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Comparative proteomic analysis of seedling leaves of different salt tolerant soybean genotypes

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ABSTRACT

Salinity is one of the major environmental constraints limiting yield of crop plants in many semi-arid and arid regions around the world. To understand responses in soybean seedling to salt stress at proteomic level, the extracted proteins from seedling leaves of salt-sensitive genotype Jackson and salt-tolerant genotype Lee 68 under 150 mM NaCl stress for 1, 12, 72 and 144 h, respectively, were analyzed by 2-DE. Approximately 800 protein spots were detected on 2-DE gels. Among them, 91 were found to be differently expressed, with 78 being successfully identified by MALDI-TOF–TOF. The identified proteins were involved in 14 metabolic pathways and cellular processes. Based on most of the 78 salt-responsive proteins, a salt stress-responsive protein network was proposed. This network consisted of several functional components, including balancing between ROS production and scavenging, accelerated proteolysis and reduced biosynthesis of proteins, impaired photosynthesis, abundant energy supply and enhanced biosynthesis of ethylene. 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.

Abbreviations: AAS, aspartate aminotransferase; ACO, 1-aminocyclopropane-1-carboxylate oxidase; ACC, 1-aminocyclopropane-1-carboxylate; ACS, 1-aminocyclopropane-1-carboxylate synthetase; Ald, aldolase; APX, ascorbate peroxidase; ASD, aspartate-semialdehyde dehydrogenase; ATP, adenosine-triphosphate; BIP, 2,3-biphosphoglycerate-independent phosphoglycerate mutase, putative; CAT, catalase; CMD, cytosolic malate dehydrogenase; DFR, dihydroflavonol reductase; EF2, elongation factor 2; EF-G, translation elongation factor EF-G; G3PD, glyceraldehyde 3-phosphate dehydrogenase; Glyox, putative glyoxalase; GRP, glycine-rich RNA-binding protein; HPD, homologous to plastidic aldolase; KEGG, kyoto encyclopedia of genes and genomes; NCBI, national center for biotechnology information; OEEP2, oxygen-evolving enhancer protein 2, chloroplastic; OX, oxidation; PB, protein biosynthesis; PDH, pyruvate dehydrogenase; PDI, protein disulfide isomerase-like; Perox, peroxiredoxin; POD, peroxidase; PP, proteolytic proteins; PS I, photosystem I; PS II, photosystem II; RBPs, RNA-binding proteins; RED, reduction; RLS, ribulose 1,5-bisphosphate carboxylase large subunit; RV, relative volume; SAMS, S-adenosylmethionine synthetase; SOD, superoxide dismutase; TatBP, rice homologue of Tat binding protein; TCA cycle, tricarboxylic acid cycle; TCTP, translationally-controlled tumor protein; Trans, transketolase.

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1. Introduction

Salinity is one of the most widespread agricultural problems in arid and semi-arid regions around the world that make fields unproductive [1]. It is estimated that about 20% of irrigated land, which yields one-third of the world's food, is affected by salinity [2]. Moreover, a significant proportion of recently cultivated agricultural land has become saline because of land clearing or irrigation [3]. As a result, the development of improved levels of tolerance to salt stress has become an urgent priority for many crop breeding programs [4], and much research effort has been applied to gain a better understanding of the adaptive mechanisms adopted by plants to combat salt stress.

Soybean is an important dicot crop due to the high content of oil and protein in its seeds [5]. More than one-third of the world's edible oils and two-thirds of the world's protein meal are derived from soybean [6]. However, soybean is subject to salinity stress that reduces its yield like many other crops. Therefore, the demand for developing salt-tolerant soybean cultivars is unavoidable. Two genotypes, Jackson and Lee 68, have been widely used to reveal soybean responses to salinity stress at physiological and agronomic trait levels by many investigators [7,8]. Pantalone et al. reported that genotype Jackson accumulated more chloride in the leaves and had significantly higher leaf chlorosis score than genotype Lee 68 at the same salt level [9]. Hamwieh et al. reported that Jackson was a salt-sensitive soybean genotype and had significantly lower salt-tolerance ratings than genotype JWS156-1 [10]. Under 150 mM NaCl stress, Cl^- was found to be more toxic than Na^+ to soybean seedlings and injury of soybean genotypes, including 'Jackson' (salt sensitive) and 'Lee 68' (salt tolerant), was positively correlated with the content of Cl^- in the leaves [8]. Valencia et al. established a rapid and effective method for screening salt tolerant soybean genotypes based on foliar symptoms with genotype Lee 68 used as one of the salt-tolerant genotypes [11]. Their results indicated that salt sensitive genotypes exhibited interveinal chlorosis while salt tolerant genotypes (including Lee 68) showed no chlorosis at the 120 mM NaCl level, and average leaf Cl^- content for salt sensitive genotypes was 1.96 times higher than for salt tolerant genotypes (including Lee 68) [11]. Taken together, all above previous results suggested that Jackson was a salt sensitive genotype while Lee 68 was a salt tolerant genotype. But their molecular mechanism in response to salt stress still remains unclear.

Proteomics is a powerful molecular tool for describing the complete proteome at organelle or tissue level and for comparing how the proteome is affected by different physiological conditions [6]. Two-dimensional polyacrylamide gel electrophoresis (2-DE) is one of the most sensitive and powerful techniques for separating hundreds of proteins [12]. It has been applied to different abiotic treatments for soybean including treatment with salt and flooding, and exposure to ultraviolet radiation [13,14]. Aghaei et al. identified seven proteins in soybean hypocotyl and root under salt stress and indicated that salinity can change the expression level of some special proteins that might play a role in the adaptation to saline conditions [15]. Sobhanian et al. identified salt responsive proteins in soybean using a proteomic technique and concluded that glyceraldehyde-3-phosphate dehydrogenase was down-regulated in the leaf/hypocotyls, and fructokinase 2 was down-regulated in the hypocotyls/root under NaCl treatment, while stem 31 kDa glycoprotein precursor was up-regulated in leaf/hypocotyls/root [6]. Xu et al. carried out a proteomic analysis of seed germination under salt stress in soybean and identified nine proteins by MALDI-TOF-MS [2]. Recently, Sobhanian et al. reviewed the changes in the plant proteome that resulted from salt stress and summarized the changes of metabolic pathways of soybean under salt stress. They indicated that photosynthesis, protein biosynthesis and ATP biosynthesis were decreased while defense protein increased in soybean in response to salt stress [16]. However, to date, still 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.

In the present study, a proteomic approach was applied to seedling leaves of two soybean genotypes, Jackson (salt-sensitive) and Lee 68 (salt-tolerant). The main objectives were to investigate the proteome expression pattern and to identify the differentially expressed proteins under salt stress; and, based on the proteomic data, the molecular mechanism in responses to salinity stress in soybean seedling leaves was discussed. Additionally, why genotype Lee 68 is more salt tolerant than genotype Jackson was also discussed at molecular level.

2. Materials and methods

2.1. Plant material

Seeds of two soybean genotypes (salt-sensitive genotype Jackson and salt-tolerant genotype Lee 68) [7,8] 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 μmol 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. Leaf 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 leaves from the unstressed plants were also collected at 1, 12, 72, and 144 h, respectively, and used as control.

2.2. Protein extraction and 2-DE

To minimize errors, three biological repeats were conducted for proteome analysis at each treated time point. For each biological repeat sample, ten leaves from ten soybean seedlings were pooled. Soybean seedling leaves were extracted with the method of TCA/ acetone precipitation according to BioRad 2-D manual with some modifications. Briefly, leaf sample powder was suspended in 10% w/v TCA/acetone containing 1 mM PMSF and 0.07% w/v β-mercaptoethanol, and held at −20 °C for 1 h. After centrifugation and rinse, the vacuum dried pellets were