



Biochemistry

A drought-sensitive barley variety displays oxidative stress and strongly increased contents in low-molecular weight antioxidant compounds during water deficit compared to a tolerant variety

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ABSTRACT

Barley displays a great genetic diversity, constituting a valuable source to delineate the responses of contrasted genotypes to environmental constraints. Here, we investigated the level of oxidative stress and the participation of antioxidant systems in two barley genotypes: Express, a variety known to be sensitive to drought, and Saïda, an Algerian landrace selected for its tolerance to water deficit. Soil-grown 15-day-old plants were subjected to water deficit for 8 days and then rewatered. We observed that upon water stress Express exhibits compared to Saïda accelerated wilting and a higher level of oxidative stress evaluated by HPLC measurements of lipid peroxidation and by imaging techniques. In parallel, Express plants also display lower levels of catalase and superoxide dismutase activity. No great difference was observed regarding peroxiredoxins and methionine sulfoxide reductases, enzymes detoxifying peroxides and repairing oxidized proteins, respectively. In contrast, upon water stress and recovery, much higher contents and oxidation ratios of glutathione and ascorbate were measured in Express compared to Saïda. Express also shows during water deficit greater increases in the pools of lipophilic antioxidants like xanthophyll carotenoids and α -tocopherol. Altogether, these data show that the differential behavior of the two genotypes involves distinct responses regarding antioxidant mechanisms. Indeed, the drought sensitivity of Express compared with Saïda is associated with oxidative damage and a lower enzymatic ROS-scavenging capacity, but in parallel with a much stronger enhancement of most mechanisms involving low-molecular weight antioxidant compounds.

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Introduction

Water availability, which is mainly influenced by factors related to soil and climate, is a major determinant of plant growth and a critical factor in agriculture (Blum, 2005; Neumann, 2008). Among

cereals, barley (*Hordeum vulgare*) is one of the most widely cultivated species around the world. Thanks to its genetic diversity, barley exhibits a remarkable adaptability to a wide range of environments from arctic latitudes to tropical areas, and also at high altitude (von Bothmer et al., 2003). This diversity is a valuable source for crop improvement, and constitutes a fundamental basis to study the processes of adaptation and to identify genes involved in stress tolerance. Barley is extensively cultivated in the Mediterranean region and is generally rain fed, therefore subjected to highly variable and often weak rainfall patterns.

Adaptation to water shortage is achieved at the molecular, biochemical, physiological and anatomical levels. Numerous studies demonstrated that changes in stomatal conductance, water and nutrient uptake, hormones, osmolytes and antioxidants play important roles in plant tolerance to drought (Ingram and Bartels, 1996). Cell growth and photosynthetic metabolism are among

Abbreviations: ASC, ascorbic acid; CAT, catalase; DHA, dehydroascorbate; GSH, glutathione; LOX, lipoxygenase; MetO, methionine sulfoxide; MSR, methionine sulfoxide reductase; Prx, peroxiredoxin; ROS, reactive oxygen species; SOD, superoxide dismutase; Trx, thioredoxin.

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the primary processes affected by water deficit (Chaves, 1991). Drought stress conditions lead generally to substantial oxidative damage (Smirnoff, 1993), which partly results from impairment in photosynthetic machinery. Indeed, a decrease in photosynthetic activity is generally observed from the first stages of water shortage due to limited CO₂ diffusion through stomata (Flexas et al., 2004). As a consequence, plants are exposed to an excess of light energy, which cannot be converted into biochemical energy and leads to the production of reactive oxygen species (ROS) (Demmig-Adams and Adams, 1992; Foyer and Noctor, 2005).

Plants have evolved a complex array of antioxidant systems to prevent oxidative injury resulting from high levels of ROS, which are able to oxidize all macromolecules within cell and impair metabolism. They display various types of well-known enzymes scavenging ROS like superoxide dismutases (SODs), catalases (CATs) and peroxidases (Apel and Hirt, 2004). Further, they also possess numerous low-molecular weight antioxidant compounds either soluble like ascorbate and glutathione, or lipophilic like carotenoids and tocopherols (Noctor and Foyer, 1998; Havaux and Niyogi, 1999; Havaux et al., 2005). Many reports demonstrated that these systems participate in responses of cereals to water deficit. For instance, Loggini et al. (1999) showed a higher content in zeaxanthin, a carotenoid involved in lipid protection and dissipation of non-radiative energy, in a sensitive wheat cultivar compared to a tolerant one. Gorantla et al. (2007) identified drought-induced genes in rice and observed that genes encoding SOD and CAT isoforms are up-regulated in response to water deficit. Accordingly, in wheat, drought induces H₂O₂ accumulation, CAT gene expression and CAT activity (Luna et al., 2005). Relatively few data are available regarding antioxidant systems in responses of barley to water deficit. Among 34 genes specifically expressed in drought-tolerant barley cultivars, several encode proteins involved in detoxification of ROS-induced compounds like aldehyde dehydrogenase, glutathione-S-transferase and ascorbate-dependent oxidoreductase (Guo et al., 2009). In other respects, thiol oxidoreductases, the activity of which is based on redox-active cysteines, have been recently found to participate in the plant antioxidant network (Rey et al., 2005; Vieira Dos Santos and Rey, 2006). These enzymes include thioredoxins (Trxs), which transfer reducing power to protein targets like peroxiredoxins (Prxs) involved in detoxification of organic peroxides. Interestingly, the CDSP32 Trx, which prevents oxidative damage (Broin et al., 2002), is induced by water deficit in Solanaceae (Rey et al., 1998) and the abundance and redox status of some Prx types are modified in response to drought in Arabidopsis (Gama et al., 2008; Rey et al., 2007). Trxs are also able to reduce methionine sulfoxide reductases (MSRs), enzymes regenerating methionine from methionine sulfoxide (MetO; Tarrago et al., 2009). Note that Met oxidation in proteins could participate in ROS scavenging (Levine et al., 1996). In cereals, the knowledge about the involvement of thiol oxidoreductases in drought stress responses is very poor. Only the expression of a *Trx h* gene has been reported to be triggered by water deficit (Gorantla et al., 2007).

In this study, we investigated the implementation of various antioxidant systems in the responses to drought of two barley genotypes reported to display a contrasted behavior: Express, a variety known to be sensitive to water deficit (Arnau et al., 1997) and Saïda, a tolerant Algerian landrace. We show that when exposed to water deficit for the same period, Express exhibits compared to Saïda accelerated wilting and oxidative stress. We then investigated how antioxidant mechanisms involving well-known ROS-scavenging enzymes, thiol reductases and low-molecular weight compounds either soluble or lipophilic are involved in the distinct responses to drought of the two genotypes.

Materials and methods

Plant material and water regime conditions

Two *Hordeum vulgare* L. genotypes were chosen based on differential tolerance to water stress. Seeds from Express, a variety sensitive to drought (Arnau et al., 1997), were obtained from S.I.S. (Via Mirandola, San Lazzaro di Savena, Italy). Seeds from Saïda, an Algerian landrace tolerant to drought, were obtained from ITGC (Institut Technique des Grandes Cultures, BP 16 El-Harrach, Alger, Algeria). After sowing in pots containing the same weight of compost soil, plants were grown under controlled conditions of temperature (25/20 °C, day/night), light (350 μmol photons m⁻² s⁻¹, 12 h photoperiod) and air humidity (60%) in a phytotron. Seven seedlings were grown in 2-L pots. All pots were similarly watered with the following frequency: 2 days with tap water and the 3rd day with a Coïc-Lesaint nutritive solution (Coïc and Lesaint, 1971). Fifteen days after sowing (4th leaf stage), three treatments were applied: one group of plants was grown under optimal watering for 8 days (control) and a second group was subjected to water stress by withholding watering for 8 days. Subsequently, some stressed plants were re-irrigated for 3 or 8 days. Leaf relative water content (RWC) was determined immediately after sampling. Pieces from young well-expanded leaves were weighed to determine fresh weight (FW) and then immersed in distilled water for 6 h at room temperature. Leaves were then blotted to remove water and weighed to measure water saturated weight (SW). Then, leaf samples were dried at 80 °C for 48 h to measure dry weight (DW). Leaf RWC was determined as follows: leaf RWC = (FW – DW)/(SW – DW).

Lipid peroxidation measurements

Lipid peroxidation was assessed by HPLC analysis of hydroxy fatty acids recovered from leaf tissues after triphenylphosphine reduction and saponification of total lipids. Leaf pieces (0.5 g) from four plants were sampled, frozen in liquid nitrogen and stored at –80 °C. Extraction was achieved according to Montillet et al. (2004). An aliquot (100 μl) was subjected to straight phase HPLC (Waters Alliance 2690 and Dual Absorbance Detector 2487, Saint-Quentin-en-Yvelines, France) using a Zorbax rx-SIL column (4.6 × 250 mm, 5-μm particle size, Hewlett Packard, Les Ulis, France), isocratic elution with 70:29.31:0.69 (v/v/v) hexane:diethyl ether:acetic acid at a flow rate of 1.5 ml min⁻¹, and UV detection at 234 nm. ROS-mediated lipid peroxidation was evaluated from the level of different hydroxyoctadecatrienoic acid (HOTE) isomers using 15-hydroxy-11,13-(Z,E)-eicosadienoic acid as internal standard (Montillet et al., 2004). LOX-induced lipid peroxidation was estimated from the level of 13-HOTE after subtraction of racemic 13-HOTE (attributable to ROS-mediated lipid peroxidation) as explained in Montillet et al. (2004).

Autoluminescence imaging

Lipid peroxidation was visualized in leaves using autoluminescence imaging, as previously described (Havaux et al., 2006; Birtic et al., 2011). Spontaneous photon emission due to spontaneous decomposition of lipid peroxides was recorded at room temperature using a highly sensitive charge coupled device camera (VersArray LN/CCD 1340-1300B, Roper Scientific), with a liquid nitrogen cooled sensor to reduce thermal noise and enable measurement of faint light by signal integration (Havaux et al., 2006). Treated plants were dark-adapted for 2 h before imaging. Acquisition time was 20 min.

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