P016

Improved Method of Rice Seed Sample Preparation for Proteome Study by Removal of Glutelin Storage Protein

Sun-Hee Woo[1*], Se-Young Kim[2], Soohyun Kim[2], Jin Young Kim[2], Yeong Hee Ahn[2], YongGu Cho[1], BeomHeon Song[1], ChulWon Lee[1], SeungKeun Jong[1], Jong Shin Yoo[2], and Young Mok Park[2]

[1]*Dept. of Agronomy, Chungbuk National University, Cheongju 361-763*

[2]*Proteomics Analysis Team, Korea Basic Science Institute, Daejeon 305-333*

Objectives

Actually analysis of rice seed proteins extracted by the most commonly used conventional protein extraction method, the trichloroacetic acid (TCA) extraction method, showed that most of proteins identified by SDS-PAGE and mass analysis techniques belong to glutelin. Thus it is urgently necessary the development of a new sample preparation method through the efficient remove of glutelin family proteins and the selective extraction of non-glutelin proteins, especially low abundant.

Materials and Methods

Rice seed (100mg) → Grind (Liquid N2 for 30min) → TCA extraction → Water extraction Proteomic analysis → In gel digestion → Identification (All MS/MS experiments for peptide sequencing were performed using a Nano-LC/MS system, composed of an Ultimate HPLC system (LC Packings, Netherlands) and a Q-TOF2 mass spectrometer (Micromass, U.K) equipped with a nano-ESI source)

Results and Discussion

In order to increase number of identified proteins in rice seed proteome study, we developed a new improved method for sample preparation by removal of glutelin. When protein samples from rice seed was extracted by the conventional trichloroacetic acid (TCA) extraction method, glutelin counts for about 60% of total rice seed proteins on SDS gels. Using our new water extraction method, glutelin consists of only about 10% of total proteins on same gels. After analyzing on a two dimensional gel electrophoresis (2-DE), 937 protein spots were detected using the conventional TCA extraction method. On the other hand, 1240 proteins could be seen using the new water extraction method. The selectivity for non-glutelin and less abundant protein by the water extraction method was also confirmed by ESI Q-TOF mass spectrometry analysis. Thus, the new water extraction method developed here can be rapidly and efficiently used to study proteome analysis of rice seed.

*Corresponding author: Tel : 043-261-2515 E-mail : shwoo@chungbuk.ac.kr

SDS-PAGE image of total rice seed proteins extracted
by conventional TCA method and new improved water method

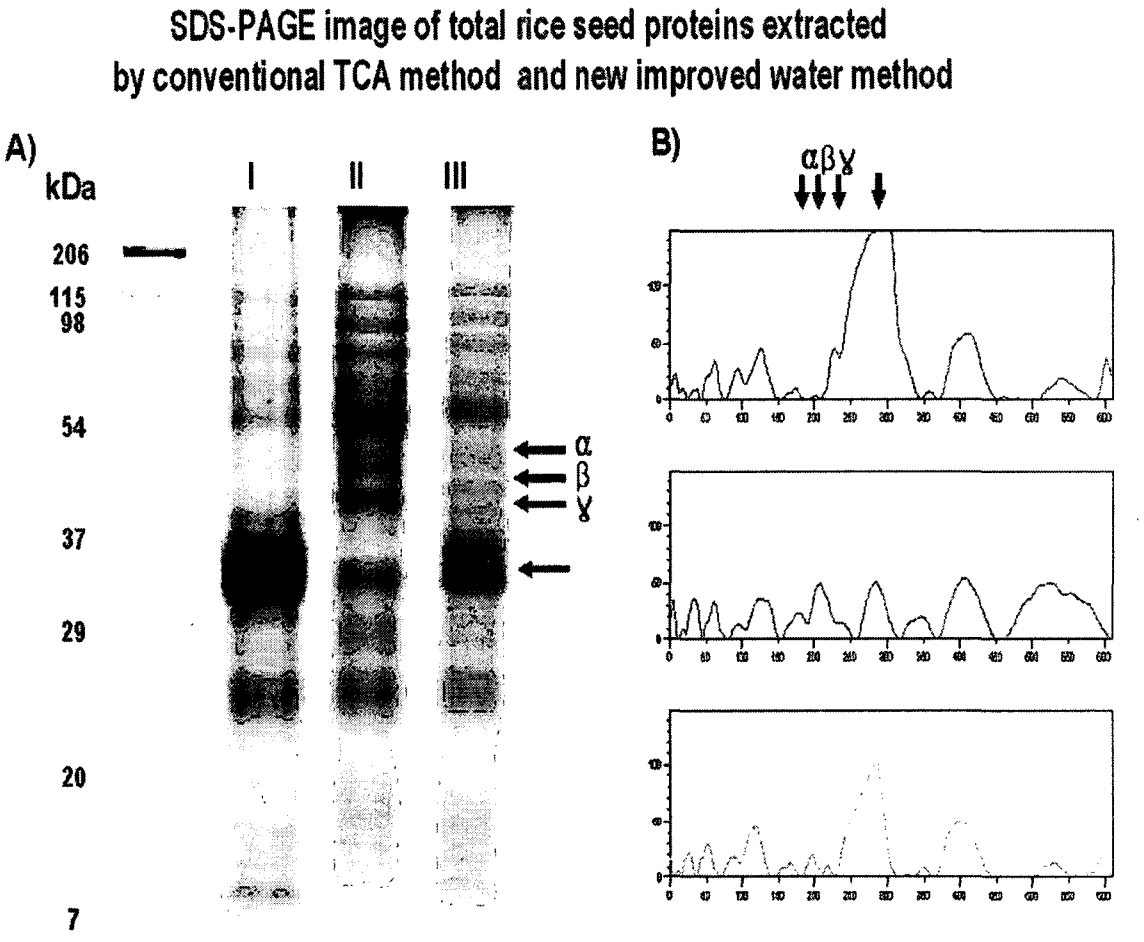

Figure 2. One half each of the protein samples shown lane I and lane II was mixed together and presented in lane III. Molecular weight standards were shown in the left. B) Scanned densitogram of SDS-PAGE image using the image analysis software ImageQuant TL (Amersham Pharmacia Biotech, Uppsala, Sweden). Arabian number I, II, and III represent the densitogram of lane I, II, II of SDS-PAGE shown in panel (A). Thick arrow represents the glutelin family proteins. Thin arrows with small letter a, b, and c indicate the bands of proteins which show most difference between lane I and II.