Priming effect of exogenous ABA on heat stress tolerance in rice seedlings is associated with the upregulation of antioxidative defense capability and heat shock-related genes

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Abstract
Heat stress is a major restrictive factor that suppresses rice production. In this study, we investigated the potential priming effect of exogenous abscisic acid (ABA) on heat tolerance in rice seedlings. Seedlings were pretreated with 10 µM ABA by root-drenched for 24 h and then subjected to heat stress conditions of 40 °C day/35 °C night. ABA pretreatment significantly decreased leaf withering by 2.5–28.5% and chlorophyll loss by 12.8–35.1% induced by heat stress in rice seedlings. ABA pretreatment also mitigated cell injury, as shown by lower malondialdehyde content, relative electrolytic conductivity, and expression of cell death-related genes OsKOD1, OsCP1, and OsNAC4, while expression of OsBI1, a cell death-suppressor gene, was upregulated by ABA pretreatment. Moreover, ABA pretreatment improved antioxidant defense capacity, as shown by an obvious upregulation of ROS-scavenging genes and a decrease in ROS content (O2− and H2O2), and downregulation of the OsRbohs genes. Application of fluridone, an ABA biosynthesis inhibitor, increased membrane injury and the accumulation of ROS under heat stress. Exogenous antioxidants (proanthocyanidins) significantly alleviated leaf withering by decreasing ROS overaccumulation and membrane injury induced by heat stress. In addition, ABA pretreatment significantly superinduced the expression of ABA-responsive genes SalT and OsWis18, the ABA biosynthesis genes OsNCED3 and OsNCED4, and the heat shock-related genes OsHSP23.7, OsHSP17.7, OsHSF7, and OsHsfA2a. Taken together, these results suggest that exogenous ABA has a potential priming effect for enhancing heat stress tolerance of rice seedlings mainly by improving antioxidant defense capacity and heat shock-related genes.

Keywords Abscisic acid (ABA) · Antioxidative defense · Heat stress · Priming · Rice (Oryza sativa L.)

Abbreviations

| ABA | Abscisic acid |
| H2O2 | Hydrogen peroxide |
| HS | Heat stress |
| HSP | Heat-shock proteins |
| HSF | Heat-shock factor |
| MDA | Malondialdehyde |
| MI | Membrane injury |
| O2− | Superoxide anions |
| PC | Proanthocyanidins |
| qRT-PCR | Quantitative real-time PCR |
| ROS | Reactive oxygen species |
| RBOH | Respiratory Burst Oxidase Homolog |

Introduction

Global warming has become a severe ecological and environmental problem due to the development of industry and population growth. Heat stress is caused by an extremely high temperature or by a lasting threshold of high-temperature weather, which has resulted from global warming (Quint et al. 2016; Xu et al. 2018). Heat stress results in a serious threat to crop production worldwide, and the yields of wheat, rice, and maize were decreased by 6.0%, 3.2%, and 7.4%...
due to 1 °C increase in the mean temperature, respectively (Zhao et al. 2017a; Janni et al. 2020). Rice (Oryza sativa L.) is a staple food crop for most of the world’s population, while the main temperature during the growing season of rice occurs during the annual long hot summer season (Shi et al. 2015). Rice production during this time suffers from heat stress which directly inhibits the survival and transplanting of late rice, as well as the heading, flowering, and grain-filling of early season rice (Yang et al. 2020). Furthermore, heat stress results in significantly decrease in grain yield, total milled rice yield, head rice yield and total milling revenue with the increasing average growing temperature (Lyman et al. 2013). Thus, the demand for an expanding population under the current situation of global warming remains a huge challenge for food production.

The effect of heat stress on rice production occurs during the entire growth stage. The germination of rice seeds was suppressed when the growth temperatures was ≥ 35 °C, as shown by a significant decrease in the germination rate and growth in buds (Yang et al. 2021). The optimal temperature of rice seedlings ranges from 22 to 28 °C, while the growth of rice seedlings is severely inhibited under growth temperature of ≥ 35 °C (Soda et al. 2018). Heat stress results in withering, browning, abnormalities, and water loss of rice leaves, suppresses the growth of seedlings and roots and causes complete wilting of rice plants (Liu et al. 2016; Kilasi et al. 2018; Li et al. 2020). Heat stress suppresses photosynthetic efficiency by decreasing chlorophyll content, disturbing the combination of chlorophyll-protein complexes, and damaging the photosystem structure (Yang et al. 2013; Fan et al. 2017). In addition, heat stress causes excess accumulation of reactive oxygen species (ROS), which results in severe damage to the membrane and cell death (Zhao et al. 2017b). Rice plants are more sensitive to heat stress at the reproductive and grain-filling stages. Heat stress results in the degradation of flowering florets, abortion of pollen, and devitalization of pollen viability of rice at the booting stage (Fu et al. 2016; Zhang et al. 2018), and suppresses the grain-filling rate in rice at the grain-filling stage (Zhang et al. 2018; Wang et al. 2019). Thus, it is indeed necessary to explore an effective pathway for the improvement of heat tolerance in rice.

Plants generate and accumulate ROS such as superoxide anions (O$_2^-$) and hydrogen peroxide (H$_2$O$_2$) under various environmental stress conditions, such as drought, salinity, alkalinity, and extreme temperature (Choudhury et al. 2017). These ROS molecules play an important role in the regulation of development and adaptation to the environment (Mittler 2017). However, excess accumulation of ROS causes oxidative stress in plants, which results in severe damage to the plant cellular membrane, RNA, DNA, and proteins (Sewelam et al. 2016). We previously reported that overaccumulation of ROS in rice roots is a main limiting factor in cell damage and plant growth inhibition in rice seedlings under alkaline stress conditions (Zhang et al. 2017). ROS accumulation is an important harmful pathway in the physiological effects of heat stress on plants (Xu et al. 2021). Heat stress causes a remarkable upregulation of respiratory burst oxidase homolog (RBOH) genes and an accumulation of ROS in rice seedlings, and excessive ROS levels disturb the balance of ROS production and scavenging, which further results in membrane lipid peroxidation, cell damage, changes in a series of antioxidant enzymes, and even plant death (Liu et al. 2019). Previous studies have shown that excessive accumulation of ROS induced by heat stress causes the decline of germination rate, pollen viability, spikelet fertility, and grain chalkiness in rice (Suriyasak et al. 2017; Zhao et al. 2017b; Yang et al. 2021), indicating that ROS level is involved in the regulation of yield formation of rice under heat stress condition and enhancing the antioxidative defense capacity promotes anther development and yield formation under heat stress conditions (Dwivedi et al. 2019). Therefore, studies on the improvement of rice tolerance to various stress factors by reducing oxidative stress induced by overaccumulation of ROS are still required for further insight and will provide a potentially useful pathway in rice field production in the future (Kerchev et al. 2015).

The phytohormone abscisic acid (ABA) plays an important role in the regulation of plant growth and adaptive to various stress factors (Dar et al. 2017; Lang et al. 2021). One important pathway of ABA action in stress tolerance is the priming effect on plants (Vishwakarma et al. 2017; Wei et al. 2017; Liu et al. 2019), which confer the potential enhanced ability to mount defense responses to impending stress factors (Aranega-Bou et al. 2014). Priming is progress that plants were pretreated with a range of chemical compounds before being treated in various external stresses and the state of chemically treated plants are referred to as “primed” (Beckers and Conrath 2007; Aranega-Bou et al. 2014). The priming effect of ABA has been demonstrated as shown by rice seeds or seedlings pretreated with ABA improved plants survival, growth, and grain yield under saline-alkaline stress conditions (Gurmani et al. 2011; Wei et al. 2015, 2017). Further analysis on the underlying mechanism of ABA priming showed that ABA priming potentiated to improve the downstream antioxidant defense capacity and stress tolerance-related gene expression for an increased adaptive response to alkaline stress (Liu et al. 2019).

ABA also functions in crops’ response to heat stress and plays a vital role in crop production under climate warming conditions (Suzuki et al. 2016; Li et al. 2021). Application of exogenous ABA improves plant heat tolerance as shown by keeping water balance, regulating stomatal conductance, and the regulation of gene expression (An et al. 2014; Li et al. 2014). Additionally, ABA also functions in the regulation of rice in the reproductive and grain-filling stages (Islam
The ROS signal pathway plays an important role in plant response to environmental stress and excess accumulation induced by various stress factors results in membrane injury, root damage, and even the death of plants (Zhang et al. 2017; Qiu et al. 2019). ABA confers to regulate ROS levels in plants' response to environmental stress factors (Wang et al. 2013; Liu et al. 2019), as well as under high-temperature conditions (Hu et al. 2010). And the ABA-deficient mutants exhibited more sensitivity to heat stress (Larkindale et al. 2005). These studies demonstrate a strong correlation between ABA application and regulation of ROS levels in plants' response to stress conditions (Suzuki et al. 2016; Liu et al. 2019). Current studies on the relationship between ABA and heat stress in rice were mainly focused on the ABA-dependent signal pathway and spraying of exogenous ABA (Zhang et al. 2019; Li et al. 2020). Research of ABA priming effect on stress tolerance in rice was mainly validated in the response to salt or alkaline stress (Wei et al. 2015, 2017; Liu et al. 2019).

This study aimed to gain insights into the effect and mechanism of ABA priming on heat tolerance in rice by focusing on the effect of ABA priming on ROS-formation or ROS-scavenging pathway. We hypothesized that the priming effect of exogenous ABA on heat stress tolerance in rice seedlings is associated with improvement of antioxidative defense capacity and expression of heat stress-tolerant genes.

**Materials and methods**

**Plant material and growth conditions**

Rice cultivar Huanghuazhan, an elite cultivar suitable to be spread in eastern China, was used in this study. It was bred by crossing ‘Huangxinzhan’ with ‘Fenghuazhan’ and was resistant to heat stress (China Rice Data Center). Rice seeds were surface-sterilized with 75% (v/v) alcohol for 5 min and rinsed with deionized water five times. After that, seeds were immersed in water for 2 days and then were sprinkled onto a petri dish with wet filter paper for pre germination in an incubator under dark conditions at 28 °C for 24 h. Eighteen uniformly germinated seeds were transplanted onto a multi-well plate floating on a 320-mL cup containing deionized water for 7 days and then grown in half-strength Kimura B nutrient solution (Miyake and Takahashi 1983) for another 7 days. All rice seedlings were grown in a controlled growth chamber under the following conditions: 28 °C day/22 °C night, 12 h photoperiod, and 350 mmol photons m⁻² s⁻¹ light intensity.

**ABA pretreatment and heat stress treatment**

ABA (Sigma, Inc., St, Louis, MO, USA) was dissolved in a small amount of absolute ethanol and then diluted with deionized water to the desired concentrations (Wei et al. 2015; Liu et al. 2019). Rice seedlings at the approximately three-leaf stage were pretreated with 10 µM ABA or deionized water by root-drench for 24 h, respectively. Then these two sets of rice seedlings were transferred to the control or heat stress conditions after being rinsed with deionized water, respectively. Thus, four treatments were set: root-drench with deionized water in unstressed condition (CK); root-drench with 10 µM ABA in unstressed condition (ABA); root-drench with deionized water in heat stress condition (HS), root-drench with 10 µM ABA in heat stress condition (ABA + HS). We used growth temperature of 40 °C to simulate heat stress. The temperature of unstressed condition was set as 28 °C day/22 °C night, and the temperature of heat stress was set as 40 °C day/35 °C night. This growth temperature was set according to the description of rice response to heat stress in South China by Shi et al. (2015) and Huang et al. (2017).

**Treatment of rice seedlings with exogenous Fluridone and Proanthocyanidins (PC)**

In this study, exogenous fluridone and PC was used to examine the mechanism of the ABA priming effect. Fluridone is an ABA biosynthesis inhibitor and inhibited rice seedlings' growth under alkaline stress (Wei et al. 2015). Proanthocyanidin is an antioxidant that effectively scavenged superoxide anion radicals and hydroxyl radicals, and alleviated alkalinity-induced suppression of rice seedling growth by inhibiting ROS overaccumulation (Rue et al. 2017; Zhang et al. 2017). Two-week-old rice seedlings were pretreated in the solution with deionized water, 10 µM fluridone, and 1% PC, by root-drench for 24 h, respectively; and then transferred to control or heat stress conditions aforementioned. The treatments were set as follows: CK, Fluridone, PC, HS, Fluridone + HS, PC + HS, respectively.

**Measurement of seedling growth**

Photograph of the growth condition of rice seedlings was taken at the indicated treatment hours. The withered leaf rate was investigated at 48, 72, and 96 h of heat stress, respectively. The withered leaf rate was recorded as 1 if the whole leaf was dry and brown, while it was recorded as 0.5 if half of the leaf was dry and brown, respectively (Liu et al. 2020). The withered leaf rate of each treatment was represented by the proportion of withered leaves of whole leaves in one cup.
Measurement of chlorophyll content

The chlorophyll content was measured according to the theory as described by Wellburn and Lichtenthaler (1984), with some modifications as described by Liu et al. (2019). Leaf samples (0.1 g) were extracted using a 10 mL mixture of ethanol (5 mL) and acetone (5 mL) under dark conditions. The absorbance of the supernatant was determined at 436 and 663 nm using a spectrophotometer (UV-2700, Shimadzu, Kyoto, Japan). The MDA concentration was calculated using the following formula: (20.29×A_645 + 8.05×A_663) V/(1000×W).

Measurement of malondialdehyde (MDA) content and relative electrolytic conductivity (REC)

The MDA content was determined by the thiobarbituric acid reaction as described by Heath and Packer (1968). Leaf samples were homogenized in 1 mL of 50 mM phosphate buffer (pH 7.8) after being smashed at the refrigerated condition with liquid nitrogen and centrifuged at 12,000×g for 15 min. Subsequently, 400 µL of supernatant was mixed with 1 mL of 0.5% thiobarbituric acid, and the mixture was boiled for 20 min. The absorbance of the resulting supernatant after cooled and centrifuged was measured at 532, 600, and 450 nm using a spectrophotometer (UV-2700, Shimadzu, Kyoto, Japan). The MDA concentration was calculated using the following formula: 6.45×(A_532 – A_600) – 0.56 × A_450. Finally, the MDA content in the leaf was calculated according to the fresh weight of the leaf of each treatment.

The relative electrolytic conductivity (REC) was an important index for evaluating the membrane injury (Tantau and Dörfling 1991; Wei et al. 2015). Relative electrolytic conductivity was represented by the electrolytic conductivity of the effusion with leaf in it before and after boiling (Wei et al. 2015). Rice leaves (2 g fresh weight) were randomly selected from each treatment group, washed with deionized water to remove surface-adhered electrolytes. Leaf samples were submerged in 15 mL of deionized water in 50 mL conical tubes and kept at room temperature for 1 h. The electrical conduction of the effusion was then measured with a DDS-12 conductivity meter (Lida Inc., Shanghai, China) and recorded as R1. The tissue samples were killed by heating tubes in a boiling bath for 40 min, cooled to room temperature, and the electrical conduction of the effusion was measured again which was recorded as R2. The REC was evaluated using the formula REC (%) = R1/R2 × 100%.

Measurement of ROS levels

The O_2^− contents were measured as described by Elstner and Heupel (1976) by monitoring nitrite formation from hydroxylamine in the presence of O_2^−, with some modifications as described by Jiang and Zhang (2001). For the determination of O_2^− contents, the fresh leaves (0.1 g) were loaded in a 2 mL tube and frozen in liquid nitrogen, then homogenized with 1 mL of 50 mM potassium phosphate buffer (pH 7.8) and centrifuged at 10,000×g for 10 min at low temperature of 4 °C, and then collected the supernatant. The incubation mixture contained 0.9 mL of 50 mM phosphate buffer (pH 7.8), 0.4 mL of 10 mM hydroxylamine hydrochloride, and 1 mL of the supernatant were mixed for incubation at room temperature for 20 min. Then, 0.3 mL of 17 mM sulfanilamide and 0.3 mL of 7 mM α-naphthylamine were added to the incubation mixture. After reaction at room temperature for 20 min, ethyl ether with the same volume was added and centrifuged at 8000×g for 5 min. The absorbance values in the aqueous solution were read at 530 nm to calculate the contents of O_2^− from the chemical reaction of O_2^− and hydroxylamine.

The H_2O_2 contents were measured as described by monitoring the A_415 of the titanium-peroxide complex (Brennan and Frenkel 1977). For the determination of H_2O_2 contents, the fresh leaves (0.1 g) were loaded in a 2 mL tube and frozen in liquid nitrogen, then homogenized with 1 mL of acetone and centrifuged at 8000×g for 10 min at low temperature of 4 °C, and collected the supernatant. The incubation mixture contained 1 mL of the supernatant, 0.1 mL of titanium sulfate, and 0.2 mL of stronger ammonia water and then was centrifuged at 4000×g for 10 min at room temperature. The precipitate was solubilized in 1 mL of 2 mol/L H_2SO_4 and then reacted at room temperature for 5 min. The absorbance values in the aqueous solution were read at 415 nm to calculate the contents of H_2O_2. The analytical reagent used to measure the H_2O_2 and O_2^− contents were acquired from the determination kit, according to the manufacturer’s instructions (Comin Biotechnology Co., Ltd. Suzhou, China) (Zhang et al. 2017; Liu et al. 2019).

RNA isolation and quantitative real-time PCR (qRT-PCR)

Rice leaves were sampled in liquid nitrogen and ground using a bench-top ball-mill (Scientz-48, Ningbo Scientz Biotechnology Co. Ltd., Ningbo, China) at 50 Hz for 30 s. Total RNA was extracted with TRIzol reagent (TaKaRa Bio Tokyo, Japan) and first-strand cDNA was synthesized using M-MLV reverse transcriptase (Thermo, Carlsbad, CA, USA) according to the manufacturer’s protocols. Quantitative real-time PCR (qRT-PCR) was performed to determine the transcriptional expression of genes, including cell death-related genes OsKOD1, OsCP1, OsNAC4 (Zhang et al. 2017), and a cell death-suppressor gene, OsBII1 (Liu et al. 2019); nine OsRboh family genes (OsRboh1-OsRboh9) (Liu et al. 2012), two ABA-response genes, OsNAC4, and OsKOD1.
genes, SalT and OsWsi18 (Liu et al. 2019); two ABA-biosynthesis genes, OsNCED3 and OsNCED4 (Zhu et al. 2009); four heat shock-related genes, OsHSP23.7 (Zou et al. 2012), OsHSP17.7 (Sato and Yokoya 2008), OsHSF7 (Liu et al. 2009) and OsHsfA2a (Wang et al. 2013); and 20 ROS-scavenging genes (R1-R20) (Liu et al. 2019). Gene-specific primers were designed using Primer 5.0 software (Tables S1–S3).

The housekeeping gene β-actin (GenBank ID: X15865.1) was used as an internal standard. PCR was conducted in a 20 µL reaction mixture containing 1.6 µL of cDNA template (50 ng), 0.4 µL of 10 mM specific forward primer, 0.4 µL of 10 mM specific reverse primer, 10 µL of 2× SYBR® Premix Ex Taq™ (TaKaRa, Bio Inc.), and 7.6 µL of double-distilled H2O in a PCR machine (qTOWER2.2. Analytic Jena. GER). The procedure was performed as follows: 1 cycle for 30 s at 95 °C, 40 cycles for 5 s at 95 °C, and 20 s at 60 °C, and 1 cycle for 60 s at 95 °C, 30 s at 55 °C, and 30 s at 95 °C for melting curve analysis. The level of relative expression was computed using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001).

Experimental design and statistical analyses

All of the experiments were conducted in a controlled growth chamber with five biological replicates, each consisting of 3 cups of rice seedlings, with eighteen seedlings each cup. Statistical analyses were performed using the statistical software SPSS 21.0 (IBM Corp., Armonk, NY). Based on a one-way analysis of variance (ANOVA), Duncan’s multiple range test (DMRT) was used to compare differences in the means among treatments. The significance level was $P < 0.05$.

Results

ABA pretreatment rescued rice seedlings from wilting and death under heat stress

There was no significant difference in leaf withering with or without ABA pretreatment at 24 and 48 h of heat stress (Fig. 1A, B). While pretreatment with exogenous ABA significantly rescued rice seedlings from wilting and death as shown by the lower withered leaf rates of seedlings pretreated with ABA at 72 and 96 h of heat stress (Fig. 1C–E), the withered leaf rate of rice seedlings was decreased by 28.5% and 15.8% by ABA pretreated under heat stress condition (Fig. 1F). And this mitigative effect was sustained to 108 h of heat stress (Fig. S1). Almost the whole leaves were withered and there was no significant difference with or without ABA application, after 120 h of heat stress (Fig. S1). Rice seedlings pretreatment with ABA significantly increased chlorophyll content by 11.3%, 25.9%, and 13.8%, compared with without ABA pretreatment under heat stress conditions (Fig. 1G).

ABA pretreatment mitigated membrane injury induced by heat stress

Exogenous ABA pretreatment significantly mitigated cell injury as shown by a lower accumulation of MDA and REC (Figs. 2A, B and S2). Compared to HS treatment, MDA content was decreased by 22.4%, 22.1%, and 10.8%, and REC was decreased by 14.1%, 13.2%, and 7.9%, at 48, 72, and 96 h of heat stress, respectively (Fig. 2A, B). In addition, a cell death suppressor, OsBI1, was significantly downregulated and the cell death-related genes, OsKOD1, OsCP1, OsNAC4, were significantly upregulated by ABA pretreated under heat stress conditions (Fig. 2C–F). The relative expression level was increased by 37.1%, 47.2%, and 50.2% with ABA pretreatment at 48, 72, and 96 h of heat stress condition, respectively (Fig. 2C).

ABA pretreatment decreased ROS accumulation and improved ROS-savaging capacity under heat stress

Pretreatment with exogenous ABA significantly inhibited ROS accumulation as shown by lower $\text{O}_2^-$ and $\text{H}_2\text{O}_2$ content in rice leaves under heat stress conditions (Fig. 3). Compared to HS treatment, the content of $\text{O}_2^-$ was decreased by 5.9%, 19.6%, and 22.2% (Fig. 3A), and content of $\text{H}_2\text{O}_2$ was decreased by 8.3%, 16.5%, and 16.1% with ABA pretreatment at 48, 72 and 96 h, respectively (Fig. 3B).

ABA pretreatment also suppressed the transcriptional expression of OsRboh genes. As shown in Fig. 4, an obvious upregulation was shown in the OsRbohs family genes by heat stress, and the relative expression level of OsRboh1, OsRboh4, OsRboh5, OsRboh6, and OsRboh7 was reached to a higher level. Among these OsRbohs family genes, OsRboh2, OsRboh3, OsRboh5, and OsRboh7 were significantly suppressed by ABA pretreatment (Fig. 4).

We further analyzed the relative expression levels of 20 ROS-scavenging genes, as shown in Fig. 5, almost all ROS-scavenging genes were upregulated by heat stress. Furthermore, ABA pretreatment significantly super-upregulated the expression level of 16 ROS-scavenging genes except for R5, R7, R13, and R20 (Fig. 5).
Exogenous ABA biosynthesis inhibitor (Fluridone) suppressed rice seedlings’ growth under heat stress

As shown in Fig. 6A, the growth condition of rice seedlings with the application of fluridone (treatment of Fluridone + HS), an ABA biosynthesis inhibitor, was similar to the HS treatment. The withered leaf rate and chlorophyll content were not statistically significant between Fluridone + HS and HS treatment (Fig. 6B, C), as well as the accumulation of O$_2^-$ and H$_2$O$_2$ (Fig. 6F, G). Accumulation of MDA and MI with the application of fluridone was significantly higher than that of HS treatment at 48, 72 h of heat stress condition, respectively (Fig. 6D, E).

Application of exogenous antioxidant (Proanthocyanidins, PC) rescued rice seedlings from leaf withering induced by heat stress

In this study, application of exogenous PC significantly rescued rice seedlings from leaf withering as shown by a decrease of 47.8%, 44.7%, and 33.5% of leaf withered rate at 48, 72, and 96 h, compared to HS treatment (Fig. 6A, B). Content of chlorophyll was increased by 13.6%, 31.3%, and 34.8% with the application of PC under heat stress conditions, compared to HS treatment (Fig. 6C). In addition, membrane injury was significantly mitigated by PC as shown by a lower accumulation of MDA content and MI in rice seedlings of PC + HS treatment (Fig. 6D, E). Consistently, the accumulation of O$_2^-$ and H$_2$O$_2$ was decreased by 25.3–41.1% and 39.8–45.6% with the application of PC, compared to HS treatment (Fig. 6F, G).

Fig. 1 ABA priming rescued rice seedlings from wilting under heat stress. Two-week-old rice seedlings were root-drenched with or without 10 µM ABA for 24 h, and then subjected to unstressed or heat stress conditions. Photographs of seedling growth (A–D) were taken at 24 h, 48 h, 72 h, and 96 h, respectively. Photograph of leaf wilting (E) was taken at 72 h. Withered leaf rate (F) and chlorophyll content (G) of leaf in rice seedlings were counted after 48 h, 72 h, and 96 h. Values are means ± SD, n = 5. Different letters on the column represent significant differences (P < 0.05) between different treatments based on Duncan’s test.
Fig. 2 ABA priming mitigated cell injury under heat stress. Two-week-old rice seedlings were root-drenched with or without 10 µM ABA for 24 h, and then subjected to unstress or heat stress conditions. Malondialdehyde (MDA) content (A) and relative electrolytic conductivity (REC) (B) of rice seedlings were measured at the indicated treatment hours. Values are means ± SD, \( n = 5 \). Expression levels of cell death-related genes, OsBI1 (C), OsKOD1 (D), OsCP1 (E), and OsNAC4 (F) were measured at the indicated treatment hours. A quantitative real-time polymerase chain reaction was performed using OsACT1 as an internal standard. The expression levels of unpretreated control (CK) were set as the unit to calculate the expression levels, shown as fold changes relative to the CK. Values are means ± SD, \( n = 3 \). Different letters on the column represent significant differences (\( P < 0.05 \)) between different treatments based on Duncan’s test.

Fig. 3 ABA priming decreased ROS accumulation under heat stress. Two-week-old rice seedlings were root-drenched with or without 10 µM ABA for 24 h, and then subjected to unstress or heat stress conditions. Accumulation of \( \text{O}_2^- \) (A) and \( \text{H}_2\text{O}_2 \) (B) in leaves were measured at the indicated treatment hours. Values are means ± SD, \( n = 5 \). Different letters on the column represent significant differences (\( P < 0.05 \)) between different treatments on Duncan’s test.
ABA pretreatment upregulated stress tolerance-related genes under heat stress

ABA signaling pathway was exactly activated by heat stress and ABA pretreatment as shown by an upregulation of two ABA-responsive genes, Salt and OsWsi18 (Fig. 7A, B). While the expression levels of Salt and OsWsi18 were significantly superinduced by 34.2–47.8% and 25.9–26.9% with ABA pretreatment, compared to HS treatment (Fig. 7A, B). The expression levels of two ABA biosynthesis genes, OsNCED3 and OsNCED4, were increased by 40.8–71.3% and 32.5–54.0% by ABA pretreatment, compared to HS treatment (Fig. 7C, D).

To gain further insights into the mechanism of ABA pretreatment for heat stress, two heat shock protein (HSP) genes, OsHSP23.7 and OsHSP17.7, and two heat shock transcription factors (HSF), OsHSF7 and OsHsfA2a, were analyzed in this study. All these stress tolerance-related genes were significantly upregulated by ABA, HS, and ABA + HS treatment, while the relative expression levels of these four genes were significantly super-upregulated by ABA pretreatment under heat stress conditions (Fig. 7E–H). The gene relative expression levels of OsHSP23.7, OsHSP17.7, OsHSF7 and OsHsfA2a was increased by 28.4–36.9%, 32.9–49.7%, 31.0–42.6% and 33.3–50.5% with ABA pretreatment under heat stress condition, respectively (Fig. 7E–H).
Heat stress is characterized by an extreme or lasting high-temperature climate for a long time, which has become an enormous meteorological disaster for crop production (Xu et al. 2021). Heat stress results in severe inhibition in crop growth and yield formation as shown by increasing leaves withering and death (Wei et al. 2012; Kilasi et al. 2018; Liu et al. 2018), damaging cell membrane and photosynthetic structure (Essemine et al. 2017; Soda et al. 2018), impairing pollen swelling (Das et al. 2014; Wang et al. 2019), reducing spikelets (Fu et al. 2016; Zhang et al. 2016, 2018) and

**Discussion**

Heat stress is characterized by an extreme or lasting high-temperature climate for a long time, which has become an enormous meteorological disaster for crop production (Xu et al. 2021). Heat stress results in severe inhibition in crop growth and yield formation as shown by increasing leaves withering and death (Wei et al. 2012; Kilasi et al. 2018; Liu et al. 2018), damaging cell membrane and photosynthetic structure (Essemine et al. 2017; Soda et al. 2018), impairing pollen swelling (Das et al. 2014; Wang et al. 2019), reducing spikelets (Fu et al. 2016; Zhang et al. 2016, 2018) and
the grain filling (Chen et al. 2017; Suriyasak et al. 2017). Recently, it was shown that the application of exogenous phytohormones alleviated heat-induced damage in plants and enhanced plant heat tolerance (Li et al. 2021). ABA plays an important role in crops’ response to environmental stress. Previous studies have reported the priming effect of exogenous ABA on tolerance to alkaline stress in rice seedlings (Wei et al. 2015, 2017; Liu et al. 2019). And we previously showed rice seeds soaked with exogenous ABA significantly improved seed growth under lasting heat stress conditions (Yang et al. 2021). In the present study, rice seedlings pretreated with exogenous ABA significantly mitigated the heat-induced leaf withering (Fig. 1), membrane injury (Fig. 2), and overaccumulation of ROS (Figs. 3 and 4), and improved ROS-scavenging capability (Fig. 5). In addition, there was some evidence that showed that application of the ABA biosynthesis inhibitor, fluridone, compromised tolerance to heat stress in rice seedlings (Fig. 6), while application of the antioxidant, PC, improved tolerance of the seedlings to heat stress (Fig. 6). Pretreatment with ABA also upregulated gene expression levels related to ABA signal and heat shock and transcription factor (Fig. 7). These data collectively suggest that pretreatment with exogenous ABA enhanced heat tolerance in rice seedlings mainly by improving ROS-scavenging capability and upregulating heat shock-related genes (Fig. 8).

ABA is an important “stress phytohormone” in plants, which has been evidenced by the action in various stress conditions such as drought, salt, alkali, cold, and high temperature (Dar et al. 2017; Vishwakarma et al. 2017). Exogenous ABA plays a vital in the improvement of stress tolerance by multiple methods, such as foliage spray, adding into solution, or seed soaking (Gurmani et al. 2011; Wang et al. 2013). An important mechanism of ABA for enhancing stress tolerance in plants is the priming effect, which helps plants to acquire a potential capacity to enhance defense response to subsequent stress factors (Aranega-Bou et al. 2014; Wei et al. 2017). This priming effect has recently been validated in rice response to salt or alkali stress as shown by seed presoaking or root drenching with exogenous ABA.
significantly improved the survival rate, plant growth, and grain yield of rice (Gurmani et al. 2011; Wei et al. 2015, 2017). Application of exogenous ABA plays an active effect in plants’ response to heat stress (Islam et al. 2018). However, few studies have reported on the priming effect of ABA in the heat stress responses. We previously reported that ABA primes rice seeds for enhanced heat stress tolerance as shown by ABA presoaking improved ROS-scavenging capacity, inhibiting ROS overaccumulation and mitigating membrane injury (Yang et al. 2021). Results of the present study showed that the ABA-responsive genes, Salt (A) and OsWsi18 (B), ABA biosynthesis genes OsNCED3 (C) and OsNCED4 (D), and stress tolerance-related genes OsHSP23.7 (E), OsHSP17.7 (F), OsHsf7 (G), and OsHsfA2a (H) were measured at 72 h. A quantitative real-time polymerase chain reaction was performed using OsACT1 as an internal standard. The expression levels of untreated control (CK) were set as the unit to calculate the expression levels, shown as fold changes relative to the CK. Values are means ± SD, n = 3. Different letters on the column represent significant differences (P<0.05) between different treatments based on Duncan’s test.
response to heat stress in rice seedlings. However, rice seedlings were withered or eventually dead induced by heat stress for more than 4 days (Fig. 1), indicating that these activation levels of ABA-induced by heat stress may not be sufficient to effectively cope with the heat stress factor. Nevertheless, ABA pretreatment upregulated the expression of ABA-responsive and ABA-biosynthesis genes to a higher degree (Fig. 7), as well as a great increase of ROS-scavenging genes (Fig. 5), heat shock-related genes (Fig. 7), and a remarkable decrease of ROS accumulation (Fig. 3) and cell death in rice seedlings under heat stress condition (Fig. 2). These results suggest that exogenous ABA enhances tolerance to heat stress in rice seeds or seedlings by the priming effect which potentiates multiple downstream pathways response to heat stress in rice

ROS plays a vital role in the regulation of plants’ response to various stress factors (Choudhury et al. 2017; Mittler 2017). ROS serves as the signaling messenger in a series of physiological processes required for the growth regulation and stress response at low levels (Sewelam et al. 2016; Mittler 2017). However, environmental stress induces overaccumulation of ROS in cells, which results in oxidative stress and even cell death in plants (Choudhury et al. 2017; Zhang et al. 2017). In rice, excessive accumulation of ROS has been identified as a key causal factor in the inhibition of seed germination and seedlings growth under various stress conditions due to the result of oxidative stress, especially for the severe cellular damage to roots (Guan et al. 2017; Zhang et al. 2017). Heat stress caused multiple physiological effects to rice including membrane and photosynthesis damage, disturbance of ROS accumulation, and carbohydrate (Xu et al. 2021). We previously showed that increasing intracellular ROS levels was the primary for the inhabitation of seed germination and bud growth under lasting heat stress conditions (Yang et al. 2021). In the present study, heat stress caused a remarkable increase of ROS in rice seedlings as shown by a gradually rising accumulation of O$_2^-$ and H$_2$O$_2$ in leaves at the indicated time (Fig. 3A, B), as well as the upregulation of a series of OsRboh genes (Fig. 4). Meanwhile, rice seedlings presented a significant membrane injury as shown by the increase of MDA and REC (Fig. 2A, B), as well as several cell death-related genes, OsKOD1, OsCP1, and OsNAC4, (Fig. 2D–F) under heat stress condition. In addition, several ROS-scavenging genes were significantly upregulated by heat stress (Fig. 5). These results indicated that the ROS signal pathway was activated in the response to heat stress in rice seedlings; however, the ROS levels were too high in turn led to severe injury to the cell membrane, and finally resulted in withering and even death of rice seedlings (Fig. 1). Application of exogenous antioxidant, PC, significantly rescued rice seedlings from death by decreasing the ROS content and membrane injury (Fig. 6), indicating that overaccumulation of ROS is an important mechanism for inhibiting rice seedlings induced by heat stress. Nevertheless, rice seedlings pretreatment with ABA significantly improved antioxidative defense capacity as shown by a series of ROS-scavenging genes (Fig. 5), and decreased the ROS accumulation (Fig. 3A, B) and membrane injury (Fig. 2), which achieved a similar effect with the PC. On the contrary, the application of fluridone was ineffective to decrease the ROS accumulation and membrane injury (Fig. 6). These data demonstrate that ABA primes for enhanced heat tolerance in rice seedlings mainly by improving the ROS-scavenging capacity (Fig. 8), which is accordant to our previous study in alkaline stress (Liu et al. 2019).

The ROS levels in plants are codetermined by the ROS formation which is mainly regulated by the RBOH genes, and the scavenging pathway that is constituted by antioxidant enzymes (Choudhury et al. 2017). The ROS formation may be induced by various stress factors, as well as ABA, while ROS levels would affect the ABA biosynthesis and catabolism (Ishibashi et al. 2015; Suriyasak et al. 2017). Thus, the “cross-effect” of ROS and ABA levels play a vital role in plants’ response to environmental stress conditions (Ye et al. 2011; Liu et al. 2019; Zhao et al. 2021). In this study, almost all these OsRboh genes were upregulated...
during the heat stress process, indicating that heat stress resulted in the accumulation of ROS by inducing the transcriptional expression of OsRboh genes in rice seedlings. Among these OsRboh genes, OsRboh1, OsRboh4, OsRboh6, OsRboh8, and OsRboh9 were induced by ABA priming and heat stress, which indicated that ABA-induced the expression of OsRboh genes to increasing ROS levels in the regulation of plant growth and response to stress factors (Li et al. 2021). Nevertheless, OsRboh2, OsRboh3, OsRboh5, and OsRboh7 was suppressed by ABA pretreatment under heat stress (Fig. 4), which may demonstrate another potential mechanism in the “cross-effect” of ROS and ABA in plants response to environmental stress, that was ABA priming may inhibit the expression of OsRboh2, OsRboh3, OsRboh5, and OsRboh7 to decrease ROS formation under heat stress condition (Fig. 8). In further studies, it would be interesting to gain further insights into the correlation between ROS formation and ABA levels by using the mutants or transgenic plants in ROS or ABA pathways.

Reprogramming the gene expression is an important pathway for plants to cope with multiply environmental stress conditions. Recently, several genes have been identified for enhancing heat tolerance in plants (Hoang et al. 2019; Su et al. 2019). Heat shock proteins and heat shock transcription factors are known as the vital defense mechanism for plants or animals to resist heat stress conditions and numerous studies have demonstrated that overexpression of the HSP or HSF genes contributed to improving stress tolerance in rice (Cheng et al. 2015; Liu et al. 2015). ABA has a potential regulation effect in the genetic network in plants’ response to the stresses (Liu et al. 2019). In this study, the ABA-responsive genes and ABA biosynthesis genes were superinduced by ABA pretreatment under heat stress (Fig. 7A–D), indicating the ABA signal pathway was indeed activated by ABA priming. In addition, two HSP genes, OsHSP23.7 and OsHSP17.7; and two HSF genes, OsHSF7 and OsHsfA2a were significantly upregulated by ABA-priming under heat stress (Fig. 7E–H). These data represented another important mechanism of the ABA-priming effect, indicating that ABA was involved in the multiple gene transcriptional regulatory network in plants under heat stress conditions.

As shown in Fig. 8, heat stress-induced the expression of OsRboh genes in rice seedlings, which caused accumulation of ROS in leaves and further resulted in severe membrane injury as shown by higher cell and plant death of rice seedlings. Exogenous ABA application super-induced the ABA signal, to inhibit expression of OsRboh2, OsRboh3, OsRboh5, and OsRboh7 and upregulate the antioxidant defense capability to decrease ROS accumulation in leaves for enhanced tolerance to heat stress, which achieved the same effect with antioxidant (PC). In addition, ABA application upregulated the expression of HSP and HSF-related genes for activating the activity of heat shock protein.

In summary, in this study, ABA priming super-increased the ABA signal in rice seedlings under heat stress, to greatly upregulate ROS-scavenging capability and expression of heat shock-related genes, for increasing adaptive response to heat stress.

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Author contributions XL and PJ designed the study; PJ, QC, FL, YY, XC, XZ, and HY performed the laboratory experiments; XL and PJ performed the data collection, statistical analysis, and figure mapping; XL and PJ wrote the manuscript; XL, CJ, and ZL participated in the modification of the manuscript; XL provided scientific expertise.

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Declarations Conflict of interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of the manuscript.

References

An Y, Zhou P, Liang J (2014) Effect of exogenous application of abscisic acid on membrane stability, osmotic adjustment, photosynthesis and hormonal status of two lucerne (Medicago sativa L.) genotypes under high temperature stress and drought stress. Crop Pasture Sci 65:274–286. https://doi.org/10.1071/CP13162

Aranega-Bou P, Leyva MDLO, Finiti I, García-Augustín P, González-Bosch C (2014) Priming of plant resistance by natural compounds. Hexanoic acid as a model. Front Plant Sci 5:488. https://doi.org/10.3389/fpls.2014.00488

Beckers GJ, Conrath U (2007) Priming for stress resistance: from the lab to the field. Curr Opin Plant Biol 10:425–431. https://doi.org/10.1016/j.pbi.2007.06.002

Brennan T, Frenkel C (1977) Involvement of hydrogen peroxide in the regulation of senescence in pea. Plant Physiol 59:411–416. https://doi.org/10.1104/pp.59.3.411
Chen Q, Zhou Y, Liu Z, Zhang L, Song G, Guo Z, Wang W, Qu X, Zhu Y, Yang D (2015) An alternatively spliced heat shock transcription factor, OsHSA2dL, functions in the heat stress-induced unfolded protein response in rice. Plant Biol 17:419–429. https://doi.org/10.1111/plb.12267

Chen JL, Tang L, Shi P, Yang BH, Sun T, Cao WX, Zhu Y (2017) Effects of short-term high temperature on grain quality and starch granules of rice (Oryza sativa L.) at post-anthesis stage. Protoplasma 254:935–943. https://doi.org/10.1007/s00709-016-1002-y

Choudhury FK, Rivero RM, Blumwald E, Mittler R (2017) Reactive oxygen species, abiotic stress and stress combination. Plant J 90:856–867. https://doi.org/10.1111/tpj.13299

Dar NA, Amin I, Wani W, Wani SA, Shikari AB, Wani SH, Masoodi KZ (2017) Abscisic acid: A key regulator of abiotic stress tolerance in plants. Gene Plant 11:106–111. https://doi.org/10.1016/j.plgente.2017.07.003

Das S, Krishnan P, Nayak M, Ramakrishnan B (2014) High temperature stress effects on pollens of rice (Oryza sativa L.) genotypes. Environ Exp Bot 101:36–46. https://doi.org/10.1016/j.envexpbot.2014.01.004

Dwivedi SK, Basu S, Kumar S, Kumar A, Jha S, Mishra JS, Bhatt BP, Kumar G (2019) Enhanced antioxidant enzyme activities in developing anther contributes to heat stress alleviation and sustains grain yield in wheat. Funct Plant Biol 46:1090. https://doi.org/10.1071/FP19016

Elstner EF, Heupel A (1976) Inhibition of nitrite formation from hydroxylamine by manganese. Anal Biochem 70:616–620. https://doi.org/10.1016/0003-2697(76)90488-7

Essemine J, Xiao Y, Qu M, Mi H, Zhu XG (2017) Cyclic electron flow may provide some protection against PSI photoinhibition in rice (Oryza sativa L.) leaves under heat stress. J Plant Physiol 211:138–146. https://doi.org/10.1016/j.jplph.2017.01.007

Fan MH, Sun X, Xu NJ, Liao Z, Li YH, Wang JX, Fan YP, Cui DL, Li P, Miao ZL (2017) Integration of deep transcriptome and proteome analyses of salicylic acid regulation high temperature stress in Ulva prolifera. Sci Rep 7:11502. https://doi.org/10.1038/s41598-017-11449-w

Fu GF, Feng BH, Zhang CX, Yang YJ, Yang XQ, Chen TT, Zhao X, Zhang XF, Jin QY, Tao LX (2016) Heat stress is more damaging to superior spikelets than inferiors of rice (Oryza sativa L.) due to their different organ temperatures. Front Plant Sci 7:1637. https://doi.org/10.3389/fpls.2016.01637

Guan QJ, Liao X, He ML, Li XF, Wang ZY, Ma HY, Yu S, Liu SK (2017) Tolerance analysis of chloroplast OsCuZn-SOD over-expressing rice under NaCl and NaHCO3 stress. PLoS ONE 12:e0186052. https://doi.org/10.1371/journal.pone.0186052

Gurmani AR, Bano A, Khan SU, Din J, Zhang JL (2011) Alleviation of salt stress by seed treatment with abscisic acid (ABA), 6-benzylaminopurine (BA) and chlormequat chloride (CCC) optimizes yield of salt stressed Oryza sativa L. Aust J Crop Sci 5:1278–1285. https://doi.org/10.1111/j.1432-2277.1996.tb01647.x

Heath RL, Packer L (1968) Photo-oxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125:189–198. https://doi.org/10.1016/0003-9861(68)90654-1

Hoang TV, Vo KT, Rahman MM, Choi SH, Jeon JS (2019) Heat stress transcription factor OsSPL7 plays a critical role in reactive oxygen species balance and stress responses in rice. Plant Sci 289:110273. https://doi.org/10.1016/j.plantsci.2019.110273

Hu XL, Liu RX, Li YH, Wang W, Tai FJ, Xue RL, Li CH (2010) Heat shock protein 70 regulates the abscisic acid-induced antioxidant response of maize to combined drought and heat stress. Plant Growth Regul 60:225–235. https://doi.org/10.1007/s10725-009-9436-2

Huang J, Zhang FM, Xue Y, Lin J (2017) Recent changes of rice heat stress in Jiangxi province, southeast China. Int J Biometeorol 61:623–633. https://doi.org/10.1007/s00484-016-1239-3

Ishibashi Y, Kasa S, Sakamoto M, Aoki N, Kai K, Yuasa T, Hanada A, Yamaguchi S, Marl I (2015) A role for reactive oxygen species produced by NDPH oxidases in the embryo and aleurone cells in barley seed germination. PLoS ONE 10:e0143173. https://doi.org/10.1371/journal.pone.0143173

Islam MR, Feng BH, Chen TT, Fu WM, Zhang CX, Tao LX, Fu GF (2018) Abscisic acid prevents pollen abortion under high-temperature stress by mediating sugar metabolism in rice spikelets. Physiol Plant 165:644–663. https://doi.org/10.1111/plp.12759

Janni M, Gulli M, Maestri E, Marmiroli M, Valliyodan B, Nguyen HT, Marmiroli N (2020) Molecular and genetic bases of heat stress responses in crop plants and breeding for increased resilience and productivity. J Exp Bot 71:3780–3802. https://doi.org/10.1093/jxb/eraa034

Jiang MY, Zhang JH (2001) Effect of abscisic acid on active oxygen species, Antioxidative defense system and oxidative damage in leaves of maize seedlings. Plant Cell Physiol 42:1265–1273. https://doi.org/10.1093/pcp/pec162

Kerchev P, De Smet B, Waszczak C, Messens J, Van Breusegem F (2015) Redox strategies for crop improvement. Antioxid Redox Sign 23:1186–1205. https://doi.org/10.1089/ars.2014.6033

Kilasi NL, Singh J, Vallejos CE, Ye C, Jagadish SK, Kusolwa P, Rathinasabapathi B (2018) Heat stress tolerance in rice (Oryza sativa L.): identification of quantitative trait loci and candidate genes for seedling growth under heat stress. Front Plant Sci 9:1578. https://doi.org/10.3389/fpls.2018.01578

Lang H, He YT, Li FC, Ma DR, Sun J (2021) Integrative hormone and transcriptome analysis underline the role of abscisic acid in seed shattering of weedy rice. Plant Growth Regul 94:261–273. https://doi.org/10.1007/s12257-021-00714-8

Larkindale J, Hall JD, Knight MR, Vierling E (2005) Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiol 138:882–897. https://doi.org/10.1104/pp.105.062257

Li H, Liu SS, Yi CY, Wang F, Zhou J, Xia XJ, Shi K, Zhou YH, Yu JQ (2014) Hydrogen peroxide mediates abscisic acid-induced HSP70 accumulation and heat tolerance in grafted cucumber plants. Plant Cell Environ 37:2768–2780. https://doi.org/10.1111/pce.12360

Li QY, Zhang CX, Zhang GH, Fu WM, Feng BH, Chen TT, Peng SB, Tao LX, Fu GF (2020) Abscisic acid negatively modulates heat tolerance in rolled leaf rice by increasing leaf temperature and regulating energy homeostasis. Rice 13:846–853. https://doi.org/10.1186/s12284-020-00379-3

Li N, Euring D, Cha JY, Lin Z, Lu MZ, Huang LJ, Kim WY (2021) Plant hormone-mediated regulation of heat tolerance in response to global climate change. Front Plant Sci 11:627969. https://doi.org/10.3389/fpls.2020.627969

Liu JG, Qin QL, Zhen Z, Peng RH, Xiong AS, Chen JM, Yao QH (2009) OsHsf7 gene in rice, Oryza sativa L. encodes a transcription factor that functions as a high temperature receptive and responsive factor. BMB Rep 42:16–21. https://doi.org/10.5489/bmbrrep.2009.42.1.016

Liu J, Zhou X, Xing D (2012) Phosphatidylinositol 3-Kinase plays a vital role in regulation of rice seed vigor via altering NDPH oxidase activity. PLoS ONE 7:e33817. https://doi.org/10.1371/journal.pone.0033817

Liu JZ, Feng LL, Li JM, He ZH (2015) Genetic and epigenetic control of plant heat responses. Front Plant Sci 6:267. https://doi.org/10.3389/fpls.2015.00267

Liu J, Zhang C, Wei C, Liu X, Wang M, Yu F, Xie Q, Tu J (2016) The RING finger ubiquitin E3 ligase OsHTAS enhances heat tolerance...
by promoting H₂O₂-induced stomatal closure in rice. Plant Physiol 170:429–443. https://doi.org/10.1104/pp.15.00879
Liu JP, Sun XJ, Xu FY, Zhang YJ, Zhang Q, Miao R, Zhang JH, Liang JS, Xu WF (2018) Suppression of OsMDHA enhancing heat tolerance by mediating H₂O₂-induced stomatal closure in rice plants. Rice 11:38. https://doi.org/10.1186/s12288-018-0230-5
Liu XL, Zhang H, Jin YY, Wang MM, Yang HY, Ma HY, Jiang CJ, Liang ZW (2019) Abscisic acid primes rice seedlings for enhanced tolerance to alkaline stress by upregulating antioxidant defense and stress tolerance-related genes. Plant Soil 438:39–55. https://doi.org/10.1007/s11104-019-03992-4
Liu XL, Xu C, Ji P, Li Q, Zhu M, Zhang ZA, Ling FL, Wang HJ (2020) Lower nitrogen levels improve growth and some physiological traits of rice (Oryza sativa) under salt stress during reproductive period. Int J Agric Biol 24:769–776. https://doi.org/10.17957/ijab.15.1499
Livak JK, Schmittgen TD (2001) Analysis of relative gene expression using real-time quantitative PCR and the 2–ΔΔCT method. Methods 25:402–408. https://doi.org/10.1006/meth.2001.1262
Lyman NB, Jagadish KS, Nalley LL, Dixon BL, Siebenmorgen T (2016) ABA is required for plant tolerance in wheat under high temperature stress. Genes 10:174. doi.org/10.3390/genes10020174
Suzuki N, Bassil E, Hamilton JS et al (2016) ABA is required for plant acclimation to a combination of salt and heat stress. PLoS ONE 11:e0147625. https://doi.org/10.1371/journal.pone.0147625
Tantau H, Dörflling K (1991) In vitro-selection of hydroxyproline resistant cell lines of wheat (Triticum aestivum): accumulation of proline, decrease in osmotic potential, and increase in frost tolerance. Physiol Plant 82:243–248. https://doi.org/10.1111/j.1399-3054.1991.tb00888.x
Vishwakarma K, Upadhyay N, Kumar N, Yadav G, Singh J, Mishra RK, Kumar V, Verma R, Upadhyay RG, Pandey M, Sharma S (2017) Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. Front Plant Sci 8:161. https://doi.org/10.3389/fpls.2017.00161
Wang GJ, Miao W, Wang JY, Ma DR, Li JQ, Chen WF (2013) Effects of exogenous abscisic acid on antioxidant system in weedy and cultivated rice with different chilling sensitivity under chilling stress. J Agron Crop Sci 199:200–208. https://doi.org/10.1111/jac.12004
Wang HT, Bian MD, Yang ZM, Lin CT, Shi WL (2013) Preliminary functional analysis of the isoforms of OsHsfA2a (Oryza sativa L.) generated by alternative splicing. Plant Mol Biol Rep 31:38–46. https://doi.org/10.1007/s11105-012-0471-1
Wang YL, Wang L, Zhou JX et al (2019) Research progress on heat stress of rice at flowering stage. Rice Sci 26:1–10. https://doi.org/10.1016/j.rsci.2018.06.009
Wei H, Liu JP, Wang Y, Huang NR, Zhang XB, Wang LC, Zhang JW, Tu JM, Zhong XH (2012) A dominant major locus in chromosome 9 of rice (Oryza sativa L.) confers tolerance to 48°C high temperature at seedling stage. J Hered 104:287–294. https://doi.org/10.1093/jhered/ess103
Wei LX, Lv BS, Wang MM, Ma HY, Yang HY, Liu XL, Jiang CJ, Liang ZW (2015) Priming effect of abscisic acid on alkaline stress tolerance in rice (Oryza sativa L.) seedlings. Plant Physiol Biochem 90:50–57. https://doi.org/10.1016/j.plaphy.2015.03.002
Wei LX, Lv BS, Li XW et al (2017) Priming of rice (Oryza sativa L.) seedlings with abscisic acid enhances seedling survival, plant growth, and grain yield in saline-alkaline paddy fields. Field Crops Res 203:86–93. https://doi.org/10.1016/j.fcr.2016.12.024
Wellburn A, Lichtenthaler H (1984) Formulæ and program to determine total carotenoids and chlorophylls A and B of leaf extracts in different solvents. Adv Photosynth Res 2:9–12. https://doi.org/10.1007/978-94-017-6368-4_3
Xu Y, Ramanathan V, Victor DG (2018) Global warming will happen faster than we think. Nature 564:30–32. https://doi.org/10.1038/s41586-018-07586-5
Xu YF, Chu CC, Yao SG (2021) The impact of high-temperature stress on rice: challenges and solutions. Crop J 9:963–976. https://doi.org/10.1007/s11105-012-0471-1
Yang WL, Huang FD, Cao ZZ, Lei BT, Hu DW, Cheng FM (2013) Effects of high temperature stress on PSII function and its relation to D1 protein in chloroplast thylakoid in rice flag leaves. Acta Agron Sin 39:1060–1068. https://doi.org/10.3724/SP.J.1001.2013.01060
Yang JY, Huo ZG, Wang PJ, Wu DR (2020) Dynamic identification of double-early rice heat and its spatiotemporal characteristics in Jiangxi Province, China. Chin J Appl Eco 31:199–207. https://doi.org/10.1093/pcp/pcr028
Zhang CX, Fu GF, Yang XQ, Yang YJ, Zhao X, Chen TT, Zhang XF, Jin QY, Tao LX (2016) Heat stress effects are stronger on spikelets than on flag leaves in rice due to differences in dissipation
capacity. J Agron Crop Sci 202:394–408. https://doi.org/10.1111/jac.12138
Zhang H, Liu XL, Zhang RX, Yuan HY, Wang MM, Yang HY, Ma HY, Liu D, Jiang CJ, Liang ZW (2017) Root damage under alkaline stress is associated with reactive oxygen species accumulation in rice (Oryza sativa L.). Front Plant Sci 8:1580. https://doi.org/10.3389/fpls.2017.01580
Zhang CX, Feng BH, Chen TT, Fu WM, Li HB, Li GY, Jin QY, Tao LX, Fu GF (2018) Heat stress-reduced kernel weight in rice at anthesis is associated with impaired source-sink relationship and sugars allocation. Environ Exp Bot 155:718–733. https://doi.org/10.1016/j.envexpbot.2018.08.021
Zhang X, Wang X, Zhuang L, Gao Y, Huang B (2019) Abscisic acid mediation of drought priming-enhanced heat tolerance in tall fescue (Festuca arundinacea) and Arabidopsis. Physiol Plant 167:488–501. https://doi.org/10.1111/plp.12975
Zhao C, Liu B, Piao S et al (2017a) Temperature increase reduces global yields of major crops in four independent estimates. Proc Natl Acad Sci U S A 114:9326–9331. https://doi.org/10.1073/pnas.1701762114
Zhao Q, Zhou LJ, Liu JC, Du XX, Asad MAU, Huang FD, Pan G, Cheng FM (2017b) Relationship of ROS accumulation and superoxide dismutase isozymes in developing anther with floret fertility of rice under heat stress. Plant Physiol Biochem 122:90–101. https://doi.org/10.1016/j.plaphy.2017.11.009
Zhao YH, Wang QW, Zhang YF, Zhang PP, Jiang MY (2021) BIP130 enhances salt tolerance through modulation of ABA synthesis and scavenging ROS in rice (Oryza sativa L.). Plant Growth Regul 93:163–173. https://doi.org/10.1007/s10725-020-00676-3
Zhu GH, Ye NH, Zhang JH (2009) Glucose-induced delay of seed germination in rice is mediated by the suppression of ABA catabolism rather than an enhancement of ABA biosynthesis. Plant Cell Physiol 50:644–651. https://doi.org/10.1093/pcp/pcp022
Zou J, Liu CF, Liu AL, Zou D, Chen XB (2012) Overexpression of OsHsp17.0 and OsHsp23.7 enhances drought and salt tolerance in rice. J Plant Physiol 169:628–635. https://doi.org/10.1016/j.jplph.2011.12.014

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