terpenoids  
42 that is important in the food, cosmetics, and pharmaceutical industries. Thus, controlled drought, in  
43 addition to water savings during cultivation, can be applied to improve the production of secondary  
44 metabolites in *S. dolomitica*.

45

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

48

## 49 **1. Introduction**

50 In combination with high levels of irradiance and increasing temperatures, drought is widely  
51 considered the most severe abiotic stressor inhibiting plant survival (Morales et al., 2013; Nogués et  
52 al., 2015). Water shortages can instigate a series of changes at the morphological, physiological,  
53 biochemical and molecular levels that adversely affect plant growth, health and productivity (Caser  
54 et al., 2016, 2017, 2018; Mitchell et al., 2013).

55 The quality of medicinal and aromatic plants (MAPs) is contingent on the composition and  
56 concentration of plant secondary metabolites (PSMs), which are themselves influenced by  
57 environmental conditions such as drought (Kleinwächter and Selmar, 2015; Mandoulakani et al.,  
58 2017). This effect occurs in all major classes of PSMs and is also dependent on plant species and  
59 cultivation practices (Kleinwächter et al., 2015).

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

65 A model was recently developed to explain how drought affects PSM production (Selmar  
66 and Kleinwächter, 2013). Its authors reported that during water-shortage conditions, stomata are  
67 closed to minimise transpiration and to preclude the entry of carbon dioxide (CO<sub>2</sub>) into the leaves.  
68 Consequently, the lower content of CO<sub>2</sub> molecules is fixed via the Calvin cycle and the fewer  
69 reduced equivalents (e.g. NADPH + H<sup>+</sup>) are consumed and re-oxidised. Thus, large amounts of  
70 NADPH + H<sup>+</sup> accumulate, generating an over-reduced state. In this condition, plants promote all  
71 reactions to consume NADPH + H<sup>+</sup>, including the biosynthesis of terpenoids and phenols. In  
72 general, PSM changes induced by drought improve the production quality of many MAPs (Caser et  
73 al., 2018; Kleinwächter and Selmar, 2014; Mandoulakani et al., 2017). However, this model has yet  
74 to be subjected to effective examination. In a recent study with *Salvia officinalis*, Radwan et al.

75 (2017) verified that the increase of monoterpene biosynthesis owed not only to a passive shift  
76 caused by stress-related over-reduced status, but also to the active biosynthesis of plant growth  
77 regulators, changes in the biochemical pathway and up-regulation of the main genes involved in  
78 terpenoid synthesis. In *S. miltiorrhiza*, Ma et al. (2012) isolated and studied the expression of  
79 several genes, coding the various enzymes involved in both the 2-C-methyl-D-erythritol 4-  
80 phosphate (MEP) and the mevalonate (MVA) pathways that lead to terpenoid biosynthesis. These  
81 enzymes, originating with the universal isoprene precursor isopentenyl diphosphate and its isomer,  
82 led to the formation of diverse terpenoids such as mono (C10)- and sesqui (C15)-terpenoids,  
83 carotenoids and chlorophylls, and bicyclic diterpenoids activated during biotic and abiotic stress  
84 responses (Prisic et al., 2004; Wenping et al., 2011).

85 The present study aims to help unravel the mechanisms behind drought stress in *S.*  
86 *dolomitica* Codd, paying particular attention to the impact on PSMs.

87

## 88 **2. Materials and methods**

### 89 *2.1. Plant material and experimental conditions*

90 A total of 120 clonally propagated plants of *S. dolomitica* Codd. were transplanted in plastic pots (9  
91 cm in diameter; 0.52 L) containing peat (Silver Torf, Agrochimica, Bolzano, Italy) and Agriperlite®  
92 (70:30 v:v). A slow-release fertiliser (Osmocote 15:11:13; Scotts Europe, The Netherlands) was  
93 used. Cultivation lasted a total of 34 days and was performed in a climate chamber with semi-  
94 controlled growth conditions (25°C, 60% air humidity, 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  of photosynthetic active  
95 radiation and 16/8 h photoperiod), located at the University of Torino (Italy, 45°06'23.21''N Lat,  
96 7°57'82.83''E Long). A complete randomised block design with three levels of irrigation was  
97 applied. The levels of irrigation were: 100% container capacity (CC) as control (well-watered,  
98 WW), 50% CC as moderate drought stress (MDS) or 0% CC as severe drought stress (SDS). For  
99 each irrigation regime, 40 plants were treated, with four replications of 10 plants each. All water  
100 amounts were kept constant throughout the experiment by gravimetric determinations as reported

101 by Caser et al. (2016). The soil moisture at the beginning of the experiment was 60% in weight.  
102 Morphological, physiological and biochemical parameters were measured after 0, 4, 7, 11, 14, 18,  
103 21, 25, 28, 32 and 34 days of cultivation to monitor the plant responses to drought over time.

104

#### 105 *2.2. Morphological parameters*

106 Plant growth (Growth Index, G.I.) was monitored by estimating the occupied volume of each plant  
107 through measurement of the height, broadest diameter, and perpendicular diameter (Demasi et al.,  
108 2017). At the end of the experiment (day 34), the roots and aerial parts of ten plants per irrigation  
109 level were weighted separately to record fresh biomass. They were subsequently oven-dried at 45°C  
110 for one week and the dry biomass was measured. The root to aerial (R:A) dry weight ratio was then  
111 calculated.

112

#### 113 *2.3. Photosynthetic pigments*

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

117 Chlorophyll and carotenoids were extracted from 50 mg of fresh, fully formed leaves from  
118 six plants per irrigation level. After an overnight extraction in 5 ml of methanol at 4°C in the dark,  
119 pigments were spectrophotometrically determined at 665, 652, and 470 nm using an Ultrospec 2100  
120 pro (Amersham Biosciences, UK) as described by Caser et al. (2013). The data were reported in mg  
121 g<sup>-1</sup> leaf fresh weight (FW).

122

#### 123 *2.4. Phenols, flavonoids and antioxidant activity*

124 One hundred mg of fresh leaves from six plants per irrigation level were powdered and  
125 homogenised with 1 ml of methanolic aqueous solution (methanol 70% v/v). Following 30 minutes  
126 on ice, the extracts were centrifuged at 10,000 rpm for 10 minutes at 25°C to recover the

127 supernatant for the following determination of phenol and flavonoid content, and the antioxidant  
128 activity.

129 The total phenols were determined colorimetrically using Folin-Ciocalteu's reagents, as  
130 described by Singleton and Rossi (1965) and indicated as mg gallic acid equivalent (GAE) g<sup>-1</sup>FW.  
131 Total flavonoid content was also determined spectrophotometrically using the colorimetric method  
132 of Kim et al. (2003), based on the formation of a complex flavonoid-aluminium and indicated as mg  
133 g<sup>-1</sup>FW. The antioxidant activity was determined using the ferric reducing antioxidant power (FRAP)  
134 method with minor modifications (Szöllôsi and Szöllôsi Varga, 2002) and indicated as  $\mu\text{mol Fe}^{2+}\text{g}^{-1}$   
135 <sup>1</sup>. The working solution was always freshly prepared and contained 7.5 mM acetate buffer, pH 3.6,  
136 0.1 mM tripyridyltriazine (TPTZ) and 0.05 mM FeCl<sub>3</sub>·6H<sub>2</sub>O. At low pH, when the tripyridyltriazine  
137 (Fe<sup>3+</sup>-TPTZ) complex is reduced to its ferrous form (Fe<sup>2+</sup>), an intensive blue colour of Fe<sup>2+</sup>-TPTZ  
138 can be monitored spectrophotometrically at 593 nm. The samples were measured in three replicates.  
139 At the end of the experiment (day 34), the total amount of total phenols, flavonoids, and antioxidant  
140 activity per plant (mg plant FW<sup>-1</sup>) was estimated on the basis of the aerial fresh biomass.

141

#### 142 *2.5. Ecophysiological evaluation*

143 The method of Scholander et al. (1965) was used to estimate the midday leaf water potential  
144 (MLWP; MPa) in three mature and fully expanded leaves per plant for six plants per irrigation level  
145 with a pressure bomb (Soil Moisture Equipment, Santa Barbara, CA, USA). Moreover, the internal  
146 CO<sub>2</sub> concentration ( $C_i$ ;  $\mu\text{mol mol}^{-1}$ ), transpiration rate ( $E$ ;  $\text{mmol m}^{-2} \text{s}^{-1}$ ), stomatal conductance ( $g_s$ ;  
147  $\text{mmol m}^{-2} \text{s}^{-1}$ ), and net photosynthetic rate ( $A$ ;  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) were measured with a portable infrared  
148 gas analyser ADC-LCPro+ (The Analytical Development Company Ltd., Hoddesdon, UK). These  
149 parameters were monitored in healthy and fully expanded leaves of six plants per irrigation level  
150 between 10:00 and 12:00 a.m., when the vapour pressure deficit (VPD) was constantly around 2.4  
151 kPa ( $\pm 0.06$  std err) with air temperature of  $26.6 \pm 0.11^\circ\text{C}$ .

152

153 *2.6. Endogenous abscisic acid determination*

154 The concentration of endogenous abscisic acid (ABA) was quantified every week in the mature  
155 leaves of six plants per irrigation level through a rapid High Performance Liquid Chromatography  
156 (HPLC) method, optimised for plant extracts and based on Solid Phase Extraction (SPE) (Bosco et  
157 al., 2013; Demasi et al., 2017). The leaves were grounded in liquid nitrogen and 0.5 g of each  
158 sample was suspended in 4 ml of the extraction solution (65% pure methanol, 25% ultrapure water,  
159 10% aqueous hydrogen chloride 1 M) for 2 h at 4°C in the dark. The samples were then filtered and  
160 the eluates were added to a SPE cartridge (Supelclean SPE LC-NH<sub>2</sub>, Supelco Analytical, USA).  
161 ABA was eluted with 5% of phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) in methanol. The procedure was carried out  
162 under artificial light with amber glassware to prevent degradation. The chromatographic analysis of  
163 the eluate was performed with HPLC 1200 Series (Agilent Technologies, Böblingen, Germany) and  
164 the signal was monitored at 265 nm with a diode array detector. Peaks identification was made on  
165 the basis of retention time, the co-injection with ABA standard along each batch samples, and the  
166 UV spectrum of the peak. The ABA content (pmol mg<sup>-1</sup> FW) was determined by peak area and was  
167 calculated based on a calibration curve constructed from the matrix-matched calibration standards.

168

169 *2.7. Analysis of biogenic volatile organic compounds*

170 The BVOCs evaluation was conducted on three grams of shoots using a Supelco Solid Phase Micro  
171 Extraction (SPME) (Supelco, Bellefonte, PA, USA) with polydimethylsiloxane (PDMS, 100 µm) at  
172 day 14. Each sample was introduced into a 100 ml glass conical flask and equilibrated for 30 min at  
173 25°C. After the equilibration time, the fibre was exposed to the headspace for 15 min at room  
174 temperature. Once sampling was complete, the fibre was withdrawn into the needle and transferred  
175 to the injection port of the Gas Chromatography–Electron Impact Mass Spectrometry (GC-EIMS)  
176 system, where the fibre was desorbed. GC-EIMS analysis was performed with a Varian CP 3800  
177 gas chromatograph (Varian, Inc., Palo Alto, CA) equipped with a DB-5 capillary column  
178 (30 m × 0.25 mm; coating thickness 0.25 µm) and a Varian Saturn 2000 ion trap mass detector

179 chromatograph (Varian, Inc., Palo Alto, CA). The analytical conditions were as follows: injector  
180 and transfer line temperature at 250°C and 240°C, respectively; oven temperature programmed  
181 from 60°C to 240°C at 3°C min<sup>-1</sup>; helium as carrier gas set at 1 mL min<sup>-1</sup>; and injection in splitless  
182 mode. Identification of the constituents was conducted via comparison of the retention times with  
183 those of the authentic samples, and computer matching against commercial (Adams, 1995) and  
184 home-made library mass spectra built from pure substances and MS literature data (Davies, 1990).

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

187

#### 188 2.8. *Essential oil isolation*

189 Twenty grams of dried leaves were hydrodistilled using a Clevenger-type apparatus (2 h), **in**  
190 **according to the procedure described in the Italian Pharmacopoeia (Farmacopea Ufficiale della**  
191 **Repubblica Italiana, 1991)**. The yields of distillation were not determined due to the low amount of  
192 the starting plant material. The EOs obtained were solubilised in *n*-hexane, dried over anhydrous  
193 sodium sulphate and filtered, and then stored in a vial at 4°C in the dark until use. GC-EIMS was  
194 used to analyse all of the EOs obtained (injection of 0.2 µL), as reported by Caser et al. (2016).

195

#### 196 2.9. *RNA isolation and RT-PCR analysis*

197 The leaves collected from six plants per irrigation level at the end of the experiment (day 34) were  
198 pooled to form three biological replicates (two plants for each biological replicate). Total RNA was  
199 extracted using the Spectrum™ Plant Total RNA extraction kit (Sigma Aldrich), starting from 80  
200 mg of material, and the RNA quantity was checked using a NanoDrop 1000 spectrophotometer  
201 (Thermo Fisher Scientific). RNA was then treated with DNase I (Invitrogen, Thermo Fisher  
202 Scientific) in accordance with the manufacturer's instructions. For each biological replicate, first-  
203 strand cDNA was synthesised, starting from 500 ng of total RNA using the High Capacity cDNA  
204 Reverse Transcription kit (Applied Biosystems, Thermo Fisher Scientific) according to the

205 manufacturer's instructions. Given the absence of the *S. dolomitica* reference genome, gene-specific  
206 primers (Table 1) were selected on the basis of the phylogenetically closest species, *S. miltiorrhiza*,  
207 and designed using Primer Express® software (v3.0, Applied Biosystems, Thermo Fisher  
208 Scientific). Reactions were carried out using Power SYBR® Green PCR Master Mix (Applied  
209 Biosystems, Thermo Fisher Scientific) as reported in Chitarra et al. (2017). Three technical  
210 replicates were run for each biological replicate, and the expression of target genes was quantified  
211 following normalisation to the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) housekeeping  
212 gene. The results were calculated as expression ratios (Relative Quantity, RQ) to control (WW). **In  
213 addition, amplified product identities were confirmed by sequencing using the dideoxy chain  
214 termination method at BioFab Research (Rome, Italy). The obtained sequences were searched in  
215 NCBI database using BLASTn tool as previously reported by Nerva et al. (2016).**

216

### 217 2.10. Statistical methods

218 The data were first tested for the variance homogeneity. All of the measured and derived data were  
219 then subjected to a post hoc test using the Ryan-Einot-Gabriel-Welsch-F test (REGW-F) and  
220 Tukey's test for gene expression analyses. The critical value for statistical significance was  $P <$   
221 0.05. All computations were conducted with SPSS statistical package (version 21.0; SPSS Inc.,  
222 Chicago, Illinois). Principal Coordinate Analysis (PCA)–biplot was subsequently performed using  
223 PAST 3.20. Eigenvalues were calculated using a covariance matrix among 34 traits as input, and the  
224 two-dimensional PCA biplot (including both drought treatments and morphological, biochemical,  
225 physiological and molecular constituents) was constructed.

226

## 227 3. Results and discussion

### 228 3.1. Plant growth

229 Moderate drought stress (MDS) and severe drought stress (SDS) reduced growth (G.I.) starting  
230 from day 21 relative to well-watered (WW) (Table 2). MDS and SDS also drastically reduced the

231 total (-74% and -83%, respectively), aerial (-80% and -82%, respectively), and root dry biomass (-  
232 60% and -85%, respectively) compared with the control (Table 3).

233 Dehydration often diminishes overall plant growth (Caser et al., 2012, 2016, 2017, 2018;  
234 Soni and Abdin, 2017) owing to the considerable reduction of photosynthesis, cell turgidity and cell  
235 growth as well as increasing evapotranspiration (Rahimi et al., 2017). This has been noted in the  
236 case of numerous MAPs belonging to Labiatae, such as *Mentha pulegium* (Hassanpour et al., 2014),  
237 *M. piperita* (Rahimi et al., 2017), *M. spicata* (Delfine et al., 2005), and *Rosmarinus officinalis*  
238 (Delfine et al., 2005). Within the genus *Salvia*, different morphological responses to drought have  
239 been reported in the literature. No differences in biomass production were observed in drought-  
240 stressed *S. officinalis* plants (Radwan et al., 2017), but a reduction was seen in other *Salvia* species  
241 such as *S. splendens* (Burnett et al., 2005), *S. miltiorrhiza* (Liu et al., 2011), and in a previous study  
242 on *S. dolomitica* (Caser et al., 2012).

243 *S. dolomitica* significantly increased the root to aerial ratio when grown in MDS in  
244 comparison to WW (+100%) and SDS treatments (+244%). Roots represent the only source of  
245 acquiring water from the soil, and so root density and size constitute key plant responses to drought  
246 stress. Mediterranean plant species typically have higher R:A ratios than plants from more mesic  
247 biomes (Valliere and Allen, 2016), potentially due to adaptation to seasonal drought. Furthermore,  
248 Mahajan and Tuteja (2005) have argued that leaves are generally more sensitive to stress than are  
249 roots. This often results in an increase in R:A when water is limited, as also seen in *S. sinaloensis*,  
250 *Allium cepa*, and *Artemisia californica* (Caser et al., 2018; Farooq et al., 2009; Valliere and Allen,  
251 2016). However, when drought conditions are excessive, a dramatic reduction of roots can also be  
252 identified, as already proven in the case of *Helichrysum petiolare* (Caser et al., 2016).

253

### 254 3.2. Photosynthetic pigments

255 A reduction in chlorophyll and carotenoid content in plants subjected to drought stress is commonly  
256 acknowledged in several species, including MAPs (Caser et al., 2016). In *S. dolomitica*, only SDS

257 significantly reduced the content of both pigments compared with WW and MDS, starting from day  
258 21 (total chlorophylls: 1.46, 1.45 and 1.19 mg g<sup>-1</sup>; carotenoids: 2.93, 2.75 and 1.54 mg g<sup>-1</sup> in WW,  
259 MDS and SDS, respectively) up to their senescence (Table 4), and combined with a simultaneous  
260 growth reduction, as previously seen in *S. sinaloensis* (Caser et al., 2018). In contrast, in *S.*  
261 *officinalis*, a considerable reduction in chlorophyll content has been observed in plants treated with  
262 MDS (-78.5%) (Bettaieb et al., 2011).

263 According to Flexas and Medrano (2002), green leaf colour in C<sub>3</sub> plants can be reduced by  
264 exacerbated drought stress. However, in the present study no significant differences in SPAD values  
265 among treatments were noted (Table 4), as previously found in *S. dolomitica* and *S. sinaloensis* by  
266 Caser et al. (2012, 2018).

267

### 268 3.3. Phenols, flavonoids and antioxidant activity

269 Drought induces oxidative stress in plants, in which reactive oxygen species (ROS) are commonly  
270 produced (Munné-Bosch and Peñuelas, 2003). Polyphenols and flavonoids are among the most  
271 adaptable natural compounds, enabling plants to scavenge ROS (di Ferdinando et al., 2014). An  
272 increase in phenolic compound biosynthesis has been noted in drought-stressed plants of *Labisia*  
273 *pumila* (Jaafar et al., 2012), *Salvia officinalis* (Radwan et al., 2017), and *S. sinaloensis* (Caser et al.,  
274 2018).

275 In the present study, the rate of total phenols, flavonoids and antioxidant activity in treated  
276 plants was monitored during the entire experiment (Table 5). *S. dolomitica* plants subjected to SDS  
277 conditions exhibited a significant decrease in the content of total phenols, flavonoids and  
278 antioxidant activity from day 4 until day 11. Subsequently, no differences occurred between  
279 treatments in total phenols and flavonoids until the end of the experiment, with the exception of day  
280 32 (29.0 and 12.7 mg GAEg<sup>-1</sup> of phenols and 8.3 and 5.7 mg g<sup>-1</sup> of flavonoids in WW and MDS,  
281 respectively). Regarding antioxidant activity, at day 25 SDS stimulated a significant increase  
282 compared with other treatments (98.7, 101.5 and 153.1 μmol Fe<sup>2+</sup>g<sup>-1</sup> in WW, MDS and SDS,

283 respectively). This time point coincided with the complete senescence of the SDS plants (Table 2).  
284 At the end of the measurements (day 25 for SDS and day 34 for WW and MDS), the total amount  
285 of total phenols, flavonoids, and antioxidant activity per plant was estimated on the basis of the  
286 fresh plant biomass (Table 5). These highlighted that all of the parameters were substantially  
287 reduced by MDS and SDS compared with WW (305.2, 53.2 and 20.5 mg GAEg<sup>-1</sup> of phenols, 105.7,  
288 17.1 and 5.3 mg g<sup>-1</sup> of flavonoids and 1815.8, 337.5 and 134.7 μmol Fe<sup>2+</sup>g<sup>-1</sup> of antioxidant activity,  
289 respectively).

290 Considering that drought-tolerant species are known to increase the accumulation of  
291 antioxidants, which help protect plant cells from ROS (Moradi et al., 2017), these results suggest  
292 that *S. dolomitica* is a drought-sensitive species. A small amount of these metabolites was produced  
293 under drought stress conditions in *S. miltiorrhiza*, too (Liu et al., 2011).

294

### 295 3.4. Ecophysiological traits

296 Diminished levels of pigments (chlorophyll and carotenoids) under increasing drought stress  
297 conditions indicated the depressed physiological needs of photosynthetic activity to limit water  
298 losses, thus resulting in lower growth. As expected, in *S. dolomitica*, water shortage affected the  
299 midday leaf water potential (MLWP), internal CO<sub>2</sub> concentration (C<sub>i</sub>), transpiration rate (*E*),  
300 stomatal conductance (*g<sub>s</sub>*), and net photosynthetic rate (*A*) (Figure 1).

301 The MLWP in the WW plants remained constant during the entire experiment (-0.34 MPa)  
302 (Fig. 1A). In MDS plants, MLWP was significantly lower on days 18, 28, 32 and 34 (-0.40, -0.52, -  
303 0.50 and -0.46 MPa, respectively) compared with the controls. Severe drought stress significantly  
304 constantly and reduced MLWP from day 7 (-0.53 MPa) until day 25 (-1.00 MPa), when complete  
305 leaf withering occurred. Within the genus *Salvia*, similar results were also found in *S. splendens*  
306 ‘Bonfire’ and *S. sinaloensis*, whose leaves reached an LWP of -1.40 and -1.10 MPa under similar  
307 severe drought conditions, respectively (Caser et al., 2018; Eakes et al., 1991). Furthermore, *S.*

308 *officinalis* and *S. mellifera* plants under the same stress conditions displayed much lower LWP (-4.8  
309 and -8.0 MPa, respectively) (Bettaieb et al., 2011; Hargrave et al., 1994).

310 No differences in  $C_i$  were observed between WW and MDS plants, ranging between 255.0  
311 and 483.4  $\mu\text{mol mol}^{-1}$  during the experiment (Fig. 1B), while a significant increase in SDS plants  
312 was observed from day 14 (423.0  $\mu\text{mol mol}^{-1}$ ) to day 25 (493.0  $\mu\text{mol mol}^{-1}$ ). Similarly, in *E* no  
313 differences between WW and MDS plants were highlighted (Fig. 1C), whereas a significant decline  
314 occurred in SDS plants on days 4, 7, 11, 14 and 25 relative to the other treatments. Regarding  
315 stomatal conductance ( $g_s$ ) (Fig. 1D), differences between WW and MDS plants occurred on days 7,  
316 21, 25, 28 and 34. SDS plants showed a significant and constant decrease starting from day 7 (0.10  
317  $\text{mmol m}^{-2} \text{s}^{-1}$ ) until complete senescence (0.01  $\text{mmol m}^{-2} \text{s}^{-1}$ ). Net photosynthetic rate ( $A$ ) (Fig. 1E)  
318 followed a similar trend in the SDS treatment, starting from day 14 (1.78  $\mu\text{mol mol}^{-2} \text{s}^{-1}$ ). The  
319 differences between WW and MDS appeared only on day 25 (15.32 and 5.16  $\mu\text{mol mol}^{-2} \text{s}^{-1}$ ,  
320 respectively). The decrease of photosynthetic activity under drought stress may be due to stomatal  
321 or non-stomatal mechanisms. In drought-tolerant species, the reduction of photosynthesis owes to  
322 stomatal closure and the limitation of water losses. In drought-sensitive plants, the reduction of net  
323 photosynthesis is primarily due to water shortage, inducing severe damage in plants. Here, SDS  
324 considerably reduced the assimilation processes, with a significant decrease of  $g_s$  and saving  
325 internal  $\text{CO}_2$ , suggesting an efficient adaptive stomatal modulation.

326

### 327 3.5. Endogenous ABA content

328 Abscisic acid (ABA) is known to be synthesised under different stress conditions, either at the root  
329 or leaf level. ABA has an inhibitory effect on cell growth and leads to the depolarisation of guard  
330 cell membranes, triggering osmotic ion efflux and the loss of guard cell turgor (McAdam and  
331 Brodribb, 2016).

332 Endogenous ABA content in the leaves of *S. dolomitica* under WW and MDS plants did  
333 not differ during the entire experiment, with a mean value equal to 0.16  $\text{pmol mg}^{-1}$  (Fig. 1F). On the

334 contrary, SDS stimulated a considerable increase in hormone concentration even at day 7 (~14 fold  
335 more than WW and MDS) until complete plant senescence (~39 fold more than WW and MDS).  
336 Endogenous ABA plays an important role in drought adaptation, and in *S. dolomitica* it rapidly  
337 increased under severe water shortage conditions, enhancing drought tolerance, as has been  
338 observed in the leaves of *Cichorium intybus* treated with similarly severe drought conditions  
339 (Ghanaatiyan and Sadeghi, 2017).

340 Within the genus *Salvia*, few studies have reported the content of ABA in response to non-  
341 optimal growing conditions. Kondrat'eva et al. (2008) found an increase of ABA in *S. sclarea* under  
342 cold stress (ranging between 5.1 and 7.1 pmol mg<sup>-1</sup>), while Asensi-Fabado et al. (2013) attained  
343 similar findings in the case of *S. officinalis* under heat stress (ranging between 3.0 and 6.0 pmol mg<sup>-1</sup>).  
344 <sup>1</sup>).

345

### 346 3.6. Biogenic volatile organic compounds production

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

355 The total emitted and identified BVOCs from the analysed shoots of *S. dolomitica* are  
356 displayed in Table 6. Overall, a number of 36, 33 and 37 compounds were recognised in WW, MDS  
357 and SDS plants, accounting for 94.43%, 81.38% and 98.18% of the total compositions,  
358 respectively. Figure 2A shows how the main volatile fractions changed in *S. dolomitica* plants  
359 subjected to different drought treatments. Well-watered plants were mainly characterised by

360 monoterpene hydrocarbons (mh); this volatile fraction considerably decreased due to increasing  
361 stress conditions (57.71%, 30.97% and 29.41% in WW, MDS and SDS, respectively). Conversely,  
362 under MDS and SDS conditions, an increase in sesquiterpene hydrocarbons (sh) was highlighted  
363 (34.09%, 47.19% and 66.32% in WW, MDS and SDS, respectively). Drought conditions also  
364 somewhat affected the production of the other reported volatile molecule class, the oxygenated  
365 monoterpene (om) (1.72%, 2.92% and 2.19% in WW, MDS and SDS, respectively).

366 Several recent reviews have addressed the role of BVOCs in enhancing the tolerance of  
367 plants to various general abiotic stressors (Possell and Loreto, 2013). However, the literature  
368 concerning BVOC emission in relation to water availability is ambiguous. *S. dolomitica*, as true of  
369 other Labiatae species, accumulate terpenes in specialised structures (i.e. glandular hairs) (Bassolino  
370 et al., 2015) and their terpene emission is deemed the consequence of terpene volatilisation from  
371 these structures, which is generally temperature-dependent (Llusia and Peñuelas, 2000). In the  
372 present study, plants under severe stress conditions demonstrated an increase in the total amount of  
373 analysed components and a substantial decline in hydrogenated monoterpenes in concomitance  
374 with a sharp increase in hydrogenated sesquiterpenes. Llusia and Peñuelas (1998) have reported that  
375 a reduction in monoterpene emission under severe drought conditions may be expected due to  
376 stomatal closure. However, sesquiterpenes are not generally emitted in large amounts (Possell and  
377 Loreto, 2013), although they can be enhanced by biotic and abiotic stresses as an indirect defence  
378 mechanism.

379 All of the investigated headspaces exhibited different amounts of the main constituents. The  
380 chemical profile in WW plants was characterised by Limonene >  $\Delta$ -3-carene > Germacrene D >  $\beta$ -  
381 Caryophyllene > (E)- $\beta$ -ocimene, in MDS plants, by Germacrene D > Limonene >  $\beta$ -Caryophyllene  
382 >  $\alpha$ -guaiene >  $\Delta$ -3-carene, and in SDS plants by Germacrene D > Limonene > Bicyclogermacrene >  
383  $\beta$ -Caryophyllene >  $\alpha$ -guaiene. Among the cited constituents, a very sharp increase (~+260%) was  
384 observed for the sesquiterpene hydrocarbons Germacrene D (from 8.57% to 22.35% and 22.16% in  
385 WW and MDS and SDS, respectively). In contrast, the monoterpene hydrocarbon (E)- $\beta$ -ocimene

386 reduced by ~60% when plants were subjected to MDS and SDS. Arey et al. (1995) have suggested  
387 that sesquiterpene emission in *S. mellifera*, which is primarily comprised of  $\beta$ -caryophyllene and  
388 Germacrene D, is not dependent on season, but any disturbance to plants may exert an influence on  
389 the total observed emission variability. Few studies have reported the impact of drought on volatile  
390 sesquiterpene emissions in MAPs, and results have to date been inconsistent. Ormeño et al. (2007)  
391 have observed a reduction in sesquiterpenes (allo-aromadendrene,  $\alpha$ -zingiberene and  $\alpha$ -cadinene) in  
392 drought-stressed *Rosmarinus officinalis* plants, while an increase in Germacrene D was observed in  
393 *Thymus vulgaris* and *T. serpyllum* (Moradi et al., 2017).

394

### 395 3.7. Essential oils

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

399 A total of 82 constituents were detected in the investigated EOs, 42, 46 and 52 of which  
400 were in WW, MDS and SDS plants, respectively (Table 7). Drought stress conditions only  
401 stimulated a slight decrease in the total amount of the identified constituents (97.3, 95.5 and 95.9%  
402 at WW, MDS and SDS plants, respectively), but affected the main chemical classes, especially the  
403 sesquiterpenes (Fig. 2B). The oxygenated monoterpenes were reduced under stress conditions (7.5%,  
404 1.1% and 1.5%, in WW, MDS and SDS, respectively), as well as the amount of the sesquiterpene  
405 hydrocarbons (53.4%, 32.9% and 33.2% in WW, MDS and SDS, respectively), while drought stress  
406 increased the oxygenated sesquiterpenes (26.0%, 53.2% and 53.1% in WW, MDS and SDS,  
407 respectively).

408 The main constituent in WW EOs was the sesquiterpene hydrocarbon,  $\beta$ -Caryophyllene.  
409 This constituent diminished considerably with drought (21.2%, 0.6% and 0.6% in WW, MDS and  
410 SDS, respectively). However, in stressed plants the main constituent comprised the oxygenated  
411 sesquiterpene Longipinalol, which increased significantly under drought conditions (0.8%, 41.9%

412 and 41.5% in WW, MDS and SD, respectively). Specifically, the chemical profile of WW plants  
413 was composed of  $\beta$ -Caryophyllene >  $\delta$ -cadinene > 1H-cyclopropanaphtalene >  $\alpha$ -eudesmol > epi- $\alpha$ -  
414 cadinol, whereas for MDS and SDS by Longipinalol > Trans- $\beta$ -guaiene >  $\beta$ -pinene >  $\alpha$ -humulene >  
415  $\delta$ -cadinene.

416 Within the genus *Salvia*, drought stress resulted in a slight increase in the total amount of  
417 EO constituents in *S. officinalis* (i.e. camphor,  $\alpha$ -thujone and 1.8-cineole) (Bettaieb et al., 2009) and  
418 *S. sinaloensis* (i.e. camphor) (Caser et al., 2018). *S. dolomitica* EOs were previously evaluated by  
419 Kamatou et al. (2007a) in South African wild plants and by Bassolino et al. (2015) in potted  
420 cultivated plants. Surprisingly, these profiles differed substantially. Wild plants primarily contained  
421 oxygenated monoterpenes (71.8%), while cultivated plants were largely composed of hydrocarbons  
422 (71.5%) and oxygenated sesquiterpenes (13.6%), with  $\beta$ -caryophyllene as the main constituent. In  
423 our study, WW plants presented a profile similar to that found by Bassolino et al. (2015). These  
424 variations in EO compositions may have arisen due to several factors (climatical, seasonal,  
425 geographical, geological and extraction method), as mentioned by González-Coloma et al. (2011) in  
426 the case of other Labiatae species.

427 Sesquiterpenes represent an extremely large and heterogeneous group of natural compounds.  
428 Given that these compounds play an essential role in plant defence response, their accumulation  
429 under abiotic stress is consistent with carbon balance theory, which states that investment in plant  
430 defence increases in response to a growth limitation. As an example, large amounts of  
431 sesquiterpenes were observed in *Inula montana* plants subjected to different abiotic stresses (i.e.  
432 altitude, drought and soil composition) (Roux et al., 2017). In this work, plants subjected to drought  
433 exhibited a reduction in hydrocarbon sesquiterpenes and an increase in oxygenated sesquiterpenes  
434 (Fig. 3). These dynamics may be considered a defence mechanism against a hostile environment,  
435 such as intense light or water shortage.

436

437 3.8. Genes involved in terpenoid biosynthesis

438 Plants adapt to biotic and abiotic stress by modulating the expression of genes responsible during  
439 both primary and secondary metabolism (Dolzhenko et al., 2010). **Dehydrin** is one of the most  
440 important genes expressed in plants during water deficit conditions (George et al., 2017). These  
441 proteins of the LEA family help maintain large amounts of water inside the plant cell during water  
442 stress, thereby protecting the plant's proteins and biomembranes (Battaglia et al., 2008).

443 Here, the expression profile of the dehydrin gene (*DH*) increased concurrently with the  
444 degree of water stress (c.a. 1.5 and 3 fold in MDS and SDS compared with WW, respectively) (Fig.  
445 3 in the box), confirming the ways in which the plants perceived the drought stress and activated  
446 particular molecular responses. A similar trend was seen for the following genes that code for  
447 enzymes involved in the terpenoid biosynthesis: geranyl diphosphate synthases (GPPS), farnesyl  
448 diphosphate synthase (FPPS), geranylgeranyl diphosphate synthase (GGPPS) and copalyl  
449 diphosphate synthases (CPS). As reported in *S. miltiorrhiza* (Wenping et al., 2011), GPPS catalyses  
450 the condensation of two units of isopentenyl pyrophosphate (IPP) and one unit of dimethyl allyl  
451 pyrophosphate (DMAPP) to form geranyl diphosphate (GPP), precursor of almost all of the  
452 monoterpenes, while FPPS catalysed the formation of farnesyl diphosphate (FPP), the precursor of  
453 almost all sesquiterpenes. Finally, GGPPS catalyses the formation of **geranylgeranyl** diphosphate  
454 (GGPP), the precursor of **diterpenes** (C<sub>20</sub>), carotenoids and chlorophylls, and CPS catalyses the  
455 cyclisation reaction that converts GGPP to form copalyl diphosphate (CPP).

456 In this study, all of the genes were upregulated in stressed plants. This was particularly  
457 evident for the FPPS and CPS2 genes (c.a. 18 and 8 fold, respectively) (Fig. 3C and F). Comparing  
458 the two drought stresses, aside from GPPS2D and CPS3 (Fig. 3B and G), the highest levels of  
459 expression were found in MDS plants. This highlights how the MDS treatment induced the  
460 transcriptional upregulation of different enzymes involved in terpenoid biosynthesis (and  
461 consequently BVOC and EO production) with greater efficiency.

462 Within the Labiatae family, CPS genes were isolated in *Salvia fruticosa*, *S. miltiorrhiza* and  
463 *R. officinalis* (Božićet al., 2015). Wenping et al. (2011) and Ma et al. (2012) have highlighted that

464 these genes have diverse expression patterns that are tightly controlled at different developmental  
465 stages (seed germination, seedling growth, vegetative stage and reproductive stage). As confirmed  
466 by our study, they also play important roles in interaction with environmental factors by inducing  
467 the biosynthesis of PSMs, as well as the other studied genes.

468

### 469 3.9 Principal Component Analysis (PCA)

470 In order to visualise congruence between WW, MDS and SDS plants on the basis of all of the  
471 morphological, physiological, metabolic and molecular variables, the whole dataset was subjected  
472 to a Principal Component Analysis (PCA; Figure. 4). The three plant groups were clearly divided  
473 by the first two components, which accounted for 52.29% and 40.58% of the variance. Well-  
474 watered plants were positively correlated to morphological parameters (growth index and dry  
475 biomass), ecophysiological traits (MLWP, *E*, *A* and *G*s), pigments (chlorophyll, carotenoids and  
476 SPAD), flavonoids and phenol content, antioxidant activity, and to the volatile monoterpene  
477 hydrocarbons and the sesquiterpene hydrocarbons, oxygenated monoterpenes, non-terpenoids and  
478 apocarotenoid components of the EOs. At the same time, the volatile oxygenated monoterpenes and  
479 the monoterpene hydrocarbons and oxygenated sesquiterpenes of EOs were mainly positively  
480 related to moderate drought conditions as well as to the expression of all of the studied genes  
481 related to the key enzymes of terpenoid biosynthesis. Finally, ABA, *Ci* and volatile sesquiterpene  
482 hydrocarbon content and the expression of the dehydrin gene were correlated to severe drought  
483 conditions. Thus, the multivariate analysis confirmed that moderate drought stress modified PSM  
484 biosynthesis without compromising the physiological status and morphological quality of *S.*  
485 *dolomitica* plants. Conversely, severe drought stress significantly reduced net photosynthesis and  
486 transpiration, while increasing endogenous ABA.

487

## 488 4. Conclusion

489 In summary, an integrated approach combining metabolomic and physiological studies facilitated  
490 the attainment of new insights regarding the mechanisms and processes involved in *S. dolomitica*  
491 drought adaptation. Plant secondary metabolites are a fascinating class of phytochemicals that  
492 exhibit immense chemical diversity. MAPs are commonly known to produce a wide range of these  
493 molecules with different industrial purposes. Here, drought stress led to modulate the expression of  
494 some of the genes involved in BVOC and EO biosynthesis, especially sesquiterpenes, a class of  
495 terpenoids of significant pertinence in the food, cosmetics and pharmaceutical industries. Given that  
496 previous studies have indicated that the EOs of *S. dolomitica* exhibit anti-plasmodial and anti-  
497 inflammatory activities (Fisher et al., 2005; Kamatou et al., 2007a, 2007b, 2008, 2010), is possible  
498 to speculate that moderate drought stress can be beneficial for PSM production in *S. dolomitica*.  
499 Furthermore, the possibility of ameliorating water-management practices in the MAP sector can be  
500 envisaged.

501

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509

510 **References**

- 511 Adams, R.P., 1995. Identification of essential oil components by gas chromatography-mass  
512 spectroscopy. Allured, Carol Stream, IL.
- 513 Arey, J., Crowley, D. E., Crowley, M., Resketo, M., Lester, J., 1995. Hydrocarbon emissions from  
514 natural vegetation in California's south coast air basin. Atmosph. Environ. 29, 2977-2988.
- 515 Asensi-Fabado, M. A., Oliván, A., Munné-Bosch, S., 2013. A comparative study of the hormonal  
516 response to high temperatures and stress reiteration in three Labiatae species. Environ. Exp.  
517 Bot. 94, 57-65.
- 518 Bassolino, L., Giacomelli, E., Giovanelli, S., Pistelli, L., Cassetti, A., Damonte, G., Bisio, A.,  
519 Ruffoni, B., 2015. Tissue culture and aromatic profile in *Salvia dolomitica* Codd. Plant Cell  
520 Tiss. Organ. 121, 83-95.
- 521 Battaglia, M., Olvera-Carrillo, Y., Garcarrubio, A., Campos, F., Covarrubias, A.A., 2008. The  
522 enigmatic LEA proteins and other hydrophilins. Plant Physiol. 148, 6-24.
- 523 Bettaieb, I., Hamrouni-Sellami, I., Bourgou, S., Limam, F., Marzouk, B., 2011. Drought effects on  
524 polyphenol composition and antioxidant activities in aerial parts of *Salvia officinalis* L. Acta  
525 Physiol. Plant. 475, 1103-1111.
- 526 Bettaieb, I., Zakhama, N., Aidi Wannes, W., Kchouk, M.E., Marzouk, B., 2009. Water deficit  
527 effects on *Salvia officinalis* fatty acids and essential oils composition. Sci. Hortic. 120, 271–  
528 275.
- 529 Bosco, R., Caser, M., Vanara, F., Scariot, V., 2013. Development of a rapid LC-DAD/FLD method  
530 for the simultaneous determination of auxins and abscisic acid in plant extracts. J. Agric.  
531 Food Chem. 61, 10940–10947.
- 532 Božić, D., Papaefthimiou, D., Brückner, K., de Vos, R.C.H., Tsoleridis, C.A., Katsarou, D.,  
533 Papanikolaou, A., Pateraki, I., Chatzopoulou, F.M., Dimitriadou, E., Kostas, S., Manzano, D.,  
534 Scheler, U., Ferrer, A., Tissier, A., Makris, A.M., Kampranis, S.C., Kanellis, A.K., 2015.  
535 Towards elucidating carnosic acid biosynthesis in Lamiaceae: functional characterization of

536 the three first steps of the pathway in *Salvia fruticosa* and *Rosmarinus officinalis*. PLoS ONE  
537 10: doi:10.1371/journal.pone.0124106.

538 Burnett, S.E., Pennisi, S.V., Thomas, P.A., van Iersel, M.W., 2005. Controlled drought affects  
539 morphology and anatomy of *Salvia splendens*. J. Amer. Soc. Hortic. Sci. 130, 775-781.

540 Caser, M., D'Angiolillo, F., Chitarra, W., Lovisolo, C., Ruffoni, B., Pistelli, Lu., Pistelli, La.,  
541 Scariot, V., 2016. Water deficit trigger changes in valuable physiological and phytochemical  
542 parameters in *Helichrysum petiolare* Hilliard & B.L. Burt. Ind. Crops Prod. 83, 680-692.

543 Caser, M., D'Angiolillo, F., Chitarra, W., Lovisolo, C., Ruffoni, B., Pistelli, Lu., Pistelli, La.,  
544 Scariot, V., 2018. Ecophysiological and phytochemical responses of *Salvia sinaloensis* Fern.  
545 to drought stress. Plant Growth Regul. 84, 383-394.

546 Caser, M., Lovisolo, C., Scariot, V., 2017. The influence of water stress on growth, ecophysiology  
547 and ornamental quality of potted *Primula vulgaris* 'Heidy' plants. New insights to increase  
548 water use efficiency in plant production. Plant Growth Regul. 83, 361-373.

549 Caser, M., Ruffoni, B., Scariot, V., 2012. Screening for drought tolerance in *Salvia* spp. and  
550 *Helichrysum petiolare*: a way to select low maintenance ornamental plants. Acta Hortic. 953,  
551 239-246.

552 Caser, M., Scariot, V., Gaino, W., Larcher, F., Devecchi, M., 2013. The effects of sodium chloride  
553 on the aesthetic value of *Buxus* spp. Eur. J. Hortic. Sci. 78, 153-159.

554 Chitarra, W., Perrone, I., Avanzato, C. G., Minio, A., Boccacci, P., Santini, D., Gilardi, G.,  
555 Siciliano, I., Gullino, M.L., Delledonne, M., Mannini, F., Gambino, G., 2017. Grapevine  
556 grafting: Scion transcript profiling and defense-related metabolites induced by rootstocks.  
557 Front. Plant Sci. 8, 654 doi:10.3389/fpls.2017.00654.

558 Davies, N.W., 1990. Gas chromatographic retention indexes of monoterpenes and sesquiterpenes on  
559 methyl silicone and carbowx20M phases. J. Chrom. 503, 1-24.

560 Delfine, S., Loreto, F., Pinelli, P. Tognetti, R., Alvino, A., 2005. Isoprenoids content and  
561 photosynthetic limitations in rosemary and spearmint plants under water stress. *Agric.*  
562 *Ecosyst. Env.* 106, 243-252.

563 Demasi, S., Caser, M., Handa, T., Kobayashi, N., De Pascale, S., Scariot, V., 2017. Adaptation to  
564 iron deficiency and high pH in evergreen azaleas (*Rhododendron* spp.): potential resources for  
565 breeding. *Euphytica* 213, 148.

566 di Ferdinando, M., Brunetti, C., Agati, G., Tattini, M., 2014. Multiple functions of polyphenols in  
567 plants inhabiting unfavorable Mediterranean areas. *Environ. Exp. Bot.* 103, 107-116.

568 Dicke, M., Loreto, F., 2010. Induced plant volatiles: from genes to climate change. *Trends Plant*  
569 *Sci.* 15, 115-117.

570 Dolzhenko, Y., Berteà, C.M., Occhipinti, A., Bossi, A., Maffei, M.E.J., 2010. UV-B modulates the  
571 interplay between terpenoids and flavonoids in peppermint (*Mentha x piperita* L.).  
572 *Photochem. Photobiol. B* 100, 67-75.

573 Eakes, D.J., Wright, R.D., Seiler, J.R., 1991. Moisture stress conditioning effects on *Salvia*  
574 *splendens* ‘Bonfire’. *J. Amer. Soc. Hort. Sci.* 116, 716-719.

575 **Farmacopea Ufficiale della Repubblica Italiana, 9th Edt, Istituto Poligrafico Zecca dello Stato,**  
576 **Roma, 1991.**

577 Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., 2009. Plant drought stress:  
578 effects, mechanisms and management. In: Lichtfouse E., Navarrete M., Debaeke P.,  
579 Véronique S., Alberola C. (eds) *Sustainable Agriculture*. Springer, Dordrecht.

580 Fisher, V.L., 2005. Indigenous *Salvia* species—an investigation of the antimicrobial activity, anti-  
581 oxidant activity and chemical composition of leaf extracts. M.Sc. Dissertation. University of  
582 the Witwatersrand, Johannesburg, South Africa.

583 Flexas, J., Medrano, H., 2002. Drought-inhibition of photosynthesis in C3 plants: stomatal and non-  
584 stomatal limitations revisited. *Ann. Bot.* 89, 183-189.

585 George, K.J., Malik, N., Kumar, I.P.V., Krishnamurthy, K.S., 2017. Gene expression analysis in  
586 drought tolerant and susceptible black pepper (*Piper nigrum* L.) in response to water deficit  
587 stress. *Acta Physiol. Plant.* 39, 104.

588 Ghanaatiyan, K., Sadeghi, H., 2017. Differential responses of chicory ecotypes exposed to drought  
589 stress in relation to enzymatic and non-enzymatic antioxidants as well as ABA concentration.  
590 *J. Hortic. Sci. Biotech.* 92, 404-410.

591 González-Coloma, A., Delgado, F., Rodilla, J. M., Silva, L., Sanz, J., Burillo, J., 2011. Chemical  
592 and biological profiles of *Lavandula luisieri* essential oils from western Iberia Peninsula  
593 populations. *Biochem. Syst. Ecol.* 39, 1-8.

594 Hargrave, K.R., Kolb, K.J., Ewers, F.W., Davis, S.D., 1994. Conduit diameter and drought-induced  
595 embolism in *Salvia mellifera* Greene (Labiatae). *New Phytol.* 126, 695-705.

596 Hassanpour, H., Khavari-Nejad, R.A., Niknam, V., Razavi, K., Najafi, F., 2014. Effect of  
597 penconazole and drought stress on the essential oil composition and gene expression of  
598 *Mentha pulegium* L. (Lamiaceae) at flowering stage. *Acta Physiol. Plant.* 36, 1167-1175.

599 Holopainen, J.K., Gershenzon, J., 2010. Multiple stress factors and the emission of plant VOCs.  
600 *Trends Plant Sci.* 15, 176-184.

601 Jaafar, H.Z.E., Ibrahim, M.H., Fakri, N.F.M., 2012. Impact of soil field water capacity in secondary  
602 metabolites, phenylalanine ammonia-lyase (PAL), malondialdehyde (MDA) and  
603 photosynthetic responses of Malaysian kacip Fatimah (*Labisia pumila* Benth). *Molecules.* 17,  
604 7305-7322.

605 Kamatou, G.P.P., Makunga, N.P., Ramogola, W.P.N., Viljoen, A.M., 2008. South African *Salvia*  
606 species: a review of biological activities and phytochemistry. *J. Ethnopharm.* 119, 664-672.

607 Kamatou, G.P.P., Van Vuuren, S.F., Van Heerden, F.R., Seaman, T., Viljoen, A.M., 2007a.  
608 Antibacterial and antimycobacterial activities of South African *Salvia* species and isolated  
609 compounds from *S. chamelaegnea*. *South Afr. J. Bot.* 73, 552-557.

610 Kamatou, G.P.P., Viljoen, A.M., Figueiredo, A.C., Tilney, P.M., Van Zyl, R.L., Van Vuuren, S.F.,  
611 Pedro, L.G., Barroso, J.G., (2007b). Glandular trichomes, essential oil composition and  
612 biological activities of *Salvia albicaulis* Benth and *S. dolomitica* Codd, two species from the  
613 Cape region of South Africa. *South Afric. J. Bot.* 73, 102-108.

614 Kamatou G.P.P., Viljoen, A.M., Steenkamp, P., 2010. Antioxidant, antiinflammatory activities and  
615 HPLC analysis of South African *Salvia* species. *Food Chem.* 119, 684-688.

616 Kim, D.O., Jeong, S.W., Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemicals from  
617 various cultivars of plums. *Food Chem.* 81, 321-326.

618 Kleinwächter, M., Paulsen, J., Bloem, E., Schnug, E., Selmar, D., 2015. Moderate drought and  
619 signal transducer induced biosynthesis of relevant secondary metabolite in thyme (*Thymus*  
620 *vulgaris*), greater celandine (*Chelidonium majus*) and parsley (*Petroselinum crispum*). *Ind.*  
621 *Crops Prod.* 64, 158-166.

622 Kleinwächter, M., Selmar, D., 2014. Influencing the Product Quality by Applying Drought Stress  
623 During the Cultivation of Medicinal Plants. In: Ahmad P., Wani M. (eds) *Physiological*  
624 *Mechanisms and Adaptation Strategies in Plants Under Changing Environment.* Springer,  
625 New York, NY.

626 Kleinwächter, M., Selmar, D., 2015. New insights explain that drought stress enhances the quality  
627 of spice and medicinal plants: potential applications. *Agron. Sust. Develop.* 35, 121-131.

628 Kondrat'eva, V.V., Voronkova, T.V., Shelepova, O.V., Olekhovich, L.S., 2008. Physiological and  
629 biochemical aspects of clary (*Salvia sclarea* L.) overwintering in central Russia. *Biol. Bull.*  
630 35, 255-261.

631 Liu, H., Wang, X., Wang, D., Zou, Z., Liang, Z., 2011. Effect of drought stress on growth and  
632 accumulation of active constituents in *Salvia miltiorrhiza* Bunge. *Ind. Crops Prod.* 33, 84-88.

633 Llusia, J., Peñuelas, J., 1998. Changes in terpene content and emission in potted Mediterranean  
634 woody plants under severe drought. *Can. J. Bot.* 76, 1366-1373.

635 Llusia, J., Peñuelas, J., 2000. Seasonal patterns of terpene content and emission from seven  
636 Mediterranean woody species in field conditions. *Am. J. Bot.* 87, 133-140.

637 Loreto, F., Dicke, M., Schnitzler, J.P., Turlings, T.C.J., 2014. Plant volatiles and the environment.  
638 *Plant Cell Environ.* 37, 1905-1908.

639 Loreto, F., Schnitzler, J. P., 2010. Abiotic stresses and induced BVOCs. *Trends Plant Sci.* 15, 154-  
640 166.

641 Ma, Y., Yuan, L., Wu, B., Li, X., Chen, S., Lu, S., 2012. Genome-wide identification and  
642 characterization of novel genes involved in terpenoid biosynthesis in *Salvia miltiorrhiza*. *J.*  
643 *Exp. Bot.* 63, 2809-2823.

644 Mahajan, S., Tuteja, N., 2005. Cold, salinity and drought stresses: an overview. *Arch. Biochem.*  
645 *Biophys.* 444, 139-158.

646 Mandoulakani, B.A., Eyvazpour, E., Ghadimzadeh, M., 2017. The effect of drought stress on the  
647 expression of key genes involved in the biosynthesis of phenylpropanoids and essential oil  
648 components in basil (*Ocimum basilicum* L.). *Phytochemistry.* 139, 1-7.

649 McAdam, S.A.M., Brodribb, T.J., 2016. Linking turgor with ABA biosynthesis: implications for  
650 stomatal responses to vapour pressure deficit across land plants. *Plant Physiol.* 171, 2008-  
651 2016. DOI: <https://doi.org/10.1104/pp.16.00380>.

652 Mitchell, P.J., O'Grady, A.P., Tissue, D.T., White, D.A., Ottenschlaeger, M.L., Pinkard, E.A.,  
653 2013. Drought response strategies derfine the relative cnotributions of hydraulic dysfunction  
654 and carbohydrate depletion during tree mortality. *New Phytol.* 197, 862-872.

655 Moradi, P., Ford-Lloyd, B., Pritchard, J., 2017. Metabolomic approach reveals the biochemical  
656 mechanisms underlying drought stress tolerance in thyme. *Anal. Biochem.* 527, 49-62.

657 Morales, C.G., Pino, M.T., del Pozo, A., 2013. Phenological and physiological responses to drought  
658 stress and subsequent rehydration cycles in two raspberry cultivars. *Sci. Hortic.* 162, 234-241.

659 Munné-Bosch, S., Peñuelas, J., 2003. Photo- and antioxidative protection, and a role for salicylic  
660 acid during drought and recovery in field-grown *Phillyrea angustifolia* plants. *Planta*. 217,  
661 758-766.

662 Nerva, L., Ciuffo, M., Vallino, M., Margaria, P., Varese, G.C., Gnani, G., Turina, M. 2016.  
663 Multiple approaches for the detection and characterization of viral and plasmid symbiont from  
664 a collection of marine fungi. *Virus Research*. 219, 22-38.

665 Niinemets, Ü., Kännaste, A., Copolovici, L., 2013. Quantitative patterns between plant volatile  
666 emissions induced by biotic stresses and the degree of damage. *Front. Plant Sci.* 4, 262.

667 Nogués, I., Muzzini, V., Loreto, F., Bustamante, M.A., 2015. Drought and soil amendment effects  
668 on monoterpene emission in rosemary plants. *Sci. Total Environ.* 538, 768-778.

669 Ormeño, E., Mévy, J.P., Vila, B., Bousquet Mélou, A., Greff, S., Bonin, G., Fernandez, C., 2007.  
670 Water deficit stress induces different monoterpene and sesquiterpene emission changes in  
671 Mediterranean species. Relationship between terpene emissions and plant water potential.  
672 *Chemosphere*. 67, 276-636.

673 Possell, M., Loreto, F., 2013. The role of volatile organic compounds in plant resistance to abiotic  
674 stresses: responses and mechanisms, in: Niinemets, Ü., Monson, R.K. (Eds.), *Biology,*  
675 *Controls and Models of Tree Volatile Organic Compound Emissions*, Springer, Berlin, pp.  
676 209-235.

677 Prsic, S., Xu, M., Wilderman, P.R., Peters, R.J., 2004. Rice contains two disparate *ent*-copalyl  
678 diphosphate synthases with distinct metabolic functions. *Plant Physiol.* 136, 4228-4236.

679 Radwan, A., Kleinwächter, M., Selmar, D., 2017. Impact of drought stress on specialised  
680 metabolism: biosynthesis and the expression of monoterpene synthases in sage (*Salvia*  
681 *officinalis*). *Phytochemistry*. 141, 20-26.

682 Rahimi, Y., Taleei, A., Ranjbar, M., 2017. Changes in the expression of key genes involved in the  
683 biosynthesis of menthol and menthofuran in *Mentha piperita* L. under drought stress. *Acta*  
684 *Physiol. Plant.* 39, 203.

685 Raut, J.S., Karuppayil, S.M., 2014. A status review on the medicinal properties of essential oils.  
686 Ind. Crops Prod. 62, 250-264.

687 Roux, D., Alnaser, O., Garayev, E., Baghdikian, B., Elias, R., Chiffolleau, P., Ollivier, E., Laurent,  
688 S., El Maataoui, M., Sallanon, H., 2017. Ecophysiological and phytochemical characterization  
689 of wild populations of *Inula montana* L. (Asteraceae) in Southeastern France. Flora. 236, 67-  
690 75.

691 Scholander, P.F., Hammel, H.T., Bradstreet, E.D., Hemmingsen, E.A., 1965. Sap pressure in  
692 vascular plants. Negative hydrostatic pressure can be measured in plants. Science. 148, 339-  
693 346.

694 Selmar, D., Kleinwachter, M., 2013. Influencing the product quality by deliberately applying  
695 drought stress during the cultivation of medicinal plants. Ind. Crops Prod. 42, 558-666.

696 Singleton, V.L., Rossi, J.A.J., 1965. Colorimetry of total phenolics with phosphomolybdic-  
697 phosphotungstic acid reagent. Am. J. Enol. Viticol. 16, 144-158.

698 Soni, P. Abdin, M.Z., 2017. Water deficit-induced oxidative stress affects artemisinin content and  
699 expression of proline metabolic genes in *Artemisia annua* L. FEBS OpenBio. 7, 367-381.

700 Szöllösi, R., Szöllösi Varga, I., 2002. Total antioxidant power in some species of Labiatae  
701 (adaptation of FRAP method). Acta Biol. Szegediensis. 46, 125-127.

702 Tholl, D., 2015. Biosynthesis and Biological Functions of Terpenoids in Plants, in: Schrader J.,  
703 Bohlmann J. (Eds.), Biotechnology of Isoprenoids. Advances in Biochemical  
704 Engineering/Biotechnology, vol 148. Springer, Cham. pp 63-106.

705 Valliere, J.M., Allen, E.B., 2016. Interactive effects of nitrogen deposition and drought-stress on  
706 plant-soil feedbacks of *Artemisia californica* seedlings. Plant Soil. 403, 277-290.

707 Wenping, H., Yuan, Z., Jie, S., Lijun, Z., Zhezhi, W., 2011. *De novo* transcriptome sequencing in  
708 *Salvia miltiorrhiza* to identify genes involved in the biosynthesis of active ingredients.  
709 Genomics. 98, 272-279.

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

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## 713 TABLES

714 **Table 1.** Oligonucleotides used in quantitative real-time PCR analysis.

Name	Putative gene description	Primer	Primer sequences 5'-3'	References
<i>DH</i>	Dehydrin	Forward	GAGGTAGAGGGGGAAAA TGG CCGATGTGTCTACGCATT	This study
		Reverse	TC GGCGTATGGGTTACACA	
<i>GPPSB</i>	Geranyl diphosphate synthase	Forward	AGC GCACCAAGGCTAGAGAG	This study
		Reverse	CTG GCTGTCCCCCAAGTTTGA	
<i>GPPS2D</i>	Geranyl diphosphate synthase	Forward	T	This study
		Reverse	CTCTCCATCACGCGAAGC GCGGGTGAGGACCTGGA	
<i>FPPS</i>	Farnesyl diphosphate synthase	Forward	GAAACAT CAGGGCCTTTACAACCAG	Ma et al. (2012)
		Reverse	CCAAGAA CCAGATTGTGGACTTGTC	
<i>GGPPS2</i>	Geranylgeranyl diphosphate synthase	Forward	GAGCGA CAACACACCTGGCGTACT	Ma et al. (2012)
		Reverse	TCCTCAA CCACATCGCCTTCAGGGA	
<i>CPS1</i>	Copalyl diphosphate synthase	Forward	AGAAAT TTTATGCTCGATTTGCT	Ma et al. (2012)
		Reverse	GCGATCT GGTTCATCGCCTTCAAC	
<i>CPS2</i>	Copalyl diphosphate synthase	Forward	GAAGAT TCCTTATCCTTTATGCTCC	Ma et al. (2012)
		Reverse	CATCCA GGAGATGCCAATTCGAA	
<i>CPS3</i>	Copalyl diphosphate synthase	Forward	CATCAGA TCAAATATAGTTGCGGCG	Ma et al. (2012)
		Reverse	GCCAAA CGGCTGCCTTGGGCTACA	
<i>CPS4</i>	Copalyl diphosphate synthase	Forward	ACAATA TCCCTGGTGACCTCCTCC	Ma et al. (2012)
		Reverse	TTCCCA ACCCTCACGGGAAGAC	
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase	Forward	CATC ACCACGGAGACGGAGGA	This study
		Reverse	CAAG	

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717 **Table 2.** Average values of growth index (G.I.) during the experiment. *S. dolomitica* plants were  
 718 well-watered (WW, 100% container capacity, CC) or subjected to moderate drought stress (MDS,  
 719 50% CC) or severe drought stress (SDS, 0% CC).

G.I. (cm <sup>3</sup> ) Treatments	Days										
	0	4	7	11	14	18	21	25	28	32	34
WW	8,134	3,186	3,525	7,114	6,464	4,788	8,355a	11,921a	12,059	12,156	12,456
MDS	8,134	5,726	5,837	4,641	4,561	4,779	4,058b	3,336b	3,451	3,587	4,002
SDS	8,143	2,951	3,011	3,611	2,874	2,786	1,534c	778c	-	-	-
<i>P</i>	ns	ns	ns	ns	ns	ns	**	*	**	**	**

720 Means followed by the same letter do not differ significantly, according to the REGW-F test (NS = non-significant;

721 \* $P < 0.05$ ; \*\*  $P < 0.001$ ).

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740 **Table 3.** Total, aerial and root dry mass production and root:aerial (R:A) ratio of treated *S.*  
 741 *dolomitica* plants at the end of the experiment. Plants were well-watered (WW, 100% container  
 742 capacity, CC) or subjected to moderate drought stress (MDS, 50% CC) or severe drought stress  
 743 (SDS, 0% CC). In parentheses are the percentage variations referred to controls.

Treatments	Dry mass production (gDW plant <sup>-1</sup> )			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%)
<i>P</i>	**	**	**	*

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

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762 **Table 4.** SPAD values, chlorophyll (a + b) and total carotenoid (Car) measured on *Salvia*  
 763 *dolomitica* plants treated with three irrigation regimes: well-watered (100% container capacity,  
 764 100% CC, WW), moderate drought stress (50% CC, MDS), or severe drought stress (0% CC, SDS).

SPAD Treatments	Days										
	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	-	-	-
<i>P</i>	ns	ns	ns	ns	ns						
Chl (a+b) (mg g <sup>-1</sup> 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	-	-	-
<i>P</i>	ns	ns	ns	ns	ns	ns	**	**	ns	ns	ns
Carotenoids (mg g <sup>-1</sup> 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	-	-	-
<i>P</i>	ns	ns	ns	ns	ns	ns	*	**	ns	ns	ns

765 Means followed by the same letter do not differ significantly, according to the REGW-F test (NS = non-significant;

766 \**P*<0.05; \*\* *P*<0.001).

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780 **Table 5.** The rate during the experiment and the total amount of leaf phenols, flavonoids and  
 781 antioxidant activity of treated *S. dolomitica* plants. Plants were well-watered (100% container  
 782 capacity, 100% CC, WW) or subjected to moderate drought stress (50% CC, MDS) or severe  
 783 drought stress (0% CC, SDS). In parentheses are the percentage variations referred to controls.

Phenols (mgGAEg <sup>-1</sup> ) 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%)
<i>P</i>	ns	*	**	**	ns	ns	ns	ns	ns	**	ns	**
Flavonoids (mg g <sup>-1</sup> ) Treatments												
	0	4	7	11	14	18	21	25	28	32	34	Total amount
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%)
<i>P</i>	ns	*	**	**	ns	ns	ns	ns	ns	*	ns	**
FRAP (μmol Fe <sup>2+</sup> g <sup>-1</sup> ) Treatments												
	0	4	7	11	14	18	21	25	28	32	34	Total amount
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%)
<i>P</i>	ns	**	**	**	ns	ns	ns	**	ns	*	ns	**

784 Means followed by the same letter do not differ significantly, according to the REGW-F test (NS = non-significant;

785 \**P*<0.05; \*\* *P*<0.001).

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792 **Table 6.** Chemical composition of volatiles emitted from *S. dolomitica* plants following well-  
 793 watered irrigation (WW), moderate drought stress (MDS) or severe drought stress (SDS). All  
 794 constituents are ordered on the basis of their linear retention index (LRI). The most relevant  
 795 constituents are underlined.

Category*	Constituents (%)	IRI	WW	MDS	SDS
mh	$\alpha$ -thujene	939	0.33	0.11	0.35
mh	$\alpha$ -pinene	953	5.75	2.97	2.76
mh	camphene	980	4.05	2.04	1.88
mh	$\beta$ -pinene	991	3.35	1.85	1.64
mh	myrcene	1031	5.09	2.01	1.76
mh	$\alpha$ -phellandrene	1040	0.57	0.18	0.27
<u>mh</u>	<u><math>\Delta</math>-3-carene</u>	<u>1050</u>	<u>9.14</u>	<u>4.16</u>	<u>4.53</u>
<u>mh</u>	<u>limonene</u>	<u>1088</u>	<u>19.80</u>	<u>13.51</u>	<u>12.24</u>
<u>mh</u>	<u>(E)-<math>\beta</math>-ocimene</u>	<u>1097</u>	<u>7.39</u>	<u>3.19</u>	<u>2.99</u>
mh	(Z)- $\beta$ -ocimene	1098	0.64	0.23	0.27
mh	$\gamma$ -terpinene	1110	0.85	0.38	0.43
om	<i>cis</i> -sabinene hydrate	1125	0.08	0.21	0.17
mh	terpinolene	1143	0.75	0.32	0.28
om	<i>trans</i> -sabinene hydrate	1165	0.18	0.21	0.24
mh	<i>allo</i> -ocimene	1189	0.91	0.29	0.26
om	isoborneol	1204	0.10	0.12	0.00
om	borneol	1285	1.36	2.39	1.79
sh	$\Delta$ -elemene	1339	0.49	0.98	1.12
sh	$\alpha$ -cubebene	1376	0.37	0.40	0.57
sh	isodene	1380	0.52	0.53	0.68
sh	$\alpha$ -copaene	1391	1.93	2.23	2.63
sh	$\beta$ -bourbonene	1398	0.22	-	1.89
sh	$\beta$ -cubebene	1418	0.21	0.47	0.40
sh	$\beta$ -elemene	1429	0.11	0.31	0.37
sh	$\alpha$ -gurjunene	1432	0.72	0.79	0.89
<u>sh</u>	<u><math>\beta</math>-caryophyllene</u>	<u>1439</u>	<u>7.86</u>	<u>9.47</u>	<u>9.09</u>
sh	$\beta$ -copaene	1454	0.92	1.29	2.00
sh	$\beta$ -gurjunene	1458	0.38	0.46	0.65
<u>sh</u>	<u><math>\alpha</math>-guaiene</u>	<u>1476</u>	<u>3.68</u>	<u>4.51</u>	<u>5.21</u>
sh	aromadendrene	1477	0.40	0.58	0.77
sh	$\alpha\beta$ -humulene	1480	0.75	1.16	1.15
sh	<i>allo</i> -aromadendrene	1485	0.46	0.72	0.94
sh	$\gamma$ -muurolene	1494	0.89	0.96	1.02
<u>sh</u>	<u>germacrene D</u>	<u>1503</u>	<u>8.57</u>	<u>22.35</u>	<u>22.16</u>
<u>sh</u>	<u>bicyclogermacrene</u>	<u>1517</u>	<u>4.21</u>	-	<u>9.27</u>
sh	$\gamma$ -cadinene	1524	1.40	-	1.84
sh	$\delta$ -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

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

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799 **Table 7.** Chemical composition of essential oils extracted from *S. dolomitica* plants following well-  
800 watered irrigation (WW), moderate drought stress (MDS) or severe drought stress (SDS). All  
801 constituents are ordered on the basis of their linear retention index (LRI). The most relevant  
802 constituents are underlined.

Category*	Constituents (%)	IRI	WW	MDS	SDS
mh	$\alpha$ -pinene	319	1.4	1.0	1.0
mh	camphene	340	0.6		
<u>mh</u>	<u><math>\beta</math>-pinene</u>	<u>386</u>	<u>0.2</u>	<u>6.1</u>	<u>6.0</u>
mh	myrcene	408	0.5	0.2	0.2
mh	$\alpha$ -phellandrene	435	0.2		
mh	$\Delta$ -3-carene	444	1.5		
mh	$\alpha$ -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)- $\beta$ -ocimene	498	0.6	0.2	0.2
mh	$\gamma$ -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	1,084			0.1
sh	lavandulyl acetate	1,111		1.4	1.3
sh	trans-pinocarvyl acetate	1,135		0.2	0.2
sh	myrtenyl acetate	1,195		0.8	0.8
om	$\alpha$ -cubebene	1,267	0.3		
sh	isolekene	1,308	0.4		
sh	$\alpha$ -copaene	1,334	2.3	0.5	0.5
sh	trans-myrtanol acetate	1,347		0.2	0.2
om	sativene	1,364	0.1		
sh	$\alpha$ -gurjunene	1,421	1.0	0.2	0.2
<u>sh</u>	<u><math>\beta</math>-caryophyllene</u>	<u>1,442</u>	<u>21.2</u>	<u>0.6</u>	<u>0.6</u>
sh	lavandulyl isobutirrate	1,452	0.9	0.2	0.2
sh	$\beta$ -copaene	1,464	0.3		
sh	$\beta$ -gurjunene	1,475	0.9		
<u>sh</u>	<u>1H-cyclopropanaphthalene</u>	<u>1,486</u>	<u>6.9</u>		-
sh	$\alpha$ -guaiene	1,491	0.8	0.2	0.2
sh	aromadendrene	1,491	0.1		
<u>sh</u>	<u><math>\alpha</math>-humulene</u>	<u>1,527</u>	<u>2.2</u>	<u>3.8</u>	<u>3.8</u>
sh	alloaromadendrene	1,546	0.9	0.5	0.5
sh	trans-cadina 1(6).4-diene	1,567	0.7		
sh	$\gamma$ -muurolene	1,586	0.8	0.6	0.6
sh	$\beta$ -selinene	1,608	0.2	0.7	0.7
sh	cis- $\beta$ -guaiene	1,621	0.5		
sh	valencene	1,624	0.3		
om	viridiflorene	1,628	3.0	0.2	0.2
<u>sh</u>	<u>trans-<math>\beta</math>-guaiene</u>	<u>1,646</u>	<u>1.9</u>	<u>18.6</u>	<u>18.5</u>
sh	$\alpha$ -bulnesene	1,658	0.1		0.1

sh	geranyl isobutyrate	1,678	1.4	1.7
sh	trans- $\gamma$ -cadinene	1,676	3.6	
<u>sh</u>	<u><math>\delta</math>-cadinene</u>	<u>1,700</u>	<u>7.1</u>	<u>3.1</u> <u>3.2</u>
os	trans-cadina-1(2).4-diene	1,718	0.7	
sh	$\alpha$ -cadinene	1,733	0.3	
os	$\alpha$ -calacorene	1,744	1.3	1.3
os	elemol	1,759	0.2	
os	germacrene D	1,786	0.5	
<u>os</u>	<u>longipinalol</u>	<u>1,801</u>	<u>0.8</u>	<u>41.9</u> <u>41.5</u>
os	caryophyllene alcohol	1,806	0.1	
os	spathunelol	1,825	0.4	1.5 1.5
os	caryophyllene oxide	1,837	3.8	0.2 0.2
os	5-epi-7-epi- $\alpha$ -eudesmol	1,894	1.6	0.3 0.3
os	humulene oxide	1,897	0.3	1.0 1.0
os	1.10-di-epi-cubenol	1,915	0.5	0.9 1.0
os	1-epi-cubenol	1,944	1.3	0.7 0.8
os	$\gamma$ -eudesmol	1,951	0.9	0.1
os	caryophylla-4(14).8(15)-dien-5-ol	1,962	0.6	
<u>os</u>	<u>epi-<math>\alpha</math>-cadinol</u>	<u>1,973</u>	<u>4.3</u>	<u>0.9</u> <u>0.9</u>
os	$\alpha$ -muurolol	1,984	0.1	0.4 0.4
os	$\beta$ -eudesmol	1,993	1.4	
<u>os</u>	<u><math>\alpha</math>-eudesmol</u>	<u>2,000</u>	<u>4.4</u>	<u>2.2</u> <u>2.3</u>
os	14-hydroxy-9-epi-(E)-caryophyllene	2,028	2.3	
os	bulnesol	2,033	0.1	0.1 0.1
os	$\alpha$ -cadinol	2,003		0.3 0.3
os	valeranone	2,047	0.9	0.4 0.4
os	cadalene	2,050		0.2 0.2
os	khusinol	2,051	0.1	0.2 0.2
os	$\alpha$ -bisabolol	2,072		0.2 0.2
os	eudesma-4(15).7-dien-1- $\beta$ -ol	2,076	0.3	
os	acorenone	2,078	0.1	
nt	trans- $\alpha$ -bergamotol	2,097	0.2	
os	$\gamma$ -atlantone	2,116		0.3 0.3
os	oplopanone	2,153	0.1	
nt	hexadecanal	2,239	0.2	0.1 0.1
os	lanceol acetate (z)	2,455		0.2 0.2
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	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

803 \*All the constituents belong to non-terpene derivatives (nt), monoterpene hydrocarbons (mh), oxygenated monoterpene

804 (om), sesquiterpene hydrocarbons (sh), oxygenated sesquiterpene (os) and apocarotenoids (ac-10).

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806 **Figures**

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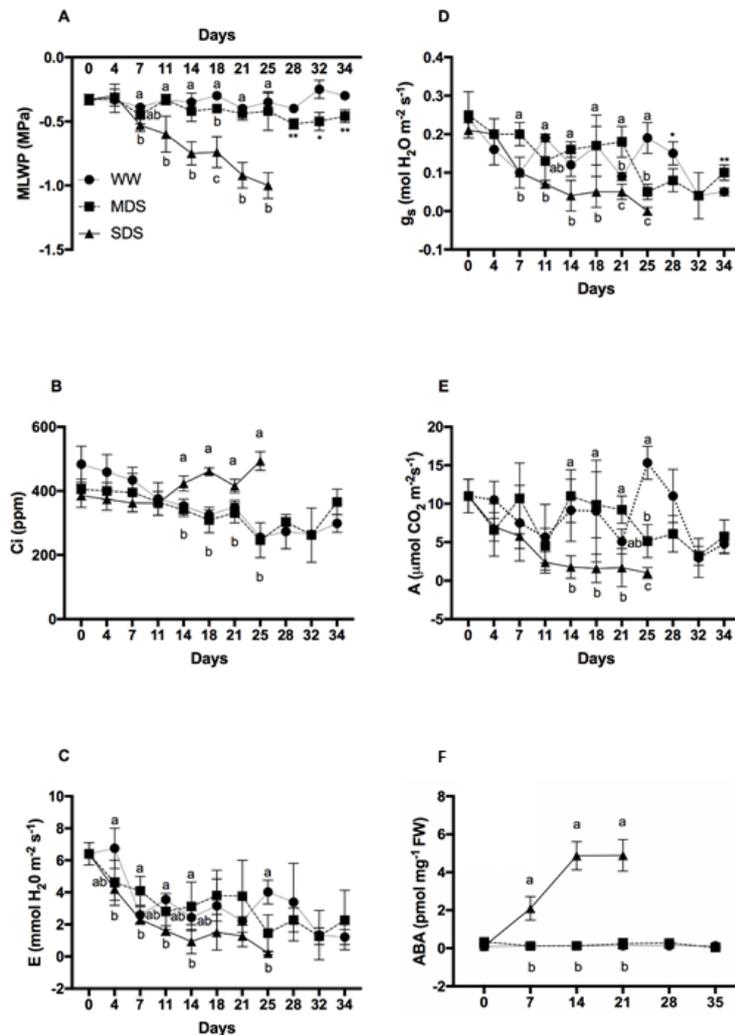
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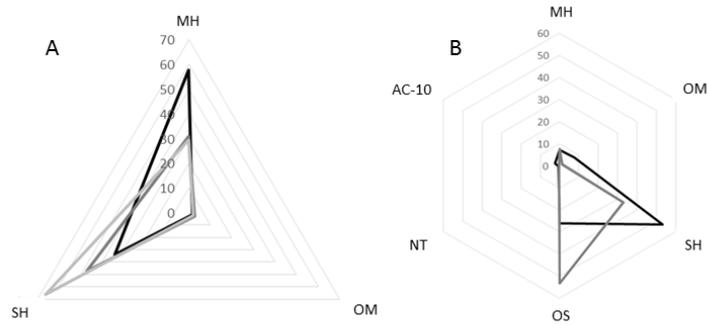


822 **Figure 1.** Midday leaf water potential (MLWP - A), gas exchange (internal CO<sub>2</sub> concentration, Ci -  
823 B; transpiration rate, E - C; stomatal conductance, *g<sub>s</sub>* - D; net photosynthetic rate, A - E) and  
824 internal abscisic acid content (ABA - F) dynamics measured on *S. dolomitica* plants treated with  
825 well-watered irrigation (WW), moderate drought stress (MDS), or severe drought stress (SDS).  
826 Mean values showing the same letter are not statistically different at  $P \leq 0.05$  according to the  
827 REGW-F post hoc test. The statistical relevance of ‘Between-Subjects Effects’ tests (ns=non-  
828 significant, \*= $P < 0.05$ , \*\*  $P < 0.001$ ) was evaluated.

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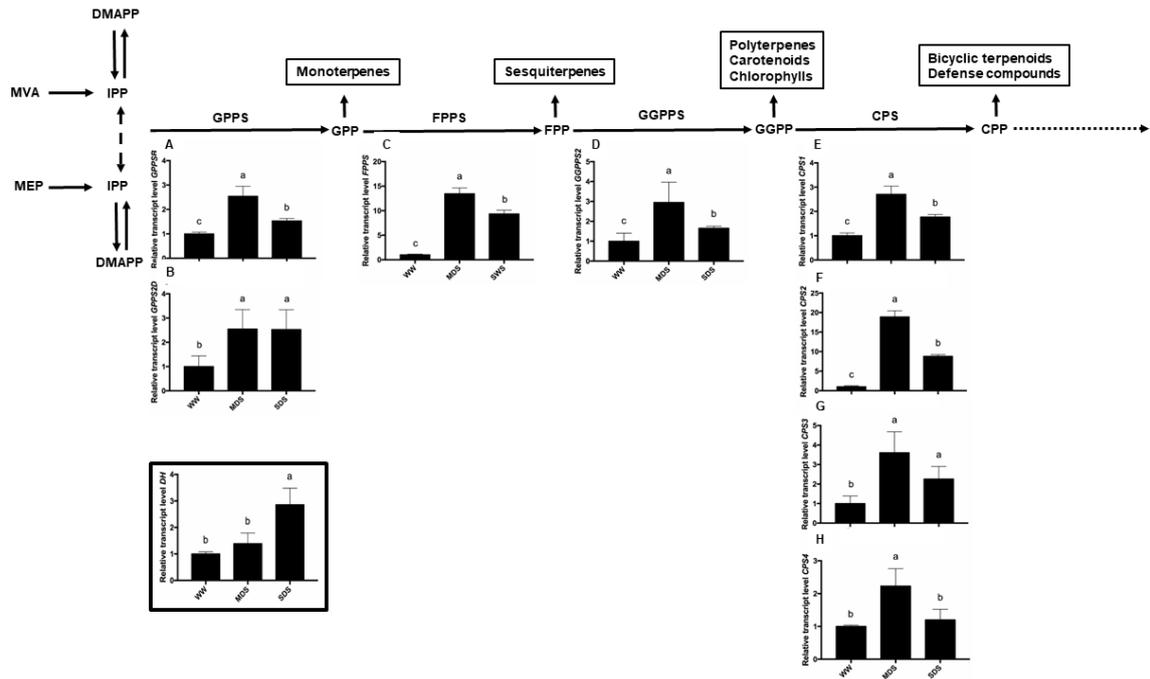
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**Figure 2.** Radar charts showing changes in terpenoid content of biogenic volatile organic compounds (BVOCs - A) and essential oils (EOs - B) of *S. dolomitica* plants in response to well-watered irrigation (WW, black line), moderate drought stress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

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**Figure 3.** Flowchart for assembling isoprenoid building blocks to produce terpenes and relative transcriptional modulation of genes involved in *S. dolomitica* terpenoid biosynthesis. Relative gene expression levels obtained by RT-qPCR analysis of the *DH* (in the box): dehydrin; *GPPSB* (A) and *GPPS2D* (B): geranyl diphosphate synthases; *FPPS* (C): farnesyl diphosphate synthase; *GGPPS2* (D): geranylgeranyl diphosphate synthase; *CPS1* (E), *CPS2* (F), *CPS3* (G) and *CPS4* (H): copalyl diphosphate synthases. In the box is displayed relative transcriptional modulation. Genes were tested on plants subjected to moderate water stress (MDS), severe water stress (SDS) or well-watered (WW) treatment. Mean values showing the same letter are not statistically different at  $P \leq 0.05$  according to the Tukey's post-hoc test. Bars represent the standard deviation of the mean (n=3). MVA, mevalonate pathway; MEP, methylerythritol phosphate pathway; IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate.

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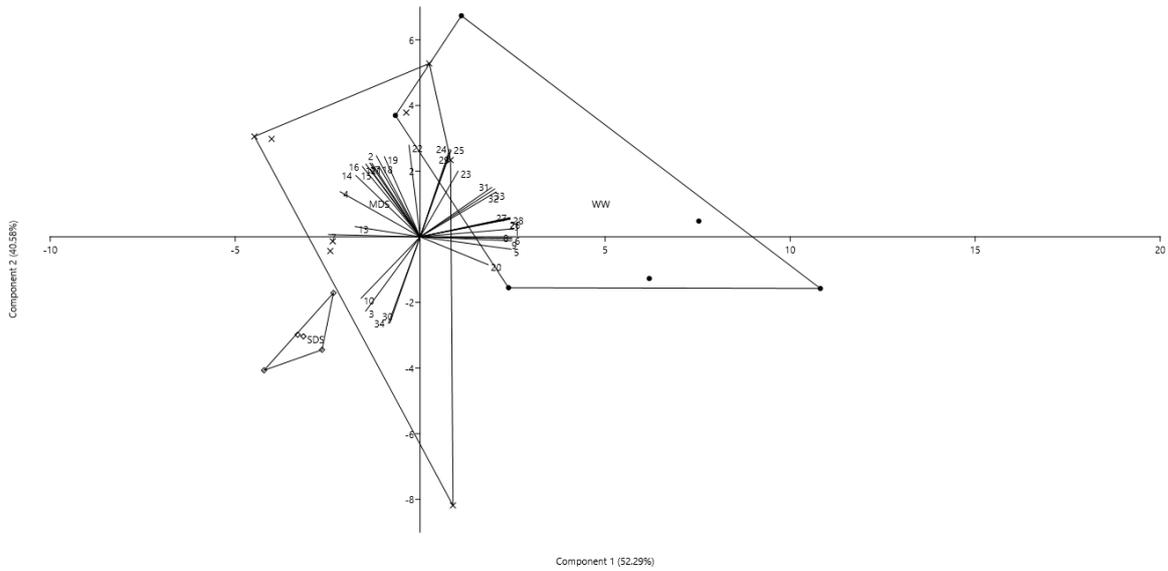
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894 **Figure 4.** Principal Component Analysis (PCA)-biplot of the plants of *S. dolomitca* under control  
895 irrigation (WW, point), moderate drought stress (MDS, cross) or severe drought stress (SDS,  
896 diamond), according to the first two principal components. Numbers indicate the analysed  
897 variables: 1. Volatile MH; 2. Volatile OM; 3. Volatile SH; 4. Oils MH; 5. Oils OM; 6. Oils SH; 7.  
898 Oils OS; 8. Oils Nt; 9. Oils Ap; 10. *DH*; 11. *Cal*; 12. *GPPSB*; 13. *GPPS2D*; 14. *FPPS*; 15.  
899 *GGPPS2*; 16. *CPS1*; 17. *CPS2*; 18. *CPS3*; 19. *CPS4*; 20. Growth index; 21. Dry biomass; 22. R:A  
900 ratio; 23. SPAD; 24. Chlorophyll; 25. Carotenoids; 26. Phenols; 27. Flavonoids; 28. FRAP; 29.  
901 MLWP; 30. Ci; 31. *E*; 32. *Gs*; 33. *A*; 34. ABA.