Introduction

Salt stress is a significant abiotic stress affecting plant physiology, as well as protein biosynthesis and modification. Salt resistance of plants is a complex phenomenon that involves biochemical and physiological changes (Munns, 2002; Pitann et al., 2009). During salt stress, Na^+ enters the cells and accumulates to a concentration that induces osmotic and ionic stress. Plant cells respond and adapt to these conditions trough signalling networks (Lee et al., 2004). It is of fundamental importance to understand the physiological differences leading to salt resistance and to get access to the molecular mechanisms underlying this response. Initial responses of plant cells after immediate stress application are of special interest for elucidating the physiological processes and signals of salt resistance. Especially post-translational modification, such as protein phosphorylation, is an important part of the stress related signal at the initial phase. The aim of the present work was to investigate the protein phosphorylation after a short-term salt exposure of maize and to elucidate the role of proteins which may contribute to leaf expansion.

Materials and Methods

A salt resistant maize hybrid was subjected to moderate short-term NaCl stress (25 mM) in nutrient solution. Plants were harvested after 1-4 h of salt treatment and proteins were extracted and separated by 2D gel electrophoresis (according to Zörb et al., 2004). Protein phosphorylation was detected using the Phos-tag system (PerkinElmer) with unparalleled selectivity and sensitivity for phospho-monoesters of tyrosine, serine and threonine. The stained 2D gels were visualised using a UV-transilluminator. Computer-assisted 2D analysis of each gel was done with Delta 2D software version 3.3 (Decodon, Greifswald). Protein spots were identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. Proteins were identified by searching the database based on MALDI TOF results of the peptide masses using the Mascot program package (http://www.matrixscience.com).

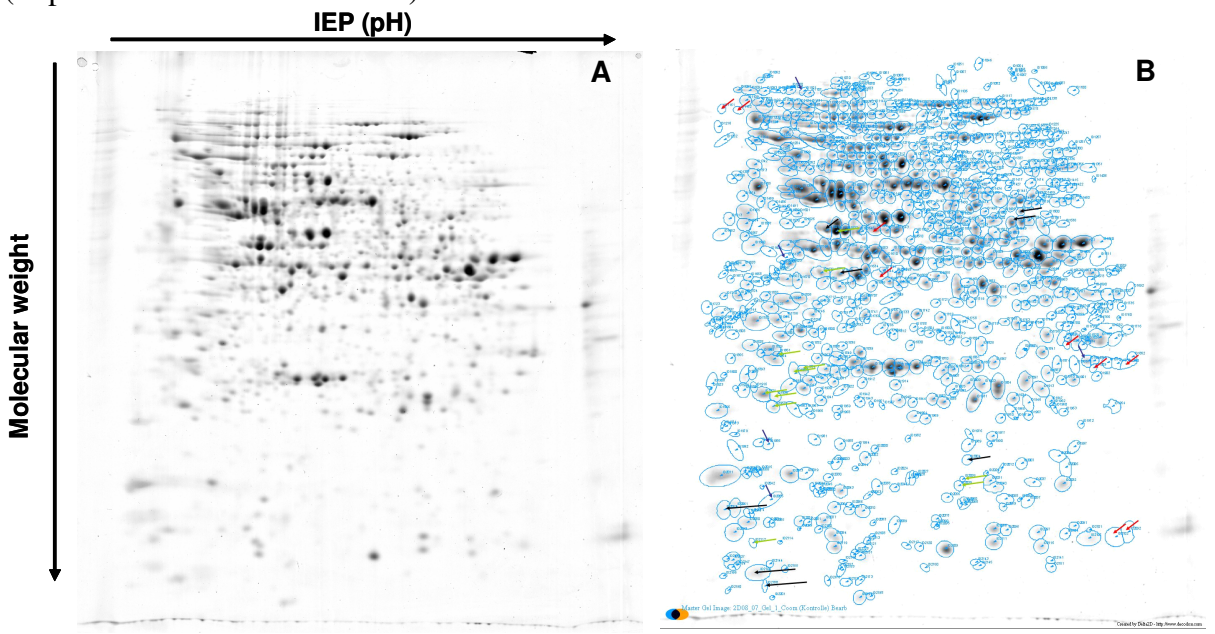

Figure 1. (A) Coomassie stained two 2D-gel showing total protein extract from maize leaves. (B) Overlay of salt treatment (25 mM NaCl, 1 h) versus control (no salt stress). Spots with arrows: green, up-regulated proteins; blue, down- regulated proteins; red, phosphorylated; black, de-phosphorylated. Remaining proteins without arrows were unchanged by salt treatment.

Result and Discussion

The use of 2D phospho-proteomics enables the analysis of hundreds of proteins simultaneously by comparing protein concentrations in treated and non-treated plants. In an earlier study the reaction of maize proteome to salt stress was characterized by a mitigation of symptoms (Zörb et al., 2004). We therefore focus on the effects of short-term salt exposure on protein phosphorylation in the initial phase of salt stress. A set of phospho-proteins of maize were detected using a two dimensional proteomics approach, but only 12 proteins were phosphorylated and 8 proteins were dephosphorylated after the application of 25 mM NaCl for 1 h (Fig. 1). Some of the phosphorylated proteins such as glucosyl transferase BX9, 2-Cys-peroxyredoxine, and xyloglucane-endotransglycosylase were enhanced after initial salt stress, whereas isocitrate-dehydrogenase, maturase and calmodulin were dephosphorylated after adjustment to saline conditions. These proteins might be involved in the growth regulation process after salt stress. In conclusion, the phospho-proteome of maize showed a distinct, fast and initial reaction under moderate saline conditions which contribute to the adaptations to salt stress of maize.

Literature

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