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Effects of high temperature on starch morphology and the expression of genes related to starch biosynthesis and degradation

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ABSTRACT

High temperature occurs frequently during grain filling in wheat (*Triticum aestivum* L.). The objective of this study was to investigate the high-temperature post anthesis effect on the starch granule morphology and gene transcripts that are involved in starch biosynthesis and degradation. Wheat plants were grown under day/night temperatures of 24/17 °C (control) and 37/28 °C (high temperature) from 5 days post anthesis until kernel dry matter accumulation ceased. Upon exposure to high temperature, the matured kernels underwent significant shrinkage, and signs of pitting on the granule surface were more pronounced. The high temperature altered the timing of the starch biosynthetic process and resulted in an earlier peak in the gene expression during starch biosynthesis. The high temperature had a distinct effect on the enhancement of the grain α-amylase activity during kernel filling, and the starch granules were susceptible to enzymatic hydrolysis. This result supported the hypothesis that increased pitting of the granule surface is related to an enhanced α-amylase activity in wheat kernels that are exposed to high temperature post anthesis.

1. Introduction

Environmental constraints are a major factor for the productivity of wheat in many regions of the world. One abiotic stress that affects wheat is high temperature. In particular, high temperature is one of the major factors that limits crop production and end use. The response to temperature depends on the wheat genotype, the specific temperature, and the time in which the temperature regimen is imposed during the developmental program (Ihsan et al., 2016). Previous studies reported that the time from anthesis to harvest maturity is notably shorter under high-temperature conditions, and prior indications of programmed cell death (PCD) of endosperm tissue became evident in US wheat 'Butte 86'. The onset and cessation of starch accumulation also occurs under high-temperature conditions, and the average kernel weight sharply decreases under these conditions (Altenbach et al., 2003).

Starch is the main component in wheat endosperm, which accounts for approximately 70% of dry grain weight. Wheat starch exists as granules with two distinct granule populations: large A-type (>10 μm) and small B-type (<10 μm) granules, which are differentiated by their chemical and morphological characteristics (Yu et al., 2015). Starch biosynthesis and the distribution of different sizes of starch granules in wheat grains have both been genetically and environmentally controlled (Li et al., 2013, 2015). Upon comparative proteome analysis of A- and B-type starch granule-associated proteins in bread wheat and *Aegilops* crassa, it was found that the phosphorylation of the starch biosynthetic enzymes is related to the formation of B-type starch granules (Cao et al., 2015). It has also been reported that starch granules in wheat grains are significantly affected by high temperature after anthesis (Wang et al., 2015). Based on the relative volume, B-type starch granules were the predominant class produced in mature grains in the 37°C/17 °C and 24°C/17 °C (day/night) regimens; however, large A type granules were predominant in the grains produced in the 37°C/28 °C regimen. A previous study was performed on two wheat cultivars at 25 °C, 35 °C, and 40 °C at different grain-filling stages. The results suggested that A-type granules were smaller and exhibited central depression and enhanced pitting at 40 °C (Wang,

Abbreviations: ADP-Glc, adenosine diphosphate glucose; *agp*, gene encoding AGPase; AGPase, ADP-glucose pyrophosphorylase; amy, gene encoding α-amylase; bam, gene encoding β-amylase; DBE, starch debranching enzyme; DPA, days post anthesis; gbss, gene encoding GBSS; HT, high temperature; SBE, starch branching enzyme; sbe, gene encoding SBE; SEM, scanning electron microscopy; ss, gene encoding SSS; SSS, soluble starch synthase.

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2008).

Pits and cavities have been shown to form on the surface of starch granules upon high temperature; however, the causes and the impacts on the starch characteristics have been rarely studied. Using confocal laser scanning microscopy, Kim and Huber (2008) revealed that wheat starch granules contain channels. In their study, A-type granules possessed both relatively large channels that originated primarily from the equatorial groove and finer channels that were located throughout the granules. The B-type starch granules possessed predominately larger, less defined, void-like channels, which were also blocked or clogged by a protein (Kim and Huber, 2008). The authors suggested that these microstructural features function as a way of transferring chemical reagents into the granule matrix, which may impact the starch properties, such as solubility capacity, swelling power, and pasting properties. Commuri and Jones (1999) observed increased pitting of starch granules using SEM (scanning electron micrograph) in maize kernels that were subjected to high temperatures, which was similar to the results of a study on the exposure of rice and wheat kernels to high temperature. The increased pitting was suggested to be due to autolysis, which is the enhanced action of starch degradative enzymes due to high temperature.

At least four classes of key enzymes have been identified as necessary for the synthesis pathway of starch granules, including ADP-glucose pyrophosphorylase (AGPase), soluble starch synthase (SSS), starch branching enzyme (SBE), and starch-debranching enzyme (DBE). Previous studies have reported that high temperature decreases metabolite levels and enzyme activities that are associated with starch biosynthesis in developing wheat grains (Asthir et al., 2012). However, the high-temperature effects on the spatiotemporal profiling of starch degradation genes that encode starch-degrading enzymes in wheat endosperm have not been examined. Different pathways exist for starch breakdown in distinct plant tissues, e.g., the breakdown of transient starch in the leaves of Arabidopsis (Zeeman et al., 2007) and in wheat pericarp (Zhou et al., 2009). Ovesná et al. (2012) detected β-amylase (β-amy1) transcript in developing barley caryopsis. MacGregor and Dushnicky (1989) also detected AMY enzyme activity in the embryo-surrounding region of the endosperm. They hypothesized that the channels on the surface of starch granules are opened via enhanced α- and β-amylase activity due to high temperature during the grain filling stage. In this paper, we survey the accumulation profiles of transcripts in developing grains for 19 genes involved in starch synthesis and degradation at various stages of grain development. By examining the exogenous enzymatic hydrolysis patterns of starch granules in the endosperm under two controlled regimens, we evaluated the high-temperature effects on the starch granule morphologies.

2. Materials and methods

2.1. Plant materials

Winter wheat (*Triticum aestivum* L.) cv. Xindong 20 was obtained from the Research Institute of Triticeae Crops of Shihezi University. Xindong 20 has an excellent yield potential (7500–8250 kg/hm^2). Wheat seeds were planted in 20-cm-diameter pots. The soil contained 63, 15, and 208 mg/kg of available N and P and exchangeable K, respectively, which were supplemented with a complete fertilizer that supplied 60, 28, and 50 mg/kg of N, P, and K, respectively. The fertilization was terminated when stress treatments were initiated. Water was added as needed at 2–3-day intervals to maintain field capacity. Plants were thinned to three per pot and were grown in a climate-controlled greenhouse at a moderate temperature with a daytime maximum temperature of 24 °C and a nighttime minimum temperature of 17 °C until they flowered. Then, the plants were transferred to controlled environment chambers for temperature treatment. A cold LED light source was supplemented to maintain a daytime length of 16 h.

2.2. Temperature treatment

Plant pots were assigned randomly in four chambers, which were set at day/night temperatures of 24/17 °C (control) and 37/28 °C (high temperature, HT), and the plants were allowed to acclimate for 5 d. Temperature treatments were maintained during periods that included 16-h days and 8-h nights from 5 DPA until the plants matured. The wheat plants were watered as needed at 2–3-day intervals to maintain the field capacity. The date of each flowering spike was tagged. The developing seeds were harvested from the middle region of the ear at 7-day intervals starting from anthesis until physical seed maturity under different growth conditions.

2.3. I_2/KI stain

Mature seeds were sectioned and stained with I_2/KI solution that contained 0.5% (w/v) I_2 and 1% (w/v) KI to localize the starch deposition. Images of the seed sections were obtained using a light microscope (Zeiss Discovery V20, Germany).

2.4. Starch isolation

To isolate the starch granules, we modified the method from Peng et al. (1999). Briefly, 2 g of seeds were steeped in H_2O at 4 °C overnight. The grains were degermed and grinded with H_2O in a mortar and pestle and screened. The slurry was transferred into a tube and centrifuged at 1700 g for 10 min. The supernatant was discarded. The sediment was treated with 2 mol/L of NaCl, vortexed, and centrifuged three times. The isolated starches were washed and centrifuged once with 0.2% (w/v) NaOH, once with 2% (w/v) SDS, and once with H_2O, and they were washed and centrifuged three times with acetone before air drying.

2.5. Scanning electron microscopy (SEM)

The isolated starch granules were tiled onto aluminum stubs using double-sided conduction adhesive tape and coated with 20 nm of gold:palladium (60:40). The granule morphology was studied using scanning electron microscopy (JEOL JFC-1600, Japan) at an accelerating voltage of 5–10 kV.

2.6. Particle size distributions of the starch granules

The prepared starch granules were fully suspended in water, and then the particle size distributions were measured using a laser diffraction particle size analyzer (Microtrac S3500, USA) according to the manufacturer's instructions.

2.7. Enzymatic hydrolysis of the starch granules

Amyloglucosidase and α-amylase treatment of the starch granules was conducted as previously reported (Tang et al., 2002). The extent of starch hydrolysis was measured by determining the reducing sugar concentrations using a colorimetric method (Bernfield, 1951). The reducing sugar concentration was calculated based on a calibration curve, which was prepared using glucose.