Environmental and Experimental Botany 86 (2013) 70–75

Contents lists available at ScienceDirect

Environmental and Experimental Botany

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

Plant response to drought stress simulated by ABA application: Changes in chemical composition of cuticular waxes

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ARTICLE INFO

Article history:
Received 30 December 2009
Received in revised form 31 May 2010
Accepted 27 June 2010

Keywords:
Abscisic acid
Carbon isotope
CER6
Chain length
Cuticular waxes
Lepidium sativum
Stomata

ABSTRACT

Plant cuticles form the interface between epidermal plant cells and the atmosphere. The cuticle creates an effective barrier against water loss, bacterial and fungal infection and also protects plant tissue from UV radiation. It is composed of the cutin matrix and embedded soluble lipids also called waxes. Chemical composition of cuticular waxes and physiological properties of cuticles are affected by internal regulatory mechanisms and environmental conditions (e.g. drought, light, and humidity). Here, we tested the effect of drought stress simulation by the exogenous application of abscisic acid (ABA) on cuticular wax amount and composition. ABA-treated plants and control plants differed in total aboveground biomass, leaf area, stomatal density and aperture, and carbon isotope composition. They did not differ in total wax amount per area but there were peculiar differences in the abundance of particular components. ABA-treated plants contained significantly higher proportions of aliphatic components characterized by chain length larger than C_{26}, compared to control plants. This trend was consistent both between and within different functional groups of wax components. This can lead to a higher hydrophobicity of the cuticular transpiration barrier and thus decrease cuticular water loss in ABA-treated plants. At both ABA-treated and control plants alcohols with chain length C_{24} and C_{26} were predominant. Such a shift towards wax compounds having a higher average chain length under drought conditions can be interpreted as an adaptive response of plants towards drought stress.

1. Introduction

Water shortage belongs to important abiotic plant stressors affecting plant growth and production. The main barrier against water loss is the plant cuticle (Kerstiens, 1996). This extracellular lipid polymer of the epidermis protects all aboveground plant organs (Hooker et al., 2007). The plant cuticle is composed of two main parts: the cuticular matrix formed by the cutin polymer layer matted by polysaccharide microfibrils and cuticular waxes which are embedded in the matrix (Kunst and Samuels, 2003). Cuticular barrier properties are mostly established by cuticular waxes which are partially crystalline (Riederer and Schreiber, 2001). The adaptation of cuticular water permeability to drought stress differs between plant species in respect to their origin (Schreiber and Riederer, 1996; Karbulková et al., 2008). The permeability differs not only between plants, their organs but also between both sides of the same leaf (Šantrůček et al., 2004; Karbulková et al., 2008). The adaxial (frequently astomatous) leaf side is often less permeable then the abaxial (stomatous) one, even after subtraction of the stomatal contribution. At favorable conditions, stomata mediate roughly 94–99% of water vapour exchange. During drought stress when stomata are closed, the plant survival depends on amounts of water lost through the cuticle. This means that the interplay between stomatal and cuticular water loss regulation is essential for plants when coping with water shortage (Kerstiens, 1996).

Both stomatal and cuticular conductance to water vapour responds to short-term changes of relative air humidity (Streck, 2003; Schreiber et al., 2001; Karbulková et al., 2008). On the other hand, the response of stomatal density and cuticular permeability to long-term (growth) relative humidity changes is rather species-specific (Geyer and Schönherr, 1990; Bakker, 1991; Hirai et al., 2002; Torre et al., 2003; Karbulková et al., 2008).

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doi:10.1016/j.envexpbot.2010.06.005

Stomatal closure during drought is mainly regulated by an abscisic acid (ABA) signaling pathway. ABA is synthesized in almost all cells containing chloroplasts or amyloplasts in both plant leaves and roots (Cutler and Krochko, 1999). ABA is also involved in promoting drought tolerance when applied exogenously (Pospíšilová et al., 1998; Wang et al., 2003; Li et al., 2004). Effects of drought stress can also be estimated from leaf carbon isotope composition (Macek et al., 2009). Stomatal closure decreases CO_2 concentration in the leaf (c_i) and thus decreases ratio between internal and ambient CO_2 concentration (c_i/c_a) which affects the discrimination against the carbon isotope ^{13}C during CO_2 fixation by Rubisco (Farquhar et al., 1982). Therefore, ABA-induced drought stress is reflected in leaf carbon isotope composition (cf. Barbour and Farquhar, 2000; Zhang et al., 2005).

Since ABA plays a key role in plant acclimation not only to drought but also to cold, salinity and development of seed dormancy, it affects the transcription of a large number of genes (Assmann, 2004). ABA-regulated genes should content ABA-responsive cis-acting elements (ABRE) in promoters (Hooker et al., 2002). Several enzymes and proteins involved in wax biosynthesis and transport are affected by ABA (Hooker et al., 2002; Luo et al., 2007; Treviño and O'Connell, 1998; Hooker et al., 2002; Luo et al., 2007). Studies focusing on ABA effects on composition of cuticular waxes are scarce; the few ones focus mainly on total wax amount but not on chemical composition (Sangtarash et al., 2009). Hence, in this study, we investigated if drought stress simulated by the exogenous application of ABA can cause significant changes in cuticular wax synthesis. More specifically, we were interested whether the effect will be quantitative, affecting the total wax amount, or qualitative affecting wax substance class composition and chain length distribution? During leaf growth and development, cuticular wax amount and composition changes relatively fast, hence, apart from stomatal closure, changes in wax composition can potentially influence plant response to drought. Waxes with chain length larger that C_{27} have been related to lower cuticular transpiration (Hauke and Schreiber, 1998). We thus hypothesize, that a preferential synthesis of longer aliphatic chains under drought conditions can be a mechanism significantly reducing water loss in plants.

2. Materials and methods

2.1. Plant material and sample size

Lepidium sativum var. *capitata* seeds were placed in 30 small pots (100 ml) with soil (about 10 seeds per pot). The pots were placed into growth chamber with controlled irradiation (800 μmol photons m^{-2} s^{-1}; 14 h day/10 h night), humidity (60%) and temperature (25 °C). Three days after germination, pots were split into two groups. The first group (control) was daily watered with 10 ml of distilled water, while the second group (ABA-treated plants) was watered with 10 ml of 10^{-4} M abscisic acid solution ((+)-Abscisic acid, Sigma–Aldrich, Germany). Seven days later, aboveground biomass from both groups was harvested.

Cotyledons were used for wax extraction and subsequently for measurements of projected leaf areas (mm^2); five samples for each of the treatment (i.e. control and ABA-treated plants) were investigated and each sample consisted of 20 individual plants pooled together. Another set of plants was used for assessing the total aboveground biomass (five samples per treatment, 10 plants per sample). Fresh and dry weighs of the plants were determined. The total aboveground biomass was expressed as amount of dry weight (mg) per plant. Powdered samples were consequently used for carbon isotope composition analyses. Stomatal density (stomatal number per mm^2) and stomatal length (μm) were estimated from leaf imprints of plants from both treatments and both leaf sides resulting in 96 imprints in total (four graticule fields per imprint were estimated to cover leaf heterogeneity). ABA content was analyzed in both ABA-treated and control samples (two samples per treatment).

2.2. Wax extraction and chemical analysis

Plants were immersed in 4 ml of chloroform ($CHCl_3$, Merck, Darmstadt, Germany) for 30 s. Chemical composition and amount of extracted cuticular waxes was measured by capillary gas chromatography with flame detection (GC-FID; CG-Hewlett-Packard 5890 series H, Hewlett-Packard, Palo Alto, CA, USA) with on-column injection (30 m DB-1 i.d. 0.32 mm, film 0.2 μm; J&W Scientific, Folsom, CA, USA) as described by Hauke and Schreiber (1998). Wax compounds were identified by gas chromatography mass spectrometry (GC–MS; quadrupole mass selective detector HP 5971, Hewlett-Packard, Palo Alto, CA, USA).

An internal standard (20 μl of tetracosane; Fluka, Ulm, Germany; chloroform solution 10 mg into 50 ml) was added to each sample directly after extraction. Hydroxyl and carboxylic groups were transformed into corresponding trimethylsilyl (TMS) derivatives by reaction with N,N-bis-trimethylsilyl-trifluoroacetamide (BSTFA; Macherey-Nagal, Düren, Germany) in pyridine (30 min at 70 °C). A sample (1 μl) was analyzed and quantification was done referring to the internal standard. Particular components are given as absolute values (μg cm^{-2}) and as percentage of total wax amount analyzed by GC-FID.

2.3. $\delta^{13}C$ isotope composition

The carbon isotope composition of leaf dry mass was determined using an elemental analyser (EA1110, ThermoQuest, Italy) linked to DeltaXLplus (ThermoFinnigan, Bremen, Germany). The ^{13}C content was calculated versus the VPDB (Vienna Pee Dee Belemnite) standard and expressed as

$$\delta^{13}C = \left(\frac{R_{sample}}{R_{std}} - 1 \right) \times 1000\,(‰)$$

where R_{sample} represents $^{12}C/^{13}C$ ratio in plant sample and R_{std} represents $^{12}C/^{13}C$ ratio in VPDB standard. The standard deviation of $\delta^{13}C$ determination in standard samples was lower than 0.1‰.

2.4. ABA determination in leaves

Cotyledon leaves (0.2–2 g of fresh weight) were used for analysis of internal ABA content in both control and ABA plants. Leaves were excised, then immediately weighted, frozen in liquid nitrogen and stored at −70 °C until analysis. Extraction and purification of plant material followed the procedure described in Dobrev and Kamínek (2002). Evaporated samples were dissolved in 200–400 μl 20% methanol in water (v/v) and 80 μl aliquot was injected into two-dimensional HPLC. ABA was determined using 2D-HPLC according to Dobrev et al. (2005).

2.5. Statistical analyses

Data were analyzed using one-way ANOVA with ABA application as a factor. When appropriate, a hierarchical design of ANOVA was used (stomatal length and density; in case of stomatal density, leaf side was used as the second factor). We further used linear regression to assess the relationship between chain length and treatment. All values in the text and tables are presented as means ± SD.