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Comparative proteomic analysis reveals molecular mechanism of seedling roots of different salt tolerant soybean genotypes in responses to salinity stress

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ABSTRACT

Salinity stress is one of the major abiotic stresses that limit agricultural yield. To understand salt-responsive protein networks in soybean seedling, the extracted proteins from seedling roots of two different genotypes (Lee 68 and Jackson) were analyzed under salt stress by two-dimensional polyacrylamide gel electrophoresis. Sixty-eight differentially expressed proteins were detected and identified. The identified proteins were involved in 13 metabolic pathways and cellular processes. Proteins correlated to brassinosteroid and gilbberellin signalings were significantly increased only in the genotype Lee 68 under salt stress; abscisic acid content was positively correlated with this genotype; proteins that can be correlated to Ca^{2+} signaling were more strongly enhanced by salt stress in the seedling roots of genotype Lee 68 than in those of genotype Jackson; moreover, genotype Lee 68 had stronger capability of reactive oxygen species scavenging and cell K^+/Na^+ homeostasis maintaining in seedling roots than genotype Jackson under salt stress. Since the genotype Lee 68 has been described in literature as being tolerant and Jackson as sensitive, we hypothesize that

Abbreviations: AAS, aspartate aminotransferase; ABA, abscisic acid; ACS, 1-aminocyclopropane-1-carboxylate synthetase; APX, ascorbate peroxidase; BES1, BRI1-EMS suppressor 1; BIN2, brassinosteroid insensitive 2; BKI1, BRI1 kinase inhibitor 1; BRI1, brassinosteroid insensitive 1; BRs, brassinosteroids; BSU1, BRI1-suppressor 1; BZR1, brassinazole resistant 1; DEPs, differently expressed proteins; EBD, 6-deoxo-24-epicastasterone; EBI, epibrassinolide; EBK, epicastasterone; EF-HCP, 39 kDa EF-Hand containing protein; GA, gilbberellin; Glyox, glyoxalase; G protein, guanine nucleotide-binding protein; GST, glutathione S-transferase; PDI, protein disulfide isomerase-like; Perox, peroxiredoxin; RLS, ribulose 1,5-bisphosphate carboxylase large subunit; ROS, reactive oxygen species; SAMS, S-adenosylmethionine; SOD, superoxide dismutase, chloroplastic; TCTP, translationally-controlled tumor protein; Trans, transketolase; VHA, V-H(+)-ATPase subunit A.

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these major differences in the genotype Lee 68 might contribute to salt tolerance. Combined with our previous comparative proteomics analysis on seedling leaves, the similarities and differences between the salt-responsive protein networks found in the seedling leaves and roots of both the genotypes were discussed. Such a result will be helpful in breeding of salt-tolerant soybean cultivars.

1. Introduction

Salinity stress is one of the major abiotic stresses that limit agricultural yield. Over 20–50% of the whole arable land is affected by salt stress every year [1]. Development of salt tolerant crops may substantially expand the word's food-producing area. Soybean is an important dicot crop due to the high content of oil and protein in its seeds, and generally considered as one of the salt-sensitive crops [2,3]. Recently, salt stress has become one of the limiting factors that reduce its yield like many other crops. As a result, development of improved levels of tolerance to salt stress has become an urgent priority for soybean breeding programs. Many previous studies have indicated that the adaptation of the plant to salt stress requires alteration in gene expression and subsequently the protein profile and is very complicated at the whole plant and cellular levels [4]. Discovering the molecular mechanism and exploring new strategies conferring salt stress to soybean will be helpful in breeding of salt-tolerant soybean cultivars. However, to date, only limited information is available about salt-response proteins in soybean. This has limited our understanding of the molecular mechanism adopted by this important crop in response to salt stress.

Two genotypes, Jackson (salt sensitive) and Lee 68 (salt tolerant), have been widely used to reveal soybean responses to salinity stress at physiological and agronomic trait levels by many investigators [2,5]. In our previous study, 78 differentially expressed proteins were identified in the seedling leaves of the two genotypes and involved in 13 metabolic pathways and cellular processes. Salt-tolerant genotype Lee 68 possessed the ability of higher ROS scavenging, more abundant energy supply and ethylene production, and stronger photosynthesis than salt-sensitive genotype Jackson under salt stress, which may be the major reasons why it is more salt-tolerant than Jackson [5]. However, due to the direct effects of soil salt stress on plant roots, plant roots are found to be more sensitive than leaves to salt stress [6]. Many processes have been reported to become dominant at the proteome level in root salt response, including salt signal perception and transduction, detoxification of ROS, salt uptake/exclusion and compartmentalization, protein translation and/or turnover dynamics, cytoskeleton/cell wall dynamics, carbohydrate and energy metabolism, and so on. These processes work together to gain cellular homeostasis in roots and determine the overall phenotype of plant growth and development under salt stress [7]. Therefore, the differentially expressed proteins identified in soybean seedling roots under salt stress may have more important roles in help us in understanding of soybean seedling's responses to salt stress.

In the present study, a proteomic approach was applied to the seedling roots of the two soybean genotypes, Jackson (salt-sensitive) and Lee 68 (salt-tolerant) under salt stress. The main objectives were (1) to identify differentially expressed proteins in seedling roots; (2) based on the proteomics and physiological and biochemical data, to discuss the molecular mechanism of soybean seedling roots in response to salinity stress; (3) combined with our previous study, to reveal the differences in metabolic pathways and cellular processes between the leaves and roots of the two soybean genotypes. Such a result will allow us to further understand and describe the possible management strategy of cellular activities occurring in salt-treated soybean seedling.

2. Experimental procedures

2.1. *Plant material*

Seeds of two soybean genotypes (salt-sensitive genotype Jackson and salt-tolerant genotype Lee 68) were sterilized with 0.1% $HgCl_2$ for 10 min. After three times of rinsing with sterilized distilled water, the seeds were germinated on wet filter paper in the dark for 72 h at 26 °C. Uniformly germinated seeds were transplanted into 1/2 Hoagland nutrient solution, which was replaced with fresh one every 3 days. The seedlings were grown in a growth chamber with 25/20 °C temperature (day/night), photon flux density of 480 $\mu mol\ m^{-2}\ s^{-1}$, 16 h photoperiod, and relative humidity of 60–80%. Thirty two-week-old uniform seedlings were selected to grow in each tank (50 cm × 40 cm × 15 cm) with 1/2 Hoagland nutrient solution including 150 mM NaCl. Root samples taken at different salt stress time points (1, 12, 72, and 144 h) were immediately used or frozen in liquid nitrogen and stored at −20 °C. The roots from the unstressed plants were also collected at 1, 12, 72, and 144 h, respectively, and used as control.

2.2. *Determination of brassinosteroid (BR), abscisic acid (ABA), and gilbberellin (GA) contents*

The contents of BR (epibrassinolide, EBI; epicastasterone, EBK; 6-deoxo-24-epicastasterone, EBD) were detected according to Aleš et al. [8]. Samples were analyzed by LC/MS using a Hewlett-Packard (Avondale, PA, USA) HP 1100 HPLC system coupled to a Micromass QuattroIItandem quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source. The capillary and cone voltages in the ESI+ mode were 3.0 kV and 16 V, respectively. Nitrogen was used for nebulization and as drying gas.

ABA was analyzed by LC-MS according to Li et al. [9]. 1000 mg of fresh plant sample were frozen in liquid N_2 and