

Expression Characteristics and Sequence Variation Analysis of Rice Starch Regulator 1 Gene in Japonica Rice With Transgressive Variation

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ABSTRACT

The parents and transgressive variation lines of hybrids with significant difference in amylose content were selected to compare and analyze the accumulation characteristics of amylose and the change of OsRSR1 expression in grains in the process of grain filling, and the PCR technology was used to clone the OsRSR1 gene base sequence of four varieties. The results showed that the amylose content in grains increased gradually with grain filling process, the amylose content of offspring and parents with high amylose content were higher than the offspring and parents with low amylose content, hybrids could obtain the transgressive variation lines through the continuous directional selection of amylose content in grain, and the accumulation of amylose content in grain was closely related to genotypes. The expression quantity of OsRSR1 gene in grain was increasing during the grain filling process, the amylose content of grain was closely related to the activity of OsRSR1 gene, and the expression of grain OsRSR1 gene could also produce transgressive variation.

KEYWORDS

Japonica Rice, RSR1 Gene Expression, Sequence Variation, Transgressive Variation

INTRODUCTION

Transcription factors are also called trans-acting factors. Typical transcription factors consist of four functional domains. Transcription factors regulate transcription and expression of genes through interaction between functional areas and promoters cis-acting elements or functional areas of other transcription factors (Liu et al., 2001; Hou, 2014; Xie, 2019). For *OsRSR1*, promoter elements may produce a marked effect in response to ABA, ethylene, and abiotic stress signals,

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thereby regulating the expression of downstream stress resistance related genes (Li, et al., 2015). The *OsRSR1* gene is affected by low temperature, drought, and NaCl stress in adversity expression. *OsRSR1* is an AP2/EREBP family transcription factor that regulates starch synthesis in rice. Its expression is negatively correlated with the expression of the genes responsible for the synthesis of starch in the endosperm in seeds and sink tissues (Fu & Hue, 2010). The study of its physiological function showed that the lack of *OsRSR1* could lead to the increase of the expression of genes related to starch synthesis in the seeds. In the deletion mutant of the *OsRSR1* gene, the expression of a class of starch synthase genes was unregulated, the amylose content in rice seed was improved, and the amylopectin structure in rice seeds was changed. Therefore, the final morphology of starch granules in rice seeds was changed, the gelatinization temperature of starch was reduced, and the microstructure of amylopectin was changed. *OsRSR1* is a transcription factor of the APETALA2/ethylene response element binding protein family. There are two AP2/ERF domains in the structure of *OsRSR1* protein, which are the typical characteristics of the AP2 family transcription factors. There is a DNA binding area of two conserved sequence blocks: YRG element and RADY element in the AP2/ERF binding domain.

The occurrence of transgressive inheritance variation in quantitative characters is a common phenomenon in sexual hybrids. The sexual hybridization between rice varieties is still one of the main ways to cultivate new rice varieties at present and in the future. However, there are few studies on the relationship between the sequence variation of the transcription factor base sequence and its transcriptional expression quantity in the sexual hybrids among the related varieties. Therefore, this study selected two rice varieties with a significant difference in the amylose content in the grains as parents. It then took the amylose as the selection index and continuously and directionally selected the transgressive variants with a significant difference in the amylose content to compare and analyze the base sequence and protein domain of the grain *OsRSR1* gene between the parent and the transgressive variant. This was to provide a theoretical basis for elucidating the relationship between the base sequence variations, the amylose content of the *OsRSR1* gene, and the molecular mechanism of transgressive inheritance variation in the grain amylose content of the hybrid progeny.

MATERIALS AND METHODS

Test Materials and Test Methods

Two japonica rice varieties with significant difference in grain amylose content were selected, i.e., parent Xixuan 1 (18.48%), Tong 769 (15.81%), and transgressive offspring stable strains Dongnong 1101 (19.71%) and Dongnong 1124 (7.40%). In 2015–2016, the pot experiment was carried out in the Agricultural College of Northeast Agricultural University. The length of the pot was 100 cm, the width was 40 cm, and the height was 60 cm. From April 1 to 15, according to the growth period of the test materials, sowing was carried out in stages to ensure the heading stage was as consistent as possible. Furthermore, plug-seedlings in trays in a greenhouse, equidistantly dibble sowing of a single sprouting seed, and dry rice-nursery management were selected. On May 15, rice seedlings with consistent growth potential were selected for transplantation. Each variety was inserted in three pots and 24 seedlings were equidistantly planted in every pot. After seedling survival, 12 seedlings were planted and treated with normal fertilizer.

At heading stage, the rice ears of the same size and extension at the same time were selected and marked with a sign. On the 10th, 20th, and 30th day after heading, eight rice ears with signs were selected, respectively. Then, 20 grains in the middle of the ear, with the same grain filling, were selected. With shells and embryos removed at a low temperature, they were put into a sterile tube, which was then quickly placed into liquid nitrogen. Finally, they were stored at -80°C to be used for quantitative analysis of fluorescence.

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