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Protein carbonylation linked to wheat seedling tolerance to water deficiency

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ABSTRACT

The appearance of the first leaf from the coleoptile in wheat seedlings (*Triticum aestivum* L.) coincides with the development of seedling susceptibility to water deficiency on the fifth day following imbibition. In dehydrated wheat seedlings, an increase in the protein carbonyl group has been observed. The coincidence of higher protein carbonylation levels with development of dehydration intolerance drew our attention. To gain more insight into the molecular basis of wheat drought tolerance, the seedling profiles of carbonylated proteins were analysed and compared. Two-dimensional gel electrophoresis (2D-PAGE) and mass spectrometry (MALDI-TOF and LC–MS/MS) were used to indicate and identify differential carbonylated proteins. Among the protein spots with at least a two-fold change in protein abundance in dehydrated seedlings in relation to control (well-watered) plants during the tolerant phase of growth, 19 carbonylated proteins increased and 18 carbonylated proteins decreased in abundance. Among 26 differentially expressed carbonylated proteins in sensitive seedlings, the abundance of 10 protein spots increased while that of 16 proteins decreased upon dehydration. We have demonstrated a link between protein carbonylation and seedling sensitivity to dehydration. The analysis of carbonylated protein profiles clearly showed that proteins with a potential role in the maintenance of dehydration tolerance in wheat seedlings are mainly linked to energy production, anti-fungal and/or insecticidal activity, or to the regulation of both protein synthesis and degradation.

1. Introduction

Water stress, one of the most common abiotic stresses, may be a consequence of primary (drought-induced) or diverse secondary (chilling-, freezing, heat- and salt-induced) or tertiary (radiation-induced) stresses (Levitt, 1980). These adverse environmental factors may induce increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), an inevitable consequence of aerobic metabolism, which with insufficient neutralization by the antioxidant system, disrupts cellular redox homeostasis and leads to the development of oxidative stress. Thus, plant tolerance to any abiotic stresses largely depends on a fine regulation of cellular redox homeostasis, which is acquired mainly via non-enzymatic and non-specific antioxidants, and a group of ROS scavenger enzymes (Mittler, 2017).

Successful plant acclimation to unfavourable environmental conditions is associated with profound changes resulting from the cell redox state (Foyer and Noctor, 2016; Anjum et al., 2016). The reaction of ROS and/or RNS with specific proteins leads to post-translational protein modifications (Spoel and Loake, 2011). Among the redox modifications, oxidation of proteins may occur through a number of mechanisms, such as reversible *S*-glutathionylation, RNS-based *S*-nitrosylation, or carbonylation of specific amino acids (Choudhury et al., 2016). Accumulating evidence suggests the occurrence of a dynamic interplay between protein carbonylation and nitrosylation. It may play an important role in the regulation of proteome remodelling during the plant response to unfavourable environmental conditions (Lounifi et al., 2013).

Carbonylation is the most common protein modification induced by ROS (Levine, 2002). The formation of protein carbonyl groups typically results from metal-catalysed oxidation of the side chain of lysine, arginine, proline, and threonine but can also be mediated by indirect reactions of lipoperoxidation products with

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cysteine and histidine residues (Choudhury et al., 2016). Consequently, the structure of proteins is modified, with a resultant loss of their biological activity and, finally, changes in cell metabolism (Nyström, 2005). Carbonylated proteins have been detected mainly in the cytosol, mitochondria (Smakowska et al., 2014) and chloroplasts (Johansson et al., 2004). The formation of carbonyl groups can be linked not only to the direct reaction to stress factors but also to abnormal translation, alternations in the chaperone system, and inhibition of enzyme activity due to the reduced availability of substrates (Nyström, 2005). The additional formation of carbonyl groups in the proteins may result in their misfolding, loss of function, and degradation by the proteasome (Levine, 2002). The extent of protein carbonylation seems to be correlated with the duration of plant exposure and the amount of ROS generated under stress conditions (Lounifi et al., 2013).

Protein carbonylation is irreversible, and thus it may serve as a biomarker of the oxidative damage to proteins in evaluations of their oxidation (Dalle-Donne et al., 2003). However, recent studies provide evidence for the existence of an enzymatic system that catalyses the elimination of formed protein carbonyls (Wong et al., 2013). To circumvent the formation of mainly insoluble, harmful protein aggregates that are toxic to cells and the accumulation of non-functional proteins, oxidized proteins are efficiently removed by several distinct proteolytic pathways that play central roles in ATP- and ubiquitin-dependent pathways with the 20S rather than the 26S proteasome (Polge et al., 2009; Kastle and Grune, 2011). The accumulation of carbonylated proteins in mitochondria and chloroplasts is prevented by proteases belonging to the ATP-dependent AAA+ family, such as Lon and FtsH proteases (Gibala et al., 2009; Rigas et al., 2012). In addition, carbonylated proteins can be removed by autophagy, a nonspecific protein-degradation pathway, which is induced by several environmental stresses (Xiong et al., 2007). In this way, the carbonylation of proteins could promote the degradation of mistranslated, damaged, and aberrant or even no longer required proteins in plant cells (Nyström, 2005).

The progression of wheat seed germination (*Triticum aestivum* L.) results in the development of seedling sensitivity to water deficiency on the fifth day following imbibition (Blum et al., 1980; Miazek et al., 2001; Corbineau et al., 2004; Farrant et al., 2004). The age-induced loss of dehydration tolerance is associated with a shift in more positive values for the half-cell reduction potential of the GSH/GSSG couple ($E_{GSSG/2GSH}$), an increase in protein carbonyl groups and a significant decrease in the ratio of protein thiol to total, protein and non-protein thiol group content (Gietler et al., 2016a). The oxidized proteins were more rapidly degraded, especially in seedlings that were sensitive to dehydration (Gietler et al., 2016b). The correlation of higher levels of carbonylation, which mediates protein quality and metabolism (Nyström, 2005), with the development of dehydration intolerance drew our attention (Gietler et al., 2016a). However, estimations of the concentration of carbonyl groups in oxidized proteins only indicate the type of post-translational protein modification; protein network alterations under water deficiency remain unknown. To gain more insight into the molecular basis of wheat drought tolerance, the seedling profiles of carbonylated proteins were analysed and compared. We used two-dimensional gel electrophoresis (2DE) and mass spectrometry (MALDI-TOF and LC–MS/MS) as tools for searching for stress-related proteins that may induce and/or may directly contribute to wheat tolerance. This approach is required to confirm one or both hypotheses that protein carbonylation in response of *Triticum aestivum* L. seedlings to dehydration might be the results of an enhanced oxidative stress, which occurs during water deficiency or it might have a physiological meaning due to different modification of metabolism in tolerant and sensitive stage of seedling growth.

2. Materials and methods

2.1. Plant material and experimental design

Seeds of spring wheat (*Triticum aestivum* L. cv. Zadra) obtained from Plant Breeding Strzelce Co., Ltd. of the Plant Breeding and Acclimatization Institute-National Research Institute (Poland) were used. The seeds were surface sterilized for 20 min with 1% sodium hypochlorite, rinsed several times with distilled water and incubated in water in the dark at 4 °C overnight. Twenty seeds were then placed side by side on a filter paper strip (25 cm × 5 cm) covered with a similar strip, rolled and placed vertically (embryos downward) into boxes containing Knop's nutrient solution supplemented with Hoagland's micronutrients (Hoagland and Snyder, 1933; Knop, 1865). The seeds were germinated, and the seedlings were grown in a climatic chamber under optimal conditions (16 h of light at 23 °C and 8 h of dark at 16 °C, a photosynthetic photon flux density (PPFD) of 260 μmole m^{-2} s^{-1}, and a relative humidity of 70–80%). After four and six days of seedling growth under optimal conditions, the seedlings were dehydrated by draining off the nutrient solution. Dehydration of seedlings was performed for 4 days under the same growth conditions. The water content in the shoots of the seedlings was determined as water saturation deficit (WSD) according to Turner (1981) and calculated using the following formula: WSD (%) = (full turgor mass-actual fresh mass)/(full turgor mass-dry mass) × 100%, where full turgor mass represented the mass after submersion in water overnight in the dark and dry mass was determined after oven drying at 80 °C overnight. The dehydration tolerance of the seedlings was evaluated based on the seedlings' survival after the four-day dehydration period and was expressed as the percent of surviving seedlings, i.e., the number of seedlings able to resume elongative growth. Three series of independent experiments were carried out and for measuring shoot water content each repetition consisted of 5 random shoots and for evaluation of seedlings survival in every biological repetition 3 sets of 20 seedlings were used.

In the proteomic experiment, well-watered four and six days old shoots of wheat seedlings were used as a control group, and seedlings of the same age were dehydrated up to 65% of WSD and used as the stressed group.

2.2. Preparation of total protein extracts

To obtain a proteome profile of carbonylated proteins, protein extraction and purification was performed. All above ground shoot tissue samples were ground into a fine powder in a mortar in liquid nitrogen. A sample of 150 mg of powder was transferred into a 2-mL test tube and purified according to Wang et al. (2006). The proteins were precipitated by addition ice-cold 10% (w/v) TCA/acetone. The sample was mixed and stored for 30 min at −20 °C. Next, it was centrifuged at 16 000 × *g* for 30 min at 4 °C, the supernatant was discarded and the pellet was washed two times with ice-cold TCA/acetone. After centrifugation, the tube was filled with 80% methanol containing 0.1 M ammonium acetate, mixed and centrifuged for 15 min. The supernatant was removed, and the pellet was washed with 80% acetone, mixed again and after centrifugation the supernatant was discarded. The remaining pellet was air-dried at room temperature to remove residual acetone and dissolved in 0.6 mL of phenol (pH 8.0) and 0.6 mL of SDS buffer (0.1 M Tris-HCl pH 8.0 containing 30% (w/v) sucrose, 5% (v/v) β-mercaptoethanol, and 2% (w/v) SDS) and mixed. After incubation at room temperature for 5 min, the water and phenolic phases were separated by centrifugation (16 000 × *g*, 4 °C, 15 min), and the upper (phenolic) phase was collected into a new tube. The extracted proteins were precipitated from the mixture by