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Comprehensive evaluation of high temperature tolerance of six rice varieties during grain-filling period based on key starch physicochemical indexes

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ABSTRACT

In order to compare the high temperature tolerance of six rice varieties, field experiments using interval sowing population were conducted to select sowing batches at high temperature and normal temperature during grain filling period. High filling temperature decreased significantly head milled rice rate, setback, amylose content and the proportion of the short chain of amylopectin, and increased obviously chalkiness, breakdown, gelatinization temperature, gelatinization enthalpy, and the proportion of the medium chain of amylopectin. Analysis of variance indicated significant differences with respect to physicochemical indexes of the starches from six rice varieties under high temperature during grain filling stage. In addition, five starch physicochemical indexes including apparent amylose content, setback, breakdown, chalkiness and gelatinization enthalpy showed more sensitivity to high temperature. These indexes could be considered as key indexes to evaluate the high temperature tolerance of these six rice varieties. The experimental results showed that the high temperature tolerance of the six varieties – Huanghuazhan, Y liangyou 957, Taiyou 390, Tianyouhuazhan, Zhongzao 39, Y liangyou NO 1 – ranged from strong to weak. The regression equation showed similar results and indicated that the high temperature tolerance of different varieties could be preliminarily evaluated using the key physicochemical indexes of starch.

1. Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops, feeding more than half of the world's population to meet their dietary requirements (Zeng et al., 2017). Starch is the major component of rice grain accounting for more than 80% of its total constituents, and therefore its physicochemical parameters such as amylose content, gelatinization temperature (GT), gelatinization enthalpy (ΔH), pasting properties including peak viscosity (PV), the trough viscosity (TV), the cool-paste viscosity (CPV), the setback (SB) and the breakdown (BD), are usually used to predict cooking and eating quality of rice (ECQ) (Kong, Zhu, Sui, & Bao, 2015; Calingacion et al., 2014). In general, the amylose content measured is mainly apparent amylose content (AAC), which cannot truly reflect the rice quality. The combined assay of AAC and pasting properties being measured using a Rapid Visco-Analyzer (RVA) were reported to be more effective method of evaluating the rice quality (Jia, Ding, Wang, & Deng, 2008). BD and SB could be used to distinguish different rice varieties with variations in AAC (Wu, Shu, & Xia, 2001). GT and ΔH measured by DSC are closely related to the crystal structure of rice, which determines the cooking time, and also two of the most important starch physicochemical indexes to evaluate ECQ (Yao et al., 2020).

Global warming has increased the mean daily average temperature at rice growing season therefore being a threat to rice production,

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especially because rice varieties are most sensitive to high temperature during the grain filling period (Fan et al., 2019). The daily average filling temperature higher than 27 °C will greatly influence the physicochemical properties of rice starch, resulting to the deterioration of rice quality (Deng et al., 2015; Wakamatsu, Sasaki, Uezono, & Tanaka, 2007; Zhu et al., 2019). Previous studies showed that high temperature at grain-filling stage could increase chalkiness degree (CHA), GT, ΔH and significantly changed the relative crystallinity, while there was no change in crystal type and the ratio of length and width (Yao et al., 2020; Yamakawa, Hirose, Kuroda, & Yamaguchi, 2007; Lin et al., 2010; Umemoto & Terashima, 2002; Zhong, Cheng, Wen, Sun, & Zhang, 2005). The starch pasting property detected by RVA analysis was affected by high temperature at grain-filling stage, but the response of RVA profiles characteristics to high temperature was different and the changes of SB and BD were more significant than PV, TV and CPV, which meant that SB and BD might be more sensitive to high temperature (Zhong et al., 2005; Chun, Lee, Hamaker, & Janaswamy, 2015; Liu et al., 2013). One of the most effective measures of preventing high temperature stress is to breed and select rice varieties that are tolerant to high temperature. Therefore, the accurate identification and evaluation of the high temperature tolerance of rice germplasms are very important for breeders, as this will help them in properly selecting which rice varieties to use. However, a lot of researches focused on the effect of high temperature on rice quality and rice starch physicochemical properties using greenhouse temperature control conditions, which was recently controversial, since extrapolating results from greenhouse could not be fully adopted in actual production (Zhang et al., 2016). We investigated the effect of high temperature on rice quality by means of natural field temperature via sowing on different dates and the results could provide more comprehensive and practical data to breeders.

Previous studies revealed that complex relationships were associated with rice quality indexes and each index had a different response to high temperature, so it was difficult to evaluate the high temperature tolerance of different rice varieties comprehensively and accurately with a single index. Multiple indexes must be adopted for comprehensive evaluation and these related indexes need to be transformed into new independent comprehensive indexes based on the principal component analysis (PCA) method, and the comprehensive evaluation can be made according to the contribution rate of each comprehensive index (Liu, Zhao, Li, & Chen, 2018; Liu, Zheng, & Ding, 2008; Maione & Barbosa, 2019). Therefore, the objective of this study was to measure physicochemical properties of rice starch at grain filling period via sowing on different dates to illustrate the effect mechanism of high temperature at grain-filling stage and further identify the key physicochemical indexes of starch being used to evaluate the high temperature tolerance among different rice varieties (Lange et al., 2019; Yang, Wang, & Zhou, 2003; Yano et al., 2019). Based on our previous work, we had reported the effect of high temperature on the physicochemical properties of rice starch during the grain filling period (Yao et al., 2020). In 2019, the ten sowing batches with six rice varieties were conducted to select sowing batches at high temperature and normal temperature during grain filling period to evaluate the high temperature tolerance of the varieties based on key starch physiochemical indexes.

2. Materials and methods

2.1. Plant material and field arrangement

Six rice varieties – Huanghuazhan, Y liangyou 957, Tianyouhuazhan, Taiyou 390, Y liangyou NO 1, and Zhongzao 39 – were used as the experimental materials in this study and respectively numbered 19Q001, 19Q002, 19Q003, 19Q004, 19Q005 and 19Q006, all of which were provided by Hunan Hybrid Rice Research Centre. In 2019, ten sowing batches were set up in order to cover sowing dates of all kinds of rice varieties and help select the maximum temperature difference between two sowing batches. Field experiments were conducted at the experimental field of the Hunan Hybrid Rice Research Center in Changsha City, Hunan Province. The rice materials were respectively sowed on March 30, April 9, April 19, April 29, May 9, May 19, May 29, June 8, June 18, June 28 and transplanted accordingly on April 27, May 4, May 15, May 27, June 5, June 14, June 25, July 4, July 14, July 23, and all varieties were arranged in three replications within each sowing batches. The field sowing, transplanting, management and taking samples methods were the same for all varieties of the three batches of rice used on our experiment (Yao et al., 2020). Air temperature was recorded by the temperature data log (HOBO, Onset Computer Corp, Bourne, MA, USA) being installed 100 cm above the soil in the field.

2.2. Experimental samples preparation

After physiological maturation, six individual plants were randomly selected for each variety in each sowing period, and all the panicles on the six individual plants were collected, then immediately placed in a 35 °C oven to dry until the rice moisture reached 14% (Zhu et al., 2019). All rice panicles were then threshed, dehulled, milled, and polished. The polished rice was analyzed for appearance and milling qualities. Starch extracted from the rice flour was used to measure a series of starch-related physicochemical indexes. Starch was extracted using the alkaline steeping method (Yao et al., 2020). In brief, the dried polished rice sample was immersed in a 0.14% sodium bisulfate solution at room temperature for 24 h. The steeped grain sample was mixed with enough 0.14% sodium bisulfate solution and ground into a slurry. The milled slurry was filtered through a 200-mesh sieve and then centrifuged at 3, 000 rpm for 20 min. The faint-colored supernatant was removed, while the remaining sediment was re-suspended in MilliQ water and centrifuged at 3,000 rpm for 20 min; this process was repeated five times. The isolated starch was placed in an oven at 55 °C to dry rapidly, and then the dried starch was ground to pass through a 200-mesh sieve and kept in a dryer.

2.3. Determination of starch physicochemical indexes

According to the temperature data, the sowing batches at high temperature and normal temperature during the filling period were screened, and the physicochemical indexes of starch of six rice varieties at high temperature and normal temperature were determined. At the same time, the response difference of the same starch physicochemical index to high temperature and normal temperature was determined by random sampling respectively in all plots of sowing batches of the high temperature and normal temperature of six rice varieties with three replicates in each sowing batch.

Brown rice rate, polished rice rate, head milled rice rate, the ratio of grain length to width, and CHA were determined to evaluate grain milling and appearance quality according to our previous method (Yao et al., 2020). Rice starch isolation, AAC, gelatinization properties, pasting property were determined to evaluate the effect of the physicochemical properties of rice starch using the methods described by Yao et al. (2020).

Starch chain length distribution was determined by ICS5000 ion chromatography system (ICS-5000, Thermo Fisher Scientific, Sunnyvale, America) as performed by Nishi, Nakamura, Tanaka, and Satoh (2001) with a minor modification. The Dionex™ CarboPac™ PA200 (3.0 × 250 mm, Thermo Fisher Scientific, Sunnyvale, America) ionic column was used according to the properties of the glucose chain. Mobile phase A (aqueous solution), mobile phase B (100 mM NaOH, 1M NaAC) and mobile phase C (100 mM NaOH) were used, the flow rate controlled at 0.4 ml/min, and the column temperature at 30 °C.

2.4. Comprehensive evaluation of high temperature tolerance

In order to eliminate the influence of variety specificity, character stress indexes (I) of physicochemical properties for all rice varieties were calculated as the experimental data to evaluate high temperature tolerance of different rice varieties. These physicochemical properties were respectively measured at high temperature and normal temperature treatment, viz character values under high temperature (PVHT) and character values under normal temperature (PVCT). Character stress indexes were calculated by means of previous methods (Nachimuthu et al., 2014; Huang, Luo, Huang, Rao, & Liu, 1999) with minor modifications as follows: I = PVHT/PVCT or I=PVCT/PVHT. If physicochemical properties under high temperature increased, the former formula was used, the latter was used if otherwise. PCA was used to change key physicochemical indexes relevant to each other into several independent comprehensive indexes by SPSS 24.0 software (Yano et al., 2019). The corresponding subordinative function value (U) of each comprehensive index was calculated by the formula: $U_X = (X-X_{min})/(X_{max}-X_{min})$. X is a comprehensive index of a certain variety in one rice varieties' group and Ux is its corresponding subordinative function value. X_{max} and X_{min} are respectively the maximum and the minimum value of the comprehensive index for all the test variety in this group (Jiang et al., 2014). Further, the weight (W) was determined by the ratio of each principal component contribution to the total contribution rate of all principal components, and the comprehensive evaluation value (D) was determined by the accumulation of the product of U_X value and W_X, and the formula: $D = \sum_{x=1}^{n} [Ux*Wx]$ (x = 1, 2, ……n), where n represented the last comprehensive index.

2.5. Statistical analysis

All parameters shown in the tables and figures used in this article represented the mean values of the experimental data obtained from triplicate tests for all varieties sown during the ten sowing batches. The analysis of all data was performed using the SPSS 24.0 statistical software program. One-way analysis of variance and Tukey's tests were used to determine whether statistically significant differences ($P < 0.05$)

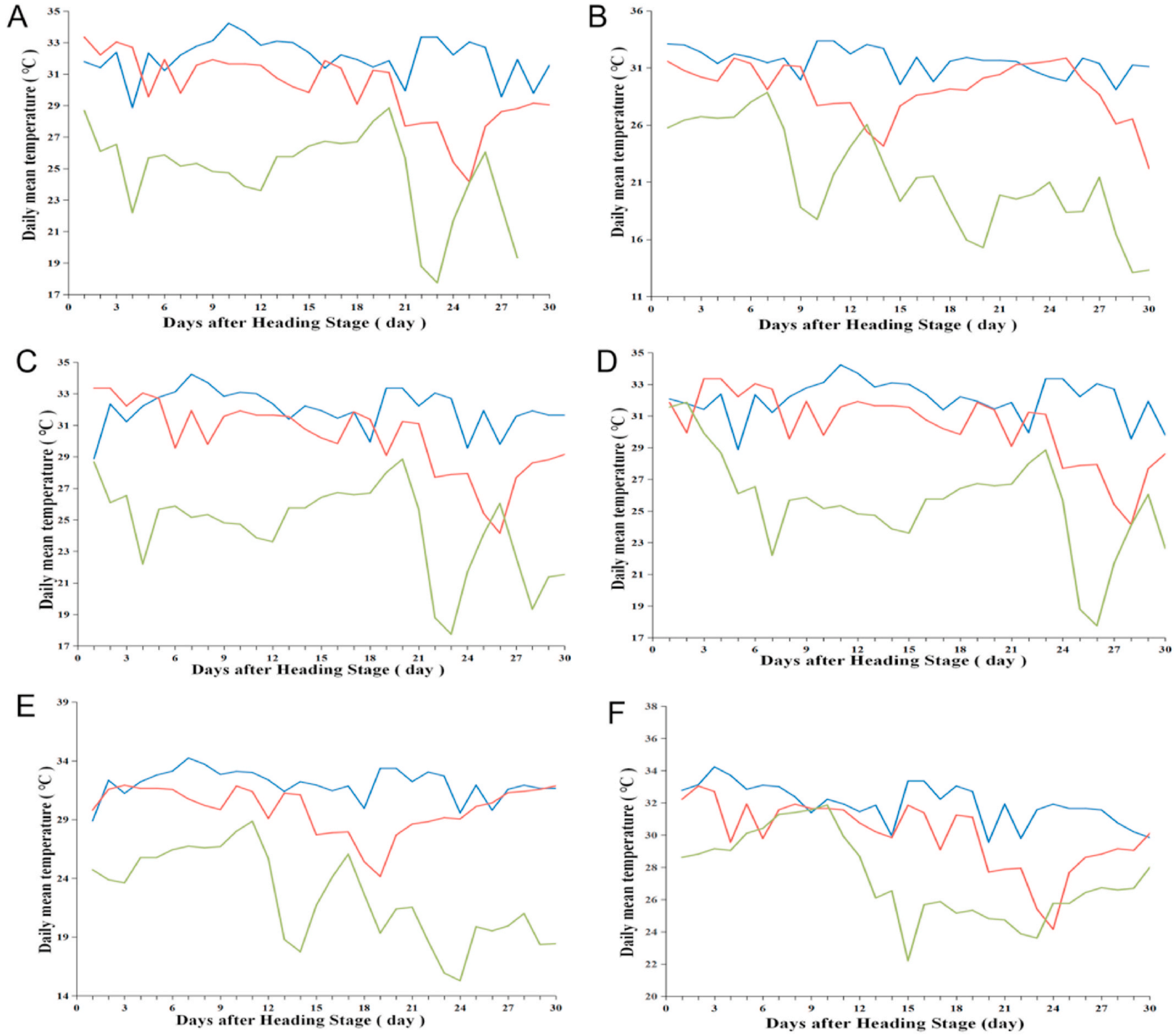

Fig. 1. The daily mean temperature of the six varieties with three sowing batches 30 days after the heading stage (MDT). A, B, C, D, E, F are MDT of Huanghuazhan, Y liangyou 957, Tianyouhuazhan, Taiyou 390, Y liangyou NO 1 and Zhongzao 39, respectively. Blue line (HT1) and red line (HT2) represent sowing batches processed under high temperature whereas the green line represents sowing batches under normal temperature (CT).(For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

existed among the means.

3. Results

3.1. Sowing and selection for the study materials

The first, the sixth, and the tenth sowing batches were selected as experimental materials based on their obviously different mean daily average temperature of 30 days (MDT) after the heading stage except 19Q006 (Fig. 1 and Supplementary Table 1). The first and sixth sowing batches of all the other varieties except 19Q006 were considered as high temperature treatment groups (HT1 and HT2) with MDT higher than 27 °C, and the MDT of the tenth sowing batch was 22–27 °C and considered as the normal temperature treatment (CT). For 19Q006 with a short growth period compared with the other five varieties, the sixth, the seventh and the tenth sowing batches were selected as the experimental materials, represented as HT1, HT2, and CT, respectively. Obviously, the daily average temperature of HT1 was higher than HT2, and the highest daily average temperature difference existed between HT1 and CT. Meanwhile, the daily mean temperature of 30 days after heading was higher than 29 °C for all varieties at HT1 treatment group, meaning that the duration time of high temperature stress lasted up to 30 days, while the continuous time of the daily mean temperature higher than 29 °C for all varieties at HT2 treatment group exceeded 12 days, showing that all varieties had suffered with continuous high temperature for more than 12 days.

3.2. Effects of high temperature on rice milling and appearance qualities

The effects of high temperature on the indexes of milling and appearance qualities were shown in Supplementary Table 2. As per our previous studies (Yao et al., 2020), high temperature at grain filling stage did not change brown rice rate and polished rice rate, but significantly increased ($P < 0.05$) CHA and decreased head milled rice rate. Except for 19Q004 and 19Q006, the ratio of grain length to width had a significant decline under high temperature, which was contradictory with our previous study showing that high temperature had no significant effect on ratio of grain length to width. Except for 19Q004, CHA showed a deterioration under both HT1 and HT2 for the other varieties. Especially, as the temperature rose, the CHA increased even more for 19Q002 and 19Q006. Head milled rice rate showed only a decreasing trend under HT1 treatment for 19Q002, 19Q003, 19Q004, 19Q005. The above results indicated that CHA was more sensitive to high temperature comparing with other indexes of milling and appearance qualities.

3.3. Effects of high temperature on the physicochemical characteristics of rice starch

Table 1
The effects of high temperature on AAC and gelatinization property.

No.	Temperature level	AAC	T_0	T_p	T_c	ΔH
19Q001	HT1	12.69 ± 0.89b	75.32 ± 0.93a	80.89 ± 0.41a	84.85 ± 0.33a	10.12 ± 0.38a
19Q001	HT2	13.80 ± 0.13 ab	66.55 ± 0.67b	71.43 ± 0.64b	77.53 ± 0.44b	9.19 ± 0.24b
19Q001	CT	15.70 ± 0.19a	62.87 ± 0.34c	67.72 ± 0.54c	71.38 ± 0.67c	8.35 ± 0.29c
19Q002	HT1	12.95 ± 0.36c	75.25 ± 0.37a	80.74 ± 0.67a	85.05 ± 0.73a	12.77 ± 0.22a
19Q002	HT2	13.69 ± 0.15b	72.46 ± 0.46b	78.53 ± 0.45b	80.93 ± 0.36b	11.07 ± 0.20b
19Q002	CT	18.35 ± 0.10a	64.30 ± 0.65c	71.90 ± 0.30c	77.80 ± 0.57c	9.34 ± 0.34c
19Q003	HT1	21.54 ± 1.13a	76.06 ± 0.22a	80.81 ± 0.54a	85.80 ± 0.63a	12.97 ± 0.41a
19Q003	HT2	22.14 ± 1.76a	74.49 ± 0.38a	79.60 ± 0.50a	85.23 ± 0.64a	12.93 ± 0.35a
19Q003	CT	24.46 ± 0.41a	71.19 ± 1.20b	75.66 ± 0.93b	81.71 ± 0.81b	9.76 ± 0.29b
19Q004	HT1	12.21 ± 0.15b	77.07 ± 0.82a	82.21 ± 0.44a	86.34 ± 0.61a	12.74 ± 0.37a
19Q004	HT2	13.14 ± 1.25 ab	75.43 ± 0.50a	80.94 ± 0.51a	85.15 ± 0.88b	12.69 ± 0.59a
19Q004	CT	16.08 ± 0.27a	71.53 ± 0.64b	78.38 ± 0.60b	82.49 ± 0.73b	10.74 ± 0.24b
19Q005	HT1	12.93 ± 1.25b	68.40 ± 0.40a	74.44 ± 0.50a	82.66 ± 0.55a	12.43 ± 0.19a
19Q005	HT2	13.43 ± 0.43b	66.37 ± 0.62b	71.99 ± 0.64b	79.03 ± 0.20b	11.22 ± 0.13b
19Q005	CT	20.00 ± 1.01a	61.55 ± 0.32c	66.22 ± 0.35c	71.68 ± 0.63c	6.82 ± 0.73c
19Q006	HT1	26.21 ± 0.08a	72.30 ± 0.30a	79.36 ± 0.61a	84.05 ± 0.18a	11.08 ± 0.11a
19Q006	HT2	26.07 ± 0.05a	71.64 ± 0.10b	77.33 ± 0.41b	81.50 ± 1.24b	10.50 ± 0.05b
19Q006	CT	26.97 ± 0.28a	71.00 ± 0.29b	75.62 ± 0.39c	81.86 ± 0.73 ab	8.40 ± 0.28c

Data are shown as the mean ± standard error of triplicate measurements. Different letters are followed after standard deviation to express significantly different ($P < 0.05$). 19Q001, 19Q002, 19Q003, 19Q004, 19Q005 and 19Q006 represent respectively the rice varieties of Huanghuazhan, Y liangyou 957, Tianyouhuazhan, Taiyou 390, Y liangyou NO 1 and Zhongzao 39. HT1 and HT2 represent the corresponding high temperature processed sowing batches, while CT represents nomal temperature sowing batch.

Table 1 showed that high temperature significantly decreased AAC for 19Q001, 19Q002, 19Q004 and 19Q005. The AAC did not obviously change under HT1 and HT2 for 19Q003 and 19Q006. Moreover, different varieties were affected on different degrees by high temperature, for example, 19Q002 showed a significant decrease as the temperature rose, while the other varieties did not exhibit this trend. High temperature caused little or no significant change in PV, TV and CPV, but significantly increased BD except 19Q003, GT (onset, T_0; peak, T_p; conclusion, T_c) and ΔH, and decreased SB for all varieties (Table 1, Table 2). SB of RVA were calculated by a relative value (setback value/peak value). Setback value equal peak viscosity minus trough viscosity. For amylopectin chain length distribution (Fig. 2), except for 19Q001, the proportion of the short chain, ranging from DP 6-18, significantly decreased with the increasing filling-grain temperature. The amount of medium chain ranging from DP 21 to 33 clearly increased, while the longer chain above DP 34 did not obviously change when the filling-grain temperature increased further. For 19Q001, the chain with DP 6-10 obviously decreased and the chain ranging from DP 11 to 20 significantly increased, and the longer chain with above DP 21 had a little change with the increasing filling-grain temperature. Similarly for 19Q005, the chain above DP 20 did not have a significant change. Therefore, the proportion of the short chain of amylopectin obviously decreased, while the proportion of the medium chain significantly increased as the filling-grain temperature increased, which promoted the formation of more perfect crystals (Xiong et al., 2021). Additionally, the proportion of the medium and long chain of amylopectin was highly positive correlated with gelatinization properties measured by DSC (Lin

Table 2
The effect of high temperature on pasting property by RVA.

NO.	Temperature level	PV	TV	BD	CPV	SB
19Q001	HT1	864.33 ± 11.02a	682.33 ± 26.65a	197.50 ± 21.92a	1029.00 ± 22.61a	0.40 ± 0.00c
19Q001	HT2	791.00 ± 22.87b	593.33 ± 29.01a	197.67 ± 10.41a	1034.00 ± 12.53a	0.56 ± 0.04b
19Q001	CT	613.33 ± 11.02c	589.33 ± 51.64a	157.33 ± 10.02b	1021.33 ± 43.32a	0.81 ± 0.03a
19Q002	HT1	930.00 ± 50.21a	766.67 ± 30.01a	189.17 ± 18.56a	1103.67 ± 42.59a	0.36 ± 0.04c
19Q002	HT2	947.33 ± 55.14a	742.67 ± 39.32a	187.33 ± 22.28a	1180.33 ± 39.70a	0.46 ± 0.03b
19Q002	CT	607.33 ± 26.73b	771.33 ± 17.67a	154.00 ± 2.83b	968.67 ± 45.35b	0.82 ± 0.02a
19Q003	HT1	870.33 ± 35.23a	768.00 ± 27.62a	102.33 ± 31.56a	1259.00 ± 46.36a	0.54 ± 0.04b
19Q003	HT2	813.00 ± 16.09a	711.33 ± 12.34 ab	101.67 ± 20.43a	1207.33 ± 25.93a	0.59 ± 0.00b
19Q003	CT	688.33 ± 38.03b	595.67 ± 70.60b	92.67 ± 40.28a	1024.33 ± 136.87a	0.76 ± 0.04a
19Q004	HT1	1010.67 ± 12.34a	861.67 ± 16.74a	226.00 ± 14.50a	1203.33 ± 27.21a	0.34 ± 0.02b
19Q004	HT2	982.00 ± 29.72a	765.67 ± 25.54b	216.33 ± 11.02a	1128.67 ± 34.53a	0.37 ± 0.00b
19Q004	CT	986.00 ± 35.09a	684.33 ± 92.40 ab	165.00 ± 10.15b	1121.00 ± 148.09a	0.44 ± 0.01a
19Q005	HT1	945.33 ± 51.81a	707.67 ± 46.61a	262.33 ± 41.20a	1324.33 ± 138.17a	0.54 ± 0.02c
19Q005	HT2	838.00 ± 29.21b	607.67 ± 25.42a	209.67 ± 28.73 ab	1104.67 ± 18.93a	0.64 ± 0.01b
19Q005	CT	850.67 ± 9.71b	603.33 ± 44.79a	114.00 ± 46.77b	1225.33 ± 59.21a	0.77 ± 0.04a
19Q006	HT1	631.50 ± 51.62a	578.00 ± 22.63a	73.50 ± 28.99a	1056.50 ± 200.11a	0.59 ± 0.02b
19Q006	HT2	565.67 ± 38.00a	535.00 ± 31.80a	59.67 ± 10.07b	969.67 ± 96.69a	0.77 ± 0.02b
19Q006	CT	599.50 ± 23.33a	547.00 ± 50.91a	33.45 ± 27.58c	988.00 ± 152.74a	0.85 ± 0.02a

Data are shown as the mean ± standard error of triplicate measurements. Different letters are followed after standard deviation to express significantly different ($P < 0.05$). 19Q001, 19Q002, 19Q003, 19Q004, 19Q005 and 19Q006 represent respectively the rice varieties of Huanghuazhan, Y liangyou 957, Tianyouhuazhan, Taiyou 390, Y liangyou NO 1 and Zhongzao 39. HT1 and HT2 represent the corresponding high temperature processed sowing batches, while CT represents nomal temperature sowing batch. SB is calculated by a relative value (setback value/peak value). Setback value equal peak viscosity minus trough viscosity.

et al., 2016), which indicated polished rice treated at high temperature during grain-filling period might need more energy and higher temperature to melt starch crystals, resulting to the polished rice being more difficult to cook.

3.4. Physicochemical indexes of rice starch closely related to high-temperature tolerance

The response difference of the same starch physicochemical index to high temperature and normal temperature was determined on 54 plots of six varieties with three sowing batches (the first, sixth and tenth sowing batches of 19Q001-19Q005 and the sixth, seventh and tenth sowing batches of 19Q006) and three replicates in each sowing batch to study the effects of HT1 and HT2 on the same physicochemical indexes (Table 3). The results showed that there were no significant changes in brown rice rate, polished rice rate and length-width ratio under the two temperature conditions (the data were not provided), while PV and CPV only increased significantly under HT1 stress, but did not change significantly under HT2 stress. However, AAC, BD and SB increased significantly under HT1 and HT2 conditions, especially AAC and SB reached an extremely significant level, indicating that AAC, BD and SB were more sensitive to high temperature than PV and CPV. From Table 3, we could find that chalkiness degree in HT2 reached significant levels and extremely significant levels by HT1 stress, while head milled rice rate, T_0, T_p, T_c and ΔH reached significant levels under these two kinds of high temperature. The analysis of variation coefficients (CV) of all the indexes measured for two consecutive years using different varieties with different sowing dates showed that AAC, BD, SB, CHA and ΔH had higher coefficients of variation (Supplementary Table 3). Meanwhile, these indexes had more significant changes under higher grain-filling temperature indicating that they were more sensitive than the other indexes. Therefore, combining the significance analysis and CV analysis under high temperature conditions, AAC, BD, SB, CHA and ΔH were considered as the key physicochemical indexes of rice starch to evaluate the high temperature tolerance of different varieties.

3.5. Comprehensive evaluation of high temperature tolerance of different varieties

PCA was used to transform multiple indexes into independent comprehensive indexes to evaluate accurately high temperature tolerance of different varieties to provide more reliable theoretical data to breeders (Deepa et al., 2009). Taking CT as control, HT1 and HT2 were considered as two high temperature treatment conditions so that the experiments could be divided into two comparison groups, namely HT1/CT and HT2/CT. The stress degree of each physicochemical index under high temperature was evaluated by character stress index under two treatment groups and the standard data analyzed using the SPSS software were shown in Supplementary Table 4. The D value was obtained by the PCA by changing ΔH, BD, SB, CHA, AAC relevant to each other into two or three independent comprehensive indexes to estimate the extent to resist high temperature. At HT1/CT treatment group, three principal components including F1, F2 and F3 were obtained by PCA with cumulative contribution rate of 96%, which could cover almost all the information of key physiological index. F1 explained 40.9% of the total variance (Table 4), and CHA and SB showed respectively high positive loading (0.958) and negative loading (−0.910), which suggested that the varieties with high principal component value of F1 exhibited high chalkiness and low SB values after high temperature treatment. F2 explained 34.4% of the total variance and the loading of BD was high on F2 (0.968), indicating that F2 was representative of BD. F3 explained 24.7% of the total variance and represented AAC (0.972). This component was also loaded with $\triangle H$ (0.669), which was consistent with the previous results that varieties with high AAC needed more energy to melt starch crystals (Lacerda, Leite, & da Silveira, 2019). Due to the high variance contribution rate of F1 and F2, CHA, SB and BD contributed the most to evaluate the high temperature tolerance of different varieties. For the HT2/CT treatment group, three principal components were obtained with cumulative contribution rate of 98%. The principal components and its coefficient were shown in Table 4 at two groups. F1, F2 and F3 represented respectively BD and SB, CHA and $\triangle H$, AAC. According to the high variance contribution rates of F1 (47.4%) and F2 (31.4%), CHA, SB and BD were the most key indexes to evaluate the high temperature tolerance of different rice varieties, which was consistent with the results from the HT1/CT treatment group. According to the standard data of character stress index and principal component coefficient of each index, the principal component value was calculated. U, W and D value was shown in Table 5. According to D value, the high temperature tolerance of the cultivars ranging from strong to weak was evaluated, viz the high temperature tolerance of the six varieties was evaluated in the same order under HT1 and HT2 treatment, with the following varieties arranged from strong to weak – 19Q001, 19Q002, 19Q004, 19Q003, 19Q006, 19Q005. The same results of high temperature tolerance were obtained in the two groups, which

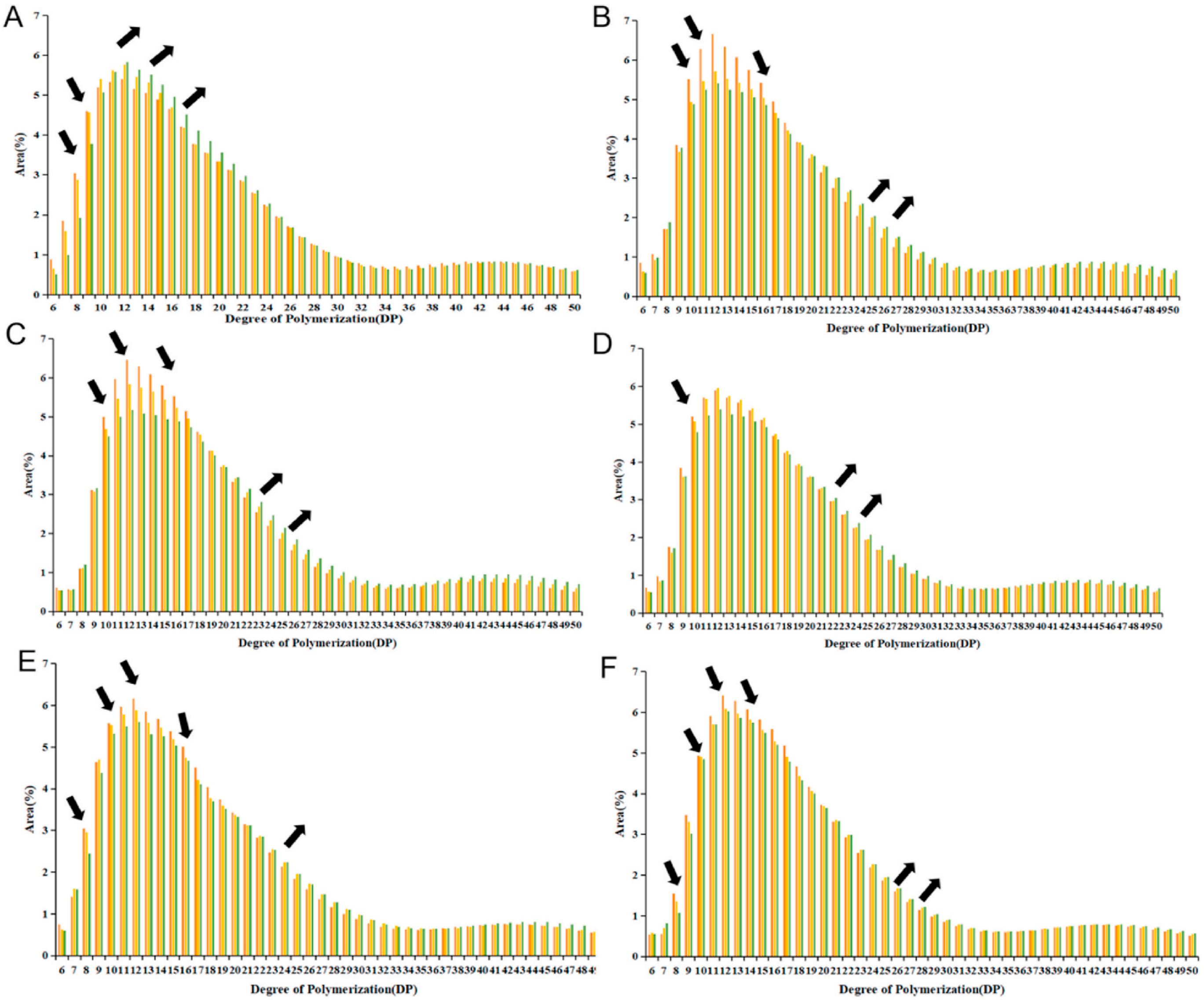

Fig. 2. Starch chain distribution of the three sowing batches of Huanghuazhan (A), Y liangyou 957 (B), Tianyouhuazhan (C), Taiyou 390 (D), Y liangyou NO 1 (E), and Zhongzao 39 (F). Green bar (HT1) and yellow bar (HT2) represent sowing batches processed under high temperature whereas the orange bar represents sowing batches under normal temperature (CT). The black arrows indicate the change in the trend of amylopectin chain length proportion with different polymerization degrees in CT, HT2 and HT1 directions. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Effects of HT1 and HT2 stress at grain filling stage on main physicochemical indexes of rice.

Treatment	Head milled rice rate/%	Chalkiness degree/%	PV	AAC	CPV	BD	SB	ΔH	T_0	T_c	T_p
HT1	61.31 ± 5.43	4.36 ± 0.93	875.36 ± 131.00	16.42 ± 5.96	1162.64 ± 118.06	175.28 ± 72.71	393.51 ± 77.08	12.02 ± 1.15	74.07 ± 3.20	84.79 ± 1.31	79.74 ± 2.75
CT	69.88 ± 2.45	2.48 ± 0.39	724.19 ± 159.58	20.26 ± 4.58	1058.11 ± 97.29	122.67 ± 51.08	520.72 ± 72.38	8.90 ± 1.36	67.07 ± 4.65	77.82 ± 5.14	72.59 ± 4.83
Difference	8.57*	1.88**	151.17*	3.84**	104.53*	52.61*	127.22**	3.11*	7.00*	6.97*	7.16*
HT2	67.69 ± 2.35	3.79 ± 0.95	822.83 ± 147.18	17.04 ± 5.61	1104.11 ± 89.51	162.06 ± 65.06	448.14 ± 56.16	11.27 ± 1.40	71.16 ± 3.88	81.56 ± 3.14	76.64 ± 4.00
CT	69.88 ± 2.45	2.48 ± 0.39	724.19 ± 159.58	20.26 ± 4.58	1058.11 ± 97.29	122.67 ± 51.08	520.72 ± 72.38	8.90 ± 1.36	67.07 ± 4.65	77.82 ± 5.14	72.59 ± 4.83
Difference	2.19*	1.31*	98.64	3.22**	46.00	39.39*	72.58**	2.37*	4.09*	3.74*	4.05*

Data are shown as the mean ± standard error of triplicate measurements. Level of significance is expressed as * for $P < 0.05$ and ** for $P < 0.01$ between temperature treatments. HT1 and HT2 represent the corresponding high temperature processed sowing batches of six rice varieties, while CT represents nomal temperature sowing batches of six rice varieties.

Table 4

Principal components and its coefficient based on character stress index of the key physiological items obtained by SPSS software under HT1 and HT2 at grain filling stage.

Treatment	Physiological items	Principal components and its coefficient			Physiological items	Corresponding variance contribution ratios of five variables		
		F1	F2	F3		F1	F2	F3
HT1	BD	0.064	0.561	0.597	BD	−0.102	0.968	−0.032
	SB	−0.573	0.401	0.191	SB	−0.910	0.351	−0.037
	AAC	0.058	0.443	−0.736	AAC	−0.142	−0.089	0.972
	CHA	0.670	−0.135	0.236	CHA	0.958	0.229	−0.074
	ΔH	0.464	0.556	−0.096	ΔH	0.392	0.686	0.669
	Contribution rate	0.409	0.344	0.247				
HT2	BD	0.432	−0.321	0.602	BD	0.974	0.107	−0.105
	SB	0.529	−0.441	0.046	SB	0.853	−0.027	0.473
	AAC	0.393	−0.119	−0.765	AAC	0.072	0.073	0.990
	CHA	0.260	0.717	0.212	CHA	−0.081	0.985	−0.131
	ΔH	0.558	0.418	−0.071	ΔH	0.266	0.871	0.411
	Contribution rate	0.474	0.314	0.212				

F1, F2, F3 are respectively three principal components obtained by PCA. HT1 and HT2 represent the corresponding high temperature processed sowing batches.

Table 5

The principal component values, U_X, W_X, comprehensive evaluation value(D), forecast value (FV) under HT1 and HT2 at grain filling stage.

Treatment	NO.	Principal component values			U_X			D	FV
		F1	F2	F3	F1	F2	F3		
HT1	19Q001	1.772	0.332	0.351	1.000	0.597	0.820	0.817	0.818
	19Q002	0.875	−0.505	0.903	0.748	0.381	1.000	0.684	0.691
	19Q003	−1.787	1.888	0.156	0.000	1.000	0.756	0.531	0.527
	19Q004	0.403	0.556	0.354	0.615	0.655	0.821	0.680	0.683
	19Q005	−1.564	−1.977	0.397	0.063	0.000	0.835	0.232	0.231
	19Q006	0.302	−0.293	−2.161	0.587	0.436	0.000	0.390	0.388
	W_X				0.409	0.344	0.247		
HT2	19Q001	1.485	0.836	0.088	1.000	0.937	0.672	0.911	0.812
	19Q002	0.640	0.448	1.072	0.804	0.823	1.000	0.851	0.686
	19Q003	0.982	−2.350	0.009	0.883	0.000	0.646	0.555	0.514
	19Q004	0.055	1.049	0.224	0.667	1.000	0.717	0.782	0.694
	19Q005	−2.814	−0.298	0.536	0.000	0.604	0.821	0.364	0.252
	19Q006	−0.348	0.314	−1.928	0.574	0.784	0.000	0.518	0.387
	W_X				0.474	0.314	0.212		

19Q001, 19Q002, 19Q003, 19Q004, 19Q005 and 19Q006 represent respectively the rice varieties of Huanghuazhan, Y liangyou 957, Tianyouhuazhan, Taiyou 390, Y liangyou NO 1 and Zhongzao 39. F1, F2, F3 are respectively three principal components obtained by PCA. HT1 and HT2 represent the corresponding high temperature processed sowing batches.

indicated that the high temperature tolerance of the varieties had similar internal response mechanism under these two kinds of high temperature conditions. Taking the comprehensive evaluation value as the dependent variable and the character stress indexes of five key physicochemical indexes as the independent variables, the multiple linear regression equation was established under HT1 stress, $D = -0.961 + 0.582*BD-0.174*SB-0.115*AAC+1.144*CHA+0.910*\Delta H$. The determining coefficient of the equation was 0.99, viz $R^2 = 0.99$,meaning that these key indexes could comprehensively evaluate high temperature tolerance of these six rice varieties. The regression equation under HT2 stress was obtained in the same way, $D = -1.893 + 1.463*BD-0.484*SB+1.430*AAC+2.604*CHA-1.454*\Delta H$, and the determining coefficient of the equation was 0.99, viz $R^2 = 0.99$. The predicted high temperature tolerance (FV) of the six rice varieties according to the regression equation was shown in Table 5 being almost the same as the D value obtained. The above results showed that the regression equation based on the character stress index of the five key indexes of the six varieties could be used to quantitatively evaluate the high temperature tolerance of the six varieties, and the reliability of the method used in this study was also demonstrated.

4. Discussion

High temperature seriously affected the physicochemical characteristics of rice starch, which resulted in poor rice quality, but different physicochemical indexes had different responses to high temperature stress (Mishra, Shekhar, Agrawal, Chakraborty, & Chakraborty, 2017). We obtained five key physicochemical indexes including AAC, BD, SB, Chalkiness and $\triangle H$ that were significantly affected by high temperature at grain filling stage and had large CV during different varieties and different sowing periods. With these five key indexes, the high temperature tolerance of six rice varieties were evaluated by means of PCA, respectively from strong to weak 19Q001, 19Q002, 19Q004, 19Q003, 19Q006, 19Q005.

The frequency of hot weather during grain filling stage dramatically decreased head polished rate, and increased CHA caused by the impairment of starch accumulation or other biosynthetic pathways in the grain (Sreenivasulu et al., 2015). At the same time, the physicochemical properties of milled rice starch, including gelatinization characteristics, pasting property, starch chain length distribution and so on, were also significantly affected by high temperature (Chun et al., 2015). Starch is the main component of milled rice and its physicochemical properties determine cooking and eating quality. The five key indexes obtained were all closely related to starch physicochemical properties in this paper, so we demonstrated that starch-related physicochemical indexes could be used to evaluate the high temperature tolerance of different rice varieties.

The composition and structure of rice starch are mainly affected by genetic and environmental factors. The changes of starch composition and structure under high temperature are mainly related to enzymes involved in starch synthesis. High temperature during grain filling stage inhibited the activities of adenosine diphosphate glucose (ADPG) enzyme, soluble starch synthase and granule-bound starch synthase (GBSS), which was not conducive to the accumulation of starch, thus leading to a significant decrease for amylose content (Yan et al., 2008). In addition, high temperature during grain-filling stage could promote the activity of soluble starch branching enzyme (soluble Q enzyme), but the activity of starch debranching enzyme (DBE) decreased. Therefore, high temperature at grain filling stage could promote short chain of amylopectin to synthesize the medium and long chains of amylopectin (Bao et al., 2020).

Changes in starch composition and structure led to the according changes in starch physicochemical properties (Bao et al., 2020). Our results showed that high temperature during grain-filling stage significantly increased chalkiness, mainly because 6–20 days after anthesis was the key period for the formation of rice chalkiness. During this period, rice varieties exposed to high temperature would increase the space between starch granules, leading to the increase of chalkiness (Sheng, Tao, & Chen, 2007). Studies have shown that GT was negatively correlated with the content of amylose, and positively correlated with the proportion of medium and long chains of amylopectin. Therefore, the increase of GT and ΔH might be due to the decrease of amylose content and the increase of the medium chains of amylopectin under high temperature (Kong et al., 2015; Dou et al., 2018). Previous studies showed that the branching structure of amylopectin mainly affected GT and crystallinity, while amylose was the main factor affecting the pasting properties measured by RVA (Rehmani et al., 2014). The amylose content of the six rice varieties used in this study was different, so the changes of relevant parameters of pasting properties under high temperature were varieties dependent, which indicated that a single index could not evaluate the high temperature tolerance of different rice varieties. Generally, rice varieties with low SB, CHA, ΔH and high BD had better ECQ (Mo et al., 2020). Meantime, BD, SB and CHA were thought to be biggest contribution in comprehesive evaluation of high temperature tolerance of different rice varieties. Therefore, PCA of the key physicochemical indexes should be carried out in the comprehensive evaluation of high temperature tolerance. The variance contribution rate of each key index in the principal component should be weighed, which can better help breeders to develop high-temperature tolerance rice varieties with good rice quality.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

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