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Experimental warming under field conditions alters starch multi-structure and flour and starch functionality of late-season *indica-japonica* hybrid rice in southern China

Taotao Yang[1,2], Ruoyu Xiong[2], Haiyuan Wang[2], Xueming Tan[2], Shan Huang[2], Jun Zhang[3], Bin Zhang[1] and Yanhua Zeng[2]*

[1]Rice Research Institute/Guangdong Key Laboratory of New Technology in Rice Breeding/Guangdong Rice Engineering Laboratory, Guangdong Academy of Agricultural Sciences, Guangzhou, China, [2]Ministry of Education Key Laboratory of Crop Physiology, Ecology and Genetic Breeding, Jiangxi Agricultural University, Nanchang, China, [3]Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, China

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KhonKaen University, Thailand

*CORRESPONDENCE
Yanhua Zeng
✉ zyh74049501@163.com

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Introduction: The effects of climate warming on starch multi-structure and flour and starch functionality of late-season *indica-japonica* hybrid rice (IJHR) in double-rice cropping systems are still unclear to date.

Methods: A 2 years field warming experiment was conducted by using free-air temperature increase facilities with an IJHR cultivar (Yongyou1538). The starch multi-structure and flour and starch functionality of IJHR were determined.

Results: Experimental warming (on average 2.1°C over the 2 years) reduced the amylose content and increased the amount of short amylopectin branch-chains (degree of polymerization 6–12), possibly due to decreased granule-bound starch synthase activity and increased starch-branch enzyme activity. Additionally, the protein content, starch granule diameter, relative crystallinity, and swelling power of IJHR were enhanced by experimental warming. The changes in rice components and starch multi-structure under warming conditions resulted in high peak viscosity, breakdown, pasting temperature, gelatinization temperatures and enthalpy and low setback of rice starch and high peak viscosity and pasting temperature and low setback of rice flour.

Conclusion: Our results indicated that climate warming might improve the pasting viscosities and enhance the thermal stability of late-season IJHR.

KEYWORDS

climate warming, *indica-japonica* hybrid rice, starch multi-structure, pasting property, thermal property

1. Introduction

Rice (*Oryza sativa* L.) is a staple food for more than half of the Chinese population (Chen et al., 2020). In southern China, double-rice cropping systems (e.g., rice–rice-fallow cropping systems and rice–rice-green manure cropping systems) have been widely developed to produce more rice on limited arable land (Liu et al., 2020). In 2019, the planting area of double-cropping rice was approximately 9.4 million hectares in China (National Bureau Statistics, 2020). The development of double-rice cropping systems plays an important role in the realization of rice self-sufficiency in China. In recent decades, Chinese breeders have overcome the problem of genetic differentiation between *indica* and *japonica* rice and successfully released a series of *indica-japonica* hybrid rice (IJHR) varieties. These varieties have strong heterosis and show high yield potential. In addition, to ensure food security, the "*indica* rice shift to *japonica* rice" engineering was implemented in double-rice cropping systems. "*Indica* rice shift to *japonica* rice" refers to the growing of *japonica* rice cultivars, including *japonica* inbred rice, *japonica* hybrid rice, and *indica-japonica* hybrid rice cultivars, instead of *indica* rice cultivars. In double-rice cropping systems, the grain yields of *japonica* rice, especially *indica-japonica* hybrid rice (e.g., Yongyou1538, Yongyou538, and Yongyou2640) are significantly higher than those of *indica* hybrid rice, which is due to the full use of temperature and light resources in late rice seasons (Hua et al., 2014).

Compared to preindustrial levels (1850–1900), the global average temperature is predicted to rise by 1.5°C by 2050 and remain within 2.0°C above preindustrial levels (IPCC, 2018). Ambient temperature plays a key role in the development and growth of rice plants. Hence, rice production in China is widely expected to be threatened by climate warming, and many studies have concentrated on investigating the influences of elevated temperatures on grain yield and rice quality in China (Xiong et al., 2017; Chen et al., 2020). Free-air temperature increase (FATI) facilities emit infrared radiation towards vegetation directly and are widely used in paddy warming experiments to assess the impact of climate warming on rice quality (Rehmani et al., 2014; Dou et al., 2017). Previous studies have indicated that experimental warming by using FATI facilities worsened the milling, appearance, and cooking qualities of *japonica* rice in middle rice cropping systems (Dou et al., 2018; Tang et al., 2019). In double-rice cropping systems, experimental warming improved the eating quality of *indica* rice in both early and late seasons but had no effect on the cooking quality of late rice (Yang et al., 2022). The influences of experimental warming on rice quality are ambiguous and variable among rice cropping systems, which may depend on the differences in ambient temperature and cultivar characteristics across China (Jing et al., 2016; Dou et al., 2018; Yang et al., 2018). Therefore, we conducted a field warming experiment by using FATI facilities with an IJHR cultivar grown in the late season. Post-anthesis warming (average 2.2°C over the 2 years) did not change the head rice rate of IJHR; it increased the chalky grain rate, protein content, trough viscosity, peak viscosity, and pasting temperature; and it decreased the amylose content and setback, which meant that the appearance and cooking qualities of late-season IJHR were deteriorated, while the nutritional and eating qualities were improved under future climate warming conditions (Yang et al., 2020b).

Starch consists of two primary components, namely, linear amylose and highly branched amylopectin, which make up approximately 90% of head rice. The contents of amylose and amylopectin are closely related to the physical and chemical properties of rice starch, which ultimately determines the quality of rice (Chen et al., 2021). Other multi-structures of rice starch, such as the fine structure of amylopectin and the morphology and size of starch granules, are also important factors affecting the eating quality of head rice and the functional characteristics of rice flour and starch (Cai et al., 2015; Bhat and Riar, 2016). The morphological and molecular structures of rice starch are determined not only by genotype but also by environmental factors, particularly the air temperature in the grain filling period (Huang et al., 2020). A previous study showed that elevated temperature increased starch granule size, crystallinity, and amylopectin branch-chain length, which induced a high peak viscosity and gelatinization temperature and a low setback of *japonica* rice flour in a middle rice cropping system (Tang et al., 2019). In double-rice cropping systems, we found that experimental warming increased the starch crystallinity and average chain length of amylopectin but resulted in low pasting viscosities of *indica* rice starch and high gelatinization temperatures (Yang et al., 2020a). However, the changes in starch multi-structure and their effects on the rice flour and starch functionality of IJHR under warming conditions are still unclear.

The formation of amylose and amylopectin molecular structures relies on several key carbon synthases, such as soluble starch synthase (SSS), granule-bound starch synthase (GBSS), and starch-branch enzyme (SBE) (Huang et al., 2020). The effects of high ambient temperature on amylose and amylopectin synthesis in rice endosperm are the main cause of the changes in grain quality. Many studies have shown that starch synthetic enzyme activities are susceptible to ambient temperature after heading, which in turn leads to changes in starch structure and rice quality (Cao et al., 2015; Jing et al., 2021a). However, the effect of experimental warming on the key enzymes of starch synthesis varied between the *japonica* and *indica* subspecies (Tang et al., 2019; Chen et al., 2023). Therefore, it is of great significance to assess the effects of experimental warming on amylose and amylopectin synthesis in IJHR endosperms, which would reveal the mechanisms of starch multi-structural changes under climate warming conditions.

As mentioned above, we hypothesized that the responses of flour and starch functionality of late-season IJHR to experimental warming may differ between the *japonica* rice in middle rice cropping systems and the *indica* rice in double-rice cropping systems due to the different growth environments and varietal characteristics, and the changes in starch multi-structure may contribute to the flour and starch functionality of late-season IJHR. Therefore, a 2 years field warming experiment was conducted by using FATI facilities to simulate midcentury climatic conditions. The aim of this study is to clarify the response characteristics of starch fine structure, crystalline structure, granule morphology, and thermal and pasting properties of IJHR to experimental warming and further analyse the roles of starch multi-structure contributing to flour and starch functionality.

2. Materials and methods

2.1. Site description and FATI facility

The FATI field experiment (Supplementary Figure S1) was established in Shanggao County (115° 09′E, 28° 31′N), a double-rice cropping area of Jiangxi Province, China. Supplementary Figure S2 shows the daily mean temperature, sunshine duration, and precipitation for the rice growing seasons in 2018 and 2019. Detailed descriptions of the soil properties and FATI facility were described in our previous study (Yang et al., 2020a). Topsoil (0–15 cm) prior to the study in 2018 contained 20.5 g kg^{-1} organic carbon and 1.9 g kg^{-1} total nitrogen at pH 5.5. The FATI facility included infrared heaters hung 75 cm above the rice canopy in each heating zone. Each infrared heater produces a influenced area of 1.5 m × 1.8 m, and the heating effect is uniform and reliable (Supplementary Figures S1B,C). From transplanting to maturity, the rice plants in the warming treatments were treated, and the canopy temperature of each subplot was monitored. The mean temperatures of the rice canopy in 2018 and 2019 was increased by 1.9°C and 2.2°C over the entire growth period, 1.8°C and 1.7°C before heading, and 1.8°C and 2.4°C after heading, respectively (Supplementary Figure S3).

2.2. Experimental design and crop management

Two temperature treatments were set as follows: ambient temperature treatment (ambient) and experimental warming treatment (warming), performed in triplicate in a completely random design. The rice cultivar Yongyou1538, a popular late-season *indica-japonica* hybrid cultivar, was tested in the double-rice cropping system. Rice seedlings were raised under ambient air in a nursing paddy. Homogeneous rice seedlings were selected, and 3 seedlings per hill were manually transplanted into subfields of 25 cm × 13 cm hill space. Sowing, transplanting, heading, and maturity dates are shown in Supplementary Table S1. Nitrogen, phosphorus, and potassium fertilizers were used for urea, calcium magnesium phosphate, and potassium chloride, respectively. The nitrogen fertilizer application rate was 210.0 kg ha^{-1}; 40% as basal fertilizer, 20% at 7 days after transplanting, and 40% at panicle initiation. The application rate of potassium fertilizers was 156.2 kg ha^{-1}; 70% was applied as basal fertilizer, and the remainder of the potassium fertilizer was applied at panicle initiation. All 46.5 kg ha^{-1} of phosphorus fertilizer was applied as basal fertilizer. From the beginning of transplanting to 20 days after transplanting, the paddy field was submerged in water approximately 3 cm deep, drained (mid-season drainage) until panicle initiation, and finally subjected wet-dry cycles through natural drainage and intermittent irrigation. Seven days before harvest, irrigation was stopped, and the paddy field was dried for the final harvest. Chemicals were sprayed to control weeds, insects, and diseases as recommended by the local department of plant protection.

2.3. Sampling and head rice components

When the rice reached maturation, 30 hills were manually harvested from each subplot. The rice grain samples were naturally air dried, stored at room temperature for 3 months, and then milled with a rice milling machine. The head rice was ground into flour and sieved (60 mesh). The total starch content in the rice flour was determined using a total starch assay kit (K-TSTA, Megazyme, Wicklow, Ireland). The amylose content in the rice flour was determined according to the national standard for rice quality evaluation GB/T 17891-2017, the People's Republic of China (PRC National Standard, 2017). The amylopectin content was calculated by subtracting the amylose content from the total starch content (Hu et al., 2020). A nitrogen analyser (Kjeltec 8400, FOSS, Copenhagen, Denmark) was used to measure the nitrogen content in the rice flour, and the conversion factor from nitrogen value to protein content was 5.95 (Hu et al., 2020).

2.4. Granules size distribution, X-ray diffraction, and amylopectin chain length distribution

Rice starch was extracted by using the sodium metabisulfite solution soaking method described in our previous study (Yang et al., 2020a). The granule size distribution of the rice starch was measured by a laser particle size analyser (Mastersizer 3000, Malvern, England) and the method was described in a previous report (Yang et al., 2020a). A 50 mg starch sample was suspended in 10 mL of ultrapure water and added to the dispersion tank. The measurement range of starch granules is from 0.1 to 2000 μm. The size of the starch granules was recorded by measuring the volume and surface distribution. The X-ray diffraction (XRD) pattern of the rice starch was obtained with a powder X-ray diffractometer (X'Pert Pro, PANalytical, Volketswil, Netherlands) under the conditions of 200 mA, 40 kV, diffraction angle (2θ) from 3 to 40°, and a scanning speed of 0.02°. The relative crystallinity (%) of the rice starch was calculated as the ratio of crystalline area to total area (MDI Jade 6 software) (Yang et al., 2020a). The XRD analyses of the rice starch (i.e., rice sample harvested from each subplot) were performed in technical duplicate. The analysis of the chain length distribution (degree of polymerization, DP) of amylopectin was performed by using high-performance anion-exchange chromatography (HPAECICS-5000, Thermo Fisher Scientific, Sunnyvale, United States) equipped with a pulsed amperometric detector as described previously (Chun et al., 2015).

2.5. Swelling power, thermal and pasting property

The swelling power of the rice starch was determined according to the method reported by Yang et al. (2020a). Differential scanning calorimetry (DSC Q2000, TA Instruments, United States) was used to determine the thermal properties of the rice starch. In brief, a starch sample of 5 mg was mixed with 10 mL of ultrapure water, sealed overnight in an aluminium pan at 4°C, and equilibrated at room temperature for 1 h before analysis. The DSC analyser was first calibrated with an empty pan as a reference and then heated from 35°C to 110°C at a rate of 10°C min^{-1}. The gelatinization temperatures, including onset temperature, peak temperature, conclusion temperature and gelatinization enthalpy of the rice starch were recorded. Samples of rice starch (i.e., rice sample harvested from each subplot) were assayed in technical duplicate. The pasting properties of the rice starch and flour were evaluated by using a rapid viscosity analyser (RVA, Newport Scientific, Australia) according to the AACC method 61-02. The peak viscosity, trough viscosity, final viscosity, breakdown viscosity, and setback viscosity were recorded.

2.6. Activity of Key carbon synthesis enzymes

In 2019, thirty panicles heading on the same day were marked in each subplot. Five representative marked panicles from each subplot were sampled every 7 days after heading. The panicles were frozen in liquid nitrogen and stored at −80°C. Rice panicles were divided evenly into three sections, including the upper, middle, and lower parts, according to length. The middle part of the panicles was hulled and the embryo and pericarp were removed from the hulled rice. The extraction of enzymes from endosperms and the measurement of enzyme activity (SSS, GBSS, and SBE) were performed according to the biochemistry reagent kits (SSS-1-Y, GBSS-2A-Y, SBE-1-Y, Suzhou Comin Biotechnology Co., Ltd., Suzhou, China).

2.7. Statistical analysis

All statistical analyses were performed using IBM SPSS ver. 24.0. The rice components, chain length distribution of amylopectin, size distribution of starch granules, relative crystallinity, swelling power, and thermal and pasting properties were analysed using two-way analyses of variance (ANOVA), and starch synthesis enzyme activities in 2019 were analysed using one-way ANOVA followed by student's *t*-test. In the present study, none of the parameters were significantly affected by the interactions between the treatments and years, and we only presented the means of the main effects (i.e., treatment and year).

3. Results

3.1. Rice components and amylopectin chain length distribution

There was no significant difference in total starch and amylopectin contents between the ambient and warming treatments (Table 1). Under warming conditions, the amylose content significantly decreased by an average of 9.0%, while protein content significantly increased by an average of 8.3% over the 2 years. Year effects were also observed for amylose and protein contents. As shown in Figures 1A,B, experimental warming changed the chain length distributions of amylopectin in 2018 and 2019. Amylopectin branches are further separated into four categories: A chains (DP6–12), B1 chains (DP13–24), B2 chains (DP25–36), and B3 chains (DP37–70). Experimental warming only increased the proportion of A chains; the proportion of B chains (DP13–70) was not changed under warming conditions in the 2 years (Table 1).

3.2. Activity of key carbon synthesis enzymes

In 2019, we identified SSS, GBSS, and SBE activities during the grain-filling stages (7, 14, 21, 28, 35 days after heading) of rice endosperms under ambient and warming conditions. At the early stage of grain filling, experimental warming significantly caused the downregulation of SSS activity (21st day) and GBSS activity (14th day) (Figures 1C,D) but increased the activity of SBE (14th day) (Figure 1E). At later grain-filling stages (28th day and 35th day), SSS, GBSS, and SBE activities showed no significant differences between the ambient and warming treatments.

3.3. Starch granules size distribution and XRD

As shown in Figures 2A–D, experimental warming significantly changed the size distribution of starch granules. The proportion of small granules ($D < 6.5\,\mu m$, volume- and surface-based) was significantly lower under warming conditions than under ambient conditions, and the proportion of large granules ($D > 6.5\,\mu m$, volume- and surface-based) was higher under warming conditions (Table 2). Furthermore, the volume-based mean diameters of starch granules were not changed, while the surface-based mean diameters were significantly increased in the warming treatments. The tested starches of ambient and warming treatments showed similar XRD patterns (Figures 2E,F). All rice starches displayed A-type crystals, including two peaks at 15° and 23° and an unresolved double peak at 17° and 18°. Under warming conditions, the relative crystallinity of rice starch significantly increased by an average of 12.3% over the 2 years (Table 2).

TABLE 1 Effects of experimental warming on rice components and amylopectin chain-length distribution (%).

	Total starch content	Amylose content	Amylopectin content	Protein content	Amylopectin chain-length category			
					A (DP6–12)	B1 (DP13–24)	B2 (DP25–36)	B3 (DP37–70)
Treatment[a]								
Ambient	85.7	15.1	70.7	6.2	27.3	45.2	13.0	14.5
Warming	85.2	13.7**	71.4	6.7**	29.9*	43.9	12.3	13.9
Year[b]								
2018	85.4	14.8	70.6	6.6	29.9	44.7	12.2	13.2
2019	85.5	14.0*	71.5	6.3*	27.3*	44.4	13.1	15.2

There were no significant two-way interactions. Significant treatment or year effects within a main category are indicated by *($0.01 < p \leq 0.05$) or **($p \leq 0.01$).

[a] Values were averaged across years.

[b] Values were averaged across warming treatments.

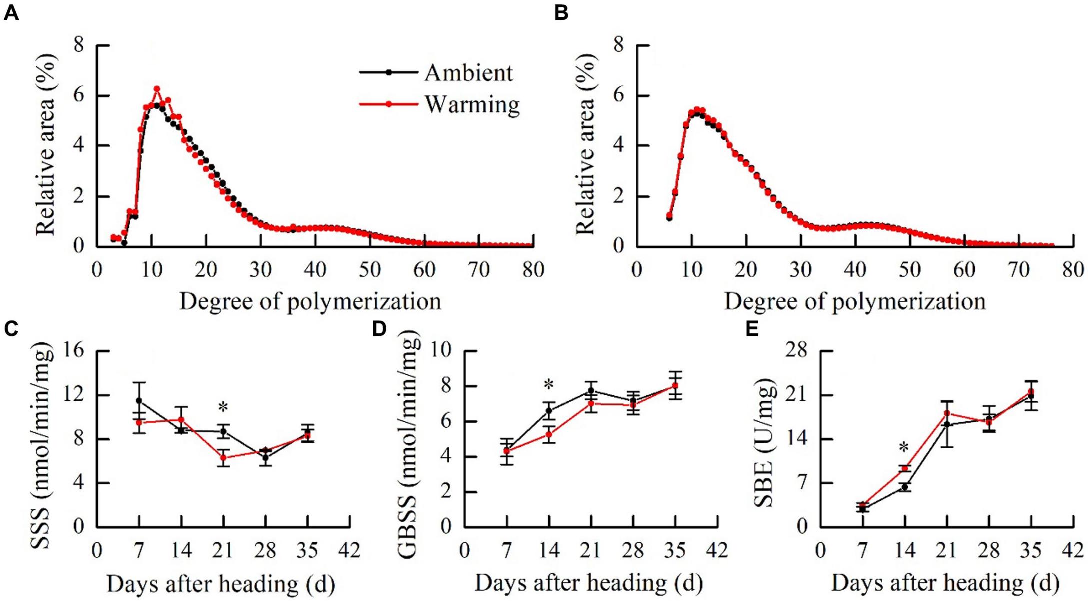

FIGURE 1
Effects of experimental warming on amylopectin chain-length distribution in 2018 **(A)** and 2019 **(B)**, and starch synthesis enzyme activity in 2019 **(C–E)**. *Significant difference at $p<0.05$ between ambient and warming. Error bars represent SD ($n=3$). SSS, soluble starch synthase; GBSS, granule-bound starch synthase; SBE, starch-branch enzyme.

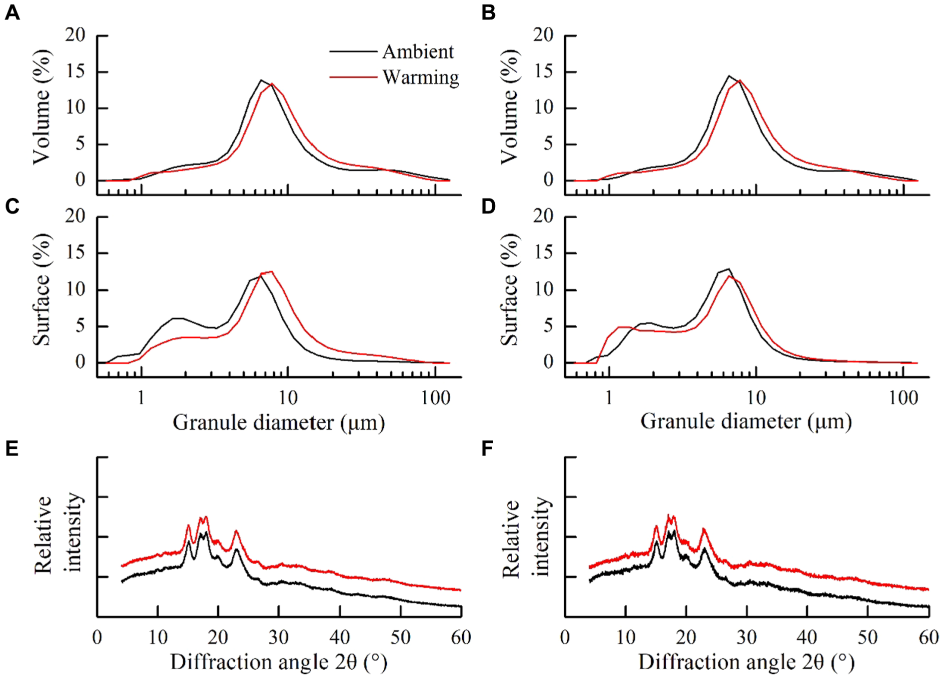

FIGURE 2
Effects of experimental warming on starch granules size distribution and starch X-ray diffraction patterns in 2018 **(A,C,E)** and 2019 **(B,D,F)**.

3.4. Starch thermal properties

In this study, experimental warming significantly affected the gelatinization temperatures and gelatinization enthalpy of rice starch (Table 3). The onset temperature, peak temperature, conclusion temperature, and gelatinization enthalpy of the rice starch were significantly increased by an average of 1.2°C, 1.3°C, 1.1°C, and 12.3%, respectively, over the 2 years under warming conditions. Moreover,

TABLE 2 Effects of experimental warming on starch granules diameter distribution (by volume- and surface-based percentages) and relative crystallinity.

	D ≤6.5		*D* >6.5		Volume-based mean diameter (μm)	Surface-based mean diameter (μm)	Relative crystallinity (%)
	Volume (%)	Surface (%)	Volume (%)	Surface (%)			
Treatment[a]							
Ambient	49.5	75.2	50.5	24.8	11.6	5.7	25.3
Warming	39.8**	60.6**	60.2**	39.4**	11.7	7.6*	28.5**
Year[b]							
2018	44.4	64.5	55.6	35.5	11.9	7.3	27.2
2019	44.9	71.2	55.1	28.8	11.4	6.0	26.6

There were no significant two-way interactions. Significant treatment or year effects within a main category are indicated by *($0.01 < p \leq 0.05$) or **($p \leq 0.01$).

[a]Values were averaged across years.

[b]Values were averaged across warming treatments.

TABLE 3 Effects of experimental warming on starch thermal properties.

	Onset temperature (°C)	Peak temperature (°C)	Conclusion temperature (°C)	Gelatinization enthalpy (Jg^{-1})
Treatment[a]				
Ambient	63.5	69.9	76.1	10.6
Warming	64.7**	71.2*	77.2**	11.9*
Year[b]				
2018	62.6	70.0	76.8	11.1
2019	65.5**	71.1*	76.6	11.4*

There were no significant two-way interactions. Significant treatment or year effects within a main category are indicated by *($0.01 < p \leq 0.05$) or **($p \leq 0.01$).

[a]Values were averaged across years.

[b]Values were averaged across warming treatments.

significant differences were also found for the onset temperature, peak temperature, and gelatinization enthalpy between years.

3.5. Swelling power and pasting properties

Experimental warming increased the swelling power of rice starch by an average of 8.0% over the 2 years (Table 4). The pasting properties of rice starch and flour were both significantly affected by year and temperature treatment, with the exception of the breakdown of rice flour (Table 4). In the warming treatments compared to the ambient treatments, the peak viscosity, breakdown, and pasting temperature of the rice starch significantly increased by 10.2%, 19.5%, and 1.4°C, respectively; and the peak viscosity and pasting temperature of the rice flour significantly increased by 2.4% and 1.6°C, respectively; and the setback of the rice flour and starch significantly decreased by 321.1% and 286.1%, respectively.

4. Discussion

4.1. Effects of experimental warming on head rice components and starch multi-structure of late-season IJHR

Experimental warming did not affect the total starch and amylopectin contents but significantly decreased the amylose content, which is in agreement with the results of previous studies conducted with FATI facilities (Dou et al., 2018; Tang et al., 2019). In this study, experimental warming only significantly increased the proportion of amylopectin A chains. The response of the chain length distribution of amylopectin to experimental warming contradicted previous studies with *japonica* rice cultivars (Chun et al., 2015; Dou et al., 2018) and our previous study with *indica* rice cultivars (Yang et al., 2020a). The components and molecular structures of rice starch are tightly associated with the activity of key carbon metabolism enzymes (e.g., SSS, GBSS, and SBE) (Huang et al., 2020). Amylose in rice endosperm is synthesized by GBSS (Huang et al., 2020). High temperatures and elevated temperatures are likely to result in decreased GBSS expression (Cao et al., 2015). Therefore, the reduction in GBSS activity during the early grain-filling stage (Figure 1B) caused a low amylose content of IJHR under warming conditions. SBE, as a key enzyme, catalyses the formation of α-1′6 glucosidic bonds in amylopectin, and GBSS is likely involved in amylopectin synthesis, particularly in the formation of long branch-chains of amylopectin (Huang et al., 2020). In our study, the increased proportion of amylopectin A chains might be due to the high SBE activity and low GBSS activity under warming (Figures 1B,C). In addition, GBSS and SBE compete for substrates during rice starch biosynthesis (Han et al., 2019). Hence, the lack of competition of GBSS at the early grain-filling stage under warming conditions might further reduce the amylose content in the present study.

In agreement with previous reports (Dou et al., 2018; Tang et al., 2018), the protein content increased under warming conditions in this

TABLE 4 Effects of experimental warming on starch swelling power and pasting properties of starch and rice flour.

	Swelling power (gg^{-1})	Starch				Rice flour			
		Peak viscosity (cP)	Breakdown (cP)	Setback (cP)	Pasting temperature (°C)	Peak viscosity (cP)	Breakdown (cP)	Setback (cP)	Pasting temperature (°C)
Treatment[a]									
Ambient	14.2	2,999	975	38	82.3	3,173	1,239	36	74.5
Warming	15.4**	3304*	1165**	−84**	83.7*	3249*	1,267	−67*	76.1**
Year[b]									
2018	14.9	2,948	1,371	304	85.9	3,087	1,256	62	74.5
2019	14.7	3356**	770**	−350**	80.1**	3336**	1,251	−93**	76.1**

There were no significant two-way interactions. Significant treatment or year effects within a main category are indicated by *($0.01 < p \leq 0.05$) or **($p \leq 0.01$).

[a] Values were averaged across years.

[b] Values were averaged across warming treatments.

study (Table 1). First, the increased protein content under warming conditions was closely associated with the increased activity of glutamine synthetase and glutamate synthetase in rice endosperms (Tang et al., 2018). In addition, the nitrogen contents in rice plants, total nitrogen uptake, and nitrogen translocation and assimilation are changed under warming conditions (Yang et al., 2019; Wang et al., 2020). Our previous study also indicated that experimental warming increased the nitrogen contents in the panicles and milled rice of late rice (Yang et al., 2022). Therefore, experimental warming might be favourable for nitrogen translocation or grain nitrogen assimilation in late-season IJHR in this study.

The changes in starch molecular structures alter its crystalline structure (Cai et al., 2015). In this study, we found that experimental warming increased starch crystallinity degrees in both years (Table 2), which was in agreement with previous studies (Chun et al., 2015; Yang et al., 2020a). Amylose contributes to an amorphous structure, while amylopectin forms a crystalline structure (Zhong et al., 2022). Additionally, a large mean granular diameter may also contribute to high relative crystallinity (Bhat and Riar, 2016). Our findings showed that experimental warming increased the proportion of large starch granules (Table 2), which was consistent with the previous report of Jing et al. (2021b). Therefore, the high relative crystallinity of the warming treatments may be associated with the low amylose content and high mean granular diameter in this study. However, Chun et al. (2015) found that the increase in relative crystallinity of rice starch with increased ripening temperature might be due to an increase in intermediate branch-chains (DP 13–34) of amylopectin in addition to a decrease in amylose content. As stated above, we speculate that the lower amylose content is responsible for the increase in starch crystallinity but minor changes in the chain length distributions of amylopectin under warming conditions.

4.2. Effects of experimental warming on flour and starch functionality of late-season IJHR

Gelatinization involves the loss of starch crystalline structure and is an endothermic process (Li et al., 2020b). As reported by previous studies, the gelatinization temperatures and enthalpy of inbred *japonica* rice starch in a middle rice cropping system and *indica* rice starch in a double-rice cropping system were increased by elevated temperature (Yang et al., 2020a; Jing et al., 2021b). The pasting temperature is the onset temperature when starch solution viscosity increases during the cooking process (Li et al., 2020b). Most studies have suggested that rice flour, whether *indica* or *japonica*, shows a tendency to have higher pasting temperatures of under warming conditions (Dou et al., 2017; Tang et al., 2019; Yang et al., 2020b). Similarly, the rice starch of late-season IJHR had high gelatinization temperatures and enthalpy and pasting temperature in the warming treatments (Table 3). Therefore, a higher cooking temperature and longer cooking time will be required to melt the starch crystalline structure, which makes IJHR grown under warming conditions more difficult to cook.

The gelatinization temperatures and enthalpy and pasting temperature are negatively associated with the apparent amylose content but positively correlated with the amylopectin content (Cai et al., 2015). A higher amylopectin content and lower amylose content stabilize the starch crystalline structure, leading to a higher gelatinization temperature (Witt et al., 2012; Li et al., 2020b). Moreover, compared to small starch granules, large starch granules have a greater barrier for heat transfer and prevent starch gelatinization because of the weaker affinity between starch granules and water molecules (Bhat and Riar, 2016). In this study, the higher thermal stability of starch (i.e., higher gelatinization temperature, gelatinization enthalpy, and pasting temperature) under warming conditions could be attributed to the lower amylose content and the higher crystallinity and larger starch granule size.

Experimental warming increased the swelling power, peak viscosity and breakdown while decreasing the setback of rice starch and flour (Table 4), and these results were similar to those of previous studies conducted under post-anthesis warming conditions (Jing et al., 2016; Dou et al., 2018). The peak viscosity and breakdown reflect starch hydration and swelling, which may be related to starch components, molecular structure, and crystalline structure (Zhong et al., 2022). In general, the amylose content is critical for determining pasting properties because amylose chains readily leach from swelling granules during heating, thereby restricting the swelling of starch granules (Wei et al., 2011). Correspondingly, amylopectin is responsible for water absorption capacity and granular swelling (Zhong et al., 2022). In addition, short amylopectin branch-chains decrease the stability of crystalline packing and make starch granules swell easily during heating (Cai et al., 2015). In this study, an increase in the proportion of short amylopectin branch-chains (A chains) and a decrease in amylose content can induce starch granule expansion, leading to greater swelling and higher occupation of space in the RVA canister, finally resulting in a higher breakdown and peak viscosity of the rice starch in the warming treatments (Kowittaya and Lumdubwong, 2014). The setback value is a measure of recrystallization or short-term retrogradation of gelatinized starch during cooling (Li et al., 2020a). The setback value is positively correlated with the amylose content and long amylopectin external chains (Li et al., 2020a). Amylopectin molecules with long external chains easily associate with amylose molecules and contribute to network formation, resulting in increases in setback during cooling (Kowittaya and Lumdubwong, 2014). In addition, rice starch with a higher proportion of large starch granules tends to have a lower setback (Jing et al., 2021a). Hence, both the low amylose content and the high proportion of large starch granules played an important role in decreasing the setback of both rice starch and flour under warming conditions in this study.

A higher setback value indicates a harder but less sticky texture of cooked rice (Li et al., 2020a). Peak viscosity and breakdown are significantly positively correlated with the taste value of cooked rice (Chen et al., 2021). Furthermore, a lower amylose content is always found in varieties with lower firmness and better taste qualities (Chen et al., 2021). According to our previous study, experimental warming did not affect the amylose content and pasting viscosity of late-season *indica* rice in a double-rice cropping system (Yang et al., 2018). However, in this study, experimental warming induced a low amylose content and setback but a high peak viscosity and breakdown of the late-season IJHR, similar to those of *japonica* rice in a middle rice cropping system (Dou et al., 2017, 2018; Tang et al., 2019). We predicted that the eating quality of the late-season IJHR was improved by experimental warming due to a lower firmness and better sensory acceptability (Jing et al., 2016). In addition, climate warming will have less of an effect on the eating quality of *indica* rice and will improve that of *japonica* rice and IJHR.

In this study, the gelatinization temperatures and most pasting viscosities of rice starch and flour from the 2 years were quite different (Tables 3,4). In the hotter year, 2019 (Supplementary Figure S3), head rice had a lower amylose content, which might cause high gelatinization temperatures and pasting viscosities of rice starch and flour. In addition, we observed minor differences between the starch pasting properties and flour pasting properties in response to experimental warming (Table 4). The pasting properties of rice flour are primarily controlled by the starch components, morphology and molecular structures. However, other components in rice flour, such as starch granule-associated proteins and non-starch polysaccharides, also alter the pasting properties (Chun et al., 2015; Zhan et al., 2020). In this study, experimental warming increased the protein content in the rice flour, which may result in the differences in the pasting properties between starch and flour (Table 4). As an important source of nutrients, a higher protein content is preferable, but rice protein competes to absorb water the during cooking process, restricting the swelling of starch granules, which in turn affects the texture and taste of cooked rice (Amagliani et al., 2017; Chen et al., 2021). The higher protein content of IJHR under warming conditions might reduce its taste value. Therefore, the effects of warming-induced changes in the amylose content, RVA characteristics, and protein content on the eating quality of late-season IJHR should be comprehensively considered in future studies.

5. Conclusion

Under experimental warming conditions, the amylose content of late-season IJHR decreased, while the short amylopectin branch-chains (DP6–12), starch granule diameter, relative crystallinity, and swelling power increased. These warming-induced changes in starch molecules and morphologic structures synergistically altered the pasting and thermal properties, such as higher gelatinization temperatures and enthalpy of rice starch but lower setback and higher pasting temperature, peak viscosity and breakdown of both rice flour and starch. Our research clarified the changes in starch multi-structures of IJHR, which contribute considerably to the pasting and gelatinization properties of rice flour and starch under warming conditions. This study provides important implications for the end-use of the IJHR and the development of adaptive strategies under future climate warming conditions.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

TY conceived the idea and wrote the manuscript. RX and HW contributed to the writing of the manuscript. XT, SH, JZ, and BZ contributed to the conception and design of the study. YZ edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2023.1203172/full#supplementary-material

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