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Physiological responses of lavender (*Lavandula angustifolia* Mill.) towater deficit and recovery

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ABSTRACT

Lavender (*Lavandula angustifolia* Mill.) plants were subjected to repeated water depletions and rehydration in order to evaluate the physiological responses to water deficit. The first cycle of water deprivation (WD) was induced by withholding water after sufficient irrigation; plants and soil samples were harvested 3 and 7 days after water deprivation (3 DAW and 7 DAW, respectively). The other half of the water-deprived plants were sufficiently re-watered once, and plants and soil samples were collected 3 and 10 days after re-watering (3 DAR and 10 DAR, respectively). WD produced strong decreases in leaf hydration and remarkable increases of H_2O_2 contents, and these changes were recovered after irrigation. Leaf hydration and hydrogen peroxide (H_2O_2) contents were linearly correlated to soil water contents. Abundance of $\delta^{13}C$ gradually increased during the experiment, indicating stomatal closure. Shoot/root ratios decreased in response to WD, particularly during the second cycle of WD. Generally, total carbon (C)and total nitrogen (N)contents decreased along the experiment, whereas, total soluble sugar contents and C/N ratios kept unchanged. Total amino acid (TAA) content dramatically accumulated at the expense of soluble protein upon WD and both nitrogen fractions fully recovered after irrigation. Compared to glutathione (GSH) that slightly increased in response to WD, ascorbate contents and dehydroascorbate (DHA) reductase activities were significantly impaired by WD, but resumed after re-watering. WD and re-watering had less pronounced effects on DHA contents and GSH reductase activities. In conclusion, lavender plants responded strongly to water deficit and the physiological alterations largely recovered to previous levels after irrigation, with the exception of total C and total N contents. Thus, episodic drought events may impact growth and productivity even in plants with high resilience.

1. Introduction

With the rapidly changing climate, severe and widespread drought events resulting from decreased precipitation and/or increased evaporation are projected to frequently occur in the next 30–90 years over many land areas (Dai 2013). Water deficiency is considered to be one of the most important ecological factors limiting plant survival and establishment (Wilson and Witkowski, 1998). To prevent damages resulting from the production of excessive levels of reactive oxygen species (ROS), antioxidant defenses must keep ROS under control (Rennenberg et al. 2006).

The ascorbate-glutathione cycle, also known as the Foyer-Halliwell-Asada cycle, plays an important role in plant protection and metabolic functioning under water deficit (Noctor and Foyer 1998). Ascorbate can accumulate to millimolar concentrations in both green and non-green tissues (Noctor and Foyer 1998), which is oxidized to dehydroascorbate (DHA) in the process of ROS detoxification. Oxidizedascorbate must be reduced to ascorbate by the action of dehydroascorbate reductase (DHAR), using glutathione (GSH) as the reducing substrate. The oxidized GSH (GSSG) is then in turn reduced by GSH reductase (GR) at the expense of NADPH (Noctor and Foyer 1998). Therefore, DHAR and GR activities are of great importance for maintaining the redox status of the ascorbate and GSH pools.

In addition to ROS detoxification, accumulation of compatible osmoprotectants can enhance dehydration tolerance during severe drought conditions, which are accompanied with increased density, elongation, depth and mass of roots for increasing the capability to access more water (Chaves and Oliveira 2004; Serraj and Sinclair 2002).

Effects of drought on plants have been well-documented for many species. However, information about the relationship between plant growth and ecophysiological performances in response to drought and re-watering is still relatively scarce (Xu et al. 2009). Understanding how plants respond to episodic drought and re-watering is, however, required for implementing vegetation management practices in a changing climate (Xu et al. 2010). In comparison to tree species, physiological responses of ornamental plants to water deficit are not yet

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well-documented, especially for herbaceous perennials, which cover a large part of landscapes with low precipitation (Mee et al. 2003).

Lavender (*Lavandula* spp.) is a popular herbaceous perennial plant that belongs to the Lamiaceae family and is native to the Mediterranean region which is characterized by increasing precipitation variability and extended summer drought (Cowling et al. 2005; Klausmeyer and Shaw 2009). Due to its high economic and ornamental potential, the species is now cultivated across the globe (Stanev et al. 2016). Several studies have reported that ascorbate and photosynthesis were strongly impacted by drought stress (Pastor et al. 1999; Stanev et al. 2016; Zhen and Burnett 2015), but little is known about the detailed physiological responses of lavender to water stress.

In the current study, physiological responses of lavender plants to water deficit and its recovery potential were characterized by subjecting *L.angustifolia* Mill. plants to repeated water deprivation cycles. In addition, foliar hydration, ROS levels, total carbon (C), total nitrogen (N), stable C isotope abundance, thiols and ascorbate contents, as well as activities of enzymes of the ascorbate-glutathione cycle together with soluble protein and total amino acid contents were analyzed. It was hypothesized that (1) withholding water supply would result in enhanced levels of ROS and decreased plant water contents, and that these changes would largely be recovered after re-watering; (2) contents of total C and N, as well as carbohydrate and N compounds, would only be slightly affected by water deficit due to the protection mediated by accumulated antioxidants and elevated enzymes activities as physiological parameters of high drought resistance of lavender plants.

2. Materials and methods

2.1. Plant materials and water deprivation

Seeds of *L.angustifolia* were sowed in pots (6 cm of diameter and 7 cm of height) filled with multiplication substrate (Floragard Vertriebs-GmbH, Oldenburg, Germany) on 9 May 2013. After germination, only one vigorous plant was kept in each pot, and seedlings were transferred to a glass house (located in Freiburg, Germany, N 48° 0′ latitude, E 7° 53′ longitude) under natural light and temperature conditions. The greenhouse was ventilated to maintain nearly ambient temperature conditions, and heat waves did not occur during the experiment. Approximate average day (10:00–14:00) air temperature and light intensity during the experiment was 26 ± 4 °C and 450–500 μmol m^{-2} s^{-1}, respectively. A total of 24 plants were randomly selected for repeated water deprivation (WD) cycles. The plants were sufficiently watered every 2 days until the last irrigation on the 31 July when the average height of the plants reached 18.5 cm. Thereafter, for the first cycle of WD, 12 individual plants were harvested 3 and 7 days after water deprivation (DAW) representing the 3 DAW and 7 DAW samples, respectively. The other 12 water-deprived plants were fully re-watered once on the 7 August. The third and fourth harvest took place on the 10 and 17 August, respectively, thus representing the 3 DAR (days after re-watering) and 10 DAR samples of the second cycle of WD. For each harvest, six individual plants were separated into leaf, stem and root sections, immediately frozen and homogenized in liquid nitrogen and stored at −20 °C. Soil water contents (SWC_{grav}) were determined immediately after each harvest gravimetrically and expressed on a dry weight basis.

$$SWC_{grav}\ (g\ H_2O\ g^{-1}\ DW) = (FW–DW)/DW.$$

Where FW is the fresh weight of the soil before drying occurs and DW is the dry weight of soil after drying at 105 °C for 48 h.

2.2. Biochemical analysis procedures

2.2.1. Leaf hydration and shoot/root ratio measurements

Leaf hydration (g H_2O g^{-1} DW) was calculated as (FW DW)/DW, where FW is the fresh mass and DW is the dry mass after drying the samples in an oven at 60 °C for 72 h. Shoot/root ratio was expressed as the DW ratio of the shoot to root (Arab et al. 2016).

2.2.2. Hydrogen peroxide contents in leaf samples

Leaf hydrogen peroxide (H_2O_2) contents were measured according to the method as described by Loreto and Velikova (2001) with modifications. About 50 mg frozen powder was extracted on ice with 1 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was then centrifuged at 4 °C, 15,000 g for 15 min. An aliquot of 300 μl supernatant was added to 300 μl 100 mmol potassium phosphate buffer (pH 7.0) and 600 μl 1 mol KI. The absorbance of the mixture was measured at 390 nm. The content of H_2O_2 was calculated according to a standard calibration curve made by different concentrations of H_2O_2.

2.2.3. Determination of total C, total N and $\delta^{13}C$

Total C,N and stable C isotope abundances in leaves were determined as described previously in Peuke et al. (2006). Briefly, 1–2 mg of oven dried (3 days at 65 °C) leaf powder was transferred into tin capsules. Samples were analyzed using an elemental analyzer (NA 2500; CE Instruments, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta Plus; Finnigan MAT GmbH, Bremen, Germany) by a Conflo II interface (Finnigan MAT GmbH, Bremen, Germany). The C isotopic values were expressed in delta notation (in per mil unit) relative to the VPDB (Vienna Pee Dee Belemnite) standard.

2.2.4. Analysis of ascorbate and thiols

Ascorbate and thiols were extracted, derivatized, and quantified as described in Du et al. (2016). Total and reduced ascorbate contents were determined photometrically using the method of Herschbach et al. (2009). Dehydroascorbate (DHA) was calculated by subtraction of reduced ascorbate from the total ascorbate.

The thiols cysteine, γ-glutamylcysteine (γ-EC) and glutathione (GSH) were extracted and quantified as described in Herschbach et al. (2009). Analysis was performed after reduction of the thiols with dithiothreitol (DTT) and derivatization with monobromobimanes on a high-performance liquid chromatography (HPLC) system equipped with a fluorescence detector as described in Schupp and Rennenberg (1988). Peaks were identified and quantified using a standard solution containing 0.2 mmol cysteine, 0.1 mmol γ-EC and 1 mmol GSH in 0.01 mol HCl.

2.2.5. Determination of invitro activities of glutathione reductase (GR) and dehydroascorbate reductase (DHAR)

The GR and DHAR invitro enzyme activities were determined as described in Arab et al. (2016). Briefly, approximately 100 mg of frozen leaf powder was extracted with 1.5 ml cold extraction buffer, containing 100 mmol potassium phosphate (pH 7.8), 80 mg polyvinylpolypyrrolidone (PVPP) and 1% Triton X-100 (v:v). To collect the protein fraction, extractswere passed through a Sephadex G-25 column (NAP-5 column, GE Healthcare Life Science) by gravity. GR activity was quantified by determining GSH dependent oxidation of 1.25 mmol NADPH at 340 nm (Polle et al. 1990). DHAR activity was determined directly by following the increase in absorbance at 265 nm, resulting from GSH-dependentascorbate production (Polle et al. 1990). Enzyme measurements were conducted at 30 °C.

2.2.6. Determination of total soluble sugar, soluble protein and total amino acid contents

To determine soluble sugar contents in leaves, 50 mg powdered frozen tissues were extracted with 1.5 ml of double-distilled H_2O with shaking at 4 °C for 60 min, then the mixture was boiled at 95 °C for 5 min. After centrifugation (10,000 g, 10 min), 200 μl of 10 times diluted supernatants was mixed with 1 ml anthrone reagent (50 mg anthrone and 1 g thiourea in 100 ml 70% H_2SO_4). The reaction solutions were boiled for 15 min and the absorbance measured at 578 nm (Carroll et al. 1956) after cooling down. Sucrose was used as a standard for quantification.