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Effects of high night temperature during grain filling on formation of physicochemical properties for *japonica* rice

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ABSTRACT

Japonica rice (*Oryza sativa*, L.), Fangxin 4 was cultured under ambient night temperature (ANT) and high night temperature (HNT) in greenhouse. The average HNTs were 4.7 °C and 8.6 °C greater than ANTs in 2009 and 2010, respectively. The effects of HNT on formation of amyloplast, crystalline structure, thermal properties, and molecular structure were investigated during grain filling stage of 10–35 d after anthesis. Results indicated that amylose contents were gradually formed along with the maturing of rice amyloplast. Moreover, amylose content of HNT treatment decreased by 7.13%–15.44% compared with that of ANT at 35 d after anthesis. T_O, T_P, T_C and ΔH_{gel} for HNT were higher than those of ANT. From 10 to 35 d, relative crystallinity presented an increase–decrease–increase pattern, and the relative crystallinity of HNT was 4.06%–14.51% higher than that of ANT at 35 d after anthesis. Amylopectin under ANT had a higher percentage of degree of polymerization (DP) 6–11, while amylopectin under HNT had a higher percentage of DP 12–23. HNT stress had an influence on the formation of amylose content and amylopectin structure, and then changed the crystalline and thermal properties of rice starch.

1. Introduction

Rice (*Oryza sativa*, L.) is one of the most important food crops for the world's human population, especially in Asia. As such, rice industry is tied directly to field yield and quality, which can be influenced by genetics and environmental conditions. The rice genetics can be altered through breeding programs, while environmental conditions are difficult to predict and can only be manipulated to some extent with the choice of planting dates. Since the early 20th century, global temperature has been continuously increasing due to the explosion of population, the development of industries, and excessive deforestation (Crowley, 2000). Particularly the increases in night temperature have been three times that of the corresponding day temperature over much of the Earth's surface (Karl et al., 1991). With these abrupt climate changes, high temperature has become one of the major disastrous factors in rice production (Prasad et al., 2006).

Recent studies in controlled-temperature and field-scale environments have established that high temperatures during critical grain filling stage affect rice kernel development, resulting in reduced yield, increased chalk, and reduced milling quality (Asaoka et al., 1985; Cooper et al., 2008; Zhong et al., 2005). Although there were a lot of studies conducted on high temperatures, few of them were related to high night temperature (HNT). The effects of HNT on rice production, especially on rice quality and fine structures of amylopectin are poorly understood (Mohammed and Tarpley, 2010). Peng et al. (2004) reported a 10% reduction in rice yield for every 1 °C increase in growing-season minimum temperature and an overall increase of 1.13 °C in night time temperatures at the International Rice Research Institute Farm from 1979 to 2003. Morita et al. (2005) and Nagarajan et al. (2010) observed that HNT resulted in decreased yields and pollen germination, increased spikelet sterility and respiration rates. The research by Cheng et al. (2010) showed that night respiration of rice plants was significantly increased by elevated high night temperature, which could adversely affect grain yields. Ambardekar et al. (2011) and Lanning et al. (2011) noted that HNT had deleterious effects on chalk levels and milling quality. Recent studies by Patindol et al. (2014) showed

Abbreviations: AC, amylose content; ANT, ambient night temperature; DSC, differential scanning calorimeter; DP, degree of polymerization; ΔH_{gel}, enthalpy of gelatinization; HNT, high night temperature; HPAEC, high pressure anion exchange chromatograph; RC, relative crystallinity; SEM, scanning electron microscopy; T_C, final temperature; T_O, onset temperature; T_P, peak temperature; XRD, X-ray diffraction.

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that high night time air temperature resulted in changes in amylopectin chain-length distribution. We have investigated the effect of HNT during grain filling on amyloplast development and grain quality in *japonica* rice (Song et al., 2013). The results indicate that HNT stress increased the rate of grain filling and decreased the accumulation of grain filling matter, resulting in appearance and milling quality traits becaming inferior. However, the HNT stress on the formation of crystalline structure, thermal properties and molecular structure need further study, which are important for rice quality.

In this study, *japonica* rice (Fangxin 4) with good uniformity was selected and divided into two groups for ambient and HNT treatments, respectively. The amyloplast development, apparent amylose content (AC), crystalline, thermal properties and molecular structure of the rice starch with ambient or HNT treatments during the period of 10–35 d after anthesis were investigated by means of scanning electron microscopy (SEM), iodine-binding method, X-ray diffraction (XRD), differential scanning calorimeter (DSC), and high pressure anion exchange chromatograph (HPAEC). The objectives of this study were to investigate the formation of crystalline, thermal properties and molecular structure for *japonica* rice during grain filling stage under HNT stress, which might provide theoretical references for rice production.

2. Materials and methods

2.1. Materials

The experiment was conducted in the experimental field of Henan Agricultural University, Zhengzhou, China in the years 2009 and 2010. Rice seeds were sown on May 5, and transplanted into plastic pots (diameter 34 cm, length 35 cm, 3 individual plants per pot) with 10 kg paddy soil on June 8. Nitrogen was applied in the form of urea, phosphorus in the form of calcium superphosphate, and potassium in the form of potassium sulphate. At planting, effective nitrogen, P_2O_5, and K_2O were applied at 2.7 g, 5.6 g and 17.5 g per pot, respectively.

2.2. Temperature treatments

Temperature treatment was conducted from 5 to 35 d after anthesis and HNT was set at 20–25 °C. The ambient night temperature (ANT) was used as the control. A plastic film covered shed equipped with electric heater and an automatic temperature control system were used for HNT treatment. The plastic film was removed in the daytime to ensure the temperature was the same as the control. HNT treatment was carried out from 19:00 to 7:00 of the next day during grain filling stage. The average temperatures for HNT and ANT during grain filling stage were 19.4 °C and 14.7 °C respectively in 2009. The average temperatures for HNT and ANT were 23.8 °C and 15.2 °C respectively in 2010. The uniform panicles at flowering day were tagged and panicles were collected at 10:00–10:30 a.m. at 5, 10, 15, 20, 25, 30 and 35 d after anthesis, respectively.

2.3. Chemical composition

Moisture content of the samples was determined according to the air-oven methods. Apparent amylose content of the rice flour was determined by iodine-binding method (Morrison and Laignelet, 1983).

2.4. Scanning electron microscopy

Rice grains from ANT and HNT treatments were examined using SEM (S–3400NII, Hitachi Inc., Japan). Before testing, the samples were transversally broken in half. Then the half-grains were fastened on a metallic sample stub with conducting silver glue and then sputtered with a layer of gold. Magnifications of 4000× were used.

2.5. Grain morphology

Grain seed morphology during the grain filling stage was taken using a digital camera (Digital Ixus 100 IS, Canon, Japan) after removing the husk.

2.6. Isolation of starch

Rice starch was isolated by the method of alkali extraction. Milled rice flour was steeped in 0.4% (w/w) NaOH solution in a ratio of 1:3. The slurry was agitated for 12 h at 35 °C, and then centrifuged at 2000× *g* for 10 min. The supernatant was discarded, and the sediment was washed three times with distilled water. The dark tailing layer atop the starch was carefully scraped away and discarded. The isolated starch was dried in a convection oven at 45 °C for 24 h, and then ground for physicochemical analysis.

2.7. Thermal properties

The thermal properties of rice starch from ANT and HNT treatments were determined using DSC (STA–449C, NETZSCH Instruments Inc., Germany). Three mg of samples were placed in an aluminum cup, and 7 μL distilled water was added. The cup was hermetically sealed and then heated from 50 to 100 °C at a rate of 10 °C/min. The major parameters of DSC profile were described as onset temperature (T_O), peak temperature (T_P), enthalpy of gelatinization (ΔH_{gel}), and final temperature (T_C).

2.8. X-ray diffraction measurement

The X-ray pattern of rice starch was obtained using an X-ray diffractometer (D8 Focus, Bruker AXS Inc., Germany) operated at 35 mA and 40 kV. The scanning region of the diffraction angle (2θ) was from 5 to 50 at 0.02° step size. The relative crystallinity (RC) was quantitatively estimated by the method described by Komiya and Nara (1986). The samples were equilibrated at 40 °C for 24 h prior to the analysis.

2.9. Branch-chain length distribution of amylopectin by HPAEC

The separated sample (50 mg) was dispersed in 5 mL of 0.05 M sodium acetate buffer (pH 3.5) by heating for 20 min with constant stirring. After cooling to room temperature, the starch solution was treated with 20 μL isoamylase (1000 U, I5284, Sigma–Aldrich Chemical Co.), and incubated at 37 °C for 48 h. After hydrolysis, it was heated in a boiling water bath for 20 min to inactivate the isoamylase. The debranched starch solution was passed through a 0.22 μm disposable syringe filter. A 25 μL sample was injected into the HPAEC (Dionex ICS–3000 chromatography system, Dionex, CA, USA) system with a pulsed amperometric detector (PAD, Dionex, CA, USA). The analytical column was a CarboPac™ PA200 column (3 mm × 250 mm, Dionex, USA). The eluent phase consisted of eluent A (water), B (100 mM NaOH), and C (1 M sodium acetate in 100 mM NaOH) using gradient elution at a flow rate of 0.5 mL/min and a column temperature of 30 °C. PAD signal was converted to carbohydrate content according to the method by Koch et al. (1998).