Postprint: A Method for Evaluating Heat Tolerance in Hybrid Rice Using Flowering Proportion

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Abstract

Selecting rice varieties with strong heat tolerance at the flowering stage is one of the effective approaches to mitigate significant yield reduction caused by extreme natural high temperatures. To improve the screening techniques for heat tolerance in rice varieties, four hybrid rice varieties were used as materials in 2013 and 2014. Using the pot cultivation method, potted rice plants were moved to an artificial climate chamber at 38.0 ℃ for 5 h at different times before and after flowering, with outdoor normal temperature (daily maximum canopy temperature of 31.2–32.8 ℃) treatment as the control, to investigate the effects of extreme high temperature periods on seed setting rate. In 2015 and 2016, five staggered sowing experiments were conducted using 40 hybrid mid-season rice combinations as materials. During the period when rice canopy temperature reached above 35 ℃, 20 panicles that started heading simultaneously and grew uniformly were selected from the five sowing dates, tagged, and the flowering proportion at different time periods was observed to study the relationship between heat tolerance ability during flowering stage and flowering proportion at different times among varieties. The results showed that the flowering proportion before 11:30 under high temperature during the flowering stage and the flowering proportion before 12:00 under normal temperature were highly significantly positively correlated with the heat tolerance index, respectively. Heat-tolerant varieties primarily enhanced their heat tolerance index indirectly through improved seed setting rate under high temperature, while seed setting rate under high temperature directly affected the heat tolerance index. Varieties that flowered early under high temperature also flowered early under normal temperature. The flowering time of hybrid rice was mainly concentrated between 9:30 and 12:30, with varying proportions of flowering at different times among varieties. Exposure to high temperature 2 h after flowering had no significant effect on seed setting rate; the earliest occurrence times of canopy temperature reaching 34 ℃ and 35 ℃ with frequency above 80% were 13:30

and 15:30, respectively. Selecting varieties with high flowering proportion 2 h before the occurrence of daily maximum temperature $ $34 ℃ or 35 ℃ can effectively avoid heat injury. Based on the above analysis, regression models for predicting and identifying the heat tolerance index were established using three indicators: flowering proportion before 11:30 under high temperature during flowering stage, flowering proportion before 12:00 under normal temperature, and seed setting rate under high temperature, with accuracy rates (predicted value/measured value) as high as 92.29%-102.73%. This provides a scientifically applicable new method for identifying heat tolerance during the flowering stage of rice varieties.

Full Text

Identification Method of High Temperature Resistance of Hybrid Rice Based on Flowering Rate

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Abstract: Selecting rice cultivars with strong high temperature resistance during the flowering period is an effective approach to mitigate yield losses caused by extreme natural heat stress. To improve identification techniques for high temperature resistance in rice varieties, we conducted experiments in 2013 and 2014 using four hybrid rice cultivars grown in pots. Potted rice plants were moved to an artificial climate chamber at 38.0°C for 5 hours at different times before and after flowering, with outdoor normal temperature (daily maximum temperature of panicle layer 31.2–32.8°C) as the control, to investigate the effects of extreme high temperature timing on seed setting rate. In 2015 and 2016, field experiments were conducted using 40 hybrid mid-season rice combinations with five sowing dates. During periods when panicle layer temperature reached above 35°C, 20 uniformly growing panicles that began heading simultaneously were selected from the five sowing dates and tagged to observe flowering proportions at different times, examining the relationship between high temperature resistance and flowering patterns among varieties. The results showed that flowering proportions before 11:30 under high temperature and before 12:00 under normal temperature were both extremely significantly positively correlated with the high temperature resistance index. High temperature-resistant varieties primarily enhanced their resistance index indirectly by improving seed setting rate under high temperature, while seed setting rate under high temperature directly affected the resistance index. Varieties that flowered early under high temperature also flowered early under normal temperature. Hybrid rice flowering was mainly concentrated between 9:30–12:30, with varying proportions among varieties at different times. High temperature exposure 2 hours after flowering had no significant effect on seed setting rate. When panicle layer temperature reached 34°C and 35°C with frequencies exceeding 80%, the earliest occurrence times were 13:30 and 15:30, respectively. Selecting varieties with high flowering proportions 2 hours before the occurrence of daily maximum temperature \$ \$34°C or 35°C could effectively avoid heat damage. Based on these analyses, regression models were established to predict the high temperature resistance index using three indicators: flowering proportion before 11:30 under high temperature, flowering proportion before 12:00 under normal temperature, and seed setting rate under high temperature. The prediction accuracy (predicted value/observed value) reached 92.29%–102.73%. This study provides a scientifically sound and practical new method for identifying high temperature resistance in rice varieties during the flowering period.

Keywords: Hybrid rice; High temperature stress; Flowering time; Flowering ratio; Seed setting rate; High temperature resistance index; Identification method

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1. Materials and Methods

Experiments were conducted at the winter-flooded paddy field and intelligent artificial climate chamber of the Rice and Sorghum Research Institute, Sichuan Academy of Agricultural Sciences in Luxian County. The experimental field had uniform soil texture with medium-to-high fertility. Luxian County is a typical high temperature and summer drought region, with \$ \$10°C effective accumulated temperature of 5,500°C and annual average temperature of 18–18.6°C. During the heading to maturity period of large-area hybrid mid-season rice, the average daily maximum temperature is 31.9–32.6°C and average daily minimum temperature is 22.5–23.6°C, with frequent high temperature damage above 35°C during the flowering period.

1.1. Effects of High Temperature Timing on Panicle Layer Temperature Dynamics and Seed Setting Rate

Pot experiments were conducted in an intelligent artificial climate chamber (manufactured by Beijing Yisheng Taihe Technology Co., Ltd.) with temperature precision of $\$\pm0.5°C.Drypaddysoil(pH6.62, organicmatter22.34g \cdot kg^{\{-1\}, totalnitrogen1.58g \cdot kg}\{-1\}, totalphosphorus0.74g \cdot kg^{\{-1\}, totalpotassium11.54g \cdot kg}\{-1\}, availablenitrogen147.6mg \cdot kg^{\{-1\}, availablephosphorus85.4mg \cdot kg}\{-1\}, availablepotassium163.1mg \cdot kg\hat{}\{-1\}\$$) was collected, crushed and mixed evenly on a drying field, then 4 kg of soil was placed in each plastic pot (rectangular prism, 33 cm high, 30 cm long, 20 cm wide).

In 2013 and 2014, four hybrid mid-season rice combinations were used as experimental materials: ‘Q You 1’ , ‘Chuangu You 7329’ , ‘Rong You 908’ , and ‘Hua Xiang 7’ . Seeds were sown on March 7, and seedlings were raised with plastic film. At 4.5-leaf stage, seedlings were transplanted to pots. Nitrogen was applied at 1.5 g per pot, with 50% as basal fertilizer, 20% as tillering fertilizer, and 30% as panicle fertilizer. Phosphorus and potassium fertilizers were applied as basal fertilizer at a ratio of N:P_2O_5:K_2O = 1:0.5:1.0. Each cultivar was planted in 13–15 pots, with 3 hills per pot and 2 plants per hill. At the heading stage, one day before high temperature treatment at 17:00, a section of panicles that would flower the next day was selected and tagged at both ends (with opened spikelets removed). High temperature treatments were applied at 5 hours before flowering (6:00), at flowering (around 11:00, when most varieties were observed to flower heavily), 1 hour after flowering (12:00), 1.5 hours after flowering (12:30), and 2 hours after flowering (13:00). Ten minutes before high temperature treatment, unopened spikelets within the tagged section from the previous day were cut off, with 1,320–1,680 spikelets marked in 6 replicates. Three replicates of pots were moved to the artificial climate chamber for 5 hours of high temperature treatment (relative humidity 85%, temperature 38.0°C), while the other three replicates were placed outdoors under normal temperature (daily maximum temperature of panicle layer 31.2–32.8°C). During high temperature treatment, a shallow water layer was maintained in the pots. After treatment, pots were moved back outdoors for management. At maturity, seed setting rate and high temperature resistance index were investigated for grains within the tagged sections (high temperature resistance index = seed setting rate under high temperature / seed setting rate under normal temperature).

1.2. Relationship Between Natural High Temperature Effects on Seed Setting Rate and Flowering Habits Among Varieties

Field experiments were conducted using 40 hybrid mid-season rice combinations that had passed regional trials. In both 2015 and 2016, sowing date experiments were established on March 5, March 25, April 20, May 4, and May 24 (to ensure at least one sowing date would encounter natural high temperature during heading). Seedlings were raised with plastic film wet-bed method and transplanted at 4.5-leaf stage. Field planting followed a 30 cm × 20 cm spacing with 2 plants per hill. Nitrogen was applied at 150 $\mathrm{kg \cdot hm^{-2}}$ (basal:tillering:panicle = 5:3:2), while phosphorus and potassium were applied as basal fertilizer at N:P_2O_5:K_2O = 1:0.5:0.8. Each cultivar was transplanted with 120 hills per sowing date in a split-plot design with three replicates, using sowing date as the main plot and hybrid combination as the subplot.

Natural high temperature treatments were selected from the sowing dates when the daily maximum temperature of the panicle layer reached 35–37.7°C during the rice flowering period in 2013–2016. Obvious high temperature damage (daily maximum temperature $ $35°C) stable periods were observed on July 15 and July 28 in 2015 and 2016. During these high temperature periods, among the five sowing dates, only the March 25, 2015 sowing and April 20, 2016 sowing had the majority of hybrid combinations at the heading stage. Therefore, 20 uniformly growing panicles that began heading simultaneously were selected and tagged from the 40 hybrid combinations sown on March 25, 2015 and April 20, 2016, with 6–8 panicles tagged per combination per replicate and three replicates. Although the 40 test varieties were identical in both years, their growth periods varied between years, resulting in only 14 common combinations that encountered high temperature simultaneously in both years. During the high temperature period, the number of flowering spikelets was recorded continuously for 4 days at three time intervals: before 11:00, 11:00–11:30, and 11:30–12:00 (with a small cut on the opened spikelet as a marker). At maturity, all tagged panicles from the 20 selected combinations were sampled to investigate panicle traits and calculate the high temperature resistance index for each hybrid combination.

To observe panicle layer temperature dynamics, mercury thermometers were hung at panicle height in the experimental field using bamboo poles during the rice heading period. Daily maximum temperatures were recorded, and when temperatures above 35°C were observed combined with sunny weather forecasts, the following day was designated as a high temperature stable period. During stable periods with temperatures above 35°C, panicle layer temperatures were observed continuously from 7:30 to 15:30 (averaged across 3 observation points in the field) and recorded hourly.

Additionally, the same 20 combinations tagged during high temperature days were sampled from the March 5 sowing (heading under normal temperature), with 5 hills sampled per combination and three replicates. Panicle traits were investigated to calculate the high temperature resistance index for each hybrid combination.

All experimental data were analyzed for variance, correlation, and regression using the DPS data processing system and Microsoft Excel.

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2. Results

2.1. Relationship Between Flowering Dynamics and High Temperature Resistance Among Hybrid Rice Varieties

The experimental results (Table 1) revealed significant or extremely significant differences among different hybrid rice varieties in seed setting rates under both normal and high temperature conditions, as well as in high temperature resistance indexes (F-values ranged from 4.23* to 10.54**). The high temperature resistance index was extremely significantly positively correlated with seed setting rate under high temperature (r-values of 0.9605**–0.9841**).** Varieties with high temperature resistance indexes above 0.8 included ‘Jingyou 127’ , ‘Q You 1’ , and ‘Chuannong You Huazhan’ .

Significant or extremely significant differences were observed among hybrid varieties in flowering proportions at different time periods under both high and normal temperature conditions (F = 3.56*–6.01**). Flowering proportions

before 11:30 and 12:00 under high temperature, and before 12:00 under normal temperature, were significantly or extremely significantly positively correlated with the high temperature resistance index (r-values of 0.4876-0.7322**) (Table 2). Regression analysis of data from Tables 1 and 2 indicated that flowering proportion before 11:30 under high temperature (X2) and flowering proportion before 12:00 under normal temperature (X6) had extremely significant effects on the high temperature resistance index (Table 3). These trends were consistent across both years.

2.2. Reasons Why Flowering Dynamics Affect High Temperature Resistance in Hybrid Rice

The experimental results (Table 4) demonstrated that high temperature 5 hours before flowering had no significant effect on seed setting. The sensitive period for high temperature damage occurred at flowering time, causing the most significant impact on fertilization and resulting in markedly reduced seed setting rates. The effects of high temperature at different times after flowering varied among varieties. Among the four tested varieties, only 'Q You 1' showed no significant effect on seed setting when high temperature was applied 1 hour after flowering, while 'Q You 1' and 'Chuangu You 7329' were unaffected at 1.5 hours after flowering. None of the four varieties were significantly affected by high temperature treatment 2 hours after flowering.

Panicle layer temperature dynamics and frequency during 16 high temperature days across the four experimental years are shown in Figure 1 [Figure 1: see original paper] and Figure 2 [Figure 2: see original paper]. Figure 1 shows that temperature increased from 7:30 to 15:30, with frequencies above 33°C, 34°C, and 35°C reaching over 80% at 11:30, 13:30, and 15:30, respectively (Figure 2). Hybrid rice flowering occurred mainly between 9:30–12:30, with different varieties showing varying flowering proportions at different times. Current research consistently indicates that daily average temperature of 30°C or daily maximum temperature $ $35°C is the critical temperature threshold for high temperature damage during rice flowering [1-2]. Therefore, selecting varieties with high flowering proportions 2 hours before the occurrence of daily maximum temperature $ $34°C or 35°C could effectively avoid heat damage.

Since varieties that flowered early under high temperature also flowered early under normal temperature (Figure 3 [Figure 3: see original paper]), varieties with high flowering proportions before 11:30 under high temperature and before 12:00 under normal temperature exhibited higher high temperature resistance indexes.

2.3. Accuracy of Using Flowering Proportion to Predict High Temperature Resistance in Hybrid Rice

To establish an indirect identification method for high temperature resistance in rice varieties, regression analysis was performed using the two-year average experimental data for the three factors that significantly influenced the high temperature resistance index: flowering proportion before 11:30 under high temperature, flowering proportion before 12:00 under normal temperature, and seed setting rate under high temperature. The coefficient of determination for the regression equations reached 71.46%-95.49%, with correlation coefficients at extremely significant levels (r-values of 0.8453-**0.9772**). Model validation using root mean square error (RMSE) between measured and predicted values [18] showed RMSE of 0.41%-1.12%, indicating good consistency between measured data and predicted values (Table 5). These regression equations can therefore be used as indirect indicators for evaluating high temperature resistance indexes.

To further verify the reliability of these regression equations, the 2015 measured data for the 14 common varieties under high temperature treatment (flowering proportion before 11:30 under high temperature, flowering proportion before 12:00 under normal temperature, and seed setting rate under high temperature) were used to predict their high temperature resistance indexes. The predicted values were then compared with the 2016 measured high temperature resistance indexes for these 14 varieties using RMSE validation, which showed RMSE of 0.79%-2.10% and prediction accuracy (predicted value/measured value) of 92.29%-102.73% (Table 6).

Multiple regression analysis of the high temperature resistance index (y) with flowering proportion before 11:30 under high temperature (x1), flowering proportion before 12:00 under normal temperature (x2), and seed setting rate under high temperature (x3) (Table 7) revealed that only the partial correlation coefficient for seed setting rate under high temperature (x3) reached extremely significant levels, while those for flowering proportion before 11:30 under high temperature (x1) and flowering proportion before 12:00 under normal temperature (x2) were not significant. The reason, as shown by path analysis (Table 8), was that although x1 and x2 were extremely significantly correlated with y, they mainly influenced y indirectly through seed setting rate under high temperature (x3), with minimal direct effects. In contrast, seed setting rate under high temperature (x3) had a substantial direct effect on the high temperature resistance index (y).

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3. Discussion

3.1. Effects of High Temperature at Different Flowering Times on Seed Setting Rate

This study demonstrated that high temperature 5 hours before flowering had no significant effect on seed setting rate, while high temperature treatment at flowering time significantly reduced seed setting rate, consistent with previous research conclusions [2,19-20]. Satake et al. [19] found that high temperature had almost no effect on spikelets that had already opened or those that would open 1 hour after treatment. Zhu et al. [20] reported that natural high temperature 1 hour after flowering had no obvious effect on seed setting rate, while Tan et al. [2] indicated that high temperature 4 hours after flowering still had substantial effects on fertilization under controlled conditions. Our study found that the effects of high temperature at different times after flowering varied among varieties. Among the four tested varieties, high temperature treatment for 5 hours (relative humidity 85%, temperature 38.0°C) applied at 1 hour, 1.5 hours, and 2 hours after flowering significantly affected seed setting in 3, 2, and 0 varieties, respectively. Consistently, high temperature treatment 2 hours after flowering had no significant effect on seed setting. These differences from previous studies may be related to different varieties and temperature/humidity conditions used.

According to our results, hybrid rice flowering occurred mainly between 9:30-12:30, with different varieties showing varying flowering proportions at different times. Higher temperatures induced earlier flowering, and hybrid rice under natural high temperature conditions suffered less damage because flowering occurred before the daily temperature peak. The earliest time when panicle layer temperature above 34°C occurred with over 80% frequency was 13:30. While daily average temperature of 30°C or maximum temperature \$ \$35°C is considered the critical threshold for high temperature damage during rice flowering, variations exist among varieties [1-2]. Therefore, to ensure greater safety of seed setting from high temperature effects during flowering, selecting varieties with high flowering proportions before 11:30 (2 hours before the occurrence of daily maximum temperature \$ \$34°C) could effectively avoid heat damage.

3.2. Identification Indicators for High Temperature-Resistant Hybrid Rice Varieties During Flowering

Numerous physiological and biochemical indicators are related to high temperature resistance during rice flowering. Varieties with low acid invertase activity in anthers and low monosaccharide content in pollen grains have poor high temperature resistance [1]. Varieties with high photosynthetic characteristics and chlorophyll content in flag leaves, high soluble sugars, soluble proteins, free proline, and heat-stable protein content, and low membrane permeability and MDA content show strong high temperature resistance [13]. Varieties with high activity of key photosynthetic enzymes and photosynthetic rate [6,14], high pollen viability and germination rate [15-16,21], and numerous pollen grains on stigmas [22] under high temperature stress also demonstrate strong resistance. While these findings are important for developing physical and chemical products to mitigate high temperature damage to seed setting, using these physiological and biochemical indicators for identifying high temperature-resistant varieties requires laboratory testing and analysis, which is not only time-lagging but also less accurate.

Seed setting rate is the best direct indicator for high temperature response, with strong reliability and perceptibility. Currently, the high temperature resistance index = (seed setting rate under high temperature / seed setting rate under normal temperature) × 100% is commonly used as a screening indicator among varieties [1,4,7]. However, both methods for obtaining this index—artificial climate chamber identification and natural high temperature identification—have limitations. The artificial climate chamber method requires potted plants to be divided into two groups during flowering: one group for high temperature treatment in the chamber and another under outdoor normal temperature, with seed setting rates investigated at maturity to calculate the resistance index. The main problems are: first, artificial climate chambers are required, which many institutions lack; second, the outdoor normal temperature group often encounters natural high temperature, causing identification failure. The natural high temperature method involves sowing identified varieties in 3–5 dates, hoping one sowing date encounters high temperature while another flowers under normal temperature, with seed setting rates investigated at maturity to calculate the resistance index. The main problems are: first, identification is limited to regions with high temperature occurrence; second, even in high temperature regions, some years lack high temperature or sowing dates miss the high temperature period, leading to identification failure; third, sowing 3–5 dates requires substantial workload. Therefore, developing more efficient and applicable identification methods is necessary.

3.3. Advantages and Application of Using Flowering Proportion for High Temperature Resistance Identification

Compared with existing methods for identifying high temperature resistance in rice varieties, the flowering proportion method offers the advantage of being applicable under either high temperature or normal temperature conditions, requiring only one sowing date. This approach not only reduces identification costs compared with traditional multi-date sowing methods but also enables large-scale variety screening. Since daily flowering time in rice is temperature-dependent, with higher temperatures inducing earlier flowering, varieties that flower early under normal temperature will flower even earlier under high temperature. Therefore, this method not only avoids identification failure due to lack of natural high temperature but also works in non-high temperature regions.

The specific identification procedure is as follows: First, classify hybrid rice varieties to be identified into groups with heading dates within 3 days of each other. Plant each group using conventional high-yield cultivation techniques, with each variety planted in 3–5 rows of 10 hills each. Second, select uniformly growing panicles that begin heading simultaneously, tag 6–8 panicles per variety per replicate with three replicates, and continuously record flowering numbers for 4 days before 11:30 (when high temperature occurs) or 12:00 (when no high temperature occurs). Finally, retrieve the tagged panicles to investigate average spikelets per panicle indoors, calculate the proportion of spikelets that flowered before 11:30 or 12:00, and determine inter-varietal differences in high

temperature resistance through analysis of variance of flowering proportions.

Flowering proportions before 11:30 under high temperature and before 12:00 under normal temperature were both extremely significantly positively correlated with the high temperature resistance index. High temperature 2 hours after flowering had no significant effect on seed setting rate. The earliest times when panicle layer temperature above 34°C and 35°C occurred with over 80% frequency were 13:30 and 15:30, respectively. Regression models for predicting the high temperature resistance index were established using three indicators: flowering proportion before 11:30 under high temperature, flowering proportion before 12:00 under normal temperature, and seed setting rate under high temperature.

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