Available online at www.sciencedirect.com
SCIENCE DIRECT
Plant Physiology and Biochemistry 44 (2006) 253–259
Plant Physiology and Biochemistry
www.elsevier.com/locate/plaphy

Short communication

Proteomics reveals elevated levels of PR 10 proteins in saline-tolerant peanut (*Arachis hypogaea*) calli

Shalu Jain [a,1], Sanjeeva Srivastava [b,1], Neera Bhalla Sarin [a], Nat N.V. Kav [b,*]

[a] *School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India*
[b] *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada*

Received 8 November 2005
Available online 15 May 2006

Abstract

The proteome of a salinity-tolerant *Arachis hypogaea* L. callus cell line was compared with its sensitive counterpart. Several low molecular weight proteins were detected by two-dimensional electrophoresis as being unique or significantly elevated in the tolerant line. The identities of several of these proteins were established as PR 10 proteins using tandem Mass Spectrometry and are shown to be phosphorylated on the basis of staining with the phosphorylation-specific stain, Pro-Q Diamond. Our results suggest that these differentially phosphorylated PR 10 proteins may play an important role in mediating salinity stress responses.

Keywords: Arachis hypogaea; Callus; Electrospray ionization; Pathogenesis related (PR) proteins; Phosphorylation; Salinity; Tandem mass spectrometry

1. Introduction

Environmental stresses such as drought and salinity severely limit agricultural productivity by reducing average crop yield by more than 50% [1]. Salinity is particularly a global concern due to the widespread salinization of soils, which is estimated to reach a staggering 50% of all arable land by the year 2050 [2]. Salinity-induced damage to plants include membrane disorganization, increase in levels of toxic metabolites, inhibited nutrient uptake and photosynthesis, generation of reactive oxygen species (ROS) and ultimately cell and plant death [3]. Due to this intricate and complex nature of plant responses to salinity, the quest for salinity-tolerant plants that are generated via conventional breeding has remained largely unsuccessful [4] whereas, biotechnological approaches have had some success [5,6].

The relatively homogenous population of cells provided by in vitro cultures of plant cells offers an excellent system to investigate the effects of salinity stress. Furthermore, the growth of plant cells in culture under prolonged salinity stress often results in tolerance and therefore may offer clues as to the molecular mechanisms that may be crucial to the development of tolerance [7–9]. In today's post-genomic era, techniques that investigate changes in the protein component of the genome, i.e. the proteome, are being increasingly used in various disciplines in order to investigate molecular changes that occur in response to stresses [10–12]. In this report we describe the characterization of proteome-level differences between a salinity-tolerant (ST) and –sensitive (SS) callus cell lines of *Arachis hypogaea* with a view of further understanding the molecular differences between the two lines that may be responsible for the higher tolerance to NaCl.

2. Results and discussion

A salinity-tolerant cell line of *Arachis hypogaea* cv. JL 24 has been obtained by exposing the callus cells to NaCl as previously described [9] and the tolerant cell line (ST) appears to grow better on NaCl than the sensitive lines (SS). Appearance of the SS and ST lines cultured on semi-solid media are shown in Fig. 1A and the effects of NaCl on fresh weight of both lines are shown in Fig. 1B. It is evident that the ST line appears to be healthier than the SS line at high concentrations of NaCl and is supported by the increased gain in fresh weight of the

* Corresponding author. Tel.: +780 492 7584; fax: +780 492 4265.
E-mail address: nat@ualberta.ca (N.N.V. Kav).
[1] These authors contributed equally to this work.

0981-9428/$ - see front matter © 2006 Elsevier SAS. All rights reserved.
doi:10.1016/j.plaphy.2006.04.006

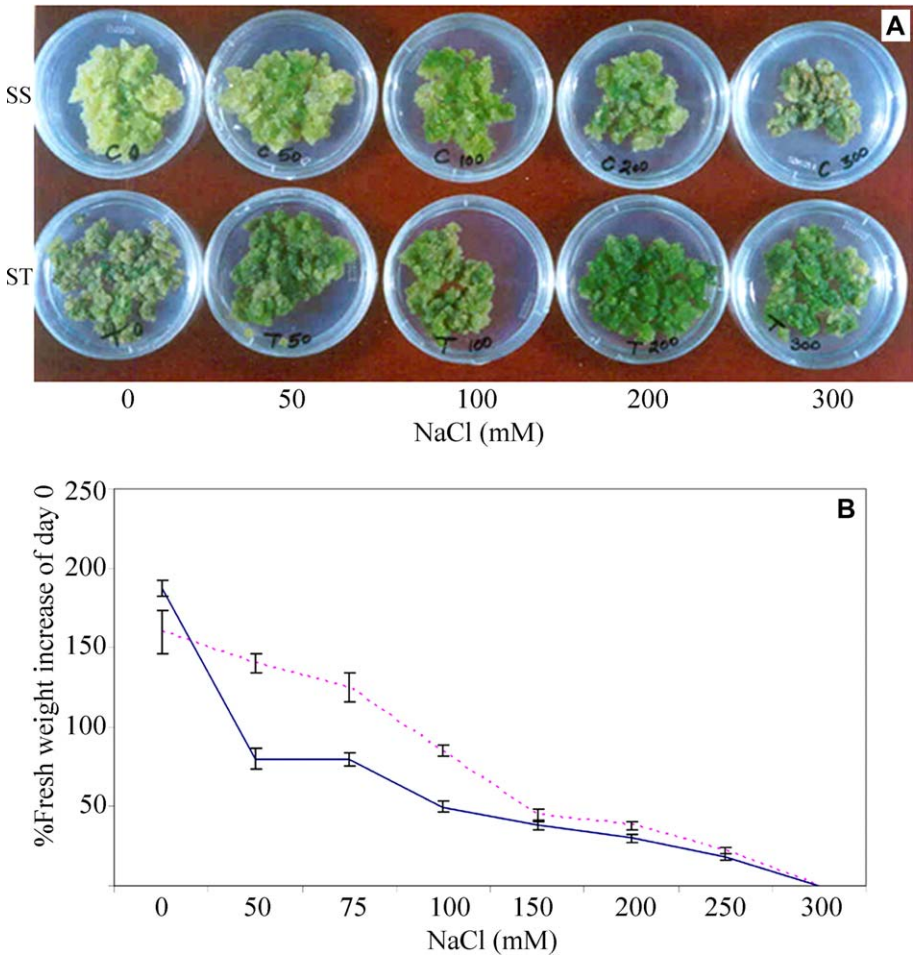

Fig. 1. Appearance of *Arachis hypogaea* callus lines (A) grown in the presence or absence of NaCl. The letters SS and ST refer to the sensitive and tolerant lines, respectively, and the numbers on the Petri dishes refer to the concentration of NaCl in mM. Effects of NaCl on the growth of the SS and ST lines (B). Fresh weights of tissues were measured 12 days after transfer to media containing different concentrations of NaCl and the change in fresh weight was expressed as a percentage of the weight on day 0. Solid and dashed lines represent SS and ST lines, respectively.

ST (Fig. 1). Fresh weight of the SS line is significantly reduced ($P < 0.01$) even at NaCl concentrations as low as 50 mM whereas in the ST line, fresh weight declined only slightly at the same concentration.

In order to identify proteins whose levels may be altered in the ST line, we subjected the proteins extracted from the SS and ST lines to two-dimensional electrophoresis. Images of two-dimensional electrophoresis gels of protein from ST and SS lines are shown in Fig. 2. Images of Coomassie blue-stained gels from the SS and ST lines cultured in the absence of NaCl are shown in Fig. 2A, B, respectively, and those stained with silver are shown in Fig. 2C, D. A detailed comparison of these images using the Student *t*-test feature of the PDQuest software (Bio-Rad) revealed at least 21 protein spots whose spot volumes were significantly higher ($P < 0.05$) or were unique in the ST gels compared to the SS gels (spots 1–13; Fig. 2A, B; spots 14–21; Fig. 2C, D). In addition, the silver-stained images of two-dimensional gels obtained with extracts prepared from SS and ST lines cultured in the presence of 200 mM NaCl are shown in Fig. 2E, F. An analysis of these gels revealed an increase in the levels of at least three proteins (Fig. 2F; spots 22–24). The MASCOT scores for most of the identified spots were higher than the threshold scores (Table 1) which is indicative of extensive identities between the MS fingerprint and the sequence in the databases and, that the observed match is not a random event.

The 24 spots (Table 1) were excised from the two-dimensional gels and identified using tandem MS. Remarkably, a large number of spots (1–15, 17–19, 21–22; Table 1) generated peptide mass fingerprints that identified them as proteins belonging to the PR 10 protein family such as the Ara h 8 allergen. Other proteins identified as being elevated in the ST line in response to salinity included an RNA-binding protein (spot 23), and a 14-3-3 protein (spot 24). Furthermore, due to this abundance of PR 10 proteins in the ST line, we have focused our discussion on this group of proteins although it is possible that the other proteins may also have important roles in mediating salinity tolerance in this line. For example, the 14-3-3 group of proteins has been shown to interact with a number of signaling molecules such as kinases and phosphatases and, may thus play vital roles in mediating important cellular processes in response to environmental changes [13].