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RESEARCH ARTICLE

Moderate soil drying mitigates the impairment of high temperature-induced spikelet opening by enhancing jasmonate accumulation in the lodicules of photo-thermosensitive male-sterile rice

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Highlights

- Moderate soil drying (MD) mitigates high temperature (HT) stress on spikelet opening in photo-thermosensitive genetic male-sterile (PTGMS) rice better than the conventional well-watered (WW) method.
- The MD regime maintains osmotic and redox homeostasis in lodicules of PTGMS rice under HT stress.
- Jasmonates mediate the effectiveness of the MD regime in alleviating HT stress on spikelet opening of PTGMS rice.

Abstract

This study investigated the role of jasmonates (JAs) in mitigating the impairment of high temperature (HT) stress-induced spikelet opening in photo-thermosensitive genetic male-sterile (PTGMS) rice under controlled moderate soil drying (MD). Two PTGMS rice varieties were grown under normal temperature (NT) and HT conditions, paired with either well-watered (WW) or MD strategies during anthesis, in both controlled-climate pot and open-air field conditions over multiple years. The MD treatment demonstrated significant protective effects compared to the conventional WW regime under HT stress, which significantly reduced the levels of JAs in lodicules and worsened spikelet opening impairment and hybrid seed yield loss. The MD regime enhanced the accumulation of JAs in lodicules, effectively alleviating HT-induced spikelet opening impairment and hybrid seed yield reduction. This protective mechanism operates through multiple pathways: (1) promoting starch hydrolysis into soluble sugars, (2) upregulating the expression of aquaporin genes, and (3) enhancing antioxidant capacity, thereby maintaining cellular osmotic and redox homeostasis in the lodicules. The crucial role of JAs in this mechanism was confirmed using JA-deficient mutants, transgenic rice lines with varying JA biosynthetic capacities, and exogenous applications of JAs. These findings indicate that MD is a more effective cultivation strategy than traditional WW in protecting PTGMS rice from HT stress, which is achieved by modulating levels of JAs to maintain osmotic and redox homeostasis in the lodicules, thus improving spikelet opening and hybrid seed yield under HT stress during anthesis.

Keywords: high temperature, moderate soil drying, jasmonates, spikelet opening, photo-thermosensitive genetic male-sterile (PTGMS), rice

1. Introduction

Climate change is exacerbating the frequency, intensity, and duration of extreme high temperature (HT) events, which are becoming an increasingly significant threat to global crop production, particularly in rice (Zhao *et al.* 2017; Lesk *et al.* 2022; Long *et al.* 2024). The “two-line method” of hybrid rice

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breeding, which involves crossing a photo-thermosensitive genetic male-sterile (PTGMS) line with its restorer line, has been pivotal in boosting global rice yields (Mou 2016; Qian *et al.* 2016). This technique surpasses the "three-line method" in several ways, including stronger hybrid vigor, the absence of a maintainer line, and a more streamlined breeding process, making it a fundamental component of modern rice farming (Mou 2016; Qian *et al.* 2016). However, PTGMS rice is particularly vulnerable to temperature fluctuations, which lead to substantial reductions in hybrid seed yield when exposed to HT stress during the anthesis stage (Yu *et al.* 2017; Zhao *et al.* 2017; Zhang *et al*. 2026).

Extended periods of HT frequently overlap with the temperature-sensitive growth phases of rice, often spanning multiple days or weeks, thereby compromising global food security (Liu *et al.* 2023). In traditional male-fertile rice, anthesis under normal temperatures typically involves lemma and palea tip separation, anther filament elongation, anther dehiscence to release pollen, and pollen dispersal and germination on the stigma (Wang 2015). However, HT stress during anthesis often causes pollen infertility, leading to reduced seed formation and grain yield in traditional male-fertile rice (Wang *et al.* 2019; Jiang *et al.* 2020; Yao *et al.* 2024). In contrast, PTGMS rice lines show total male infertility when the temperature climbs above 23°C or 24°C (Mou 2016; Zhao *et al.* 2017). The primary factor contributing to the lower seed yield in "two-line method" hybrid rice under HT stress is the poor or reduced opening rate of spikelets in PTGMS rice lines (Mou 2016; Yang *et al.* 2020).

The opening of rice spikelets is typically triggered by the expansion of lodicules within the lemma (Wang 2015; Balcerowicz 2024; Gou *et al.* 2024). This expansion is primarily due to starch degradation into soluble sugars by amylase enzymes and the influx of water and nutrients from neighboring tissues (Wang 2015; Yang *et al.* 2020). Our earlier research revealed that under HT stress, the soluble sugar content in the lodicules of PTGMS rice lines was significantly lower than that under lower temperatures, which was strongly correlated with the reduced spikelet opening rate (Yang *et al.* 2020). This suggests that the reduced soluble sugar levels in the lodicules under HT stress hinder the spikelet-opening process. Aquaporins, membrane channels that regulate water movement, play a significant role in influencing the water homeostasis in cells and tissues of plants by modulating water flux (Wang *et al.* 2021; Sun *et al.* 2024). Alternatively, one study has shown that the excessive accumulation of reactive oxygen species (ROS) caused by HT stress leads to an imbalance in the redox homeostasis in lodicule cells, subsequently causing difficulties in spikelet opening in PTGMS rice (Yang *et al.* 2020). However, the specific mechanism by which HT stress disrupts the redox and water homeostasis in lodicules, leading to spikelet opening difficulties in PTGMS rice, remains unclear.

To combat the adverse effects of HT stress on rice production, effective water management is typically seen as the primary solution (Guo *et al.* 2021). Traditionally, well-watered (WW) irrigation characterized by continuous flooding or maintaining high water levels in rice fields has been widely used to counteract the impacts of HT stress. This approach is thought to reduce the intensity of HT extremes by increasing moisture levels, which helps to cool the environment around the rice plants and mitigate HT stress (Guo *et al.* 2021). However, the effectiveness of traditional WW irrigation in reducing HT stress is debatable, and its implementation is often challenging due to the high costs of controlled irrigation systems, which many farmers cannot afford. Moreover, extreme HT events often coincide with periods of low rainfall in rice-growing regions, leading to severe water shortages (Shi *et al.* 2023). Recent studies have shown that controlled moderate soil drying (MD) can more effectively increase rice grain yields under normal temperature conditions (Zhang *et al.* 2022, 2024; Bo *et al.* 2024). Therefore, it is crucial to investigate the potential of implementing a controlled MD regime as an alternative to traditional WW irrigation for mitigating the impact of HT stress on spikelet opening in PTGMS rice.

Jasmonates (JAs), including jasmonic acid (JA) and methyl jasmonate (MeJA), are vital phytohormones that play critical roles in plant responses to biotic and abiotic stresses, as well as in regulating plant growth and development processes such as floret development, filament elongation, and lodicule swelling and senescence (Wasternack and Hause 2013; Cai *et al*. 2014; Yuan and Zhang 2015; Goossens *et al.* 2016; Guo *et al.* 2022; Song *et al.* 2022; Waadt *et al.* 2022; Muzaffar *et al.* 2024). Studies have documented that deficiencies in JAs impede lodicule expansion by reducing the accumulation of osmotic regulatory substances and water, resulting in varied floret opening times in rice (Liu *et al.* 2017; Ding *et al.* 2024; Wang *et al.* 2024). Furthermore, JAs have been found to positively modulate HT stress tolerance by activating the JA signaling pathway in plants (Tian *et al.* 2020; Yang *et al.* 2020, 2021; Li *et al.* 2023; Guo *et al.* 2024). In contrast, some studies report reduced JA levels and the downregulation of JA biosynthetic genes in rice under HT stress conditions (Zhu *et al.* 2021; Yao *et al.* 2024). However, limited information exists on the potential role and mechanism of JAs in mediating the impact of controlled MD irrigation on spikelet opening in PTGMS rice lines, particularly when subjected to HT stress during anthesis.

This study aimed to validate the hypothesis that a controlled MD regime can more effectively mitigate HT stress on spikelet opening by stabilizing the osmotic and redox homeostasis in lodicules of PTGMS rice lines compared to the conventional WW regime. Temporal patterns of multiple traits, including levels of JAs, α-amylase activity, starch and soluble sugar contents, antioxidant capacity, hydrogen peroxide (H_2O_2) content, key aquaporin gene expression levels, and osmolality level in the lodicules, root activity, plant water status, and leaf photosynthesis of PTGMS rice, were analyzed to decipher the underlying biological mechanisms. In addition, JA-deficient mutants, JA-overproducing lines, and exogenous JA and MeJA applications were employed to further validate and elucidate the specific mechanisms by which JAs regulate this process.

2. Materials and methods

2.1. Plant materials and growth conditions

This study was conducted at an experimental farm of

Yangzhou University, located in Jiangsu Province, China (32°30′N, 119°25′E), and spanned the rice-growing seasons from May to October in 2021 and 2022. It involved two inbred *indica* PTGMS rice lines, Quan 211S and Xin'an S, as well as an inbred *indica* restorer line, Yangdao 6 (YD-6, also known as 9311), a traditional rice line used as a regular pollen donor. Seeds of Quan 211S and Xin'an S were sown on May 12, while YD-6 seeds were sown on May 7, 12, 17, and 22 to align their flowering times with those of the PTGMS lines. After germination, the seedlings were initially grown in a paddy field. Once they reached 28-d-old, they were transplanted into porcelain pots measuring 25 cm in diameter, 30 cm in height, and holding a volume of 14.7 L. Each pot contained three hills, with two seedlings per hill. The porcelain pots were filled with 13 kg of sandy loam soil sourced from the surface tillage layer of the local paddy field. The day before transplanting, a base fertilizer containing 0.8 g of N (from urea) and 0.5 g of KH_2PO_4 was added to each pot and mixed into the soil. In addition, 0.4 g of N (from urea) was applied to each pot during three critical growth stages: mid-tillering (MT), panicle initiation (PI), and pistil and stamen formation (PSF). All PTGMS rice lines were cultivated in pots with a water level maintained at 3 to 4 cm and under open-air conditions, except during the anthesis periods when specific temperature and irrigation treatments were applied.

2.2. Temperature and irrigation treatments

Once 10% of the panicles emerged from the flag leaf sheaths, the two pot-grown PTGMS rice lines were relocated to four intelligent phytotrons equipped with an intelligent control system for meteorological conditions. These phytotrons were used to administer specific temperature and irrigation regimes. The NT and HT settings were calibrated to follow the typical daily temperature variations of the local climate, as outlined in Table 1. In addition, the intelligent phytotrons were set to match all other critical meteorological parameters of a standard rice-growing season to ensure that the PTGMS rice lines could successfully complete their life cycle. The six-day temperature treatment ended when 95% of the panicles had fully emerged from the flag leaf sheaths, marking the completion of the anthesis phase.

For each temperature treatment described above, two different irrigation techniques were employed concurrently: the conventional WW method and the controlled MD method. In the WW method, pots were manually filled with tap water to maintain a water layer of 3 to 4 cm, following standard agricultural procedures, up until 7 days before the final harvest. Conversely, the MD method kept the soil water potential (SWP) at a depth of 15 to 20 cm within the range of –10 to –15 kPa. To accomplish this, each pot was outfitted with a tensiometer that included a sensor to measure the SWP at the specified depth. Tensiometer readings were documented every 2 h, covering the period from 06:00 a.m. to 18:00 p.m. When the SWP reached the pre-set value, each MD pot received an additional 350 mL of tap water. After the temperature treatment, the MD pots were transitioned to the traditional WW method, with a 3 to 4 cm water layer maintained until 7 d before the final harvest. Both the WW- and MD-treated rice plants were transferred to open-air conditions until they reached the maturity stage and were harvested. Each treatment included 66 replicate pots. Within each treatment group, the pistils of PTGMS rice from six pots were hand-pollinated with pollen from the restorer line (YD-6) grown in open conditions to assess the fertilization rate, grain yield, and other yield-related traits of the hybrid rice. When extreme outdoor temperatures exceeded 39°C after the temperature treatment in the artificial climate chamber, the rice plants were kept in the chamber under normal conditions (control temperature in Table 1) until the external HT subsided, then they were moved outdoors to ensure that the effectiveness of the temperature and irrigation treatments remained uncompromised.

Table 1 Settings for the temperature treatments at different time periods during a day in the intelligent climate chambers[1)]

Treatment period (h)	NT (°C)	HT (°C)	LD (μmol m^{-2} s^{-1})
06:01–08:00	26	30	800
08:01–10:00	29	35	1,000
10:01–14:00	32	39	1,000
14:01–16:00	30	35	1,000
16:01–18:00	28	32	800
18:01–20:00	26	30	100
20:01–06:00	24	26	0

[1)] NT, normal temperature; HT, high temperature; LD, light density.

2.3. Sampling

During the treatment period, opening spikelets (where the angle between the lemma and palea was ≥10°) were collected from 14 pots of each PTGMS rice line on the 2nd, 4th, and 6th d, designated as DA-2, DA-4, and DA-6, respectively. After collection, the lodicules were carefully extracted from each opening spikelet using fine-tipped tweezers, and this operation was performed on the surface of a box filled with ice. Simultaneously, the roots of those rice plants were carefully and meticulously extracted intact from the soil in the pots. Portions of the freshly collected roots and lodicules were immediately used to measure root physiological activity and osmolality, respectively. Sufficient remaining samples were quickly immersed in liquid nitrogen for 5 min and then stored at –80°C for future assessments.

2.4. Measurements

Observation of spikelet opening During anthesis, when the spikelets began to open, the opened spikelets were identified and labeled. The number of opened spikelets and total number of spikelets (including both opened and unopened) on the tagged panicles were then recorded. A spikelet was considered open if the angle between the lemma and the palea was at least 10°. The spikelet opening rate was calculated as the percentage of open spikelets relative to the total number of spikelets per pot. The spikelet opening rates of the two PTGMS rice varieties under various temperature and irrigation conditions were measured on DA-2, DA-4, and DA-6.

Extraction and quantification of JAs in lodicules Endogenous JAs (JA and MeJA) in lodicules were extracted

and quantified using a modified method from Liu *et al.* (2010). Fresh lodicules (15–20 mg, 40–50 pairs) were frozen in liquid nitrogen and ground. The powder was mixed with 200–300 μL of methanol, incubated overnight at 4°C, and centrifuged at 4,800×g for 10 min. The supernatant was collected, and the residue was re-extracted with 200 μL of methanol. The combined supernatant was filtered through a 0.22 μm membrane and eluted with 300 μL of 100% methanol from a Waters Sep-Pak C18 cartridge. JA and MeJA were quantified using an LC-MS/MS system (Thermo Fisher Scientific, USA). The contents of JAs in the lodicules of two PTGMS rice varieties were measured under various temperature and irrigation conditions on DA-2, DA-4, and DA-6.

Determination of redox homeostasis in lodicules Total antioxidant capacity (T-AOC) indicates the ability to quickly and effectively clear ROS. Lipid peroxidation was assessed by measuring malondialdehyde (MDA), and protein oxidative damage was evaluated by analyzing protein carbonyl groups (PCG). The levels of T-AOC, MDA, and PCG in lodicules were determined using a commercial kit (Comin Biotechnology Co., Ltd., Suzhou, China). The H_2O_2 content in lodicules was quantified according to the method described by Brennan and Frenkel (1977). These parameters in the lodicules of the two PTGMS rice varieties were measured under the various temperature and irrigation conditions on DA-2, DA-4, and DA-6.

Determination of starch and soluble sugar contents, and α-amylase activity in lodicules Soluble sugar and starch levels in the lodicules were quantified following the protocol outlined by Fu *et al.* (2011). The α-amylase (endo-amylase) activity in the lodicules was evaluated using the techniques described by McCleary and Sheehan (1987). Under different temperature and irrigation conditions, the starch and soluble sugar contents, along with the α-amylase activity, were assessed in the lodicules of the two PTGMS rice varieties on days DA-2, DA-4, and DA-6.

Determination of osmolality and photosynthesis Osmolality in the lodicules and photosynthesis performance (including leaf photosynthetic rate, leaf transpiration rate, and leaf stomatal conductance) of the two PTGMS rice varieties were documented during the late morning (10:30 a.m.–12:00 p.m.) on DA-2, DA-4, and DA-6 using the Vapro 5520 osmometer (ICT, Wescor, USA) and a LI-COR 6400 system (LI-COR, Lincoln, NE, USA), respectively, in all treatment groups.

Determination of root physiological activity The root active absorption area of fresh root samples was quantified using the methylene blue staining technique, as described by Zhang (1998). Root oxidation activity was assessed by measuring the oxidation rate of alpha-naphthylamine (α-NA), following the protocol outlined by Ramasamy *et al.* (1997). Xylem sap was collected from the roots overnight, from 19:00 p.m. to 07:00 a.m., according to the methods detailed in our earlier publication (Zhang *et al.* 2022).

qRT-PCR analysis Expression levels of aquaporin genes (*OsPIP1;1*, *OsPIP1;2*, *OsTIP1;1*, and *OsTIP2;1*) in the lodicules of the two PTGMS rice varieties were measured under various temperature and irrigation conditions on DA-2, DA-4, and DA-6. This procedure entailed RNA extraction, cDNA synthesis, and qRT-PCR, following the methods outlined by Miao *et al.* (2020), with *OsActin* serving as the internal control gene. Details of the primers used for each gene, including their specifications, are provided in Appendix A.

Final harvest The hybrid seeds generated by crossing the PTGMS rice lines (Quan 211S and Xin'an S) with the restorer line (YD-6) were collected at maturity from three pots, each containing a fully representative plant per treatment. These samples were used to assess the spikelet fertilization rate and yield-related parameters. The spikelet fertilization rate is defined as the percentage of fertilized spikelets (comprising unfilled grains and fully filled grains with a specific gravity of ≥1.0 g cm^{-3}) to the total spikelets (sterile grains (i.e., empty grains), unfilled grains, and fully filled grains) per pot. In contrast, the seed-setting rate is defined as the percentage of fully filled grains to the total spikelets per pot. The seeds from three extra pots per treatment were harvested to evaluate the hybrid seed yield.

Exogenous JA and MeJA application This study evaluated the effects of JA and MeJA on two PTGMS rice lines, Xin'an S and Quan 211S, grown in pots under the NT and HT treatments in well-watered conditions, as described above. On the first day of temperature treatment implementation, between 9:30 and 10:00 a.m., 100 μmol L^{-1} JA and 100 μmol L^{-1} MeJA were applied to fully emerged panicles using a paintbrush. Partially emerged panicles were carefully injected with the solution using a 10 mL syringe. Both solutions contained ethanol at 0.05% (v/v). Control plants were treated with deionized water containing the same ethanol concentration. Based on preliminary experiments, the concentration and timing of exogenous JA and MeJA applications were determined as the spikelet opening effect in PTGMS rice was optimal with a single spraying under the mentioned conditions.

Spikelets undergoing anthesis were collected 2 d after treatment, and the lodicules were carefully stripped from the spikelets using tweezers and placed into centrifuge tubes filled with liquid N. Samples from each treatment were stored at –80°C and used to determine JAs (JA and MeJA) and other physiological traits in the lodicules. In addition, the spikelet opening characteristics and hybrid seed yield formation of the two PTGMS lines (Xin'an S and Quan 211S) were comprehensively assessed under each experimental treatment.

2.5. Field experiment in open-air conditions

One PTGMS rice line, Quan 211S, was used in an open-field trial conducted from May to October in 2023, spanning the entire rice-growing season. Pre-germinated Quan 211S seedlings were initially planted in a seedbed within the paddy field. Then when they reached 28-d-old, they were transplanted into the main field at a hill spacing of 0.25 m by 0.16 m, with each hill containing two seedlings. Before transplanting, the soil was enriched with a basal fertilizer consisting of 96 kg ha^{-1} N from urea, 30 kg ha^{-1} P from calcium superphosphate, and 40 kg ha^{-1} K from potassium chloride, applied 1 d in advance. In addition, supplemental N at 48 kg ha^{-1} from urea was added during the MT, PI, and PSF stages. Irrigation was maintained to keep the

water levels between 3 and 4 cm throughout the growing period, except for drainage periods immediately after the end of tillering and 1 wk before harvest, following standard agricultural practices.

The experiment featured four treatment scenarios: normal ambient temperature with well-watered conditions (NT-WW), normal ambient temperature with moderate soil drying (NT-MD), high temperature with well-irrigated conditions (HT-WW), and high temperature with moderate soil drying (HT-MD). In the open-field setting, Quan 211S was exposed to HT conditions from dawn (05:30 a.m.) to dusk (18:30 p.m.), while NT conditions served as the control. These treatments were applied from the onset of heading to the end of anthesis, lasting 6 d as previously specified, and were repeated three times. The infrared heating system, measuring 3 m×1.5 m, included five independently adjustable infrared lamps (1,000 W, 59 cm×20 cm) positioned 1.5 m above the rice plants and capable of height adjustment. In the NT plots, a non-functional heater of the same dimensions was used to mimic the shading effect of the infrared lamps. To ensure the independence of the NT and HT zones, they were separated by approximately 10 m.

In conjunction with each temperature modification, two irrigation methods were used: standard WW, where plots were maintained with a consistent water depth of 7–10 cm; and MD, where plots were kept at a SWP of –10 to –15 kPa at a depth of 15–20 cm. The SWP was monitored using the aforementioned tensiometers, and tap water was added as required to maintain the necessary water potential levels. The infrared lamp heating system, or a similar model, was set up in a fully isolated area measuring 4.5 m×5.0 m. A mechanized, movable steel frame canopy was prepared to protect the field from potential rain, although no precipitation was recorded during the experiment. Following the treatments, all plots that experienced temperature and irrigation changes were maintained at a water depth of 3–4 cm until 7 d before the harvest, adhering to standard agricultural practices. The air temperature within the canopy for Quan 211S was monitored in real time during the experiment. On the 5th d after the temperature and irrigation treatments, the levels of JA content, α-amylase activity, starch, soluble sugar content, aquaporin gene expression, and the T-AOC, H_2O_2, MDA, and PCG contents were quantified in the lodicules. Mature hybrids resulting from the cross of Quan 211S with 9311 were harvested from a 2 m^2 area of plants per treatment to evaluate the spikelet fertilization rates and hybrid seed yields. The methods for these evaluations are detailed above.

2.6. Creation and cultivation of genetically modified rice lines exhibiting various JA biosynthesis capacities

In this study, transgenic rice lines with altered levels of JA biosynthesis were employed to evaluate the impact of JAs on the spikelet opening capacity in PTGMS rice. The CRISPR/Cas9 Genome Editing System (provided by Biogle Genetech Co., Ltd., Jiangsu, China) was used to knockout the *OsAOS1* gene in the *japonica* rice cultivar Zhonghua 11 (ZH11), resulting in the generation of two JA-deficient mutants (*osaos1-KO#1* and *osaos1-KO#2*). To generate *OsAOS1*-overexpressing (OE) transgenic plants, we amplified the coding region of *OsAOS1* using wild-type ZH11 as the template and cloned it into the p1301UbiNOS vector driven by the maize ubiquitin promoter (Ubi) and containing the NOS terminator. Two transgenic overexpression lines (*OsAOS1-OE#1* and *OsAOS1-OE#2*) were obtained through *Agrobacterium*-mediated transformation of rice calli. Overexpression of the *OsAOS1* gene at the mRNA level in the transgenic lines was confirmed by qRT-PCR analysis.

Genetically modified rice lines with diverse JA biosynthesis capabilities were sown in the field from May to October 2024. After 28 d, the seedlings were transplanted into three randomized plots in the field, each measuring 4.5 m×5.0 m with a hill spacing of 0.25 m×0.16 m and one seedling per hill, and grown under open-air conditions. Before transplanting, the soil was enriched with a basal fertilizer (96 kg ha^{-1} N from urea, 30 kg ha^{-1} P from calcium superphosphate, and 40 kg ha^{-1} K from potassium chloride) applied 1 d earlier. In addition, 48 kg ha^{-1} of N from urea was added during the MT, PI, and PSF stages. The field was drained at the end of tillering and 1 wk before harvest, following standard practices; otherwise, the water level was maintained at 3–4 cm. This study examined the contents of JAs and osmolality in the lodicules during anthesis, as well as spikelet opening and fertilization status, and seed yield at maturity in the genetically modified rice lines. These analyses were conducted on the 4th d after the initiation of heading, using previously described methodologies.

2.7. Statistical analysis

Variance analysis was performed using the SAS/STAT statistical software (version 9.2; SAS Institute). Means were compared using the least significant difference test at the $P<0.05$ level ($LSD_{0.05}$). Bar charts were generated using SigmaPlot 10.0, and radial bar charts were produced using Origin 2021.

The results of the pot experiments conducted in the controlled climate chamber in 2022 were consistent with those from 2021. Therefore, the main text below primarily presents the findings from the 2021 experiments, while key results from the 2022 experiments are provided in the Appendices B and C.

3. Results

3.1. Plant water and photosynthetic status, spikelet opening and fertilization, and hybrid seed yield formation

Compared to the WW regime, the MD regime enhanced the root oxidation activity and root active absorption area, regardless of temperature conditions. The xylem sap flow rate from the root and the leaf photosynthetic capacity (including leaf photosynthetic rate, leaf transpiration rate, and leaf stomatal conductance) were only slightly suppressed in the MD scenario compared to the WW scenario under NT settings. However, under HT stress, these parameters were significantly higher in the MD scenario for both PTGMS rice lines. In the same irrigation treatment, these root-related and leaf-related parameters showed marked reductions at HT

compared to NT (Appendices D and E).

The HT stress caused marked declines in the spikelet opening rate, spikelet opening angle, spikelet fertilization rate, seed setting rate, and seed yield compared to NT conditions under the same irrigation regime. However, the MD irrigation regime significantly improved the spikelet opening rate, spikelet opening angle, spikelet fertilization rate, seed setting rate, and seed yield of hybrid rice compared to the WW regime under both NT and HT conditions. Since the irrigation and temperature treatments were applied only at the heading stage of rice, they did not significantly affect the number of spikelets per pot or the 1,000-grain weight for each PTGMS rice line (Fig. 1-A–J; Appendices B and C).

3.2. JA and MeJA contents in lodicules

The HT stress during anthesis significantly reduced the levels of JA and MeJA in the lodicules of PTGMS rice lines compared to the NT treatment under the same irrigation regime. The JA and MeJA levels in the lodicules were significantly higher under the MD treatment than under the WW treatment, with consistent trends observed in the two PTGMS rice lines regardless of NT or HT conditions (Fig. 2-A–D; Appendix C).

3.3. Osmotic homeostasis in lodicules

The osmolality level in lodicules exhibited a marked enhancement under HT compared to NT, while under the same temperature conditions, the MD regime led to a reduction in osmolality when contrasted with the WW regime (Fig. 3-A and B). Conversely, the soluble sugar content and α-amylase activity in lodicules notably diminished under HT compared to NT within the same irrigation regime. Moreover, both soluble sugar content and α-amylase activity were significantly elevated in the MD regime relative to the WW regime, regardless of whether the conditions were NT or HT. The starch content in lodicules did not show significant variation between the MD and WW treatments under NT conditions; however, under HT stress, the starch content in the MD treatment was notably higher. Furthermore, the starch content in lodicules consistently and significantly decreased under HT stress compared to the NT treatment, irrespective of the irrigation regime applied (Fig. 4-A–F).

3.4. Aquaporin gene expression in lodicules

Under the WW treatment, HT stress significantly reduced the expression levels of *OsTIP1;1*, *OsTIP2;1*, *OsPIP1;1*, and *OsPIP1;2* in the lodicules of both PTGMS rice lines compared to NT conditions. Under the NT treatment, MD treatment significantly increased the expression levels of *OsPIP1;1*, *OsPIP1;2*, *OsTIP1;1*, and *OsTIP2;1* compared to the WW treatment. Under HT stress, MD treatment significantly increased the expression levels of *OsTIP1;1* and *OsTIP2;1* in the lodicules of both Xin'an S and Quan 211S, and the expression of *OsPIP1;1* in the lodicules of Quan 211S compared to the WW treatment. In Xin'an S, the expression levels of *OsPIP1;1* in the lodicules were significantly higher

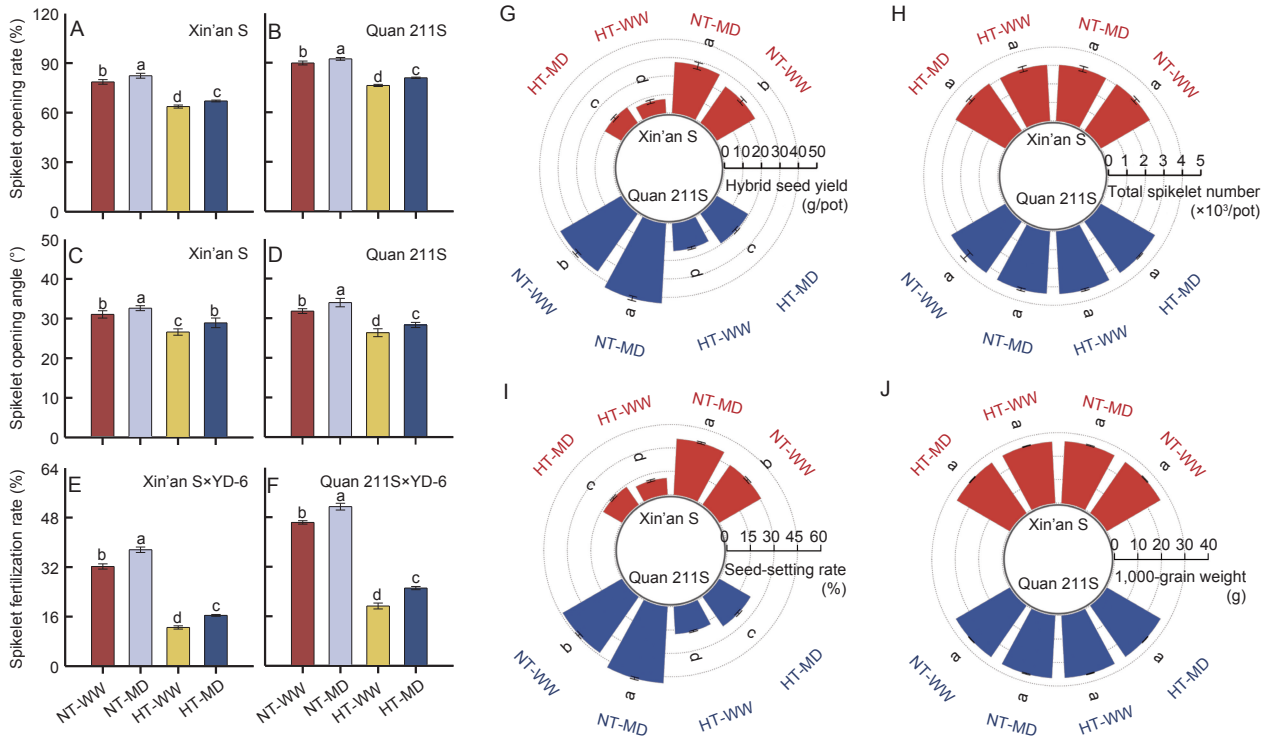


Fig. 1 Spikelet opening, fertilization, and hybrid seed yield formation of photo-thermosensitive genetic male-sterile (PTGMS) rice under multiple irrigation and temperature settings during anthesis. NT, normal temperature; HT, high temperature. WW, well-watered; MD, moderate soil drying. YD-6, Yangdao 6. Bars mean SD (*n*=3). Different lowercase letters above the error bars indicate significant differences (*P*=0.05) among treatments within the same rice line.

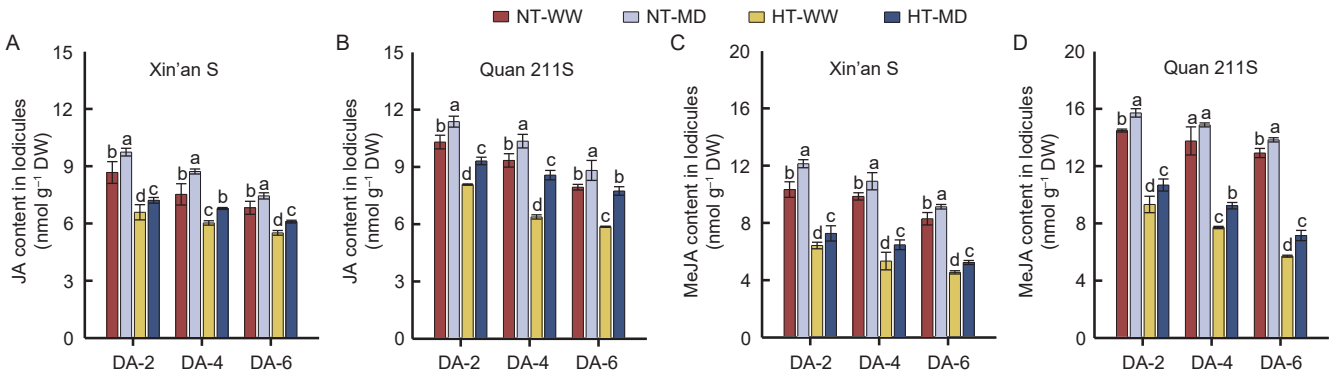


Fig. 2 Jasmonate contents in the lodicules of photo-thermosensitive genetic male-sterile (PTGMS) rice under multiple irrigation and temperature treatments during anthesis. NT, normal temperature; HT, high temperature. WW, well-watered; MD, moderate soil drying. DA-2, DA-4, and DA-6 represent the 2nd, 4th, and 6th d following treatment initiation, respectively. JA, jasmonic acid; MeJA, methyl jasmonate. Bars mean SD (n=3). Different lowercase letters above the error bars indicate significant differences (P=0.05) among treatments within the same measurement time point.

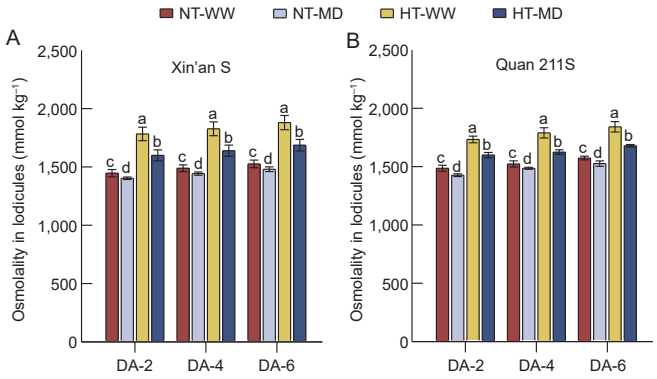


Fig. 3 Osmolality levels in the lodicules of photo-thermosensitive genetic male-sterile (PTGMS) rice under multiple irrigation and temperature treatments during anthesis. NT, normal temperature; HT, high temperature. WW, well-watered; MD, moderate soil drying. DA-2, DA-4, and DA-6 represent the 2nd, 4th, and 6th d following treatment initiation, respectively. Bars mean SD (n=3). Different lowercase letters above the error bars indicate significant differences (P=0.05) among treatments within the same measurement time point.

in the later stages of the MD treatment compared to the WW treatment. In both Xin'an S and Quan 211 S, the expression levels of *OsPIP1;2* in the lodicules were significantly increased under the MD treatment, particularly during the early stages of temperature treatments. Overall, the MD treatment significantly increased the expression levels of *OsPIP1;1*, *OsPIP1;2*, *OsTIP1;1*, and *OsTIP2;1* in the lodicules of the two PTGMS rice lines compared to the WW treatment (Fig. 5-A–H).

3.5. Redox homeostasis in lodicules

When exposed to HT stress, the T-AOC levels in the lodicules steadily decreased, whereas the levels of H_2O_2, MDA, and PCG in the lodicules of the two PTGMS rice lines were significantly elevated compared to NT conditions, regardless of whether the treatment was conventional WW or MD. Generally, the MD approach resulted in higher T-AOC levels and lower H_2O_2, MDA, and PCG levels in the lodicules of the two PTGMS rice lines compared to conventional WW, across both temperature scenarios (Fig. 6-A–H).

3.6. Effect of exogenous JA and MeJA application on spikelet opening in PTGMS rice

Compared to the CK, the application of exogenous JA or MeJA significantly increased the endogenous levels of JA and MeJA, T-AOC, the soluble sugar content, α-amylase activity, and the expression levels of aquaporin genes (*OsTIP1;1*, *OsTIP2;1*, *OsPIP1;1*, and *OsPIP1;2*), while significantly reducing osmolality, as well as the H_2O_2, MDA, and PCG levels in the lodicules. This treatment also significantly increased the spikelet opening rate, spikelet opening angle, spikelet fertilization rate, seed-setting rate, and hybrid seed yield in both PTGMS rice lines, regardless of whether they were grown under NT or HT conditions (Figs. 7-A–D and 8-A–F; Appendices F–J).

3.7. Effect of modifying the JA synthesis gene on spikelet opening in rice

Compared to the WT (ZH11), the JA-deficient rice mutants (*osaos1-KO#1* and *osaos1-KO#2*) exhibited significantly lower levels of JAs (JA and MeJA) in the lodicules, a significantly smaller spikelet opening angle, and a significantly lower spikelet opening rate. In contrast, the levels of JAs (JA and MeJA) in the lodicules, spikelet opening angle, and spikelet opening rate were significantly higher in the JA-overproducing rice lines (*OsAOS1-OE#1* and *OsAOS1-OE#2*) compared to the WT (ZH11). In addition, the osmolality level in the lodicules was significantly higher in the JA-deficient mutants and significantly lower in the JA-overproducing rice lines (Fig. 9-A–M).

3.8. Effect of controlled MD on spikelet opening in field-grown PTGMS rice under HT stress during anthesis in open-air conditions

Similar to the patterns observed in pot-grown PTGMS rice lines (Quan 211S and Xin'an S) within the phytotron, field-grown PTGMS rice (Quan 211S) under the MD regime showed significant improvements in spikelet opening angle,

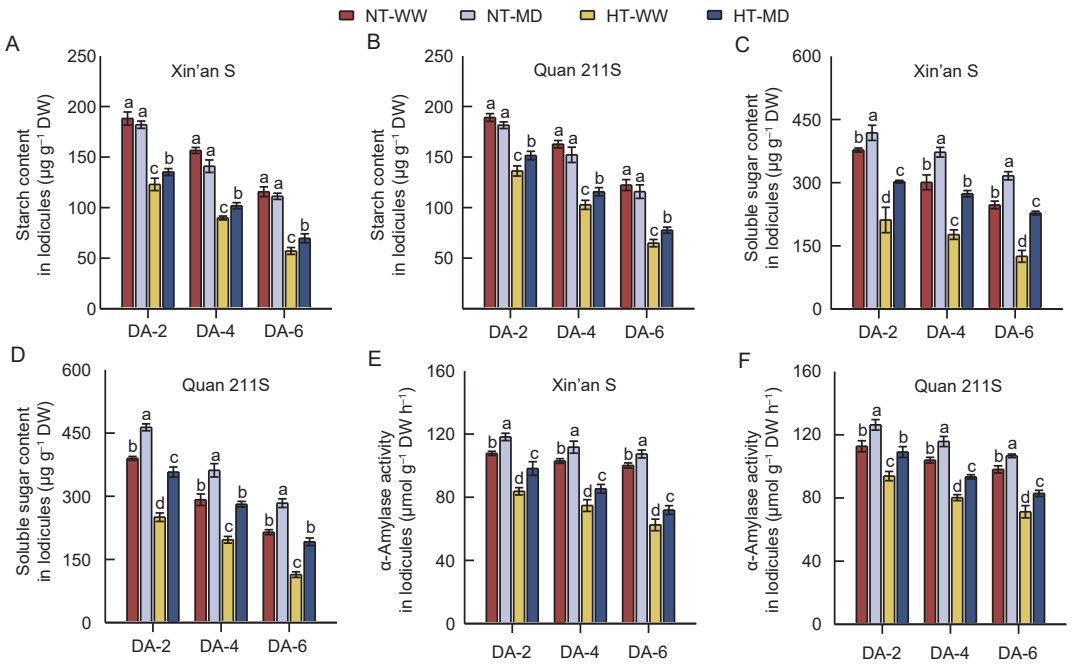


Fig. 4 Starch hydrolysis status in the lodicules of photo-thermosensitive genetic male-sterile (PTGMS) rice under multiple irrigation and temperature treatments during anthesis. NT, normal temperature; HT, high temperature. WW, well-watered; MD, moderate soil drying. DA-2, DA-4, and DA-6 represent the 2nd, 4th, and 6th d following treatment initiation, respectively. Bars mean SD (*n*=3). Different lowercase letters above the error bars indicate significant differences (*P*=0.05) among treatments within the same measurement time point.

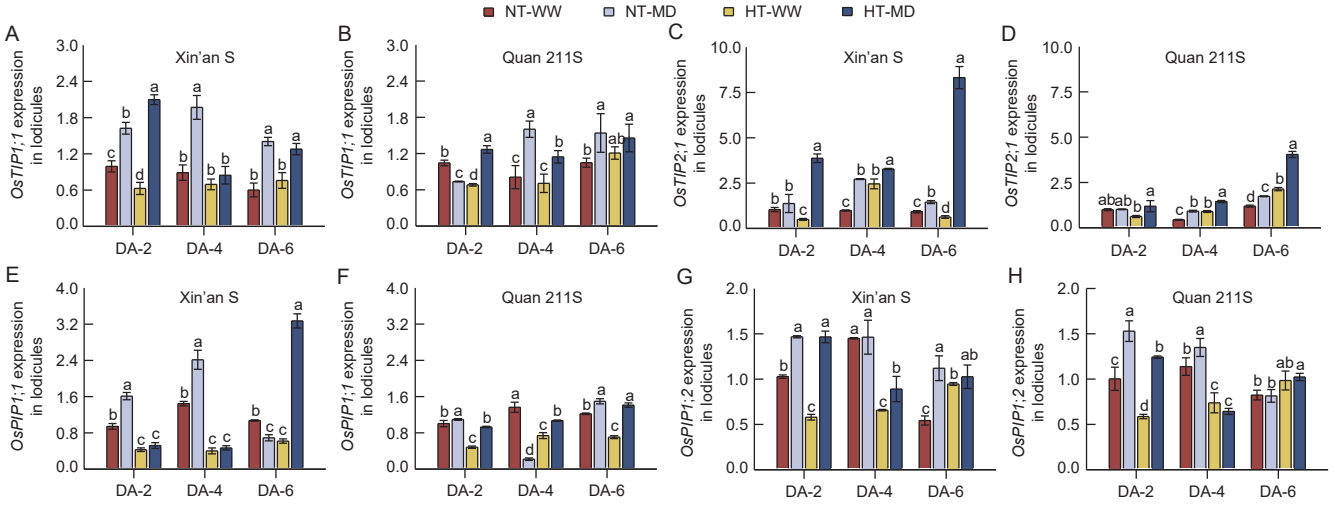


Fig. 5 Aquaporin gene expression in the lodicules of photo-thermosensitive genetic male-sterile (PTGMS) rice under multiple irrigation and temperature treatments during anthesis. NT, normal temperature; HT, high temperature. WW, well-watered; MD, moderate soil drying. DA-2, DA-4, and DA-6 represent the 2nd, 4th, and 6th d following treatment initiation, respectively. Bars mean SD (*n*=3). Different lowercase letters above the error bars indicate significant differences (*P*=0.05) among treatments within the same measurement time point.

spikelet opening rate, spikelet fertilization rate, hybrid seed yield, and the contents of JAs (JA and MeJA), as well as increased α-amylase activity and soluble sugar content in the lodicules, compared to the conventional WW regime across all temperature conditions. Overall, the positive effects of the MD regime on spikelet opening in PTGMS rice (Quan 211S) were particularly pronounced under HT stress in open-air conditions (Fig. 10-A–O).

4. Discussion

4.1. Role of the MD regime in mitigating HT stress impairment of spikelet opening in PTGMS rice

Combining HT and water-deficit stress is known to significantly worsen their negative impact on crop reproduction, leading to substantial yield losses (Lesk *et al.* 2022). To combat HT stress, the conventional WW regime is widely used in rice farming (Guo *et al.* 2021). However, our observations showed that a

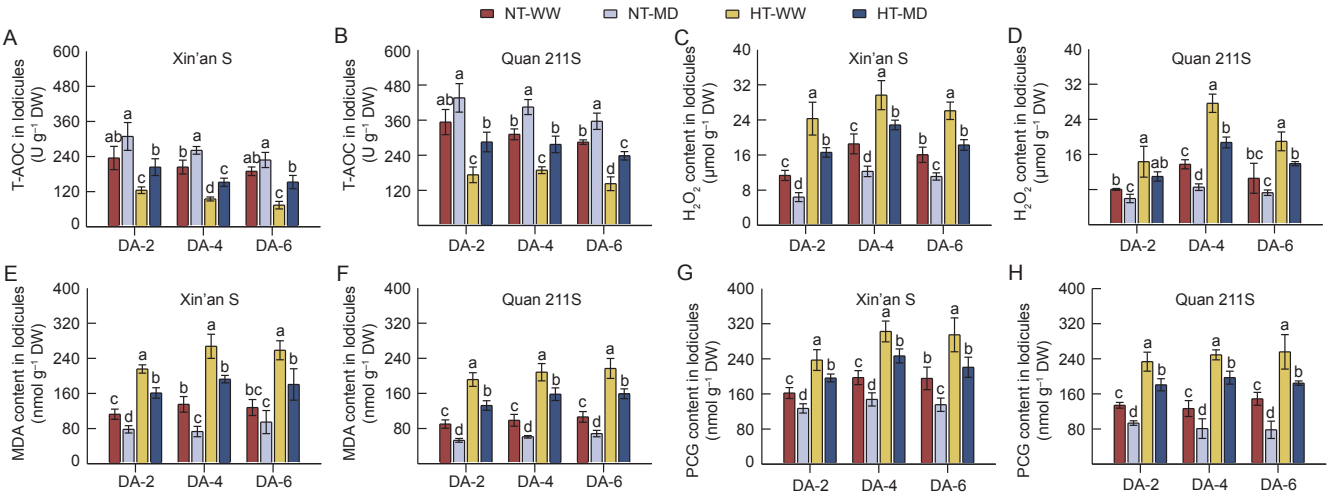


Fig. 6 Redox homeostasis status in the lodicules of photo-thermosensitive genetic male-sterile (PTGMS) rice under multiple irrigation and temperature treatments during anthesis. NT, normal temperature; HT, high temperature. WW, well-watered; MD, moderate soil drying. DA-2, DA-4, and DA-6 represent the 2nd, 4th, and 6th d following treatment initiation, respectively. T-AOC, total antioxidant capacity; H_2O_2, hydrogen peroxide; MDA, malondialdehyde; PCG, protein carbonyl groups. Bars mean SD (n=3). Different lowercase letters above the error bars indicate significant differences (P=0.05) among treatments within the same measurement time point.

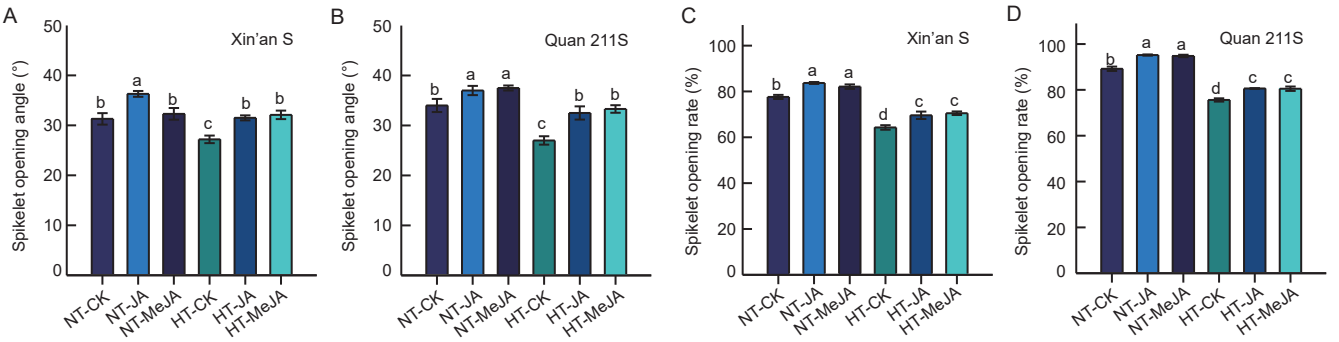


Fig. 7 Effects of exogenous jasmonates on the spikelet opening angle and rate in photo-thermosensitive genetic male-sterile (PTGMS) rice during anthesis. JA, jasmonic acid (100 μmol L^{-1} for exogenous application); MeJA, methyl jasmonate (100 μmol L^{-1} for exogenous application). NT, normal temperature; HT, high temperature. Bars mean SD (n=3). Different lowercase letters above the error bars indicate significant differences (P=0.05) among treatments within the same rice line.

controlled MD regime during anthesis in PTGMS rice effectively reduced spikelet opening issues and minimized the loss in hybrid seed yield compared to the conventional WW regime, especially under HT stress (Figs. 1 and 10; Appendices B and C). Notably, we found that rice plants maintained a consistent water status across different soil types when SWP was the same, despite variations in soil water content (Zhang *et al.* 2021). These findings suggest that a controlled MD regime with SWP above –15 kPa can serve as a universal indicator, independent of soil type, for monitoring and managing plant and soil water status and mitigating HT stress in rice. This approach is significant for enhancing rice production in the face of global warming and water scarcity, and for improving irrigation strategies to better handle HT stress.

4.2. Role of JAs in mediating the MD regime's mitigation of spikelet opening impairment by HT stress in PTGMS rice

Until now, limited information has been available on how a controlled MD can alleviate HT stress on spikelet opening in PTGMS rice lines. JAs are commonly regarded as pivotal for plant responses to a diverse array of biotic and abiotic stressors (Wasternack and Hause 2013; Goossens *et al.* 2016; Song *et al.* 2022; Waadt *et al.* 2022; Muzaffar *et al.* 2024). However, their role in regulating plant tolerance to HT stress remains a topic of debate (Clarke *et al.* 2009; Du *et al.* 2013; Kazan 2015; Yang *et al.* 2021). The results of this study showed significant reductions in the spikelet opening rate, spikelet opening angle, spikelet fertilization rate, and hybrid seed yield, along with a concurrent reduction in JAs (JA and MeJA) in the lodicules of PTGMS rice under HT stress compared to NT conditions, especially under the WW regime. Conversely, the MD regime notably enhanced the levels of JAs (JA and MeJA) in the lodicules, as well as the spikelet opening rate, spikelet opening angle, spikelet fertilization rate, and hybrid seed yield, with this positive effect being further amplified under HT stress (Figs. 1, 2, and 10; Appendices B and C). Furthermore, the spikelet opening rate, spikelet

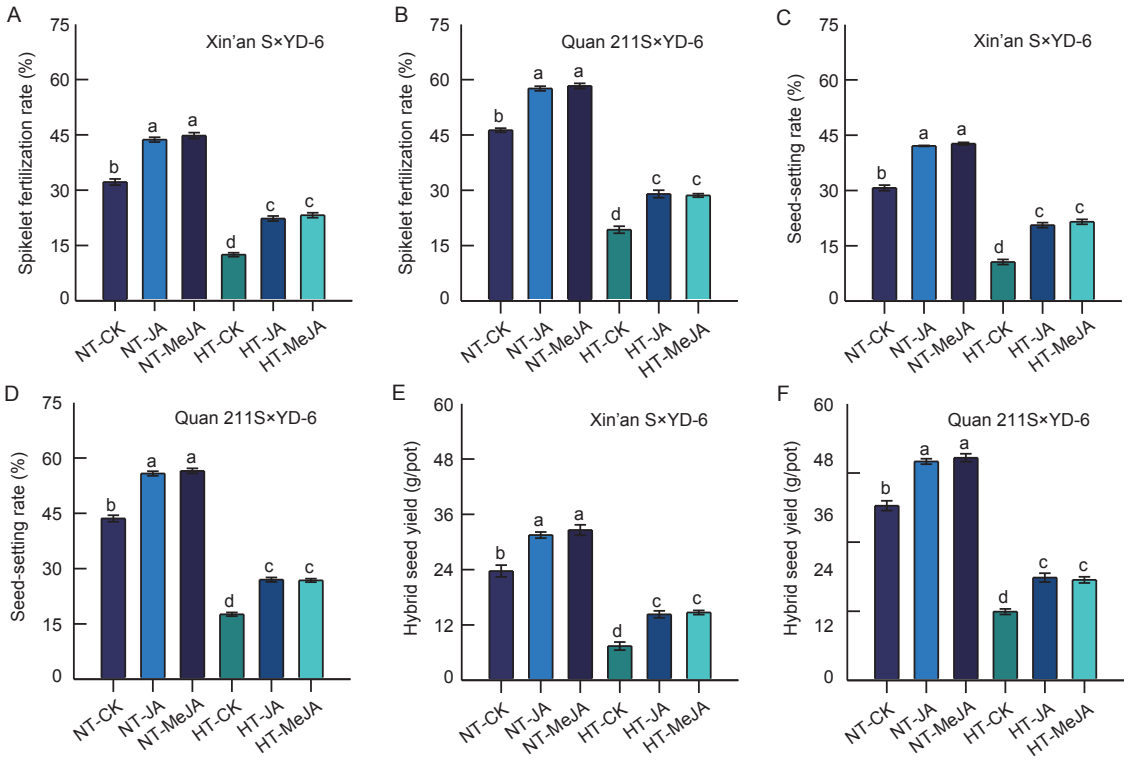


Fig. 8 Effects of exogenous jasmonates on the spikelet fertilization rate, seed-setting rate, and hybrid seed yield in photo-thermosensitive genetic male-sterile (PTGMS) rice. JA, jasmonic acid (100 μmol L^{-1} for exogenous application); MeJA, methyl jasmonate (100 μmol L^{-1} for exogenous application). NT, normal temperature; HT, high temperature. Bars mean SD (n=3). Different lowercase letters above the error bars indicate significant differences (P=0.05) among treatments within the same rice line.

opening angle, spikelet fertilization rate, and hybrid seed yield in the PTGMS rice were significantly increased when JA or MeJA was applied to the panicles under HT stress (Figs. 7 and 8). Genetic evidence further indicated that the spikelet opening rate, spikelet opening angle, spikelet fertilization rate, and seed yield were significantly reduced in JA-deficient mutants (*osaos1-KO#1* and *osaos1-KO#2*), while they were notably increased in JA-overproducing lines (*OsAOS1-OE#1* and *OsAOS1-OE#2*) compared to their WT (ZH11) (Fig. 9). These data demonstrate that JAs (JA and MeJA) are highly effective in promoting the MD method controlled alleviation of HT-induced spikelet opening difficulties in PTGMS rice.

4.3. Mechanism by which JAs mediate the MD regime's mitigation of HT stress-induced spikelet opening impairment in PTGMS rice

However, the mechanism by which JAs mediate the facilitation of spikelet opening in PTGMS rice by controlled MD under HT stress during anthesis remains unclear. Accumulating osmoregulatory compounds, primarily soluble sugars, in rice lodicules is essential for ensuring successful spikelet opening (Wang *et al.* 2015; Yang *et al.* 2020; Ding *et al.* 2024). Studies have reported that on the 1st d of relatively mild HT stress (max. 35°C) implementation, the content of JAs in the lodicules increased significantly or slightly, while on the 2nd and 3rd d, they decreased significantly; while the starch and soluble sugar contents in the lodicules only decreased greatly on the 1st and 2nd d of relatively mild HT stress implementation and recovered to NT levels on the 3rd d for most PTGMS rice lines (Yang *et al.* 2020). In our observations, under the traditional WW regime and relatively severe HT stress (max. 39°C), PTGMS rice exhibited reduced α-amylase activity, and starch and soluble sugar contents, along with a sharp decline in the levels of JAs, and a higher osmolality level in the lodicules throughout the temperature treatment period, which coincided with significant drops in the spikelet opening rate and spikelet opening angle (Figs. 1–4 and 10). The specific differences in the effects of HT stress on the contents of JAs, starch, and soluble sugar in the lodicules in various studies may be attributed to the intensity and duration of HT stress treatments and the differences in PTGMS rice lines. The specific mechanisms are worthy of further in-depth exploration.

Notably, the MD regime significantly increased the levels of JAs, α-amylase activity, starch content and soluble sugar content, while reducing the osmolality level in the lodicules and boosting the spikelet opening rate and spikelet opening angle under HT stress in PTGMS rice (Figs. 1–4). Notably, under HT stress, the starch and soluble sugar contents in the lodicules of PTGMS rice decreased significantly, while MD alleviated these HT-induced reductions. This may be mainly due to HT severely inhibiting photosynthesis, while MD effectively alleviates this inhibitory effect (Appendix E), leading to corresponding changes in the starch and soluble sugar contents in lodicules under HT stress. The application of JA or MeJA significantly elevated the α-amylase activity

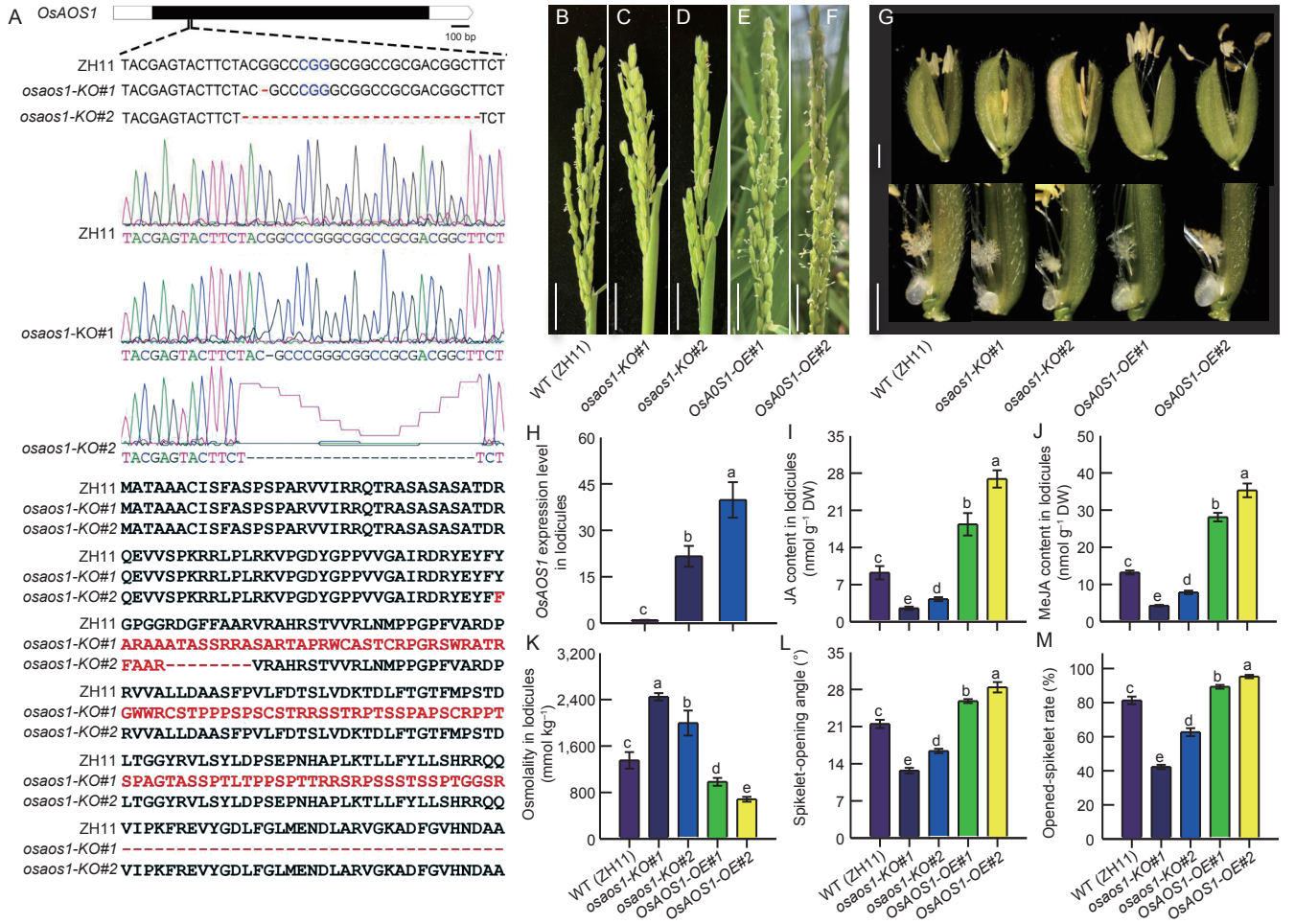


Fig. 9 Effect of modifying the jasmonates synthesis gene (*OsAOS1*) on spikelet opening in rice. WT, wild type; ZH11, Zhonghua 11; *osaos1-KO#1* and *osaos1-KO#2*, JA-deficient rice mutants; *OsAOS1-OE#1* and *OsAOS1-OE#2*, JA-overproducing rice lines. JA, jasmonic acid; MeJA, methyl jasmonate. Scale bars=2 cm in B–G. Bars in H–M mean SD (n=3). Different lowercase letters above the error bars indicate significant differences (P=0.05) among rice lines.

and soluble sugar content and reduced osmolality in the lodicules, while increasing the spikelet opening rate and spikelet opening angle under either NT or HT conditions (Figs. 7 and 8; Appendices F, G, and I). In addition, the JA-deficient mutants (*osaos1-KO#1* and *osaos1-KO#2*) exhibited reduced spikelet opening rates and spikelet opening angles, coupled with higher osmolality in the lodicules, whereas these parameters showed the opposite trends in the JA-overproducing lines (*OsAOS1-OE#1* and *OsAOS1-OE#2*), as compared to the WT (ZH11) (Fig. 9). These results suggest that controlled MD enhances spikelet opening by elevating JA-driven starch hydrolysis into soluble sugars in lodicules, thereby stabilizing the osmotic regulation-driven water homeostasis in the lodicules of PTGMS rice under HT stress.

An alternative way JAs can contribute to alleviating spikelet opening issues under HT stress in the context of controlled MD could be attributed to their involvement in modulating the cellular redox regulatory capacity and water homeostasis in the lodicules of PTGMS rice. Maintaining an optimal and stable cellular redox homeostasis is a crucial physiological foundation for plants to effectively cope with various stressors (Foyer and Hanke 2022; Mittler *et al.* 2022; Shekhawat *et al.* 2022). On the other hand, studies indicate that HT stress can suppress the expression of multiple *OsTIPs* and *OsPIPs* genes in panicles during anthesis, leading to reduced panicle water content and reduced pollen and stigma activity, which in turn results in a lower seed set and yield of rice (Hu *et al.* 2020). Other research suggests that under HT stress, the expression levels of *OsTIPs* and *OsPIPs* increase in anthers but decrease in glumes, and thermo-tolerant rice varieties exhibit higher aquaporin gene expression levels compared to thermo-sensitive varieties (Wang *et al.* 2021).

Our findings indicated that a lower T-AOC level and, in most cases, lower expression of aquaporin genes (*OsPIP1;1*, *OsPIP1;2*, *OsTIP1;1*, and *OsTIP2;1*) were concurrently associated with a sharp decline in JAs levels, a surge in oxidative damage (i.e., higher H_2O_2, MDA, and PCG) and tissue water deficit (i.e., higher osmolality) in the lodicules, as well as significant reductions in the spikelet opening rate and spikelet opening angle in PTGMS rice under the traditional WW regime when exposed to HT stress. Notably, the MD regime significantly mitigated the inhibitory effects of HT stress on the contents of JAs, T-AOC, and aquaporin gene expression levels in the lodicules. It concurrently

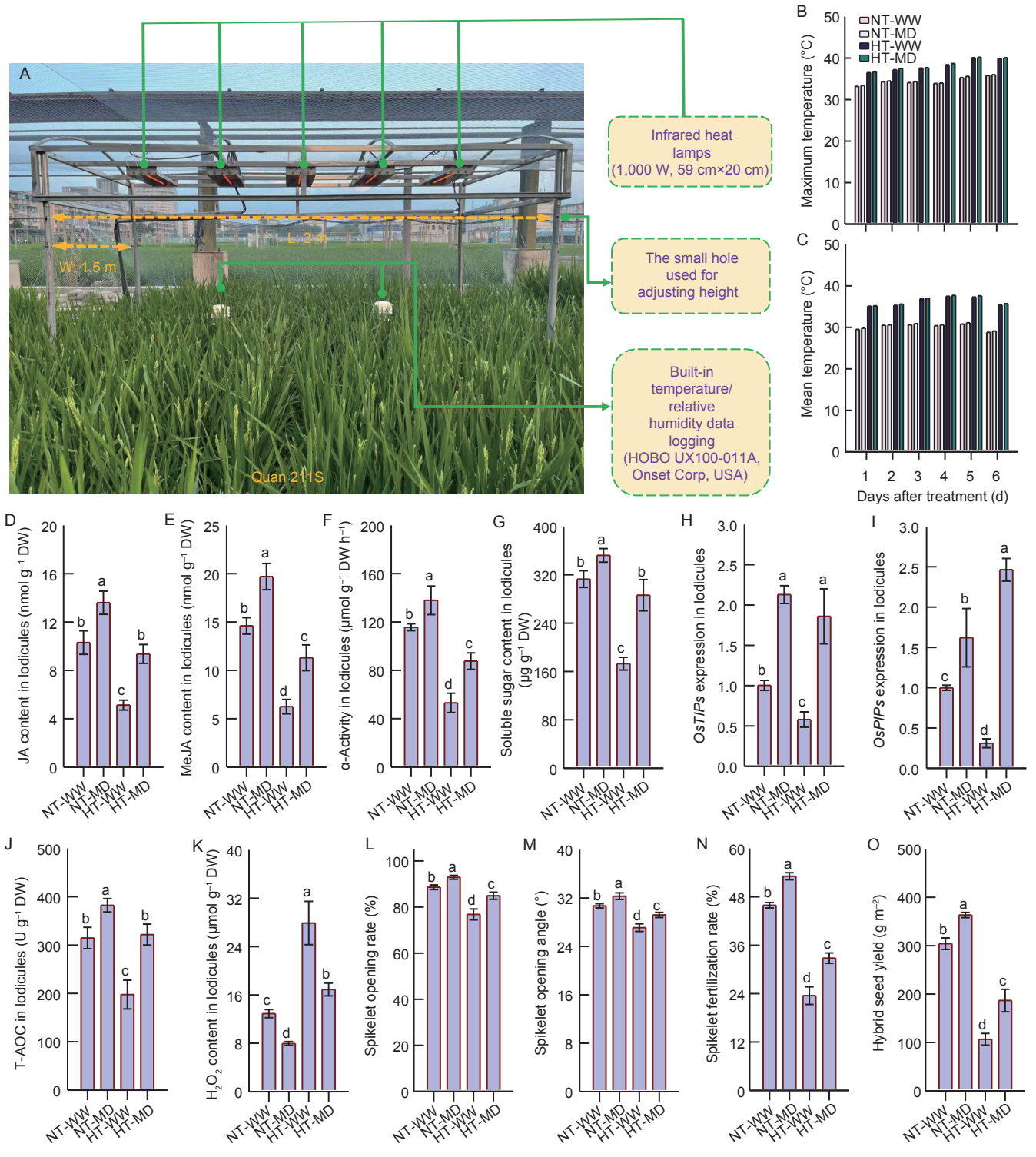


Fig. 10 Effects of various irrigation and temperature treatments on the photo-thermosensitive genetic male-sterile (PTGMS) rice Quan 211S in open-air field conditions. NT, normal ambient temperature; HT, high temperature. WW, well-watered; MD, moderate soil drying. JA, jasmonic acid; MeJA, methyl jasmonate; T-AOC, total antioxidant capacity. *OsTIPs* represents the average expression levels of *OsTIP1;1* and *OsTIP2;1*. *OsPIPs* represents the average expression levels of *OsPIP1;1* and *OsPIP1;2*. Bars mean SD (n=3). Different lowercase letters above the error bars indicate significant differences (P=0.05) among treatments.

reduced oxidative damage (i.e., lower H_2O_2, MDA, and PCG) and improved tissue water status (i.e., lower osmolality), thus preventing declines in the spikelet opening rate and spikelet opening angle of PTGMS rice (Figs. 1–3, 5, 6, and 10). Furthermore, the exogenous application of JAs further substantiated the positive roles of JAs in maintaining cellular redox homeostasis, aquaporin gene expression, and promoting spikelet opening in PTGMS rice under both NT and HT conditions (Figs. 7 and 8; Appendices F, H, I, and J). The data suggests that under HT stress, suppressed T-AOC and

aquaporin gene expression levels lead to severe oxidative damage and water imbalance in the lodicules. This results in a low spikelet opening rate and spikelet opening angle due to reduced JAs levels, especially in the traditional WW regime. Conversely, the controlled MD regime increases the levels of JAs in lodicules, enhancing T-AOC and aquaporin gene expression and maintaining optimal and stable cellular redox and water homeostasis, thereby improving spikelet opening and fertilization and boosting the hybrid seed yield in PTGMS rice under HT stress.

This study proposes that JAs, including JA and MeJA, are vital for mitigating spikelet opening issues in PTGMS rice, especially under HT stress. This is supported by the observation that Quan 211S, with higher levels of JAs in its lodicules, consistently displayed a greater spikelet opening rate, spikelet opening angle, spikelet fertilization rate, and hybrid seed yield compared to Xin'an S, regardless of irrigation or temperature conditions (Figs. 1 and 2; Appendices B and C). In addition, the application of exogenous JAs significantly increased the spikelet opening rate, spikelet opening angle, spikelet fertilization rate, and hybrid seed yield in PTGMS rice under both NT and HT stress during anthesis (Figs. 7 and 8). Therefore, the capacity to produce robust JAs, particularly under HT stress, may serve as a critical physiological indicator for developing thermo-tolerant PTGMS rice. Furthermore, applying exogenous JA or MeJA as a chemical treatment might provide an efficient approach for alleviating HT stress on spikelet opening in PTGMS rice. Notably, varying ROS levels in plant tissues are often closely associated with abscisic acid (ABA)-related events in response to drought adversity and/or heat injury (Qiu *et al.* 2021; Zhao *et al.* 2023; Sato *et al.* 2024). In this study, the osmolality (water status) in lodicules of PTGMS rice lines changed regularly with differential increases and reductions under the irrigation and/or temperature treatments. Thus, this system is worthy of further in-depth exploration to determine the role of ABA in this process in PTGMS rice, especially whether and how ABA and JAs may interact.

A well-developed and active root system can maintain normal physiological activities in plants and help them withstand adverse environmental stresses (Oldroyd and Leyser 2020; Li *et al.* 2021; Maqbool *et al.* 2022). Studies have shown that HT stress during anthesis significantly reduces root vigor in rice, with thermo-tolerant varieties exhibiting higher root vigor under HT stress compared to thermo-sensitive varieties (Cao *et al.* 2009). Moreover, waterlogging reduces soil oxygen levels, which hampers root functions and adversely affects plant growth and development (Pedersen *et al.* 2021). The data presented here revealed that HT stress significantly reduced the water acquisition ability (i.e., lower root oxidation activity and root active absorption area, and xylem sap flow rate from root) and photosynthetic capacity (i.e., lower leaf stomatal conductance, leaf transpiration rate, and leaf photosynthetic rate) of PTGMS rice under all irrigation treatments compared to the NT treatment. Importantly, the controlled MD treatment was more effective than the WW treatment in improving root water uptake under HT stress and enhancing both plant water homeostasis and photosynthetic capacity (Appendices D and E). These improvements may help JAs mitigate HT-induced spikelet opening impairment of PTGMS rice at the whole-plant level, and the relevant action mechanisms are worthy of further exploration.

5. Conclusion

The controlled MD regime emerges as an innovative irrigation strategy, offering superior protection against HT stress-induced spikelet opening impairment and hybrid seed yield loss during anthesis in PTGMS rice, compared to the traditional WW regime. The reduction in hybrid seed yield caused by spikelet opening impairment under HT stress is predominantly attributed to reduced JAs levels in the lodicules. In contrast, the MD regime effectively mitigates spikelet opening impairment and associated hybrid seed yield losses by enhancing the accumulation of JAs in the lodicules of PTGMS rice. The protective role of JAs under the MD regime is primarily mediated through the maintenance of cellular redox homeostasis and water homeostasis in the lodicules of PTGMS rice under HT stress during anthesis (Fig. 11).

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used generative AI tools to assist in improving the clarity and readability of the language. All content generated was carefully reviewed and revised by the authors as necessary, and the authors take full responsibility for the final content of the article.

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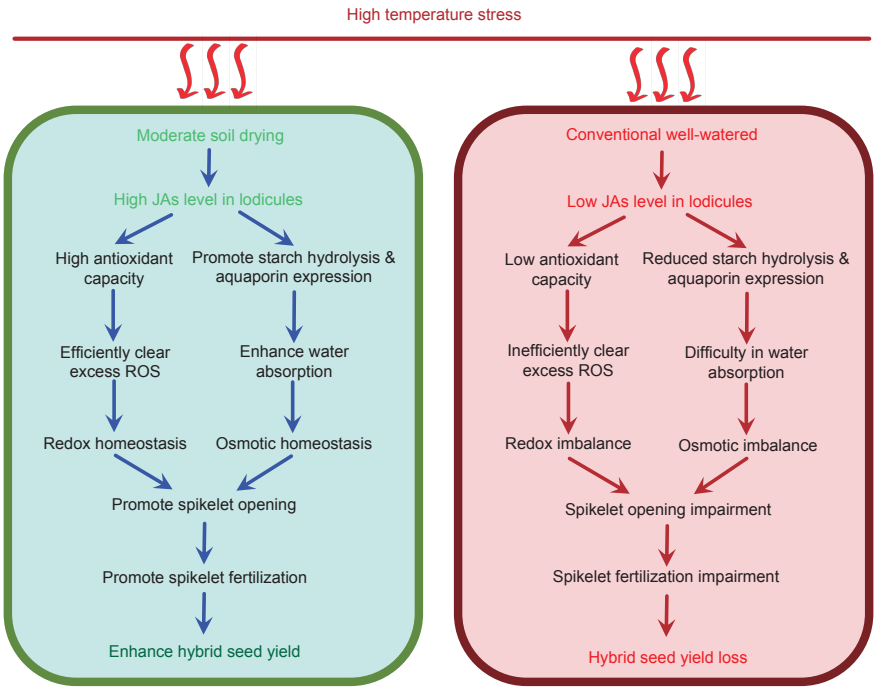


Fig. 11 Proposed model for the jasmonate response to moderate soil drying and conventional well-watered regimes in affecting the impairment of spikelet opening in photo-thermosensitive genetic male-sterile (PTGMS) rice under high temperature stress during anthesis. JA, jasmonic acid; ROS, reactive oxygen species.

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