Proteome Analysis of Wheat Seedlings Roots under Aluminum Stress

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Introduction

Abiotic stress is the major limiting factor of plant growth and crop yields and harmful factor concerning the growth and productivity of crops worldwide. Aluminum is a light metal that makes up 7% of the earth's crust dissolving ionic forms and is the third most abundant element after oxygen and silicon. Aluminum toxicity is regarded as one of the most serious agricultural problems for crop production on acid soils. Among various Al toxicity symptoms, the most sensitive response is the inhibition of root elongation. Globally wheat is the leading source of vegetable protein (8–12%) in human food, having a higher protein content than that of other major cereals. Wheat is the most widely studied plant species with regard to Al tolerance and is the best-characterized genetic system for analyzing Al tolerance. Proteomics approach is a powerful tool for the analysis of wheat physiological function. This study was performed to understand physiological response of abiotic stress in particularly aluminum stresses and to identify proteins responsible to aluminum stress responsive proteins in wheat using mass spectrometry.

Materials and Methods

Seed of wheat (Triticum aestivum L. cv. Keumkang), after sterilization by a 70% ethanol and 70% sodium hypochlorite solution, were germinated on petri dishes (140 x 25 mm) for 5 days under dark condition at 18 °C and 70% relative humidity in a growth chamber (GC-300TLH, Jeiotech, Korea). And then transferred hydroponic apparatus (20 X 27 cm) containing 5X diluted Hoagland solution and treated with 0 uM AlCl3, 100 uM AlCl3 and 150 uM AlCl3 (the pH of solution was adjusted to 5.0) for 5 days for 16 h photoperiod (1,500 lux/0 lux) at 20 °C/18 °C (day/night) and 70% relative humidity in a growth chamber. After 5 days, seedlings were collected and used for measuring physiological parameters including fresh weight and length of roots and shoots and analyzing proteomics. Concentrations of positive ions and aluminum in the roots were determined by inductively coupled plasma-optical emission spectrometry (ICP-OES; Optima 5300 V; Perkin-Elmer, Inc., USA). For confocal microscopy, wheat roots were carefully cut and collected in petri dishes (60 x 15 mm) containing double-distilled water for rinsing. After rinsing, the roots were stained with a 100 uM aqueous solution of morin for 30 min. Then they were washed twice with double-distilled water for 5 min each. They were placed on slides, mounted in mounting solution and observed with confocal microscopy (LSM 410; Carl Zeiss, Jena, Germany). Proteins were extracted from wheat seedlings roots and its concentration was determined by RC/DC assay. Then we carried out 2-DE followed by image analysis and in-gel digestion. And, finally we confirmed MS/MS analysis and bioinformatics.

Results and Discussions

Seeds of wheat cv. Keumkang (Korean cultivar) were germinated on petridish for 5 days and then transferred hydroponic apparatus which was treated with 0 uM AlCl3 (control), 100 uM AlCl3 and 150 uM AlCl3 for 5 days. The length of roots, shoots and fresh weight of wheat seedlings were decreased under aluminum stress. The concentrations of K+, Mg2+ and Ca2+ were decreased whereas Al3+ and P2O5 concentration was increased under aluminum stress. Using confocal microscopy, the fluorescence intensity of aluminum was increased with morin staining. In this study, a proteome analysis was performed to identify proteins, which is responsible to aluminum stress in wheat roots. In 10-day-old seedlings, proteins were extracted from roots and separated by 2-DE, stained by CBB. Using image analysis, a total of 47 differentially expressed protein spots were selected, whereas 19 protein spots were significantly up-regulated and 28 protein spots were significantly down-regulated by aluminum stress following protein spots analyzed by LTQ-FTICR-MS. S-adenosylmethionine, oxalate oxidase, malate dehydrogenase, cysteine synthase, ascorbate peroxidase were up-regulated and heat shock protein 70, O-methyltransferase 4, enolase, amylogenin were down-regulated under Al stress which is treated as target protein in this study.

References


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Figure 1. Physiological difference under aluminum stress in wheat seedlings. (A) Morphological symptom of wheat seedling, (B) Effect on length and fresh weight of wheat seedlings

Figure 2. Ion concentration and aluminum uptake in wheat roots under aluminum stress. (A) Ca$^2+$ (B) K$^+$ (C) Mg$^{2+}$ (D) $P_2O_5^-$ (E) AF$^+$

Figure 3. 2-DE patterns of proteins from wheat roots under aluminum stress.

Figure 5. Relative protein intensity of differentially expressed proteins under aluminum stress.