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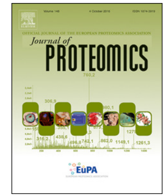

# Lysine acetylproteome profiling under water deficit reveals key acetylated proteins involved in wheat grain development and starch biosynthesis

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ABSTRACT

Lysine acetylation is a widespread protein posttranslational modification in all organisms. However, quantitative acetylproteome characterization in response to water deficit during crop grain development remains unknown. In the study, we performed the first large-scale acetylproteome analysis of developing wheat grains under water-deficit using label-free quantitative proteome approach. In total, 716 acetylated sites corresponding to 442 acetylated proteins were identified, of which 106 acetylated sites representing 93 acetylated proteins (including 88 non-histones) showed significant changes under water-deficit. The functional classification showed that 57% and 20% of acetylated proteins were related to metabolic and cellular processes, respectively. Water-deficit caused widespread functional crosstalk between protein acetylation and other PTMs. Particularly, both acetylation and phosphorylation occurred in two key enzymes involved in starch biosynthesis, sucrose synthase (SuSy) and ADP glucose pyrophosphorylase (AGPase). Their crosstalk could play important roles in starch biosynthesis and yield formation under drought conditions. Western blot analysis combined with tandem mass spectrometry identification further verified the reliability of the acetylproteome results. Most of the acetylated proteins showed consistences between transcription and post-translation levels by quantitative real-time PCR. A putative metabolic pathway was proposed to dissect the roles of protein acetylation in regulation of drought response and defense during wheat grain development.

*Significance:* Lysine acetylation is a widespread modification in all organisms. We performed the first large-scale acetylproteome analysis of developing wheat grains under water-deficit and revealed key acetylated proteins involved in wheat grain development and starch biosynthesis.

## 1. Introduction

Wheat, as the basis of bread, pasta, and breakfast cereals, is the undisputed number one cereal crop due to its value as a staple food and protein source [1]. With the continual increase in the world population, the global demand for wheat has risen [2], and feeding the growing population under deteriorating climatic conditions is one of the greatest challenges facing humans in the near future. Drought, as a major abiotic stress, could induce significant changes to both the yield and quality of many crop species [3]. An estimated 60% of land used for wheat cultivation is suffering moderate-to-extreme drought stress, even in arid climates. Therefore, developing drought-resistant crops with improved water-use efficiencies and enhanced productivities is the most promising approach for attenuating future threats to food security [4].

In recent years, based on transcriptomics, metabolomics and proteomics many studies have advanced our knowledge about the molecular mechanisms of the drought response and wheat seed development [3,5–9]. Plants have progressively developed various regulatory mechanisms to deal with drought stress at molecular, cellular, and other whole-plant levels [10,11]. The phytohormone abscisic acid (ABA) regulates plant growth and development processes, such as seedling growth, seed maturation and germination, and plant adaptation to

*Abbreviations:* AGPase, ADP glucose pyrophosphorylase; BiFC, bimolecular fluorescence complementation; DAF, days after flowering; HOP, HSP70-HSP90 organizing protein; MDH, malate dehydrogenase; PPI, protein – protein interaction; PPP, pentose phosphorylation pathway; PTM, protein posttranslational modification; RLK, receptor-like serine/threonineprotein kinases; SEM, scanning electron microscope; SOM, stereoscopic optical microscope; SuSy, sucrose synthase; TEM, transmission electronmicroscope; TWK, thousand kernels weight; UBE, ubiquitin-conjugating enzyme

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diverse environmental stresses [12]. In addition, receptor-like kinases (RLKs) play a crucial part in optimizing the response of plant to water stress [13]. Moreover, it is widely accepted that protein posttranslational modification (PTM) of protein targets is a critical process in transmiting environmental stresses to physiologically diverse processes such as cell cycle regulation, growth, autophagy, and apoptosis [14].

Recent technological advances, combined with high-specific antibodies have made it possible to study alternate PTMs, such as sumoylation (lysine), methylation (arginine), acetylation (lysine) and ubiquitination (lysine), on a global scale [14–16]. Among PTMs, modifications on the ε-amino groups of lysine residues, identified over 50 years ago in histones, are involved in gene expression regulation [17]. Subsequent studies have indicated that many modifications could occur at lysine residues in both histone and non-histone proteins [18–20], of which lysine acetylation is increasingly popular as a major PTM because of its reversibility and the presence of ideal candidate regulators for different signaling and regulatory pathways [21]. Recently, numerous cases of acetylation in non-histone proteins have been identified in higher plants, such as *Brachypodium*, *Oryza*, *Glycine* max, and *Arabidopsis* [22–25]. Acetylation appears to be a dynamic modification that regulates diverse protein properties, including subcellular localization, transcriptional activity, DNA–protein interactions and protein stability [26]. However, the functional basis for acetylation is the chemical change made to the modified amino acid. Lysine acetylation could neutralize the positive charge of the amine group of the lys residue, and enhances the hydrophobicity and increases the size of the lys side chain, which not only impact the ability of the lys side chain to form hydrogen bonds and to have electrostatic interactions with negatively charged residues but also increase van der Waals interactions with other proteins [27]. Also, acetylation is a covalent modification, in which an acetyl group is transferred from acetyl-coenzyme A (CoA) by acetyltransferases, either affecting the α-amino group or the ε-amino group of a lysine residue [23]. In some non-histone proteins, acetylation mingles with phosphorylation, ubiquitination and other PTMs to form complex regulatory programs within or across proteins.[15] Increasing evidence indicates an unexpected importance of lysine acetylation in metabolic control and coordination of different metabolic pathways [28,29].

The seeds of wheat (2n = 6 × = 42, AABBDD) are mainly composed of proteins and starch [30]. Starch serves as the major storage carbohydrate in the cereal endosperm and deposits in the starch granule as a semi-crystalline structure [31]. Based on their diameters, two distinct forms of granules are present, A-type (10–50 μm) and B-type (5–9 μm) granules. Based on their structural composition, starch granules are usually composed of amylose and amylopectin [30,32]. Amylose is synthesized by granule-bound starch synthase (GBSS), while amylopectin synthesis is controlled by four enzyme classes: ADP glucose pyrophosphorylase (AGPase), starch synthase (SS), starch-branching enzyme (SBE), and starch-debranching enzyme (DBE) [32,33]. Starch biosynthesis related enzymes are regulated by phosphorylation, the only known covalent modification that occurs naturally in starch biosynthesis [32]. Protein phosphorylation is indispensable to form starch-synthesizing protein complexes [34].

To date, comprehensive proteome-wide analyses of PTMs in wheat, including leaves and developing grains, have mainly focused on phosphoproteomic analysis [12,15,35,36]. In terms of acetylproteome characterization in wheat, only one qualitative analysis of the lysine acetylproteome in seedlings has been reported [37]. Therefore, the role of protein acetylation modification in developing wheat grains under water deficit remains unclear. In this study, we performed the first acetylproteome analysis of developing wheat grains under water deficit using label-free quantification. Our results revealed the prevalence and importance of lysine acetylation in regulating wheat grain development and yield and quality formation under water-deficit conditions.

## 2. Materials and methods

### 2.1. Plant materials and water-deficit treatment

Chinese winter wheat cultivar “Jingdong 17” (*Triticum aestivum L.*, 2n = 6 × = 42, AABBDD) was planted in the Wuqiao experimental station at Hebei Province (37°41′02″N and 116°37′23″E), China Agricultural University in 2015–2016 wheat growing season. The average annual amount of sunshine was 2690 h, the average annual temperature was 12.6 °C, and the mean rainfall during the 2014–2015 wheat growing season was 128 mm. The field experiment included two different treatments: well-watered condition (CK) and water-deficit condition (DS). Each group had three biological replicates. The well-watered group was subject to normal water irrigation of 750 $m^3/hm^2$ at the jointing and flowering stages as local cultivation management, while the water-deficit group was set as rain-fed region without artificial water irrigation. Soil samples at maturity were collected at 20 cm increments to a depth of 200 cm using a soil corer, and the soil water content was determined using oven-drying method. The field growth dynamics was regularly observed and the plants were marked after flowering. The developing grains at 10, 15, 20, 25 and 30 days after flowering (DAF) from two treatments were collected and stored at −80 °C prior to subsequent analyses.

### 2.2. Measurement of main agronomic traits and physiobiochemical parameters

The main agronomic traits of mature plants were tested, such as plant height, panicle length, tiller number, spikelet number, kernel number, kernels per spike, grain hardness, thousand kernels weight (TWK) and grain yield. Relative water content (RWC), total chlorophyll content and the activities of malondialdehyde (MDA), glycine betaine, SuSy and AGPase were meatured based on the published procedure [12,38]. Datas were analyzed using SPSS statistics software (Version17.0) via independent Student's *t*-tests and two-tailed test.

### 2.3. Optical microscope and electron microscope observation of grain structures

The observation of grain structures by optical microscope, scanning electron microscope (SEM) and transmission electron microscope (TEM) was performed based on the previous studies [17,39,40].

### 2.4. Protein extraction

Total proteins were extracted based on the previous procedures [41] with minor modifications. The extraction buffer contained l mM DTT, l mM PMSF and Protease Inhibitor Cocktail (1 tablet/10 mL; Roche, Basel, Switzerland) followed by 3 μM TSA (BBI, CAS: 58880-19-6) and 50 mM NAM (Sangon, CAS: 98-2-0) provisionally. After freeze-drying at 4 °C, the pellets were added to 300 μL of solubilization buffer at room temperature for 2 h. Finally, the extracted proteins were determined with a 2-D Quant Kit (Amersham Bioscience, USA) and adjusted to the same concentration for further analysis.

### 2.5. Trypsin digestion and immunoaffinity enrichment of lysine acetylated peptide

In total, 10 mg proteins were reduced using 10 mM DTT for 2.5 h at 37 °C and alkylated for 30 min at normal temperature with 50 mM IAA away from light. And then the dilution and digestion of the protein samples were performed by adding 100 mM $NH_4CO_3$ (adjusting to urea concentration < 1.5 M) and trysin (1:50 trypsin-to-protein mass ratio for a 18 h) in turn, respectively. Tryptic peptide mixture were collected and desalted by a 100 mg of SEP-PAK (C18, Waters) and lyophilized for next enrichment. To enrich acetylated peptides, tryptic peptides were

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