Proteome Analysis of Grain Filling and Seed Maturation in Rice

Sun-Hee Woo, Jung-Hee Ko, Hee-Young Jang, Seong-Woo Cho, Jong-Soon Choi, Hae-Chun Choi, Young-Mok Park, Je-Kyu Kim and Chul-Won Lee*

1Dept. of Crop Science, Chungbuk National University, Cheong-ju 361-763, Korea
2Mass Spectrometry Research Center, Korea Basic Science Institute, Chungbuk 863-883, Korea
3Lab. of Molecular Breeding, Arid land Research Center, Tottori University, Japan
4Division of Life Science, Korea Basic Science Institute, Daejeon 305-333, Korea
5National Crop Experiment Station, RDA, Suwon 441-100, Korea
6Dept. of Plant Life and Environmental Sciences, Hakyong National University, Anseong, 456-749, Korea

E-mail: cwlee@chungbuk.ac.kr

Introduction

To achieve the breeding goals for high yield and high protein rice, innovative tools are essential to identify the huge information hidden in the rice proteome. In this study, we set out to define and characterize the expressed proteome of the grain filling and different developmental stages of seed maturation in rice, with the aim of using the knowledge of which proteins are expressed to gain insights into the mechanisms of some of the important in grain filling and seed maturation.

Materials and methods

A rice wild type Illpum (japonica) and mutant type Suwon 464 (same name cultivar, Goami 2) were cultivated in the experiment field of the National Crop Experiment Station, Suwon, South Korea. Controlled environment facilities were used for rice growth and grain sampling. Grain samples were collected at 7 days, 21 days and 35 days during grain filling and seed maturation and were used for this experiment. 2-DE was performed according to previously reported methods. Silver staining was carried out by the method of Heukeshoven and Dernick using silver staining kit of Amersham Biotech. The protein contents were determined according to the method of Bradford. After protein measurement, silver stained gels were scanned using the PowerLook III image scanner (UMAX data system Inc.). The stained protein spots were excised from the gel and digested with trypsin (Promega, Madison, WI, USA). Then proteins were identified by peptide mass fingerprinting (PMF) using MALDI-TOF mass spectrometry.

Results and discussion

The progress of seed development was accompanied by a change in the pattern of soluble proteins as visualized by two-dimensional gel electrophoresis (Fig.1). Approximately 1,000 well defined spots could be resolved on the stage 80 gel, and about 900 spots could be resolved on the stage 87 gel in the pH 4 to 7 region. Many spots in the four cultivars changed in intensity during the 5-week development period of the present survey. About one-half of the most abundant spots at stage 87 were absent at stage 80, whereas 80% of the most abundant protein spots at stage 80 were also present at stage 87. This apparent discrepancy arises from the fact that some of the proteins that appear during the development process are extremely abundant at stage 87. The amount of salt-extractable protein in the seeds also changed slightly during development, in accordance with the idea that the proportion of proteins in an inaccessible stored from should increase during development. This could also influence the protein patterns seen on the two-dimensional gels; however, we decided to compare gels with equal...
protein load to visualize as many protein spots as possible at the late stage of development without losing resolution at the earlier stage.

Fig. 1. 2-DE of proteins extracted from rice seeds of 1 week after fertilization (A), 3 weeks after fertilization (B), matured stage (C) and functional classification of rice seed proteins (D).

Thus, the metabolic enzymes for the starch biosynthesis are up-regulated whereas the sucrose synthesis and glycolytic enzymes are down-regulated. The enzymes responsible for carbon partitioning are systematically regulated during grain filling and seed maturation with a concerted fashion.