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Relationship between chalkiness and the structural and thermal properties of rice starch after shading during grain-filling stage

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ABSTRACT

Chalkiness is a major concern in rice production and its acceptance and is increased by shade stress. However, the relationship between rice chalkiness and the structural and thermal properties of starch is unclear. Here, we investigated the effect of shade stress on rice starch properties. The chalky grain rate and chalkiness degree significantly decreased with the amylose content, Mn, and ΔH and increased with surface area- and volume-weighted mean diameters, branching degree, ratio of 1022/995 cm^{-1}, and molecular weight polydispersity. Shade stress significantly increased the volume- and surface area-weighted mean diameters and Mw and decreased the amylose content, A chain proportion of amylopectin, Mn, and regularity of starch. These effects led to an increase in the molecular weight polydispersity and branching degree and a decrease in the crystallinity degree and 1045/1022 cm^{-1} ratio, thereby reducing starch ΔH and uniformity. These factors contributed to increased chalkiness of rice under shade stress.

1. Introduction

Rice is a staple crop that serves as the main carbohydrate source for more than half of the world population, with approximately 500 million tons of milled rice produced annually (Tao, Yu, Prakash, & Gilbert, 2019). Rice quality is controlled by the physicochemical characteristics of grains that provide specific uses for customers (Deng et al., 2018; Wangcharoen et al., 2016). As the major form of stored carbohydrate in the endosperm, starch accounts up to 90 % of the dry weight and plays a key role in determining the grain quality of rice (Bao et al., 2020; Fitzgerald, Mccouch, & Hall, 2009; Zhang et al., 2020).

Wangcharoen et al. (2016) have suggested that rice quality is negatively associated with amylose content and textural characteristics such as hardness. Functional characteristics such as the thermal and viscosity properties of starch are closely associated with rice texture quality (Lee, Lee, & Chung, 2017; Singh, Pal, Mahajan, Singh, & Shevkani, 2011; Wang, Deng, Ren, & Yang, 2013). Additionally, the functional properties of rice are based on starch molecular structural parameters (Bao et al., 2020; Cai et al., 2015). For example, the pasting, gelatinization, and retrogradation properties of starch are closely associated with the branch chain-length distribution of amylopectin (Jane, Chen, Lee, Mcpherson, & Kasemsuwan, 1999; Lee et al., 2017; Zhou et al., 2020). However, the structural and physicochemical starch properties of cereal grains differ under varying environmental conditions (Almeida, Batista, Di-Medeiros, Moraes, & Fernandes, 2019; Lu, Sun, Wang, Yan, & Lu, 2013; Shi, Gu, Lu, & Lu, 2018; Singh et al., 2011).

A 4%–6% general decrease in sunlight over land surfaces has occurred from 1960 to 1990 (Wild, Gilgen, Roesch, Ohmura, & Tsvetkov, 2005). Known as global dimming and low light, shade stress has been an ongoing worldwide phenomenon for the past few decades, with climate change and environment pollution strongly impacting both crop yield and quality (Gommers, Visser, St Onge, Voesenek, & Pierik, 2013; Shao et al., 2020). It is estimated that heavy haze and aerosol pollution may further decrease solar irradiance by 28 %–49 % (Tie et al., 2016).

Abbreviations: A, short chain; B1, middle chain; B2, long chain; B3, very long chain; d(0.1), d(0.5), and d(0.9) represent granule sizes at the 10th percentile, median, and 90th percentile by volume, respectively; Mn, number average molecular weight; Mp, peak molecular weight; Mw, weight average molecular weight; Mz, Z average molecular weight; Tc, conclusion temperature; To, onset temperature; Tp, peak temperature; ΔH, gelatinization enthalpy.

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The Sichuan basin is a representative rice production region with low light intensity, and rice plants in this region experience shade stress caused by rainy and cloudy conditions that occur during the grain-filling stage (July to September). This leads to decreased photosynthate production in the leaf lamina that arises due to reduction of the net photosynthetic rate, electron transport rate, and photochemical efficiency (Dai et al., 2009; Wang, Deng, & Ren, 2015), as well as decrease in the chlorophyll a/b ratio and key enzyme activities in photosynthesis (e.g. phosphoenolpyruvate carboxylase) (Jia, Li, Dong, & Zhang, 2010). Shade stress inhibits the development of rice amyloplasts by reducing the activities of starch synthesis enzymes (e.g. granule bound starch synthase and soluble starch synthase) (Li, Ryu, Tohru, & Haruto, 2005); this results in decreased starch and amylose content and short chains of amylopectin but increases amylopectin long chains and variation in starch viscosity (Deng et al., 2018; Wang et al., 2013).

A major quality concern affecting the production and acceptance of rice is chalkiness, which is caused by deficient amyloplast development and by increased interspaces between loosely packed starch granules and leads to a decrease in the milling and texture qualities of rice (Ishimaru et al., 2009; Yoshioka, Iwata, Tabata, Ninomiya, & Ohsawa, 2007). Previous studies demonstrated that shade stress significantly increased the chalkiness of rice (Deng et al., 2018; Ren, Yang, Xu, Fan, & Ma, 2003). However, the effect of shade stress on the structural and thermal characteristics of starch and their relationship with rice chalkiness remains unclear. We hypothesized that shade stress during the grain-filling stage increases rice chalkiness by reforming starch granule size and the fine structure of rice.

Therefore, a field light control experiment (rice grain grown under full sunlight and 53 % shade stress) was conducted in Hanyuan and Wenjiang, Sichuan, China. The main objectives of this study were to evaluate the effect of shade stress on the (a) fine structural properties of rice starch granules, (b) thermal properties of rice starch, and (c) their respective relationships with chalkiness. Our findings provide information for efficiently utilizing rice starch and decreasing rice chalkiness in regions affected by shade stress.

2. Materials and methods

2.1. Plant materials and experimental design

Field experiments with three replicates were conducted in Liujia village (29°29′N, 102°37′E), Hanyuan county and Huihe village (30°43′N, 103°52′E), Wenjiang district, Sichuan province, China in 2018 and 2019. The climatic data and soil properties from transplantation to maturity are shown in Table 1 (Li et al., 2020). The elite Chinese bread indica rice variety, Huanghuazhan, was used as it is broadly adaptable, has a high yield potential and quality, and is widely cultivated in China. Seedlings were raised in a seedbed for 30 days, then two plants were transplanted per hill and spaced 33.3 × 20.0 cm apart. Control plants were not shaded. A 30-day shade stress treatment was applied to the test groups after the heading stage using a single layer of white cotton yarn screen. The screen was placed 0.5 m above the rice canopy to provide good ventilation and block approximately 53 % of the solar radiation (Deng et al., 2018). The dimensions of each plot were 4.0 × 4.0 m^2 (2018) and 3.0 × 8.0 m^2 (2019) in Hanyuan and 3.0 × 10.0 m^2 in Wenjiang. Approximately 2 kg of rice grain was randomly sampled from each plot, at the maturity stage, for observing the chalkiness over the two years and determining the starch properties in 2019.

2.2. Determination of chalky grain rate, chalkiness degree, and protein and starch contents of head rice

The head rice chalky grain rate and chalkiness degree were measured with three replicates using a JMWT12 rice appearance quality tester (Dongfujiuheng Instrument Technology Co., Ltd., Beijing, China). The chalky grain rate and chalkiness degree were calculated as follows:

$$\text{Chalky grain rate (\%)} = \text{Number of chalky grains}/\text{Number of observed grains} \times 100 \quad (1)$$

$$\text{Chalkiness degree (\%)} = \text{Chalky area}/\text{Total area of observed grains} \times 100 \quad (2)$$

Protein content was measured from the total nitrogen content of head rice with a conversion index of 5.95 following the protocol of Tao et al. (2019). Starch content was measured following the method described by Shi et al. (2018).

2.3. Starch isolation

Three starch samples from each treatment were isolated according to the method previously described by Lu and Lu (2012), with minor modifications. Head rice (20 g, 13.5 % moisture content) was soaked in 100 mL of ultrapure water containing sodium metabisulfite and 10 mg g^{-1} alkaline protease at 42°C for 24 h. The samples were homogenized with a blender and sifted using a 200-mesh sieve. The slurry was collected and allowed to stand for 12 h. This step was repeated 5–8 times until the settled starch layer was purified. The starch samples were collected and naturally dried. Moisture content was then equalized in a thermotank at 37°C for 7 days. Samples were stored at room temperature until further use.

2.4. Determination of amylose and amylopectin contents

Amylose and amylopectin contents of starch samples were measured according to the method described by Shi et al. (2018). Amylopectin content was calculated as the difference between the starch and amylose contents.

2.5. Determination of starch granule morphology

Starch granule morphology was imaged based on the protocol described by Zhou et al. (2020). Samples (100 mg) were mounted on a metal stub, covered with gold, and then observed and photographed with a Zeiss Merlin Compact scanning electron microscope (SEM, Zeiss, Oberkochen, Germany).

2.6. Determination of granule size

One hundred milligram starch samples were ultrasonically blended

Table 1
Climate data and soil properties from transplanting to maturity.

Year	Mean temperature (°C)	Precipitation (mm)	Solar radiation (MJ m^{-2})	Organic matter (g kg^{-1})	Total nitrogen (g kg^{-1})	Total phosphorus (g kg^{-1})	Total potassium (g kg^{-1})
Hanyuan							
2018	21.0	403	1375	25.5	2.20	1.62	28.1
2019	21.3	314	1583	36.5	1.89	1.33	29.2
Wenjiang							
2018	24.4	633	1041	28.5	1.50	0.94	17.5
2019	24.0	332	1093	29.9	1.47	1.04	16.4

in 1 mL of 75 % alcohol. Granule size was estimated using a Mastersizer 3000 laser diffraction particle size analyzer (Malvern Instruments Ltd., Worcestershire, UK) at a range of 0.1–3500 μm. The number-, volume-, and surface-weighted mean diameters, and the 10th percentile (d(0.1)), median (d(0.5)), and 90th percentile (d(0.9)) of volume were calculated following the protocol previously described by Lin et al. (2016).

2.7. Analysis of X-ray diffraction (XRD) pattern and branching degree

Starch samples (100 mg) were scanned with an X'Pert Pro X-ray diffractometer (PANalytical, Almelo, Netherlands) to determine XRD. CuK α was used as the X-ray source with 0.154-nm filtered radiation. A 100-mg sample was scanned at scattering angles of 5°–60° (2θ) using a scanning rate of 4° min^{-1}. Crystallinity degree was calculated using the MDI-Jade 5.0 software (Material Data, Inc., Livermore, CA, USA). A Bruker BioSpin GmbH NMR spectrometer (Bruker, Rheinstetten, Germany) was used to determine the branching degree of starch samples.

2.8. Determination of branch chain-length distribution

The branch chain-length distribution of amylopectin was measured with an ICS-5000 high-performance anion-exchange chromatograph (Thermo Fisher Scientific, Waltham, MA, USA) using a Dionex™ CarboPac™ PA10 anion-exchange column according to the method described by Li et al. (2019).

2.9. Fourier transform infrared (FTIR) spectrum measurements

Starch Fourier transform infrared spectra were scanned using a Nicolet iZ-10 FTIR instrument (Thermo Fisher Scientific). Starch samples (5 mg) were mixed with 250 mg KBr and pressed into film-coated tablets. The KBr was considered as the background of the tablet. Wavenumbers from 400 to 4000 cm^{-1} were measured at 4 cm^{-1} spectral resolution over 32 scans.

2.10. Molecular weight distribution analysis

According to the method described by Zou, Xu, Wen, and Yang (2020)), starch molecular weight was measured using a gel permeation chromatography-refractive index-multiangle laser light scattering detector (GPC-RI-MALLS) with Optilab Trex (Wyatt, Santa Barbara, CA, USA) and DAWN HELEOSII (Wyatt, Santa Barbara, CA, USA). The data were analyzed with the Astra version 6.1 software (Wyatt, Santa Barbara, CA, USA).

2.11. Measurement of thermal properties

Starch thermal properties were determined using a Q2000 differential scanning calorimeter (TA Instruments, New Castle, DE, USA) following the protocol previously described by Gong et al. (2017). A 10-mg starch sample and 30 μL of deionized water were weighed into a DSC pan which was hermetically sealed and balanced at room temperature for 24 h. Then, the sample was heated from 30 °C to 95 °C at a rate of 10 °C min^{-1}. The data were analyzed using the Universal Analysis software (TA Instruments, New Castle, DE, USA).

2.12. Statistical analysis

Two-way analysis of variance was used to analyze the data with SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA). The least significant difference ($P = 0.05$) was used to measure the difference between the means of each treatment. Figures were drawn using GraphPad Prism 5.0 (GraphPad Software, Inc., CA, USA).

3. Results and discussion

3.1. Effect of shade stress on rice chalkiness

Both chalky grain rate and chalkiness of head rice are shown in Fig. 1A and B. Consistent with previous studies (Deng et al., 2018; Ren et al., 2003), shade stress significantly increased both the chalky grain rate and chalkiness in both 2018 and 2019. Chalky grain rate (183 %–201 % and 131 %–132 %) and chalkiness (378 %–820 % and 184 %–193 %) increased in both Hanyuan and Wenjiang, respectively.

3.2. Effect of shade stress on protein, amylose, amylopectin, and starch contents

Shade stress significantly increased the head rice protein content (Fig. 1C). Consistent with the results reported by Deng et al. (2018), more protein was observed in endosperm grown under shade stress. Shade stress applied after the heading stage significantly increased the amylopectin content while decreasing the amylose content of starch and the starch content of head rice (Fig. 1D, E, and F). Similar results have been demonstrated for cereal crops, such as maize (Lu et al., 2013; Shi et al., 2018), wheat (Liu, Zhang, Li, Zhang, & Cai, 2017), and rice (Wang et al., 2013) and may result from lower starch synthesis enzyme activities caused by an insufficient sucrose supply from the leaf lamina (Jia et al., 2010; Li et al., 2005; Wang et al., 2015). Reduction in amylose content, therefore, contributed to the increased chalkiness (Zhao et al., 2019).

3.3. Effect of shade stress on the structural properties of rice starch

3.3.1. Starch granule morphology

The morphological characteristics of starch granules vary according to starch source and environmental stress (Almeida et al., 2019; Gong et al., 2017; Shi et al., 2018; Wani et al., 2012). In the current study, the morphological characteristics of extracted starches were evaluated by SEM and the micrographs have been shown in Fig. 2. The morphology of control starch granules from both sites showed irregular and polyhedral shapes with edges and sharp angles. Most starch granules produced under shade stress possessed a morphology similar to that of the control samples. However, rice granule uniformity was decreased by shade stress; some round or oval granules were observed. Additionally, shade stress also increased the number of pinholes on the smooth surface of the starch. These may be because of the delayed development of the endosperm and amyloplasts (Deng et al., 2018) and may contribute to increased chalkiness under shade stress.

3.3.2. Size distribution of starch granules

Different climate and agronomic processing conditions that cause natural variability in amylose and amylopectin formation may contribute to granule size diversity (Ma, Wang, Wang, Jane, & Du, 2017; Wani et al., 2012; Zou et al., 2020). The effects of shade stress on the granule size distribution characteristics of starch are presented in Fig. 3 and Table 2. Generally, the number, volume, and surface area distributions of starch granules displayed a unimodal trend with peaks at approximately 3.5, 6.0, and 5.0 μm, respectively. Shade stress significantly increased both volume- and surface area-weighted mean diameters, along with d(0.5) and d(0.9), in both sites while decreasing the number-weighted mean diameter of starch in Wenjiang. This indicated that the development of existing starch granules was restricted by shade stress (Shi et al., 2018). In general, control treatments showed higher number, volume, and surface area size distribution percentage of granules from 3.5 to 8.5 μm, whereas shade stress increased the percentage of granules with a size distribution below 3.5 μm and above 8.5 μm. These results suggested that starch granule regularity was decreased by shade stress, which might contribute to increased rice chalkiness.

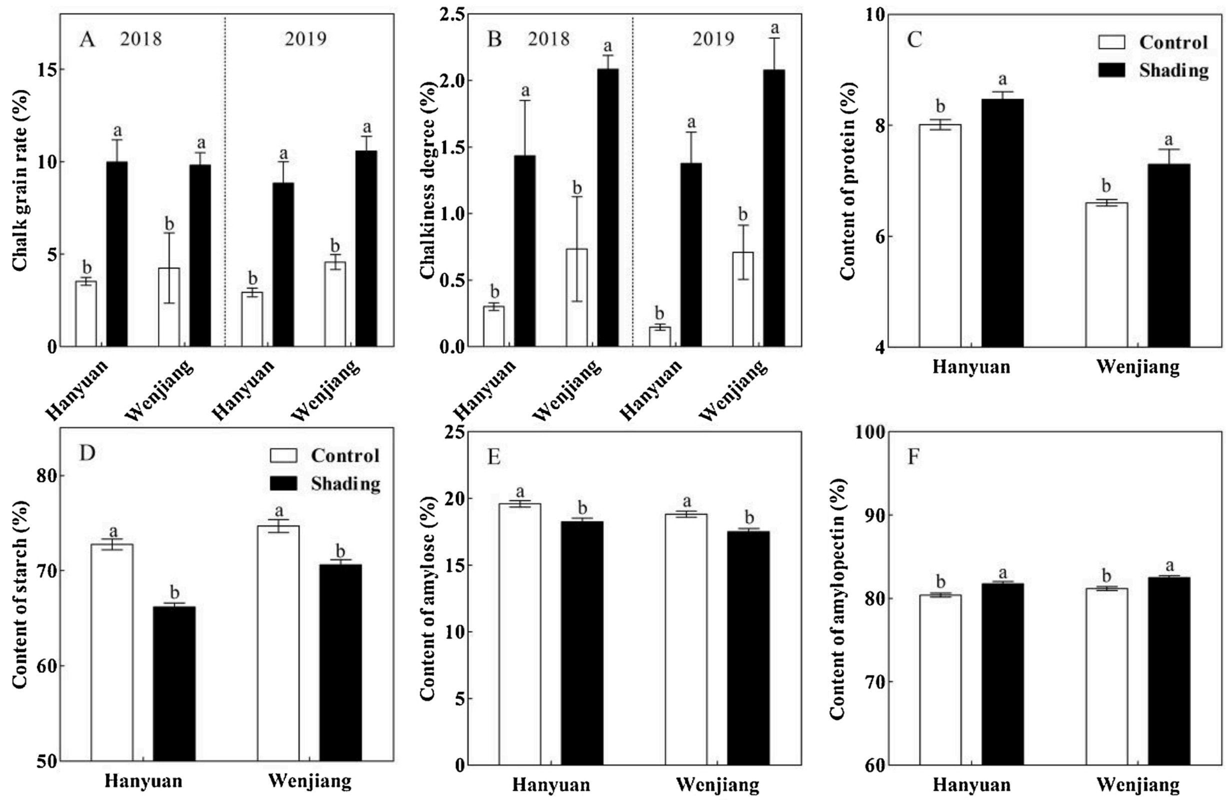

Fig. 1. Effect of shade stress on chalky grain rate (A), chalkiness degree (B), protein (C) and starch (D) contents of rice, and amylose (E) and amylopectin (F) contents of starch. Mean ± SD with similar lowercase letters did not differ significantly at $p = 0.05$ when compared to the control.

3.3.3. *Crystallinity and branching degree*

The highly ordered crystalline structure is conferred by the intra- and inter-molecular hydrogen bonds of starch (Garg & Jana, 2011). Therefore, XRD is recommended as an effective method for determination of the crystal structure of starch (Almeida et al., 2019). All samples in the current study displayed typical A-type diffraction patterns (Fig. 4A and B), suggesting that shade stress did not alter the polymorphic structure type of rice starch. Crystallinity degree calculated from the XRD patterns is shown in Table 2. Shade stress significantly decreased the stability of rice starch crystals, probably due to the reduction in short chains that form the crystalline zone of starch (Zhu & Liu, 2020). However, the reduction in crystallinity degree was higher in samples from Wenjiang (10.2 %) than in those from Hanyuan (7.34 %), which may be attributed to the different climatic conditions. Furthermore, shade stress significantly increased the branching degree in both sites, likely because of the decreased short chain, but increased long chains proportion of amylopectin (Table 3), which can be used to predict the extent of amylopectin branching (Deng et al., 2018; Zhang, Zhu, Shao, Gu, & Liu, 2013). Therefore, shade stress decreased the crystallinity degree but increased the branching degree, thereby contributing to the increased chalkiness.

3.3.4. *Fourier transform infrared spectrum*

The 1045/1022 and 1022/995 cm^{-1} ratios are important in the FTIR spectrum of starch, in which peaks at the 1045 and 1022 cm^{-1} respectively represent the crystalline and amorphous regions (Zhang et al., 2020; Zou et al., 2020). Thus, the ratios of 1045/1022 and 1022/995 cm^{-1}, respectively, have been used to estimate the internal changes of the order degree and formation of double helix of starch molecules (Zhou et al., 2020). The transmittance-FTIR spectra of rice starch are shown in Fig. 4C, and the 1045/1022 and 1022/995 cm^{-1} ratios are shown in Table 2. Similar absorption peaks were observed under control and shade stress treatments in both sites, suggesting that shade stress did not lead to new groups. The results of Almeida et al. (2019) suggested that a high amylose content led to a high 1045/1022 cm^{-1} ratio but a low 1022/995 cm^{-1} ratio. Moreover, shade stress significantly reduced the 1045/1022 cm^{-1} ratio but increased the 1022/995 cm^{-1} ratio, which agreed with the lower crystallinity degree inferred from our XRD results (Table 2). The 1045/1022 cm^{-1} ratio significantly increases with the crystallinity degree and amylopectin short branch chain (Cai et al., 2015).

3.3.5. *Starch granule molecular weight*

Information on the molecular weight of starch is shown in Table 3 and Fig. 5A and B. Shade stress significantly increased the Mw, Mz, and Mp of rice starch in both sites, in agreement with the decreased A chain proportion and increased long chains proportion of amylopectin (Table 3). Polydispersity, a measure of the broadness of the molecular weight distribution, can be calculated as the ratio of Mw/Mn and Mz/Mn (Cai et al., 2015; Hu et al., 2020). A lower polydispersity indicates a narrower molecular weight distribution of starch (Zou et al., 2020). In this study, shade stress significantly increased Mw/Mn and Mz/ Mn in both study sites. Greater molecular weight distribution may restrain the crystallization of amylose (Hu et al., 2020). Therefore, shade stress led to a reduced crystallinity degree of rice starch in both sites, which agreed with the reduced regularity of starch granules shown in Fig. 3.

3.4. *Effect of shade stress on the thermal properties of rice starch*

As an endothermic transition of starch, gelatinization corresponds to the dissociation of the double helical order of amylopectin as it changes to an amorphous conformation from a semi-crystalline structure (Li, He, Dhital, Zhang, & Huang, 2017), in which the transition temperatures represent the double helical order and ΔH reflects its content (Cooke & Gidley, 1992; Gong et al., 2017). The thermal characteristics of rice starch calculated from the DSC analysis (Fig. 5C and D) are shown in Table 4. The To, Tp, Tc, and ΔH were 62.0 ℃–63.5 ℃, 67.1 ℃–68.4 ℃,

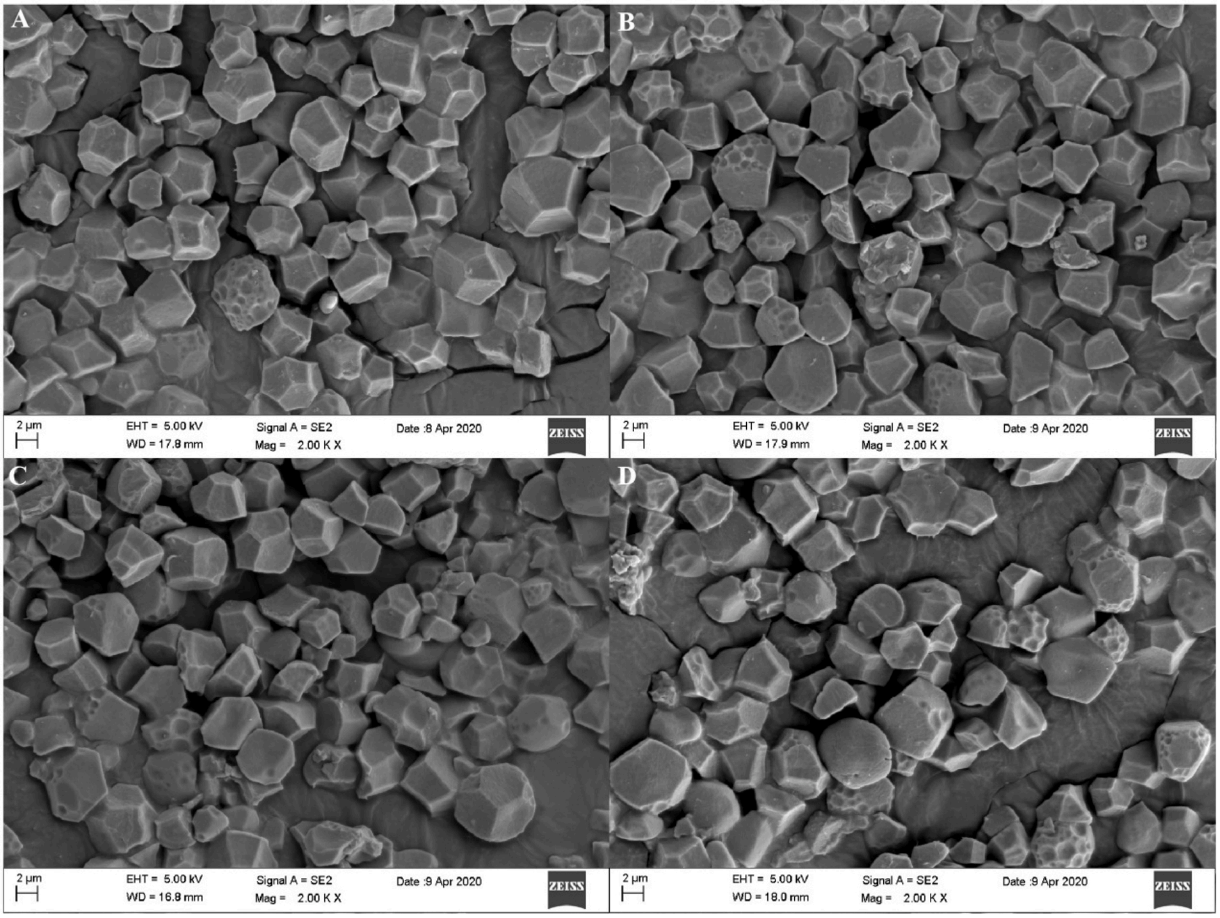

Fig. 2. Scanning electron microscope photographs of starch granules from control (A and C) and shade stress (B and D) grown in Hanyuan (A and B) and Wenjiang (C and D).

72.7 ℃–73.8 ℃, and 10.0–11.7 J g^{-1}, respectively. Shade stress significantly decreased the Tp in Hanyuan and ΔH and To in both study sites compared to the controls. The decrease in transition temperatures suggested that a lower temperature was required to disrupt the double helical order, while the decrease in ΔH indicated a reduction in the crystallinity degree of starch by decreasing the double helical order content (Almeida et al., 2019; Gong et al., 2017). This may be because of the decrease in amylose content and A chain proportion of amylopectin that occurred under shade stress, thereby contributing to decreased thermal stability of starch (Shi et al., 2018).

3.5. Relationship between chalkiness and structural and thermal properties of rice starch

Chalkiness, the white opaque portion of rice endosperm, is regulated by the synthesis of starch, as well as the fine structure and arrangement of the starch granule (Deng et al., 2018; Ishimaru et al., 2009). We found that rice chalkiness was closely related to the structural and thermal properties of starch. Both chalky grain rate and chalkiness degree were significantly and negatively correlated to the amylose content, Mn, and ΔH, and significantly increased with increasing amylopectin content, surface area- and volume-weighted mean diameters, d(0.5), d(0.9), branching degree, ratio of 1022/995 cm^{-1}, Mw/Mn, and Mz/Mn (Table 5). This was in agreement with the results reported by Deng et al. (2018), who suggested that shade stress increased rice chalkiness by impeding caryopsis development and regulating the starch characteristics of rice. Zhao et al. (2019) demonstrated that the increased longer chain proportion of amylopectin but reduced amylose content and short chain proportion of amylopectin contributed to the increase in chalkiness of rice. The chalky grain rate was significantly decreased with starch content, crystallinity degree, ratio of 1045/1022 cm^{-1}, and A chain proportion of amylopectin, whereas the chalkiness degree was increased with B3 chain proportion of amylopectin. These findings indicated that increase in regularity of starch is important for controlling chalkiness; considerably large starch granules adversely affect the reduction of chalkiness.

4. Conclusions

An analysis of the changes in the structural and thermal properties of rice starch is indispensable to understand the mechanism by which shade stress increases rice chalkiness. We compared the multi-scale structural properties, as well as the thermal characteristics of rice starch under shade stress with full sunlight control. The results showed that chalkiness was closely related to the amylose content, surface area- and volume-weighted mean diameters, d(0.5), d(0.9), branching degree, ratio of 1022/995 cm^{-1}, Mn, Mw/Mn, Mz/Mn, and ΔH. Shade stress significantly increased the amylopectin content, surface area- and volume-weighted mean diameters, and Mw, but decreased the amylose content, A chain proportion of amylopectin, and Mn, as well as the regularity of starch granules. These effects contributed to the increased Mw/Mn, Mz/Mn, and branching degree and decreased crystallinity degree and 1045/1022 cm^{-1} ratio of rice starch, thereby reducing ΔH and starch uniformity. Therefore, the chalkiness of head rice was significantly increased by shade stress. Our results provide useful guidance for improving rice grain quality in low-light regions.

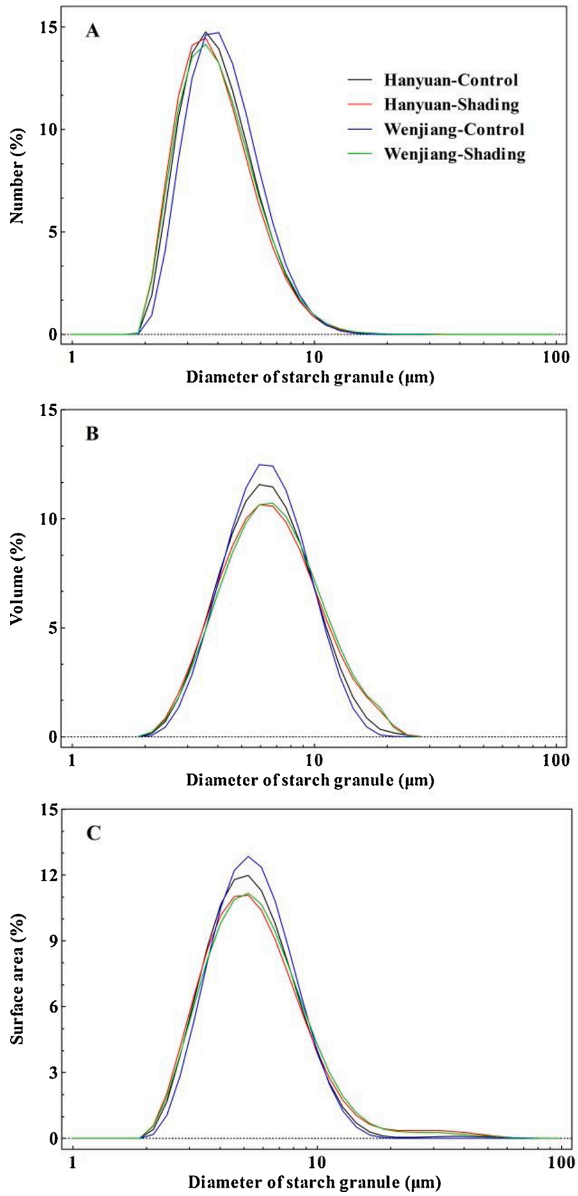

Fig. 3. Effect of shade stress on the number (A), volume (B), and surface area (C) distribution of rice starch granules.

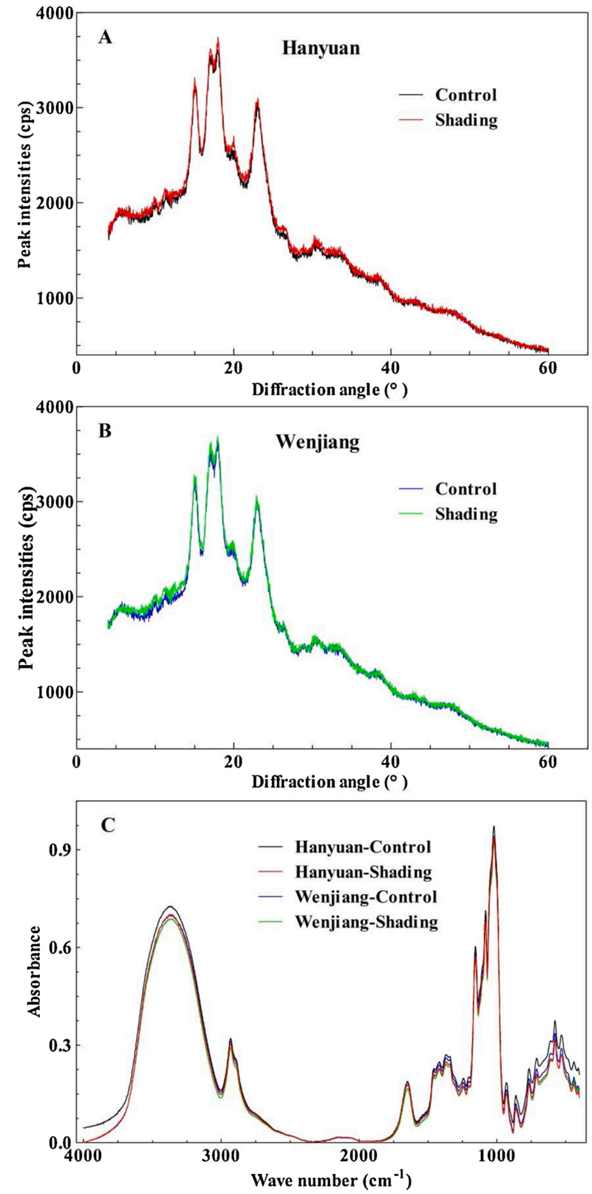

Fig. 4. Effect of shade stress on XRD patterns (A and B) and transmittance-FTIR spectra of rice starch (C).

Table 2
Effect of shade stress on the granule diameter, degree of crystallinity and branching, and ratio of 1045/1022 and 1022/995 cm^{-1} of rice starch.

Treatment	Number weighted mean diameter (μm)	Volume weighted mean diameter (μm)	Surface area weightedmean diameter (μm)	d(0.1)	d(0.5)	d(0.9)	Crystallinity degree (%)	Branching degree (%)	Ratio of 1045/1022 cm^{-1}	Ratio of 1022/995 cm^{-1}
Hanyuan										
Control	4.29 ± 0.01a	6.88 ± 0.29b	5.99 ± 0.38b	3.97 ± 0.02a	6.94 ± 0.29b	14.3 ± 0.48b	35.4 ± 0.49a	3.85 ± 0.09b	0.86 ± 0.00a	1.22 ± 0.00b
Shade	4.22 ± 0.05a	7.26 ± 0.17a	6.54 ± 0.04a	3.99 ± 0.04a	7.46 ± 0.03a	27.6 ± 2.98a	32.8 ± 1.42b	4.17 ± 0.14a	0.85 ± 0.00b	1.25 ± 0.01a
Wenjiang										
Control	4.48 ± 0.03a	6.76 ± 0.09b	5.85 ± 0.04b	4.08 ± 0.03a	6.79 ± 0.02b	11.7 ± 0.46b	38.4 ± 0.60a	4.17 ± 0.05b	0.87 ± 0.01a	1.23 ± 0.00b
Shade	4.30 ± 0.15b	7.38 ± 0.14a	6.51 ± 0.03a	4.03 ± 0.10a	7.43 ± 0.05a	21.8 ± 3.19a	34.5 ± 0.25b	4.26 ± 0.05a	0.84 ± 0.01b	1.24 ± 0.01a

Table 3
Effect of shade stress on the amylopectin chain-length distribution and molecular weight of rice starch.

Treatment	A (DP6–12)	B1 (DP13–24)	B2 (DP25–36)	B3 (DP > 36)	Mn (10^4 kDa)	Mp (10^4 kDa)	Mw (10^4 kDa)	Mz (10^4 kDa)	Mw/Mn	Mz/Mn
Hanyuan										
Control	29.4 ± 0.17a	46.6 ± 0.78a	11.5 ± 0.15b	12.4 ± 0.11a	5.01 ± 0.33a	133.8 ± 4.07b	29.49 ± 1.58b	160.8 ± 5.70b	5.89 ± 0.07b	32.16 ± 0.96b
Shade	28.5 ± 0.17b	46.7 ± 0.01a	11.9 ± 0.18a	12.9 ± 0.09a	4.49 ± 0.25b	253.3 ± 4.08a	35.49 ± 1.22a	198.9 ± 7.59a	7.92 ± 0.17a	44.35 ± 0.79a
Wenjiang										
Control	30.7 ± 2.03a	44.3 ± 0.73b	11.6 ± 1.00a	13.4 ± 0.30a	3.70 ± 0.05a	17.13 ± 0.78b	20.22 ± 0.59b	119.2 ± 3.32b	5.47 ± 0.24b	32.22 ± 1.35b
Shade	27.7 ± 0.31b	47.0 ± 0.27a	11.8 ± 0.23a	13.5 ± 0.35a	2.60 ± 0.11b	35.28 ± 1.61a	25.14 ± 2.64a	137.1 ± 5.72a	9.64 ± 0.60a	52.73 ± 0.08a

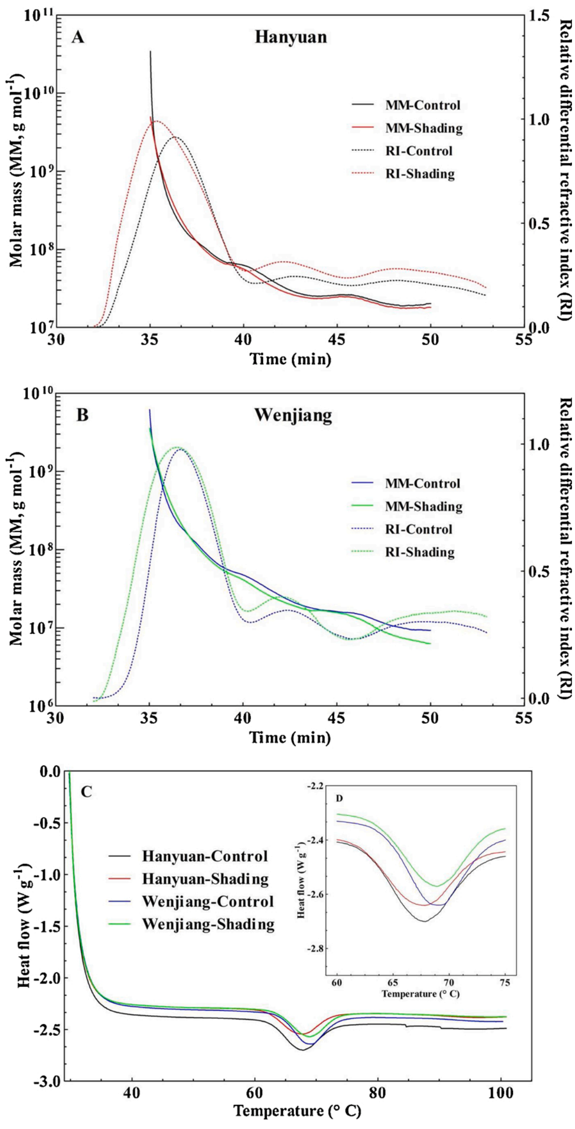

Fig. 5. Effect of shade stress on starch granule molecular weight (A and B) and DSC curves from 30 °C to 95 °C (C) and 60 °C to 75 °C (D).

CRediT authorship contribution statement

Fei Deng: Data curation, Investigation, Writing - original draft. **Qiuping Li:** Data curation, Investigation, Writing - original draft. **Hong Chen:** Methodology. **Yuling Zeng:** Investigation. **Bo Li:** Investigation. **Xiaoyuan Zhong:** Software, Conceptualization. **Li Wang:** Software, Conceptualization. **Wanjun Ren:** Conceptualization, Supervision, Writing - review & editing.

Table 4
Effect of shade stress on the thermal properties of rice starch.

Treatment	To (℃)	Tp (℃)	Tc (℃)	ΔH (J/g)
Hanyuan				
Control	62.5 ± 0.02a	67.4 ± 0.14a	72.9 ± 0.14a	11.7 ± 0.13a
Shade	62.0 ± 0.11b	67.1 ± 0.10b	72.7 ± 0.21a	10.0 ± 0.25b
Wenjiang				
Control	64.0 ± 0.08a	68.4 ± 0.11a	73.8 ± 0.06a	11.2 ± 0.15a
Shade	63.5 ± 0.09b	68.3 ± 0.12a	73.5 ± 0.07a	10.0 ± 0.05b

Table 5
Relationship between chalkiness and structural and thermal properties of rice starch.

Index	Chalky grain rate	Chalkiness degree
Starch content	−0.65*	−0.5
Amylose content	−0.94**	−0.93**
Amylopectin content	0.94**	0.93**
Surface area-weighted mean diameter	0.89**	0.80**
Volume-weighted mean diameter	0.92**	0.85**
d(0.5)	0.88**	0.78**
d(0.9)	0.79**	0.66*
Crystallinity degree (%)	−0.55*	−0.43
Branching degree (%)	0.69**	0.74**
Ratio of 1045/1022 cm^{-1}	−0.61*	−0.49
Ratio of 1022/995 cm^{-1}	0.78**	0.70**
A	−0.60*	−0.49
B3	0.47	0.56*
Mn	−0.64*	−0.73**
Mw/Mn	0.92**	0.89**
Mz/Mn	0.94**	0.91**
ΔH (J/g)	−0.95**	−0.90**

* and ** represent the significance at $p = 0.05$ and $p = 0.01$, respectively.

Declaration of Competing Interest

The authors report no declarations of interest.

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