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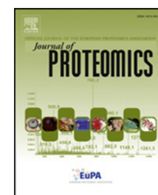

Identification of the proteins associated with low potassium tolerance in cultivated and Tibetan wild barley

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ABSTRACT

In previous studies, we found Tibetan wild barley accessions with high tolerance to low K. In this study, ionomics and proteomics analyses were done on two wild genotypes (XZ153, tolerant and XZ141, sensitive), and a cultivar (B1031, tolerance to low K) to understand the mechanism of low-K tolerance. XZ153 was much less affected by low K stress than the other two genotypes in plant biomass and shoot K content. A total of 288 differentially accumulated proteins were identified between low-K and normal K treated plants. Among them, 129 proteins related to low-K tolerance were mainly involved in defense, transcription, signal transduction, energy, and protein synthesis. The analysis of tandem mass tag (TMT) detected 51 proteins which were increased in relative abundance under low K in XZ153, but unaltered or decreased in XZ141. The proteomics results showed that XZ153 is highly capable of rearranging ion homeostasis and developing an antioxidant defense system under low-K stress. Moreover, ethylene response and phenylpropanoid pathways could determine the genotypic difference in low-K tolerance. The current results confirmed the possibility of Tibetan wild barley providing low-K tolerant germplasm and identified some candidate proteins for use in developing the cultivars with low-K tolerance.

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1. Introduction

As one of the most important and abundant nutrient ions in living plant cells, potassium (K) constitutes 2%–10% of plant dry weight [1]. It plays crucial roles in many physiological and biochemical processes, including enzyme activation, protein synthesis, photosynthesis, stomatal movements, ion homeostasis, etc. [2,3]. However, the available K level in most soils is not sufficient for plant requirements, similar to the case that happened in China. Moreover, with increasing cereal crop yields mainly caused by the development and planting of super-rice or super-wheat cultivars, more K is required and taken off from the soil for normal growth and yield formation [4]. As a whole, the low K level in the soil or the shortage of K fertilizer is becoming a global issue affecting crop yield and sustainable production, particularly in developing countries. K deficiency not only causes the inhibition of plant growth, photosynthesis, and transpiration, but also makes the plants more susceptible to pests and diseases [5–8], finally resulting in a reduced yield and deteriorated quality of crops.

It is a basic and also the most efficient approach to develop crop cultivars with high low-K tolerance or K use efficiency in coping with low K availability in the soil and insufficient K fertilizer supply [8,9]. It has been well documented that there is a dramatic difference in K use efficiency among plant species or genotypes within a species [10–12], indicating that K nutrition in plants is a genetically controlled trait, and can be improved by genetic manipulation. However, a narrower genetic diversity in cultivated barley has become a bottleneck for genetic improvement [13]. Thus, it is imperative for us to collect and exploit the barley genotypes with a wide genetic diversity. It has been reported that Tibetan annual wild barley, growing under extremely harsh environments with an altitude of higher than 3500 m, contains the accessions with high tolerance to some abiotic stresses, such as drought and salinity [14–17]. Additionally, wild barley is rich in genetic variation and shows generally better adaption to poor soil fertility, including K deficiency. In fact, we have identified some wild barley genotypes with high low-K tolerance in a previous study [18]. Therefore, it is significant to understand the underlying mechanisms of low K tolerance in wild barley.

To date available proteomic studies on plants were mostly limited to 2D gel electrophoresis analysis. However, the low-abundance proteins, such as membrane proteins and hydrophobic proteins, are difficult to detect on 2D gel electrophoresis [19–21]. To overcome the disadvantage of this technique, non-gel-based quantitative proteomic methods have been developed in recent years. Isobaric mass tagging (e.g., TMT and iTRAQ) is a precise and sensitive multiplexed peptide/protein quantification technique in mass spectrometry [22], which has been intensively

Abbreviations: AGC, automatic gain control; CPR, cytochrome P450 reductase; CYP, cytochrome P450; DW, dry weight; HCD, high-energy collision-induced dissociation; ICP-OES, inductively coupled plasma-optical emission spectroscopy; iTRAQ, isobaric tag for relative and absolute quantification; NCE, normalized collision energies; PAL, phenylalanine ammonia lyase; PMSF, phenylmethanesulfonyl fluoride; PVPP, polyvinylpolypyrrolidone; TMT, tandem mass tag.

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used for revealing the proteins being differentially expressed under any given conditions, including biotic and abiotic stresses, such as heat stress [23] and Al stress [24]. Recently, some omics methods have been performed on some plants, for instance, rice [25], soybean [26] and watermelon [27], to investigate the underlying tolerance mechanism of plants to K deficiency, mostly at the transcriptional level. The barley proteomics can be mainly summarized as (a) industry driven biotechnology, including seed germination, beer proteomes, and malting proteomes and (b) biology driven proteomics, including abiotic stress tolerance and nitrogen use efficiency [28].

However, little is known about the molecular nature of adaptive responses at the level of proteins, in particular by means of the isobaric tag method. Thus, it is imperative for us to reveal the mechanism or to explore the relevant proteins of high K use efficiency in barley.

In our previous research, some barley genotypes differing in low K tolerance were indentified [18]. Additionally, in view of the difference among genotypes in K content and deficient symptom under low K stress, the hypothesis could be proposed that some proteins related to K utilization and stress responses might be associated with low K tolerance. In this study, a comprehensive proteomics study was conducted on two wild barley genotypes (XZ153, low K tolerant and XZ141, low K sensitive) and one cultivar (Lu Dao Mai, low K tolerant, referred to as B1031 thereafter). The main objectives of the current study are to (1) characterize the proteomes of barley leaves using a gel-free proteomics approach, (2) identify differentially accumulated proteins in different barley genotypes by applying an isobaric TMT technique, and (3) determine the proteins and the signaling pathways in response to low K stress, which are useful for making molecular insights into K-efficient or low K tolerant mechanisms.

2. Materials and methods

2.1. Plant materials and K treatments

Two Tibetan wild barley accessions (XZ153, low K tolerant and XZ141, low K sensitive) and one cultivar (B1031, low K tolerant) were used in this study according to a previous study [18]. Seeds were sterilized with 2% H_2O_2 for 30 min and rinsed with distilled water for three times, then soaked for 6 h at room temperature. The seeds were germinated on moistened filter papers in the germination boxes, and placed into a plant growth chamber (22/18 °C, day/night). After 10 days of germination, uniform seedlings were transplanted into plastic pots (5 L) for hydroponic incubation. The experiment was conducted in a greenhouse with aerated hydroponic solution according to Zeng et al. [29]. Plants were supplied with a half-strength hydroponic solution in the first week and then changed into a full strength solution, and renewed every five days. Three-leaf-stage seedlings were subjected to K treatments with two levels: 0.01 mM (low K) and 1 mM (normal K as control).

2.2. Biomass and element content determination

At 16 days after K treatments, the plants of each treatment were sampled, and roots were thoroughly rinsed with tap water, and dried with tissue papers. Then shoots and roots of seedlings were separated, and dried at 80 °C for 72 h, and weighed. Dry shoots and roots were ground into powder, and an approximately 0.1 g tissue sample was used for the measurement of element content using inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Optima 8000DV, PerkinElmer, USA), according to the equipment operation manual.

2.3. Protein extraction and digestion

At 16 days after K treatment, three biological replicates of the leaves for each treatment were pooled for TMT analysis. Leaves (approximately 500 mg fresh weight) were powdered and homogenized in extraction buffer with cold 10% v/v TCA in acetone, 50 mM DTT, 1 mM phenylmethanesulfonyl fluoride (PMSF) and polyvinylpolypyrrolidone (PVPP). Then, the samples were incubated at −20 °C for 2 h. After centrifugation at 20,000 *g* and 4 °C for 10 min, the supernatant was discarded. The precipitate was washed with cold acetone supplemented with 50 mM DTT and 1 mM PMSF for three times. The dried pellets were re-suspended with lysis buffer (7 M urea, 2 M thiourea, 5 mM EDTA, 10 mM DTT and 1 mM PMSF). The suspended samples were then dissolved by ultrasonics for 10 min. After dissolution, the solution was centrifuged at 20,000 *g* for 10 min at 4 °C to remove the remnant. Subsequently, the supernatant was transferred to a new tube, reduced with 10 mM DTT at 56 °C for 1 h and alkylated with 55 mM iodoacetamide for 45 min at room temperature in darkness. The protein was precipitated with 4 volumes of chilled acetone at −20 °C for 30 min. After centrifugation, the pellet was then dissolved in 0.5 M TEAB and sonicated for 5 min. The centrifugation step was repeated and the supernatant was collected. Protein concentrations were determined with 2-D Quant kit (GE Healthcare) according to the manufacturer's instructions. Approximately 100 μg protein of each sample was digested with trypsin (Promega, USA) at a 1:20 mass ratio to the sample, and then incubated at 48 °C overnight [30].

2.4. TMT labeling

After trypsin digestion, peptide was desalted by a Strata C18 SPE column (Phenomenex, USA) and vacuum-dried. TMT six-plex Isobaric Label Reagent Set (Pierce, Idaho, ID, USA) was used according to the manufacturer's instructions. Briefly, samples were added with two units of the different isobaric chemical reagents and dissolved in 41 μL acetonitrile. The peptides from samples of three genotypes XZ153, XZ141 and B1031 (treatment and control) were labeled with TMT reporter 126, 127, 128, 129, 130 and 131 respectively, followed by an incubation step for 2 h at room temperature. The peptide mixtures were finally pooled, desalted and lyophilized.

2.5. LC–MS/MS analysis by Q-Exactive

LC–MS/MS analysis was carried out accounting to Tse et al. [31] and Wu et al. [32]. The general workflow was described as follows: the labeled peptide was redissolved in buffer A (2% acetonitrile and 0.1% formic acid) and centrifuged at 20,000 *g* for 2 min. The supernatant was transferred into new sample tubes and loaded onto an Acclaim PepMap 100 C18 trap column (Dionex, size 75 μm × 2 cm, USA) equipped with EASY nLC1000 nanoUPLC (Thermo Scientific, USA) and the peptide was eluted onto an Acclaim PepMap RSLC C18 analytical column (Dionex, size 50 μm × 15 cm, USA). Each fraction was independently separated with an 85 min gradient comprised of 5 min to 35% buffer B (0.1% formic acid in acetonitrile), followed by 5 min linear gradient to 90% B, and maintained at 90% B for 5 min at a constant flow rate of 300 nL/min. The peptides were submitted to a nanoESI source followed by tandem mass spectrometry (MS/MS) in Q-Exactive (Thermo Scientific, USA) that was coupled online to the HPLC [33]. Peptides were sprayed into the Q-Exactive MS/MS system with a spray voltage of 1.8 kV. A full mass spectra scan range of 350 to 2000 (m/z) was performed and intact peptides were acquired in the Orbitrap at a resolution of 70,000, and the automatic gain control (AGC) was set to 1,000,000 ions. Dynamic exclusion was activated for this process to prevent repetition, with a repeat count of 2, exclusion duration of 45 s, and ±5 ppm mass tolerance [31]. The fragment intensity multiplier was set to 20. In high-energy collision-induced dissociation (HCD) the following parameters were used: a resolution of 7500 in a centroid mode, a target value of 100,000 ions, normalized collision energies (NCE) of 40% and an activation time of 10 ms.

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