Proteomic Analysis of TC65 vs. gluP6 RNA Binding Proteins Associated with RNA Localization

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Introduction

Storage proteins found in developing rice endosperms have been found to be RNA-localized in special subdomains of the endoplasmic reticulum (Crofts et al., 2004). Vps9 is a conserved domain and protein found in rice plants, such as the Tai Chung (TC65) strain and Kitaake strain of rice most commonly consumed in Japan; a protein partially responsible and involved in RNA localization and endocytosis. gluP6 (the Vps9 mutant strain), has been the focus of this research. By studying conserved domains and proteins involved in RNA localization of storage proteins, one can better understand the importance and mechanisms involved in RNA sorting and RNA localizing of prominent storage molecules in developing rice endosperms, such as prolamine, glutelin, and globulin. The following research evaluates gluP6 mutant against a wild type strain, TC65, via 2D-DIGE analysis. 2D-DIGE is two-dimensional difference in gel electrophoresis analysis, a technique that allows for the analysis of two protein samples simultaneously; then a comparison of which proteins are up/down regulated can be determined amongst the mutant strain and WT. With the aide of Delta2D imaging software, 14 protein spots were found to be expressed with a significant degree of difference on the gels, which will indicate statistically significant protein spots. These results aim to provide further insight on RNA localization of storage proteins and perhaps respective chaperons in developing rice endosperms.

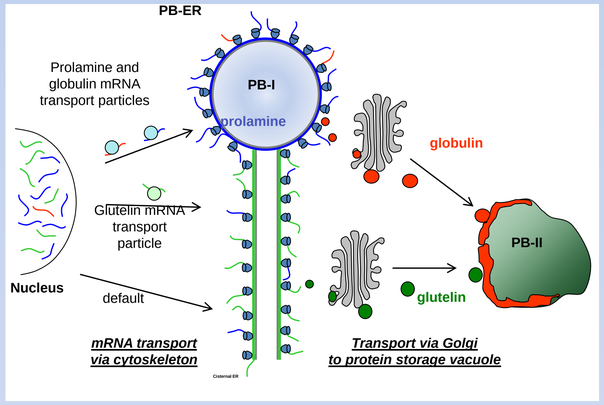

Figure 1. After transcription in the nucleus, targeted storage protein mRNAs are transported to different subdomains within the cell of a developing rice endosperm, which will determine protein synthesis and localization. The globulin and prolamine targeted protein storage mRNAs are transported to the protein body endoplasmic reticulum (PB-ER) via the actin cytoskeleton, while the glutelin mRNAs are transported to the cisternal endoplasmic reticulum. Posttranslational localization of prolamine polypeptides accumulate in intracisternal inclusion granules called protein body type I (PB-I). Glutelin and globulin polypeptides maneuver through the Golgi apparatus and accumulate in storage protein vacuoles called protein body type II (PB-II). The localization and transport of these storage protein mRNAs is dependent on *cis*-localization elements, which are hypothesized to have an important role in interacting with RNA-binding proteins critical for their transport and final destination posttranslationally, which will ultimately determine their functional efficacy.

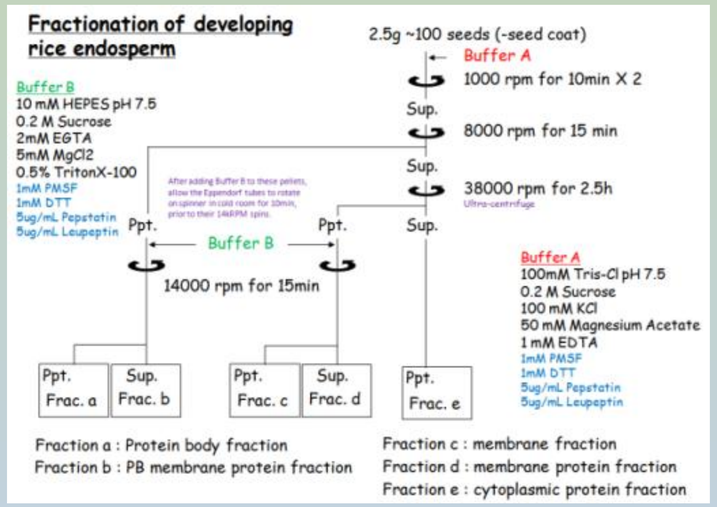

Figure 2. Schematic representation of the fractionation protocol performed on developing rice endosperms. The cytosolic fraction E of both wild type and gluP6 was protein assayed to quantify yield and concentration of protein.

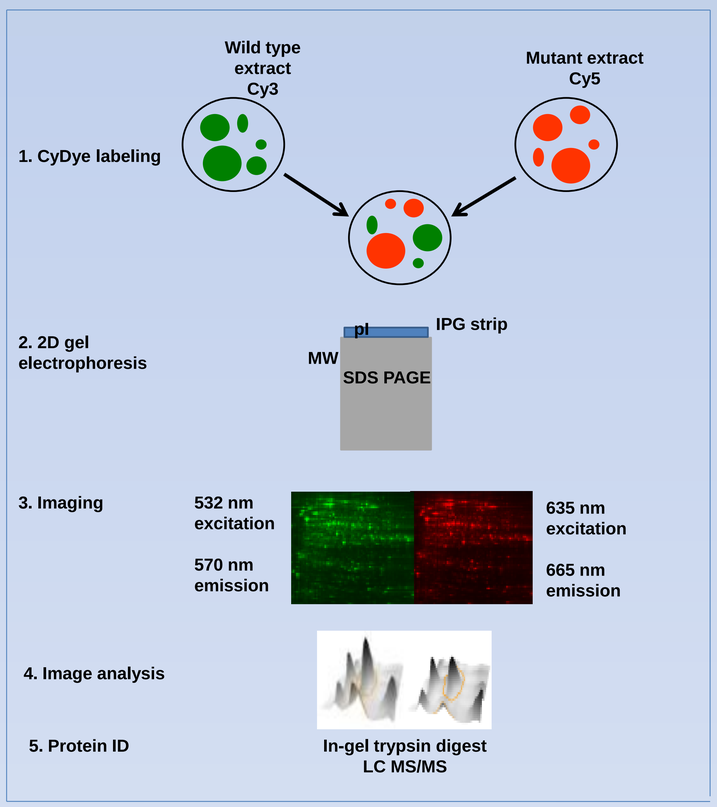

Figure 3. The diagram shows a schematic representation of the 2D DIGE analysis performed on wild type and *gluP6* mutant developing rice seed extracts using CyDye fluors (GE Healthcare). Differentially expressed protein spots were exised, in-gel trypsin digested, and analyzed by liquid chromatography tandem mass spectrometry.

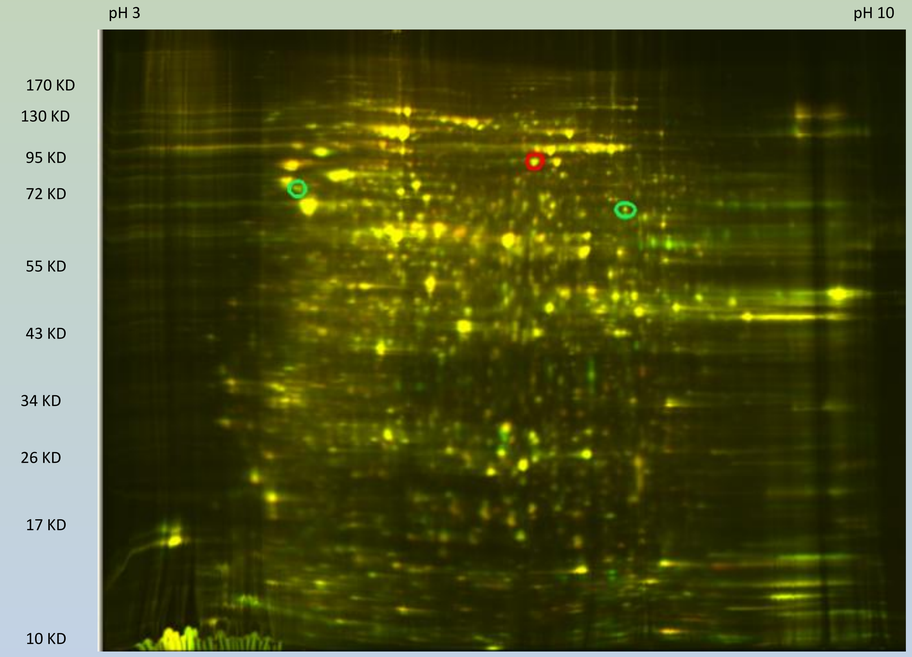

Figure 4. 2D DIGE comparing the soluble fractions of wild type TC65 (green) and gluP6 mutant (red) 14 day old developing rice endosperms. Protein samples were first focused along pH3-10 24cm immobilized pH gradient strips for approximately 50kVhrs after being labled with CyDyes, in order to separate proteins by their isoelectric points, the first dimension in separation. Then the strips were ran across a large 10-18% gradient SDS-PAGE to separate according to their molecular weight, the second dimension of separation. Experiment was performed in biological triplicate. To analyze the protein spots, Delta2D imaging software was used to perform statistical tests to determine which spots were present in 1.5 fold increase, illustrating statistical significance ($p<0.05$). 14 spots were found to be significant, but only three of these were scored for MS.

Table 1. Significant protein spots in gluP6 mutant

Spot #	Protein Description	MW	pI	Function	cDNA accession #	up/down regulation
4	glucose-6-phosphate isomerase	62.4 kDa	6.93	carbon metabolism	AK068236	down
9	5-methyltetrahydropteroyltriglutamate	84.6 kDa	6.22	amino acid metabolism	AK067726	up
9	5-methyltetrahydropteroyltriglutamate	84.6 kDa	6.23	amino acid metabolism	AK065255*	up
12	protein disulfide isomerase-like protein	64.4 kDa	4.56	protein modification	AK243646	down

Conclusions

- 3 distinct proteins were found to be differentially expressed in the TC65 wild type and gluP6 mutant developing rice endosperm extracts. These differences may be attributed to mutating the conserved Vps9 domain important for RNA localization and endocytosis, which will further affect storage protein gene expression in developing rice endosperms.
- MS results indicate that the three protein spots excised were all involved in either metabolite metabolism or posttranslational modifications.
- In the future, another triplicate of 2D-DIGE gels should be focused and ran with a much larger protein sample with the intent of analyzing and scoring the other 7 protein spots that were significantly different, and perhaps finding new significant spots.

Acknowledgements
This work was generously supported by National Science Foundation Grant NSF DBI-0605016 and USDA-CSREES-NRI Grant 2006-35301-17043. We thank the University of Idaho Environmental Biotechnology Institute for mass spec analysis.

References
Crofts AJ, Washida H, Okita TW, Ogawa M, Kumamaru T, Satoh H. 2004. *Plant Physiol*,136:3414-3419.