REGULATING EFFECT OF NITROGEN ON GRAIN YIELD AND ANTIOXIDANT DEFENSE OF SUPER AND NON-SUPER FRAGRANT RICE UNDER HIGH SOIL TEMPERATURE

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Abstract. The purpose of this study was to investigate the effects of nitrogen (N) application on the grain yield and antioxidant response in fragrant rice under high soil temperature during the flowering stage. A pot experiment was conducted with two conventional fragrant rice varieties, i.e., super rice variety (Yuxiangyouzhan) and non-super rice variety (Xiangyaxiangzhan) grown under two nitrogen application levels (ZN, 0 g N per 12 kg soil and AN, 1.75 g N per 12 kg soil) as well as two temperature levels (Ambient soil temperature, AT and high soil temperature, HT). The grain yield and antioxidant defense of super and non-super fragrant rice were evaluated. The results showed that significant differences in grain yield and antioxidant defense parameters with regard to the variety, temperature treatment, nitrogen treatment, and their interactions were detected. Compared with ZN, the AN treatment significantly increased the grain yield of Yuxiangyouzhan and Xiangyaxiangzhan by 55.06% and 13.32%, respectively, under AT conditions and by 32.62% and 29.19%, respectively, under HT condition. Besides, the antioxidant parameters varied by variety and temperature treatment at AN treatment. Nitrogen application had a significant effect on Superoxide dismutase (SOD) activity and Catalase (CAT) activity in the leaves. The results of this study confirmed that the N application could regulate grain yield and antioxidant capacity of fragrant rice at flowering stage under HT condition.

Keywords: *growth, antioxidant enzyme, flowering stage, heat stress, climate change*

Introduction

Rice (*Oryza sativa* L.) is the major cereal crop for half of the world's population, and demand is expected to double by 2050 (Ray et al., 2015). China is the largest rice producer, with nearly 30 million hectares of rice cropland (Song et al., 2022). Global temperatures have increased by about 0.2°C per decade due to the greenhouse effect (Kim et al., 2013; Ray et al., 2015). High temperatures have been reported to led to a decline in rice yields (Peng et al., 2004). Rice production in China has been seriously affected owing to the severe impact of intensified, extreme heat. For example, Xiong et al. (2017) and Ali et al. (2019) noted a 39.6% reduction in cereal yields at high temperatures. Similarly,

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antioxidant parameters are also affected by high temperatures (Cooper et al., 2008; Han et al., 2009).

The flowering stage of rice is one of the most sensitive periods, during which rice is highly vulnerable to high temperature stress (Satake and Yoshida, 1978). Previous studies have shown that abnormal cracking of anthers (Matsui and Omasa, 2002) and a decreased number of pollinated and germinated pollen grains on the stigma (Satake and Yoshida, 1978) lead to increased spikelet sterility at high temperature. Jagadish et al. (2007) found that temperatures exceeding 35°C for more than one hour during the flowering stage can significantly reduce spikelet fertility. Studies have shown that high temperature stress causes physiological damage to rice plants, leading to reduced metabolism of enzyme activities, and thereby reducing rice yield (Cooper et al., 2008; Han et al., 2009). There are previous studies that suggest that high temperatures in the air and temperature differences in the soil may affect carbohydrate metabolism in plants (Xu and Huang, 2000). Additionally, some studies have revealed that soil temperature appears to be more critical than air temperature in controlling plant growth (Aldous and Kaufmann, 1979; Kuryanagi and Paulsen, 1988; Martin et al., 1989; Ruter and Ingram, 1990; Xu and Huang, 2000). What’s more, soil temperature impacts root growth (McMichael and Burke, 1998), such as significantly affecting the root vigor, root proline content, and the Malondialdehyde (MDA) concentration (Bai et al., 2017). Soil temperature affects photosynthesis (Wu et al., 2012); high or low temperatures in air, soil, or irrigation water during the day or night may result in different growth responses in rice plants (Beauchamp and Lathwell., 1967). Therefore, it is necessary to understand the response of rice to high temperatures and to take relevant crop management measures to alleviate the adverse impacts of high temperatures on rice yield in order to maintain food security.

The temperature of plant tissues and organs can reflect the degree of high-temperature stress on plants. Some studies have reported that reasonable N application can decrease the temperature of plant principles, leaves, and canopy, which is also conducive to increasing N uptake and dry matter accumulation in rice, and ultimately improving the high temperature tolerance of rice (Yan et al., 2008; Coast, 2015). Besides, it has been reported that N applications increased grain yield eventually by influencing spike number and seed-setting rate (Zhou et al., 2021). The generation of reactive oxygen species (ROS) is one of the indicators of plants responses to adverse environmental conditions (Chinchilla et al., 2007; Hedrich, 2012; Jwa and Hwang, 2017). In general, plants have developed various mechanisms to cope with the accumulation of ROS, including the activation of the antioxidant enzyme systems, non-antioxidant enzyme systems, and detoxification processes (Apel and Hirt, 2004). Superoxide dismutase (SOD), peroxidase (POD), and Catalase (CAT) as the three major antioxidant enzymes, play a crucial role in scavenging ROS (Gill and Tuteja, 2010). Moreover, the Malondialdehyde (MDA) is one of the end products of lipid peroxidation (Marnett, 1999), and its presence indicates oxidative stress occurs in plants (Yamauchi et al., 2008). Moreover, proline accumulation is common in many plant species to reflect environmental stress and plays an important role in osmoregulation in particular (Szabados and Savoure, 2010). Hence, the aim of present study was to explore the effects of nitrogen fertilizer application on rice yield and antioxidant systems at high temperatures.

Material and methods

Experimental details

A pot experiment was performed in 2018 (March-July) in the greenhouse at the Experimental Research Farm, South China Agricultural University, Guangzhou, China. Super rice variety (Yuxiangyouzhan) and non-super rice variety (Xiangyaxiangzhan) were used as experimental materials. At the three-leaf stage, uniform seedlings were manually selected and transplanted into plastic pots (25 cm in diameter and 23 cm in height) in June 2018. The pot was filled with 12 kg dry soil. The experimental soil was a sandy loam containing 20.45 g kg^{-1} soil organic matter, 1.09 g kg^{-1} total nitrogen, 1.03 g kg^{-1} total phosphorus, and 19.68 g kg^{-1} total potassium. There were 5 hills per pot and 3 seedlings were transplanted per hill. The water layer of 2-4 cm was maintained from transplanting to harvest. This experiment was arranged in a completely randomized design (CRD). There were 48 pots in total.

Experimental treatment

The present study was subjected to two nitrogen application levels (ZN, 0 g N/12 kg soil, and AN, 1.75 g N/12 kg soil) and the calcium superphosphate (3.20 g) and potassium chloride (1.11g) were applied to each pot as basal fertilizer. At the flowering stage (50% of the rice plant panicle begin flowering), two temperature levels (Ambient soil temperature, AT and high soil temperature, HT) were conducted. The pot group for the HT treatments were subjected to high temperature treatment for 5 consecutive days during the day from 9:00 to 12:00, respectively, and lasted for 5 days. In the temperature treatment, the original water in the pot was heated to a corresponding temperature of 35-42°C, and then water was poured back into the pots until the temperature in the 2-4 cm soil depth reached 36°C. The daytime temperatures were recorded during the experimental treatment period (*Figure 1*).

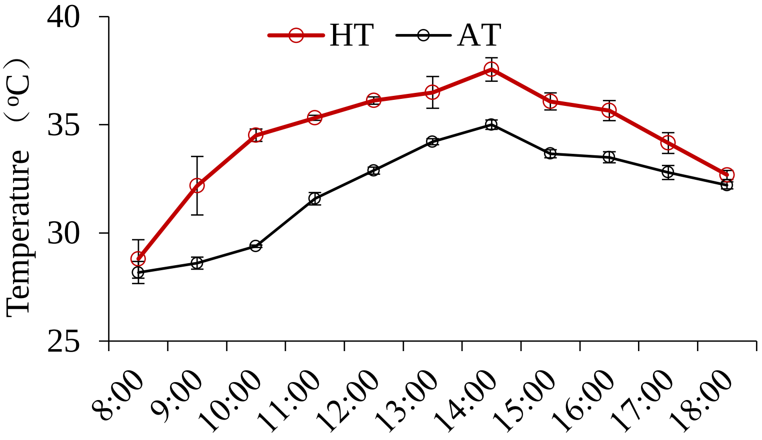

Figure 1. *Soil temperature conditions during the treatment period*

Sampling and measurements

Determination of yield and yield components

At mature stage, the six represents mature plants were selected from each treatment to measure the grain yield and the yield components. Grain yield was determined by weight

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after manual threshing and sun-drying of the grains. The number of panicles per pot, the total number of grains per panicle and the number of filled grains per panicle were recorded for each treatment to calculate filled grain percentage and 1000-grain weight.

Measurement of the antioxidant parameters

The uniform leaves were selected for the antioxidant parameter determination on the day 4 after the HT treatment. The leaves samples were frozen immediately in liquid nitrogen for 3 minutes and kept at -80 °C until biochemical analysis was performed. The crude enzyme solution is extracted as follows: Grind the fresh leaves (0.3 g) with liquid nitrogen and add 5 ml pH 7.8 phosphate buffer solution (PBS) to prepare homogenate in an ice bath. Homogenate was centrifuged at 8000 rpm for 15 minutes at 4 °C.

The Superoxide dismutase (SOD) activity was determined by the nitro blue tetrazolium (NBT) method (Li et al., 2021). Add 1.50 ml sodium phosphate (50 mM), 0.3 ml Met (130 mM), 0.3 mL EDTA Na (100 μ M) 0.3 ml riboflavin (20 μ M) and 0.25 ml distilled water, determination was conducted by a spectrophotometer (Shimadzu, Japan UV2600 UV), the absorbance was read at 560 nm. SOD activity per unit was defined as the amount of enzyme required to inhibit 50% NBT photoreduction reaction, and the active unit (U) is expressed as U g^{-1} FW.

Peroxidase (POD) activity was estimated by using guaiacol oxidation (Zou, 2003). The determination solution contains crude enzyme extract (50 μl), 0.3% H2O2 (1 ml), 0.2% guaiacol (0.95 ml) and 1 ml sodium phosphate buffer (50 mM). Determination was conducted by a spectrophotometer (Shimadzu, Japan UV2600 UV). The absorbance was read at 470 nm. Set a unit (U) to increase the light absorption value by 0.01 per minute, and the active unit is expressed as U g^{-1} min^{-1} FW.

Catalase (CAT) activity was measured according to Zhang et al. (1980). The reaction mixture consisted of 1.95 ml distilled water, 1.0 ml 0.3% hydrogen peroxide solution, and 0.05 ml enzyme extract. Determination was conducted by a spectrophotometer (Shimadzu, Japan UV2600 UV). The absorbance was read once at 240 nm at 30 second intervals for 2 minutes. One unit (U) is defined as the absorbance decreasing by 0.01 per minute, and the active unit is expressed as U g^{-1} min^{-1} FW.

Malondialdehyde (MDA) content was using the thiobarbituric acid (TBA) method (Hao et al., 2007). Add 0.4ml 0.5% thiobarbituric acid (TBA) to the crude enzyme extract (0.2 ml) and boiled in the water bath at 100°C for 30 min. The boiled samples were then cooled down, centrifuge at 3000 rpm for 15 minutes and determination was conducted by a spectrophotometer (Shimadzu, Japan UV2600 UV), the absorbance of the supernatant was recorded at 450 nm, 532 nm and 600 nm, and the content was expressed in nmol g^{-1} FW.

Proline (Pro) content was gauged by using G-250 (Bradford, 1976). Leaf samples (0.3 g) were homogenized in 3% sulfosalicylic acid(W/V) and homogenate was soaked in boiling water for 10 min. A mixed solution was made by using supernatant (0.4 mL), glacial acetic acid (0.4 mL) and chromogenic agent (0.4 mL) and soaking the mixture in boiling water for 30 min. Add toluene (0.8 mL) after cooling down, and the upper toluene extract (1 mL) was centrifuged at 10000 rpm for 3 min. Determination was conducted by a spectrophotometer (Shimadzu, Japan UV2600 UV). The absorbance at 520 nm was measured, the proline content was expressed in μg g^{-1} FW.

Statistical analysis

The experimental data were analyzed by Statistix version 8 (analysis software, Tallahassee, Florida, USA) and Microsoft Excel 2010 (Microsoft, New Mexico, USA). At the 0.05 probability level, the mean comparison among different treatments was estimated according to the least significant difference (LSD) test ($P<0.05$).

Results

Grain yield and yield components

Grain yield

Temperature treatment (T), Nitrogen treatment (N), V×N, and T×N caused a markedly effects on grain yield (*Table 1*). Compared with the ZN treatment, the AN treatment resulted in a pronounced increase in the grain yield by 55.06% and 13.32% for Yuxiangyouzuhan and Xiangyaxiangzhan under the AT condition, respectively. The AN treatment notably increased the grain yield by 32.62% and 29.19% for Yuxiangyouzuhan and Xiangyaxiangzhan, as compared with the ZN treatment under the HT condition, respectively. On average, the AN treatment caused a striking increase of 8.61% and 17.06% in the grain yield for Yuxiangyouzhan and Xiangyaxiangzhan, respectively, as compared with the ZN treatment. On average, the grain yields of Yuxiangyouzhan and Xiangyaxiangzhan at HT condition were lower (64.16% and 66.90%, respectively) than those under AT conditions. Hence, at ambient soil temperature, the N treatment caused a significant increase in the grain yield (*Figure 2*).

Table 1. *ANOVA of the investigated parameters*

Parameters	V	T	N	V×T	V×N	T×N	V×T×N
Yield	0.08ns	1163.10**	65.23**	0.84ns	12.10**	16.35**	11.23ns
Panicle per pot	2.06ns	4.17ns	31.56**	0.39ns	4.06ns	0.01ns	0.11ns
Grain number per panicle	53.26**	75.91**	57.77**	2.50ns	26.10**	0.41ns	1.85ns
Filled grain percentage	28.72**	786.23**	0.45ns	21.54**	0.06ns	2.12ns	0.49ns
1000-grain weight	163.60**	295.24**	3.49ns	0.61ns	0.49ns	1.24ns	0.16ns
SOD activity	0.38ns	13.79**	0.24ns	4.67ns	6.78*	1.05ns	0.76ns
POD activity	1.72ns	0.47ns	34.82**	0.36ns	7.17**	11.87**	10.47ns
CAT activity	94.57**	22.44**	11.08**	1.03ns	0.01ns	1.78ns	0.01**
MDA content	7.42ns	16.45**	4.66ns	0.09ns	1.11ns	1.11ns	9.67ns
Proline content	21.92*	2.91ns	3.73ns	0.93ns	10.00**	23.02**	3.88ns

V, Variety; T, Temperature treatment; N, nitrogen treatment. * and ** indicates significant difference at $P < 0.05$ and $P < 0.01$, respectively; ns indicates no significant difference

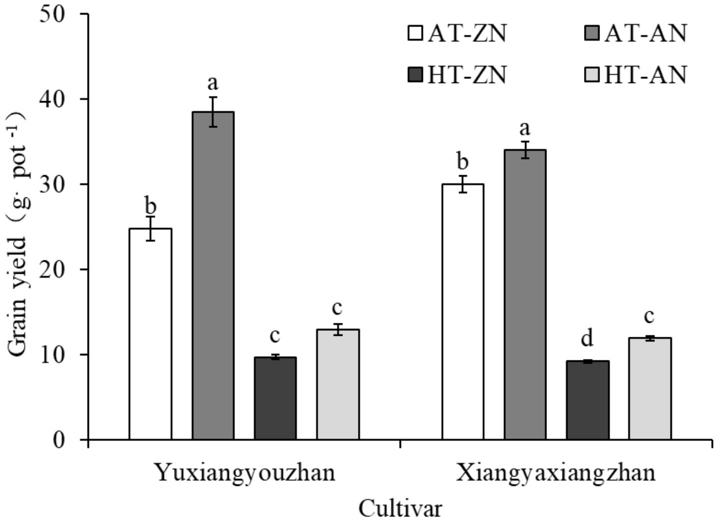

Figure 2. *Effect of nitrogen application on grain yield of super and non-super fragrant rice under high soil temperature. Vertical bars with different lower case letters above are significantly different at p = 0.05 by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; AN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

Panicle per pot

Nitrogen treatment (N) showed a pronounced increase in the panicle per pot (*Table 1*). Under the AT and HT conditions, the AN treatment produced an obvious increase in the panicle per pot by 14.52% and 15.00% for Yuxiangyouzhan and by 5.97% and 7.17% for Xiangyaxiangzhan, respectively, in comparison to the ZN treatment. Additionally, when temperature conditions were ignored, the AN treatment resulted in a pronounced increase in panicle per pot by 14.75% and 6.55% for Yuxiangyouzhan and Xiangyaxiangzhan, respectively (*Figure 3*).

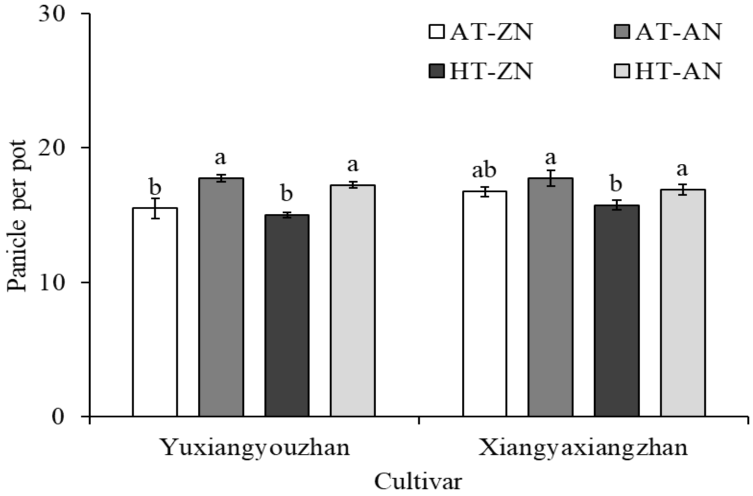

Figure 3. *Effect of nitrogen application on panicle per pot of super and non-super fragrant rice under high soil temperature. Vertical bars with different lower-case letters above are significantly different at p < 0.05 by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; AN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

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Grain number per panicle

Variety (V), Temperature treatment (T), Nitrogen treatment (N), and V×N significantly influenced the grain number per panicle (*Table 1*). Under the AT and HT conditions, the AN treatment significantly enhanced the grain number per panicle for Yuxiangyouzhan by 31.21% and 24.37%, respectively, compared to the ZN treatment. Also, the AT and HT conditions had no influence on the grain number per panicle in the AN treatment for Xiangyaxiangzhan. Moreover, when comparing only the nitrogen treatment, the AN treatment resulted in a notable increase of 27.89% and 5.68% in the grain number per panicle for Yuxiangyouzhan and Yuxiangyouzhan, respectively. Compared with the AT treatment, a striking decrease in the grain number per panicle was detected at the HT treatment by 8.43% and 15.15% for Yuxiangyouzhan and Xiangyaxiangzhan, respectively. When compared to Yuxiangyouzhan, Xiangyaxiangzhan increased the grain number per panicle by 13.12% (*Figure 4*).

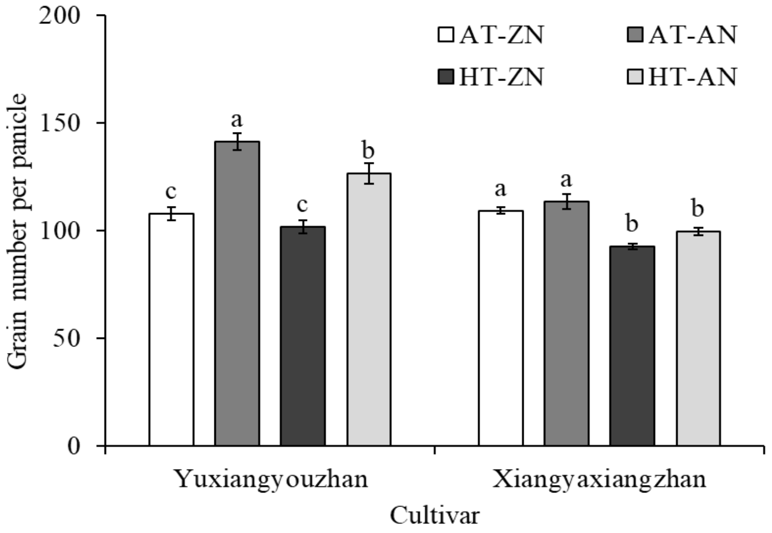

***Figure 4.** Effect of nitrogen application on grain number per panicle of super and non-super fragrant rice under high soil temperature. Vertical bars with different lower-case letters above are significantly different at $p < 0.05$ by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; AN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

Filled grain percentage

Variety (V), Temperature treatment (T), and V×T significantly influenced the filled grain percentage (*Table 1*). Compared with the ZN treatment, the AN treatment did not affect the filled grain percentage for Yuxiangyozuhan and Xiangyaxiangzhan. Furthermore, when temperature conditions were not considered, the AN treatment caused a significant increase in the filled grain percentage by 2.85% and 1.11% for Yuxiangyouzhan and Xiangyaxiangzhan, respectively. Besides, when considering only the effect of temperature treatment on the percentage of filled grains of the varieties, there was a reduction of 46.97% and 52.58% for Yuxiangyouzhan and Xiangyaxiangzhan under AT and HT conditions, respectively. In addition, the difference in the filled grain percentage between varieties was manifested as Xiangyaxiangzhan being 20.22% higher than Yuxiangyouzhan (*Figure 5*).

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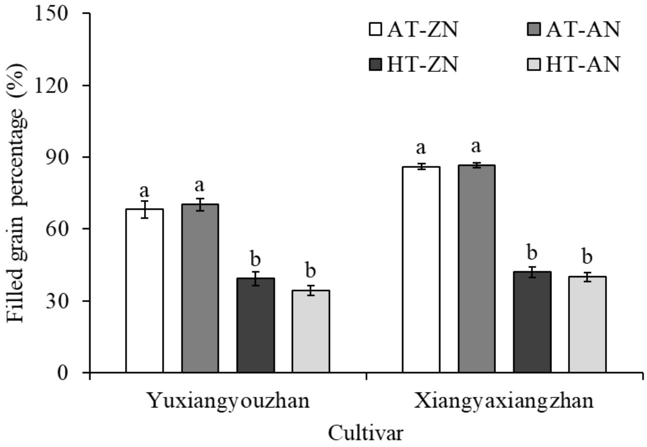

Figure 5. *Effect of nitrogen application on filled grain percentage of super and non-super fragrant rice under high soil temperature. Vertical bars with different lower-case letters above are significantly different at p < 0.05 by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; HN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

1000-grain weight

Variety (V) and Temperature treatment (T) significantly affected the 1000-grain weight (*Table 1*). When compared to the ZN treatment, the AN treatment significantly increased in the 1000-grain weight by 4.90% for Yuxiangyouzhan under HT conditions, while there was no significant effect under AT conditions. The AN treatment did not affect the 1000-grain weight for Xiangyaxiangzhan under AT and HT conditions. Additionally, as compared to the ZN treatment, a significant increase was detected in the AN treatment without taking into account the temperature condition (2.86% and 1.44%). However, only temperature conditions were considered; Yuxiangyouzhan in the HT condition was lower by 13.40% than that in the AT condition; Xiangyaxiangzhan was lower by 13.53% than that in the AT condition. Besides, when the difference between the varieties was considered only in the 1000-grain weight, the result showed that Xiangyaxiangzhan was 9.55% higher than Yuxiangyouzhan (*Figure 6*).

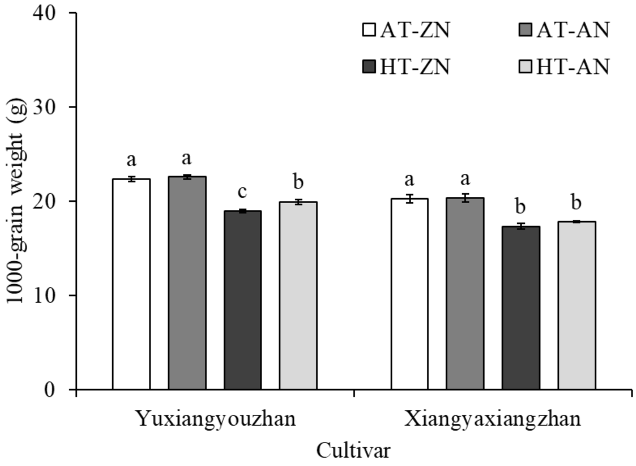

Figure 6. *Effect of nitrogen application on 1000 grain weight of different rice varieties under high temperature. Vertical bars with different lower-case letters above are significantly different at p < 0.05 by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; AN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

Antioxidant parameters

SOD activity

Temperature treatment (T) and V×N significantly affected the SOD activity (*Table 1*). Compared with the ZN treatment, the SOD activity for Yuxiangyouzhan and Xiangyaxiangzhan under the AT condition was not affected pronouncedly by the AN treatment. The AN treatment sharply increased the SOD activity by 6.39% for Xiangyaxiangzhan under HT conditions as compared with the ZN treatment. Additionally, AN treatment was decreased the SOD activity by 2.50% for Yuxiangyouzhan when temperature conditions were not considerated, however, the SOD activity increased by 3.73% for Xiangyaxiangzhan. Moreover, merely considering the temperature condition, a striking decrease was shown in SOD activity by 1.71% and 6.26% for Yuxiangyouzhan and Xiangyaxiangzhan under the AT and HT condition, respectively (*Figure 7*).

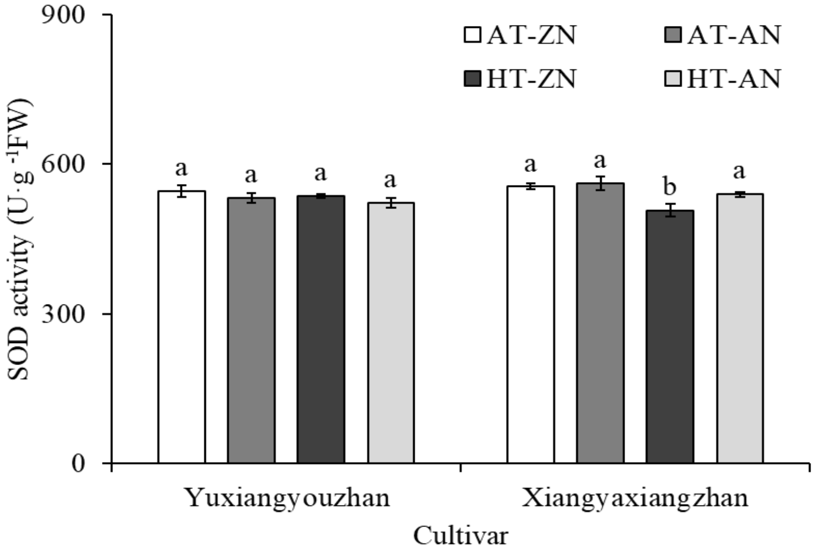

Figure 7. *Effect of nitrogen application on SOD activity in different rice varieties under high temperature at flowering stage. Vertical bars with different lower-case letters above are significantly different at $p < 0.05$ by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; AN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

POD activity

Nitrogen (N), V×N, and T×N significantly affected the POD activity (*Table 1*). Compared with ZN, the AN treatment had no significant effect on the POD activity for Yuxiangyouzhan and Xiangyaxiangzhan under the AT condition. Nevertheless, the AN treatment significantly increased the POD activity by 42.56% for Xiangyaxiangzhan under HT conditions. Furthermore, AN treatment significantly increased by 7% and 20.73%, respectively, as compared with ZN treatment for Yuxiangyouzhan and Xiangyaxiangzhan when temperature conditions were not considered. (*Figure 8*).

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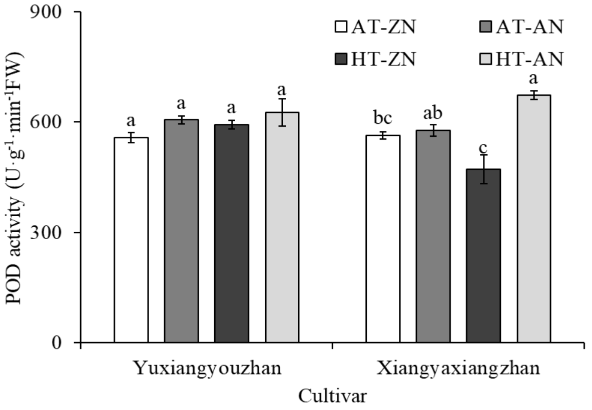

Figure 8. *Effect of nitrogen application on POD activity in different rice varieties under high temperature at flowering stage. Vertical bars with different lower-case letters above are significantly different at p < 0.05 by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; AN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

CAT activity

Variety (V), Temperature treatment (T), and Nitrogen treatment (N) significantly influenced the CAT activity (*Table 1*). Under the AT condition, the AN treatment increased the CAT activity by 11.09% and 11.52% for Yuxiangyouzhan and Xiangyaxiangzhan, respectively. Compared with the ZN treatment, the AN treatment significantly increased the CAT activity for Xiangyaxiangzhan under the HT condition by 48.15%, while having no obvious effect on Yuxiangyouzhan. Besides the ambient influence of the AT and HT conditions, compared with the ZN treatment, the AN treatment notable increased for Yuxiangyouzhan and Xiangyaxiangzhan by 19.47% and 2.41%, respectively, However, merely taking the temperature condition into consideration, Yuxiangyouzhan and Xiangyaxiangzhan decreased by 1% and 2% under the AT and HT conditions, respectively. What's more, without considering temperature condition and nitrogen treatment, a notable increase in the CAT activity was observed by 25.83% for Yuxiangyouzhan as compared with Xiangyaxiangzhan (*Figure 9*).

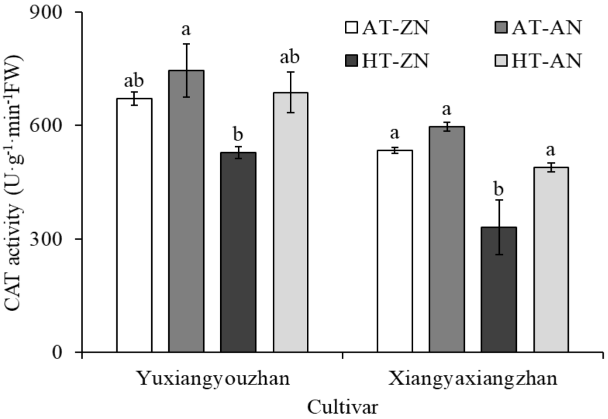

Figure 9. *Effect of nitrogen application on CAT activity of different rice varieties under high temperature. Vertical bars with different lower-case letters above are significantly different at p < 0.05 by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; AN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

MDA content

Temperature treatment (T) effected significantly on MDA content (*Table 1*). Besides, the MDA content showed a negative correlation between grain yield and its component, especially with the 1000-grain weight, which was significantly correlated. Compared to the ZN treatment, the AN treatment had not affected under AT and HT conditions for Yuxiangyouzhan. Compared with ZN treatment, AN treatment obviously reduced the MDA content by 26.04% under the AT condition for Xiangyaxiangzhan. Additionally, the AN treatment resulted in a pronounced reduction by 14.13% and 4.73% in the MDA content for Yuxiangyouzhan and Xiangyaxiangzhan, respectively. Besides, the effect of temperature results in a 12.20% and 20.43% decrease in MDA content for Yuxiangyouzhan and Xiangyaxiangzhan under AT and HT conditions, respectively (*Figure 10*).

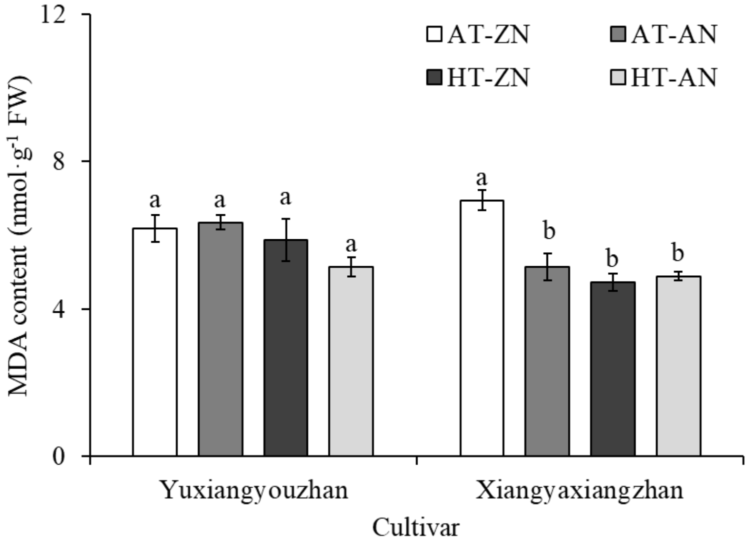

***Figure 10.** Effect of nitrogen application on MDA activity of different rice varieties under high temperature. Vertical bars with different lower-case letters above are significantly different at $p < 0.05$ by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; AN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

Proline content

Variety (V), V×N and T×N had a significant influence on proline content (*Table 1*). For Yuxiangyouzhan, compared with ZN treatment, AN treatment caused a significantly increased proline content by 19.04% under the AT condition, however, there was no effect in the HT condition. For Xiangyaxiangzhan, as compared with the ZN treatment, the AN treatment decreased by 7.26% in the AT condition and significantly reduced by 15.40% in the HT condition. Moreover, when ignoring the influence of AT and HT conditions, AN treatment resulted in an increase of 2.47% for Yuxiangyouzhan and a decreased of 11.63% for Xiangyaxiangzhan. Besides, comparing only the proline content of different varieties, Xiangyaxiangzhan had a notable decrease of 18.30% compared to Yuxiangyouzhan (*Figure 11*).

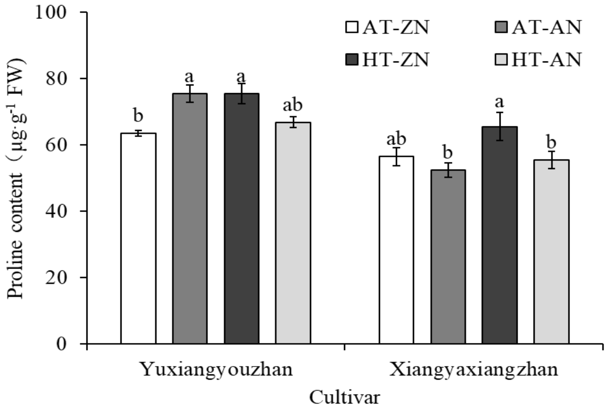

Figure 11. *Effect of nitrogen application on proline content of different rice varieties under high temperature. Vertical bars with different lower-case letters above are significantly different at p < 0.05 by LSD tests. Capped bars represent SD. ZN, 0g N in 12 kg soil; AN, 1.75 g N in 12 kg soil; AT, Ambient soil temperature; HT, high soil temperature*

Correlation analysis

The result showed that the grain yield had a significant positive correlation with the filled grain percentage (*P*<0.01) and 1000-grain weight (*P*<0.05). Temperature treatment significantly affected SOD and CAT activity, as well as MDA contents. Nitrogen application show a remarkable influence on POD and CAT activity. For T×N interaction significantly affected POD activity and proline content. In a word, the parameters of the antioxidant defense showed positive or negative with the grain yield and yield components (*Table 2*).

Table 2. *Correlation analysis of the investigated parameters*

Parameters	Grain yield	Panicle per pot	Grain number per panicle	Filled grain percentage	1000-grain weight	SOD activity	POD activity	CAT activity	MDA content
Panicle per pot	0.6058								
Grain number per panicle	0.6363	0.6812							
Filled grain percentage	0.8989**	0.3934	0.2729						
1000-grain weight	0.7931*	0.2933	0.7335*	0.6092					
SOD activity	-0.4965	-0.0105	0.1322	-0.6246	-0.4350				
POD activity	0.4437	0.7755*	0.5367	0.2212	0.0353	0.2958			
CAT activity	-0.3439	0.3485	-0.3043	-0.2960	-0.7709*	0.3335	0.4830		
MDA content	-0.6747	-0.5283	-0.6775	-0.4948	-0.7996*	0.4503	-0.0172	0.4472	
Proline content	0.0774	-0.0175	-0.5841	0.3836	-0.2951	-0.5746	-0.0954	0.2769	0.2119

* and ** indicates significant difference at $p < 0.05$ and $p < 0.01$, respectively

Discussion

Nitrogen is one of the major limiting plant nutrients required by agricultural crops (Yang et al., 2015). As is known to us, high temperature is a major abiotic stress that has a strongly negative impact on rice growth and development (Yang et al., 2015). Previous researchers have provided evidence with experiments that the high temperature leads to decreased rice yield (Huang et al., 2016; Ali et al., 2019). Furthermore, Sano et al. (2008) found that nitrogen fertilization increased grain yield. This study aimed to investigate the effects of N application on grain yield and antioxidant defense in two rice varieties under high rhizosphere temperatures during the flowering stage.

In the event of continuous high temperature weather above 35 ℃, heat damage will occur. In addition, high temperature reduced the filled grain percentage and thus ultimately affected grain yield (Matsui, 2001; Jagadish, 2010). With the continuous intensification of climate change, the negative effects on grain yield under high temperatures are increasing (Pachauri et al., 2014). It has been estimated that the grain yield may decrease by 10% for every 1 ℃ increase in the temperature during the rice growing season (Peng et al., 2004). Our finding demonstrated that grain yield and its components, including filled grain percentage, 1000-grain weight, panicle per pot and panicle per pot declined under HT condition, while the N treatment increased grain yield. Besides, our study also observed that the filled grain percentage of two rice varieties was lower than that under the AT condition, which is basically consistent with previous studies.

Zhou et al. (2022) revealed that optimized N application improved the number and structure of panicles. In addition, another study revealed similar consequences in that the number of panicles and filled grain percentage had a greater influence on grain yield after N treatment (Zhou et al., 2021). Similar results have also been found in this study, and correlation analysis indicated that the panicle per pot, grain number per panicle, and the filled grain percentage were positively correlated with grain yield at the N treatment (*Table 2*). Therefore, the increase in the grain yield in the N application resulted from the increase in the number of panicles per pot, total grain per panicle, filled grain percentage, as well as the change in the 1000-grain weight of different rice varieties.

Moreover, plants have formed a complex system of enzymatic and non-enzymatic antioxidant defenses in order to alleviate reactive oxygen species (ROS) produced under stress (Ashraf et al., 2015). It has been reported that SOD, CAT, and POD play an important role in the oxygen scavenging system of plants, which are involved in the detoxification of superoxide, hydrogen peroxide, and peroxide respectively (Xie et al., 2009; Jiang et al., 2014). In addition, Peng (2015) showed that an appropriate increase in nitrogen nutrition can improve the activity of the enzymatic defense system in plant cells.

This study revealed that N application had no significance on POD and SOD activity under the AT condition. Nevertheless, non-super rice demonstrated a significantly different yield under HT conditions at AN treatment (*Figures 6-7*). In addition, SOD activity showed a remarkable influence on temperature conditions and the interaction of variety and nitrogen (V×N) (*Table 1*). Besides, POD activity is obviously affected by N application as well as the interaction of variety and nitrogen (V×N) and the interaction of temperature and nitrogen (T×N) (*Table 1*). It is interesting to note that POD activity and panicle per pot were positively correlated, whereas SOD activity showed a negative correlation with panicle per pot (*Table 1*). Furthermore, a previous study revealed that the CAT activity of rice flags showed no significance in response to HT condition (Zhang et al., 2015). In this study, the CAT activity had a significant influence among the V and T condition and N treatment, however, no significant effect was revealed among the interactions of variety and temperature (V×T), variety and nitrogen (V×N) and temperature and nitrogen (T×N) (*Table 1*). In addition, we found that the accumulation of MDA in two rice varieties under AN treatment at HT condition, changed inconsistently (*Figure 9*), in which the MDA content of super rice varieties decreased while non-super rice increased. As an end product of membrane lipid peroxidation, MDA can inhibit the activity of cell protective enzymes and reduce the content of antioxidative enzymes (Jędrzejuk et al., 2018). Therefore, it can be seen from this experiment that the degree of membrane lipid damage was lower in super rice than that in non-super rice. In addition, V×N, V×T, and T×N showed no significantly influenced MAD activity (*Table 1*). Besides, previous findings showed that proline (Pro) as an important osmotic regulator, and its accumulation plays an important role in plant stress tolerance under stress conditions (Tang, 1984; Kaur and Asthir, 2015). Proline induced the stress tolerance and prevented the electrolyte leakage by inhibiting lipid peroxidation and scavenging reactive oxygen (ROS) (Taylor, 1996; Kaur and Asthir, 2015). Our study found that the content of proline in the super rice variety increased significantly at AN treatment under AT conditions. However, we were surprised to find that the content of proline decreased with AN treatment.

From the perspective of yield and antioxidant defense performance, this study showed that nitrogen application did alleviate the damage caused by high temperature stress (*Figure 12*).

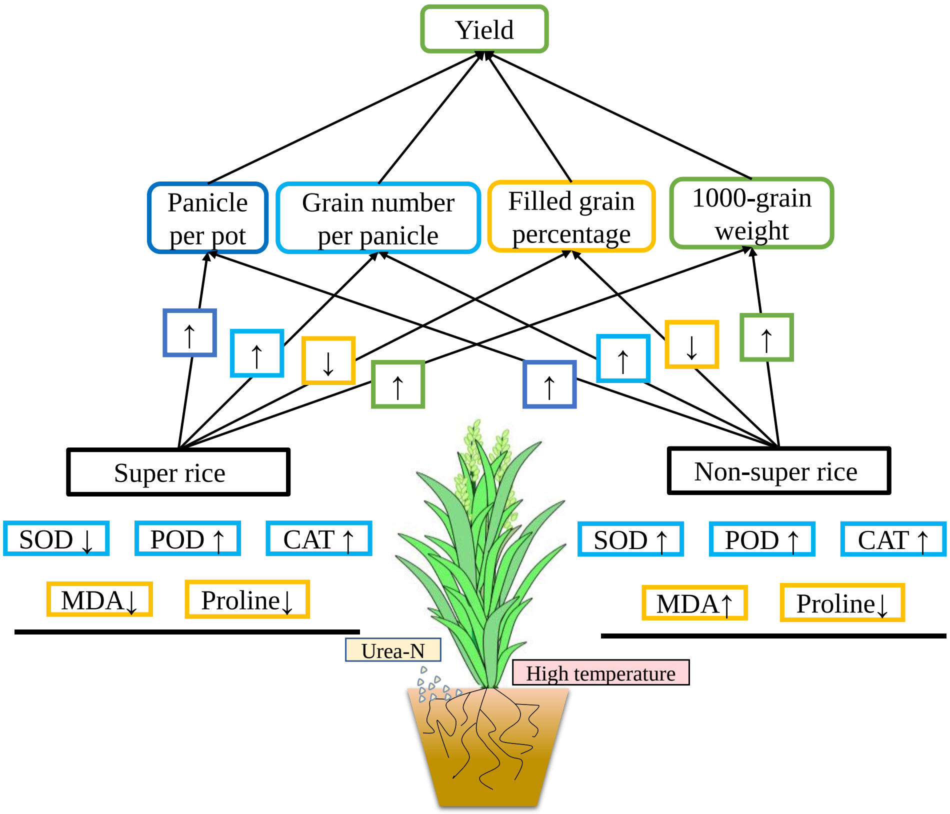

Figure 12. *The mechanism for the regulation effect of nitrogen on the yield formation of super and non-super rice under high soil temperature*

Conclusion

Compared with the AT condition, the HT condition showed a significant reduction in the grain yield. On the contrary, the interaction of N application and temperature had a remarkably impact on grain yield. In a word, N application under HT conditions increased grain yield and affected the yield components of both fragrant rice varieties. In addition, the influence of nitrogen application improved the POD and CAT activities but decreased the proline content. Overall, N application under HT conditions can improve rice yield and biochemical properties during the flowering stage.

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