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PROTEIN LYSINE ACETYLATION, MALONYLATION, SUCCINYLATION, CROTONYLATION, AND 2-HYDROXYISOBUTYRYLATION IN DEVELOPING RICE SEED AND THEIR ROLES IN SEED METABOLIC REGULATION AND NUTRIENT RESERVOIR DEVELOPMENT

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Introduction: Protein lysine malonylation, succinylation, crotonylation, and 2-hydroxyisobutyrylation have been recognized as new Post-Translational Modifications (PTMs) in recent years. However, the proteins harboring these modifications and their corresponding functions of the modifications remain largely unknown in cereal plants. Using antibody-based affinity enrichment of modified peptides followed by nano-HPLC/MS/MS analyses, we identified from a few hundreds to over nine thousand modification sites for these four modifications together with acetylation in developing rice (*Oryza sativa*) seeds, respectively. Distinct sequence motifs at the modification sites were identified for each of the modifications, respectively. Proteins with different sequence motifs were shown to be favorably associated with unique metabolic pathways or protein function domains. Many of the modified proteins and the corresponding modification sites were conserved from *E. coli*, human, to plants and many of the modification sites can be modified by multiple different acyl groups, especially in the key enzymes of essential metabolic pathways. Rice proteins with co-modifications of succinylation, malonylation, crotonylation, 2-hydroxyisobutyrylation, acetylation, ubiquitination, and phosphorylation were studied through a comprehensive analysis. The potential cross-talks among different modifications in the regulation of cellular metabolic processes are

examined. In addition, the impact of heavy lysine modifications on lysine bioavailability in rice storage proteins has been investigated. Our study delivers a platform for expansive investigation of the molecular networks administrating cereal seed development via post-translational modifications.

Methodology: Using antibody-based affinity enrichment of modified peptides followed by nano-HPLC/MS/MS and bioinformatics analyses

Conclusions & Significance: The first proteome wide 2-hydroxyisobutyrylation study in plant will be presented, which represents the largest post translational modification sites identified in plants. The first proteome wide malonylation study in plants will be presented. The distinct sequence motifs of succinylation, malonylation, crotonylation, and 2-hydroxyisobutyrylation, respectively, will be presented. Rice proteins with co-modifications of succinylation, malonylation, crotonylation, 2-hydroxyisobutyrylation, acetylation, ubiquitination, and phosphorylation will be presented. The potential roles of the post translational modifications in seed and seed nutrition reservoir development will be discussed.

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