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RESEARCH ARTICLE

Proteomic analysis of salt and osmotic-drought stress in alfalfa seedlings

MA Qiao-li[1, 3*], KANG Jun-mei[2*], LONG Rui-cai[2], CUI Yan-jun[2], ZHANG Tie-jun[2], XIONG Jun-bo[2, 4], YANG Qing-chuan[2], SUN Yan[1]

[1] *College of Animal Science and Technology, China Agricultural University, Beijing 100193, P.R.China*
[2] *Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing 100193, P.R.China*
[3] *School of Chemistry & Chemical Engineering, Ningxia University, Yinchuan 750021, P.R.China*
[4] *Institute of Animal Science, Hubei Academy of Agricultural Sciences, Wuhan 430064, P.R.China*

Abstract

Alfalfa is widely grown and is one of the most important forage crops in the world, but its growth and biomass production are markedly reduced under salt and drought stress, particularly during the early seedling stage. To identify the mechanisms behind salt and drought responsiveness at the alfalfa seedling stage, the proteins expressed were analyzed under no-treatment, 200 mol L^{-1} NaCl and 180 g L^{-1} PEG treatment conditions during the seedling stage. Out of more than 800 protein spots detected on two-dimensional electrophoresis (2-DE) gels, 35 proteins showed statistically significant responses (P<0.05) to NaCl and PEG stress, which were selected for tandem mass spectrometric identification, owing to their good resolution and abundance levels, and 32 proteins were positively identified. The identified proteins were divided into seven functional categories: photosynthetic metabolism, protein biosynthesis, folding and assembly, carbohydrate metabolism-associated proteins, stress defense related protein, metabolism of nucleic acid, other function categories and unknown proteins. Our results suggested that these proteins may play roles in alfalfa adaptation to salt and drought stress. Further study of these proteins will provide insights into the molecular mechanisms of abiotic stress and the discovery of new candidate markers in alfalfa.

Keywords: alfalfa, salinity stress, osmotic stress, seedling growth, proteomics

1. Introduction

Drought and salinity are major abiotic stresses that affect plant growth, development and crop productivity (Wang *et al.* 2009). Drought and salinity stress impose osmotic stresses which lead to loss of cell turgor. Then membranes may become disorganized, proteins may be inactivated or denatured and excess levels of reactive oxygen species (ROS) may cause oxidative damage (Krasensky and Jonak 2012). Therefore, plant adaption to drought and salt stress is the result of various inter-connected physiological, biochemical and molecular processes (Munns and Tester 2008). Salinity problems are very similar to drought or water stress. The detrimental effects of salt not only lead to water deficits at relatively high solute concentrations in the soil,

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MA Qiao-li, E-mail: mql_2008@126.com; KANG Jun-mei, E-mail: kangjmei@126.com; Correspondence SUN Yan, Tel: +86-10-62732793, E-mail: cts-china@cau.edu.cn
* These authors contributed equally to this study.

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but also cause specific Cl^- and Na^+ toxicities (Manaa *et al.* 2011). It has been suggested that the plant suffers from osmotic stress first and then is later affected by salt-specific effects (Munns *et al.* 2002). High salt concentrations may cause ion imbalances, hyper osmotic stress and oxidative damage (Zhu 2002) and can have different effects on a plant depending on the plant development stage. It has been reported that many plants are sensitive to abiotic stress during the early young seedling stage, and that the stage also has important effects on plant growth and production (Kerepesi and Galiba 2000). Salt and drought responses by plants are complex processes and the exact structural and functional modifications caused by these stresses are poorly understood. Thus, understanding the different adaption mechanisms to environmental stresses may lead to novel strategies for plant improvement.

Alfalfa (*Medicago sativa* L.) is the most important perennial forage legume for hay production. It is well adapted to a wide range of climate and soil conditions, but grows best on deep, loam soils that are well drained. It is relatively drought tolerant but also responds very well to irrigation. It tolerates some alkalinity, but does not do well on highly-alkaline or salty soils (Munns and Tester 2008). Large-scale studies intended to identify drought and salt stress-related mechanisms have been undertaken and the impact of salt and drought stress on alfalfa plants has been investigated using physiological, genomic and agronomic approaches (Wang *et al.* 2009; Long *et al.* 2013; Tang *et al.* 2013). However, the proteomic data are very limited. Protein quantities are determined not only by transcription, but also by translation and post-translational modifications and it is well known that, in some cases, there is a poor or no correlation between mRNA and protein abundance (Gallardo *et al.* 2007). Hence proteomics analysis is needed in order to reveal the plasticity of gene expression because this allows for the global analyses of gene products and the physiological state of the plant. Proteomic analysis, as a complement to genome and transcriptome analysis, has been proved to be a powerful method for offering a more direct analysis of cellular responses (Thomas and VanBogelen 2000). Two-dimensional electrophoresis (2-DE) means that stress-induced proteins in plants can be subjected to expression analysis and this may help to investigate the roles of these proteins under a variety of physiological and environmental conditions (Komatsu and Tanaka 2005). In such studies, comparative proteomics has been proved to be one of the best approaches and has emerged as an efficient tool for the global analysis of protein expression levels in recent years (Cánovas *et al.* 2004). Many recent proteomic studies have been performed on various tissues in alfalfa, such as roots (Daher *et al.* 2010), leaves (Soares *et al.* 2007), seeds (Alkhalfioui *et al.* 2007), embryos (Imin *et al.* 2005) and cytoplasmic organelles (de Jong *et al.* 2007). These studies have identified many proteins and laid a good foundation for the construction of some regulatory networks. Moreover, even within one tissue type, a large number of unique proteins can exist or be differentially regulated at various stages of development (Nozu *et al.* 2006). However, little is known about the protein networks that are affected by salt and osmotic drought stress in alfalfa. The objective of this study was to identify proteins which may act differently when under salt and osmotic drought stress using 2-DE and MS technologies. We chose equivalent osmotic stress conditions, where the drought stress levels were similar to the osmotic water stress caused by salt stress. The combination of physiological and proteomic analysis may constitute an original contribution to understanding the salt and osmotic drought effect during the alfalfa seedling growth stages. It is hoped that the proteins identified will increase knowledge about resistance to drought and salt stress in alfalfa and offer new insights that can be used when researching the difference between salt and drought stress.

2. Materials and methods

2.1. Plant materials, growth conditions and stress treatments

The alfalfa (*Medicago sativa* L. *cv*. Zhongmu 3) used in this study was cultivated and registered as salt-tolerant cultivar by Institute of Animal Sciences of Chinese Academy of Agricultural Sciences in 2006. The seeds were surface-sterilized with 10% sodium hypochlorite solution for 15 min and rinsed seven times in sterile, distilled water. The seeds were germinated in a growth chamber (Jiangnan, China) at (25±2)°C under a 16 h-light (90 μmol m^{-2} s^{-1})/8 h-dark regime on Petri dishes (9.0 cm in diameter) containing two layers of wet filter paper and grown on until radicle initiation. Twenty uniformly germinated seeds per sample were transferred to new Petri dishes on filter paper soaked with 2.5 mL of Hoagland solution. The seedlings were grown under a 16 h photoperiod (150 μmol m^{-2} s^{-1}) at 25°C/70% relative humidity and with an 8 h dark period at 20°C/90% relative humidity. After the plants had been left to grow for 20 days in the growth chamber, 200 mol L^{-1} NaCl or 180 g L^{-1} PEG4000 was added to the Hoagland solution and Hoagland solution without NaCl or PEG4000 served as the control treatment. After 48 h, the seedlings were harvested for physiological investigation and proteomic analysis.

2.2. Relative electrolyte leakage measurement

Seedling samples (0.3 g) were placed in tubes containing 10 mL double deionized water. The tubes were then evacu-