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Comparative proteomic analysis of salt response proteins in seedling roots of two wheat varieties

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ABSTRACT

A comparative proteomic analysis was made of salt response in seedling roots of wheat cultivars Jing-411 (salt tolerant) and Chinese Spring (salt sensitive) subjected to a range of salt stress concentrations (0.5%, 1.5% and 2.5%) for 2 days. One hundred and ninety eight differentially expressed protein spots (DEPs) were located with at least two-fold differences in abundance on 2-DE maps, of which 144 were identified by MALDI-TOF-TOF MS. These proteins were involved primarily in carbon metabolism (31.9%), detoxification and defense (12.5%), chaperones (5.6%) and signal transduction (4.9%). Comparative analysis showed that 41 DEPs were salt responsive with significant expression changes in both varieties under salt stress, and 99 (52 in Jing-411 and 47 in Chinese Spring) were variety specific. Only 15 and 9 DEPs in Jing-411 and Chinese Spring, respectively, were up-regulated in abundance under all three salt concentrations. All dynamics of the DEPs were analyzed across all treatments. Some salt responsive DEPs, such as guanine nucleotide-binding protein subunit beta-like protein, RuBisCO large subunit-binding protein subunit alpha and pathogenesis related protein 10, were up-regulated significantly in Jing-411 under all salt concentrations, whereas they were down-regulated in salinity-stressed Chinese Spring.

1. Introduction

Soil salinity is a prevalent abiotic stress, which seriously impairs crop production on at least 20% of irrigated land worldwide [1]. Salinity stress leads to slow growth, wilting or even death of plants, especially in high salt concentrations. Ion toxicity, nutrient constraints, hyperosmotic stress and oxidative stress caused by salt stress may be the primary causes of severely disrupted protein synthesis and act by interfering with normal enzyme activity [2,3]. Under salt stress, plants accumulate ion and reactive oxygen species (ROS) that are harmful to plant cells, especially under high salt concentrations [4]. These toxic by-products can decrease enzyme activity or even degrade some proteins. Due to genotypic difference and environmental condi-

Abbreviations: DEPs, differentially expressed protein spots; CS, Chinese Spring; RWC, relative water content; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; SAM2, S-adenosylmethionine synthetase 2; PR10, pathogenesis related protein 10; TCTPs, translationally controlled tumor-associated proteins.

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tions, plants have adaptive mechanisms to minimize salt injury, and different plants have developed different abilities to survive salt stress. In barley, S-adenosylmethionine (SAM) synthase and peroxidase involved in the detoxification of reactive oxygen species (ROS) were more abundant in salt-tolerant cultivar (cv) Steptoe than in salt-sensitive cv Morex, while proteins involved in iron uptake were expressed at a higher level in the sensitive cv Morex [5]. Salt-tolerant barley cultivars may have greater ability to sequester Na^+ into sub-cellular compartments and/or maintain K^+ homeostasis during salt stress [6]. Higher levels of vacuolar H^+-ATPase might play a pivotal role in salinity tolerance of plant roots.

To date, a number of salt-responsive genes involved in membrane transport, signal transduction, redox reaction and other processes have been identified. Many stress-related genes such as *AtNHX1*, *AVP1*, *AtSOS1* and *AtSOS2* are required for Na^+ sequestration and extrusion to maintain intracellular Na^+/K^+ homeostasis [7–9]. Such genes also include *AgNHX1* and *SKC1* in rice [10], and *GhNHX1* in tobacco [11]. Some small molecular compatible solutes were synthesized by a series of genes closely related to abiotic stress responses. For example, the *BADH* gene in *Suaeda liaotungensis* kitag, which encodes betaine aldehyde dehydrogenase, the key enzyme of glycinebetaine synthesis, was introduced into maize to improve salt tolerance [12–14]. Sorbitol dehydrogenase encoded by the *PmSDH1* gene over-accumulates mannitol to regulate salt stress in transgenic *Plantago major* plants [7]. Similarly, some transcriptional factors, such as *OsNAC5* and *JERF3* in rice and *TaSTRG* in wheat, can regulate stress responses and transgenic plants had improved tolerance to abiotic stresses such as salt, drought and cold [15,16]. Despite these examples, salinity response remains a very complicated quantitative trait, causing many proteins to undergo removal of signal peptides, RNA splicing and post-translational modifications (PTMs) such as phosphorylation and glycosylation. This leads to poor correlations between transcriptomes and proteomes in different wheat cultivars under abiotic stresses [17,18].

Recently, proteomic analysis has become one of the best strategies to reveal the dynamics of expression under salt stress. Comparative analysis of root proteomes between two durum wheat varieties with different tolerance levels to NaCl showed that the net synthesis of a 26 kDa polypeptide was significantly changed, being more evident in the more tolerant variety under 200 mM salt stress [19]. Wang et al. [20] identified 49 salt-responsive DEPs between seedling-roots of wheat cultivars Shanrong No. 3 and Jinan 177 under 200 mM salt treatments for 24 h. A wheat V-H^+-ATPase E subunit protein was enhanced by salt stress, evidently more so in a wheat salt tolerant cultivar, under 137 mM salt stress [21]. However, proteomic studies on wheat roots at different levels of salt stress are rather limited; the few reports in this area were all focused on a very narrow range of salt concentrations [19–21]. Thus, comparisons of proteomic dynamics between salt-tolerant and salt-sensitive wheat varieties are yet to be studied, especially under a range of salt concentrations.

Wheat, the second major crop in the world, is a salt-sensitive glycophyte significantly affected by soil salinity. Since the root plays important roles in plant positioning, water absorption, and mineral uptake, it is also considered to be the primary site of salinity perception and the main organ responsible for tolerance to salt stress [2]. A comprehensive survey of the root proteome in response to salinity stress will help in understanding salt tolerance in wheat. Common wheat cv Chinese Spring (CS), which is widely used in experimental studies is sensitive to salt [22] and other stresses such as heat [23]. Chinese cv Jing-411, widely cultivated in the Beijing area in the 1990s, has characteristics of high yield, lodging resistance and abiotic stress tolerance. However, the protein dynamics of salt tolerance have not been investigated. In the present work, we undertook a comparative proteomic analysis of roots of wheat cv Jing-411 and Chinese Spring after exposure to a gradient of salinity conditions.

2. Materials and methods

2.1. Plant materials and salt treatment

The experiments were carried out on common wheat varieties (*Triticum aestivum* L., 2n=6x=42, AABBDD), Jing-411 and Chinese Spring. Seeds were germinated on wet filter paper in the dark at room temperature. Uniformly pregerminated seeds were grown in 16 h light and 8 h dark at 23 °C–25 °C. Two-leaf seedlings were transferred to Hoagland's solution containing 5 mM KNO_3, 2 mM $MgSO_4$, 1 mM KH_2PO_4, 5 mM $Ca(NO_3)_2$, 50 μM $FeNa_2(EDTA)_2$, 50 μM H_3BO_3, 10 μM $MnC1_2$, 0.8 μM $ZnSO_4$, 0.4 μM $CuSO_4$, and 0.02 μM $(NH_4)_6MoO_{24}$. The nutrient solution in the chamber was changed every 3 days. Salt stress was conducted on seedlings starting from three-leaf stage under NaCl concentrations of 0, 0.5%, 1.5% and 2.5% for 48 h. After treatment, the seedling roots were harvested and frozen at −80 °C. Three biologically independent replicates were performed at different times to ascertain reproducibility.

2.2. Relative water content (RWC) of leaf, chlorophyll content and root Na^+ measurement

The fresh weights (FW) of second leaves were measured immediately after harvesting. The leaves were floated on deionized water for 24 h at 4 °C under low irradiance. The turgid leaves were then quickly weighted (TW) and dry masses were determined after oven-drying at 70 °C for 48 h. The relative water content (RWC) was calculated as: RWC (%) = (FW − DW) / (TW − DW) × 100 (DW = dry weight) [24].

To measure leaf chlorophyll, 0.2 g of second leaf material was ground into a powder in liquid nitrogen with added 80% acetone, followed by incubation in water at 60 °C water for 5 h. The supernatant was transferred to a new tube and 80% acetone was added to a final of 25 ml volume for measurement of optical density values under 663 nm and 645 nm (Amersham Biosciences ultrospec 3100 *pro*).

The root sodium content was determined from the samples after oven-drying for 5 days. The roots were ground twice in liquid nitrogen into a fine powder, 0.1 g of which was added to 5 ml nitric acid and incubated for 1 h at room temperature. The resulting solution was filtered and its Na^+ concentration was assayed by Agilent 7500Ce (USA) and calculated as per unit of dry mass. All measurements were