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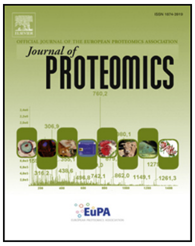

Comparative proteomics analysis of superior and inferior spikelets in hybrid rice during grain filling and response of inferior spikelets to drought stress using isobaric tags for relative and absolute quantification

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ABSTRACT

The biological functions of the differentially abundant proteins between superior and inferior spikelet grains were investigated based on the isobaric tags for relative and absolute quantification to further clarify the mechanism of rice grain filling at the proteomic level, as well as the response of inferior spikelets to drought dress (−20 kPa or −40 kPa). Compared with superior spikelets, inferior ones had lower sink strength due to the lower sink activities (lower abundances of ADP-glucose pyrophosphorylase, granule-bound starch synthase, starch branching enzyme and pullulanase) and smaller sink sizes (lower abundances of structural proteins). The slower and later grain filling resulted from the weaker decomposition and conversion of photoassimilate and the slower cell division. Moderate drought stress (−20 kPa) promoted the grain filling of inferior spikelets through regulating the proteins associated with photoassimilate supply and conversion. These proteins may be important targets for rice breeding programs that raise the rice yield under drought condition. The findings offer new insights into rice grain-filling and provide theoretical evidences for better quality control and scientific improvement of super rice in practice.

Biological significance

Rice cultivars with large panicles do not always guarantee high yield and grain quality probably due to the slow grain filling and many unfilled grains of inferior spikelets. In general, earlier-flowering superior spikelets, which are usually located on apical primary branches, fill faster and produce larger and heavier grains. In contrast, later-flowering inferior spikelets located on proximal secondary branches are either sterile or fill slowly and poorly, and the differences are more significant in large panicle rice or super rice. The increase of rice yield has been limited by the unsatisfactory grain filling of inferior spikelets,

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and the inferior spikelets are more prone to environmental factors during grain filling. Thus, we herein investigated the biological functions of differently abundant proteins between superior and inferior spikelet grains by using iTRAQ to unravel the mechanism of rice grain filling and the response of inferior spikelets to drought stress at proteomic level. This study offers new insights into rice grain-filling and provides valuable evidences for better quality control and scientific improvement of super rice in practice.

1. Introduction

Rice sink capacity and grain filling efficiency significantly impact the yield that has been tentatively elevated by breeding of large panicle hybrid rice or super rice mainly through increasing the number of full-filling spikelets per panicle [1]. However, cultivars with large panicles do not always guarantee high yield and grain quality probably due to the slow grain filling and many unfilled grains of inferior spikelets [2,3]. In general, earlier-flowering superior spikelets, which are usually located on apical primary branches, fill faster and produce larger and heavier grains. In contrast, later-flowering inferior spikelets located on proximal secondary branches are either sterile or fill slowly and poorly, and the differences are more significant in large panicle rice or super rice [4–6]. The increase of rice yield has been limited by the unsatisfactory grain filling of inferior spikelets [7–10]. Compared with numerous studies focused on sucrose-to-starch conversion [5,11–19], limitations in carbon supply and sink capacity [20–23], imbalance of phytohormones [7,10,24–27], enzyme activities [13,28–30], and gene expression [31–33] have seldom been referred. However, grain filling is a complex biological and molecular process, with the exact mechanism remaining elusive.

Rice quality is determined by both genotype and environment. The two peaks of grain filling and two environmental-sensitive periods, especially those of large panicle rice, are determined by the ear filling characteristics of different grains under normal conditions [34]. The degree and the rate of grain filling as well as the grain weight of rice spikelets differ largely depending on their positions on a panicle, and the inferior spikelets are more prone to environmental factors during grain filling, indicating that effectively regulating cultivation can augment the yield by improving the grain filling of inferior spikelets [35]. Soil water status, particularly that during grain filling, influences grain quality dramatically. Proper water stress can promote the remobilization of prestored carbon reserves to the grains [36], increase the ratio of abscisic acid (ABA) to ethylene [19], and enhance the activities of sucrose synthase (SuSase), soluble and insoluble invertase, starch branching enzyme (SBE) and soluble starch synthase (SSSase). It may also facilitate the starch accumulation during grain filling, especially that in inferior spikelets during the grain filling of wheat [37].

In the last decade, proteomics has become an indispensable complement of transcriptome in life science. Proteomics is able to analyze simultaneous changes and to classify the temporal patterns of protein accumulation in complex developmental processes [38]. Rice is the main grain crop for human and one of the *Poaceae* plants with the smallest genome (about 430 MB) [39]. Besides, rice is an eligible model plant for molecular biology because its gene is easily operatable and similar to those of other monocot plants [40]. Since the rice genome map has been finished in 2002, the proteomics of rice has been highlighted [41–44], mainly on the protein abundance patterns of tissues/organs and subcellular components. Zhang et al. [43] employed 2-D gel-based comparative proteomic and phosphoproteomic analyses to search the differentially abundant proteins in inferior spikelets under exogenous ABA treatment, and found that 111 such proteins were related with defense response as well as carbohydrate, protein, amino acid, energy and secondary metabolisms, revealing that the grain filling of rice inferior spikelets was regulated by ABA through the proteins and phosphoproteins participating in carbon, nitrogen and energy metabolisms. Zi et al. [45] analyzed the stress responsive proteins during rice embryogenesis by using isobaric tags for relative and absolute quantification (iTRAQ) and shotgun techniques, and found that most of the up-regulated proteins, including heat shock-, lipid transfer-, and reactive oxygen species-related proteins, were functionally categorized as stress responsive. The proteomics of superior or inferior spikelets, especially that of large panicle rice or super rice, has scarcely been analyzed, and the differences of protein abundances and functions between superior and inferior spikelets remain unclear. Although 2-DE provides a visual representation of the proteome in which distinct protein isoforms resulting from the changes in Mr and/or pI can be observed, it does not apply to detection of low-abundance proteins and more accurate quantification [46]. iTRAQ, one of the mass-based quantitative approaches, has become prevalent in the field of crop proteomics [47] by simultaneously identifying and quantifying proteins from multiple samples with high coverage. Thus, we herein investigated the biological functions of the differentially abundant proteins between superior and inferior spikelet grains by using iTRAQ to unravel the mechanism of rice grain filling and the response of inferior spikelets to drought stress at proteomic level. This study offers new insights into rice grain-filling and provides valuable evidences for better quality control and scientific improvement of super rice in practice.

2. Materials and methods

2.1. Rice cultivation

Field experiments were carried out in an experimental farm of Taihu Area Institute of Agricultural Sciences, Su Zhou, Jiangsu province, China in 2011, with large-panicle hybrid Japonica rice Yongyou 8 (according to the test for an average of 181 grains with 26.3 grams weight per panicle) as the material. Seedlings were sown on 20th May and transplanted on 25th June at a hill spacing of 0.3 m × 0.15 m with 1 seedling per hill. The soil of the field was paddy soil that contained 2.42% organic matter and 158.4, 8.4 and 127.0 mg·kg^{-1} available N–P–