scientific reports

Check for updates

OPEN

Physiological and biochemical mechanisms of taste decrease in high tasting japonica rice under warming at growth stages

Ke Ma & Yuanyuan Zhou

In order to better understand the response of the taste of high-tasting japonica rice in cool regions to climate warming, we have set up warming treatments at six growth stages in the field in two years: the before heading stage (T1), the whole growth stage (T2), the grain-filling stage (T3), the early grain-filling stage (T4), the middle grain-filling stage (T5) and the late grain-filling stage (T6). Except for T6, which showed no significant changes, all other treatments had varying degrees of negative effects on the taste value of milled rice, with T2 and T3 being the most serious, followed by T1, and finally T4 and T5. The taste value of milled rice was positively correlated with amylopectin content, total starch content, peak viscosity (PV), breakdown (BD), and negatively correlated with protein content, amylose content, hot pasting viscosity (HPV) and setback (SB). There was a close relationship between carbon and nitrogen metabolism of rice grains. The protein content was negatively correlated with the total starch content and amylopectin content, and positively correlated with the activity of glutamic oxalo-acetic transaminase (GOT) and glutamate pyruvate transaminase (GPT). The total starch content had no significant relationship with ADP-glucose pyrophosphorylase (AGPP) activity. The amylopectin content was positively correlated with soluble starch synthase (SSS) activity, and not significantly correlated with starch branching enzyme (SBE) and starch debranching enzyme (DBE) activity. The amylose starch content was positively correlated with granule-bound starch synthase (GBSS) activity and negatively correlated with SSS activity. Under warming conditions, the increase of nitrogen metabolism level promoted the increase of protein content and the decrease of amylopectin content in milled rice, leading to a decrease in taste quality.

The Intergovernmental Panel on Climate Change Sixth Assessment Report (AR6): Climate Change 2023 showed that due to the increase of the greenhouse gas emissions caused by human activities, the average temperature of the Earth was rising[1]. According to the estimation results of multi-model, the annual average surface temperature in China would increase by 2.7 °C and 5.4 °C respectively by the end of the twenty-first century under SSP2-4.5 and SSP5-8.5 scenarios compared with the observed data during 1995–2014, which both higher than the global average level[2]. Rice, one of the most important food crops on Earth, its response to climate change directly affects the dietary status of nearly half of the world's population[3]. Rice taste quality is one of the most important indicators for evaluating its quality[4]. At present, the methods for evaluating the rice cooking quality are mainly divided into the following 4 categories[5], the first is to determine the physicochemical components of rice with the aid of instruments to indirectly determine the taste of rice; the second is to evaluate the quality of rice through the characteristics of rice starch Rapid Visco Analyzer (RVA) profiles; the third is to evaluate with a special taste analysis instrument; the fourth is to use the sensory evaluation that based on the taste of the taster, which mainly evaluate from the appearance, color, aroma, taste, viscosity, hardness and comprehensive indicators of rice. Because starch and protein are the main components of milled rice, the physiological mechanism of quality traits such as the taste value of milled rice is generally based on carbon and nitrogen metabolism at the grain-filling stage of rice[6,7]. In previous studies, the research on the relationship between warming and the taste of milled rice mostly focused on warming at the whole grain-filling stage[8,9], and the research on warming at different growth stages on eating taste is still rare. In addition, the superior grains (SG) and inferior grains (IG) of rice had great differences in physiological and biochemical indicators due to the difference in growth processes[10,11], so it is necessary to systematically analyze the changes in different parts of the whole panicle affected by high temperature.

Jilin Agricultural Science and Technology University, Jilin 132101, China. ✉email: zhouyy9911@163.com

Jiyang 100, a high-tasting japonica rice broadly cultivated in Northeast China, was used as the experimental material. We have adopted warming treatment at six growth stages of rice in the field. By determining the taste value, RVA profiles and biochemical compositions of the tested variety of SG, IG and overall grains under each treatment, to clarify the law of the effect of warming at different stages on the taste quality. Then, the changes of carbon and nitrogen metabolism enzyme activities at the grain-filling stage were determined. Finally, we discussed the mechanism of the effect of warming on the taste of high-tasting japonica rice. This study provided a theoretical basis for the changes in taste quality caused by climate warming that may be faced in the production of high-tasting japonica rice.

Materials and methods

Site description and experimental design

The experiment was set up in the experimental field of Jiyang Academy of Agricultural Sciences, Wanlong Town (125°45′E, 42°36′N), Meihekou City, Jilin Province from 2019 to 2020. In this experiment, we set warming at six growth stages after transplanting with rice grown under natural conditions was taken as a control (CK). These six growth stages were the before heading stage (T1), the whole growth stage (T2), the grain-filling stage (T3), the early grain-filling stage (T4), the middle grain-filling stage (T5), the late grain-filling stage (T6). Among them, the full heading stage was the beginning of grain-filling, and the grain-filling stage was calculated as 45 days. The early grain-filling stage was 1–15 days, the middle grain-filling stage was 16–30 days, and the late grain-filling stage was 31–45 days. The experiment used a combination of passive and active methods to increase temperature in the field[12]. Passive warming relied on a semi open transparent greenhouse with a length of 8 m, width of 6 m, and height of 2.5 m in the north–south direction. Active warming method relied on a heater, and the setting referred to the method of Shah et al.[13]. An 8 m long PVC ventilation pipe was connected to the end of the heater, which passed through the rice field 20 cm above the water surface. Small holes were drilled in the pipe at different distances to allow the warm air could be evenly discharged from the pipe to increase the temperature of the plot. We used passive warming from 6:00 to 19:00 on sunny days, and active warming from 19:00 at night to 6:00 the next day, and on cloudy and rainy days. Temperature changes were recorded using a temperature data logger (ZDR-41, Hangzhou Zeda Instruments Co., Ltd., and the measuring range was − 40–100 °C). Four probes were placed in each main area, and the height of the probe was continuously adjusted to keep pace with the growth of the plant and maintain consistency with the plant canopy. The experiment adopted a randomized block design, with each treatment repeated three times. The planting areas for each repetition in 2019 and 2020 were 9 m^2 and 15 m^2, respectively. The warming situation of each treatment was showed in Fig. 1 and Table 1.

Crop variety and field management

Jiyang 100 is a nationally approved rice variety, which was also one of the agricultural dominant varieties in Jilin Province in 2022. It has high-tasting taste characteristics and broadly cultivated in northeastern China. The field management methods were the same in 2019–2020.The pre-germinated seeds were sown on April 13, and seedlings were transplanted (spacing 20 cm × 30 cm) on May 25. The drainage and irrigation were separate for each treatment. The basic soil characteristics of the experimental field were as follows: organic matter 5.7%, alkali-hydrolysed nitrogen 192 mg·kg^{-1}, available phosphorus 34 mg·kg^{-1}, available potassium 90 mg·kg^{-1}, Na^+ 100 mg·kg^{-1}, Mg^{2+} 525 mg·kg^{-1}, electrical conductivity 0.18 ms/cm, and pH 6.5. The 75 kg hm^{-2} of N fertilizer, the 75 kg hm^{-2} of P fertilizer and the 67.5 kg hm^{-2} of K fertilizer were used as the base fertilizers on May 20 (before harrowing). The 45 kg hm^{-2} of N fertilizer was used as a top dressing at the early tillering stage on June 15. The 30 kg hm^{-2} of N fertilizer and the 7.5 kg hm^{-2} of K fertilizer were used as a top dressing at the panicle initiation stage on July 11. After rice maturation, all three replicated plots of each treatment were harvested separately. The grain numbers of the half of distance from the neck to the tip of panicles were defined as the inferior grains (IG), and the remaining half were the superior grains (SG). After harvesting, half of the rice panicles were separated into SG and IG for threshing, while the other half were mixed and threshed to collect the total grains. The total grains, SG, and IG were stored in the sample drying chamber (temperature: approximately 20 °C; relative humidity: approximately 20%) for two months. After stabilization of their physicochemical properties, they were determined for taste quality value, RVA profiles, and chemical composition indicators.

Determination of taste quality and appearance quality of milled Rice

After milling, the total grains, SG, and IG from each treatment replicate were determined for quality parameters in triplicate. The taste characteristics of milled rice were determined using STA1B-RHS1A device (Satake Corp., Hiroshima, Japan). 30 g of milled rice was accurately weighed and placed in a stainless steel cup of the STA1B device. The rice was rinsed repeatedly with water until the rinse was clear. After the rice was finally drained, 42 g of water was added, the cup was covered with filter paper and the rice was allowed to soak for 30 min. Then, the samples were steamed for 30 min and kept warm for 10 min. After braising the rice, gently stirred the rice in the cup, covered the cup with filter paper and put it in the cooling device until it cooled down. 8 g of cooked rice was removed from the cup, placed it in a stainless steel sample ring with a diameter of 30 mm and a height of 9 mm, and a rice cake sample was formed using a cooked rice press (repeat 3 times). Placed the sample in a measuring tank and inserted into the eating meter within the device. Each side of the rice sample was evaluated once. The food quality, hardness, viscosity and other indicators were determined. The rice appearance quality tester JMWT 12 (Satake Corp., Hiroshima, Japan) was used to detect the chalkiness rate and chalkiness degree in rice quality indicators.

Determination RVA profiles of milled rice

After milling, the total grains, SG, and IG from each treatment replicate were determined for RVA profiles in triplicate. The viscosity characteristics of milled rice was determined by a fast viscosity analyzer (TecMaster),

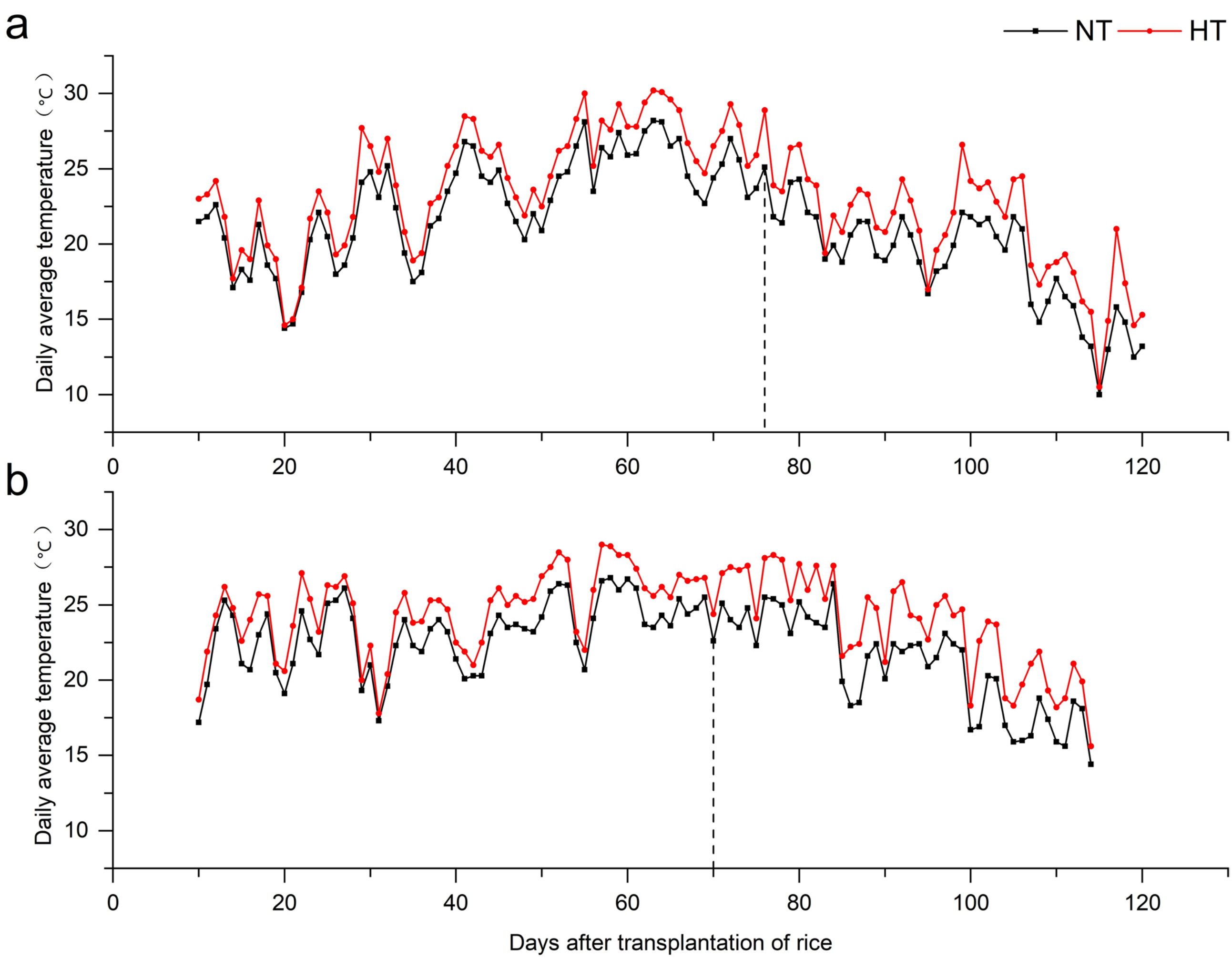

Fig. 1. Comparison between daily average temperature under warming treatment and ambient temperature. A and b represent the temperature change maps for 2019 and 2020 respectively. NT refers to the normal temperature state, HT refers to the warming state; The black dashed line in the temperature diagram represents the heading stage.

Weighed 3 g samples of milled rice flour into RVA aluminum bucket and then added 25 ml distilled water. First, the mixture was kept at 50 °C for 1 min, and then heated to 95 °C in 3.8 min, and held for 2.5 min. Then cooled to 50 °C in 3.8 min and held for 2.5 min. Stirred at a rotation speed of 960 rpm for 10 s, and then maintained a constant speed of 160 rpm until the end of the experiment. Starch gelatinization characteristics were then measured, including PV, HPV, cold pasting viscosity (CPV), breakdown (BD = PV – HPV), setback (SB = CPV – PV), consistency viscosity (CSV = CPV – HPV) and pasting temperature (PaT).

Determination of biochemical and physiological indexes

After milling, the total grains, SG, and IG from each treatment replicate were determined for protein content, amylose content, and amylopectin content in triplicate. The dual-wavelength method[14] was used to determine the amylose, amylopectin contents of milled rice. The specific procedures for determining amylose and amylopectin contents by dual-wavelength were as follows: Weighed 10 g of rice sample ground it into powder with a hammer-type cyclone mill and passed it through an 80-mesh sieve. Extracted the fat with 85% methanol at a rate of 5–6 drops per second for 6 h. Placed the defatted rice powder on a plate and spreaded it out in a fume hood for 2 days to allow the residual methanol to evaporate and reach moisture balance. Weighed 100 mg of defatted rice powder in a 50 mL beaker. Added 10 mL of 1.0 mol/L KOH solution, and heated in a water bath at 85 ± 1 °C for 20 min. Then, made the volume up to 50 mL with distilled water and let it stand. Drawed 5 mL of the sample solution, added 25 mL of distilled water, adjusted the pH to 3.0 with 2 mol/L and 0.1 mol/L HCL solutions, added 0.5 mL of iodine reagent, and made up to 50 mL with distilled water. Determined the absorption values $A_{\lambda 2}$, $A_{\lambda 1}$, $A_{\lambda 4}$, and $A_{\lambda 3}$ at the determination wavelengths and reference wavelengths of the two starches (λ1, reference wavelength of amylose; λ2, determination wavelength of amylose; λ3, reference wavelength of amylopectin; λ4, determination wavelength of amylopectin). Obtained ΔA (amylose) = $A_{\lambda 2} - A_{\lambda 1}$ and ΔA (amylopectin) = $A_{\lambda 4} - A_{\lambda 3}$. Based on the standard curves of the two starches, calculated the contents of amylose and amylopectin in rice. The Kjeldahl method[15] were used to determine the protein content of milled rice. From 2019 to 2020, after the heading of rice,

Year	Treatment	Date range	AT (°C)	DAT (°C)	DPAT (°C)	DAAT (°C)
2019	T1	6/5–7/31	24.1 ± 4.0	1.6 ± 0.5	0.9 ± 0.4	0.7 ± 0.1
	T2	6/5–9/23	23.1 ± 4.1	1.9 ± 0.7	1.1 ± 0.6	0.8 ± 0.2
	T3	8/10–9/23	21.0 ± 3.8	2.2 ± 0.9	1.3 ± 0.7	0.9 ± 0.2
	T4	8/10–8/24	23.4 ± 2.5	2.1 ± 0.7	1.4 ± 0.6	0.7 ± 0.1
	T5	8/25–9/8	22.5 ± 2.3	2.3 ± 0.8	1.3 ± 0.6	1.0 ± 0.2
	T6	9/9–9/23	17.4 ± 3.2	2.4 ± 1.0	1.4 ± 0.8	1.0 ± 0.2
2020	T1	6/5–7/24	24.4 ± 2.6	1.6 ± 0.7	0.9 ± 0.6	0.7 ± 0.2
	T2	6/5–9/17	24.3 ± 2.9	2.1 ± 1.0	1.3 ± 0.8	0.8 ± 0.3
	T3	8/4–9/17	23.6 ± 3.3	2.7 ± 1.0	1.7 ± 0.8	1.0 ± 0.3
	T4	8/4–8/18	26.8 ± 1.4	2.5 ± 0.8	1.6 ± 0.5	0.9 ± 0.3
	T5	8/19–9/2	24.0 ± 1.6	2.7 ± 1.1	1.7 ± 0.8	1.0 ± 0.3
	T6	9/3–9/17	20.1 ± 2.3	2.9 ± 1.3	1.8 ± 0.9	1.1 ± 0.3

Table 1. Warming date and air temperature changes of each treatment. AT, average daily temperature; DAT, difference in daily average temperature between treatment and control; DPAT, daily increase temperature by passive warming; DAAT, daily increase temperature by active warming; T1, warming at the before heading stage; T2, warming at the whole growth stage; T3, warming at the grain-filling stage; T4, warming at the early grain-filling stage; T5, warming at the middle grain-filling stage; T6, warming at the late grain-filling stage. The data in the table represent the average values ± SD.

we collected SG and IG every 5 days on the panicles with the same growth process. After husking, wrapped every 25 grains with tin foil, marked the harvest information, and quickly freezed them in liquid nitrogen and stored them at − 80 °C. After annual harvest, took 25 SG or IG samples as the determination unit to determine the activities of related carbon and nitrogen metabolism enzymes, and repeated three times. Through the enzyme activities of SG and IG, calculated the enzyme activities of the whole panicles. Among them, ADP-glucose pyrophosphorylase (AGPP, EC 2.7.7.21), soluble starch synthase (SSS, EC 2.4.1.21), starch branching enzyme (SBE, EC 2.4.1.18), starch debranching enzyme (DBE, EC 3.2.1.68), granule-bound starch synthase (GBSS, EC 2.4.1.21), glutamic oxalo-acetic transminase (GOT, EC 2.6.1.1) and glutamate pyruvate transminase (GPT, EC 2.6.1.2) were determined using analytical kits, which provided by Qingdao Sci-tech Innovation Quality Testing Co., Ltd., China.

Data analysis
Analysis of variance was performed using the IBM® SPSS® 20.0. Means were tested using the least significant difference at $P < 0.05$ (LSD 0.05). Path analysis referred to the previous method[16]. R-Studio was used to calculate the Pearson correlation coefficient and perform graphing. Image software of Origin 2018 was used to perform graphing. The graphical data in the paper were comprehensive results from two years.

Results
Taste value and RVA of milled rice
The results showed that T6 had no significant effect on the taste value of milled rice, while T1, T2, T3, T4 and T5 had significant negative effects ($P < 0.05$) (Fig. 2). Each warming treatment had a different degree of negative effect on the taste value of milled rice, with T2 and T3 being the most serious, followed by T1, and finally T4 and T5 (Fig. 2). The average range of the taste value of milled rice was between 73.5 and 84.3. The taste value of SG milled rice was higher than that of IG except that of T1 (Fig. 2). Compared with CK, T1 and T5 significantly widened the difference of taste value between SG and IG milled rice, T2 significantly narrowed this gap, and T3, T4, and T6 slightly increased the difference at the average level (Fig. 2). In terms of RVA profiles, each warming treatment mainly had negative effect on PV, BD, and CSV of milled rice, positive effect on HPV, SB, and CPV, and different effects on PaT (Fig. 2).

Chemical compositions of milled rice
T1, T2, T3, T4, T5 and T6 significantly increased the protein content of milled rice. The changes in protein content of T1, T2, T3 and T4 milled rice was affected by the combined effects of SG and IG, while T5 and T6 were mainly affected by IG (Fig. 3). Among the changes in the total starch content of milled rice in each treatment, T1 and T5 were significantly increased, T2 and T3 were significantly decreased, and there was no significant difference between T4, T6 and CK. The changes in total starch content of milled rice of T1, T2 and T3 was affected by the combined effects of SG and IG, while T5 was mainly affected by SG (Fig. 3). T1, T2, T4 and T5 significantly increased the amylose content of milled rice, T3 and T6 were significantly decreased. The changes in amylose content of T1, T2, T4 and T5 milled rice was affected by the combined effects of SG and IG, while T5 and T6 were mainly affected by IG (Fig. 3). According to the results of the amylopectin content of milled rice in each treatment, T6 was significantly increased, while T1, T2, T3, T4 and T5 were significantly decreased. Among them, the changes in amylopectin content of T1, T2 and T4 milled rice was affected by the combined effects of SG and IG, T3 was mainly affected by SG, while T5 and T6 were mainly affected by IG (Fig. 3).

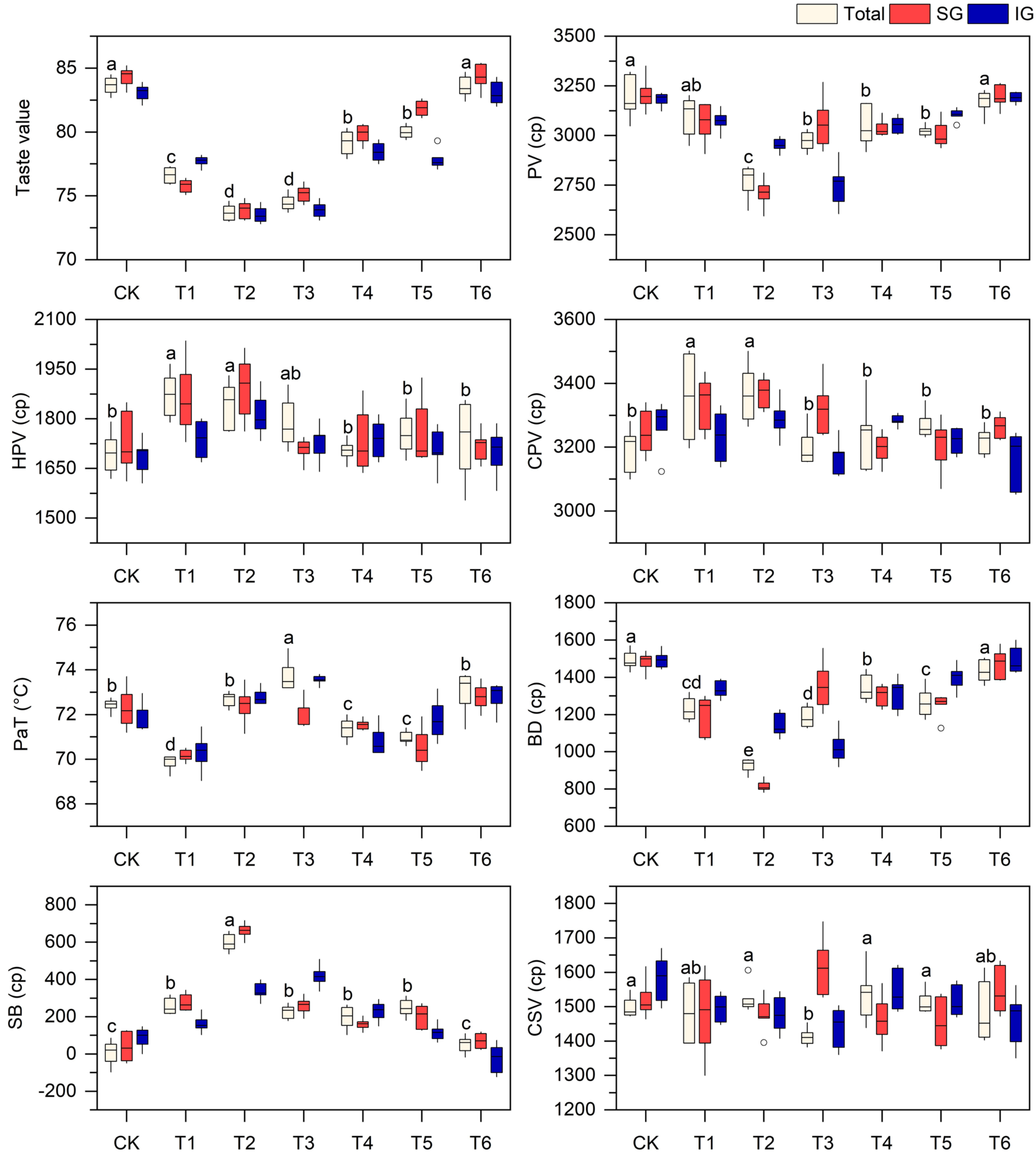

Fig. 2. The taste value and Rapid Visco Analyzer (RVA) profiles of milled rice under warming at different growth stages of high-tasting japonica rice. SG, superior grains; IG, inferior grains. Different letters indicate significant differences at the $P < 0.05$ level. The box boundaries indicate the 25th and 75th percentiles; the black line in the box marks the median; whiskers below and above the box indicate the minimum and maximum values, respectively; ∘ indicates outlier.

Appearance quality of milled rice

The chalky grain rate of total grains in T2, T3, T4, and T5 increased significantly by 129.3%, 74.5%, 34.8%, and 47.8%, respectively, while the chalkiness degree increased significantly by 201.7%, 132.8%, 27.6%, and 96.6%, respectively (Fig. 4). In contrast, the chalky grain rate of total grains in T1 and T6 decreased significantly by 21.7% and 23.4%, respectively, and the chalkiness degree decreased significantly by 36.2% and 39.7%, respectively

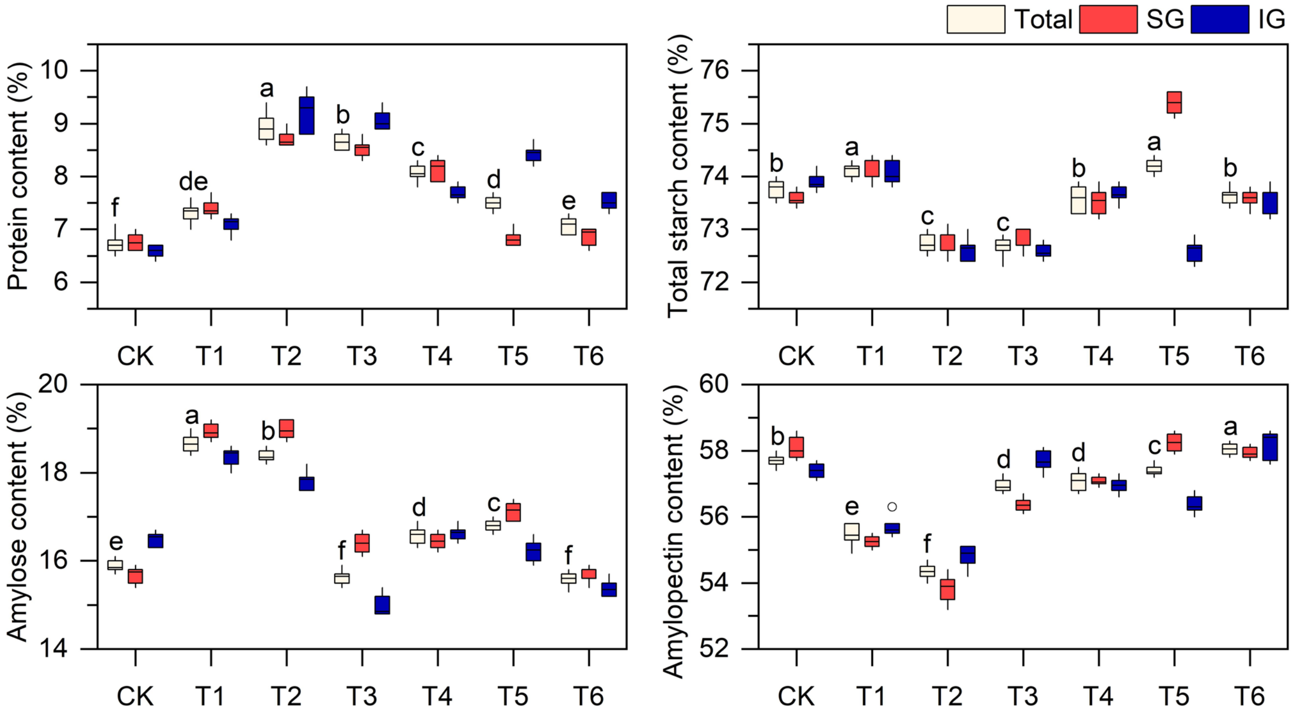

Fig. 3. The chemical compositions of milled rice under warming at different growth stages of high-tasting japonica rice. SG, superior grains; IG, inferior grains. Different letters indicate significant differences at the $P < 0.05$ level. The box boundaries indicate the 25th and 75th percentiles; the black line in the box marks the median; whiskers below and above the box indicate the minimum and maximum values, respectively; ○ indicates outlier.

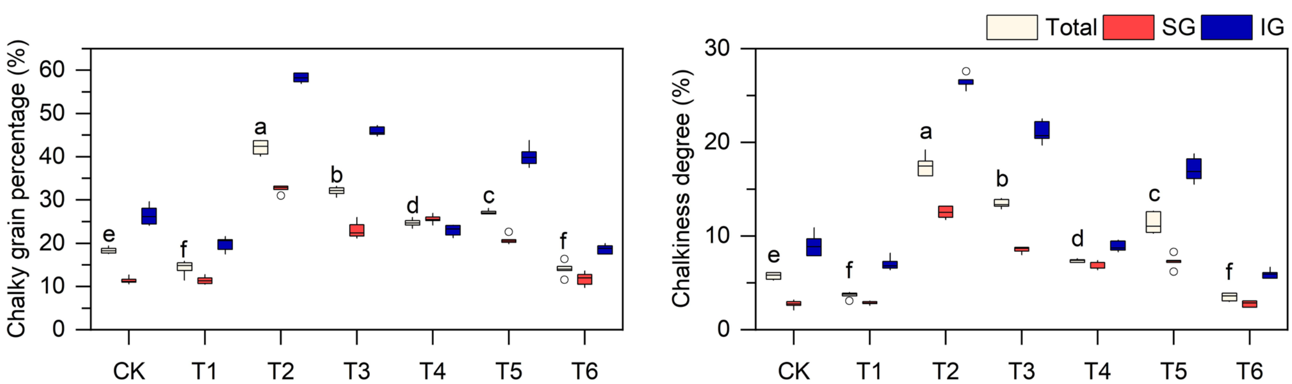

Fig. 4. The appearance quality of milled rice under warming at different growth stages of high-tasting japonica rice. SG, superior grains; IG, inferior grains. Different letters indicate significant differences at the $P < 0.05$ level. The box boundaries indicate the 25th and 75th percentiles; the black line in the box marks the median; whiskers below and above the box indicate the minimum and maximum values, respectively; ○ indicates outlier.

(Fig. 4). Among them, the changes in the appearance quality of total grains in T2, T3, and T5 were jointly influenced by SG and IG, while T4 was effected by SG, and T1 and T6 were influenced by IG (Fig. 4).

Carbon and nitrogen metabolisms of rice grains

Compared with CK, T1, T2, and T3 had varying degrees of negative effect on the average AGPP activity of the total grains at the grain-filling stage, decreasing by 3.4%, 12.1%, and 1.7%, respectively; T6 had positive effect, increasing by 3.4%; T4 and T5 had no significant effect (Fig. 5). T1, T2, T3, T4, and T5 all had varying degrees of negative effect on the average SSS vitality of the total grains at the grain-filling stage, decreasing by 13.8%, 18.2%, 4%, 4%, and 2%, respectively; T6 had positive effect, increasing by 1.2% (Fig. 5). T2, T3, T4, and T5 had varying degrees of positive effect on the average SBE activity of the total grains at the grain-filling stage, increasing by

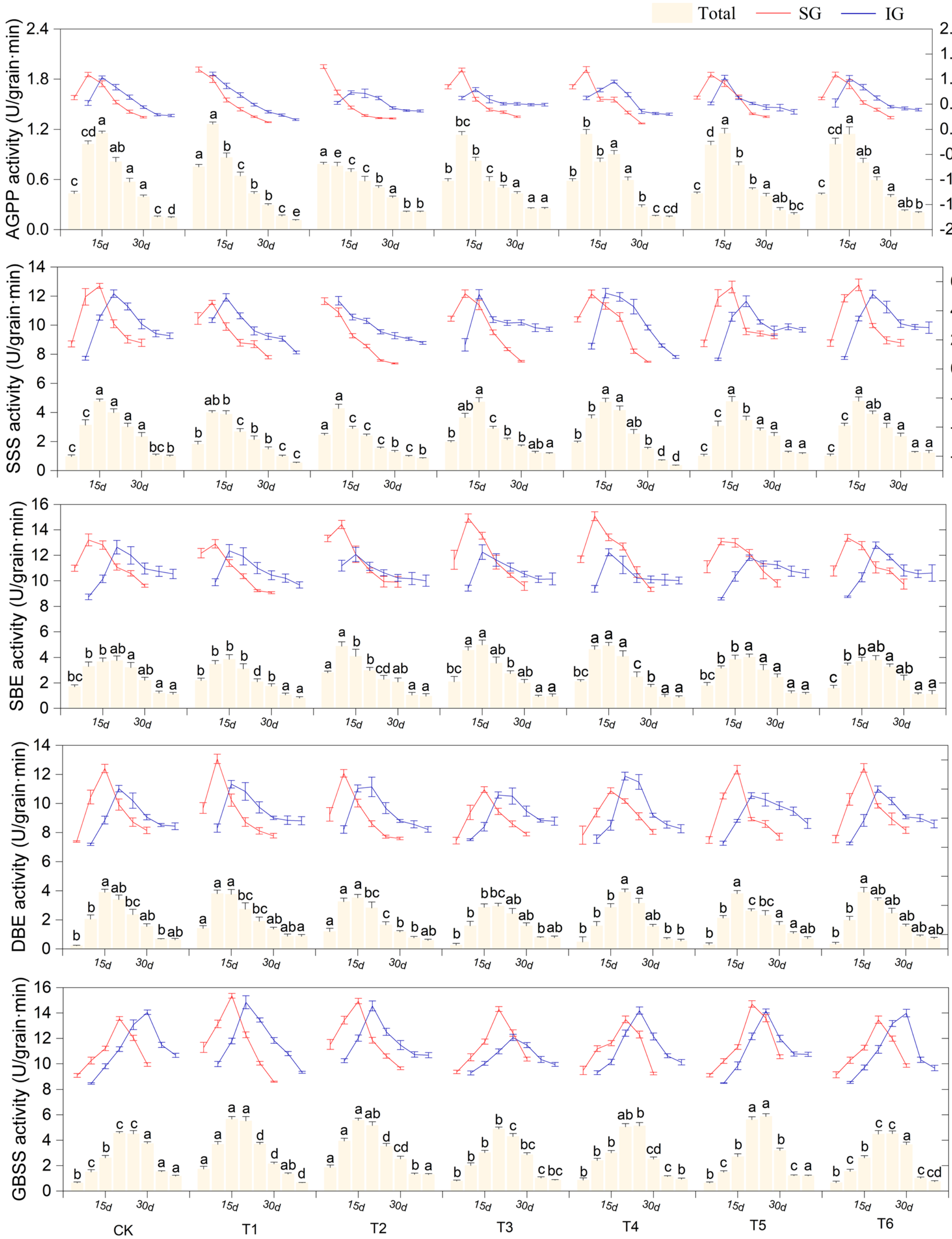

Fig. 5. Effect of warming at different growth stages on the ADP-glucose pyrophosphorylase (AGPP) activity, the soluble starch synthase (SSS) activity, the starch branching enzyme (SBE) activity, the starch debranching enzyme (DBE) activity and granule-bound starch synthase (GBSS) activity in grains of high-tasting japonica rice. Different letters indicate significant differences ($P < 0.05$) among treatments at the same stage.

4.8%, 8%, 8%, and 2.4%, respectively; T1 had negative effect, decreasing by 9.2%; T6 had no significant effect (Fig. 5). T1, T2, and T6 had varying degrees of positive effect on the average DBE activity of the total grains at the grain-filling stage, increasing by 13.6%, 1.1%, and 2.2%, respectively; T3 and T5 had varying degrees of negative effect, decreasing by 10.3% and 1.1%, respectively; T4 had no significant effect (Fig. 5). T1, T2, T4, and

T5 had varying degrees of positive effect on the average GBSS activity of the total grains at the grain-filling stage, increasing by 21.1%, 25.1%, 4.4%, and 8.8%, respectively; T3 and T6 had negative effect, decreasing by 2.4% and 4.8%, respectively (Fig. 5).

Each warming treatment had varying degrees of positive effect on the average GOT activity of the total grains at the grain-filling stage, with T1, T2, T3, T4, T5, and T6 increasing by 1.2%, 25.3%, 21.7%, 8.4%, 3.6%, and 3.6%, respectively (Fig. 6). T2, T3, T4, T5, and T6 had varying degrees of positive effect on the average GPT activity of the total grains during the grain-filling stage, increasing by 7.7%, 13.2%, 7.7%, 4.4%, and 5.5%, respectively; T1 had negative effect, decreasing by 6.6% (Fig. 6).

Chemical compositions and RVA profiles in relation to the taste value of milled rice

The values of PV, HPV, BD and SB had a significant correlation with the taste value of milled rice. The taste value was significantly positively correlated with PV and BD, and significantly negatively correlated with HPV and SB (Fig. 7).

The taste value of milled rice had a significant correlation with the amylose, amylopectin, total starch and protein contents. The amylose and protein contents were significantly negatively correlated with the taste value, and the amylopectin and total starch contents were positively correlated with the taste value (Fig. 7). The decrease in taste values of T1, T2, T3, T4, and T5 was related to the increase in protein content and decrease in amylopectin content (Figs. 2, 3, 7). And the correlation coefficients between protein content, amylopectin content and taste value were relatively high, which was the most important chemical composition that affected the taste value of milled rice by warming (Fig. 7).

Path analysis results showed that protein content, amylose content, and BD value can explain the changes in taste value well (adjusted $R^2 = 0.820$) (Table 2). The influence of protein content on the taste value through BD ($r = -0.114$) was slightly greater than that of amylose content ($r = -0.092$) (Table 2).

Relationship between carbon and nitrogen metabolism in grains at grain-filling stage and chemical compositions of milled rice

There was no significant correlation between the total starch content of milled rice and the AGPP activity of grains (Fig. 7). The content of amylopectin in milled rice was significantly positively correlated with the SSS activity of grains, but not significantly correlated with the SBE and DBE activity (Fig. 7). The amylose content in milled rice was significantly positively correlated with the GBSS activity of grains and significantly negatively correlated with SSS activity (Fig. 7). There was significant positive correlation between the protein content of milled rice and the GOT and GPT activity of grains (Fig. 7). There was significant negative correlation between the amylopectin and amylose content in milled rice (Fig. 7). There was significant negative correlation between protein content and total starch content as well as amylopectin content (Fig. 7). Therefore, the SSS and GBSS activity of grains had a capacity competition relationship in regulating the synthesis of amylopectin and amylose.

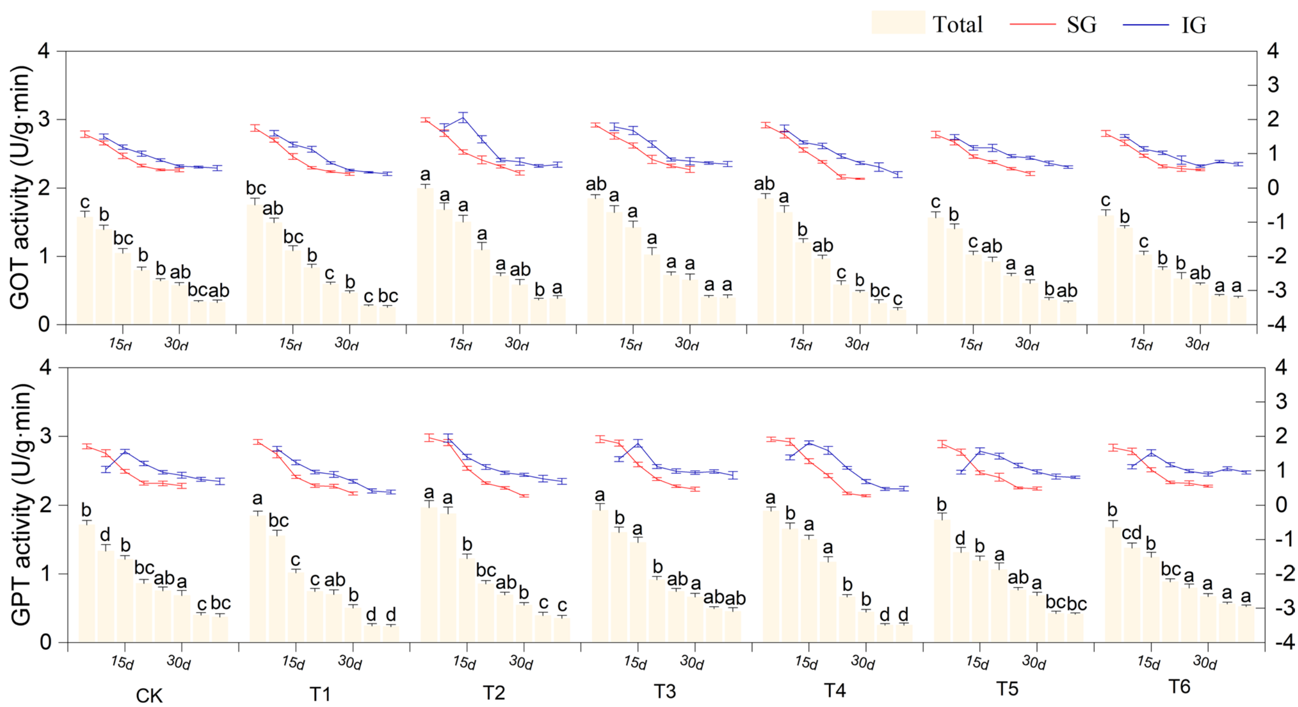

Fig. 6. Effect of warming at different growth stages on the glutamic-oxaloacetic transaminase (GOT) activity and the glutamic-pyruvic transaminase (GPT) activity in grains of high-tasting japonica rice. Different letters indicate significant differences ($P < 0.05$) among treatments at the same stage.

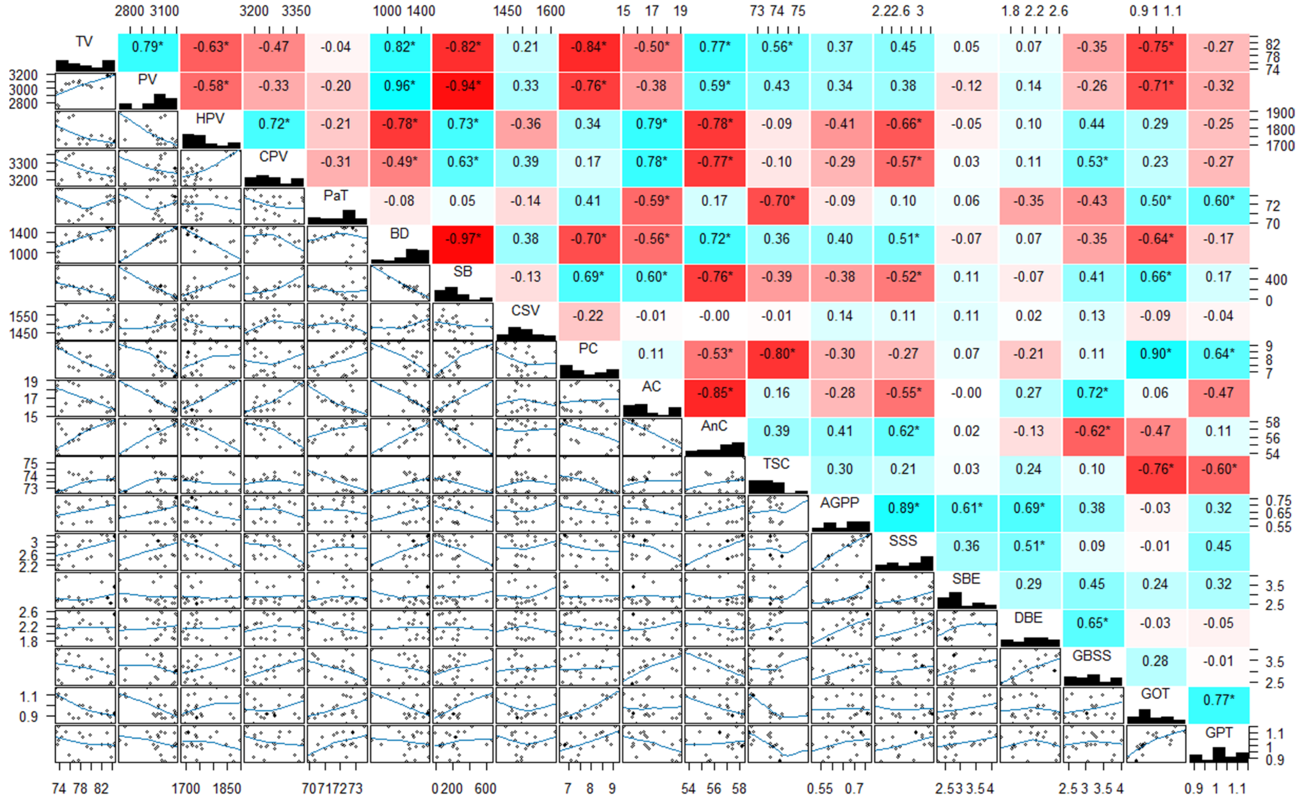

Fig. 7. Correlation between taste value, rapid visco analyzer (RVA) profiles, chemical composition, and enzyme activity.

The optimal regression equation	R^2	P	Independent variable	Direct path coefficient	Indirect path coefficient		
					x_1	x_2	x_3
$y = 114.014 - 2.96x_1 - 0.996x_2 + 0.003x_3$	0.82	< 0.01	x_1	− 0.656	–	− 0.042	− 0.114
			x_2	− 0.318	− 0.087	–	− 0.092
			x_3	0.176	0.424	0.166	–

Table 2. Path analysis between chemical compositions, RVA profiles and taste value of milled rice. Taking taste value of milled rice as dependent variable, chemical compositions and RVA profiles were used as independent variables. x_1, protein content of milled rice; x_2, amylose content of milled rice; x_3, breakdown (BD) value of milled rice.

There was a capacity competition relationship between the synthesis process of the protein and starch of milled rice, among which the competition for amylopectin synthesis is the main one.

Discussion

Effects of warming at different growth stages of high-tasting japonica rice in cool regions on the taste value of milled rice

In studies investigating the effects of warming on rice, greenhouses have been the most widely used facilities. Early greenhouse systems primarily relied on solar radiation to elevate internal temperatures, but such devices exhibited limited warming capacity during cloudy/rainy days and nighttime. With technological advancements, modern greenhouses allow precise temperature control, yet still inadequately simulate atmospheric gas composition, wind speed, and other environmental factors[17]. This study adopted a combined passive and active warming in the field, which not only achieved warming effects during cloudy/rainy days and nighttime but also maintained air circulation, effectively simulating the effects of climate warming on rice production to a significant extent. Notably, the passive mode demonstrated a more pronounced daily mean temperature increase compared to the active mode (Table 1). However, while this integrated approach offers significant advantages, it concurrently alters air humidity levels. Such humidity variations may influence rice grain filling, paddy field pest/disease dynamics and ultimately grain quality which requires further investigation. Previous studies on the influence of temperature on the taste quality of milled rice mainly focused on the grain-filling stage[18,19]. It is generally accepted that the temperature of rice at grain-filling stage is closely related to the taste quality of rice, too high or low is not good for the production of high-tasting rice[20,21]. However, the suitability of temperature also affects the growth and development of rice, such as the important stages of tillering and booting[22]. Therefore, only study on warming at the grain-filling stage could not explain the effect of the complexity of climate warming on quality traits. This study found that T1 also affected the taste quality of milled rice and changed the rule that milled rice of SG had a higher taste value than IG (Fig. 2). Although there was no significant difference in the taste value of milled rice between T2 and T3, the average value of T2 was smaller than that of T3. This indicated that T2 may be affected by the cumulative effect of T1 and T3, but this cumulative negative effect would not continue to decrease the taste value of milled rice after reaching a certain level. At the same time, the average taste values of SG and IG milled rice of T2 were also lower than those of T1 and T3, further verified this speculation (Fig. 2). The previous studies believed that the temperature at the early grain-filling stage had the most critical effect on rice quality[23,24]. We found that T4 and T5 had a negative effect on the taste value of milled rice except T6, and there was no significant difference between T4 and T5 (Fig. 2). This indicated that the change in the taste value of milled rice of T3 may be affected by the cumulative effect of T4 and T5. In addition, the effects of T4 and T5 on the taste of milled rice were related to the grain-filling progress. The degree of negative influence of T4 on SG was greater than that of T5, and the degree of negative influence of T5 on IG was greater than that of T4 (Fig. 2). The climatic temperature during the grain-filling stage of rice in cool region was conducive to the production of high-tasting rice[25]. The average value of milled rice taste of the tested varieties under the normal climate reached 83.7. However, due to the affect by warming at different growth stages, the taste value of milled rice of T1, T2, T3, T4 and T5 was not higher than 80 points. It can be seen that climate warming was generally not conducive to the taste of high-tasting japonica rice in cool regions, which was different from Chen's conclusion[25]. In our simultaneous experiment, we also had three varieties: Akita-Komachi, Beizuo 189, and Jijing 816. The trend of each treatment was the same as that of Jiyang 100, further verifying our conclusion. The data graph of taste quality changes for three varieties under different treatment can be found as Supplementary Figure S1 online.

Biochemical mechanism of the effect of warming at different growth stages on the taste of high-tasting japonica rice

The higher protein and amylose contents, the lower amylopectin content and lower the taste quality[26,27]. Our conclusions are consistent with the results of previous extensive studies. Li et al.[28] believed that amylose content of milled rice might be the most important factor affecting taste quality. However, the negative effect of warming on the taste value of milled rice was mainly related to the increase of protein content[29], which is consistent with the conclusion of this study. Regarding the effect of high temperature on amylose content of milled rice, different scholars had different results. The high temperature during the grain-filling stage or the early grain-filling would cause the amylose content of milled rice decrease[30]. Zhong et al.[31] believed that high temperature caused the amylose content of milled rice rise or fall, which was related to the rice varieties. In this study, the amylose content of milled rice had complex changes due to different stages of warming treatment. T1 and T2 would significantly increase the amylose content of milled rice, which should be caused by changes in economic or agronomic traits such as tiller, grains per panicle or thousand-grain weight due to warming before grain-filling. Same as the previous results[32], we found that T3 and T6 decreased the amylose content of IG, which led to the decrease of the total amylose content of milled rice. T4 and T5 increased the amylose content of milled rice of SG and IG, which eventually led to the increase of amylose content of the whole milled rice, which was different from Wang's conclusion[33]. Therefore, warming in different regions or different growth stages in the same region has different effects on amylose content of milled rice. Under the background of unique genotype, there was a trade-off relationship between starch and protein content, amylose and amylopectin content under different treatments (Fig. 2). This was conducive to better understand the influence mechanism of warming on taste quality.

RVA profiles are widely used to evaluate food crop taste quality. The chemical composition of milled rice is closely related to the RVA profiles. Previous studies have shown that RVA profile values were significantly correlated with amylose and protein content ($P < 0.05$)[34], and also related to amylopectin content and branching[26]. The hardness, cohesiveness, stickiness and resilience of milled rice were significantly negatively correlated with amylose or protein content. The hardness, cohesiveness, gumminess and chewiness of rice were negatively correlated with CPV and SB levels[26]. This is consistent with our conclusion that warming cause the increase in protein or amylose content, which result in a decrease in the taste value. Path analysis showed that both protein and amylose could affect the taste quality by changing BD (Table 2). These three indicators could explain the effect of warming on the taste quality of milled rice.

Tang et al.[35] investigated the effects of chalkiness on the cooking and taste quality of japonica rice using 10 japonica varieties. They found that chalkiness traits had no significant effect on the physicochemical indices of rice cooking and the taste of rice. Zhou et al.[36] reported that the chalky grain rate and chalkiness degree of late-season indica rice in South China exhibited highly significant negative correlations with taste quality. In this study, warming before heading stage (T1) significantly reduced chalky grain rate and chalkiness degree, but the taste value still decreased markedly. Warming during the late grain-filling stage (T6) significantly increased chalky grain rate and chalkiness degree, but the taste value remained unchanged. Warming at the early to middle grain-filling stages (T2, T3, T4, and T5) substantially increased chalky grain rate and chalkiness degree, the increased gaps between starch granules in rice, and ultimately led to reduced taste value. Therefore, under warming at different growth stages of rice, the relationship between taste quality and appearance quality exhibited changes, which is closely linked to factors such as warming-induced acceleration of grain-filling at early to middle grain-filling stages.

Physiological mechanism of the effect of warming at different growth stages on the taste of high-tasting japonica rice

Studies have shown that in the photosynthetic or non-photosynthetic organs of higher plants, AGPP, SSS, SBE, DBE and GBSS are the key enzymes of starch synthesis and metabolism in grains of milled rice, regulating the synthesis and accumulation of starch[37]. Among them, AGPP is responsible for the synthesis of total starch, SSS, SBE and DBE are responsible for the synthesis of amylopectin, and GBSS is responsible for the synthesis of amylase[37]. The conclusions in previous studies about the effects of high temperature on carbon metabolizing enzymes were not consistent. Jin et al.[30] believed that AGPP and SSS were insensitive to the environmental temperature, but SBE was comparatively sensitive to the temperature, and its activity declined when temperature was too high or too low. Li et al.[38] found the AGPase, SSS, SS and R enzymes were key enzymes affecting the rate of starch accumulation at high temperature. The results of Cheng et al.[39] showed that effect of temperature on amylose/total starch was more attributable to GBSS rather than SS, SSS, AGPP, SBE and DBE. Shen et al.[40] found that the correlation between SSS activity and taste value during the whole grain-filling stage was not significant, while the correlation between AGPP and SBE activity and taste value was significant. In our study, the changes in enzyme activity related to carbon metabolism in rice grains under warming at different growth stages were complex. The amylopectin content in milled rice was affected by the SSS activity of grains, but not significantly correlated with the SBE and DBE activity (Fig. 6). The GBSS of grains was the key enzyme in the amylose synthesis of milled rice, but there was a capacity competition between GBSS and SSS in the amylopectin synthesis. It was worth mentioning that there was no significant correlation between the total starch content of milled rice and the AGPP activity of grains and the sucrose content of leaves under warming, indicating that the total starch content of grains was also affected by nitrogen metabolism or other metabolic regulation.

In the study of nitrogen metabolism during the grain-filling stage of rice, GOT and GPT have been extensively studied as the key enzymes. It would increase the GOT or GPT enzyme activity to varying degrees under warming at the grain-filling stage, which is consistent with the results of previous studies[29]. Warming at the grain-filling stage led to an increase in the level of nitrogen metabolism, and increased the protein content of milled rice, which was closely related to the decrease in the taste value of the rice. Unlike other treatments, T1 had already stopped warming before filling, and the increase in protein content of milled rice was not caused by the increase in average levels of GOT and GPT at the whole grain-filling stage. The reason for this may be related to other nitrogen metabolism pathways in the grains, as well as the transaminase activity during the higher stage of grain-filling rate. The coordinated increase in grain filling rate and nitrogen metabolism level may have an important impact on improving the protein content of rice. There was significant negative correlation between the total starch content, amylopectin content, and protein content of milled rice under each warming treatment (Fig. 7). This indicated that there was capacity competition relationship between the process of protein and starch synthesis of grains. According to the response of carbon and nitrogen metabolism, the increase in protein content of rice in each warming treatment was due to the increase of its nitrogen metabolism level, rather than the decrease in carbon metabolism level. In summary, the negative effect of nitrogen metabolism response on rice taste under warming was more pronounced compared to carbon metabolism.

Conclusions

T1, T2, T3, T4 and T5 had significant negative effect on the taste of milled rice, while T6 had no significant effect. Each warming treatment had a different degree of negative effect on the taste value of milled rice, with T2 and T3 being the most serious, followed by T1, and finally T4 and T5. The taste value of milled rice was positively correlated with amylopectin content, total starch content, PV, BD, and negatively correlated with protein content, amylose content, HPV and SB. Among them, protein content, amylose content and BD value can well explain the changing law of taste value (adjusted $R^2 = 0.820$). In carbon and nitrogen metabolism, the protein content was negatively correlated with the total starch content and amylopectin content, and positively correlated with the activity of GOT and GPT. The total starch content had no significant relationship with AGPP activity. The amylopectin content was positively correlated with SSS activity, and not significantly correlated with SBE and DBE activity. The amylose starch content was positively correlated with GBSS activity and negatively correlated with SSS activity. The increase in protein content of rice in each warming treatment was due to the increase of its nitrogen metabolism level, rather than the decrease in carbon metabolism level. Meanwhile, the increase in protein content of milled rice led to a decrease in amylopectin content. In summary, the negative effect of nitrogen metabolism response on rice taste under warming was more pronounced compared to carbon metabolism.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article.

Received: 6 February 2025; Accepted: 13 May 2025
Published online: 19 May 2025

References

1. IPCC. Climate change 2021: The physical science basis Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change. (Cambridge University Press, 2022).
2. Yang, X. L., Zhou, B. T., Xu, Y. & Han, Z. CMIP6 evaluation and projection of temperature and precipitation over China. *J. Adv. Atmos. Sci.* **38**, 817–830 (2021).
3. Priyadarshini, D. P. et al. Rice bran extraction and stabilization methods for nutrient and phytochemical biofortification, nutraceutical development, and dietary supplementation. *J. Nutr. Rev.* **00**, 1–21 (2024).

4. Zhang, S. Q. et al. Effect and identification of different allele combinations of SSIIa and Wx on rice eating and cooking quality. *J. Euphytica* **220**, (2024).
5. Zhang, Y. R., Zhou, X. Q. & Yang, L. L. Present situation and expectation on methods for taste evaluation of rice. *J. Chin. Cereals Oils Assoc.* **24**, 155–160 (2009).
6. Ma, Z. T. et al. Effect of starch and protein on eating quality of japonica rice in Yangtze River Delta. *Int. J. Biol. Macromol.* **261**, 129918 (2024).
7. Zheng, C. K. et al. Moderate salinity stress affects rice quality by influencing expression of amylose- and protein-content-associated genes. *Int. J. Mol. Sci.* **25**, 4042 (2024).
8. Lin, C. J. et al. Influence of high temperature during grain filling on the accumulation of storage proteins and grain quality in rice (*Oryza sativa* l.). *J. Agric. Food Chem.* **58**, 10545–10552 (2014).
9. Tsukaguchi, T. & Iida, Y. Effects of assimilate supply and high temperature during grain-filling period on the occurrence of various types of chalky kernels in rice plants (*Oryza sativa* l.). *J. Plant Prod. Sci.* **11**, 203–210 (2008).
10. Lu, B. et al. Comparative performance of superior and inferior grains for quality parameters following diversified temperature during grain filling stage. *J. Cereal Sci.* **115**, 103812 (2024).
11. Ge, J. L., Chen, X., Zhang, X. B., Dai, Q. G. & Wei, H. H. Comparisons of rice taste and starch physicochemical properties in superior and inferior grains of rice with different taste value. *J. Food Res. Int.* **169**, 112886 (2023).
12. Ma, K., Zhou, Y. Y., Ma, Y. & Zhang, T. R. Increased rice yield by improving the stay-green traits and related physiological metabolism under long-term warming in cool regions. *Int. J. Plant Prod.* **18**, 175 (2024).
13. Shah, F. et al. Rice grain yield and component responses to near 2°C of warming. *J. Field Crops Res.* **157**, 98–110 (2014).
14. Zhu, T., Jackson, D. S., Wehling, R. L. & Geera, B. Comparison of amylose determination methods and the development of a dual wavelength iodine binding technique 1. *J. Cereal Chem.* **85**, 51–58 (2008).
15. AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists, fifteenth ed. Association of Official Analytical Chemists, Washington, DC, USA (1990).
16. Du, J. J. & Chen, Z. W. A method for path analysis using SPSS linear regression. *J. Biol. Bull.* **45**, 4–6 (2010).
17. Dou, Z. *Effects of free-air warming during grain filling stage on rice grain filling and quality and the regulation effects of nitrogen spikelet fertilizer*. Doctoral dissertation. Nanjing Agricultural University (2017).
18. Gong, J. L., Zhang, H. C., Hu, Y. J., Long, H. Y. & Huo, Z. Y. Effects of air temperature during rice grain-filling period on the formation of rice grain yield and its quality. *Chin. J. Ecol.* **32**, 482–491 (2013).
19. Zhang, G. L. et al. Effects of high temperature stress during grain-filling period on physiological characteristics in flag leaves and grain quality of rice. *Chin. J. Agrometeorol.* **35**, 650–655 (2014).
20. Choi, K. J., Park, T. S., Lee, C. K., Kim, J. T. & Kang, H. W. Effect of temperature during grain filling stage on grain quality and taste of cooked rice in mid-late maturing rice varieties. *Korean J. Cropence* **56**, 404–412 (2011).
21. Meng, X. F. et al. Effect of air temperature during grain filling stage on rice quality. *J. Mt. Agric. Biol.* 13–17 (2019).
22. Ma, K., Zhou, Y. Y., Zhang, Z. A., Chen, Q. Y. & Jing, N. Photosynthetic response and yield formation mechanisms of rice under warming at different growth stages in cool region. *J. Int. J. Plant Prod.* **16**, 223–233 (2022).
23. Wang, J. K. *Mechanism and regulation of high temperature at the early stage of grain filling on changes in carbon and nitrogen metabolism of Japonica rice grains*. Master's dissertation. Chinese Academy of Agricultural Sciences (2021).
24. Nagata, K., Takita, T., Yoshinaga, S., Terashima, K. & Fukuda, A. Effect of air temperature during the early grain-filling stage on grain fissuring in rice. *J. Jpn. J. Crop Sci.* **73**, 336–342 (2004).
25. Chen, M. J. et al. Dissecting the meteorological and genetic factors affecting rice grain quality in northeast China. *J. Genes Genomics* **43**, 975–986 (2021).
26. Tao, K., Yu, W. W., Prakash, S. & Gilbert, R. G. High-amylose rice: starch molecular structural features controlling cooked rice texture and preference. *J. Carbohydr. Polym.* **219**, 251–260 (2019).
27. Chen, H. et al. Correlation of taste values with chemical compositions and rapid visco analyser profiles of 36 indica rice (*Oryza sativa* l.) varieties. *J. Food Chem.* **349**, 129176 (2021).
28. Li, H. Y., Prakash, S., Nicholson, T. M., Fitzgerald, M. A. & Gilbert, R. G. The importance of amylose and amylopectin fine structure for textural properties of cooked rice grains. *J. Food Chem.* **196**, 702–711 (2016).
29. Liang, C. G. et al. High temperature at grain-filling stage affects nitrogen metabolism enzyme activities in grains and grain nutritional quality in rice. *J. Rice Sci.* **18**, 210–216 (2011).
30. Jin, Z. X., Qian, C. R., Yang, J., Liu, H. Y. & Jin, X. Y. Effect of temperature at grain filling stage on activities of key enzymes related to starch synthesis and grain quality of rice. *J. Rice Sci.* **12**, 261–266 (2005).
31. Zhong, L. J., Cheng, F. M., Wen, X., Sun, Z. X. & Zhang, G. P. The deterioration of eating and cooking quality caused by high temperature during grain filling in early-season indica rice cultivars. *J. Agron. Crop Sci.* **191**, 218–225 (2010).
32. Li, T. *Eeffets of temperature and light on carbohydrate metabolism and quality in rice grain*. Doctoral dissertation. Sichuan Agricultural University (2005).
33. Wang, J. K. *Mechanism and Regulation of High Temperature at the Early Stage of Grain Filling on Changes in Carbon and Nitrogen Metabolism of Japonica Rice Grains* Master's dissertation, Chinese Academy of Agricultural Sciences (2021).
34. Balet, S., Guelpa, A., Fox, G. & Manley, M. Rapid visco analyser (rva) as a tool for measuring starch-related physiochemical properties in cereals: A review. *J. Food Analyt. Methods* **12**, 2344–2360 (2019).
35. Tang, S. Z. et al. The effect of chalkiness on the cooking and taste quality of japonica rice. *J. Jiangsu Agric. Sci.* **04**, 4–5 (2003).
36. Zhou, S. Z. et al. Study on the correlation between cooking, appearance, milling quality and taste quality of late made indica rice in South China. *J. Hybrid Rice* **02**, 56–58 (2002).
37. Umemoto, T., Nakamura, Y. & Ishikura, N. Activity of starch synthase and the amylose content in rice endosperm. *J. Phytochem.* **40**, 1613–1616 (1995).
38. Li, M. Y., Shi, Q. H., Hu, Z. H., Pan, X. H. & Tan, X. M. Effects of high temperature stress on activity of amylosynthease in endosperm of early indica rice varieties. *J. Sci. Agric. Sin.* **40**, 1622–1629 (2007).
39. Cheng, F. M., Zhong, L. J. & Sun, Z. X. Effect of temperature at grain-filling stage on starch biosynthetic metabolism in developing rice grains of early-indica. *J. Sci. Agric. Sin.* **36**, 492–501 (2003).
40. Shen, P., Qian, C. R., Jin, Z. X., Luo, Q. X. & Jin, X. Y. Relationship between variation in activities of key enzymes related to starch synthesis during grain filling period and quality of eating and cooking in rice. *J. Rice Sci.* **13**, 43–50 (2006).

Acknowledgements
This study was supported by an earmarked fund for the Jilin Provincial key research and development project (20230202009NC).

Author contributions
Conceptualization, K.M. and Y.Z.; methodology, K.M. validation, K.M. and Y.Z.; formal analysis, K.M. and Y.Z.; investigation, K.M. and Y.Z.; resources, K.M. and Y.Z.; data curation, K.M. and Y.Z.; writing original draft preparation, K.M.; writing review and editing, K.M. and Y.Z.; supervision, K.M.; project administration, K.M.; funding acquisition, K.M. and Y.Z. All authors have read and agreed to the published version of the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-02328-w.

Correspondence and requests for materials should be addressed to Y.Z.