Journal of Integrative Agriculture 2015, 14(4): 642–659

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RESEARCH ARTICLE

Effect of high temperature on the expressions of genes encoding starch synthesis enzymes in developing rice endosperms

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Abstract

High temperature is the major environmental factor affecting grain starch properties of cooking rice cultivars. In this study, two non-waxy *indica* rice genotypes, *cv*. 9311 and its mutant with extremely high amylose phenotype (9311*eha*) were used to study the differential expressions of genes in starch synthesis and their responses to high temperature (HT). Significant increase in apparent amylose content and hot-water-soluble starch content in mutant 9311*eha* were genetically caused by a substitution from AGTTATA to AGGTATA at the leader intron 5′ splice site in *Wx* gene. This mutation resulted in different mRNA transcript levels, mRNA splicing efficiencies and protein levels of *Wx* between the two rice genotypes, which also lead to the genotype-dependent alteration in the temporal pattern of *Wx* transcription and granule-bound starch synthase (GBSS) activity in response to HT. However, changes in the activities of other starch synthesizing enzymes and their expressions of distinct isoform genes were not significant with the *Wx* gene mutation, thus only minor difference in the particle size of starch granule, chain-length distribution and gelatinization enthalpy were found between the two genotypes. The temporal-specific expression of multiple isoform genes responsive to different temperature regiments indicated that the reduction of GBSS transcript expression under HT was generally accompanied by the decreased expressions of *SSSIIa*, *SSSIIIa* and *SBEIIb*. Consequently, high temperature-ripened grains in 9311*eha* showed high proportion of intermediate and long B chains and somewhat lower level of short A chain compared to the wildtype. The temperature-dependent alteration of amylose content was not only attributed to the reduced expression of GBSS, but also associated with the complimentary effect of *SSSIIa* and *SBEIIb*.

Keywords: rice, starch synthesis, gene expression, amylopectin strucrute, high temperature

1. Introduction

Rice (*Oryza sativa* L.) is a starch-rich staple food that provides 35–60% of the caloric intake for over 3 billion people in the world. Starch reserve in rice grains accounts for 80–90% of final endosperm weight (Vandeputte *et al*. 2004). Grain-filling in cereal endosperms is actually a biological process of starch accumulation that ultimately affects the yield of rice and grain quality (Fitzgerald *et al*. 2009). Rice grain-filling is vulnerable to the change environmental temperatures with respect to starch biosynthesis and accumulation (Asaoka *et al*. 1985). Specifically, high temperature (HT) during grain-filling period cause the formation of loosely packed starch granules, and diminish the amount of starch deposited in discrete granules, thereby

Received 13 March, 2013 Accepted 19 May, 2014
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doi: 10.1016/S2095-3119(14)60782-6

leading to the reduction in grain weight together with more production of chalky grains (Geigenberger *et al*. 2011). More importantly, the palatability property of cooking rice cultivars may be remarkably compromised due to the alteration of starch composition in rice endosperms after rice plants exposure to HT during filling period (Fitzgerald *et al*. 2009).

The storage starch in nonwaxy rice endosperms consists of two major components: linear α-polyglucan amylose and branched α-polyglucan amylopectinch components in nonwaxy rice endosperms. The ratio of amylose to total starch content, commonly expressed as amylose content (AC, %) in milled rice grains, widely considered as one of the most important parameters for assessing the physicochemical properties of starches, and the chain length distribution of amylopectin are also responsible for the eating and cooking quality of rice cultivars (Asaoka *et al*. 1985; Reddy *et al*. 1993). In developing rice endosperms, the amylose synthesis is mainly regulated by granule-bound starch synthase (GBSS), a genetic target of *Wx* gene, whereas the amylopectin synthesis is coordinated by soluble starch synthase (SSS), starch branching enzyme (SBE) and starch debranching enzyme (DBE) (Tetlow 2011). Recent studies have also revealed the role of GBSS in amylopectin synthesis. Hanashiro *et al*. (2008) reported that GBSS is necessary for the biosynthesis of extra-long unit chains (ELC) of amylopectin in rice endosperms. The importance of GBSS in the formation of the ELC fraction in amylopectin is also observed in wheat (Yoo and Jane 2002) and sweet potato (Kitahara *et al*. 2007). In some cases, the amylose synthesis is a result of synergistic roles by certain specific isoforms of SSS and SBE, for instance, *SSSIIa* and *SBEIIb*. A combination of the deficient mutation of *SSSIIa* (*alk*) and *SBEIIb (amylose-extender*, *ae*) genes can substantially increase the amylose content, change chain length structure of amylopectin and decrease gelatinization temperatures (GT) of starch granules (Nishi *et al*. 2001; Nakamura *et al*. 2005). So far, at least two or more distinctive isoforms have been identified for each of four key enzymes (GBSS, SSS, SBE and DBE) in starch synthesis. These isoforms have either unique or overlapping roles in the synthesis of amylose and amylopectin, depending on their substrate specificity, preferential expression in certain organs/tissues, at development stages (Ohdan *et al*. 2005; Yamakawa *et al*. 2007). Recent studies on molecular genetic and gene profile expression analyses, integrated with biochemical characterizations, have significantly improved our understanding of the unique functions of the isoforms of those key enzymes and their regulating network for starch biosynthesis in higher plants (Ohdan *et al*. 2005; Yamakawa *et al*. 2007; Lin *et al*. 2010). Nevertheless, our understanding is relatively poor on how the environment temperatures impact the expression the enzyme isoform-encoding genes and the consequent variations in starch structure and composition.

In past decades, studies with wheat, rice and maize and other plants indicate that the activities of SSS, and/or even the transcriptional expression for SSS genes, are impaired at the elevated temperatures (Yamakawa *et al*. 2007). In rice, the increase in starch gelatinization temperature under HT is mainly attributed to reduced proportion of long B chains and increased proportion of intermediate B chains in amylopectin (Asaoka *et al*. 1985). The enzyme activities and mRNA expression levels of *GBSSI* and *SBEIIb* are decreased at HT, leading to lower AC and the higher gelatinization resistance (Jiang *et al*. 2003). The effects of HT on the transcriptional expression and enzymatic activity of *GBSSI* are genotype-dependent. In the low-amylose cultivar, a substitution at the putative leader intron 5′ splice site may impact the splicing efficiency of *Wx* gene transcript (Ayres *et al*. 1997), which is closely correlated with the diversity of rice genotypes in AC and their responses of *GBSSI* expression to varying environmental temperatures. Recent studies on high temperature-induced chalky appearance, starch accumulation and storage protein indicate that there are marked differential expressions for various isozyme genes of SSS and SBE enzymes in response to high temperature during filling period (Yamakawa *et al*. 2007). It is also suggested that the expression patterns and their responses of multiple starch-synthesis genes to HT were isoform-dependent in rice endosperm and leaf tissues, both of which have high expressions of *GBSSI*, SSS*I*, *SSSIIa*, *SSSIIIa*, *SBEI*, *SBEIIb*, and *ISA2* (Hirose and Terao 2004; Ohdan *et al*. 2005). But so far, the relationships between HT-induced isoform expression patterns and starch quality variation, and the isoform interaction in the course of starch synthesis are still not clear, especially for *indica* genotypes.

In this study, two non-waxy *indica* rice genotypes, *cv*. 9311 and its mutant with extremely high amylose phenotype 9311*eha*, are utilized to compare their temporal mRNA expression patterns of various isoform genes of GBSS, SSS, SBE and DBE enzymes (isoamylase and pullulanase) in developing endosperm under different temperature treatments. The enzymatic activities of GBSS, SSS, SBE and DBE in developing endosperms and starch physicochemical structure in milled grains are also analyzed with the rice plants grown at well defined temperature condition. Our aims are to examine the accompanying alteration in the expression patterns of major isozyme genes responsible for the starch biosynthesis in rice endosperm caused by the mutation of *GBSSI* gene locus, and make some efforts to elucidate their distinctive sensitivity and stage-specificity of these isozyme genes preferentially expressed in rice endosperm and their relations to starch structure variation in response to HT environmen. Such results will provide helpful knowledge for understanding the possible interaction among different