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Abstract

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

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

The quality of medicinal and aromatic plants (MAPs) is contingent on the composition and concentration of plant secondary metabolites (PSMs), which are themselves influenced by environmental conditions such as drought (Kleinwächter and Selmar, 2015; Mandoulakani et al., 2017). This effect occurs in all major classes of PSMs and is also dependent 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 a characteristic aroma and particular biological properties (Caser et al., 2016; Loreto et al., 2014; Moradi et al., 2017; Nogués et al., 2015; Radwan et al., 2017; Raut and Karuppayil, 2014).

A model was recently developed to explain how drought affects PSM production (Selmar and Kleinwächter, 2013). Its authors reported that during water-shortage conditions, stomata are closed to minimise transpiration and to preclude the entry 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 equivalents (e.g. NADPH + H^+) are consumed and re-oxidised. Thus, large amounts of NADPH + H^+ accumulate, generating an over-reduced state. In this condition, plants promote all reactions to consume NADPH + H^+, including the biosynthesis of terpenoids and phenols. In general, PSM changes induced by drought improve the production quality of many MAPs (Caser et al., 2018; Kleinwächter and Selmar, 2014; Mandoulakani et al., 2017). However, this model has yet to be subjected to effective examination. In a recent study with *Salvia officinalis*, Radwan et al.

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

The present study aims to help unravel the mechanisms behind drought stress in *S. dolomitica* Codd, paying 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 *S. dolomitica* Codd. were transplanted in plastic pots (9 cm in diameter; 0.52 L) containing peat (Silver Torf, Agrochimica, Bolzano, Italy) and Agriperlite® (70:30 v:v). A slow-release fertiliser (Osmocote 15:11:13; Scotts Europe, The Netherlands) was used. Cultivation lasted 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} of photosynthetic active radiation 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 randomised block design with three levels of irrigation was applied. The levels of irrigation were: 100% container capacity (CC) as control (well-watered, WW), 50% CC as moderate drought stress (MDS) or 0% CC as severe drought stress (SDS). For each irrigation regime, 40 plants were treated, with four replications of 10 plants each. All water amounts were kept constant throughout the experiment by gravimetric determinations as reported

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

2.2. Morphological parameters

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

2.3. Photosynthetic pigments

The relative quantity of chlorophyll was measured on six leaves per plant, randomly selected in six plants per irrigation level, 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 overnight extraction in 5 ml of methanol at 4°C in the dark, pigments were spectrophotometrically determined at 665, 652, and 470 nm using an 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 from six plants per irrigation level were powdered and homogenised with 1 ml of methanolic aqueous solution (methanol 70% v/v). Following 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 using the ferric reducing antioxidant power (FRAP) method with minor modifications (Szôllôsi and Szôllôsi Varga, 2002) and indicated as µmol $Fe^{2+}g^{-1}$. The working solution was always freshly prepared and contained 7.5 mM acetate buffer, pH 3.6, 0.1 mM tripyridyltriazine (TPTZ) and 0.05 mM $FeCl_3 \cdot 6H_2O$. At low pH, when the tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to its ferrous form (Fe^{2+}), an intensive blue colour of Fe^{2+}-TPTZ can be monitored spectrophotometrically at 593 nm. The 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 per plant for 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}), transpiration rate (*E*; mmol m^{-2} s^{-1}), stomatal conductance (g_s; mmol m^{-2} s^{-1}), and 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

The concentration of endogenous abscisic acid (ABA) was quantified every week in the mature leaves of six plants per irrigation level through a rapid High Performance Liquid Chromatography (HPLC) method, optimised for plant extracts and based on Solid Phase Extraction (SPE) (Bosco et al., 2013; Demasi et al., 2017). The leaves were grounded in liquid nitrogen and 0.5 g of each sample was suspended in 4 ml of the extraction solution (65% pure methanol, 25% ultrapure water, 10% aqueous hydrogen chloride 1 M) for 2 h at 4°C in the dark. The samples were then filtered and the eluates were added to a SPE cartridge (Supelclean SPE LC-NH_2, Supelco Analytical, USA). ABA was eluted with 5% of phosphoric acid (H_3PO_4) in methanol. The procedure was carried out under artificial light with amber glassware to prevent degradation. The chromatographic analysis of the eluate was performed with HPLC 1200 Series (Agilent Technologies, Böblingen, Germany) and the signal was monitored at 265 nm with a diode array detector. Peaks identification was made on the basis of retention time, the co-injection with ABA standard along each batch samples, and the UV spectrum of the peak. The ABA content (pmol mg^{-1} FW) was determined by peak area and was calculated based on a calibration curve constructed from the matrix-matched calibration standards.

2.7. Analysis of biogenic volatile organic compounds

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

chromatograph (Varian, Inc., Palo Alto, CA). The analytical conditions were as follows: injector and transfer line temperature at 250°C and 240°C, respectively; oven temperature programmed from 60°C to 240°C at 3°C min^{-1}; helium as carrier gas set at 1 mL min^{-1}; and injection in splitless mode. Identification of the constituents was conducted via comparison of the retention times with those of the authentic samples, and 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), in according to the procedure described in the Italian Pharmacopoeia (Farmacopea Ufficiale della Repubblica Italiana, 1991). The yields of distillation were not determined due to the low amount of the starting plant material. The EOs obtained were solubilised in *n*-hexane, dried over anhydrous sodium sulphate and filtered, and then stored in a vial at 4°C in the dark until use. GC-EIMS was used to analyse all of the EOs obtained (injection of 0.2 µL), as reported by Caser et al. (2016).

2.9. RNA isolation and RT-PCR analysis

The leaves collected from six plants per irrigation level 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 the RNA quantity was 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 synthesised, 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. Given the absence of the *S. dolomitica* reference genome, gene-specific primers (Table 1) were selected on the basis of the phylogenetically closest 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 Scientific) as reported in Chitarra et al. (2017). Three technical replicates were run for each biological replicate, and the expression of target genes was quantified following normalisation to the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH)* housekeeping gene. The results were calculated as expression ratios (Relative Quantity, RQ) to control (WW). In addition, amplified product identities were confirmed by sequencing using the dideoxy chain termination method at BioFab Research (Rome, Italy). The obtained sequences were searched in NCBI database using BLASTn tool as previously reported by Nerva et al. (2016).

2.10. Statistical methods

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

3. Results and discussion

3.1. Plant growth

Moderate drought stress (MDS) and severe drought stress (SDS) reduced growth (G.I.) starting 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

significantly reduced the content of both pigments compared with 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), and combined with a simultaneous growth reduction, as previously seen in *S. sinaloensis* (Caser et al., 2018). In contrast, in *S. officinalis*, a considerable reduction in chlorophyll content has been observed 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 exacerbated drought stress. However, in the present study no significant differences in SPAD values among treatments were noted (Table 4), as previously found in *S. dolomitica* and *S. sinaloensis* by Caser et al. (2012, 2018).

3.3. Phenols, flavonoids and antioxidant activity

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

In the present study, the rate of total phenols, flavonoids and antioxidant activity in treated plants was monitored during the entire experiment (Table 5). *S. dolomitica* plants subjected to SDS conditions exhibited a significant decrease in the content of total phenols, flavonoids and antioxidant activity from day 4 until day 11. Subsequently, no differences occurred between treatments in total phenols and flavonoids until the end of the experiment, with the exception of day 32 (29.0 and 12.7 mg $GAEg^{-1}$ of phenols and 8.3 and 5.7 mg g^{-1} of flavonoids in WW and MDS, respectively). Regarding antioxidant activity, at day 25 SDS stimulated a significant increase compared with other treatments (98.7, 101.5 and 153.1 µmol $Fe^{2+}g^{-1}$ 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 $GAEg^{-1}$ of phenols, 105.7,
288 17.1 and 5.3 mg g^{-1} of flavonoids and 1815.8, 337.5 and 134.7 µmol $Fe^{2+}g^{-1}$ 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_2 concentration (*C*i), transpiration rate (*E*),
300 stomatal conductance (g_s), 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.*

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

No differences in *C*i were observed between WW and MDS plants, ranging between 255.0 and 483.4 µmol mol^{-1} during the experiment (Fig. 1B), while a significant increase in SDS plants was observed from day 14 (423.0 µmol mol^{-1}) to day 25 (493.0 µmol mol^{-1}). Similarly, in *E* no differences between WW and MDS plants were highlighted (Fig. 1C), whereas a significant decline occurred in SDS plants on days 4, 7, 11, 14 and 25 relative to the other treatments. Regarding stomatal conductance (g_s) (Fig. 1D), differences between WW and MDS plants occurred on days 7, 21, 25, 28 and 34. SDS plants showed a significant and constant decrease starting from day 7 (0.10 mmol m^{-2} s^{-1}) until complete senescence (0.01 mmol m^{-2} s^{-1}). Net photosynthetic rate (*A*) (Fig. 1E) followed a similar trend in the SDS treatment, starting from day 14 (1.78 µmol mol^{-2} s^{-1}). The differences between WW and MDS appeared only on day 25 (15.32 and 5.16 µmol mol^{-2} s^{-1}, respectively). The decrease of photosynthetic activity under drought stress may be due to stomatal or non-stomatal mechanisms. In drought-tolerant species, the reduction of photosynthesis owes to stomatal closure and the limitation of water losses. In drought-sensitive plants, the reduction of net photosynthesis is primarily due to water shortage, inducing severe damage in plants. Here, SDS considerably reduced the assimilation processes, with a significant decrease of g_s and saving internal CO_2, suggesting an efficient adaptive stomatal modulation.

3.5. Endogenous ABA content

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

Endogenous ABA content in the leaves of *S. dolomitica* under WW and MDS plants did not differ during the entire experiment, with a mean value equal to 0.16 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^{-1}), 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^{-1}
344).

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 amd
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

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

All of the investigated headspaces exhibited different amounts of the main constituents. The chemical profile in WW plants was characterised by Limonene > Δ-3-carene > Germacrene D > β-Caryophyllene > (E)-β-ocimene, in MDS plants, by Germacrene D > Limonene > β-Caryophyllene > α-guaiene > Δ-3-carene, and in SDS plants by Germacrene D > Limonene > Bicyclogermacrene > β-Caryophyllene > α-guaiene. Among the cited constituents, a very sharp increase (~+260%) was observed for the sesquiterpene hydrocarbons Germacrene D (from 8.57% to 22.35% and 22.16% in WW and MDS and SDS, respectively). In contrast, the monoterpene hydrocarbon (E)-β-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 β-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, α-zingiberene and α-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 monoterpens 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, β-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 β-Caryophyllene > δ-cadinene > 1H-cyclopropanaphtalene > α-eudesmol > epi-α-
414 cadinol, whereas for MDS and SDS by Longipinalol > Trans-β-guaiene > β-pinene > α-humulene >
415 δ-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, α-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 β-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 pyrophospahate (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 (C20), 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

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 also play important roles in interaction with environmental factors by inducing the biosynthesis of PSMs, as well as the other studied genes.

3.9 Principal Component Analysis (PCA)

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

4. Conclusion

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

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

713 **TABLES**

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

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	

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

G.I. (cm^3)	Days										
Treatments	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	-	-	-
P	ns	ns	ns	ns	ns	ns	**	*	**	**	**

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

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

Treatments	Dry mass production (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	**	**	**	*

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

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	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

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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Table 5. The rate during the experiment and the total amount 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 parentheses are the percentage variations referred to controls.

Phenols (mgGAEg^{-1})	Days											
Treatments	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 the REGW-F test (NS = non-significant; *P<0.05; ** P<0.001).

Table 6. Chemical composition of volatiles emitted from *S. dolomitica* plants following 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). The most relevant constituents are underlined.

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
om	*cis*-sabinene hydrate	1125	0.08	0.21	0.17
mh	terpinolene	1143	0.75	0.32	0.28
om	*trans*-sabinene hydrate	1165	0.18	0.21	0.24
mh	*allo*-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	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 *S. dolomitica* plants following 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). The most relevant constituents are underlined.

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	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	α-cubebene	1,267	0.3		
sh	isoledene	1,308	0.4		
sh	α-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	α-gurjunene	1,421	1.0	0.2	0.2
sh	β-caryophyllene	1,442	21.2	0.6	0.6
sh	lavandulyl isobutirate	1,452	0.9	0.2	0.2
sh	β-copaene	1,464	0.3		
sh	β-gurjunene	1,475	0.9		
sh	1H-cyclopropanaphtalene	1,486	6.9		
sh	α-guaiene	1,491	0.8	0.2	0.2
sh	aromadendrene	1,491	0.1		
sh	α-humulene	1,527	2.2	3.8	3.8
sh	alloaromadendrene	1,546	0.9	0.5	0.5
sh	trans-cadina 1(6).4-diene	1,567	0.7		
sh	γ-muurolene	1,586	0.8	0.6	0.6
sh	β-selinene	1,608	0.2	0.7	0.7
sh	cis-β-guaiene	1,621	0.5		
sh	valencene	1,624	0.3		
om	viridiflorene	1,628	3.0	0.2	0.2
sh	trans-β-guaiene	1,646	1.9	18.6	18.5
sh	α-bulnesene	1,658	0.1		0.1

sh	geranyl isobutyrate	1,678		1.4	1.7
sh	trans-γ-cadinene	1,676	3.6		
sh	δ-cadinene	1,700	7.1	3.1	3.2
os	trans-cadina-1(2).4-diene	1,718	0.7		
sh	α-cadinene	1,733	0.3		
os	α-calacorene	1,744		1.3	1.3
os	elemol	1,759	0.2		
os	germacrene D	1,786	0.5		
os	longipinalol	1,801	0.8	41.9	41.5
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-α-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	γ-eudesmol	1,951	0.9		0.1
os	caryophylla-4(14).8(15)-dien-5-ol	1,962	0.6		
os	epi-α-cadinol	1,973	4.3	0.9	0.9
os	α-muurolol	1,984	0.1	0.4	0.4
os	β-eudesmol	1,993	1.4		
os	α-eudesmol	2,000	4.4	2.2	2.3
os	14-hydroxy-9-epi-(E)-caryophyllene	2,028	2.3		
os	bulnesol	2,033	0.1	0.1	0.1
os	α-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	α-bisabolol	2,072		0.2	0.2
os	eudesma-4(15).7-dien-1-β-ol	2,076	0.3		
os	acorenone	2,078	0.1		
nt	trans-α-bergamotol	2,097	0.2		
os	γ-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
	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).

Figures

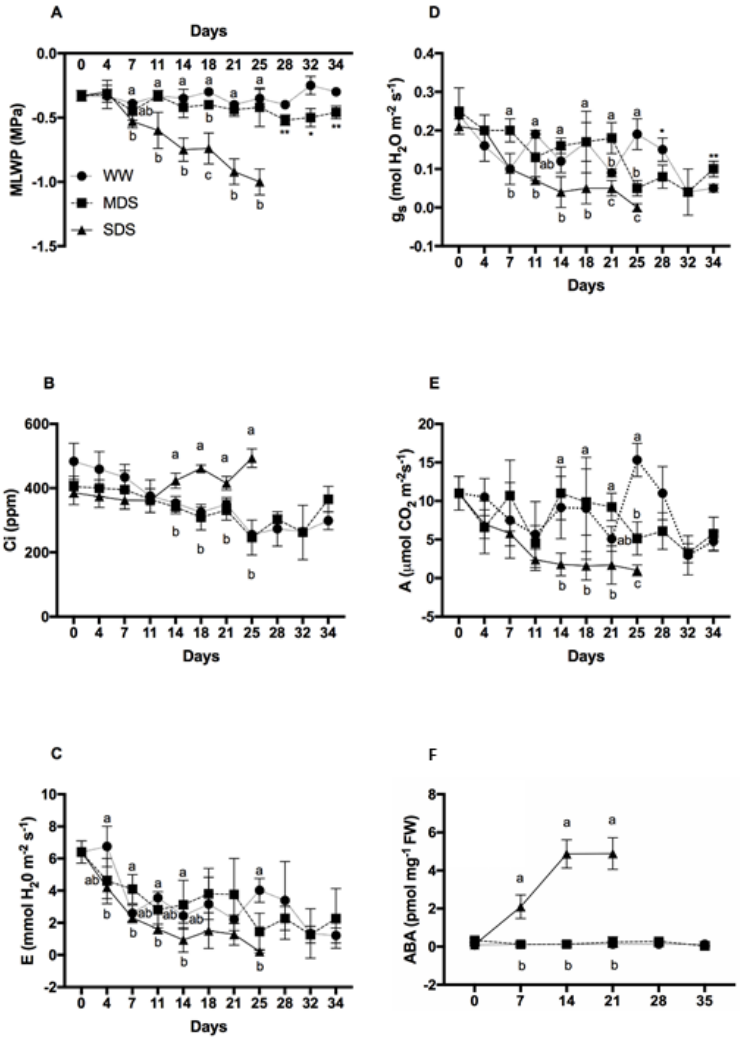

Figure 1. Midday leaf water potential (MLWP - A), gas exchange (internal CO_2 concentration, C*i* - B; transpiration rate, *E* - C; stomatal conductance, *gs* - D; net photosynthetic rate, *A* - E) and internal abscisic acid content (ABA - F) dynamics measured on *S. dolomitica* plants treated with well-watered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

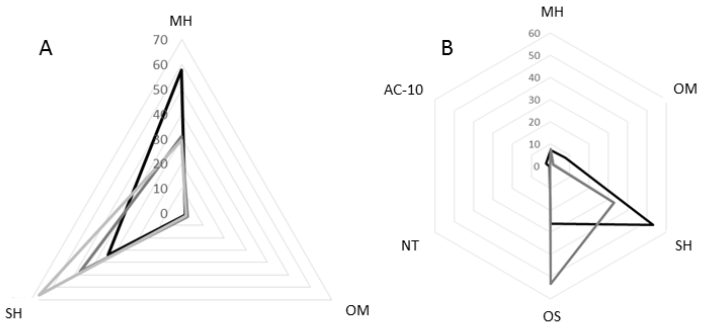

Figure 2. Radar charts showing changes in terpenoid content of biogenic volatile organic compounds (BVOCs - A) and essential oils (EOs - B) of *S. dolomitica* plants in response to well-watered irrigation (WW, black line), moderate drought stress (MDS, dark grey line) or severe drought stress (SDS, light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

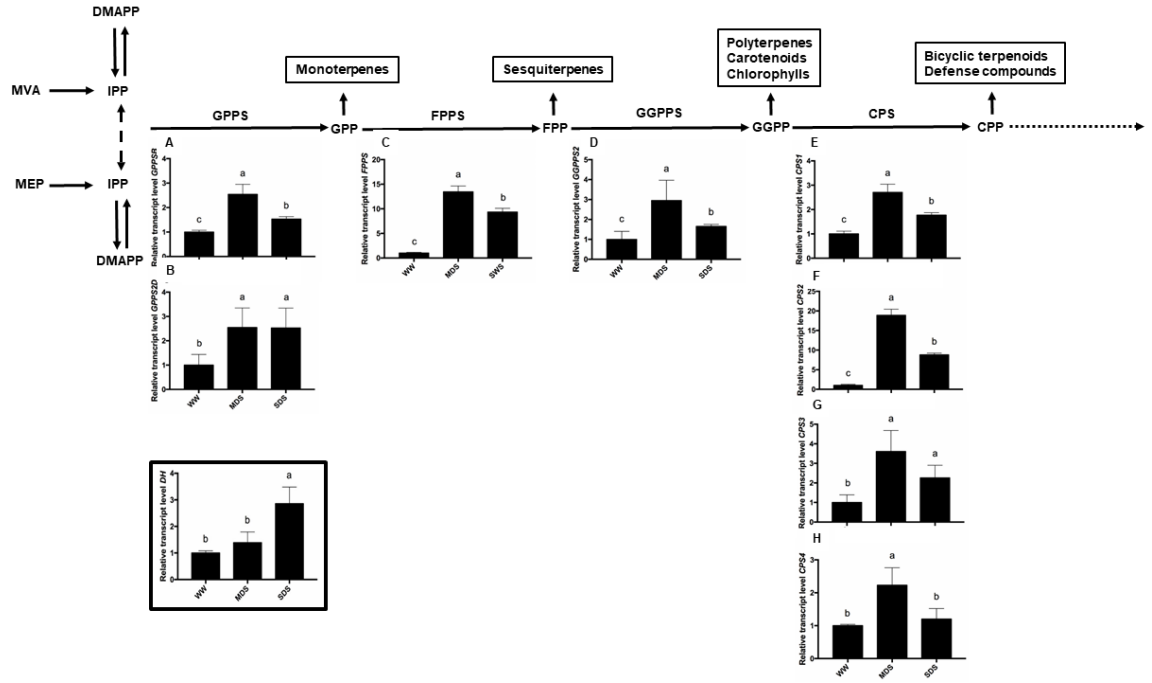

Figure 3. Flowchart for assembling isoprenoid building blocks to produce terpenes and relative transcriptional modulation of genes involved in *S. dolomitica* terpenoid biosynthesis. Relative gene expression levels obtained by RT-qPCR analysis of the *DH* (in the box): dehydrin; *GPPSB* (A) *and GPPS2D* (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 patway; IPP, isopenthenyl diphosphate; DMAPP, dimethylallyl diphosphate.

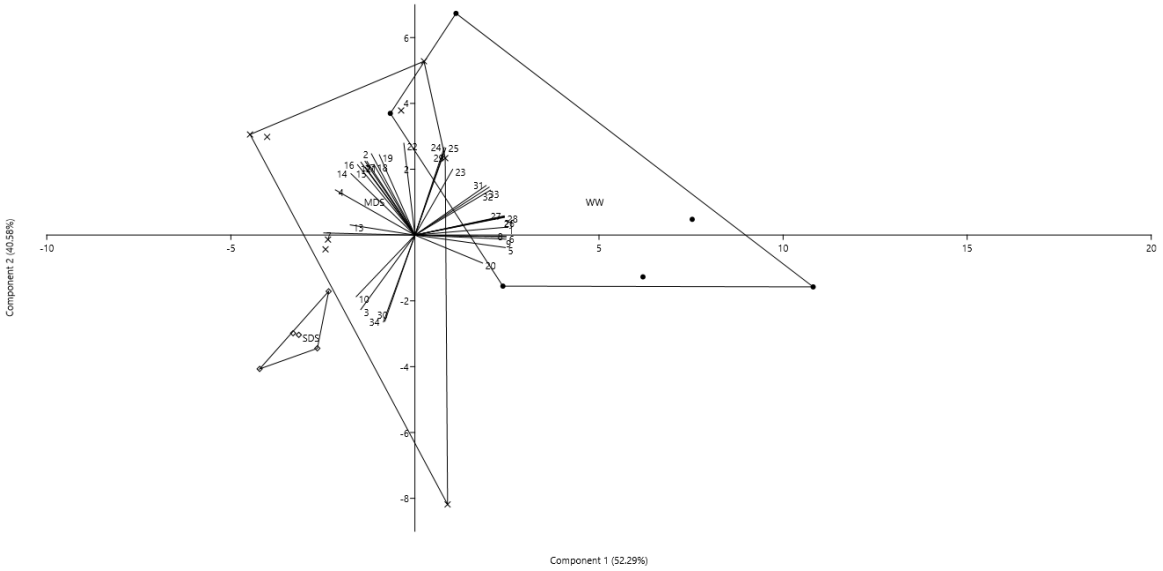

Figure 4. Principal Component Analysis (PCA)-biplot of the plants of *S. dolomitca* under control irrigation (WW, point), moderate drought stress (MDS, cross) or severe drought stress (SDS, diamond), according to the first two principal components. Numbers indicate the analysed variables: 1. Volatile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