Scientia Horticulturae 120 (2009) 271–275

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Water deficit effects on *Salvia officinalis* fatty acids and essential oils composition

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ARTICLE INFO

Article history:
Received 7 July 2008
Received in revised form 30 October 2008
Accepted 30 October 2008

Keywords:
Water deficit
Salvia officinalis
Aerial part
Growth
Fatty acid
Essential oil

ABSTRACT

The effects of water deficit on vegetative growth, fatty acids and essential oil yield and composition of *Salvia officinalis* aerial parts were investigated. Plants were treated with different levels of water deficit (C, MWD and SWD). Results showed important reductions of the different growth parameters. Drought decreased significantly the foliar fatty acid content and the double bond index (DBI) degree. This later was provoked mainly by a strong reduction of linolenic acid proportion and the disappearance of palmitoleic acid. Besides, moderate deficit increased the essential oil yield (expressed as g/100 g on the basis of dry weight) and the main essential oil constituents were camphor, α-thujone and 1.8-cineole which showed an increasing under moderate water deficit.

1. Introduction

Abiotic stress (drought, high soil salinity, chilling, deforestation, urbanization, climate change and agricultural malpractice) are responsible for the world-wide deterioration of plant cover and the erosion of soils (Boyer, 1982). Moreover, plants secondary metabolites can be altered by environmental factors and water deficit is considered to be a major one affecting many aspects of plant physiology and biochemistry (Charles et al., 1994). In the case of aromatic crops, drought may cause significant changes in some metabolites yield and compositions (Petropoulos et al., 2008). Membranes are main targets of degradative processes induced by drought and it has been shown that, under water deficit, a decrease in membrane lipid contents is correlated with an inhibition of lipid biosynthesis (Pham-Thi et al., 1987; Monteiro de Paula et al., 1990, 1993).

Sage (*Salvia officinalis* L.) is a perennial woody sub-shrub native to the Mediterranean region that is now extensively cultivated all over the world mainly to obtain dried leaves to be used as raw material in medicine, perfumery, and food industry (Bruneton, 1999). Many Salvia subspecies are used as herbal tea and for food flavouring as well as in the cosmetic, perfumery and pharmaceutical industries (Tisserand and Balacs, 1995). The data show that the composition of the sage essential oil varies significantly depending on soil mineral fertilization (Piccaglia and Marotti, 1993), light intensity (Li et al., 1996), organ age (Langer et al., 1993), climate conditions (Mathé et al., 1992), season (Grella and Picci, 1988), organ and culture site (Santos-Gomes and Fernandes-Ferreira, 2001; Perry et al., 1999). Because of such variation, the sage essential oil composition sometimes does not match the profile defined by standard ISO 9909 for official sage essential oil, which, according to Bruneton (1999), is cis-thujone (18–43%), trans-thujone (3–8.5%), camphor (4.5–24.5%), 1,8-cineole (5.5–13%), α-humulene (0–12%), α-pinene (1–6.5%), camphene (1.5–7%), limonene (0.5–3%), linalool, and bornyl acetate (2.5% maximum).

However, no data have been collected regarding *S. officinalis* lipids and essential oils changes under drought. So, in this paper we present results obtained from experiments dealing with some biochemical responses of this plant submitted to water deficit.

2. Materials and methods

2.1. Plant material and water deficit treatment

The plant used in this work was obtained by cutting propagation. The mother plants were cultivated in a plant nursery, 35 km north-east of Tunis. Ten-centimeter-length stems with two nodes and four opposite leaves were cut from mother plants and transplanted to 10 l pots (four plants per pots) filled with agricultural soil containing 0.22, 0.34, 0.05 and 0.08 mequiv. $(100\ g)^{-1}$ of dry soil of Na^+, K^+, Ca^{2+}, Fe^{2+}, respectively. During 30 days of pretreatment, plants were irrigated with tap water, and then divided into three lots subjected to different water

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doi:10.1016/j.scienta.2008.10.016

levels: 100% (control (C)), 50% (moderate water deficit (MWD)) and 25% (severe water deficit (SWD)) of field capacity (FC). This later was determined in the pots by weight. Experiments were carried-out in a greenhouse with a 14 h photoperiod (photosynthetic photon flux density, PPFD: 400 mol m^{-2} s^{-1}) and lasts 2 months from April 2007 to June 2007. Mean temperature and relative humidity were, respectively, 30 ± 5 °C, 55 ± 5% day and 16 ± 2 °C, 90 ± 5% night.

2.2. Growth and water potential measurements

For each water treatment, measurements of plant height and fresh and dry weights were monitored. Plants were harvested at the soil surface, immediately weighed (fresh weight) and then oven-dried at 75 °C for 48 h and reweighed (dry weight). Measurements of water potential were realized on the aerial parts of different plants (C, MWD and SWD) at the beginning, 15 and 30 days of treatments. Water potential was measured by Scholander pressure chamber (Scholander et al., 1965).

2.3. Total lipids extraction

Total lipids from leaves were extracted by the method described by Bligh and Dyer (1959). One gram of fresh leaves was fixed for 5 min in boiling water in order to inactivate the phospholipases (Benson and Strickland, 1960). Samples were ground in a mortar into a mixture of chloroform/methanol (2:1, v/v). After washing with water of fixation and decantation during 24 h at +4 °C, the organic phase containing total lipids was recovered and dried under a nitrogen stream. Finally, the residue was dissolved in a known volume of toluene–ethanol (4:1, v/v) and stored at −20 °C for further analyses. Total lipid extractions were repeated three times.

2.4. Fatty acid methylation and analysis

Total fatty acids were converted into their methyl esters using the sodium methylate at 3% in methanol according to the method described by Cecchi et al. (1985). Methyl heptadecanoate (C17:0) was used as an internal standard in order to quantify fatty acids. Methyl esters obtained were analyzed by gas chromatography using a Hewlett-Packard 6890 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame-ionization detector (FID), an electronic pressure control (EPC) injector and a capillary column (HP Innowax): 30 m × 0.25 mm × 0.25 μm with a stationary phase made of polyethylene glycol. Analyses were performed at split mode (60:1) under oven temperature program: isotherm at 150 °C during 1 min, from 150 °C to 200 °C at the rate of 15 °C/min, from 200 °C to 242 °C at the rate of 2 °C/min and isotherm at 225 °C during 2 min. Injector and detector temperatures were respectively held at 250 and 275 °C. Carrier gas was nitrogen at a flow rate of 1.6 ml/min.

2.5. Essential oil isolation

Essential oils were isolated from 50 g of the fresh aerial parts by conventional hydrodistillation for 3 h; this time was fixed after a kinetic survey during 30, 60, 90, 120, 150, 180 and 210 min. The hydrodistillation was performed by a simple laboratory Quik-fit apparatus which consisted of a 1000 ml steam generator flask, a distillation flask, a condenser and a receiving vessel. The obtained distillate was extracted using diethyl–ether as solvent (1/1, v/v) and dried over anhydrous sodium sulphate. The organic layer was then concentrated at 35 °C using a Vigreux column and the essential oil stored at −20 °C prior to analysis. In order to quantify essential oils and their constituents, γ-terpinolene was used as internal standard. Essential oil isolation was done in triplicates.

2.6. Essential oil gas chromatography analysis

Essential oils were analyzed by gas chromatography using the apparatus described above and two columus: a polyethylene glycol capillary one (HP Innowax: 30 m × 0.25 mm, 0.25 μm film thickness) and a 5% diphenyl, 95% dimethylpolysiloxane capillary column (HP-5: 30 m × 0.25 mm, 0.52 μm film thickness); the flow of the carrier gas nitrogen was 1.6 ml/min. The split ratio in the injector was 60:1. Analysis was performed using the following temperature program: oven temps isotherm at 35 °C for 10 min, from 35 to 205 °C at the rate of 3 °C/min and isotherm at 205 °C during 10 min. Injector and detector temperature were held, respectively, at 250 and 300 °C.

2.7. Gas chromatography–mass spectrometry

GC–MS analyses were carried out on a gas chromatograph HP 5890 (II) coupled to a HP 5972 mass spectrometer (Agilent Technologies) with electron impact ionization (70 eV). A HP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was used. The column temperature was programmed to rise from 50 °C to 240 °C at a rate of 5 °C/min. The carrier gas was helium with a flow rate of 1.2 ml/min; split ratio was 60:1. Scan time and mass range were 1 s and 40–300 m/z, respectively.

2.8. Compound identification

Essential oil components were identified by comparison of their retention indices (RI) relative to (C_8–C_{22}) *n*-alkanes with those of authentic compounds under the same conditions (Davies, 1990). Further identification was made by matching their recorded mass spectra with those stored in the Wiley/NBS mass spectral library and other published mass spectra (Adams, 2001). Fatty acids were identified by comparison of their retention times with those of pure reference standards. Essential oil and fatty acid standards were obtained from Fluka and Sigma Aldrich.

2.9. Statistical analysis

Data were subjected to statistical analysis using statistical program package STATISTICA (Statsoft, 1998). Percentage of each volatile compound and fatty acids was the mean of three replicates ± S.D. and the differences between individual means were deemed to be significant at $p < 0.05$.

3. Results and discussion

3.1. Effect of water stress on growth

The depressive effect of drought on plant morphology is observed from the second week of treatment and is more pronounced with the intensity of water constraint. Plants subjected to severe water deficit presented thinner stems with fewer, dry and smaller leaves than the control ones. On the other hand, our results showed that water treatments reduced significantly plant height and this effect was more pronounced with the severity of drought. Thus, under SWD and MWD, the aerial part height was significantly reduced respectively by 46.2 and 23%, compared with the control. This constraint induced by MWD and SWD led to a substantial decline of water potential (−1.2 to −4.8 MPa) (Table 1). Furthermore, fresh and dry matter weights were significantly affected by drought and were reduced