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Proteome Profiling Analysis of Grain Filling and Seed Maturation in Rice by SELDI-TOF MS and Affinity Chromatography

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Objectives

In order to gain better understandings for the developmental process in rice seed at different stages of grain filling and maturation, we performed two-dimensional electrophoresis in combination with MALDI-TOF or nLC-ESI Q-TOF/MS and SELDI-TOF/MS with SDS-PAGE after Q-Sepharose purification followed by MALDI-TOF MS/MS.

Materials and Methods

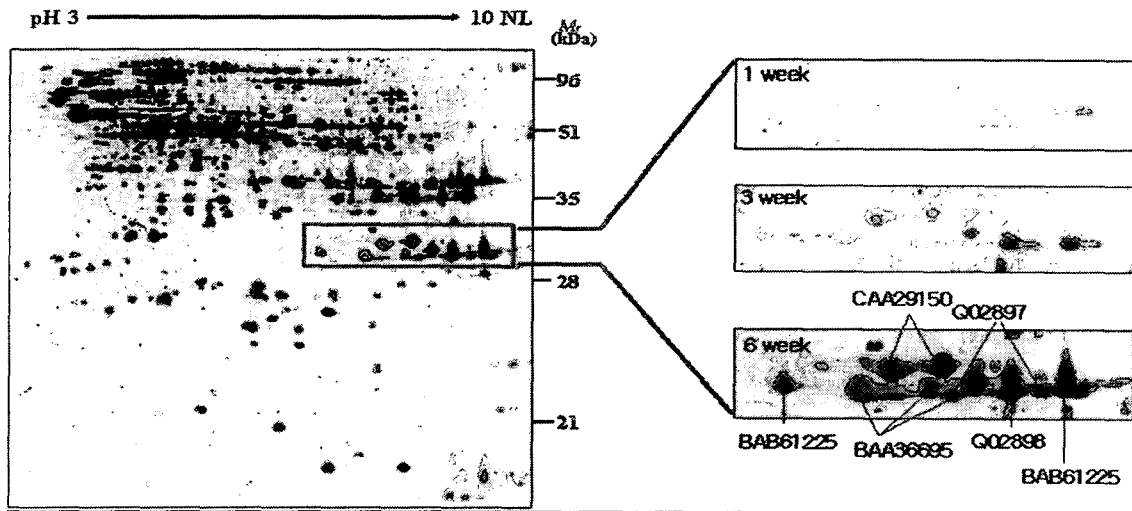
Sampling rice seed grains at 1, 3, 6 WAF at National Crop Experiment Station → Preparation of rice total proteins → 2-DE Proteomic analysis → Sample preparation for protein chip analysis → SCX column purification and protein identification

Results and Discussion

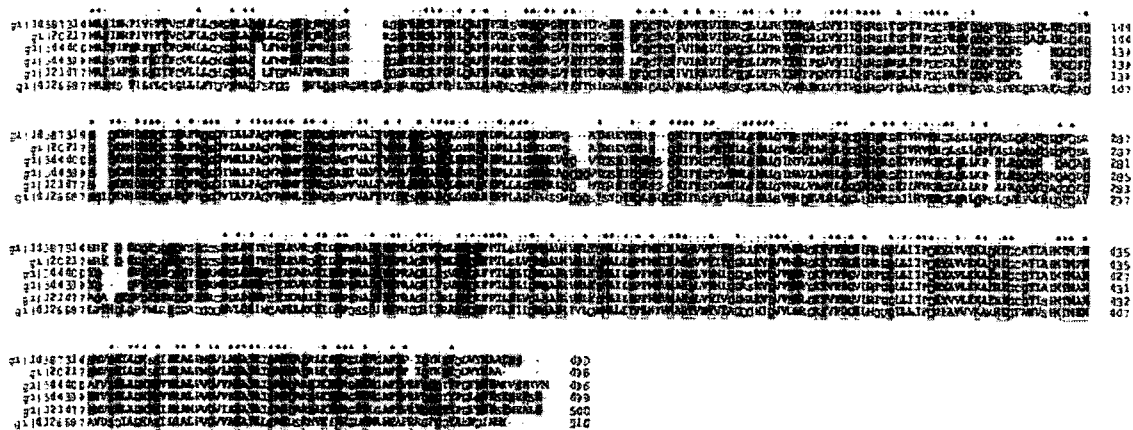
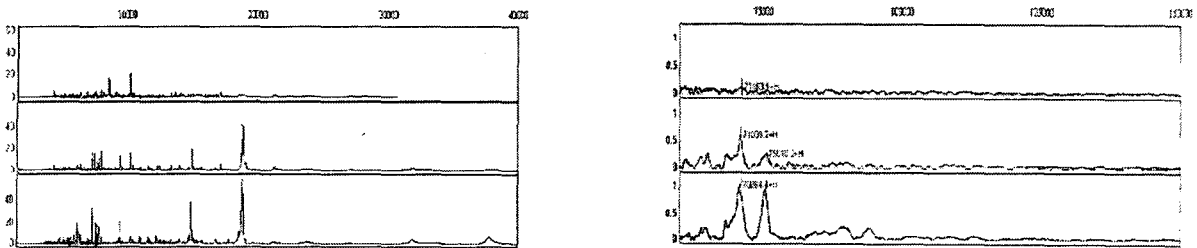
The present study describes the deeper analysis of novel proteins involved in rice seed maturation using protein chip-based technology called "surface-enhanced laser desorption/ionization-time of flight-mass spectrometry" (SELDI-TOF MS). Total soluble proteins of rice seed grain prepared from 1-week (1-WAF), 3-week (3-WAF) and maturation stages (6-WAF) were overlaid on SAX2 protein chip to exclude the highly abundant glutelins observed mostly in basic region and subsequently applied on SELDI-TOF MS for the proteome profiling. With a newly challenging proteomic method, we identified 20 individual polypeptides in the range of m/z 4,411 to 75,376 expressed noticeably at the specific stage. In particular, the mass fragments of m/z 50,973 and 56,678 presume to be the same profiling pattern during seed maturation as observed as 2-DE/MS. Three out of 20 candidate polypeptides were further verified as r40c1 protein, putative NAM protein and glutelin precursor by Q-Sepharose chromatography purification and SDS-PAGE analysis. Profiling analysis by SELDI-TOF MS provides a robust and quick proteome profiling and in particular, enables us to get information of low molecular weight polypeptides hard to detect on 2-DE/MS.

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Two-dimensional electrophoresis analysis of glutelins from mature seed of *Oryza sativa* cv. Ilpumbyeo



Full scan of SELDI-TOF MS rice seed protein profiles analyzed on SAX2 Protein-chip array



Multiple protein sequence alignment of glutelin proteins identified from 2D-PAGE and SELDI-TOF/MS analyses.