Effects of Enhanced Ultraviolet-B (UV-B) Radiation and Antioxidative-type Plant Growth Regulators on Rice (Oryza sativa L.) Leaf Photosynthetic Rate, Photochemistry and Physiology

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Abstract
Elevated UV-B radiation deleteriously affects rice yields. The impacts of plant growth regulator (PGRs; α-tocopherol, glycine betaine [GB] and salicylic acid [SA]) applications on higher plants have been the subject of many studies. However, little or no work has been carried out on rice responses to α-tocopherol, GB or SA under UV-B stress conditions. This study determined the effects of α-tocopherol (2.3 kg ha⁻¹), GB (2.0 kg ha⁻¹) or SA (12.9 g ha⁻¹) application on rice leaf photosynthetic rate (PN), photochemistry and physiology under ambient and elevated UV-B conditions. Elevated UV-B decreased PN (17%), quantum yield (8%), electron transport rate (9%), total chlorophyll concentration (8%), plant height (12%), number of leaves (17%), pollen viability (6%), phenolic concentration (46%) and yield (21%). The applications of α-tocopherol, GB or SA increased yield by 23%, 18% and 29%, respectively, under elevated UV-B. Application of PGRs increased leaf phenolic content thus rendering protection against elevated UV-B.

Keywords: α-Tocopherol, glycine betaine, phenolic content, rice, salicylic acid, UV-B

1. Introduction
Global stratospheric ozone depletion is elevating surface ultraviolet-B (UV-B) levels (Russell et al., 1996). Increase in UV-B levels can alter crop productivity as it affects photosystem II, the electron transport systems, enzymes, pigments, nucleic acids and growth regulators (Sullivan & Teramura, 1989; Caldwell & Flint, 1994). UV-B radiation can affect plants by inhibiting photosynthesis, damaging DNA, pollen and pollen tube development, and changing accumulation of biomass and partitioning (Caldwell et al., 1998; Feng et al., 2000).

Elevated UV-B decreased leaf photosynthetic rate (PN), thereby decreasing rice yields (Dai et al., 1994; Kumagai et al., 2001; Mohammed & Tarpley, 2009a, 2010, 2011a). Elevated UV-B decreases leaf stomatal conductance (Dai et al., 1992), chlorophyll content (He et al., 1993; Huang et al., 1993), rubisco content (Ziska & Teramura, 1992), nitrogen concentration, protein content (Hidema et al., 1996), chlorophyll fluorescence, and/or altered photosynthesis-related gene expression (Strid et al., 1996a,b), thereby decreasing PN.

Apart from PN, enhanced UV-B radiation can negatively affect plant morphology and phenology (Mohammed & Tarpley, 2011a), pollen viability, pollen germination, pollen tube growth, fertilization and fruit set, thereby decreasing yield (Feng et al., 2000, Koti et al., 2005). Carotenoids can protect the photosynthetic apparatus against enhanced UV-B by quenching highly reactive singlet oxygen and dissipating excess excitation energy (Nonnengiesser et al., 1996; Rakhimberdieva et al., 2004). Phenolics in the epidermal layer also play an important role in protecting the photosynthetic apparatus against UV-B (Meijkamp et al., 1999). Hence, an increase in carotenoid and/or phenolic concentrations protects photosynthetic tissues from enhanced UV-B radiation.

Alpha-tocopherol, GB or SA application enhances plant tolerance to abiotic stresses (DeLong & Steffen, 1998; Mohammed & Tarpley, 2011b, 2011c). The UV-B radiation has been shown to increase the peroxidation of lipids in plants (Predieri et al., 1995). The α-tocopherol present in the thylakoid membrane protects the structure and function of photosynthetic membranes by scavenging active O₂⁻ species and peroxyl radicals produced as a result of stress (Fryer, 1992; Hess, 1993). In addition, exogenous application of α-tocopherol increases membrane stability under elevated UV-B (Pelle et al., 1990). Glycine betaine enhances stress tolerance by protecting enzymes (Paleg et al., 1981), photosystem II (Allakhverdiev et al., 1996), membrane integrity and increasing the antioxidant status...
of the plant (Mohammed & Tarpley, 2009b). Salicylic acid enhances resistance to biotic and abiotic stresses (Lopez-Delgado et al., 1998) by increasing antioxidant capacity and phenolic content in plants (Rao et al., 1997; Mohammed & Tarpley, 2009, 2011a; Ghasemzadeh & Jaafar, 2012).

Genetic improvement and breeding for UV-B tolerant rice cultivars can be beneficial for rice adaptation to future climate conditions. However, genetic improvement and breeding for UV-B tolerance are long-term approaches. A short and easy way to negate the negative effects of enhanced UV-B is through the use of PGRs. The use of PGRs for the prevention and/or amelioration of various environmental stresses are a viable approach to make rice production more resilient to UV-B stress. Glycine betaine, SA, vitamin E, proline and choline are some of the PGRs which can induce stress-tolerance (thermotolerance, drought tolerance, cold tolerance and/or salinity tolerance) in various crop plants (Mohammed & Tarpley, 2011b). The research presented herein addresses the effects of \( \alpha \)-tocopherol, GB or SA on rice leaf photosynthetic rate, photochemistry and physiology under UV-B conditions.

2. Material and Methods

2.1 Plant Material and Growing Conditions

Three independent experiments were laid out in complete randomized design. In each experiment there were three replications per UV-B and PGR combination. Rice inbred cultivar ‘Cocodrie’, was used in all three experiments. Plants were grown in pots (15 cm diameter x 17.5 cm height) filled with a clay-rich soil and were placed in square boxes lined with 6.0 mm thickness black plastic (FILM-GARD, Minneapolis, Minnesota, USA). Four seeds per pot were sown at a 2.5-cm depth. After emergence, plants were thinned to one plant per pot, which were maintained until maturity. The boxes were filled with water to approximately 2 cm above the top of the soil in each pot, 20 days after emergence (DAE). Nitrogen was applied at planting, 20 DAE and at the panicle-differentiation stage as described by Mohammed et al., (2007). At planting, urea-N was applied at the rate of 113.5 kg ha\(^{-1}\) along with 45.4 kg ha\(^{-1}\) of phosphorus (P\(_2\)O\(_5\)). The remaining nitrogen fertilizations were applied at the rate of 79.5 kg ha\(^{-1}\) of nitrogen in the form of ammonium sulfate at 20 DAE and at the panicle-differentiation stage. Mean day temperature and humidity in the greenhouse were monitored using standalone sensor/loggers (HOBOs, Onset Computer Corporation, Bourne, Massachusetts, USA). The greenhouse temperature and absolute humidity ranged between 27-35 \(^\circ\)C and 14-16 g/m\(^3\), respectively. The light intensity and CO\(_2\) in the greenhouse during the day were measured using a light quantum meter (Quantum Meter, Apogee Instruments, Logan, Utah, USA) and LI-6400 (LI-6400, LI-COR Inc., Lincoln, Nebraska, USA), respectively. The light intensity in the greenhouse ranged between 600-800 \(\mu\)mol m\(^{-2}\) s\(^{-1}\).

2.2 UV-B Radiation Treatments

In all three experiments, UV-B radiation from fluorescent sun-lamps were delivered to plants for nine hours from 0800 to 1700 h by UV-313 lamps (Q-Panel Company, Cleveland, Ohio, USA) driven by 40 W dimming ballasts in a square wave fashion. The lamps were wrapped with cellulose diacetate film (solarized 0.07 mm, JCS Industries Inc., La Mirada, California, USA) to filter out radiations below 280 nm. The cellulose diacetate on the lamps was changed at regular intervals to account for the degradation of the cellulose diacetate properties. The lamps were arranged on the aluminum frame to provide a uniform UV-B radiation over the canopy. Four UV-B lamps were used to supply the required dosage. The UV-B energy delivered at the top of the canopy was monitored daily with a UV meter (UVM, Apogee Instruments Inc. Utah, USA). Plants were exposed to UV-B dose of 5 (ambient) or 10 (enhanced) kJ m\(^{-2}\) d\(^{-1}\), 20 DAE.

2.3 Plant Growth Regulator (PGR) Treatments

The PGRs, \( \alpha \)-tocopherol (2.3 kg a.i. ha\(^{-1}\)), GB (2.0 kg ha\(^{-1}\)), and SA (12.9 g ha\(^{-1}\)) were applied at the rate of 300 \(\mu\)L per plant at boot stage of rice plant using a pre-calibrated perfume-bottle sprayer. The PGRs were dissolved in de-ionized water with 0.5% (v/v) surfactant (Latron AG-98 spreader activator, Rohm and Haas Company, Philadelphia, Pennsylvania, USA). The \( \alpha \)-tocopherol and SA were purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and GB was supplied by Capstone Food Ingredients (Marion, Massachusetts, USA).

2.4 Leaf Photosynthesis

The net photosynthetic rate (\( P_N \)) of the penultimate leaves was measured using a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, Nebraska, USA), 10 days after PGR treatments (DAT). The \( P_N \) was measured between 1000 h and 1200 h. When measuring \( P_N \), the light intensity, temperature and CO\(_2\) concentration in the leaf cuvette were set to 1500 \(\mu\)mol m\(^{-2}\) s\(^{-1}\), 25\(^\circ\)C and 390 ppm (ambient CO\(_2\) concentration in the greenhouse), respectively.
2.5 Chlorophyll Fluorescence

Chlorophyll fluorescence is often used to evaluate the functionality of the photosynthetic system in chloroplast membranes under various stresses (Chen et al., 2010). Chlorophyll a fluorescence parameters, maximum quantum efficiency of photosystem-II (Fv/Fm), thylakoid membrane stability (Fo/Fm), quantum yield of PSII (Y), electron transport rate (ETR) and non-photochemical quenching (NPQ) were assessed by measuring fluorescence with a pulse-modulated fluorometer (OS5p, Opti-Sciences, Hudson, NH, USA). The minimal fluorescence (Fo), maximum fluorescence (Fm) and Fv/Fm were measured in 30 min dark-adapted leaves. For Y and ETR, plants were under a steady state of photosynthesis (plants were exposed to ambient sunlight for more than 5 hours), a prerequisite for measuring Y and ETR. A photosynthetically active radiation (PAR) clip (OS5p PAR Clip, Opti-Sciences, Hudson, NH, USA) provides the PAR measurements while measuring Y and ETR. While measuring the Y and ETR the range of PAR was 600-700 $\mu$mol m$^{-2}$ s$^{-1}$. The coefficient of non-photochemical quenching of excitation energy (NPQ) was calculated using Klughammer and Schreiber’s equations, where NPQ is $[(Fm-Fms)/Fms]$ (Klughammer & Schreiber, 2008). The leaf chlorophyll fluorescence was measured 10 DAT.

2.6 Leaf Pigments

Avoiding the mid-vein, three leaf discs (0.65 cm diameter) were obtained from mid-blade of the penultimate leaves for chlorophyll and carotenoid determination, 10 DAT. The three leaf discs were placed in a 10 mL vial with 5 mL of dimethyl sulphoxide (DMSO) and incubated for 24 h in darkness. From the 10 mL vials, 200 $\mu$L of the extract was transferred to microtiter plates of polypropylene material. The absorbance of the extract was measured using a PowerWave$_{x}$ microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) at 480, 648 and 664 nm (Chappelle et al., 1992) to calculate the carotenoid, chlorophyll a and chlorophyll b concentrations using equations described by Lichtenthaler (1987). Values for total chlorophyll were obtained by summing up the values of chlorophyll a and chlorophyll b. The pigment concentrations were expressed on a leaf area basis, $\mu$g cm$^{-2}$.

2.7 Morphology and Pollen Viability

At harvest, plant height was measured, the numbers of productive tillers and viable leaves were recorded and dry weights were determined.

Pollen viability was measured using the staining procedure from Virmani et al. (1997) with minor modifications. The 1% iodine potassium iodide (IKI) stain was prepared by dissolving 1 g iodine and 2 g potassium iodide in 100 mL de-ionized water. Pollen was dusted from the plants on the Petri dish, 4-5 mL of 1% IKI stain was applied per Petri dish, followed by incubation for 12 hours. After incubation the pollen grains were observed under a microscope. The pollen grains were classified based on their shape and the extent of staining. The viable pollen grain is round and deep red stained (Virmani et al., 1997). The total numbers of pollen grains and sterile pollen grains were counted and pollen viability was expressed as percentage.

2.8 Grain Characteristics

Grain length, width, volume, surface area and chalkiness of brown (dehulled) rice were determined using a Winseedle (Regent Instruments, Inc. Quebec, Canada), which uses image analysis of scanned color images of the grain to calculate these parameters.

2.9 Leaf Phenolic Concentration

Avoiding the mid-vein, three leaf discs (0.65 cm diameter) were obtained from mid-blade of the penultimate leaves for chlorophyll and carotenoid determination, 10 DAT. The three leaf discs were placed in a 10 mL vial with 5 mL phenolic extractant, which is a mixture of methanol, water and hydrochloric acid in 7:2:1 ratio by volume (Mirecki & Teramura, 1984) and incubated for 24 h in darkness. From the 10 mL vials, 200 $\mu$L of the extract was transferred to microtiter plates of polypropylene material. The absorbance of the extract was measured using a PowerWave$_{x}$ microplate spectrophotometer (Bio-Tek Instruments, Inc., Winooski, Vermont, USA) at 300 nm (Kakani et al., 2004), and the phenolic concentration was calculated using the equation, $C = 16.05 \times A$, where A is absorbance at 300nm and C is the phenolic concentration (g/mL of extract). The phenolic concentrations were expressed on a leaf area basis, $\mu$g cm$^{-2}$.

2.10 Data Analysis

Observations were analyzed using the Proc GLM procedure of SAS (SAS statistical analysis package version 9.2, SAS Institute, Cary, NC, USA) to test significant differences among the experiments (three repeats of an experiment), UV-B (two UV-B levels) and PGR treatments (4; untreated + 3 PGRs) for the parameters measured. Duncan’s Multiple-Range Test (alpha level of 0.05) was used to separate the means. For the parameters measured, there were no significant differences among the experiments. Hence, for a parameter measured, values from
three experiments were used to obtain the mean and standard error (n = 9). The standard errors of the means are presented in the graphs as error bars.

Figure 1. Effects of UV-B and plant growth regulators on rice leaf photosynthetic parameters. Bars with different letters for a particular parameter differed at P ≤ 0.05

3. Results

3.1 Leaf Photosynthetic Parameters

There was no difference among the experiments for leaf photosynthetic parameters. The untreated plants grown under 10 kJ UV-B showed decreased Pₙ (17%) and increased leaf transpiration (33%), compared to untreated plants grown under 5 kJ UV-B (Figure 1a, d). The α-tocopherol-treated plants grown under 5 kJ UV-B showed 12% increased Pₙ, compared to untreated plants grown under ambient UV-B (Figure 1a). In addition, α-tocopherol-, GB- and SA-treated plants grown under 10 kJ UV-B showed 27%, 10% and 10% increases in Pₙ, compared to untreated plants grown under 10 kJ UV-B (Figure 1a). The GB- and SA-treated plants grown under 10 kJ UV-B showed 13% and 20% decreases in stomatal conductance, compared to untreated plants grown under 10 kJ UV-B (Figure 1b). The α-tocopherol-treated plants grown under 10 kJ UV-B showed 3% decrease in internal CO₂ concentration, compared to untreated plants grown under 10 kJ UV-B (Figure 1c).

3.2 Chlorophyll Fluorescence

There was no difference among the experiments for chlorophyll fluorescence. In addition, there was no difference between the UV-B treatments or among the PGR treatments for Fv/Fm and Fo/Fm (Figure 2a, b). The untreated plants grown under 10 kJ UV-B showed decreased Y (8%) and ETR (9%) and increased NPQ (15%),
compared to untreated plants grown under 5 kJ UV-B (Figure 2c, d, e). The SA-treated plants showed 12% and 10% increases in Y at 5 kJ and 10 kJ UV-B, compared to untreated plants (Figure 1c). In addition, SA-treated plants grown under 10 kJ UV-B showed 14% increase in ETR, compared to untreated plants grown under 10 kJ UV-B (Figure 2d).

![Figure 2](image)

**Figure 2.** Effects of UV-B and plant growth regulators on rice leaf photochemistry. Bars with different letters for a particular parameter differed at P ≤ 0.05

### 3.3 Leaf Pigments

There was no difference among the experiments for leaf chlorophyll or carotenoid concentration. In addition, the untreated plants showed no difference between the UV-B treatments for chlorophyll a, chlorophyll b, carotenoids concentrations or chlorophyll a/b ratio (Figure 3a, b, c, d). However, there was 8% decrease in total chlorophyll concentration at 10 kJ UV-B (Figure 3e). The α-tocopherol-treated plants showed 55%, 67% and 58% and 26%, 17% and 24% increases in chlorophyll a, chlorophyll b and total chlorophyll concentrations under 5 kJ and 10 kJ UV-B, respectively.
UV-B, respectively (Figure 3a, b, e). In contrast, GB-treated plants showed 28%, 13% and 25% and 30%, 22% and 29% decreases in chlorophyll a, chlorophyll b and total chlorophyll concentrations under 5 kJ and 10 kJ UV-B (Figure 3a, b, e). The GB-treated plants grown under 5 kJ UV-B and SA-treated plants grown under 10 kJ UV-B showed 26% and 28% decreases in carotenoids, compared to untreated plants (Figure 3c).

3.4 Rice Morphology, Pollen Viability and Dry Weight

There was no difference among the experiments for rice morphology or pollen viability. In addition there was no difference among the PGR treatments with respect to rice morphology or pollen viability (Figure 4a, b, c, d). However, untreated plants grown under 10 kJ UV-B showed 12%, 17% and 6% decreases in plant height, number of viable leaves per plant and pollen viability, compared to untreated plants grown under 5 kJ UV-B (Figure 4a, c, d). The untreated plants grown under 10 kJ UV-B showed 23% increase in shoot dry weight and
21% decrease in yield, compared to untreated plants grown under 5 kJ UV-B (Figure 5a, b). The SA-treated plants grown under 5 kJ UV-B showed 22% and 17% increases in shoot dry weight and yield, compared to untreated plants grown under 5 kJ UV-B (Figure 5a, b). The \( \alpha \)-tocopherol-, GB- and SA-treated plants grown under 10 kJ UV-B showed 24%, 18% and 29% increases in yield, compared to untreated plants grown under 10 kJ UV-B (Figure 5b).

![Graph showing the effects of UV-B and plant growth regulators on rice morphology and pollen viability. Bars with different letters for a particular parameter differed at \( P \leq 0.05 \).](attachment:image.png)
Figure 5. Effects of UV-B and plant growth regulators on rice shoot dry weight and yield. Bars with different letters for a particular parameter differed at P < 0.05.

3.5 Grain Characteristics

There was no difference among the experiments for grain parameters. In addition there was no difference between the UV-B treatments or among the PGR treatments for grain volume or grain surface area (Figure 6c, d). However, untreated plants grown under 10 kJ UV-B showed 3% and 37% increases in grain width and grain chalkiness, compared to untreated plants grown under 5 kJ UV-B (Figure 6b, e). The GB-treated plants showed 2% and 3% decreases in grain length under 5 kJ and 10 kJ UV-B, compared to untreated plants (Figure 6a). The α-tocopherol- and GB-treated plants grown under 5 kJ UV-B showed 5% and 1% increases in grain width, compared to untreated plants grown under 5 kJ UV-B (Figure 6b). The α-tocopherol-, GB- and SA-treated plants grown under 10 kJ UV-B showed 2% increase and 3% and 1% decreases in grain width, respectively, compared to untreated plants grown under 10 kJ UV-B (Figure 6b). The SA-treated plants grown under 5 kJ UV-B showed 41% decrease in grain chalkiness, compared to untreated plants grown under 5 kJ UV-B (Figure 6e). The α-tocopherol-, GB- and SA-treated plants grown under 10 kJ UV-B showed 23%, 22% and 22% decreases in grain chalkiness, compared to untreated plants grown under 10 kJ UV-B (Figure 6e).
3.6 Leaf Phenolic Concentration

There was no difference among the experiments for leaf phenolic concentration. However, untreated plants grown under 10 kJ UV-B showed 46% decrease in leaf phenolic concentration, compared to untreated plants grown under 5 kJ UV-B (Figure 7). The α-tocopherol-, GB- and SA-treated plants showed 213%, 57% and 29% and 476%, 157% and 352% increases in leaf phenolic concentration under 5 kJ and 10 kJ UV-B, respectively, compared to untreated plants (Figure 7).
4. Discussion

The present study was conducted to improve our understanding of rice responses to α-tocopherol, GB and SA applications under UV-B stress conditions. Our results indicated beneficial effects of α-tocopherol, GB and SA application under UV-B stress conditions. In the present study, UV-B inhibited PN. However, this decrease in PN under enhanced UV-B was not associated with stomatal conductance or internal CO₂ concentration. In this study, PN decreased due to decreased photosynthetic quantum yield and total chlorophyll concentration. The decrease in photosynthetic quantum yield under enhanced (10 kJ) UV-B might be due to damaged D1 protein in PSII (Gao & Ma, 2008) and decrease in total chlorophyll concentration might be due to chlorophyll degradation (Kakani et al., 2003). Previous studies have reported chlorophyll degradation as a result of enhanced UV-B (Huang et al., 1993; Kakani et al., 2003). The damage to chloroplasts and changes in photosynthetic pigments result in reduction of PN (Teramura et al., 1990; Sullivan & Rozema, 1999).

In this study, enhanced UV-B decreased plant height and pollen viability. Decrease in plant height is one of the indicators of UV-B damage (Fiscus et al., 1999). Previous studies have shown decreased plant height as a result of enhanced UV-B in monocots and dicots (Tevini & Teramura, 1989; Dai et al., 1994). Decreased plant height under enhanced UV-B is due to decreased carbohydrate content (Zhao et al., 2003), damaged cell components, and interaction of growth regulators (Ensminger & Schafer, 1992). Enhanced UV-B also decreases pollen production, viability and germination (Feng et al., 2000, 2003; Koti et al., 2005), which are essential for seed/fruit set. Decreased seed/fruit production under enhance UV-B can occur due to decreased pollen production or viability (Koti et al., 2005). In this study, rice yield decreased under enhanced UV-B. Similar results were reported by previous studies with respect to yields under enhanced UV-B (Kumagai et al., 2001; Mohammed & Tarpley, 2009a, 2010, 2011a). In this study, enhanced UV-B increased grain chalkiness. Tsukaguchi and Iida (2008) stated that decreased carbon supply to the grain due to stress can lead to chalky kernels.

The epidermal layer is known to accumulate secondary metabolites, such as phenolics and flavonoids that absorb/screen UV-B and shield the underlying tissues against harmful UV-B radiation (Cen & Bornman, 1993; Olsson et al., 1998). In this study, leaf phenolic concentration decreased in plants grown under enhanced UV-B radiation. Previous studies have stated that leaf UV-B absorbing compounds, such as phenolic concentration, decreased when the plants are grown in relatively high PAR/UV-B (Wilson & Greenberg, 1993; Alexieva et al., 2001). The reduction in secondary metabolites (phenolics) might be due to reduction in photo-assimilation. Decreased photo-assimilation lowers the efficacy of the biosynthetic system to produce secondary metabolites (phenolics). Zhao et al. (2003) stated that UV-B-induced reduction of assimilate production leads to lower production of secondary metabolites.
In this study, application of α-tocopherol, GB or SA increased rice yield. The application of α-tocopherol, GB or SA increased leaf photosynthetic rate and pollen viability, thereby resulting in higher yield. Most of the abiotic stresses, including UV-B, produce reactive oxygen species (ROS). The ROS can increase lipid peroxidation, protein degradation, and DNA fragmentation leading to cell death (Farooq et al., 2008). Application of α-tocopherol, GB or SA can alter antioxidant levels in plants and detoxify superoxide radicals, thus preventing oxidative damage and protecting the membranes and enzymes (Pelle et al., 1990; Farooq et al., 2008; Mohammed & Tarpley, 2009b). Previous studies have shown that GB or SA can increase photosynthetic rate by increasing photosynthetic pigments and carboxylase activity of Rubisco (Singh & Usha, 2003; Farooq et al., 2008). In this study, application of α-tocopherol, GB or SA increased leaf phenolic content, thus rendering protection to photosynthetic apparatus.

In conclusion, enhanced UV-B negatively affected leaf photosynthetic rate, photochemistry and physiology, thereby reducing rice yield; application of α-tocopherol, GB or SA increased rice yield under UV-B stress conditions. The application of α-tocopherol, GB or SA application increased leaf photosynthetic rate, pollen viability and leaf phenolic concentration, thus increasing rice yield under UV-B stress conditions.

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