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Exogenous spermidine enhances the photosynthetic and antioxidant capacity of rice under heat stress during early grain-filling period

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Abstract. High temperature has adverse effects on rice growth by inhibiting the flag leaf photosynthetic and antioxidant capacity, which can be alleviated by various exogenous chemicals such as spermidine (Spd). However, the role of Spd in conferring heat tolerance in rice is not well documented. Conventional japonica rice varieties Wuyunjing 24 and Ningjing 3 were treated with high temperatures at 37.5/27.0°C (day/night) and foliar sprayed with 1 mmol L^{-1} Spd after flowering. Results showed activities of superoxide dismutase (SOD) and peroxidase (POD) activities were deceased during high temperature treatment and eventually lead to the malondialdehyde (MDA) accumulation. Exogenous Spd significantly increased both SOD and POD activities at the later stage of high-temperature treatment, and reduced MDA accumulation were identified in both rice varieties. Application of Spd further increased the amount of soluble sugars during high temperature stress and that maintained the osmotic equilibrium of rice leaves. Spd significantly increased photosystem II (ΦPSII), photosynthetic electron transport rate (ETR), variable fluorescence/maximum fluorescence ratio (F_v'/F_m'), stomatal conductance and the photochemical reaction of light energy ratio (P_r), and ultimately improved the photosynthetic and transpiration rate during high temperature stress. In conclusion, exogenous Spd can effectively alleviate the adverse consequences of high temperature and could be further applied to provide strategies in mitigating the challenges of global warming-induced yield loss and other possible relevant issues.

Additional keywords: antioxidant defence, antioxidant defense, high temperature, *Oryza sativa*, photosynthesis.

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Introduction

Rice originates from tropical low latitude regions, and has thermophilic physiological features. However, the growth of rice requires an optimum range of temperature. According to the latest IPCC synthesis report (IPCC 2013), global surface temperature is supposed to increase by 0.3–4.8°C in 2081–2100 and that would bring an uncertainty for rice production. Previous studies have suggested rice at the reproductive stage is most sensitive to heat stress, and exposure to high temperatures above 35°C results in reduced chlorophyll content. Although the antioxidant system is activated during heat stress, prolonged exposure to high temperature damages the antioxidant capacity of rice, resulting in accumulation of reactive oxygen species (ROS) and subsequent oxidative damage to the cell membrane system and PSII, as well as reduced Rubisco enzyme activities. These changes lead to a subsequent decrease in photosynthetic rate in rice (Tang *et al*. 2005; Teng *et al*. 2008; Mohammed *et al*. 2015).

Grain filling is a process that occurs when the panicle accepts assimilates from other vegetative organs and transmutes sucrose into starch, which directly determines grain weight and rice yield. Rice grain-filling rate would be regulated by the external ambient temperature and eventually leads to the reduced grain length and width, subsequently poor quality during grain-filling stage (Mohammed and Tarpley 2010) and yield (Zhang *et al*. 2006; Krishnan *et al*. 2011; Liu *et al*. 2013). With the intensification of global warming, the frequency and intensity of high temperature encountered in China's lower reaches of Yangtze River delta have been significantly strengthened, especially in the early stage of rice grain filling. Therefore, it is necessary to conduct theoretical research into these aspects, and results may further provide a theoretical basis for proposing appropriate measures in mitigating the adverse effects of global warming.

Chemical control is an important cultivation measures in regulating plant growth and development in adverse environment. Polyamines participate as a general plant growth

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regulator in a plurality of biological processes, including morphogenesis floral organs, flowering process, root absorption and abiotic stress defence responses (Galston and Kaur-Sawhney 1995; Shi and Chan 2014). Spermidine (Spd) is an important polyamine widely distributed in plant tissues that is involved in almost all processes of adaptation to stresses in plants including salinity (Duan *et al*. 2006; Liu *et al*. 2006), cold (Zheng *et al*. 2008), drought (Yang *et al*. 2004; Farooq *et al*. 2009) and heat (Sagor *et al*. 2013; Das *et al*. 2014). In most of the above cases, Spd-mediated protection has a close association with enhanced levels of antioxidant capacity and reduced rate of superoxide anion and malondialdehyde (MDA) content to maintain the stability of plant cell membrane (Liu *et al*. 2006; Farooq *et al*. 2009; Tian *et al*. 2009). In a study conducted by Mostofa *et al*. (2014), rice seedlings pre-treated with exogenous Spd showed reduced propionaldehyde, hydrogen peroxide and proline contents, but increased FW and ascorbic acid, glutathione and chlorophyll contents.

As one of the most important cereal crops, rice production will face the aberrant changes in temperature caused by the intensified climate change in the future. To better cope with these adverse conditions, it will be necessary to apply effective cultivation control measures. However, this should be based on the understanding of the physiological changes of rice plant when exposed to heat stress and the recovery stages. Based on the alleviating effects of exogenous Spd involved in heat stress tolerance, in this study we evaluated the heat induced damages in rice plants and investigated the effects and regulatory mechanisms of the exogenous Spd on the physiological features of rice under heat stress, assuming that Spd could be further applied in mitigating the challenges of global warming induced rice yield loss and other relevant food security issues.

Materials and methods

Plant materials, growth condition and treatments

Wuyunjing 24 and Ningjing 3 are the main conventional japonica rice varieties cultivated in the region of Yangtze River delta in China. In this study, those two varieties were selected as the test materials based on their sensitivities to temperature increasing (data not shown). Experiments were conducted in Baolin farm (119°27′57″E, 31°54′25″N), Danyang City, Jiangsu Province in 2015. Soil texture was clay loam and organic matter content is 20.2 g kg^{-1}. Total nitrogen content of field is 1.85 g kg^{-1} with available phosphorus content of 13.23 mg $kg^{-1}$and available potassium content of 119.4 mg kg^{-1}. Sowing was conducted on 23 and 25 May 2015. Transplanting was conducted on 28 and 30 June in 2015. Cultivation measures were applied according to the technical requirements of the local field production.

To investigate the alleviation of heat stress by Spd, high-temperature treatments were conducted (i.e. high-temperature + water spraying (CK) and high-temperature + Spd spray (Spd + HT)). Furthermore, a normal-temperature + water spraying (NT) control treatment were designed to evaluate the high-temperature damage caused by CK treatment in the heat stress group. A total of six treatments with three replicates for each treatment were conducted. Rice plants were treated for 12 consecutive days after flowering and were removed from the greenhouse to continue growing. Rice samples were sprayed with Spd purchased from Sigma-Aldrich on the day of high-temperature treatment and the day after between 16 : 00 and 17 : 00 hours. Tween-20 (0.01% final concentration) was added to Spd solution to enhance the foliar adhesion. Temperatures used for heat stress treatment were 37.5°C during the day and 27.0°C at night inside the greenhouse, whereas the average temperatures were 26.1°C during the day and 22.4°C at night outside the greenhouse.

Yield components

Total grain number, seed setting rate and 1000-grain weight per panicle at maturation were measured in each treatment and data were used to calculate the final yield for each treatment.

Chlorophyll content determination

Acetone (80%) was used as the extraction liquid and absorption value was analysed at the 663 and 645 nm in the spectroscopy. Relative content of chlorophyll was calculated based on the formula Chl *a* (mg L^{-1}) = 12.72 A663 – 2.59 A645 and Chl *b* (mg L^{-1}) = 22.88 a645 – 4.67A663.

Measurement of photosynthetic and chlorophyll fluorescence parameters

Photosynthetic and chlorophyll fluorescence parameters were measured using a LI-6400 portable photosynthesis and fluorescence system (LI-COR BioSciences). Photosynthetic rate, stomatal conductance and transpiration rate in the middle of flag leaves were measured on a sunny morning from 09 : 00 to 11 : 00 hours. To measure the chlorophyll fluorescence parameters, the photosynthetic leaf chamber of an LI-6400 portable photosynthesis system was replaced with the fluorescence measuring leaf chamber. Dark reaction was measured by covering the middle of the leaves for 15 min before measurement. The maximum quantum yield of PSII was then measured (F_v/F_m). The measurement light source was subsequently opened to continuously light activate all parameters for ~20 min until they stabilised, after which the following parameters were measured: variable fluorescence/maximum fluorescence ratio (F_v'/F_m'), photochemical quenching (qP), actual PSII efficiency (ΦPSII), non-photochemical quenching (NPQ), and photosynthetic electron transport rate (ETR). Formulae proposed by Demmig-Adams *et al*. (1996),

$$P(\%) = \mathrm{qP} \times F_v'/F_m', \tag{1}$$

$$D(\%) = 1 - F_v'/F_m', \tag{2}$$

and

$$\mathrm{E}(\%) = (1 - \mathrm{qP}) \times F_v'/F_m' \tag{3}$$

were used to calculate the photochemical reaction (*P*), heat dissipation of antenna pigments (*D*), and excitation energy dissipation in the PSII reaction centre (*E*) respectively. Chlorophyll fluorescence parameters were measured one day before high-temperature treatment (denoted as post-high-temperature treatment day 0), 6 and 12 days after high-temperature treatment, and 10 days after the study (denoted as post-high-temperature treatment day 22), giving a total of four measurements.

Measurement of antioxidant enzyme activities and MDA content

To measure the antioxidant enzyme activities and MDA content, three leaves were collected on days 1, 3, 6, 9, 12 and 22 after high-temperature treatment. Upon collection, leaves were snap frozen in liquid nitrogen for 30 s and stored at −40°C for subsequent analyses. All leaf samples were homogenised in phosphate buffer solution (pH 7.0) in an ice bath, after which they were subjected to refrigerated centrifugation to obtain crude enzyme solution to measure the antioxidant enzyme activity and MDA content. The superoxide dismutase (SOD) activity was measured by the nitroblue tetrazolium (NBT) photoreduction method. Additionally, peroxidase (POD) activity was measured by the guaiacol method and MDA content was measured by the thiobarbituric acid (TBA) method described in a study conducted by Lee (2000).

Measurement of soluble sugar

To measure the soluble sugar in rice plants, leaves were collected on days 1, 3, 6, 9, 12 and 22 after high-temperature treatment. Leaf samples were dried in a circulation oven at 105°C for 30 min, after which they were stored in an oven at 80°C. The anthrone colourimetry method described by Zhang (1992) was used to measure soluble sugar in rice crops.

Statistical analysis

Data obtained for seed-setting rate, single-grain weight, enzyme activities, NSC content were analysed as a completely randomised design following ANOVA and the mean values were compared by Duncan's multiple range test (DMRT) based on the least significant difference (l.s.d.) test at a 5% probability level. Different letters indicate significant differences between treatments at $P < 0.05$. Data sorting and analysis and figure preparation were performed using Microsoft Excel 2010 and OriginPro 8. SPSS 20.0 (SPSS Inc.) was used for statistical analysis.

Results

Effects of Spd on rice crop yield under heat stress during the early grain-filling period

Heat stress induced significant changes in seed-setting rate and grain weight and eventually leads to a yield loss in both tested rice varieties. Spd spraying of rice under heat stress improved the seed-setting rate by 17.71 and 3.56% in Wuyunjing 24 and Ningjing 3 respectively. Significant difference was found between the Spd + HT group and NT group of Wuyunjing 24 (Table 1). In addition, Spd treatment under heat stress improved the 1000-grain weight by 0.9 and 1.41% in Wuyunjing 24 and Nigjing 3. Significant differences were also observed between the CK group and Spd + HT group of Ningjing 3. Total yield for both varieties were significantly reduced in high temperature treated groups (CK and Spd + HT) compared with plants under normal temperature. Furthermore, significant effects of Spd on relieving yield loss under high temperature stress were detected.

Effects of Spd on chlorophyll content of leaves of rice under heat stress

The chlorophyll a concentration of Wuyunjing 24 under heat stress did not differ significantly compared with the NT group on days 1–6. However, this trend was significant increased on days 9–22 when compared with that in NT group. The chlorophyll *b* contents were similar to the chlorophyll *a* contents. Foliar Spraying of Spd effectively reduced the chlorophyll a contents in leaves compared with those of the CK group on days 12–22 in Wuyunjing 24 rice variety under heat stress. In addition, Spd reduced the chlorophyll *b* contents to 6.67% lower than the CK group on day 22. For variety Ningjing 3, the chlorophyll *a* content was higher compared with the NT group from the third day of high-temperature treatment. Accumulation of aging and stress damage led to significant differences between these two groups on day 22. The chlorophyll *b* contents in Ningjing 3 were consistent with those of chlorophyll *a*. Spd treated Ningjing 3 rice variety under heat stress reduced chlorophyll *a* contents to lower than those of the CK group from day 3, and this reduction became significant on day 22. Spd + HT treatment on Ningjing 3 rice significantly reduced the chlorophyll b contents to below those of CK group (Fig. 1).

Effects of Spd on net photosynthetic rate, stomatal conductance, and transpiration rate of rice under heat stress during the early grain-filling period

As shown in Fig. 2, the net photosynthetic rates (P_n) of both rice plants were significantly reduced at the 12th day under heat stress. Spd spraying on both varieties significantly recovered the low P_n due to heat stress. When compared with the CK group, P_n

Table 1. Comparison of rice yield and yield components of different treatments

CK, high-temperature treatment + foliar water spraying; Spd + HT, high-temperature treatment + foliar Spd spraying; NT, normal temperature treatment + foliar water spraying. Different letters after each number represent significant differences: *, $P < 0.05$

Variety	Treatment	Panicles	Grains per panicle	Filled grain rate (%)	1000-grain weight (g)	Grain yield (g pot^{-1})
Wuyunjing24	CK	28.00a	160.8a	36.74c	26.21b	43.68c
	Spd + HT	27.00a	158.6a	44.02b	26.77b	51.02b
	NT	27.40a	158.2a	97.89a	27.68a	115.52a
Ningjing3	CK	32.09a	148.4a	40.83c	23.36c	46.01c
	Spd + HT	32.33a	150.4a	45.95b	23.88b	53.29b
	NT	32.60a	151.0a	98.98a	25.29a	118.98a

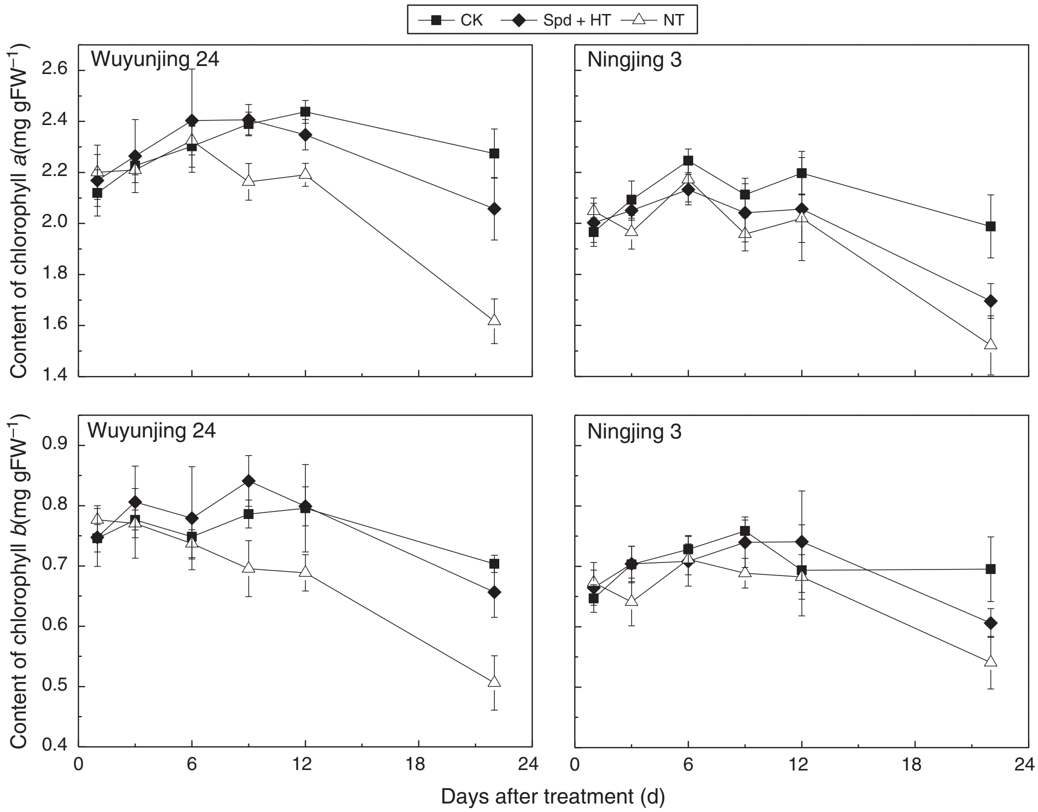

Fig. 1. Effects of exogenous spermidine (Spd) on contents of chlorophyll in rice leaf under heat stress during early grain filling stage.

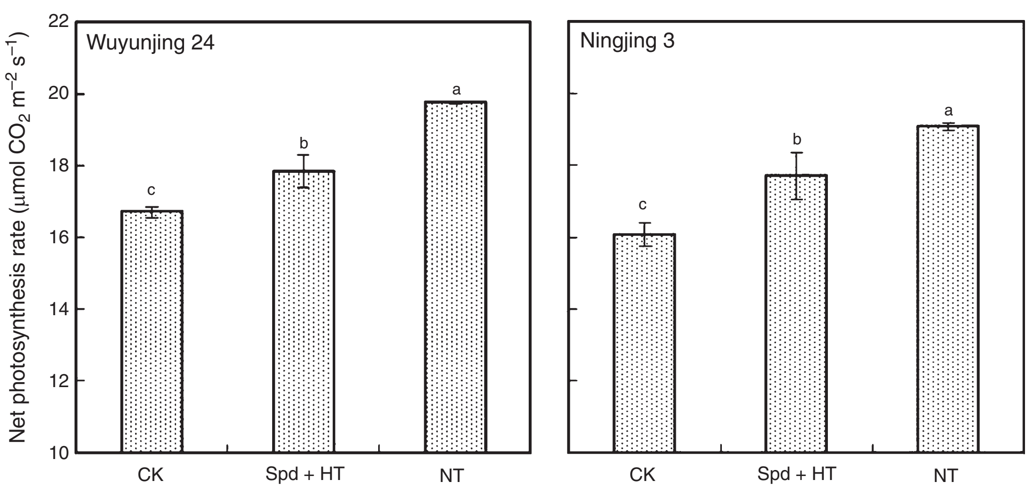

Fig. 2. Effects of spraying spermidine (Spd) on net photosynthesis rate of rice leaf under heat stress.

of Wuyunjing 24 variety under heat stress increased by 6.85% after Spd spraying, whereas that of Ningjing 3 under heat stress increased 10.09% after Spd spraying. As shown in Fig. 3, stomatal conductance of Wuyunjing 24 at the 12th day did not differ significantly from that of the NT group, whereas stomatal conductance of Ningjing 3 was significantly higher than in the NT group. Spd spraying significantly increased stomatal conductance by 10.29 and 50.20% in Wuyunjing 24 and Ningjing 3 respectively. Changes in the transpiration rate of both species were inconsistent with stomatal conductance. The transpiration rate of rice leaves was significantly reduced at the late stage of high temperature treatment. Spd + HT treatment significantly

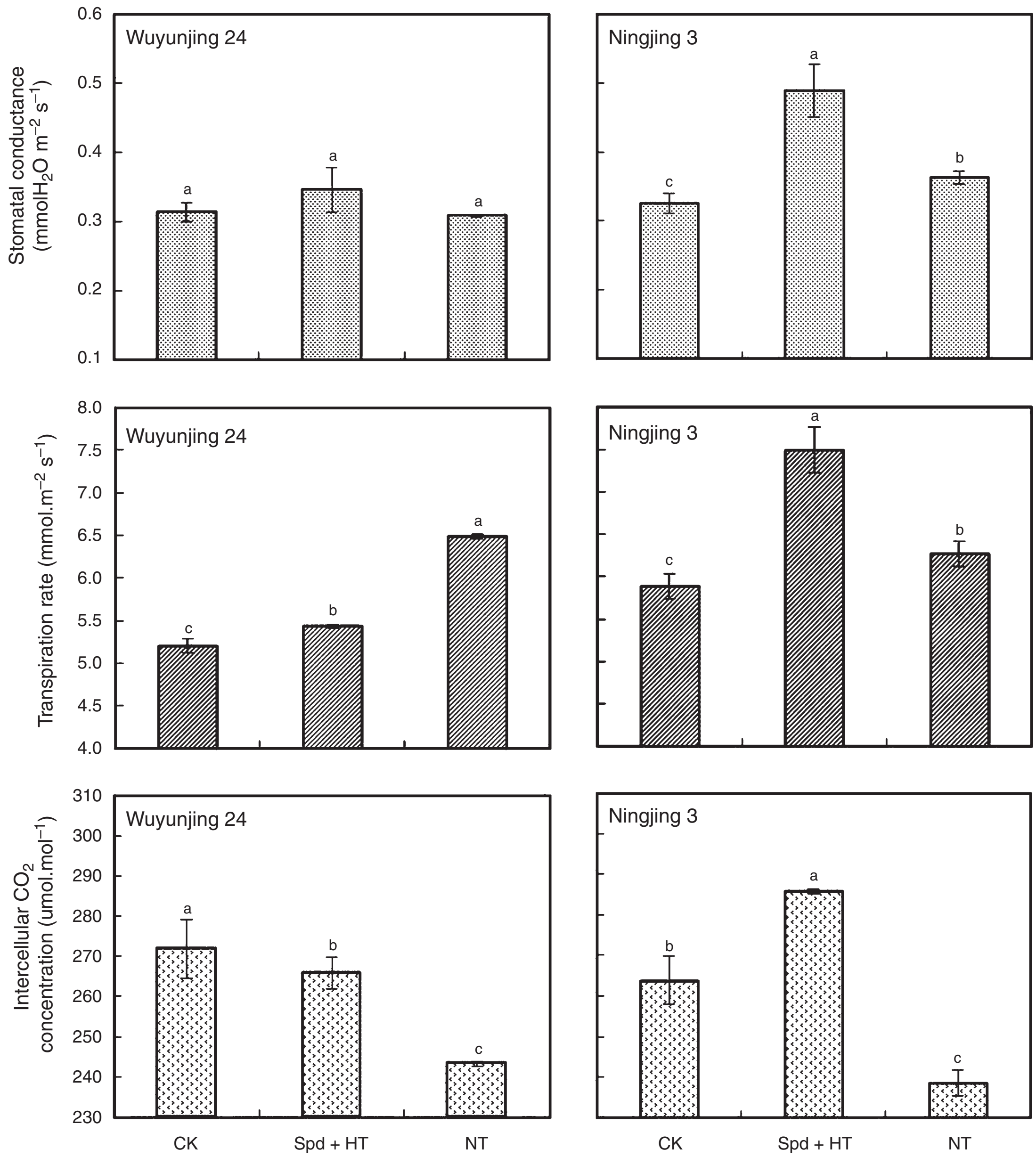

Fig. 3. Effects of spaying spermidine (Spd) on stomatal conductance, transpiration rate and intercellular CO_2 concentration of rice leaf under heat stress.

increased the transpiration rate compared with the CK group. These findings were consistent in both tested rice varieties.

Effects of Spd on chlorophyll fluorescence parameters in rice under heat stress during the early grain-filling period

ΦPSII, ETR and F_v/F_m

As shown in Fig. 4, rice leaf ΦPSII and ETR exhibited different degrees of reduction under heat stress in Wuyunjing 24 and Ningjing 3. The ΦPSII and ETR in Wuyunjing 24 and Ningjing 3 decreased with growth and development, whereas high-temperature treatment with water spraying of the CK group significantly reduced ΦPSII and ETR on day 6, resulting in a significantly lower ΦPSII and ETR compared with the NT group on day 12 of heat stress. In Wuyunjing 24, the F_v/F_m on days 6 and 12 of heat stress were significantly lower than those of the NT group. In Ningjing 3, the F_v/F_m on day 6 of heat stress was significantly lower than that of the NT group, and was relatively lower than that of the NT group (non-significant) on day 12 of heat stress. The F_v/F_m in both rice plants recovered from heat stress after the recovery period.

After treated with Spd, the reduction of ΦPSII and ETR in the Spd + HT group on day 6 after heat stress was significantly less than that in the CK group, resulting in a significantly higher ΦPSII and ETR of the Spd + HT group in Wuyunjing 24 (63.52 and 38.44% respectively) and Ningjing 3 (30.14 and

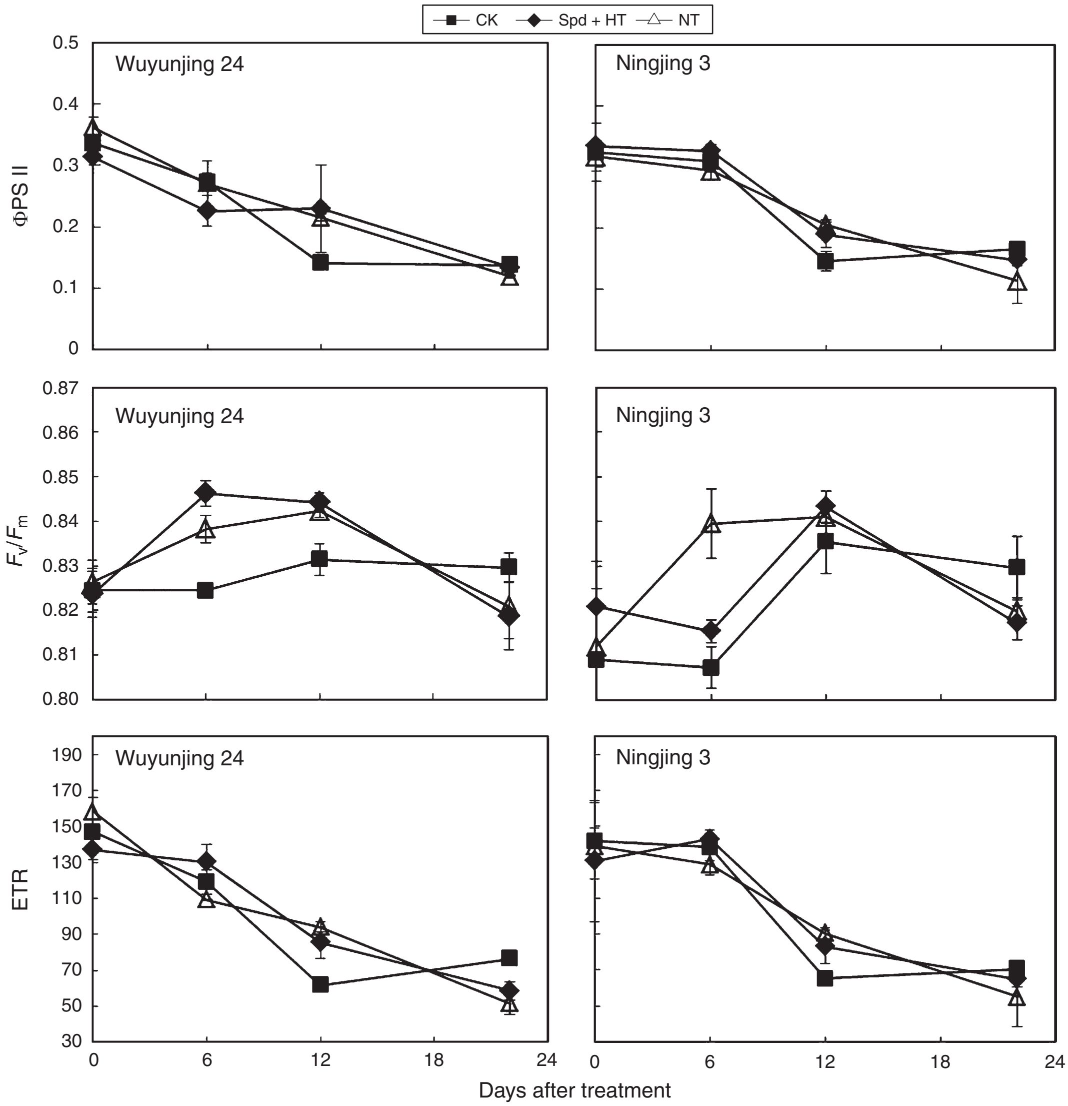

Fig. 4. Effects of spermidine on variable fluorescence/maximum fluorescence ratio (F_v'/F_m'), photosystem II (ΦPSII) and photosynthetic electron transport rate (ETR) of rice flag leaf under stage heat stress during early grain filling.

27.58% respectively) on day 12 relative to the CK group. Results indicated exogenous Spd not only improved ΦPSII and ETR after heat stress, but also had the same effect on F_v/F_m. In Wuyunjing 24, F_v/F_m of the Spd + HT group on days 6 and 12 were significantly increased by 2.65 and 1.53% respectively, after treated with Spd compared with the CK group. Similarly, in Ningjing 3, the F_v/F_m of the Spd + HT group on day 6 and day 12 increased by 1.01 and 0.87%, respectively, compared with the CK group, and significant differences were found between the Spd + HT group and CK group on day 6.

Light energy distribution captured on rice leaves

In Wuyunjing 24, the *P* (photochemical reactions) distribution during the photochemical reaction did not differ between two rice varieties under heat stress and normal temperature treatment on day 6. Prolonged exposure to heat stress significantly decreased photochemical reactions on day 12. No significant difference in *D* (thermal dissipation of antenna pigment) was observed in response to high-temperature treatment among groups, whereas *E* (excitation energy dissipation) was significantly increased on day 12 of heat stress. Under heat stress, this trend is consistent in performance between the two varieties. Excitation energy dissipation under heat stress treatment was significantly higher than those under normal temperature treatment on days 6 and 12 (Fig. 5).

In Wuyunjing 24, no significant difference in photochemical reactions, thermal dissipation of antenna pigment or excitation energy dissipation was observed on days 6 and day 22 in exogenous Spd + HT treatment compared with the CK. On day 12, the photochemical reactions were increased by 38.52% in Spd + HT treated plants compared with CK, but no significant difference was found in thermal dissipation of antenna pigment between those two groups. Furthermore, exogenous Spd reduced

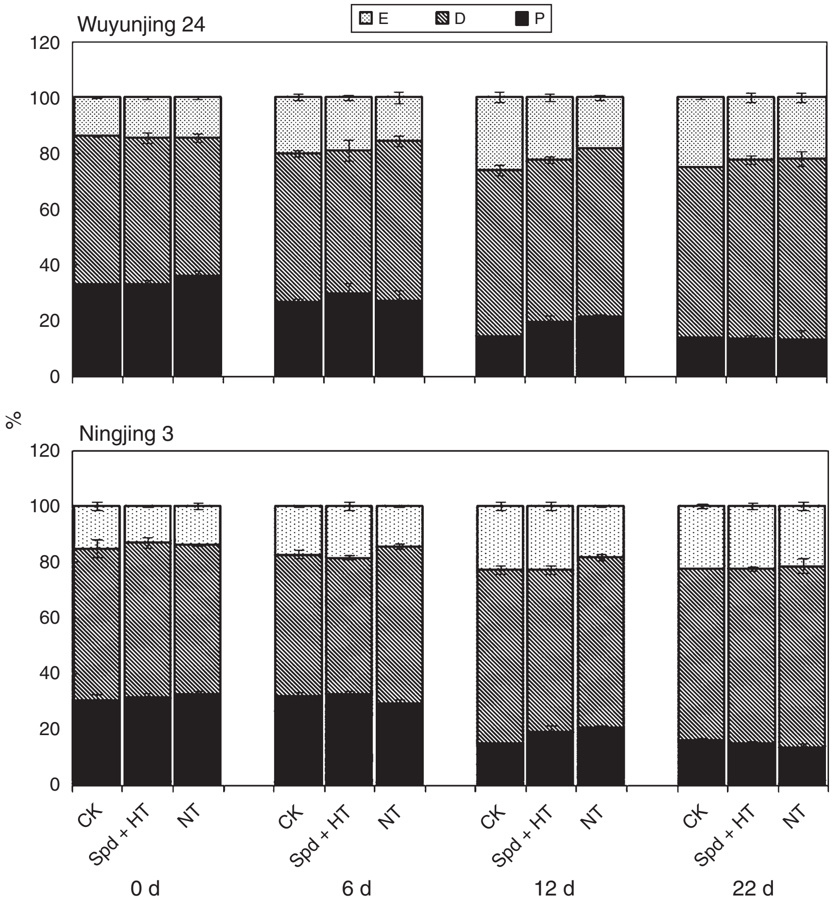

Fig. 5. Effect of spermidine (Spd) on allocations of absorbed-light energy of rice leaf under heat stress during early grain-filling stage.

E by 13.70% compared with the CK group. Spd treatment of Ningjing 3 had a similar effect in the Spd + HT and CK groups. Individually, the photochemical reactions of the Spd + HT group was 27.49% higher than that of the CK group on day 12, whereas the thermal dissipation of antenna pigment light energy of the Spd + HT group was reduced by 6.78% and the excitation energy dissipation showed no significant change. On day 22, all parameters had a certain degree of recovery in different temperature-treatment groups.

Effects of Spd on SOD, POD and MDA under heat stress during the early grain-filling period

SOD activities in both rice varieties showed an initial increase followed by a decrease (Fig. 6). In Wuyunjing 24, SOD activity on day 9 of heat stress was significantly lower than in the NT group. Spd spraying significantly inhibited the reduction of SOD activity caused by heat stress, inducing increases of 77.95 and 78.90% in SOD activity on days 9 and 12 compared with the CK respectively. After 10 days of recovery in normal temperature, the SOD activity of the Spd + HT group was still maintained at above that of the CK group. In Ningjing 3, the SOD activity of the CK group on day 6 of heat stress was significantly higher than that of the NT group. This phenomenon lasted through the high-temperature treatment and recovered to the level of the NT group after the recovery period. Spd treatment of Ningjing 3 further enhanced the SOD activity by 37.43 and 21.86% on days 9 and 12, respectively, compared with the CK group. The SOD activities were maintained at levels above those of the CK group even during the recovery period.

The POD activity of Wuyunjing 24 under heat stress in the CK group was significantly lower than that of rice crops in the NT group. The POD activity of the Spd + HT group was significantly higher than that of the CK group from day 3 to day 22. Under heat stress, the POD activity of Ningjing 3 was opposite to that of Wuyunjing 24. In Ningjing 3, the POD activity of the CK group was significantly higher than that of the NT group during day 3–9. POD activity was significantly elevated in the Spd + HT group during days 6–22, suggesting that Spd led to a significant increase in POD and SOD activities in different rice species.

Heat stress significantly increased the MDA accumulation content of Wuyunjing 24 in the CK group on days 6–22. When compared with the CK group, Spd + HT treatment significantly reduced the MDA accumulation by 18.89 and 15.90% on days 6 and 12 respectively. In Ningjing 3, MDA accumulation in the CK group during high-temperature treatment and the recovery

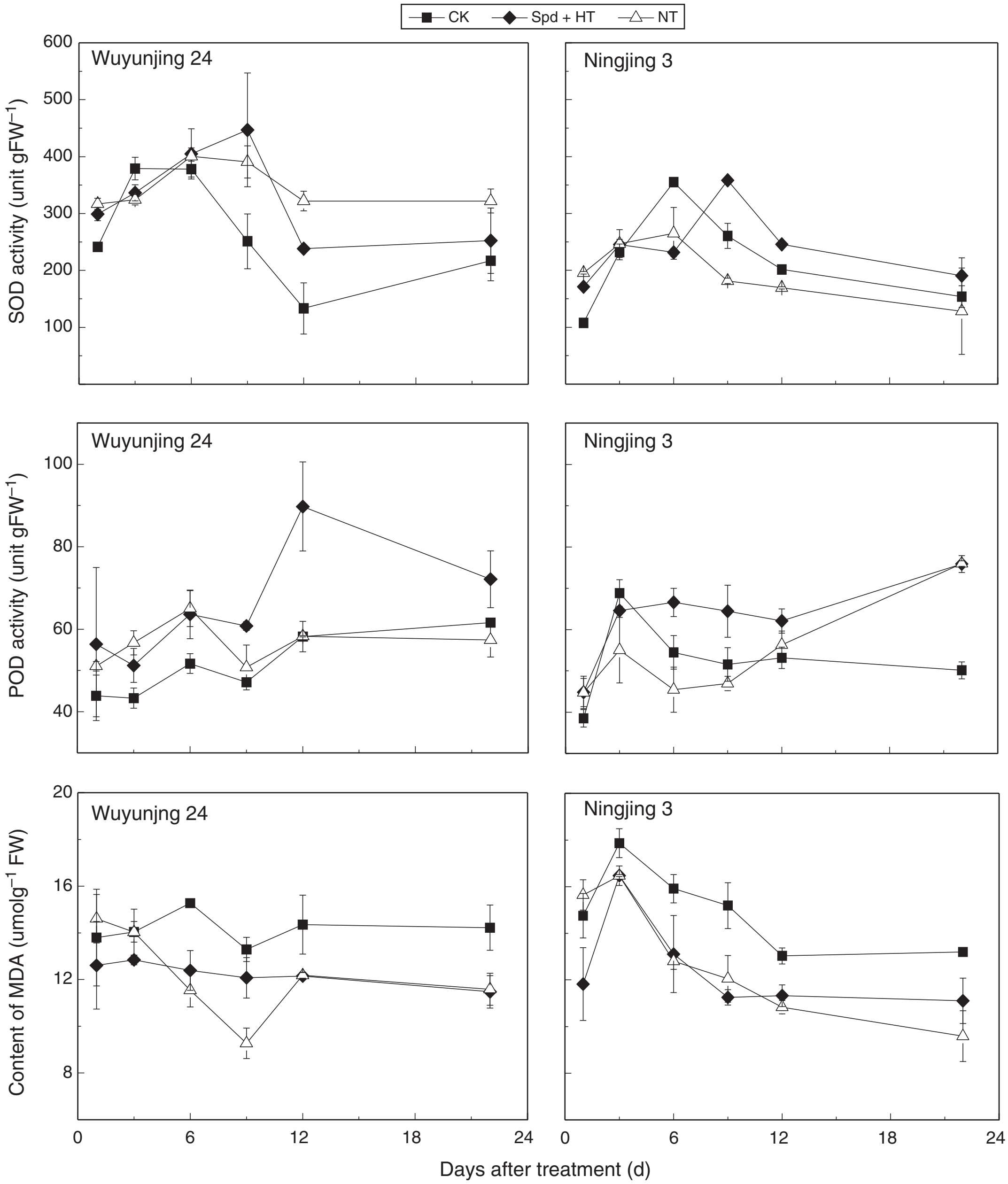

Fig. 6. Effects of spermidine (Spd) on the activity of superoxide dismutase (SOD), peroxidase (POD) and the content of malondialdehyde (MDA) of rice leaf under heat stress during early grain-filling stage.

period was significantly increased. Exogenous Spd spraying significantly reduced the MDA accumulation content in the Spd + HT group on days 3–22 compared with the CK group, suggesting that Spd reduced MDA accumulation under heat stress.

Effects of Spd on soluble sugar accumulation under heat stress during the early grain-filling period

As shown in Fig. 7, prolonged heat stress increased the soluble sugar accumulation in rice leaves of both varieties. Soluble sugar accumulation increased significantly on day 9 of heat stress in Wuyunjing 24 and on day 12 of heat stress in Ningjing 3. These high soluble sugar accumulations lasted through the whole recovery period in both varieties. Spd spraying further elevated the soluble sugar accumulation under heat stress, whereas soluble sugar accumulation in Wuyunjing 24 was significantly higher compared with the CK group on day 22.

Discussion

Ambient temperature affects the plant growth and development by raising the organogenesis temperature, and thus temperature of organs reflects the degree of high temperature stress on plants. Exogenous Spd significantly reduced the 1st leaf temperature in Wuyunjing 24 variety under high temperature stress compared

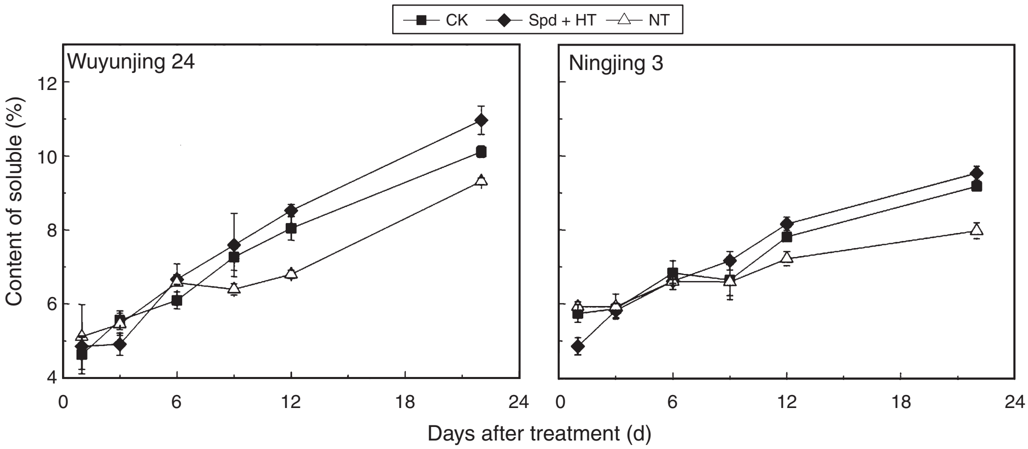

Fig. 7. Effects of spermidine (Spd) on soluble sugar content of rice leaf under heat stress during early grain-filling stage.

with the CK. However, effects on reducing temperature stress of the 2nd and 3rd leaf of Wuyunjing 24 and the top three leaves of Ningjing 3 were not significant. Similarly, Spd significantly reduced the panicle temperature of Wuyunjing 24 compared with the CK treatment, whereas this effect on Ningjing 3 was not significant (see Fig. S1, available as Supplementary Material to this paper). Based on the yield analysis, Spd effectively alleviated the seed setting rate loss in Wuyunjing 24 under high temperature stress and this effect was not detected in Ningjing 3, which may suggest those two varieties have different sensitivities to high temperatures and exogenous Spd.

Photosynthesis is a chemical reaction that forms organic materials while releasing oxygen but using light energy, carbon dioxide and water (Barber 2009). Light energy required in photosynthesis is first absorbed by chlorophyll molecules. Light absorption via chlorophyll can be divided into three parts: first, absorbed light is transferred to a light reaction centre for photochemical reaction (i.e. *P*); second, absorbed light energy is dissipated through heat radiation (i.e. *D*); and finally, absorbed light energy is dissipated through fluorescence radiation energy in PSII (i.e. *E*) (Krause and Weis 1991). Therefore, measurement of fluorescence is an effective method to study photosynthesis (Logan 2005). The photosynthesis assimilation process requires electron transfer. The first protein complex of the electron-transport chain is PSII, which is an important element in the photosynthetic system (Liao *et al*. 2013). Previous studies have shown that the chlorophyll content of crop leaves decreased under heat stress (Wu 2003; Efeoglu and Terzioglu 2009), and that induced the impairment of PSII function (Song *et al*. 2013) These changes were exhibited as reductions of F_v/F_m and ΦPSII and increased initial fluorescence. Reduction of chlorophyll contents and damage to PSII are important factors leading to a declined photosynthetic rate. This study demonstrated that prolonged heat stressed ultimately increased the chlorophyll content (Fig. 1), which differed from the previous findings. One possible explanation for this was that heat stress resulted in relatively large damage to the panicles during the early grain-filling period, thereby interrupting transportation from the leaves to the panicles. Further studies would be necessary to confirm these findings. However, reduction of the light energy ratio of F_v/F_m, ΦPSII, ETR and photochemical reactions indicated damage to the photosynthetic system, resulting in a decline in photosynthetic rate (Fig. 2). These results were consistent with those of previous reports. Exogenous Spd spraying reduced the accumulation of chlorophyll content, indicating that Spd alleviated the disruption of photosynthetic matter transport due to heat stress. Spd also protected PSII by effectively increasing the F_v/F_m, ΦPSII, ETR and light energy used in the photochemical reaction. These findings suggest that exogenous Spd optimised energy distribution and protected the structure and function of the photosynthetic system, thereby promoting initial electron transfer in PSII. These results are consistent with those of previous studies using tomato seedling (Su *et al*. 2013) and ginger (Li *et al*. 2014). Moreover, heat stress reduced the transpiration rate of two rice species, whereas exogenous Spd increased the stomatal conductance of rice leaves, thereby increasing the transpiration rate. Spd may reduce leaf temperature by increasing the transpiration rate and therefore protect the photosynthetic structure and related membrane system of rice leaves.

When plants are subjected to environmental stress they regulate the coding of related isozyme expressions to change antioxidant enzymes SOD and POD activities (Shah and Nahakpam 2012; Tian *et al*. 2012). These two enzymes promote scavenging of free radicals and ROS to protect the membrane systems of the plant from oxidative damage. MDA is a product of oxidative damage in the plant cell membrane that reflects the degree of stress damage in the plant. In the present study, SOD and POD activities were initially elevated in both rice species during heat stress, after which they declined under prolonged heat stress. This finding is in accordance with the variation patterns of antioxidant enzyme activity under heat stress reported by Zhang *et al*. (2005). However, the SOD and POD activities of Ningjing 3 during later stages of heat stress were significantly higher than those of the NT group at the same treatment time point, whereas the SOD and POD activities of Wuyunjing 24 during the late stage of heat stress was significantly lower than that of the NT group. MDA accumulation in both species was significantly elevated under heat stress, among which MDA accumulation of Wuyunjing 24 in the CK group was maintained at a relatively high level throughout the high-temperature treatment (until the day 22 of the treatment) that was significantly higher than that of Ningjing 3 at the same time point. These findings suggest that changes in antioxidant enzyme activities and MDA accumulation contents varied among rice species, which might involve genotypic differences in their heat-resistance. When compared with CK, exogenous Spd spraying significantly elevated SOD and POD activities in both rice species, and prolonged high-temperature treatment further elevated the enzyme activities, resulting in damage to the antioxidant defences in the plants and initiating declines in antioxidant enzyme activities. Spd spraying significantly improved the enzymatic activities of SOD and POD, which promoted significant reduction of the MDA contents in the middle to late stage of high-temperature treatment. These findings were consistent with those of a study conducted by Tian *et al*. (2012) with cucumber seedlings. Specifically, exogenous Spd enhanced SOD and POD activity, reduced membrane lipid peroxidation, and lowered MDA accumulation content. The Spd-enhanced oxidant enzyme activities might originate from the characteristics of polycations, which promoted the binding with protein receptor and improved its stability. In addition, polyamine, a signal transduction substance, could promote the synthesis of related functional proteins, thereby enhancing the activities of antioxidant enzymes and protecting the photosynthetic structures in plants.

Soluble sugar is a substance for osmotic adjustment to maintain osmotic balance in the cells and play an important role in plant resistance. Previous studies had different views in soluble sugar variation in rice crops under heat stress. An investigation by Lu *et al*. (2009) showed that soluble sugar accumulation increased under heat stress, and studies by Liu *et al*. (2008) and Liao *et al*. (2013) demonstrated decreased soluble sugar accumulation under heat stress. This reduction might have been due to the physiological and metabolic diseases caused by heat stress. These two opposite results might be associated with the different intensities of heat stress. In the present study the soluble sugar contents (Fig. 7) matched those reported by Lu *et al*. (2009) Specifically, the soluble sugar content of the two rice species under heat stress were significantly higher than in the NT group, and exogenous Spd spraying further elevated the accumulation of soluble sugar when compared with the CK group. These findings were consistent in both rice species. However, soluble sugar levels in Wuyunjing 24 were more sensitive to treatment and therefore reached a significant level at the day 22 of treatment.

In the present study the elevated seed-setting rates and the weight of both rice species were significantly reduced under heat stress, which was consistent with the results of a study conducted by Duan *et al*. (2013). Spd spraying of two rice species effectively improved the seed-setting rates and grain weight under heat stress, especially those that showed a significant effect in both species. However, seed-setting rates of both rice species were not sensitive to Spd treatment, although Wuyunjing 24 was relatively more sensitive than Ningjing 3. Heat stress can induce the declines in both seed-setting rates and grain weight in rice crops and one possible mechanism would be the damages to the reproductive organs caused by high temperatures. Results indicate that heat stress could greatly reduce the rice pollen activity and exogenous Spd alleviated such effects to a certain extent (Fig. S2) (Endo *et al*. 2009). Furthermore, high temperatures caused some of the tapetum functions required for pollen adhesion and germination on the stigma and exogenous Spd obviously recovered such influence (Fig. S3). That may explain the significant decrease of spikelet sterility detected in this study under heat stress. However, we observe no obvious morphological changes in ovary size or pollen tube length caused by heat or exogenous Spd in rice (Fig. S4) and that is mainly due to the formation of rice pistil organs had been accomplished before exposure to high temperature treatment.

Conclusion

Heat stress during the early grain-filling period disrupted the antioxidant capacity and the membrane osmotic balance in rice leaves and further damaged the photosynthetic apparatus. Exogenous Spd effectively alleviated damage to the antioxidant, osmotic balance and photosynthetic systems caused by heat stress and further improved the cellular defence capability of rice crops. In addition, Spd improved the stomatal conductance and transpiration rate to lower the leaf temperature of rice crops during heat stress, thereby maintaining a more suitable temperature for the plant and exhibiting a better seed-setting rate and grain weight. In the present study Spd alleviated the damage caused by heat stress in both rice species; however, genotypic differences in the sensitivity of Spd existed in both rice species.

Conflicts of interest

The authors declare no conflicts of interest.

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