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EFFECTS OF HIGH TEMPERATURE ON YIELD, QUALITY AND PHYSIOLOGICAL COMPONENTS OF EARLY RICE

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High temperature negatively impacts rice production, and the heat-tolerant cultivars could improve the resistance to high temperature stress. In the present study, six rice varieties were exposed to high temperatures stress (35°C and 38°C) for 3 days during the early filling stage. The differences among two high-temperature treatments were analyzed in terms of yield components, grain quality and physiological parameters of rice. The results showed that high temperature decreased seed setting rate, 1000-grain weight, plumpness, single plant yield, brown rice rate, milled rice rate and amylose content while increased unfilled grain rate of all rice varieties compared with the control. However, the yield components of different rice varieties in response to high temperature exhibited significant genotypic differences. In addition, the influence of high temperature on the hybrid rice variety Jinyou402 was greater than that on Ganxin203, and the influence of high temperature on the conventional rice variety E134 was greater than that on Zhong531. The 38°C treatment was more damaging to early rice than the 35°C treatment. Meanwhile, the influences of high temperature on the physiological parameters of flag leaves were consistent with the influence on yield components and grain quality.
Keywords: amylose content, early filling stage, high temperature, rice, seed setting rate.

INTRODUCTION

Rice (*Oryza sativa* L.) is a major cereal crops for nearly half of the world population in terms of direct consumption, especially in Asia (Cantrell and Reeves, 2002). Rice cultivation is highly vulnerable to natural environment deterioration and global warming (Peng *et al*., 2004; Mohammed and Tarpley, 2009a; Kim *et al*., 2013; Ray *et al*., 2015; Rahman *et al.*, 2017). Air temperature was predicted to increase in the future due to climate change, which could decrease rice productivity (Walbot, 2011). Most rice production comes from tropical regions wherein high temperature is a major constraint to the sustainable food security, especially when extreme temperature coincides with critical stages of plant growth and development (Jagadish *et al*., 2007; El-Kereamy *et al*., 2012; Schiermeier, 2015).

Rice yield and quality were declined when high temperature coincides with the grain filling stage (Yamakawa *et al*., 2007; Mitsui *et al*., 2013). High temperature stress during the grain filling stage resulted in a decreased grain size, grain weight, poor milling quality, low amylose content, and an increased degree of chalkiness in rice (Morita *et al*., 2005; Yamakawa and Hakata, 2010; Madan *et al*., 2012). High temperature stress caused physiological damage to rice plants with a reduced light perception, enzymatic activity, and carbohydrate metabolism, which decreased rice yield and quality (Cooper *et al*., 2008; Han *et al*., 2009).

High temperature negatively impacts rice production, and the heat-tolerant cultivars could improve the resistance to high temperature stress. Compared to the non-heat resistant varieties, heat-tolerant *Indica* rice N22 and IR64 showed significant spikelet fertility (Prasad *et al*., 2006; Jagadish *et al*., 2010; Madan *et al*., 2012). It was reported that the decrease ration of grain weight and net photosynthetic rate in the heat-sensitive line (XN0437S) decreased far more than in the heat-tolerant line (XN0437T) under high temperature (Liao *et al*., 2014). However, the specific differences under high temperature are not synthetically analyzed with more evidence. Considering recent global warming, understanding the physiological processes underlying this decrease is of great importance. Meanwhile, comprehending the mechanisms by which rice responds to heat stress will facilitate the development of heat-tolerant cultivars with improved productivity under warmer global climates. The overall objective of this study was to quantify comprehensively the effects of high temperature on physiological parameters to provide a theoretical basis for high-temperature tolerant rice breeding.

MATERIALS AND METHODS

***Plant material*:** Four early rice varieties (*Oryza sativa* sp. *indica*), the two hybrid rice varieties Ganxin203 (RongfengA / R3) and Jinyou402 (Jin23A / R402), and the two conventional rice strains (referred to as variety in the study) Zhong531 and E134 with stable characteristics were used as experimental materials.

***Crop husbandry*:** The trials were performed from March to August (2011 and 2012, experimental data taken from 2012) in a net house at the science and technology park of Jiangxi Agricultural University (latitude: 28° 46′ N, longitude: 115° 50′ E, altitude: 48.80 m), Nanchang, China. The seeds were selected before sun drying, sterilized with hydrogen peroxide and soaked in tap water. The seeds were germinated inside a plant incubator at 28°C / 24°C (12 h day / 12 h night cycles) for 3 d and were sown by hand on March 15, 2012. The seeds were sown at about 2 cm depth into plastic basins containing rice nursery culture soil. The rice seedlings were cultivated in a greenhouse to maintain the heat. The seedlings were irrigated in the late afternoon each day to maintain the soil water content. Upon reaching the three–leaf stage, the uniformly sized seedlings were selected and transplanted into plastic pots (16.50 cm internal diameter × 16.50 cm depth) on April 13, 2012. Each pot with only one seedling was placed directly into the experimental rice field. The planting density was 25.50 cm × 24.50 cm. The plants were fully watered throughout the crop growth cycle. Fertilizer was applied as follows: 165 kg nitrogen per hectare as urea, 180 kg K_2O per hectare as potassium chloride, and 90 kg P_2O_5 per hectare as calcium magnesium phosphate. Basal dressing, tiller fertilizer, and panicle fertilizer were respectively supplied 2 d before transplantation, at 15 d after transplant, and at approximately 15 d to 20 d before heading (i.e., panicle differentiation stage). The proportion of basal dressing, tiller fertilizer, and panicle fertilizer was 4:2:4. Other field management, disease, and pest prevention were conducted using local conventional high-yield cultivation techniques. No major pests or diseases were noted.

***High temperature treatment*:** Two different high-temperature treatments were performed inside a plant growth cabinet. Based on the characteristics of natural high temperature during summer season in double-rice cropping area, such as Jiangxi, Hunan, and Zhejiang provinces in central China, two temperature gradients (i.e., daily maximum temperature: 35°C and 38°C) were set. The running days for high temperature treatment were 3 d. The randomized rice plants that had essentially identical growth and development in the field pots were selected to tab labels at late panicle-differentiation stage. Each temperature treatment combination had six labelled plants. When the main and secondary tillers of the rice plants grew at the stage between full heading and grain filling period (7 d to 10 d after heading of 10% rice plants, i.e. early filling stage), all labelled random plants for two high-temperature treatments were transferred at 08:00 a.m. into the growth cabinet (PRX－1500B, internal size: length 1.91 m × width 0.76 m × height 1.83 m, Shanghai Bilon Instruments Co., Ltd., China). The treatments and genotypes appeared randomized in the growth cabinet. The high-temperature treatment for Ganxin203 and Jinyou402 were from June 19 to June 22, whereas that for Zhong531 and E134 was from June 26 to June 29 and from June 29 to July 2, respectively. The diurnal variation of air temperature was based on the temperature variation curve 1 d before simulation to control the high temperature in the programmable growth cabinet. The installations of other climatic factors for different high-temperature treatments in two plant growth cabinets were kept similar. The illumination time was 14 h based on weather conditions at that time. Lamps were balanced to ensure uniform flux densities throughout the cabinet. Illumination time, intensity, and relative humidity were kept unified during the treatment process.

The specific condition inside the plant growth cabinet was as follows. The duration of daily maximum temperature was maintained for 2 h (13:00 p.m. to 15:00 p.m., Beijing time). The diurnal range of temperature simulated the temperature variation curve as follows: daily minimum temperature was from 00:00 a.m. to 06:00 a.m. Then the temperature increased by 1°C per 1 h by 13:00 p.m. and high temperature was maintained until 15:00 p.m. The temperature gradually decreased by 1°C for 1 h increase between 15:00 p.m. and

Table 1. The setting of meteorological factors during high temperature treatment.

Meteorological factors		O′ clock																
		00:00-5:00	6:00	7:00	8:00	9:00	10:00	11:00	12:00	13:00	14:00	15:00	16:00	17:00	18:00	19:00	20:00	21:00	22:00-00:00
T (°C)	35	27	28	29	30	31	32	33	34	35	35	34	33	32	31	30	29	28	27
	38	30	31	32	33	34	35	36	37	38	38	37	36	35	34	33	32	31	30
RH (%)		82	81	80	79	78	77	76	75	74	74	75	76	77	78	79	80	81	82
II (μ mol m^{-2} s^{-1})		0	324	324	324	432	432	540	540	540	540	540	432	432	324	324	0	0	0

T: temperature; RH: relative humidity; II: illumination intensity. Two different high-temperature treatments (35°C and 38°C) were conducted. The meteorological factors contained temperature, relative humidity and illumination intensity. Illumination time, intensity and relative humidity were kept similar for each treatment during treatment processing. Time (Beijing Time) was an automatic cycle every 24 hours. The duration for treatment was 3 d.

22:00 p.m. Then, the temperature returned to daily minimum temperature from 22:00 p.m. to 00:00 a.m. The setting programs of temperature were automatically repeated every 24 h. The meteorological factors of high-temperature treatments are listed in Table 1.

Another 10 random pots of labelled plants in the paddy field was used as the control. The meteorological data in the experimental field were obtained from the agricultural meteorological station located within 1 km of the field. After high-temperature treatment, plants were immediately moved back to the rice field and removed the pots for natural growth. The temperature variation of high-temperature treatments and the controls in the field during treatment processing is described in Table 2. This table showed that the mean minimum temperature of all high-temperature treatments for the four early rice varieties was significantly higher than that of the control (all at $P < 0.01$). The mean daily temperature and mean maximum temperature of high-temperature treatments for Ganxin203 and Jinyou402 were obviously higher than those of the control (all at $P < 0.01$). The mean daily temperature and mean maximum temperature for 35°C treatments of Zhong531 and E134 were insignificant, whereas those for 38°C treatments were higher than those of the control.

***Chlorophyll content [soil plant analysis development (SPAD value)]*:** Four second leaves from the top of main tillers (each from separate plant) were tagged for identification in each high-temperature treatment and the control. In addition, the leaf chlorophyll content of the four rice varieties was measured. Each SPAD value is the mean of three replicates determined from the central area of the second leaf from the top, with the SPAD-502 chlorophyll meter. The SPAD values were measured at around 10:00 a.m. at 1 d before the temperature treatment (i.e., background value) and at 0, 1, 4, 7, and 10 d after the high-temperature treatment.

***Yield and its components*:** After removing the abnormal rice plants, three replicates (three random plants) from each high-temperature treatment and the control were harvested individually at physiological maturity. The yield and its components of these plants were measured, including seed setting rate, unfilled grain rate, 1000-grain weight, plumpness, and single plant yield. All panicles were threshed by hand and their grain weights were recorded. The seed setting rate was estimated as the ratio of the number of filled grains to the total number of reproductive sites (grains) and expressed as a percentage. Each grain was pressed between the forefinger and thumb to separate the filled grains from the empty grains. The filled grains included both completely and partially filled grains (i.e., solid grains and unfilled grains). After withdrawing empty grains, the solid grains were separated from the unfilled grains by immersing in tap water. The grains that settled were the solid grains, whereas those that remained afloat were the unfilled grains. Plumpness was estimated as the ratio of average weight of the fertilized grains to the weight of the completely filled grains, with density above 1.0.

***Brown rice rate, milled rice rate and amylose content*:** The brown rice rate and the milled rice rate were determined for each temperature treatment and the controls using the standard method NY147-88 supplied by the Chinese Ministry of Agriculture. The grains were milled into brown rice using a JLGJ-4.5 type rice huller (Xinfeng, Food Instrument Factory, Taizhou, Zhejiang Province, China). The brown rice rate was estimated as the ratio of the average weight of brown rice to the weight of grain samples and expressed as percentage. The brown rice was milled into milled rice for 70 s using a JNMJ-3 type rice mill (Xinfeng). The milled rice rate was calculated as the ratio of the average weight of milled rice multiplied by the brown rice rate to the weight of samples from brown rice and expressed as a percentage. Amylose

Table 2. The temperature variation during high temperature treatment (°C).

Cultivar	Treatment	CK			High temperature			Mean daily temperature difference		
		MDT	MAT	MIT	MDT	MAT	MIT	MDT difference	MAT difference	MIT difference
Ganxin203	35°C	26.73	31.33	22.68	30.33	35.00	28.00	3.61**	3.68**	5.33**
	38°C				33.33	38.00	31.00	6.61**	6.68**	8.33**
Jinyou402	35°C	26.73	31.33	22.68	30.33	35.00	28.00	3.61**	3.68**	5.33**
	38°C				33.33	38.00	31.00	6.61**	6.68**	8.33**
Zhong531	35°C	28.33	32.58	23.20	30.33	35.00	28.00	2.01	2.43	4.80**
	38°C				33.33	38.00	31.00	5.01**	5.43*	7.80**
E134	35°C	30.35	35.15	25.23	30.33	35.00	28.00	-0.02	-0.15	2.78**
	38°C				33.33	38.00	31.00	2.98**	2.85**	5.78**

MDT: mean daily temperature; MAT: mean maximum temperature; MIT: mean minimum temperature. Values followed by *or **are significantly different at 0.05 or 0.01 probability levels according to Student′ s t test, ns is non-significant, respectively. The temperature of the control was observed from the agricultural meteorological station located within 1 km of the field. Mean daily temperature difference was calculated as the remainder of the subtraction from the high temperature to the control.

content was determined based on the methods in the national standard GB/T17891-1999 and GB7648-1987. The samples from milled rice were ground into powder using hammer cyclone grinding JXFM110 type (Shanghai Jiading Grain and Oil Equipment Co., Ltd., China) to measure the amylose content. The samples for measuring amylose content consisted of the polished powder after passing through 0.25 mm mesh. Amylose content was determined via the iodine blue colorimetric method with four replicates.

***Other physiological components*:** During the grain filling stage, the flag leaf was detached at approximately 2 cm at 0, 2, and 4 d after the high-temperature treatment. The samples were immediately frozen in liquid nitrogen, and then stored at -80°C for subsequent analysis. Physiological biochemical components were estimated using a ultraviolet–visible spectrophotometer TU-1810D type (Purkinje General Instrument Co., Ltd., Beijing, China), including soluble protein content (Coomassie brilliant blue method, G-250), Malondialdehyde (MDA) content (thiobarbituric acid method, TBA), and proline content. Each measurement was repeated thrice. The character stress index of all items under the high-temperature treatments was calculated as the ratio of the characteristic value under high temperature to that of the control.

***Statistical analysis*:** The rice plants were harvested at maturity. All yield parameters, grain quality components, and physiological items were observed. The differences among the means for the plant growth cabinet and natural field experiments were analyzed using a Student's t-test (SPSS software ver. 17.0).

RESULTS

The seed setting rate, 1000-grain weight, plumpness, and single plant yield of the four early rice varieties declined under the high-temperature treatments, whereas their unfilled grain rate increased compared with that of the control during the early filling stage (Table 3). The variation range of the rice varieties increased when subjected to high-temperature treatment intensity. The high temperature responses of the yield components significantly differed among the different early rice varieties. Single plant yield of hybrid rice Jinyou402under high temperature was more affected than that of Ganxin203, whereas the effects on conventional rice E134 exceeded those on Zhong531. High temperature did not significantly affect the yield and components of Zhong531 compared with those in the control (except the unfilled grain rate). Hence, Zhong531 has high temperature tolerance. The high temperature treatments significantly decreased the single plant yield of Jinyou402 (35.98%), Ganxin203 (21.03%), and E134 (18.07%) compared with the controls ($P < 0.05$), which indicates that these genotypes are sensitive to high temperature. Meanwhile, the single plant yield of the four early rice varieties grown at 35°C showed slight decreases compared with those grown under the control conditions.

Similar to the influence of high temperature on yield components, the high temperature treatment also decreased the brown rice rate, milled rice rate, and amylose content, with the decline increasing with increasing temperature. The temperature responses of the brown rice rate, milled rice rate, and amylose content to were different among the four early rice varieties. The decrease in the brown rice rate, milled rice rate, and amylose content of Jinyou402 and Zhong531 at 35°C did not significantly differ from those of the control, which proved that the high temperature only caused slight heat injury (Table 4).

The chlorophyll content of the four early rice varieties in the controls gradually declined with time. After high-temperature treatment, the chlorophyll content in the second leaves from

Table 3. Effects of high-temperature treatment on grain yield and its components of early rice during the early filling stage.

Cultivar	Treatment	Seed setting rate (%)	Unfilled grain rate (%)	1000-grain weight (g)	Yield per plant (g)	Plumpness (%)
Ganxin203	CK	95.15±1.53aA	6.07±1.32aA	26.66±0.08aA	25.78±0.63aA	91.37±0.10aA
	35°C	76.49±2.14bA	6.26±0.04aA	26.45±0.05aA	25.08±0.06aA	79.66±2.08bB
	38°C	62.55±1.65cB	6.99±0.52aA	24.42±0.02bB	20.36±2.65bA	71.50±2.36cB
Jinyou402	CK	88.72±0.66aA	7.91±1.05aA	26.69±0.89aA	26.97±1.11aA	81.45±5.60aA
	35°C	76.74±0.57bAB	10.48±2.44aA	26.52±0.80aA	22.38±2.33aA	79.38±5.22aA
	38°C	73.94±2.83bB	11.40±1.93aA	26.48±0.42aA	17.27±1.83bB	74.59±5.00aA
Zhong531	CK	91.47±0.54aA	12.24±3.32aA	28.28±0.40aA	18.61±2.08aA	87.72±3.41aA
	35°C	88.88±0.52aA	15.47±3.49abA	25.94±0.34aA	18.18±1.94aA	85.29±0.97aA
	38°C	84.07±4.45aA	20.39±1.34bA	25.33±0.33aA	17.59±2.11aA	83.15±3.42aA
E134	CK	83.76±1.10aA	19.93±1.44aA	25.34±0.44aA	19.04±1.06aA	81.26±2.60aA
	35°C	76.66±1.83aAB	23.56±0.38aAB	22.65±0.15abA	15.77±1.21aA	76.01±1.88aA
	38°C	66.26±1.98bB	25.01±1.49bB	22.06±0.78bA	15.60±0.61bA	74.79±2.62aA

The data are means ± standard error; data within the same column with the same letters indicate no significant difference at 1 % or 5% level according to Student′s t-test.

Table 4. Effects of high-temperature treatment on brown rice rate, milled rice rate, and amylose content of early rice during the early filling stage.

Cultivar	Treatment	Brown rice rate (%)	Milled rice rate (%)	Amylose content (%)
Ganxin203	CK	78.63±0.45aA	66.86±0.84aA	19.21±0.19aA
	35°C	76.90±0.37aAB	61.47±0.65bAB	16.69±0.32cB
	38°C	74.80±0.52bB	58.48±1.13bB	17.51±0.08bB
Jinyou402	CK	79.04±0.12aA	65.58±0.78aA	18.88±0.24aA
	35°C	77.51±0.33aAB	63.73±1.55aA	18.17±0.09abA
	38°C	73.94±0.39bB	60.90±2.84bA	17.84±0.24bA
Zhong531	CK	72.29±0.06aA	64.87±0.24aA	11.26±0.13aA
	35°C	69.74±0.26abAB	62.77±0.15aA	10.65±0.11abAB
	38°C	68.32±0.37bB	58.12±0.76bB	9.96±0.31bB
E134	CK	72.47±0.22aA	61.37±0.34aA	25.88±0.18aA
	35°C	71.69±0.23aAB	60.97±0.28aA	22.56±0.18bB
	38°C	70.82±0.13bB	57.82±0.98bB	22.48±0.06bB

The values are the mean of three replicates ± standard error. Data within the same column with the same letters during each phase indicate no significant difference at 1 % or 5% level according to Student′s t-test. Amylose content was measured using the iodine colorimetric method with four replicates.

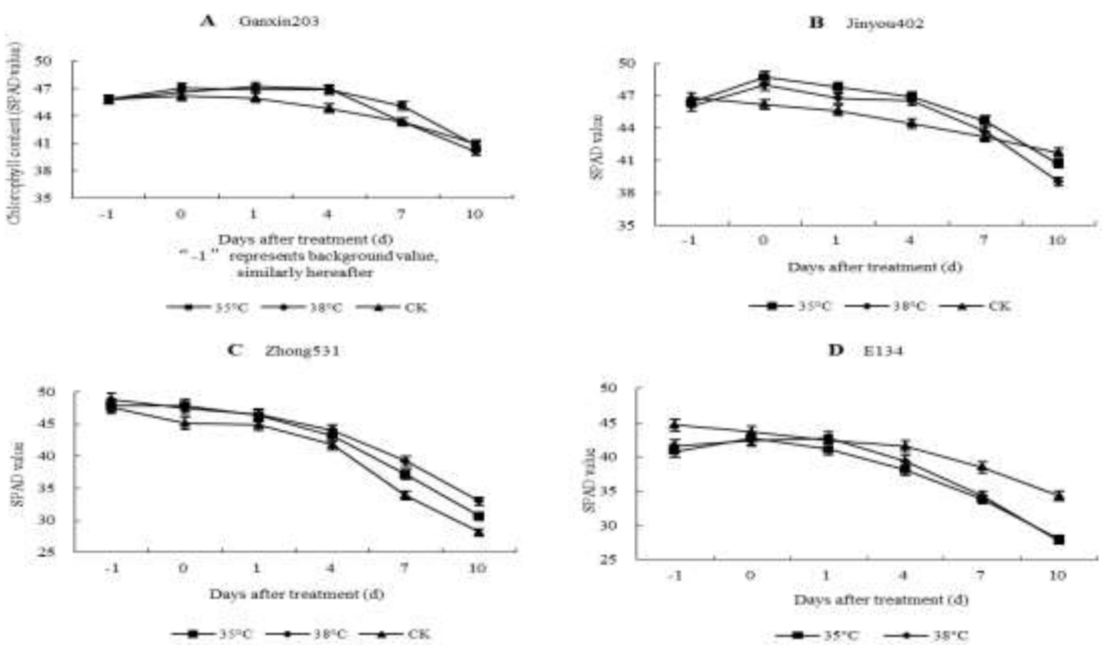

Figure 1. Dynamic changes of chlorophyll content (SPAD value) in early rice second leaves from the top after high-temperature treatment during the early filling stage. A: Ganxin203; B: Jinyou402; C: Zhong531; D: E134. SPAD value represents the chlorophyll content. "-1" on the abscissa represents background value. Diamond symbols represent the treatment of 35°C, while square symbols and triangles represent the treatment of 38°C and CK, respectively. Data are expressed as the mean of four replications.

the top of the plants increased within a narrow range. After ward, the chlorophyll content decreased compared with that of the control. At 10 d after high-temperature treatment, the chlorophyll content of two hybrid rice varieties was lower than that of the control, and the chlorophyll content at 35°C was higher than that at 38°C at the same time points. At 10 d after high-temperature treatment, the chlorophyll content of Ganxin203 in the control and the two treatments were significantly decreased ($P<0.05$). The SPAD of Jinyou402 in the control was significantly decreased at 10 d. By contrast, SPAD value was obviously lower at 7 d ($P<0.05$), which clearly indicate that the effects of high temperature on

Table 5. Effects of high-temperature treatment on proline content (μg/g. FW) in early rice flag leaves during the early filling stage.

Cultivar	Treatment	Days after treatment (d)			Average
		0	2	4	Treatment/CK
Ganxin203	CK	26.97±0.07cC	24.33±0.29cB	22.62±0.42cB	
	35°C	66.24±0.11aA	39.46±0.99aA	32.87±0.77aA	1.84±0.31aA
	38°C	35.40±0.50bB	28.54±0.50bB	27.65±0.32bAB	1.24±0.04aA
Jinyou402	CK	28.40±0.36bA	24.76±0.14aA	22.76±0.43aA	
	35°C	34.11±0.07aA	22.69±0.07bB	19.69±0.09aA	0.99±0.10aA
	38°C	34.54±0.07aA	23.19±0.15bB	20.83±0.53aA	1.02±0.10aA
Zhong531	CK	26.83±0.07cC	24.90±0.57aA	15.83±0.22bA	
	35°C	32.61±0.15aA	17.55±0.04bB	17.33±0.86abA	1.00±0.16aA
	38°C	29.19±0.29bB	22.69±0.83aAB	19.26±0.36aA	1.07±0.09aA
E134	CK	24.76±0.14aA	21.62±0.12aA	18.83±0.50aA	
	35°C	20.40±0.21bB	20.26±0.36aA	15.26±0.78aA	0.86±0.04aA
	38°C	19.40±0.50bB	17.69±0.07aA	12.48±0.42aA	0.75±0.05aA

The values are the mean of three replicates ± standard error. Data within the same column with the same letters during each phase indicate no significant difference at 1 % or 5% level according to Student's t-test.

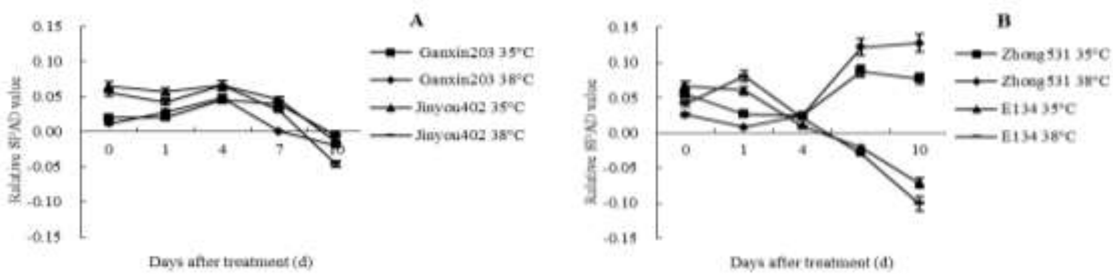

Figure 2. Dynamic changes in relative chlorophyll content (SPAD value) in the second leaves of the early rice after high-temperature treatment during the early filling stage. A: Ganxin203 and Jinyou402; B: Zhong531 and E134. Relative chlorophyll content = [(SPAD value of treatment - background value of the treatment) - (SPAD value of the CK - background value of the CK)]/CK SPAD value. Average values from four independent measurements are shown with error bars.

Jinyou402 is greater than that on Ganxin203 (Fig. 1A and B). The chlorophyll content of the control and two conventional rice varieties in the treatments were lower than those of the two hybrid rice varieties, which indicates that the functional period of the leaves in conventional rice is shorter than that of the hybrid rice. The chlorophyll content of the conventional rice variety E134 was generally low, and the background value at 38°C was higher than that at 35°C, causing the chlorophyll content at 35°C to be lower than that at 38°C at the same time point. However, the chlorophyll content of the conventional rice variety Zhong531 ripened under high temperature was always higher than that of the control, which demonstrates that E143 is more sensitive to high temperature than Zhong531 (Fig. 1 C and D).

The changes in relative chlorophyll content (SPAD value) in the four early rice varieties after the high-temperature treatment are shown in Figure 2. To reduce the effects of background, the relative chlorophyll content was calculated as the relative SPAD value = [(SPAD value of treatment - background value of treatment) - (SPAD value of CK - background value of CK)]/CK SPAD value. The results showed that the relative chlorophyll content of Ganxin203 and Jinyou402 was above zero ($P < 0.05$) within 7 d after the high-temperature treatment, which suggest that chlorophyll content increased within a narrow range. At 10 d after high-temperature treatment, the chlorophyll content of Ganxin203 and Jinyou402 was lower than that of the control, which is consistent with the changes in the relative chlorophyll content. The relative chlorophyll content of the two hybrid rice varieties was also higher at 35°C than at 38°C (Fig. 2A). The relative changes in chlorophyll content in E134 were similar to that of the two hybrid rice varieties, and was less than zero at 7 d after the high-temperature treatment. By contrast, the high-temperature treatment during early filling stage positively affected the relative chlorophyll content of Zhong531 (with the content always greater than zero). This finding suggests that Zhong531 tolerates the high temperature (Fig. 2B).

Table 5 shows the proline content of all treatments and the controls in the four early rice flag leaves gradually declined with the growth period. Proline content significantly differed among the four early rice varieties in response to high temperature stress. After high-temperature treatment, the proline content of Ganxin203 was always significantly greater than that of the control. At 0 d after high-temperature treatment, the proline content in Jinyou402 flag leaves was significantly higher than that of the control, but became lower than that of the control thereafter. The proline content in Zhong531 only changed slightly, but continuously deceased in E134 compared with the control. This finding indicates that E134 is heat tolerant. The proline content in Jinyou402 and Zhong531 under the 35°C treatment was lower than that under the 38°C treatment (Table 5).

Generally, the soluble protein content in rice flag leaves during the grain filling stage gradually declined with growth period progression. As shown in Table 6, the soluble protein content of all treatments and the controls decreased, with the decline in soluble protein content after high-temperature treatment higher with the treatment ratio to CK higher than zero (except Ganxin203 at 35°C). The most prominent decrease was in Jinyou402, with an average decrease in soluble protein content of 17.16% after the high-temperature treatment during the early filling stage.

The changes in MDA content in the control during the early

Table 6. Effects of high-temperature treatment on soluble protein content (mg/g. FW) in the flag leaves of early rice during the early filling stage.

Cultivar	Treatment	Days after treatment (d)			Average
		0	2	4	Treatment/CK
Ganxin203	CK	1.24±0.03bB	1.00±0.01bB	0.90±0.00bB	
	35°C	2.24±0.07aA	2.18±0.01aA	2.14±0.01aA	2.12±0.17aA
	38°C	0.93±0.07cC	0.90±0.05cC	0.58±0.07cC	0.76±0.08bB
Jinyou402	CK	1.18±0.03aA	1.16±0.05aA	1.13±0.04aA	
	35°C	0.96±0.02bA	0.91±0.03bA	0.87±0.06bA	0.79±0.01aA
	38°C	1.19±0.05aA	0.92±0.04bA	0.89±0.02abA	0.86±0.07aA
Zhong531	CK	1.41±0.02aA	1.26±0.16aA	0.98±0.03aA	
	35°C	1.09±0.00bB	1.02±0.00aA	0.72±0.10aA	0.77±0.04aA
	38°C	1.14±0.04bB	1.01±0.02aA	0.97±0.01aA	0.87±0.10aA
E134	CK	1.23±0.02aA	1.13±0.05aA	1.00±0.03aA	
	35°C	1.23±0.01aA	1.02±0.07aA	1.00±0.12aA	0.97±0.03aA
	38°C	0.89±0.02bB	1.02±0.04aA	1.15±0.01aA	0.93±0.13aA

The values are the mean of three replicates ± standard error. Data within the same column with the same letters during each phase indicate no significant difference at 1 % or 5% level according to Student′s t-test. The soluble protein content was determined using the Coomassie brilliant blue method (G-250).

Table 7. Effects of high-temperature treatment on MDA content (nmol/g FW) in early rice flag leaves during the early filling stage.

Cultivar	Treatment	Days after treatment (d)			Average
		0	2	4	Treatment/CK
Ganxin203	CK	10.85±0.25aA	12.70±0.08aA	13.65±0.26aA	
	35°C	8.43±0.40bA	10.24±0.12bB	10.75±0.14bB	0.79±0.01bA
	38°C	10.71±0.83abA	11.36±0.05bB	12.60±0.45aAB	0.93±0.02aA
Jinyou402	CK	11.31±0.20aA	12.81±0.23aA	13.44±0.14aA	
	35°C	12.15±0.09aA	13.01±0.22aA	13.72±0.16aA	0.96±0.01aA
	38°C	11.96±0.10aA	13.22±0.38aA	13.93±0.41aA	1.00±0.01aA
Zhong531	CK	10.45±0.10aA	12.13±0.10aA	11.86±0.32aA	
	35°C	10.75±0.07aA	9.85±0.14bB	8.54±0.07bB	0.85±0.09aA
	38°C	8.77±0.32bA	8.92±0.09cB	10.83±0.21aAB	0.83±0.05aA
E134	CK	13.88±0.10aA	14.81±0.30aA	16.68±0.28aA	
	35°C	11.40±0.15cB	13.15±0.49aA	13.44±0.70bA	0.84±0.03aA
	38°C	12.66±0.09bA	13.18±0.53aA	14.35±0.25abA	0.89±0.02aA

The values are the mean of three replicates ± standard error. Data within the same column with the same letters during each phase indicate no significant difference at 1 % or 5% level according to Student′s t-test. The soluble protein content was determined using the thiobarbituric acid method (TBA).

filling stage indicated that the MDA content of early rice flag leaves gradually increased, and decreased after high-temperature treatment (Table 7). The MDA content of the flag leaves was more influenced by the 35°C treatment than the 38°C treatment during the grain filling stage. Meanwhile, the two high-temperature treatments did not significantly affect the MDA content of Zhong531, with an average decrease of 15.40% in the 35°C treatment and 17.19% in the 38°C treatment, respectively. This result suggests that Zhong531 was tolerant to high temperature.

DISCUSSION

Transient or sustained high temperature causes a series of changes that affect plant growth and development. Although rice plants can withstand certain heat stress, most reports provide direct evidence of decreased rice yield and grain quality because of the increased temperature (Huang *et al*., 2016). The negative effects on rice plant caused by high temperature were a result of decreased spikelet fertility, faster grain filling, fewer filled grains, and lower grain weight, which affect grain yield and quality during the grain filling stage (Prasad *et al*., 2006; Mohammed and Tarpley, 2009b; Lin *et al*., 2010; Fahad *et al*., 2016). Rice grains ripened under high temperature exhibited low weights, chalky appearance, and low amylose content (Yamakawa *et al*., 2007; Mitsui *et al*., 2013). Our findings demonstrated that yield components, including seed setting rate, 1000-grain weight, plumpness, and grain quality, as well as brown rice rate, milled rice rate, and amylose content of rice declined under high temperature compared with those under the control. The heat damage at 38°C on early rice, as measured in terms of yield components, was greater than that at 35°C treatment. The differences in mean daily temperature and mean maximum temperature in E134 at 34°C were negative, whereas the mean minimum temperature (i.e., night time temperature) was obviously higher than that of the control thereby, decreasing the yield. In the present study, significant genotypic differences were observed between high temperature responses to yield components among different rice varieties. The influence of high temperature on the hybrid rice Jinyou402 was more than that on Ganxin203, and the influence on the conventional rice E134 exceeded that on Zhong531.

In the current study, we found the effects of high temperature on physiological parameters of rice flag leaves were consistent with the influence on yield components and grain quality. High temperature negatively affected chlorophyll content, and the decreased rate of chlorophyll content in heat-tolerant varieties was lower than that in heat-sensitive varieties (Mohammed and Tarpley, 2009a). In the present research, the chlorophyll content of rice increased within a narrow range under high temperature, and then decreased compared with the control. Meanwhile, the effect of high temperature on the hybrid rice Jinyou402 was greater than that on Ganxin203. Previous studies have shown that high temperature could break the balance of starch content, protein storage in mature grains, reactive oxygen species, antioxidant enzyme activity, and proline content in rice leaves (Morita *et al*., 2005; Fitzgerald and Resurreccion, 2009; Hasanuzzaman *et al*., 2013). Proline, as a plant osmotic regulator, protects the membrane and enzyme activity under abiotic stress (Cha-um and Kirdmanee, 2008). Our findings clearly indicate that Jinyou402 had lower proline content than those of Ganxin203 under high temperature. By contrast, the proline content of the conventional rice variety E134 was lower than that of the control, which indicates that E134 is sensitive to heat. Proteins are important to plant life activities. Soluble protein plays a highly protective role in plants that are exposed to abiotic stresses (Yamakawa and Hakata, 2010). High temperature improved enzyme activity during source–sink transportation and enhanced leaf protein synthesis, which accelerated leaf soluble protein transport to the grains and promoted grain protein synthesis thereby, lowering the soluble protein levels in the leaves. In the present study, the soluble protein content of the flag leaves of the four early rice varieties decreased under high temperature.

Different rice varieties exhibit different responses to high-temperature stress. Based on decreases in spikelet fertility under high temperature, N22, IR64, BKN6624-46-2, Nipponbare and Huanghuazhan were heat-tolerant cultivars, whereas Shuanggui 1, Akitakomachi, Minamihikari, Koshihikari, IR-8, and Hinohikari were heat-sensitive cultivars (Prasad *et al*., 2006; Cao *et al*., 2009; Jagadish *et al*., 2010; Chen *et al*., 2011). The japonica varieties Koshiibuki and Tentakaku produced less chalky grains under high temperature (high temperature tolerant), whereas Hatsuboshi and Sasanishiki produced severely chalky grains (high temperature sensitive) (Yamakawa *et al*., 2007). Our results indicated that Zhong531with higher seed setting, 1000-grain weight and yield was heat tolerant, while E134 was susceptible to high temperature. The heat tolerance of rice is related to *in vivo* reactive oxygen generation, membrane lipid peroxidation, as well as the balance between removal and defense mechanism. Huanghuazhan with higher yield had stronger oxidation and antioxidant protection capability in the roots, and higher adenosine triphosphate enzyme activity in rice grains under high temperature (Cao *et al*., 2009). Yangdao 6 maintained higher chlorophyll content, free proline content, soluble protein content, and lower MDA content, which may contribute to greater heat resistance (Xie *et al*., 2012). In the present work, we demonstrated that Zhong531 had higher chlorophyll content, proline content, soluble protein, and lower MDA content, which may be important for maintaining its high yield under high temperature during the early filling stage.

***Conclusion*:** In conclusion, the yield components of different early varieties in response to high temperature exhibited significant genotypic differences. The influence of high temperature on the hybrid rice variety Jinyou402 was greater than that on Ganxin203, and the influence of high temperature on the conventional rice variety E134 was greater than that on Zhong531. The influences of high temperature on the physiological parameters of flag leaves were consistent with the influence on yield components and grain quality.

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