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Water deficit regimes trigger changes in valuable physiological and phytochemical parameters in *Helichrysum petiolare* Hilliard & B.L. Burtt

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ABSTRACT

The genus *Helichrysum* Miller is a source of many bioactive metabolites commonly used in traditional medicine. In particular, *Helichrysum petiolare* Hilliard & B.L. Burtt shows activities as antiseptic, anti-inflammatory and in the control of anxiety disorder. Biosynthesis and accumulation of secondary metabolites is a defense mechanism of plants and it is strictly influenced by the surrounding environmental conditions. In this study, drought was imposed on *H. petiolare* (HEL008 clone CREA-Sanremo collection) to understand the effect of water stress on the dynamics of plant biomass and secondary metabolites production, and the morphological and physiological mechanisms involved in plant responses. *H. petiolare* was cultivated for 34 days under three water regimes: 100% of container capacity (CC, control), 50% CC (moderate water stress), and 0% CC (severe water stress). Plant growth traits, leaf water potential, gas exchange parameters, phenol, flavonoid, and anthocyanin content, and antioxidant activity changes were determined twice a week, while the volatile organic compounds (VOCs) and essential oils (Eos) at the end of the trial. Severe water stress dramatically reduced aerial and root dry weight, chlorophyll and carotenoid content, leaf water potential, water use efficiency (WUE, A/E), transpiration rate (E), stomatal conductance (gs), net photosynthetic rate (A) and antioxidant activity. Moderate water stress induced only slight changes and led to an increase of WUE at the end of the experiment. The total amount of VOCs and Eos was not affected by water stress while their quality changed. Moderate water stress increased the main constituents of both VOCs, i.e. the monoterpene hydrocarbons, and Eos, i.e., the oxygenated sesquiterpenes. In conclusion, this *H. petiolare* cultivation under the applied moderate drought condition could lead to a double benefit i.e., water-saving irrigation practice and high quality metabolite production.

1. Introduction

Plants produce a huge and diverse assortment of secondary metabolites. Their biosynthesis is largely influenced by the surrounding environmental conditions (Croteau et al., 2000).

Drought is well known to affect the secondary metabolite content, solute accumulation, and enzymes activities (Bettaieb et al., 2009; Selmar and Kleinwächter, 2013). Accumulation of secondary metabolites is a defense mechanism of plants to adapt to the water stress by altering their cellular metabolism (Gulen and Eris, 2004). Moderate and severe water stress conditions may cause the formation of reactive oxygen species and photoinhibitory damage (Asada, 1996). In the chloroplasts of the plant cells, protection against oxidative damages is provided by both enzymatic and non-enzymatic antioxidants (Asada, 1999). Thus, water stress induces physiological and molecular defense responses by increasing antioxidant concentrations (Eskling et al., 1997) and osmoprotectants in plant tissues.

Many plants have been identified as source of antioxidants and their consumption have been recommended (Liu and Ng, 2000; Lee et al., 2003). Worldwide the consumption of herbal medicines and use of natural antioxidants are continuously increasing with an estimated market value of 700 million US $ (Raut and Karuppayil, 2014). The increase in biosynthesis and accumulation of such

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metabolites could improve the production of herbal medicines and natural antioxidants for human health (Lubbe and Verpoorte, 2011; Raut and Karuppayil, 2014). Therefore, it is very important to understand the effect of environmental factors on the dynamics of biomass and productivity of secondary metabolites and the morphological and physiological mechanisms involved in plant's innate immune responses (Hsiao, 1973; Levitt, 1980; Davies and Zhang, 1991; Close and Bray, 1993; Kramer and Boyer, 1995).

The beneficial effects on human health of Volatile Organic Compounds (VOCs) and Essential oils (Eos), as herbal remedies produced by aromatic plants, are largely documented, raising interest in the medicinal chemistry community. To date, over 1700 volatile compounds have been identified and characterized for their biological activity (Muhlemann et al., 2014). Therefore, VOCs and Eos are the most studied class of plant secondary metabolites. VOCs emitting and Eos production are influenced by numerous biotic and abiotic factors and researchers are focused to improve their quality through the study of the ecological relevance and the molecular basis involved in plant-environment interactions (Maffei et al., 2011).

Different studies have shown that plants exposed to drought stress produced higher concentration of secondary metabolites than those cultivated under well watered conditions (Selmar and Kleinwachter, 2013; Alinian et al., 2016). In medicinal plants, water stress condition lead to increase the content of artemisinin in *Artemisia annua* L. (Charles et al., 1993), betulinic acid, quercitin, and rutin in *Hypericum brasiliense* Choisy (de Abreu and Mazzafera, 2005), and hyperforin in *Hypericum perforatum* L. (St. John's wort) plants (Zobayed et al., 2007).

In this study we focused our attention on *Helichrysum petiolare* Hilliard & B.L. Burtt. The genus *Helichrysum* Miller, belonging to the family of Asteraceae, consists of approximately 500 species, some of which are endemic to the Mediterranean area. Plants belonging to the *Helichrysum* genus have been traditionally a source of many bioactive compounds (Bremner and Meyer, 2000; Mathekga et al., 2000). Numerous species are commonly used by Mediterranean and South African populations in the treatment of wounds, infections, and respiratory conditions (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; van Wyk et al., 1997; Scott et al., 2004). Some of them showed also biological activities in in vitro assays. *H. foetidum*, *H. italicum*, and *H. nudifolium* showed anti-oxidant (Czinner et al., 2000; Tirillini et al., 2013), anti-microbial (Meyer and Afolayan, 1995; Taglialatela-Scafati et al., 2013) and anti-inflammatory activity (Jäger et al., 1996), respectively. *H. italicum* ssp. *microphyllum* and *H. zivojinii* appeared to have antifungal, antiviral, anti-HIV, and anti-cancer properties (Angoini et al., 2003; Nostro et al., 2003; Appendino et al., 2007; Matic et al., 2013). Leaves of *H. petiolare* are commonly used to treat coughs, colds, catarrh, headache, fever, menstrual disorders, and urinary tract infections (Lourens et al., 2008). Moreover, *H. petiolare* showed activities as antiseptic, anti-inflammatory and in the control of anxiety disorder (Eliovson, 1984; Arnold et al., 2002; Lourens et al., 2004, 2008, 2011).

The present study aimed to understand the morphological, physiological, and biochemical changes in *H. petiolare* plants induced by different water irrigation regimes and to evaluate their influence on the so-called volatilome (VOCs emitted) and Eos profiles.

2. Materials and methods

2.1. Plant material, experimental design and treatments

Plants of *H. petiolare* Hilliard & B.L. Burtt were provided by the CREA-FSO collection (clone ID = HEL008; located in Sanremo, Imperia, Italy—43°81′60.28″N Lat, 7°76′67.38″E Long), grown in the glasshouse of the University of Torino (Italy, 45°06′23.21″N Lat, 7°57′82.83″E Long), clonally multiplied by cuttings, and placed in pots of 9 cm in diameter (one plant per pot) filled with peat (Silver Torf, Agrochimica, Bolzano, Italy) and Agriperlite® (70:30). Fertilization took place with a slow-release fertilizer (Osmocote 15:11:13; Scotts Europe, The Netherland). When plants reached at least 20 cm in height were transferred in a climatic chamber with controlled growth conditions (25 °C, 60% air humidity, 300 μmol m^{-2} s^{-1} and 16/8 h photoperiod) for all the experiment.

The experimental design was a split-plot design with three treatments and four replications per treatment. A total of 120 plants were randomly divided in three groups and subjected to irrigation at 100% of container capacity (CC, control), 50% CC (moderate water stress), and 0% CC (severe water stress). The value of 50% CC was set to determine moderate water stress in *H. petiolare* plants in a previous study (Caser et al., 2012). No irrigation treatment (0% CC) was applied to induced severe water stress. All the water contents were kept constant throughout the experiment. Gravimetric determinations of water contents were made by weighing soil samples before and after oven-drying to constant weight at 80 °C for one week. These values were used to calibrate all measurements of the moisture content of the substrate in the container. Container capacity was determined 48 h after irrigation and was calculated according to the equation of Paquin and Mehuys (1980). The soil moisture levels were maintained by manual irrigation and checked by weighing individual container every two days. The experiment lasted for a total of 34 days.

2.2. Plant growth parameters

Height and diameters of each plant were measured twice a week and used to calculate the growth index (GI; $\Pi^*\{[(D' + D'')/2]/2\}^2 \times H$, where D′ is the widest width, D″ is the perpendicular width and H is the height; Hidalgo and Harkess, 2002). At the end of the experiment, ten plants per treatment were harvested and roots and aerial parts were separated. After recording their fresh biomass, they were oven-dried at 65 °C for one week and dry biomass was weighted.

2.3. Pigments analysis

The concentration of pigments in 50 mg of fresh fully formed leaflets per treatment was evaluated twice a week. The chlorophyll and carotenoids were over night extracted in 5 ml of pure methanol at 4 °C in the dark. The absorbance of the extracts at 665, 652, and 470 nm was spectrophotometrically determined using a Ultrospec 2100 pro (Amersham Biosciences, UK), and the content of Chl a, Chl b, and carotenoids, respectively, were determined using the method described by Lichtenthaler (1987). The relative quantity of chlorophyll present in leaf tissue was also measured twice a week on 10 randomly selected leaves per treatment using the Chlorophyll Meter SPAD-502 (Konica Minolta Sensing Inc., Osaka, Japan).

2.4. Leaf water potential and gas exchange parameters

One hour before the beginning of the measurements (10–12 a.m.), the plants were transferred in lab for adaptation to ambient light intensity and temperature.

Midday leaf water potentials (LWP, Ψw) were determined twice a week, in three fully expanded leaves of six plants per treatment using a Scholander-type pressure chamber (Soil Moisture Equipment, Santa Barbara, CA, USA) (Scholander et al., 1965).

The measurement of internal CO_2 concentration (C_i), transpiration rate (E), stomatal conductance (g_s), and net photosynthetic rate (A) were performed on adult leaves twice a week, using a