Proteomic analysis of rice caryopsis development and its response to high temperature

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Abstract

Rice caryopsis proteins were profiled by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE), and differentially expressed proteins were analyzed by Liquid chromatography/tandem mass spectrometry (LC\MS\MS). In results more than 400 polypeptide spots were monitored during development, and more than 70 of them were analyzed by LC\MS\MS. Fifty four proteins with function annotations were identified. Among them 21 were carbohydrate metabolism related proteins, 14 were protein synthesis and sorting related proteins, 9 were stress response related proteins. Wx proteins and glutelins were the most significant spots showing increase during development. Allergen-like proteins, PPDK (Pyruvate, orthophosphate dikinase) and NADH-SDH (NAD-dependent sorbitol dehydrogenase) were also expressed along the development, implying their physiological roles in caryopsis. Expression of large isoforms of Wx proteins were correlated with the amylose content of rice caryopsis. One protein, with high GC content in its DNA sequence, showed correlation with chalky trait of kernels. High temperature (35/30 ℃) decreased expressions of Waxy proteins, allergen-like proteins and elongation factor 1 β; but increased expression of small heat shock proteins (sHSP), glyceraldehydes-3-phosphate dehydrogenase and prolamin. Abundance of sHSP seemed to be positively related to the chalky appearance of kernels. Wx proteins and glutelins were phosphorylated, and glutelins were glycosylated during development, suggesting the existence of post-modification on the molecules.

Key words

rice, proteomic, high temperature, grain quality, Wx protein, heat shock protein.

Media summary

Identification of high temperature responsive genes in rice kernels may benefit the improvement of grain quality in tropical and subtropical areas.

Introduction

The currently emerged proteomic technology allows a global investigation of the functions of caryopsis proteins during development, and provides an integrated information especially for the responses to environmental factors. Rice proteomic studies have been conducted for profiling and identifying proteins involved in metabolic pathways of various organs (Komatsu *et al.* 2003; Koller *et al.* 2002), responses to hormones (Rakwal & Komatsu 2000; Komatsu *et al.* 2003), and response to water stress (Salekdeh *et al.* 2002). In the present study we attempted to obtain a global observation for the dynamic change of protein expression patterns during rice caryopsis development and find out candidate proteins that may be crucial to grain quality especially under high temperature environment. Differential expression proteins during development or in response to high temperature were selected by 2D-PAGE and identified by a LC/MS/MS system. The potential functions of identified proteins were further investigated and discussed.

Methods

A high temperature tolerant indica type cultivar (TN 1) and a high temperature sensitive cultivar (TNG 67) was grown in a phytron. Kernels were sampled at 3, 6, 9, 12, and 15 days after anthesis (DAA). High temperature treatment was imposed at the day of anthesis (35/30 ℃). Changes in fresh weight, dry weight, endosperm nuclei number, and soluble protein content were determined. Caryopsis proteins were

analyzed by IEF/SDS-PAGE with silver staining. For IEF electrophoresis, proteins were resuspended with sample buffer containing 8.5 M urea, 1% (v/v) Triton X-100, 2.5 % (w/v) DTT, 1% (v/v) Pharmalyte, and tracking dye. IPG gel strips (13 cm, pH 3-10, Immobiline DryStrip, Amersham Biosciences, Uppsala, Sweden) were equilibrated overnight with 25 µl of the sample solution. IEF was conducted with a Multiphore II unit (Amersham Pharmacia Biotech), run at 3500 V for 8 h. In addition, IPG gels were used for SDS-PAGE with 15% (w/v) separation and 5% (w/v) stacking gels. Protein spots were visualized with a silver staining kit (Amersham Pharmacia Biotech).

Proteins differentially expressed during development and in response to high temperature were dissected from the gels and analyzed by a LC\MS\MS system. Proteins and genes identification and annotation were searched on the web sites of related rice gene banks. Expressions of target proteins were further confirmed by immunoanalysis. Possibility of protein modification was also determined using glycosylation- or phosphorylation-assay kits. The phosphorylation (Pro-Q Diamond Phosphoprotein Gel Stain Kit P-33300, Molecular Probes, Eugene, Oregon, USA) and glycosylation (Pro-Q Emerald 300 Glycoprotein Gel and Blot Stain Kit P-21857, Molecular Probes) assays were performed according to the manufacturer's instructions. Stained gels were visualized with an UV transilluminator at 300 nm.

Results and Conclusion

1. During early developmental stage, more then 400 protein spots could be visualized in the gel by silver-staining. And more than 1200 spots could be monitored by a computer-image analysis system. More than 80 differentially expressed spots were successfully identified by LC\MS\MS and with annotated functions from the survey of software. These proteins are involved in important physiological and biochemical pathways for grain development.

2. Amounts of 4 proteins were decreased by high temperature treatment imposed at grain filling stage, while abundances of 6 proteins were enhanced by the high temperature.

Proteins decreased at high temperature	Proteins increased at high temperature
Allergen-liked protein	16.9 KDa class I heat shock protein
Nucleoside diphosphate kinase 1	17.9 KDa heat shock protein
Elongation factor 1-beta	18 KDa maize heat shock protein
WB gene	Ribulose 1,5-biphosphate caboxylase/oxygenase (large chain)
	G-3-P dehydrogenase
	Prolamine 7

3. More than 6 of Waxy protein isoforms (granule bound starch synthase) were identified in the gels, and could be classified into two major groups by molecular mass. The abundance of Waxy proteins was remarkable high in seeds of the varieties having high amylose content (TN1 and IR36). Few or even no amount of Waxy protein isoforms expressed in the rice varieties of less than 5% amylose content. The abundance of high Waxy protein isoforms positively correlated with the amylose content of tested varieties.

4. One protein showed differential expression between chalky and translucent kernels. The gene has 74% GC content. The protein expressed after 9 DAF (days after flowering) and gradually increased up to 5 times higher until maturation. The protein expressed significantly higher in translucent region than in chalk region of endosperm.

5. Expression of class 1 heat shock proteins (sHSP) was significantly enhanced in endosperm high temperature grown kernels. The abundance of sHSP was lower in good-quality rice varieties (Koshihikari, Jasmin and TK 9) than in chalky-rice varieties (TNG 67, Pegonia, and Arboria). The expression of sHSP may affect the grain quality in response to high growing temperature.

6. Using recently developed commercial kits, we examined the status of post-translational modification of rice kernel proteins. In the phosphorylation assay, only the regions in the gel with Wx proteins and glutelin yielded positive signals for protein expression at 12 DAA . Four Wx protein isoforms and seven protein spots in the glutelin protein region exhibited signals for phosphorylation staining. The four Wx isoforms detected corresponded to the high Wx isoforms. Spot no 24, identified as a DNA fragment, gave a strong phosphorylation signal in the gel and can be used as an internal check to confirm that phosphorylation is being detected. In the protein glycosylation assay, seven proteins in the glutelin region gave positive signals.

References

Koller A., Washburn M.P., Lange B.M., Andon N.L., Deciu C., Haynes P.A., Hays L., Schieltz D., Ulaszek R., Wei J., Wolters D. & Yates J.R. (2002) Proteomic survey of metabolic pathways in rice. Proceedings of the National Academy of Sciences **18,** 11969-11974

Komatsu S., Konishi H., Shen S. & Yang G. (2003) Rice proteomics: a step toward functional analysis of the rice genome. Molecular and Cellular proteomics **2,** 2-10.

Rakwal R. & Komatsu S. (2000) Role of jasmonate in the rice (*Oryza sativa* L.) self-defense mechanism using proteome analysis. Electrophoresis 21, 2492-2500.

Salekdeh G.H., Siopongco J., Wade L.J., Ghareyazie B. & Bennett J. (2002) Proteomic analysis of rice leaves during drought stress and recovery. Proteomics **2,** 1131-45.