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Heat Stress Affects Seed Set and Grain Quality of Vietnamese Rice Cultivars during Heading and Grain Filling Period

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Abstract

Environmental stress trigger a variety of rice plant response, ranging from alters seed set, grain yield and grain quality during flowering and grain filling stage. Efforts are required to improve our understanding of the impact of heat stress on rice production, which are essential strategies in rice cultivation. This article investigated the seed set, yield components and grain yield of Vietnamese rice cultivars (*Indica* germplasm) under high temperature environment during the flowering and grain filling stage. Six rice cultivars, including popular cultivars and new cultivars of Cuu Long Delta Rice Research Institute, and one popular extraneous cultivar with differences in maturing time, were grown in pots at high temperature (HT) and natural temperature condition as control (CT). All rice cultivars were subjected to the high temperature starting from the heading stage to the harvest maturity, applied by greenhouse effect. The greenhouse has about 25 cm window opening on 3 sides for air ventilation. The seed set rate of the heat-sensitive rice genotypes decreased significantly under HT, leading to a significant reduction in grain yield. The lowest seed set was recorded in "OM4900" (44.3%) and "OM18" (39.9%) under high temperature environment. The lower yield in all rice cultivars at an elevated temperature resulted in a dramatic decrease of filled grains and contributed to a loss of 1000-grain weight. "OM892" is a potential rice cultivar for heat tolerant breeding program due to the seed set percentage was above 80% in both HT and CT conditions. High temperature during the grain filling stage resulted in a decreased amylose and increased chalkiness for all OM cultivars.

Keywords: grain quality, high temperature, OM rice, seed set

Introduction

Rice is one of the most important crops in the world, especially in Asia, where approximately 90% of the rice production is consumed (FAO, 2008). Rice consumption has been increasing in Africa and Latin America as well (Jagadish et al., 2007). Unfortunately, climate change is predicted to have negative impacts on food production, food quality and food security (Ceccarelli et al., 2010). High temperature caused by climate change is exposing most of the world's crops, including rice, at least at some stages of their life cycle. Global warming might increase agricultural production in some temperate countries, but possibly reduce it in most Asian countries (Rosenzweig et al., 1993). In the report of Shah et al. (2014), the increase in the daily mean temperature of 2°C would make a dramatic reduction in rice grain yield. Wheat and rice are more sensitive to high temperature during reproductive stages compared to vegetative stages (Djanaguiraman et al., 2020).

Many studies have discussed the impact of the rising temperature on rice cultivation. The critical temperature differs according to variety, growth stage, duration of critical temperature and diurnal change (Sridevi and Chellamuthu, 2015). Plant reproductive processes are complex and sensitive to environmental changes, including high temperatures, which ultimately affect fertilization and post-fertilization processes leading to decreased yields. High temperature affects anther dehiscence, pollination and pollen germination which then leads to spikelet sterility and yield loss (Yoshida et al., 1981). Flowering is one of the most susceptible stages in the life cycle of rice, and rice spikelet at anthesis exposed to above 35 °C for 4–5 days induces sterility, with no seed produced (Thuy et al., 2020)

High temperature during the grain filling period limits grain yield and quality in rice. High night temperature

and day high temperature adversely affect rice productivity (Haixian et al., 2011). Rice quality is mainly evaluated by several traits, including milling, appearance, cooking, eating, nutritional qualities and other indicators. In addition to genetic factors, these traits are also affected by the grain positions on the rachis and environmental factors such as water status and high temperature. Previous studies showed that grain quality was significantly affected by the different positions of grains, which is particularly relevant to heavy-panicle rice varieties (Cheng et al., 2000).

In rice production and breeding practices, heading date and yield traits are generally considered as key indicators for the response to temperature variation; for instance, early heading and yield loss have been commonly observed at high temperatures. The identified heat-tolerant varieties based on high seed-set rate and the phenotype protocols could be used in future genetic studies and breeding programs focusing on heat tolerance. Throughout this study, we determined the heat tolerant cultivars for future warmer circumstances by phenotype protocols. In addition, our investigation presented yield and grain quality parameters response to the temperature of Cuu Long Delta Rice Research Institute cultivars which could provide a reference for building crop index based weather.

Materials and Methods

Crop Cultivation

Six cultivars of Cuu Long Delta Rice Research Institute (OM cultivars) and one popular extraneous cultivar of CLRRI in Mekong Delta were used in this experiment (Table 1). The selected cultivars include three popular cultivars, three new cultivars

and one heat tolerant cultivar. Depending on the growth duration, the sowing date of each cultivar was adjusted to make the synchronization of anthesis in all cultivars. Three hills (3 plants/hill) of each cultivar were sowed to pot (25 cm height x 25 cm diameter x 25 bottom diameter) filled with soil. Rice plants were given additional fertilizer at the ratio N-P₂O₅-K₂O=10-6-4 (g/m²). Twenty pots for each cultivar were placed in the net house until heading, i.e., when the first panicle was extruded out of its flag leaf sheath.

High Temperature Treatment

The experimental design was a split-plots with ten replications. The main plots and the subplots were two temperature regimes (high temperature and control) and 6 cultivars (Table 1), respectively. This study was conducted in the net house; control (CT) and in the greenhouse; high temperature treatment (HT). In HT condition, heat stress was applied by using greenhouse effect to increase the temperature inside which opened about 25 cm on 3 sides for air ventilation. In CT condition, the temperature inside the net house was similar to the natural condition. The temperature treatment started at heading stage, half pots of each cultivar were transferred to HT while half of it remained in CT. The temperature treatments were applied from heading to harvest maturity.

Measurement of Air Temperature

The air-temperature in net house and green house was measured with thermo recorders 'Ondotori' (TR-55i-Pt, T AND D, Japan). The temperature sensors were installed in a force-ventilated radiation shield and placed at 2 m height above the ground. The diurnal air-temperature was recorded every 10-minutes from the heading stage to harvest maturity.

Table 1. Details on the growth duration, sowing date and the heading date of seven cultivars in the net house of Cuu Long Delta Rice Research Institute, Can Tho, Vietnam.

No.	Rice cultivar	Growth duration (days)	Sowing date	Heading date	Note
1	"OM5451"	99	20-Nov-20	25-Jan-21	Popular cultivar of CLRRI in Mekong Delta
2	"OM8923"	99	20-Nov-20	25-Jan-21	Heat tolerant cultivar of CLRRI (Thuy and Shaitoh, 2017)
3	"OM8"	99	20-Nov-20	25-Jan-21	New cultivar of CLRRI
4	"OM426"	99	20-Nov-20	25-Jan-21	New cultivar of CLRRI
5	"OM18"	99	20-Nov-20	25-Jan-21	New cultivar of CLRRI
6	"OM4900"	103	15-Nov-20	25-Jan-21	Popular long growth duration cultivar of CLRRI
7	"Jasmine85"	103	15-Nov-20	25-Jan-21	Popular extraneous cultivar of CLRRI in Mekong Delta

Yield and Yield Components

At physiological maturity, 30 hills were sampled diagonally from each treatment to determine grain yield/ hill and yield components; i.e., the number of panicle per hill, 1000-grain weight (g), number of spikelet per panicle, and percentage of filled grain (%). The grain yield was adjusted to the standard moisture (14%).

Heat Tolerance Analysis

The heat tolerant cultivars were classified base on the spikelet fertility (seed set) at the maturity stage according to IRRI (2002). Heat tolerance scoring system in rice was defined base on the percentage of seed set (fertility) with the following scale; high tolerant (>80%), tolerant (61-80%), moderately tolerant (41-60%), susceptible (11-40%), highly susceptible (<11%).

Chalky Percentage

Three replications (20-30g each) of milled grains of each treatment were used for the determination of chalky grains. Grains covering white parts covering more than 10% of their total surface area, for example, a white belly, white center, or white back, were recorded chalky grains.

Amylose Content

Three replications (0.1g) of rice starch of each treatment were prepared from rice seeds by diluted alkali method and gelatinized by treatment with 1 mol.L⁻¹ NaOH. Amylose content was measured by a colorimetric method using an iodine-potassium iodide solution (Rolando et al., 2004).

Statistical Analysis

Data of yield, yield components and grain quality were analyzed as two-way completely randomized design. Tukey's HSD at a probability level of 0.5 and 1.0 % was used to compare the difference between treatment and genotype. SAS version 9.2 (SPSS Inc.) was used for data analysis.

Results

All rice cultivars were subjected to high temperature environment after heading. The maximum temperature in heading time in HT and CT was higher and less than 35°C, respectively. The physiological effects of high temperature on sterility and grain yield when the temperature over 35°C had been documented (Matsui et al., 2001). During the day, the temperature in green house (HT) was always around 4.0-8.5°C higher than net house (CT) (Figure 1). The temperature of both in HT and CT were changed by the natural air temperature. The max-temperature was 35.8-40.0°C and 29.5-34.9°C in HT and CT, respectively. The minimum temperature was not much different between HT and CT.

The seed set rate decreased in all tested cultivars under high temperature (Table 2). The seed set rate of OM cultivars ranged from 73.4% to 89.4% in the max-temperature in day around 30.5-31.4°C during the anthesis. When the max-temperature in day increased to 37.8-38.6°C the seed set of these cultivars ranged from 55.7-80.8%. Most OM cultivars were tolerant and moderate tolerant to high temperature. "Jasmine85" was also the same as OM cultivars in heat tolerant score. "OM8923" kept the

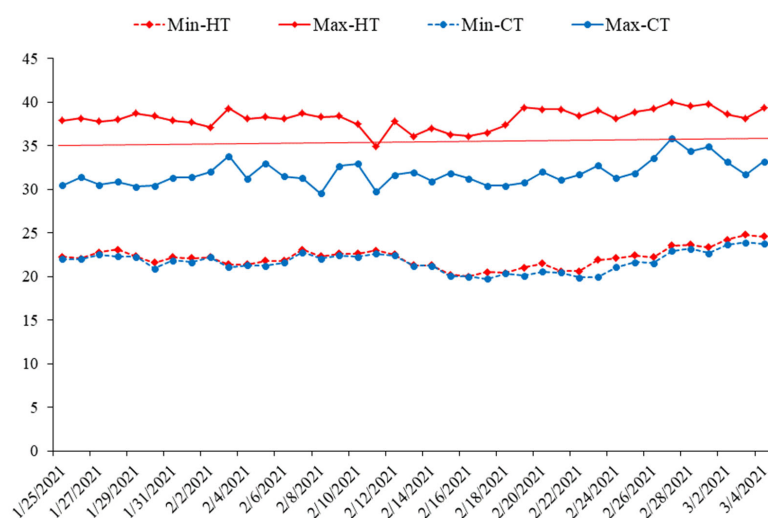


Figure 1. The maximum and minimum temperature in the high temperature and control environment after the rice heading stage.

percentage of seed set in the range of high tolerant score under both conditions.

The magnitude of genotypic effects on yield traits varied greatly, showing an association of stronger effects with increased daily-mean temperature (Table 3). The grain yield of all cultivars decreased significantly in the range 13.5-46.3% under the high temperature condition. The highest reduction was in "OM5451" with 46.3% yield loss by heat stress. The grain yield in "OM8923" was the least affected by high temperature. The number of panicle per hill and the number of spikelet per panicle were not affected by the temperature regime. The significant decrease in filled grains and 1000-grain weight under high temperature condition lead to lower yield of all cultivars under high temperature condition.

The percentage of grain chalkiness in most cultivars increased significantly under high temperature (Figure 2A). The difference in the percentage of grain chalkiness between CT and HT varied widely among six cultivars, and ranged from 0.2-12.2% and 3.4-40.6% in CT and HT, respectively. The chalkiness in "OM5451" and "OM426" were strongly affected by high temperature during the grain filling stage. The chalky ratio of "Jasmine85" on HT and CT was similar in 7.6 and 6.6%, respectively. High temperature during grain filling had a considerable influence on amylose content in hulled rice endosperm (Figure 2B). There is a significant reduction of amylose content in HT compared to CT. Amylose content at maturity of OM cultivar was 19.5 and 20.4 % at HT and CT condition, respectively. "OM8" was the most affected in amylose by heat stress with 10.9% lower in HT compared to CT.

Discussions

The optimum temperature range for rice development fluctuates between 27-32°C (Xinyou et al., 1996). The temperatures of >35°C negatively affect to flowering pattern and spikelet fertility (Matsui et al., 2001). In this study, the maximum temperature after the heading in HT was remained higher than 35°C and lower than 35°C in HT and CT, respectively. The temperature in HT reached over 35°C from 09.00 to 16.30 that is the anthesis time of *indica* rice group. The temperature in HT was always higher than the critical temperature for rice development during flowering and grain filling stage. In CT, air temperature was in the optimum range to rice development.

The flowering stage in most rice genotypes occur over a 5 days period, and the anthesis duration varies in different rice genotype (Prasad et al., 2006). During the flowering stage, temperatures higher than the optimal temperature induce spikelet sterility and thus decrease the rice yield (Nakagawa et al., 2003). The anthesis time in day is important due to the fact that spikelet sterility is induced by high temperature during or soon after anthesis (1~3 h in rice) (Satake and Yoshida, 1978). In this study, the fertility of OM rice cultivars decreased when the temperature reached over 35°C. These results were consistent with the studies of Yoshida et al. (1981) in that the temperature above 35°C at the flowering stage induces floret sterility. Spikelet sterility was induced by high temperature particularly on the flowering day. During the flowering day, high temperature during anthesis time was the most detrimental to spikelet fertility, followed by high temperature right before anthesis. High temperature after anthesis had little influence on spikelet fertility as reported by Satake and Yoshida (1978) that spikelets exposed to high temperature one hour after

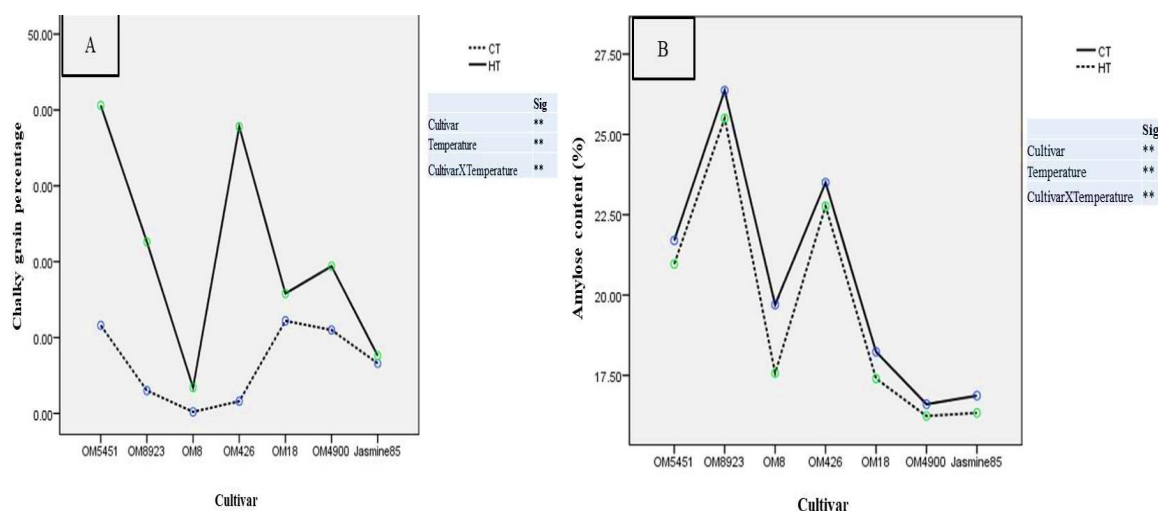


Figure 2. The quality of different cultivars of rice in the high temperature and control condition. * and ** show significant differences based on ANOVA at $\alpha=0.05$ or $\alpha=0.01$, respectively.

Table 2. The seed set rate and scale of heat tolerance of several rice cultivars under high temperature and control condition

Rice cultivar	Temperature condition	Seed set rate (%)	Scale of heat tolerance
"OM5451"	CT	89.3	High tolerant
	HT	56.8	Moderately tolerant
"OM8923"	CT	89.4	High tolerant
	HT	80.2	High tolerant
"OM8"	CT	84.8	High tolerant
	HT	61.5	Tolerant
"OM426"	CT	84.1	High tolerant
	HT	70.5	Tolerant
"OM18"	CT	73.4	Tolerant
	HT	60.1	Tolerant
"OM4900"	CT	75	Tolerant
	HT	55.7	Moderately tolerant
"Jasmine85"	CT	90.1	High tolerant
	HT	69.9	Tolerant

Note: the maximum temperature during anthesis was 37.8-38.6°C in the high temperature environment and 30.5-31.4°C in the control condition.

Table 3. Yield and yield components of seven rice cultivars in the high temperature and control condition.

Rice cultivar		Number of panicle/ hill	Number of spikelets/ panicle	1000-grain weight (g)	Filled grains (%)	Yield/hill (g)
"OM5451"	CT	13	118	25.1	89.3	34.3
	HT	12	109	24.8	56.8	18.4
"OM8923"	CT	12	138	25.5	89.4	37.8
	HT	12	134	25.0	80.2	32.2
"OM8"	CT	16	89	26.5	84.8	32.0
	HT	15	86	26.2	61.5	20.7
"OM426"	CT	13	89	24.9	84.1	24.3
	HT	12	86	24.7	70.5	18.0
"OM18"	CT	11	165	26.4	73.4	35.2
	HT	12	162	26.1	60.1	30.4
"OM4900"	CT	12	135	25.0	75	30.3
	HT	12	127	25.0	55.7	21.2
"Jasmine85"	CT	11	106	25.4	90.1	26.7
	HT	12	99	25.2	69.9	20.9
Cultivar		**	ns	**	**	**
Temperature		ns	ns	**	**	**
Cultivar*Temperature		ns	ns	**	**	ns

Note: * and ** show significant difference based on ANOVA at $\alpha=0.05$ or $\alpha=0.01$, respectively; ns = not significant.

anthesis had normal fertility. The major causes of high temperature-induced sterility were attributed to the disturbed pollen shedding and the decreased viability of pollen grains, resulting in decreased number of germinated pollen grains on a stigma (Thuy et al., 2020).

In this study, yield losses under high temperature were attributed to spikelet sterility and reduction of grain weight (Table 3.) which was the same finding of Prasad et al. (2006). Flowering is the growth stage that is the most susceptible to high temperature in rice, and poor germination of pollen grains on stigma is partially responsible for the spikelet sterility (Satake and Yoshida, 1978). The decrease of grain weight is related to the formation of imperfect grains and the reduction in dry mass accumulation and translocation (Tashiro and Wardlaw, 1991).

Chalky, an opaque in the rice grain, is an important quality characteristic in rice and occurs most commonly when rice is exposed to high temperature during development (Areum et al., 2009). The primary cause for chalky grain is an imbalance between sink and source abilities of carbohydrate metabolism, as a result of high temperature at the ripening stage (Wang et al., 2006). Endosperm chalkiness is both qualitative and quantitative characteristics of improper grain filling in determining grain appearance quality (Peng et al., 2014). Copper et al. (2008) showed that high ambient temperature from the anthesis (R4) to single grain maturity stage (R8) in reproductive development induced increases in chalky kernels. In this study, the mean daily temperature from R4 to R8 in HT was higher than that in CT with the increases of 2.1-4.8°C. Thus, the percentage of chalky grains observed the same trend as the previous report. In the report of Tabassum et al. (2021), the number of days with max-temperature above 34°C was more causative to chalky ratio than days with lower temperature. In this study, the max-temperature was always above 35°C corresponding to the increase of chalkiness percentage in all OM cultivars (Figure 2A).

Amylose content in all OM cultivars was reduced under high temperature conditions (Figure 2B.). Our findings are in agreement with the previous findings that high temperature during grain filling affects the total starch content and reduced amylose content in endosperm starch (Cheng et al., 2000). The previous study suggested that the change in amylose/amylopectin ratio under high temperature attributed to reducing in activity of granule-bound starch synthase and the role enzyme responsible for amylose biosynthesis (Ahmed et al., 2015).

Conclusions

This study suggested that the reduction of grain yield in OM cultivars was due to the negative effects of high temperatures on filled grain (seed set) rate and grain weight. High temperatures at the anthesis time have a negative impact on the seed set ability of OM cultivars. All OM cultivars were in moderately to high tolerant range to high temperature. The most sterile-tolerant cultivar to heat stress was "OM892". Consistent with the previous reports, the grain quality of OM cultivars parameter during grain filling stage changed at high temperature. "OM8923" is sterile-tolerant when subjected to high temperatures during the anthesis stage, therefore this cultivar could be used as a breeding material to develop heat tolerant cultivars for the future warmer circumstances.

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