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Screening of Heat-Tolerant *Indica* Rice Varieties in the Middle and Lower Yangtze River

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Abstract: Rice growth is constantly challenged by severe environmental conditions, especially high-temperature stress, brought about by global warming. How to increase rice yields is a current issue in many countries. By analyzing the meteorological data from Anhui Province over the past 60 years, the frequency and level of high-temperature damage were comprehensively analyzed. On this basis, five *indica* rice varieties with strong high-temperature tolerance, relatively high yield and stable yield were selected by laboratory-simulated high-temperature and field high-temperature experiments: Longliangyou Huazan (LLYHZ), 9you-6 (9Y-6), Longliangyou-1988 (LLY-1988), Fengliangyou-4 (FLY-4) and Huanghuazan (HHZ). These are suitable for planting in the middle and lower reaches of the Yangtze River. This study provides a theoretical reference and scientific basis for the study of rice tolerance to high-temperature stress.

Keywords: *Oryza sativa*; high temperature; variety screening; yield

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1. Introduction

The average global surface temperature increased by 0.74 °C from 1906 to 2005 and by 1.09 °C from 2011 to 2020 [1]. With global warming, the frequency and level of extreme megathermal climates are increasing. Rice is an important crop in China, and with the increase in global greenhouse effects, high temperature has become one of the major adverse conditions that are harming rice production. Anhui Province is one of the main rice production provinces in China, with a perennial planted area of more than 2.27 million hectares. In 2018, the rice planting area in Anhui Province reached 2.54 million hectares, ranking fourth in China, and the total production reached 16.812 million tons, ranking sixth in China (Agricultural Statistics of China, http://cnki.nbsti.net/CSYDMirror/Trade/home/index/Z009, accessed on 11 September 2022). Rice cultivation in Anhui Province is dominated by one-season *indica* rice. According to previous studies, high temperatures in Anhui Province from 1961 to 2013 mainly occurred from late July to mid-August, when *indica* rice is heading and blooming in the first season. The heading and flowering period of rice is a sensitive period vulnerable to high temperatures. At this time, high-temperature stress can lead to floret sterility, resulting in fertilization obstacles and reduced anther dehiscence rate, and seriously affecting seed setting percentage and yield [2].

Many previous studies have explored the adaptation of rice to high-temperature damage. At present, the effective measures mainly include the screening of high-temperature tolerance varieties [3], the reasonable adjustment of sowing date and the spraying of plant growth regulators [4]. During the growing period of rice, meiosis, flowering and grain filling are very sensitive to high temperatures. A continuous high temperature will affect the seed setting rate and 1000-grain weight of rice. Therefore, the identification and screening of high-temperature-resistant varieties usually focus on these periods. Many studies have confirmed the molecular responses and physiological and ecological characteristics of rice

under high-temperature stress. Under high-temperature stress, the photosynthetic rate, respiration rate, hormone level, cell membrane stability and physiological metabolism of rice are significantly inhibited [5]. Research has confirmed that enzymes such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) have a high correlation with plant heat tolerance [6,7]. Non-enzymatic antioxidants such as ascorbate, phenolic compounds, glutathione, alkaloids, α-tocopherols, carotenoids and some nonprotein amino acids have also been found to play important roles in the process of high-temperature tolerance [8,9]. The response of rice to high temperature is a complex biological process, not only involving a physiological level, but one which is also closely related to gene coding, protein translation, metabolic expression and so on. Previous studies have found that high temperatures induce the expression of several genes and proteins, such as heat stress proteins (HSPs) [10], heat shock transcription factors (HSFs) [11] and heat-tolerance-related genes SBPase [12] and FAD7 [13]. In conclusion, in order to screen out high-temperature-resistant varieties, comprehensive studies have been conducted on the gene, protein, physiological and metabolic levels.

Determining the suitable sowing date under different ecological conditions is the key to enforcing a rational cultivation management plan [14]. A reasonable sowing date can not only ensure that all stages of rice growth and development are consistent with the local optimal temperature and light conditions and make reasonable and efficient use of local resources, but can also reduce the occurrence of disasters during the growing period and increase rice yield [15]. The influence of sowing date on rice yield is mainly reflected in yield components. Khalifa found that the optimum sowing date was beneficial to chlorophyll content, leaf area index, storage capacity, spikelet number, grains per panicle and 1000-grain weight [16].

In recent years, the annual frequency of megathermal climate days and the degree of high-temperature damage have shown a gradual increasing trend. The tolerance degree of rice to high temperatures is different among germplasms. Breeding high-temperature-tolerant varieties is one of the important measures to resist heat damage. Generally, *indica* rice has more heat tolerance than *japonica* rice. High temperature mainly affects the pollen activity of *indica* rice and influences pollen germination and fertilization of *japonica* rice [2]. At present, farmers often choose rice varieties with yield as the main index, ignoring the high-temperature tolerance of rice varieties. An unreasonable sowing date means that the rice heading and flowering times overlap with megathermal climates. In the face of extreme heat, there is a lack of technical measures for prevention and control. Therefore, the creation of *indica* rice varieties adapted to high-temperature stress is of great significance for reducing the impact of high-temperature damage on rice production. In view of the above problems, this project analyzes meteorological data over nearly 60 years in four regions of Anhui Province (Jianghuai (JH), Yanhuai (YH), Yanjiang (YJ), Jiangnan (JN), Table S1) to reveal the level of occurrence of high-temperature damage. *Indica* rice varieties widely used in the middle and lower reaches of the Yangtze River were collected, and rice varieties with a strong tolerance to high temperature were screened by artificial simulation in the greenhouse and high-temperature stress in the field, to provide a basis for the breeding of rice varieties with heat tolerance and high, stable yields.

2. Materials and Methods

2.1. Data Investigation and Analysis

The daily maximum and average temperature values and other meteorological data from 1961 to 2018 were obtained from 45 ground meteorological stations in the Jianghuai area of Anhui Province (http://ah.cma.gov.cn/, accessed on 11 September 2022). The average annual number of high-temperature-damage days and the average annual number of stations in the Jianghuai region were statistically analyzed, as well as the trends in the annual historical series of high-temperature-damage data of the average annual number of days and the average annual number of stations.

2.2. Pot Experiment Design

This experiment was carried out in the Hefei Branch of the National Rice Improvement Center, Anhui Academy of Agricultural Sciences in 2017 (117°14′27″ E, 31°53′42″ N). The cultivars tested were 13 middle-*indica* rice varieties widely grown in the middle and lower reaches of the Yangtze River, including Fengliangyou-4 (FLY-4), Hui Liangyou-898 (HLY-898), Hui Liangyou-858 (HLY-858), Hui Liangyou-996 (HLY-996), Hui Liangyou-6 (HLY-6), Long Liangyou Huazan (LLYHZ), Long Liangyou-1988 (LLY-1988), Quan Liangyou-2118 (QLY-2118), Y Liangyou-900 (YLY-900), Y Liangyou-2 (YLY-2), Huang Huazan (HHZ), II You-838 (IIY-838) and ShenLiangyou-1813 (SLY-1813). Seeds were sown on May 14, seedlings were raised in the field, and seedlings with the same growth were transplanted to plastic buckets (length, width and height 24 cm × 22.5 cm × 21.5 cm, respectively) on June 11, with 2 holes in each bucket and 2 plants in each hole. Each bucket contained 10 kg of sun-dried and crushed paddy soil. We added water (10 L) 1 week before transplanting to activate the soil. Then, 1 day before transplanting, pure nitrogen 1.0 g, pure phosphorus 1.5 g and pure potassium 0.7 g were applied to each bucket and mixed as the base fertilizer. At 7 days after transplanting, 0.4 g pure nitrogen was applied per bucket as tiller fertilizer. At the young panicle differentiation stage, 0.6 g of pure nitrogen and 0.7 g of pure potassium were applied per bucket as a panicle fertilizer. Other management measures should be carried out according to the requirements of high-yield rice planting.

During the growth of the rice, when the panicle heads leaked from the leaves, the tillers or main stems with the same growth were selected for labeling. The plants were transferred to the greenhouse and subjected to high-temperature treatment for 7 days; normal temperature was used as the control (Table 1). At the end of treatment, the plants were grown to maturity at room temperature.

Table 1. The temperature settings at different times between high-temperature and normal- temperature treatment.

Treatment	07:00–09:00	09:00–11:00	11:00–16:00	16:00–19:00	19:00–07:00
High temperature	29.0 °C	35.0 °C	37.0 °C	32.0 °C	27.0 °C
Normal temperature	29.0 °C	32.0 °C	32.0 °C	32.0 °C	27.0 °C

2.3. Measurement Index and Pot Method

2.3.1. Seed Setting Percentage

The seed setting percentage of each variety treated with high temperature and normal temperature was determined using the water drift method at the mature stage. The seed setting percentage = filled grains/(filled grains + partially filled grains + unfilled grains) × 100%.

2.3.2. Relative Heat Tolerance Coefficient (HT)

See Equation (1):

$$HT = HTx/HTck \quad (1)$$

HTX: Identification of high-temperature seed setting percentage of varieties, unit %; HTCK: seed setting percentage of heat-tolerant control varieties at high temperature; the control cultivar was FLY-4, a medium *indica* rice with strong heat tolerance.

According to the identification standards of rice variety heat tolerance identification technical regulations [17] (Table 2), the heat tolerance of the varieties to be tested was determined according to the value of the relative coefficient HT.

Table 2. Classification standard for heat tolerance of rice varieties.

Classification	HT	Heat Tolerance
1	HT $\geq$ 1.1	Strong
3	0.9 $\leq$ HT < 1.1	Relatively strong
5	0.7 $\leq$ HT < 0.9	Normal
7	0.5 $\leq$ HT < 0.7	Relatively weak
9	HT < 0.5	Weak

2.4. Natural High-Temperature Experiment

This experiment was carried out in 2018 at the Lujiang Guohe Experimental Base, Rice Research Institute, Anhui Academy of Agricultural Sciences. Fifteen varieties, namely Fengliangyou-4 (FLY-4), Wandao-153 (WD-153), Huang Huazan (HHZ), 9You-6 (9Y-6), Shenliangyou-1813 (SLY-1813), Long Liangyou-1988 (LLY-1988), Long Liangyou Huazan (LLYHZ), Jiuliangyou Huanglizhan (JLYHLZ), Y Liangyou-900 (YLY-900), Y Liangyou-2 (YLY-2), II You-838 (IIY-838), Hui Liangyou-6 (HLY-6), Hui Liangyou-858 (HLY-858), Hui Liangyou-898 (HLY-898) and Hui Liangyou-996 (HLY-996), were selected. The experimental ground was an idle winter field, using moist seedling raising. According to the meteorological data and the growth period of varieties, the high-temperature stress was set by adjusting the sowing date. A total of 5 treatments were set for the sowing date, 7 days apart for each treatment (Table 3). There were 2 replicates for each variety $\times$ sowing date treatment, arranged in random blocks. The planting specifications of each plot were 4 rows $\times$ 10 plants, and the row spacing was 30 $\times$ 15 cm. A sampling investigation was carried out for two intermediate behavioral targets. The amount of nitrogen fertilizer applied in each treatment was 225 kg·ha^{-1}, the ratio of base fertilizer: tiller fertilizer: panicle fertilizer was 5:2:3, and all nitrogen fertilizer was applied in the form of urea. The application rate of P fertilizer was 75 kg·ha^{-1}, which was applied once in the form of superphosphate as the base fertilizer. The application rate of potassium fertilizer was 120 kg·ha^{-1}, which was applied twice in the form of potassium chloride as base fertilizer and spike fertilizer. Base fertilizer, tiller fertilizer and panicle fertilizer were applied 1 d before transplanting, and early tiller fertilizer 7 d after the transplanting and young panicle differentiation period. The high-yield cultivation method was adopted for water management, and disease, insect and grass damage were strictly controlled during the whole growth period to prevent yield loss.

Table 3. The rice sowing date and planting specifications.

Treatment	Seeding Time (Month.Day)	Transplanting Time (Month.Day)	Planting Specification
I	4.20	5.20	30 $\times$ 15 cm
II	4.27	5.27	30 $\times$ 15 cm
III	5.4	6.4	30 $\times$ 15 cm
IV	5.11	6.11	30 $\times$ 15 cm
V	5.18	6.18	30 $\times$ 15 cm

2.5. Measurement Index and Method

2.5.1. Record of Growth Period

The exact dates of sowing, transplanting, jointing, heading, ripening and harvest were recorded.

2.5.2. Record of Meteorological Data

The meteorological data included daily average temperature, maximum temperature, minimum temperature, rainfall, sunshine hours and photosynthetically active radiation.

2.5.3. Determination of Yield and Its Constituent Factors

Yield measurement at maturity stage was conducted according to the average number of panicles per single hole in each treatment, where two holes were selected to determine the number of grains per panicle. The water drift method was used to remove the empty grain and obtain the seed setting percentage. Then, 1000 solid seed samples (dry seeds) were weighed in triplicate (error not exceeding 0.05 g) to obtain 1000-seed weight. The mature period of each field cut square measured as real yield.

2.6. Statistical Analysis

We carried out three biological replicates for all measurements. The differences among the treatments were calculated and statistically analyzed using a one-way analysis of variance (ANOVA) by the least-significant-difference multiple-range test (LSD, $p < 0.05$). The statistical package for OriginPro 8.0 (OriginLab, Northampton, MA, USA) and the Data Processing System (DPS) version 7.05 (Zhejiang University, Hangzhou, China) was used for the statistical analysis.

3. Results

3.1. The time Regularity and Changing Trend of High-Temperature Damage

Based on the meteorological data of 45 ground meteorological stations in the Jianghuai rice region of Anhui Province, the megathermal climate from 1961 to 2018 was analyzed. The results showed that the megathermal climate along the Huaihe region (Yanhuai, YH) and the central part of the Jianghuai (JH) region mainly occurred from late July to early August and extended to the middle of August in the Jiangnan (JN) region and along the Yangtze River (Yanjiang, YJ) (Figure 1). Based on these meteorological data, climate series data of high-temperature damage during the heading and flowering period (late July to mid-August) of middle-rice were constructed. The results show that the average number of days and average station times of high-temperature damage show a single valley pattern, that is, the degree of high-temperature damage decreased from 1961 to 1980 and increased after 1980. The fluctuation term shows great interannual differences, but after 2000, the fluctuation terms of the average annual number of days of high-temperature damage, the average annual number of stations with a gradual development trend and the fluctuation range of the average annual number of stations become significantly smaller (Figure 2). This indicates that since the 21st century, especially since 2010, the occurrence frequency and spatial coverage of high-temperature damage have increased.

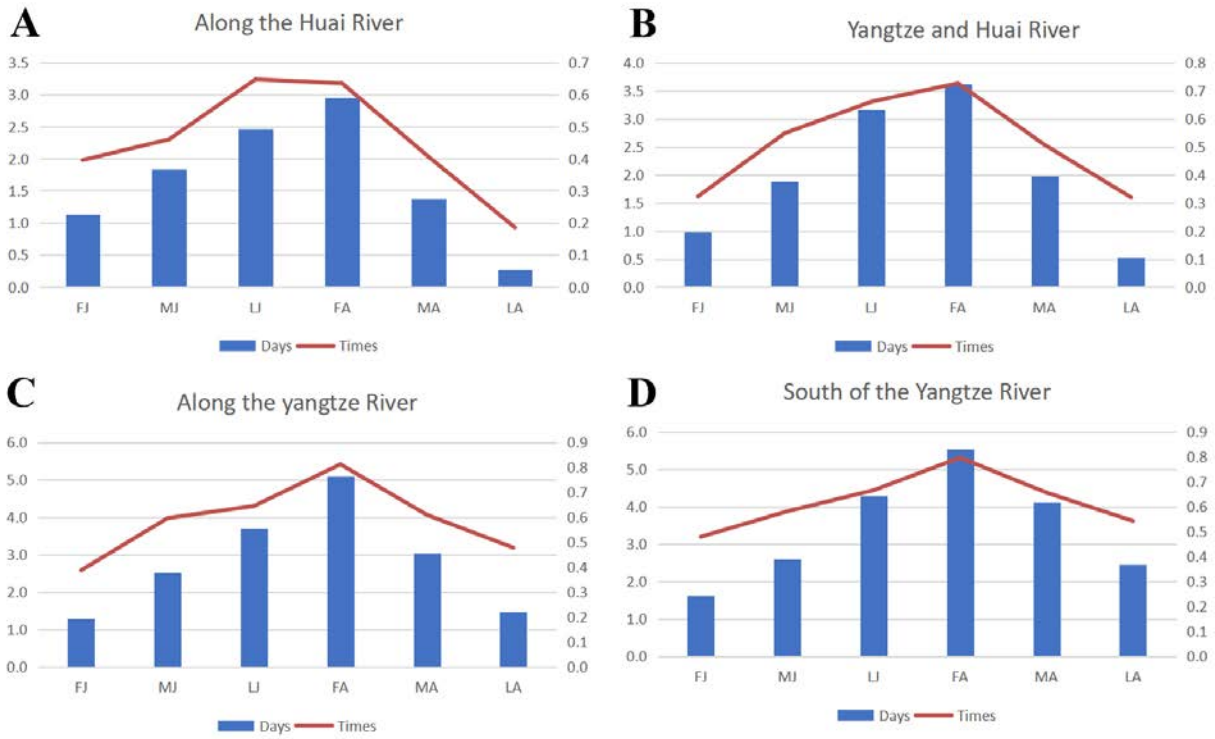

Figure 1. Timing and frequency of high-temperature damage in different subareas of rice at the flowering and panicle stages. (**A**) The area along the Huai River (Yanhuai, YH); (**B**) the area in Yangtze and the Huai River (Jianghuai, JH); (**C**) the area along the Yangtze River (Yanjiang, YJ); (**D**) the area south of the Yangtze River (Jiangnan, JN).

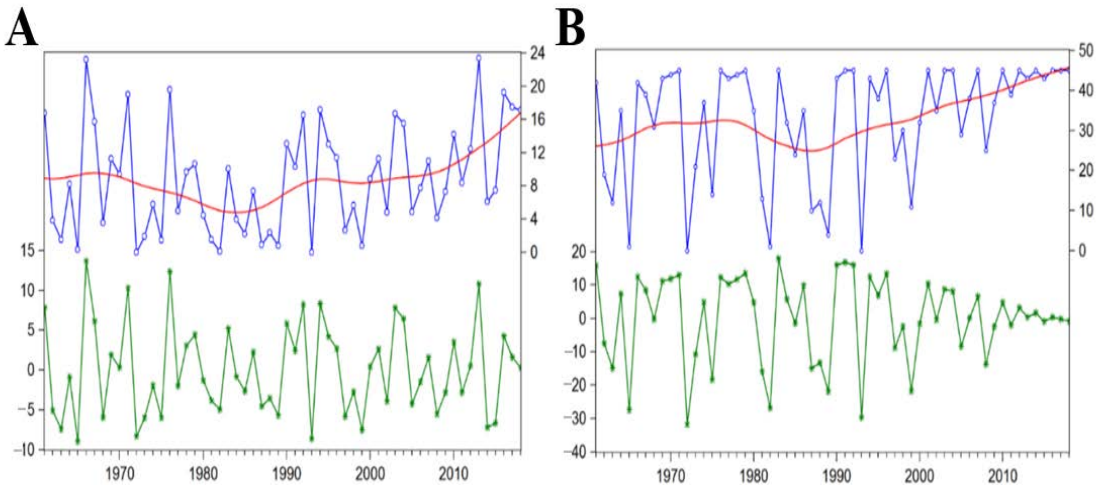

Figure 2. Interannual variation of high-temperature damage during heading and flowering of middle rice in Jianghuai from 1961 to 2018. The abscissa represents the year. (**A**) Average day per year. The green line indicates the fluctuation term. The red line indicates the trend term. The blue line indicates the average days per year. (**B**) Annual average number of sites. The green line indicates the fluctuation term. The red line indicates the trend term. The blue line indicates the annual average number of sites.

3.2. Spatial Regularity and Changing Trends of High-Temperature Thermal Damage

There were some differences in the degree of high-temperature damage in different regions of Jianghuai. The annual frequency of high-temperature damage over more than 3 days and the annual average number of thermal damage days showed obvious zonal

distribution. The annual frequency of high-temperature damage was 0.7–1.1 times in the Huaihe region, 1.0–1.3 times in the central Jianghuai region and 1.4–1.5 times along the Yangtze River. Days of occurrence per year were 4–7 days in the Huaihe region, 8–13 days in the central Jianghuai region and 11–16 days along the Yangtze River (Figure 3). In other words, the annual frequency and average annual days of high-temperature damage were higher in the south and lower in the north.

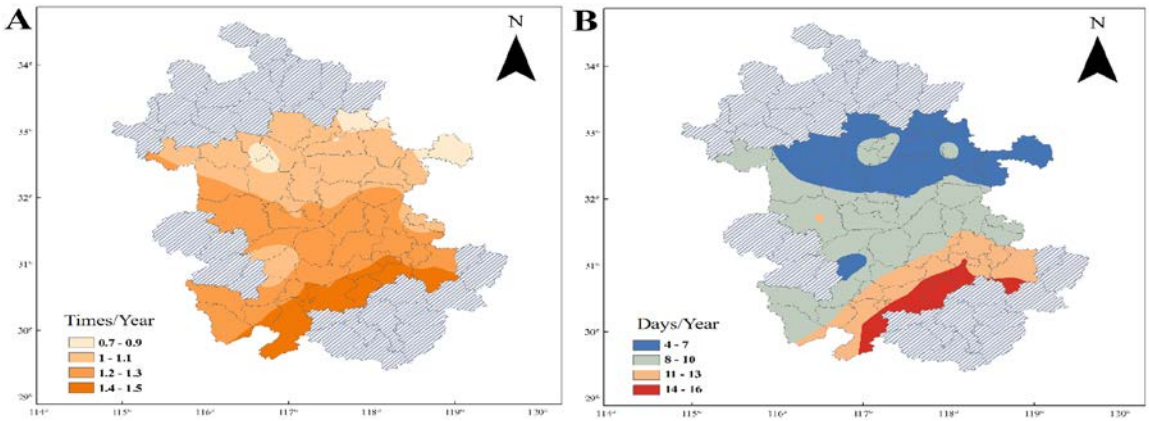

Figure 3. Spatial distribution of high-temperature damage during heading and flowering of mid-season rice in Jianghuai from 1961 to 2018. (**A**) Frequency per year. (**B**) The number of days per year.

3.3. Effect of High Temperature on Seed Setting Percentage of Potted Rice

High temperature significantly reduced the seed setting percentage of the tested varieties. Compared with normal-temperature treatment (CT), the seed setting percentage of the tested varieties under high-temperature treatment (HT) decreased by 31.6%. There are some differences in the high-temperature tolerance of different varieties. Under normal-temperature treatment, the seed setting percentage of the tested varieties was 83.5–87.5%, and there were no significant differences (Figure 4). Compared with normal temperature, the seed setting percentage of all varieties under high temperature decreased by 15.9–50.2%. Among them, FFLY-4, HHZ, LLYHZ, LLY-1988 and HLY-996 had relatively small decreases of 15.9–24.2%. SLY-1813 and HLY-6 decreased by 42.2% and 50.3%, respectively.

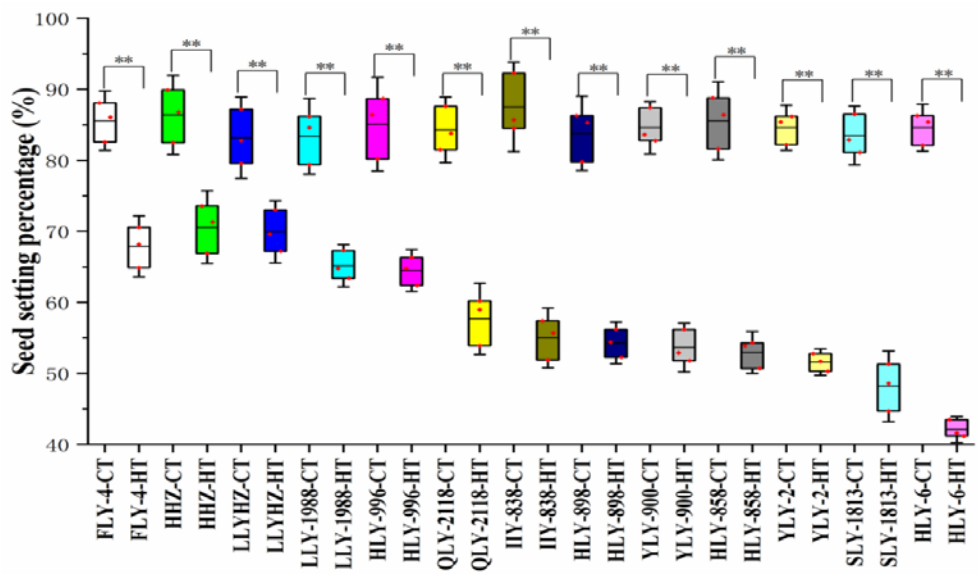

Figure 4. Seed setting percentage of different varieties under high-temperature and normal-temperature treatment. NT: Normal-temperature treatment. HT: High-temperature treatment. Error bars are the standard deviation (±SD) of three biological replicates. ** $p < 0.01$ by Student's *t*-test.

3.4. Relative Heat Tolerance Coefficient of Different Varieties

Under high-temperature treatment, the seed setting percentage of the tested varieties ranged from 42.1% to 70.6%, and the variation was 15.3%. Taking Fengliangyou-4 as the control, the relative heat tolerance coefficients of different varieties were different. The HT values of HHZ, LLYHZ, LLY-1988 and HLY-996 were between 0.9 and 1.1, showing strong heat tolerance. The HT of Huiliangyou-6 was 0.62, less than 0.7, indicating that the heat tolerance of this variety was weak. The heat tolerance of other varieties was of a general grade (Figure 5, Table S2).

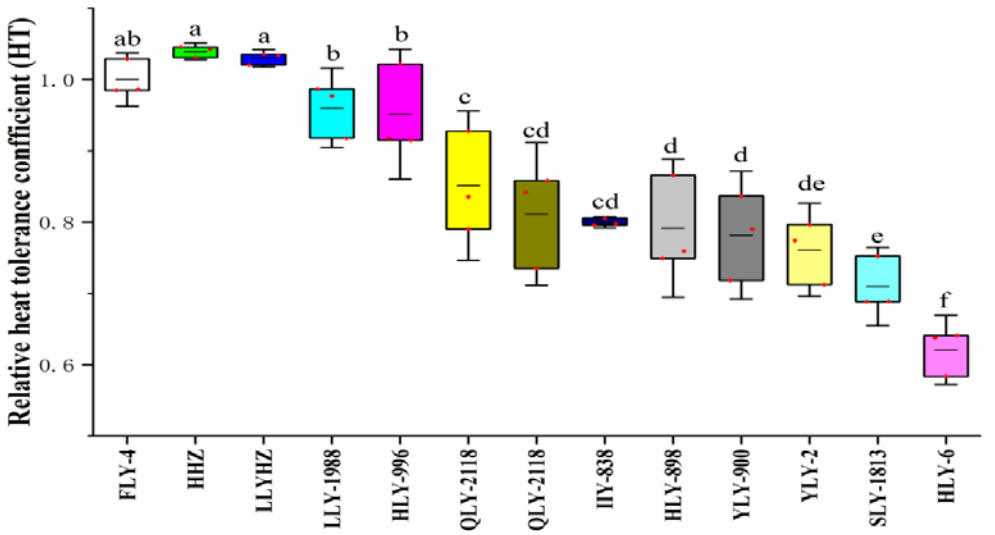

Figure 5. Relative heat tolerance of different varieties. Error bars are the standard deviation (±SD) of three biological replicates. Columns with different letters are significantly different (LSD test, $p < 0.05$). Error bars are the standard deviation (±SD) of three biological replicates.

3.5. Effects of High Temperature on Rice in the Field

3.5.1. Meteorological Data of Rice at Full Heading Stage

Rice heads were concentrated from July 20th to August 20th; high temperatures occur from late July to mid-August, with daily average temperatures above 30 °C and daily maximum temperatures above 35 °C (Figure 6).

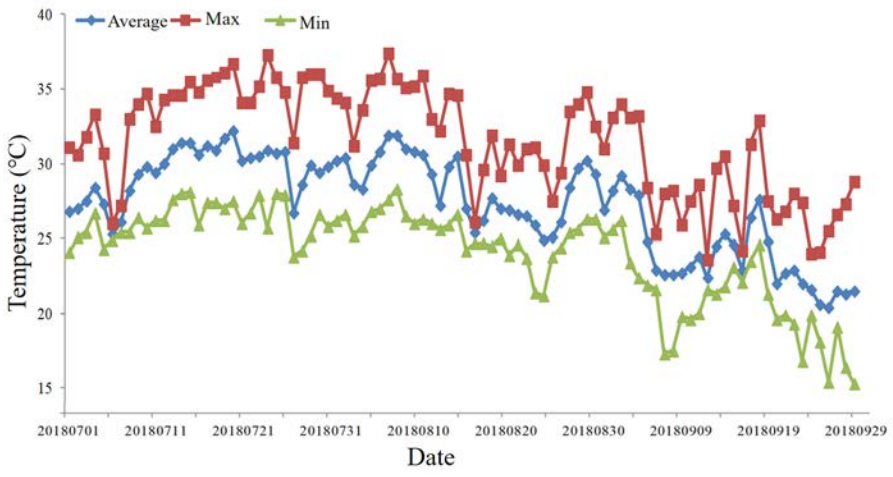

Figure 6. Meteorological data of rice at full heading stage. Average: average temperature. Max: maximum temperature. Min: minimum temperature.

3.5.2. Effects of Different Sowing Date Treatments on Main Growth Period of Rice

With the delay of sowing date, the jointing stage, heading stage and maturity stage were delayed, and the whole growth period was shortened (Table S3). The full heading stage of rice was distributed from 20 July to 22 August, and the mature stage was distributed from 5 September to 25 September. Compared with the first sowing date (20 April) and the fifth sowing date (18 May), the full heading stage was 12–15 d different, the mature stage was 5–11 d different and the entire growth period was 16–23 d shorter. The full heading to maturity period was shortened by 3–7 days.

3.5.3. Effect of Sowing Date on Yield of Different Rice

With the delay of sowing date, the yield of different varieties first decreased, then increased and then decreased again (Table 4). LLY-1988, LLYHZ, 9Y-6, FLY-4, HHZ and YLY-2 had the highest yield, and the average yield reached more than 675 kg·mu^{-1}.

Table 4. Yield and variation of different varieties at different sowing dates.

Varieties	Yield (kg·ha^{-1})					Average (kg·ha^{-1})	CV (%)
	T1	T2	T3	T4	T5		
LLYHZ	11,407.5	10,906.5	10,396.5	11,083.5	10,843.5	10,927.5 ± 368.8	3.38
9Y-6	11,886.0	10,174.5	10,828.5	10,242.0	10,234.5	10,673.1 ± 728.4	6.82
LLY-1988	11,277.0	9999.0	10,681.5	10,354.5	10,641.0	10,590.6 ± 471.1	4.45
FLY-4	11,151.0	9429.0	9666.0	10,855.5	11,662.5	10,552.8 ± 965.7	9.15
HHZ	10,264.5	8706	10,249.5	10,621.5	10,812.0	10,130.7 ± 831.7	8.21
YLY-2	9831.0	10,186.5	10,777.5	10,237.5	9618.0	10,130.1 ± 831.69	4.37
HLY-898	11,508.0	9240.0	9544.5	10045.5	9099.0	9887.4 ± 975.9	9.87
SLY-1813	11,359.5	9049.5	9970.5	9990.0	9027.0	9879.3 ± 952.2	9.64
WD-153	10,368.0	9238.5	9885.0	9360.0	9865.5	97,473.4 ± 454.7	4.67
HLY-858	11,541.0	9091.5	9268.5	10,258.5	8062.5	9644.4 ± 1315.6	13.64
HLY-996	10,761.0	9208.5	9361.5	9708.0	8619.0	9531.6 ± 791.9	8.31
YLY-900	10,689.0	8119.5	9171.0	9145.5	9910.5	9407.1 ± 959.0	10.19
JLYHLZ	8778.0	10,035.0	8227.5	10,089.0	9286.5	9283.2 ± 803.8	8.66
HLY-6	11,005.5	8650.5	9073.5	9526.5	7414.5	9134.1 ± 1308.7	14.33
IIY-838	8883.0	8469.0	8781.0	9322.5	8764.5	8844.0 ± 308.8	3.49

Note: As the differences between rice varieties are compared in this study, it is not suitable to use standard deviation (±SD) for comparative analysis, because they are all absolute indicators, and their value is not only affected by the degree of differences among different rice varieties. Therefore, we used the coefficient of variation to eliminate the effect of the degree of variation of different rice varieties, and we listed the mean and standard deviation (±SD) together.

According to the coefficient of variation yield for different varieties at different sowing dates, LLYHZ, 9Y-6, LLY-1988, FLY-4 and HHZ had strong high-temperature tolerance and high and stable yields. Their average yields at different sowing dates were 10,927.5, 10,673.1, 10,590.6, 10,552.8 and 10,130.7 kg·ha^{-1}, respectively. The coefficients of variation of yield were 3.38%, 6.82%, 4.45%, 9.15% and 8.21%, respectively (Table 4).

3.5.4. Response Characteristics of Yield Composition to Temperature of Varieties with Different Heat Tolerance

The yield compositions for five heat-tolerant cultivars and five heat-sensitive cultivars were compared under different sowing dates to analyze the response characteristics of yield composition of different heat-tolerant cultivars to temperature. The coefficient variation of effective panicle number per acre and 1000-grain weight of heat-tolerant varieties such as LLYHZ, 9Y-6, LLY-1988, FLY-4 and HHZ was larger than that of heat-sensitive varieties under different sowing dates, but the coefficient of variation of grain number per panicle and seed setting percentage was smaller (Table 5).

Table 5. Yield composition characteristics of heat-tolerant cultivars under different sowing dates.

Varieties	PNM (104)	CV (%)	KPS	CV (%)	SSP (%)	CV (%)	TSW (g)	CV (%)
Heat-tolerant varieties								
LLYHZ	18.3 ± 1.98	10.8	207.2 ± 16.78	8.1	85.8 ± 20.00	4.3	23.3 ± 0.35	1.5
9Y-6	17.6 ± 1.90	10.8	181.0 ± 20.63	11.4	88.1 ± 23.08	5.1	26.2 ± 4.32	16.5
LLY-1988	18.7 ± 1.27	6.8	187.0 ± 13.65	7.3	85.1 ± 21.87	4.4	25.7 ± 0.59	2.3
LLY-4	16.4 ± 0.67	4.1	182.3 ± 4.74	2.6	85.3 ± 24.14	4.0	28.3 ± 0.53	1.9
HHZ	17.6 ± 1.21	6.9	194.3 ± 23.31	12.0	85.2 ± 20.62	4.6	24.2 ± 3.34	13.8
Mean		7.9		8.3		4.5		7.2
Heat-sensitive variety								
YLY-900	15.9 ± 1.16	7.3	238.5 ± 20.03	8.4	76.2 ± 17.75	3.7	23.3 ± 0.09	0.4
HLY-858	16.8 ± 1.18	7.0	201.9 ± 28.06	13.9	84.0 ± 20.41	4.7	24.3 ± 0.66	2.7
YLY-2	17.5 ± 1.07	6.1	219.0 ± 9.20	4.2	81.6 ± 19.18	8.6	23.5 ± 0.63	2.7
SLY-1813	18.0 ± 1.49	8.3	218.0 ± 40.77	18.7	76.3 ± 18.31	6.9	24.0 ± 1.06	4.4
HLY-6	17.1 ± 0.68	4.0	178.1 ± 30.99	17.4	78.5 ± 21.59	7.8	27.5 ± 0.99	3.6
Mean		6.6		12.5		6.3		2.8

Note: PNM: panicle number per mu. KPS: kernels per spike. SSP: seed setting percentage. TSW: thousand-seed weight. As the differences between rice varieties are compared in this study, it is not suitable to use standard deviation (±SD) for comparative analysis, because they are all absolute indicators and their value is not only affected by the degree of differences among different rice varieties. Therefore, we used the coefficient of variation to eliminate the effect of the degree of variation of different rice varieties, and we listed the mean and standard deviation (±SD) together.

According to the comprehensive analysis, LLYHZ, 9Y-6, LLY-1988, FLY-4 and HHZ were the single-season *indica* rice varieties with high yield and high-temperature tolerance suitable for planting in the Yangtze–Huaihe River basin.

4. Discussion

4.1. Occurrence Regularity of High-Temperature Damage in Anhui Rice Region and Reasonable Growth Process of Rice in Different Subregions

Based on the analysis of meteorological data from 1961 to 2018, it was clear that heat damage caused by high temperatures mainly occurred in late July and mid-August in the rice area of Anhui Province. Since 2000, heat damage caused by high temperatures at the heading and flowering stage of middle-rice in the Jianghuai region has shown an increasing trend. The heading and flowering period is the most sensitive period to high temperatures and the most serious period of high-temperature damage. The booting to anthesis temperature mainly affects the normal development of rice pollen; a high temperature reduces completion of the rice floral organ development, and causes decreases in pollen dysplasia, pollen life expectancy and pollen vitality. High temperatures also cause poor powder and fertilization and reduce the fertile pollen rate and anther dehiscence rate, resulting in pollen on the stigma becoming blocked. This causes new sterility and fertilization rates, and finally, the husk of the rice is increased, the seed setting percentage is decreased and the yield is decreased [18,19]. Mohammed et al. [20] found that compared with a normal night temperature (27 °C), a higher night temperature (32 °C) decreased the pollen germination rate of rice, resulting in lower seed setting percentage and yield. The spikelet setting rate of rice was not only related to the intensity of high-temperature treatment, but also decreased gradually with the increase in sustained high temperature [21]. Therefore, previous studies have confirmed that high-temperatures seriously harm the growth and development of rice at the heading and flowering stage. The period of high-temperature damage in the rice area of Anhui Province is close to the heading and flowering period of rice, which greatly threatens the safety of rice production. Therefore, the breeding of rice varieties with high-temperature tolerance is very important.

4.2. A number of Heat-Tolerant Varieties were Screened out by the Pot Experiment

The grain-filling period of rice is both the key period of yield formation and the sensitive period of heat damage caused by high-temperatures [22]. Our results showed that high temperatures during the grain filling period would lead to a reduction in leaf assimilation ability, the acceleration of rice senescence, the shortening of the grain filling period, an increase in the empty hull rate and a decrease in the seed setting percentage and yield [23]. Morita et al. studied endosperm cells under high-temperature treatment and found that high-temperature treatment reduced the duration of grain growth, i.e., the grain filling period was shortened. High-temperatures also reduced the size of endosperm cells, resulting in lower grain weight at high temperatures [21]. Grain fullness decreased after high-temperature treatment, but there were significant genotypic differences among different cereal crop varieties in response to high temperatures [24]. In this study, the relative heat tolerance coefficient of different varieties was selected through the effect of high temperature on seed setting percentage of potted rice. Of course, all research should be reflected in the integration and application of technology models and systems. On this basis, the effects of high-temperature stress on yield of different rice varieties were further investigated in this study.

4.3. Effects of High-Temperature Stress on Yield of Different Rice Varieties

At present, the research on the heat damage index of high-temperature stress focuses on two aspects: temperature and time of damage. Previous studies have found that compared with the vegetative growth stage, the reproductive growth stage is more vulnerable to high-temperature damage, and most studies focus on the late stage of young panicle differentiation [25], the flowering stage [26] and the grain filling stage [27]. High temperatures during these periods will seriously affect the yield and quality of rice. Peng et al. [28] found that for every 1.0 °C increase in the average minimum temperature during the dry season in tropical regions, the yield decreased by 10%. Previous studies have confirmed that high-temperature stress affects pollen development, anther dehiscence and spikelet fertility [20], as well as physiological and biochemical processes such as assimilate accumulation and transport, grain filling and starch synthesis, ultimately leading to a decrease in seed setting percentage and yield [29,30]. This study also found that there were significant differences in yield components among rice varieties with different heat tolerances under different sowing dates. This result is consistent with previous studies. The rice yield is affected by the strength of "source", "sink" and "flow" and the degree of coordination among them [31,32]. Therefore, the effects of high-temperature stress on rice yield and its components vary with regions, varieties and time of occurrence.

5. Conclusions

Analyzing the meteorological data of Anhui Province in the past 60 years showed that high temperatures in the Huaihe region and along the central part of the Yangtze River mainly occur from late July to early August and extend to the middle of August in the Jiangnan region along the Yangtze River. Since 2000, the frequency and degree of high-temperature damage in the middle rice heading and flowering period in the Yangtze River have been increasing. Five *indica* rice varieties with strong high-temperature tolerance, relatively high yields and stable yields were selected after laboratory-simulated high-temperature experiments and natural high-temperature experiments in the field: LLYHZ, 9Y-6, LLY-1988, FLY-4 and HHZ. These are suitable for planting in the middle and lower reaches of the Yangtze River (Figure 7). This study provides a theoretical reference and scientific basis for the study of rice under high-temperature stress.

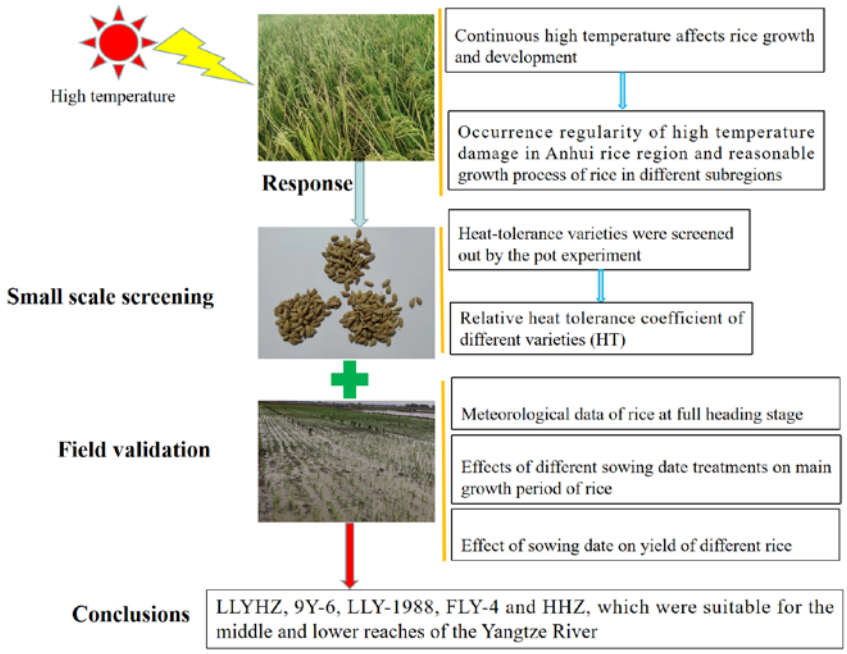

Figure 7. Screening of heat-tolerant *indica* rice varieties in the middle and lower reaches of the Yangtze River.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12102462/s1. Table S1: The four regions of Anhui Province. Table S2: The HT of different varieties. Table S3: Survey of rice growth progress at different sowing dates.

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Data Availability Statement: All data generated during this study are included in this published article and its Supplementary Information Files, and the raw data used or analyzed during the current study are available from the corresponding author on reasonable request.

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