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RESEARCH

Effects of elevated air temperature on physiological characteristics of flag leaves and grain yield in rice

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As an indispensable environment element for crop growth, air temperature has brought challenge for the sustainable development of rice (*Oryza sativa* L.) production. Elevated air temperature led to great loss in rice grain yield in many districts suffering from heat stress due to the greenhouse effect worldwide, which has received more and more attention from researchers. A field experiment was conducted to investigate impacts of high air temperature (HAT) after rice heading stage on dynamics of SPAD values, soluble sugar, soluble protein, and malondialdehyde (MDA) contents of flag leaves, and grain yield attributes. The results showed that HAT significantly reduced SPAD values, soluble sugar and protein contents, seed-setting rate, number of filled grains per panicles, 1000-grain weight, and grain yield, while increased MDA content. There exists strong correlation between each physiological parameter and days from heading stage to maturity, which can be simulated by quadratic curve equation or linear regression equation. Under HAT, the enhanced MDA content and decreased soluble sugar content demonstrated the damage of membrane structure and photosynthesis function of rice flag leaves, which was partially attributed to the reduced SPAD value and soluble protein content. In the present experiment, rice seed-setting rate was more vulnerable to HAT than grain weight. The disturbance of physiological metabolism in flag leaves was a fundamental reason for the reduction of rice grain yield under HAT.

Key words: Physiological parameters, dynamics, yield attributes, *Oryza sativa*.

INTRODUCTION

Temperature is one of the most important environmental factors determining crops development and yield formation. The continuous rise in air temperature incurred by green house effect inevitably affects crops production on the globe. Rice (*Oryza sativa* L.) is a staple food crop, feeding a large population in the world. However, extremely high air temperature (HAT) climate frequently occurs in recent years, which not only results in great loss in rice yield but also severely threatens food security. Now it has become a major impediment for the acquisition of stable rice grain yield in many rice production districts. In the south of China, high air temperature brings about significant decrease in rice seed-setting rate, 1000-grain weight and yield due to abnormal seeding and poor grain-filling. Especially in the years with sustained high air temperature weather, grain yields of some *indica* rice cultivars were considerably reduced with the sharp decrease of seed setting rate by 65% (Wang and Zhong, 2004).

To clarify the physiological mechanism regarding HAT stress inhibiting the formation of rice yield, related experimentations have been carried out (Tashiro and Wardlaw, 1991; Peng et al., 2004; Morita et al., 2005; Jagadish et al., 2007). Owing to rice productivity is vulnerable to high air temperature from booting to grain-filling stage; emphases of former researches have been largely on rice pollen activity, flowering behavior, and grain-filling characteristics under high air temperature (Nagato and Ebata, 1965; Yoshida and Hara, 1977; Matsui et al., 2000; Tao et al., 2008). It has been reported that rice pollen fertility, flowering duration and net photosynthetic rate of flag leaves decreased rapidly when air temperature was over 35 °C (Matsui et al., 2001; Zhang et al., 2007; Tao et al., 2008). Rice grain filling rate increased while grain-filling duration was shortened obviously due to insufficient assimilation and the nutrition competition among grains under high air temperature conditions (Nagato et al., 1966; Kobata and Uemuki, 2004). Certainly, such responses to high air temperature for rice plants were closely correlated with the reduced grain yield.

High air temperature destroys the balance of secreting and scavenging reactive oxygen species, and results in lipid peroxidation in rice plants, which inevitably hampers normal physiological metabolism, such as the production of chlorophyll, osmotic adjustment substance and photosynthesis (Zhang et al., 2007). Xie et al. (2012) reported that high air temperature during heading stage negatively influenced SPAD value (relative content of chlorophyll) in rice flag leaves, significant reduction occurring with the continuous increment of air temperature. As the most important source organs, rice

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flag leaves play a dominant role in providing assimilates for the development of sink organs (grains). Nevertheless, limited reports regarding physiological responses of rice flag leaves to high air temperature are available now (Zhang et al., 2007; Xie et al., 2012), especially the lack of researches on dynamics of physiological parameters after heading stage. In addition, most previous experiments with respect to high air temperature affecting rice development were implemented in green house or climate incubator based on simulative air temperature increment, which cannot better reflect daily weather variation in rice production, such as temperature, precipitation and light intensity (Tao et al., 2008; Mohammed and Tarpley, 2009; Li et al., 2011). Hence, we designed a field experiment to conduct further investigation by setting effective temperature increment treatment, aiming at providing useful information for the establishment of suitable rice cultivation practices coping with heat stress.

The objectives of the experiment were (i) to investigate effect of high air temperature after heading stage on dynamics of SPAD value, soluble sugar, soluble protein and MDA contents in rice flag leaves, and yield attributes; and (ii) to discuss the relation between the reduction of yield and the variation of physiological characteristics of flag leaves under high air temperature.

MATERIALS AND METHODS

Site and experimental design

The field experiment was carried out on the farm of Sichuan Agricultural University, Yaan (29°59' N, 102°59' E), China, in 2006 and 2007. The basal fertility conditions of the soil in the trial are as follows: organic matter 19.16 g kg^{-1}, total N 1.19 g kg^{-1}, available N 100 mg kg^{-1}, available P 20 mg kg^{-1}, available K 80 mg kg^{-1}, pH 6.50. A hybrid *indica* rice cultivar currently cultivated in local production, Gangyou 527, was grown in the paddy field. Rice seeds were sown on 12 April 2006 and 15 April 2007 and their seedlings were transplanted to paddy fields on 18 May 2006 and 20 May 2007. The heading date (50% of all plants) was on 6-7 August and plants were harvested on 20-21 September. The precipitation during key stage of rice grain-filling (25 d after heading stage) was 161.1 mm in 2006 and 263.2 mm in 2007. Field management was carried out according to local practice.

The experiment was arranged as a randomized complete block design with three replicates. Two temperature treatments were set, natural air temperature (NAT) and high air temperature (HAT). Dimension of each plot was 3 × 6 m. Rice plants were exposed to HAT treatment from heading stage to maturity. We designed HAT treatment according to the method stated by Kobata and Uemuki (2004) with some modifications. Steel frames 2 m high were placed on trial plots as HAT treatments. Their tops were covered by polyester films as proofs, with a small hole (0.02 m in diameter) in the center of each proof, the sides from 0.7 m above ground to the top were wrapped with flimsy polyethylene films, and the rest parts remained uncovered to permit natural ventilation. These films had high transparency over 90% light transmittance. Plots without HAT treatment were arranged as control (NAT). The HAT treatment effectively increased air temperature, with other climatic conditions unchanged.

Plant sampling

Rice plants with uniform development process were tagged at heading stage in each plot. Tagged plants of three hills from each plot were sampled at 7, 13, 19, 25, 31, and 37 d after heading stage, with flag leaves removed from plants for partial physiological parameters measurements. Part flag leaves were dried at 105 °C for 1 h and then at 80 °C until constant weight in a forced-air oven, subsequently milled to powder for measurements of soluble sugar content. The remainder (fresh sample) was used for assay of malondialdehyde (MDA) and soluble protein contents.

Determination of SPAD value

Chlorophyll relative content was expressed by SPAD value. Twenty flag leaves of tagged plants from each plot were sampled to measure chlorophyll relative content by SPAD chlorophyll meter (SPAD 502 Plus, Spectrum Technologies, Plainfield, Illinois, USA) at 7, 12, 17, 23, 28, 33, and 38 d after heading stage. Measurements were made three times through nipping the top, middle, and bottom parts of each flag leaf, and these data were averaged as SPAD value of each leaf.

Assay of soluble sugar content

A 0.15 g powder of flag leaves was transferred into a centrifuge tube with scale and then 7 mL 80% alcohol was added to extract soluble sugar. The determination was made according to the method of Xiong (2003).

Measurements of soluble protein and MDA contents

A 200 mg of flag leaves was put into a mortar with the supplement of 5 mL of phosphate buffer (pH 7.0) subsequently mixed and ground into homogenate in ice-bath. The extracting solution was prepared for the determination of soluble protein content. A 0.5 g of flag leaves was transferred into a mortar with the addition of 50 mmol L^{-1} phosphate buffer (pH 7.8), then mixed and ground into homogenate in ice-bath. Extracting solution was used to measure MDA content. Test methods were described by Xiong (2003).

Assay of yield and yield components

At maturity, rice plants of five hills were sampled in each plot for the determination of yield components (1000-grain weight, the number of filled grains per panicle and effective panicles). Rice plants of 3 m^2 quadrat (except border plants) in each plot were harvested to estimate grain yields at moisture content of 140 g kg^{-1}.

Statistical analysis
Data were analyzed by SPSS (Statistical Package for the Social Sciences) base software version11.5 (2001). Least significant difference (LSD) at $p = 0.05$ probability level was used to distinguish among treatments. There were no significant differences in grain yield and yield attributes across the two study years and the interaction between year and temperature treatments (Table 1). Similar results were obtained in other physiological parameters of flag leaves (Table 2). Therefore data from both study years were averaged.

RESULTS AND DISCUSSION

Differences in experimental factors
In the experiment, from rice heading stage to maturity (6 August to 19 September 2006 and 7 August to 20 September 2007), mean air temperatures of daytime (from 07:00 to 19:00 h) and nighttime (from 19:00 to 07:00 h) under HAT were approximately 2.0 and 0.3 °C higher than those under NAT (Figure 1).

Effects of HAT on physiological parameters of rice flag leaves
SPAD values and soluble sugar content increased initially then fell gradually for both treatments after heading stage (Figures 2 and 3). HAT significantly reduced the averages of SPAD values and soluble sugar content by 31.34% and 16.29%, respectively, after heading stage. The relationship between the two physiological parameters and the days after heading stage was well described by quadratic curve equation, with days as independent variable (Figures 2 and 3). Under HAT stress, the average of soluble protein content significantly decreased by 9.88% while MDA content increased by 7.78%, when compared with those under NAT. There was a strong correlation between soluble protein and MDA contents and the days after heading stage, which could be simulated by linear regression equation (Figures 4 and 5). It is widely accepted that chlorophyll plays a pivotal role in regulating photosynthesis, including the capture of sunlight and the conversion of luminous energy (Oh et al., 1997). In general, chlorophyll content is positively correlated with photosynthetic rate in rice leaves (Liu, 1980). SPAD value represents the relative content of chlorophyll, which is convenient and effective for the research of chlorophyll level without damaging rice plants organs. A strong correlation between chlorophyll content and SPAD value has been identified, namely, the higher the SPAD value, the higher chlorophyll content is found in rice leaves (Xie et al., 2012). As a result, SPAD value is an important element reflecting photosynthesis ability of rice leaves. In the present experiment, diminished SPAD values suggest impaired photosynthesis in rice leaves under HAT.

Soluble proteins in leaves have been identified as crucial enzymes related to biological metabolism, such as protease determining photosynthesis in photosynthetic apparatus. Soluble sugars are the

Table 2. ***P*** **values from ANOVA results of the effects of temperature treatments and years and their interactions on SPAD value, soluble sugar, soluble protein and MDA contents in rice flag leaves.**

Sources of variance	df	SPAD	Soluble sugar content	Soluble protein content	MDA content
Temperature treatments (T)	1	0.005	0.002	0.007	0.029
Year (Y)	1	0.226	0.344	0.574	0.642
T×Y	1	0.181	0.667	0.228	0.304

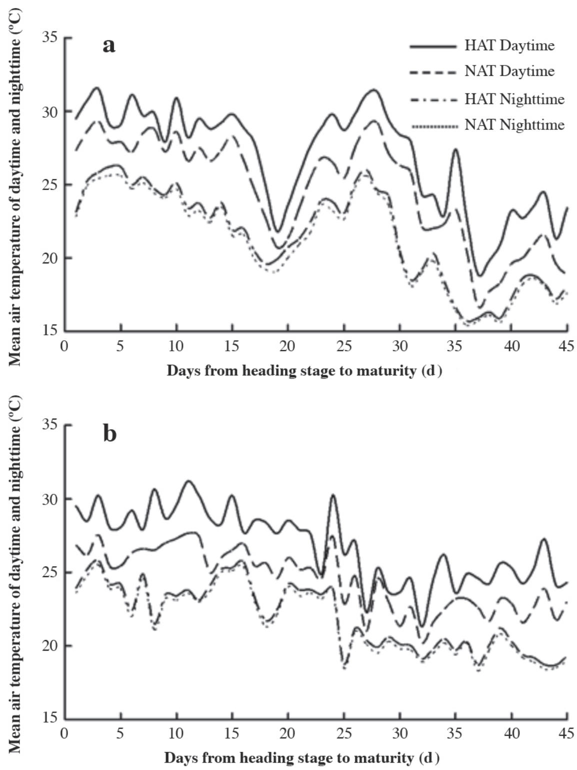


Figure 1. Mean air temperature of daytime from rice heading stage to maturity under natural air temperature (NAT) and high air temperature (HAT) in 2006(a) and 2007(b).

Table 1. ***P*** **values from ANOVA results of the effects of temperature treatments and years and their interactions on grain yield, yield attributes.**

Sources of variance	df	Number of effective panicles m^{-2}	Number of filled grains $panicle^{-1}$	1000-grain weight	Seed-setting rate	Grain yield
				g	%	g m^{-2}
Temperature treatments (T)	1	0.230	0.008	0.004	0.008	< 0.001
Year (Y)	1	0.696	0.577	0.500	0.930	0.179
T×Y	1	0.203	0.101	0.392	0.423	0.675

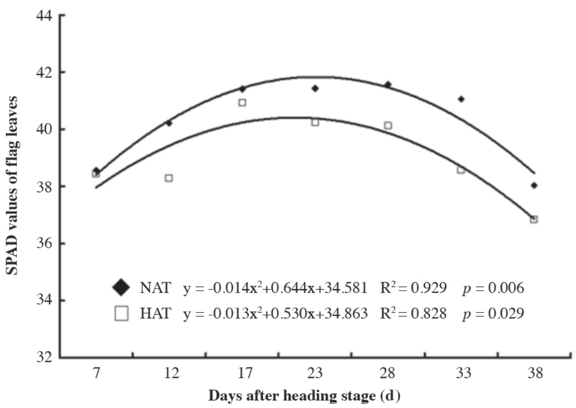
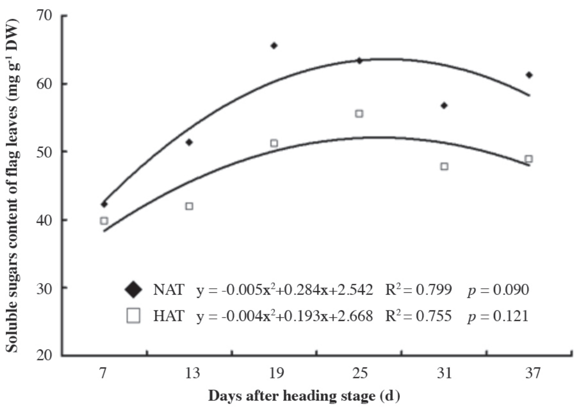


Figure 2. Dynamics of SPAD values of flag leaves after heading stage under natural air temperature (NAT) and high air temperature (HAT).

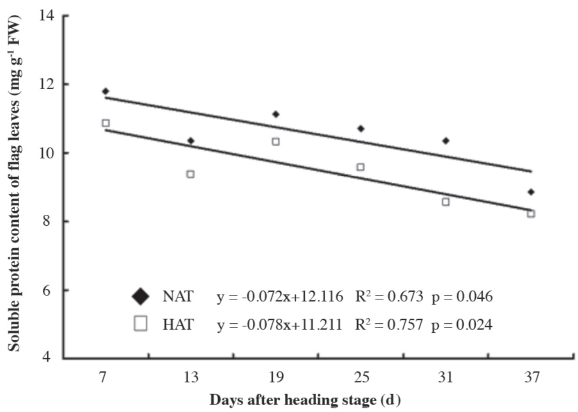


DW: Dry weight.

Figure 3. Dynamics of soluble sugar content of flag leaves after heading stage under natural air temperature (NAT) and high air temperature (HAT).

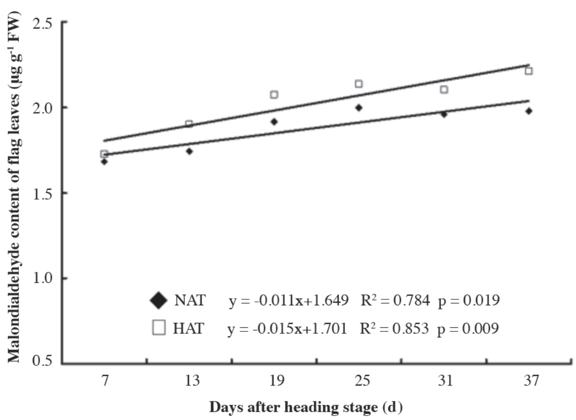
products of photosynthesis in leaves, being an important parameter evaluating photosynthetic function. In general, approximately 60% of assimilates demanded by rice grain-filling are derived from post-anthesis photosynthetic production produced by flag leaves, which prominently contributes to grain filling. Therefore, the photosynthetic ability of flag leaves is crucial for the determination of grain yield. In the present experiment, HAT triggered a significant decrease in SPAD values, soluble protein and sugar contents of flag leaves, implying that photosynthetic mechanism was severely impaired along with the reduction of photosynthesis under HAT, which was in agreement with the reports by Zhang et al. (2007), who found that the impaired net photosynthetic rate by HAT was mainly attributed to the reduction of chlorophyll content as well as activities of activating enzyme (RuBisCO) and carboxylase (RuBP) involved in photosynthesis in flag leaves.

Previous studies have demonstrated that soluble sugars and proteins excreted by plant are also main substances for the osmotic regulation and the protection of stable cell membrane structure under diverse stress conditions, which is beneficial to maintain normal turgor pressure and avoid serious dewatering for cell metabolism (Zhang et al., 2007). Normally, the generation and erasion of reactive oxygen species remains balanced in plant cell; however, the balanced system was disturbed in adversity stress (Kreiner et al., 2002; McDonald and Vanlerberghe, 2005). Thus the enhancement of the amount of reactive oxygen species certainly results in lipid peroxidation with MDA as terminal products, accompanied by the injury of cell membrane structure and function (Apel and Hirt, 2004; Volkov et al., 2006; Hu et al., 2008). MDA content is a vital symbol defining degree of lipid peroxidation, the higher the MDA content, the more severely the cell membrane damaged (Heath and Packer, 1968). In the present experiment, MDA content of flag leaves was enhanced significantly under HAT, suggesting that HAT increased cell membrane permeability, precluded physiological metabolism and expedited senescence of



FW: Fresh weight.

Figure 4. Dynamics of soluble protein content of flag leaves after heading stage under natural air temperature (NAT) and high air temperature (HAT).



FW: Fresh weight.

Figure 5. Dynamics of malondialdehyde content of rice flag leaves after heading stage under natural air temperature (NAT) and high air temperature (HAT).

flag leaves, which was also one of the main reasons for the decline of dry matter production in rice under HAT.

Effects of HAT on rice grain yield and yield attributes

Since the HAT treatment was conducted from rice heading stage, there was no significant difference in effective panicles per m^2 between NAT and HAT (Table 3). When compared to NAT, significant decreases of 25.70%, 22.09%, 5.17%, and 31.24% in number of filled grains per panicle, seed-setting rate, 1000-grain weight and grain yield under HAT were observed (Table 3). Matsui et al. (2000) reported that the decline of pollen activity, due to the disturbance of pollen swelling, was the main factor limiting seed-setting rate in rice. Insufficient assimilation products from vegetable organs to developing grains rather than the decrease of sink capacity induced the reduction of rice grain weight under HAT, as described by Kobata and Uemuki (2004), who demonstrated it through thinning treatment. Results in the experiment also confirmed that the decreased soluble sugar content as main photosynthesis assimilates in flag leaves restricted nutritional absorption of grains, decreasing final grain weight. In addition, our research showed that the fall of the grain yield caused by HAT was primarily attributed to the reduced seed-setting rate and grain weight. It is notable that HAT generated more influence on seed-setting rate than grain weight. These results coincided with the conclusions made by Jagadish et al. (2007); Li et al. (2011); and Madam et al. (2012).

Table 3. Effects of high air temperature (HAT) on rice grain yield and yield attributes.

Treatment	Effective panicles m^{-2}	Number of filled grains per panicle	Seed-setting rate	1000-grain weight	Grain yield
			%	g	g m^{-2}
NAT	198.75a	187.66a	86a	27.85a	1007.83a
HAT	195.39a	139.43b	67b	26.41b	692.98b

Numbers followed by different letters are significantly different (p = 0.05). Data are the averages for the 2 yr study.
NAT: natural air temperature.

CONCLUSIONS

High air temperature (HAT) significantly decreased SPAD values (relative content of chlorophyll), soluble sugar and protein contents of leaves and grain yield, while enhanced malondialdehyde content in leaves under field condition. Therefore, we can conclude that HAT stress reduces the amount of assimilates produced by photosynthesis and accelerates the senescence of flag leaves, which are key causes of the decline of rice grain yield.

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