Rice Flag Leaf Physiology, Organ and Canopy Temperature in Response to Water Stress

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Abstract: Using two rice cultivars, the effect of severe, mild and no water stress, W3, W2 and W1, respectively, on flag leaf physiology, the ecological characteristics of canopy and organ temperatures were studied in 2008 and 2009. The grain yield was reduced under W3 due to decreased seed setting rate and 1000 grain weight but not under W2. Water stress had a significant effect on the flag leaf physiological characteristics along with the soluble sugar and amino proline content. Catalase and peroxidase activities, photosynthetic and transpiration rates, and stomatal conductance in W2 were significantly higher than in W3 and similar to those in W1. The organ and canopy temperatures were significantly higher in W3 than in either W1 or W2, and there was no significant difference between W1 and W2. This study clearly showed that water stress had a significant effect on leaf physiology, temperature of organs and canopy. Mild water stress (soil water potential maintained at -15- -20 kPa) could construct a population that is water-saving and resistant to heat stress. This facilitates access to a high yield as well.

Key words: Organ and canopy temperature, Physiology of flag leaf, Rice, Water stress.

Water is an important limiting factor for crops, adversely affecting their overall growth and development (Boonjung and Fukai, 1996; Kato et al., 2007), accumulation and revolution of dry material and photosynthetic characteristics (Kumar et al., 2006; Li et al., 2008; Wang et al., 2008), along with their yield and quality (Wang et al., 2006; Yang et al., 2002). However, Zheng et al. (2006) reported that water stress after heading had little effect on the yield of rice, and Wang et al. (2004) reported that moderate water stress at the heading and filling stages significantly promoted the grain filling and increased the seed setting rate and grain weight.

In rice, the response to water stress during the vegetative phase resulted in a reduced interception of photosynthetically active radiation (PAR) (Inthapan and Fukai, 1988). This reduction was due to a declined leaf expansion rate and a decrease in height, leaf area and biomass production, and tiller abortion. This in turn reduced leaf elongation, which promoted leaf death (Cutler et al., 1980; Farooq et al., 2009a; Hsiao et al., 1984; Turner et al., 1986).

The productivity of rice depends not only on the accumulation, but also the effective portioning of dry matter to the grain (Passioura, 1982). Drought stress at the reproductive stage significantly increased dry matter portioning from leaves and stems to grains (Kumar et al., 2006). Water stress reduces photosynthesis (Beck et al., 2007; Chaves, 1991), and hampers nutrient transport and disrupts membrane function (Farooq et al., 2009b). Severe drought conditions resulted in limited photosynthesis due to a decline in Rubisco activity (Bota et al., 2004). Dehydration causes cell shrinkage and a subsequent decline in cellular volume leading to viscous cellular contents. Increased concentrations of solutes may become toxic, thereby affecting the functioning of some enzymes, including those required for photosynthetic machinery (Hoekstra et al., 2001). Substantial inhibition of stomatal conductance and net photosynthesis by water stress causes metabolic limitations and oxidative damage to the chloroplast (Zhou et al., 2007). Malondialdehyde (MDA) is the product of lipid peroxidation and its content is related to the degree of lipid peroxidation and membrane damage, while catalase (CAT) and peroxidase (POD) are important enzymes for scavenging active oxygen species, thus protecting biological membranes. During drought stress, the activities of the antioxidative defense system; including superoxide dismutase (SOD), CAT and POD, are decreased; leading to increased active oxygen species. Membrane damage caused by reactive oxygen species (ROS) will produce more MDA, causing further damage (Hung et al., 2005, Shi et al., 2007).

Mackill and Coffmam (1983) and Xu et al. (1999) found a strong physiological activity at a lower panicle temperature and canopy temperature. A lower organ temperature indicated slight heat injury, and lower canopy
temperatures enhanced resistance to heat injury. Crops respond to water stress during the growth and development phase resulted in a reduced leaf area, tiller abortion, accumulation and revolution of dry material, along with their yield and quality. As an effect of water stress on crop organ temperature, the thermal image showed genotypic variation of crop organ temperature in response to drought (Jones and Leinonen, 2003; Leinonen and Jones, 2004). Heading is a stage most sensitive to soil moisture, and drought will impede the physiological activities of root, leaf photosynthesis, dry matter accumulation, and transpiration rate. The yield and quality of the rice are also affected (Cai et al., 2002; Tao et al., 2004; Wang et al., 2006). In the present study, water treatments were started within 7 days after heading. Flag leaf physiology, organ temperature and canopy temperature were determined during this period. Furthermore, we obtained infrared images of the rice canopy under each water stress treatment. Our objectives were (1) to understand the effect of water stress on the yield and the physiology of the flag leaf, (2) to investigate the effect of water stress on organ and canopy temperature, and (3) to find feasible irrigation levels to construct a population with water-saving characteristics and resistance to heat stress, and facilitate access to high yield.

Materials and Methods

1. Plant material and drought treatments

Field experiments were performed to evaluate the effect of water stress on the physiology of flag leaf, and organ and canopy temperatures using 2401 (japonica rice) and IIyou107 (indica rice) in 2008, Zhejing22 (japonica rice) and IIyou7954 (indica rice) in 2009. Experiments were conducted at the Longyou Experimental Station (119°10′E, 29°02′N), of the Zhejiang Academy of Agricultural Sciences, Hangzhou, Zhejiang Province, China.

Three levels of water stress were imposed at heading for 7 days: W1, no stress (the field had a 4 cm water layer, soil water potential was 0 kPa); W2, mild water stress (the soil surface had no water layer, but the artificial revamp water was supplied at least 3 times daily to maintain the soil water potential at -15-20 kPa); W3, severe water stress (soil water potential was maintained at -40-50 kPa). The soil water levels were measured with a soil moisture meter (6440FS, Spectrum, USA), and soil water potential at 0-20 cm below the surface of the paddy field.

Seeds were sown on 25 May 2008 and 29 May 2009. Plant spacing was 30.0 cm between rows and 13.3 cm between plants, the plot area was 24 m² (6 m × 4 m), replicated 4 times. During the growing season, except the treatment period, the plants were fully irrigated under the field condition. The weather was fine during the period of water stress, although a removable shelter was prepared to prevent the rain. We applied fertilizer consisting of 225 kg N ha⁻¹ (as carbamide), 115 kg P₂O₅ ha⁻¹ (as superphosphate), and 180 kg K₂O ha⁻¹ (as potassium chloride).

2. Measurement

(1) Air temperature, relative humidity (RH) and organs temperature measurement

The observed heading dates of the plots were 5 Sep 2008 (2401), 27 Aug 2008 (IIyou107), 6 Sep 2009 (zhejing22) and 2 Sep 2009 (IIyou7954). After heading, we obtained 10 random samples per plot of four replications. The mean surface temperature of each organ (panicle, flag leaf, second leaf and third leaf) in each plot was measured with a hand-held infrared thermometer (Foodpro, Raytek, USA) between 1300-1330. To avoid the effect of soil temperature and other environmental factors on the measurement of the organ temperatures, we held the instrument parallel to the horizontal plane, and perpendicular to the plant organ (using a long clamp to make the organ erect). The ratio of the distance to the spot size was 8:1, which is a specification of the thermometer. Measurements were carried out when the infrared rays gathered to a focus, which was when the distance between the sensor and the organ was about 10.0 cm and spot size was about 1.5 cm. Organ temperature was calculated as the average of the dorsal and the ventral surfaces. Air temperature and RH were measured at 200 mm above the canopy continuously with solid-state sensors (ZDR-20, Zeda, China and Model DHM-2, Aspiration Psychrometer, China). Values were recorded at 10 min intervals. Solar radiation and wind speed were also recorded at 10 min intervals with a data logger (Delta-T Devices, UK).

(2) Thermal image acquisition

Canopy temperature was measured at the top of the rice population. Thermal images were taken with a Thermacam camera (model P25, FLIR systems, USA) from 1200 to 1300; the view of the angle was 45° off the horizontal at a distance of 1 m from the lens to the upper leaves, and ambient conditions were 29°C, 50% RH, and wind speed 1.9 m s⁻¹ (wind speed was almost constant during measurement in different treatment plots). Canopy temperature is shown as the area-averaged temperature of all dots of a thermal image, and each value is the average of several thermal images taken at 1200-1300.

(3) The net photosynthetic rate, transpiration rate and stomatal conductance

The net photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (C) of flag leaf were measured at heading and 7 days after heading with an LI-6400 photosynthetic system (LI-COR Inc. Lincoln, NE, USA) at 1030-1130.

(4) Total soluble sugar and free proline measurement

Proline was quantified by the acid-ninhydrin procedure of Bates et al. (1973). Leaf samples (0.5 g) were ground
with 3% sulphosalicylic acid (10 mL) and clarified by centrifugation. The supernatant (2 mL) was mixed with the same volume of ninhydrin in acetic acid, the mixture was oven incubated at 100°C for 1 hr, and the reaction was finished in an ice bath. The reaction mixture was extracted with toluene (4 mL) and the absorbance was read at 517 nm, using toluene as a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis. Three replicates were measured for each sample, and mean values were displayed.

Free sugar content of the samples, was determined by high pressure liquid chromatography (HPLC) basically according to the analytical method reported by Labaneiah and Luh (1981). The flag leaves cut from the plants and with midribs removed were extracted in 10 volumes of 80% (v/v) ethanol at 75°C for 30 min. After cooled, xylose (0.5% of the sample weight) was added as an internal standard, and the sample was homogenized. The homogenate was centrifuged at 1,500 xg for 10 min. The pellet was re-extracted twice in 5 volumes of 80% (v/v) ethanol, and the three supernatants were combined and dried in vacuum. The residue was dissolved in 1 mL of distilled water. The sample was passed through a SEPAK C18 cartridge (Millipore Corporation, MA) which had been equilibrated with water, and it was eluted with 2 mL of distilled water. An aliquot (20 mL) of the eluate was analyzed by HPLC using an 830-RI refractive index detector (JASCO, Tokyo, Japan) and a Shodex SUGAR SP0810 column (Showa Denko, Tokyo, Japan). The column temperature was 80°C, and the mobile phase was distilled water at a flow rate of 0.8 mL min⁻¹. The measurements were repeated three times.

(5) Antioxidants

CAT was measured according to Zhang and Kirkham (1994). POD activity as guaiacol peroxidase was determined spectrophotometrically by monitoring the formation of tetraguaiacol (extinction coefficient ε = 26.6 mM⁻¹ cm⁻¹) from guaiacol in the presence of H2O2. MDA content was determined by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Approximately 1 g leaves was homogenized with 2 mL of 0.1% trichloroacetic acid (TCA) and centrifuged at 14,000 rpm for 15 min. After centrifugation, 1 mL of supernatant was mixed with 2.5 mL 0.5% TBA in 20% TCA and incubated in boiling water for 30 min. It was cooled immediately on ice to stop the reaction and centrifuged at 10,000 rpm for 5 min. Absorbance at 532 and 600 nm was determined, and MDA concentration was estimated by subtracting the non-specific absorption at 600 nm from the absorption at 532 nm using an absorbance coefficient of extinction, 156 mM⁻¹ cm⁻¹.

(6) Seed setting rate and 1000 grain weight

At maturity, the seed setting rate and 1000 grain weight of 30 panicles per treatment, chosen at random, were measured. These panicles were harvested at physiological maturity and the numbers of filled and unfilled grains per panicle were counted. ‘Seed setting’ was estimated as the ratio of the number of completely filled grains to the total number of reproductive florets (expressed as a proportion). Each floret was pressed between the forefinger and thumb and the starch observed (filled material was observed by peeling husk) to determine whether the grain was filled or not. The number of filled grains included both completely and partially filled grains. A 1000-grain random bulk sample was used to determine normal kernel weight. The sample was oven dried at 105°C for 72 hr to determine its weight and adjusted to normal grain weight, dividing by 86.5% and 85.5% for indica and japonica rice, respectively. The fresh seeds were seeds harvested recently and contained 25%–35% moisture. The dry seeds had no free water. The normal seeds contained 13.5% and 14.5% moisture for indica and japonica rice, respectively. Four replicates were used for each treatment.

3. Data analysis

The experiment was arranged in a randomized block design with four replications. The surface temperature was measured on 10 random samples and the mean values were used for comparison. Significant differences between treatments were determined based on the ANOVA of three treatments with two cultivars in each year using the SPSS 11.5 statistical software.

Results

1. Effect of water stress on yield

Water deficit affected growth, development and the physiological activities of rice plants resulting in reduced yield. Flowering stage is very sensitive to water deficit and is the critical stage for rice cultivation. Table 1 shows the effects of severe, mild and no water stress, W3, W2, and W1, respectively, given at flowering stage on the yield and yield components. W3 reduced seed setting rate and 1,000 grain weight significantly, resulting in reduced grain yield as compared with W1, but W2 did not, at a level of 0.05 (p<0.05). Thus, W2 had no negative impact on the yield. The cultivar x water stress interaction was not statistically significant indicating that the effect of water stress on rice yield did not vary with the cultivar.

2. Net photosynthetic rate, transpiration rate and stomatal conductance

Water stress had a significant effect on net photosynthetic rate (PN), transpiration rate (Tr) and stomatal conductance (Cs) (Table 2). Net photosynthetic rate in the flag leaf was the highest in W2 followed by W1 and W3. The photosynthetic rate in W3 was significantly different from that in W1 and W2, but the difference between W1 and W2 was not significant. The transpiration rate (Tr) and
| Year | Cultivar | Treatment | Panicles \((\times 10^3 \text{ ha}^{-1})\) | Spikelets per panicle | Seed setting rate \( (%) \) | 1000 grain weight \( (\text{g}) \) | Grain weight \( (\text{kg ha}^{-1}) \) |
|------|----------|-----------|---------------------------------|----------------------|-----------------|-----------------|-----------------|
| 2008 | 2401     | W1        | 204.74 a                         | 129.8 b              | 90.5 a          | 27.18 a         | 8983.5 a        |
|      |          | W2        | 205.16 a                         | 133.6 ab             | 92.5 a          | 27.07 a         | 9447.1 a        |
|      |          | W3        | 204.91 a                         | 135.5 a              | 74.2 b          | 25.77 b         | 7206.0 b        |
|      | Ilyou107 | W1        | 253.35 a                         | 196.4 a              | 80.2 a          | 26.53 a         | 10161.3 a       |
|      |          | W2        | 251.86 a                         | 198.6 a              | 82.4 a          | 26.76 a         | 10602.0 a       |
|      |          | W3        | 252.90 a                         | 193.1 a              | 66.1 b          | 25.26 b         | 7728.8 b        |
|      | Zhejing22| W1        | 272.75 a                         | 134.4 a              | 92.1 a          | 27.50 a         | 9010.5 a        |
|      |          | W2        | 278.40 a                         | 138.0 a              | 93.4 a          | 27.78 a         | 9241.3 a        |
|      |          | W3        | 276.60 a                         | 135.9 a              | 79.6 b          | 26.04 b         | 7365.7 b        |
| 2009 |          | Ilyou7954 | W1                                | 240.15 a             | 201.2 a         | 82.5 a          | 25.62 a         | 10144.2 a       |
|      |          | W2        | 236.22 a                         | 199.4 a              | 84.3 a          | 26.77 a         | 10203.6 a       |
|      |          | W3        | 238.05 a                         | 202.5 a              | 70.2 b          | 25.19 b         | 8097.0 b        |

W1, W2 and W3 were different water treatments (W1, no stress; W2, mild water stress; W3, severe water stress). The values followed by a different letter within a column for the same cultivar are significantly different at \( P = 0.05 \).

| Year | Cultivar | Treatment | \( P_n \) (\( \mu \text{mol m}^{-2} \text{s}^{-1} \)) | \( T_r \) (\( \text{mmol m}^{-2} \text{s}^{-1} \)) | \( C_s \) (\( \text{mmol m}^{-2} \text{s}^{-1} \)) |
|------|----------|-----------|---------------------------------|-----------------|-----------------|
| 2008 | 2401     | W1        | 25.6 a                           | 16.3 a           | 1.27 ab         | 1.21 a          |
|      |          | W2        | 26.7 a                           | 17.4 a           | 1.39 a          | 1.28 a          |
|      |          | W3        | 24.2 a                           | 14.5 a           | 1.15 b          | 0.94 b          |
|      | Ilyou107 | W1        | 27.7 a                           | 17.7 a           | 1.36 a          | 1.25 a          |
|      |          | W2        | 29.3 a                           | 18.6 a           | 1.44 a          | 1.35 a          |
|      |          | W3        | 25.2 a                           | 15.4 a           | 1.29 a          | 0.84 b          |
|      | Zhejing22| W1        | 24.6 a                           | 15.0 a           | 1.26 ab         | 1.13 a          |
|      |          | W2        | 25.4 a                           | 16.2 a           | 1.32 a          | 1.27 a          |
|      |          | W3        | 23.6 a                           | 14.7 a           | 1.15 b          | 0.84 b          |
| 2009 |          | Ilyou7954 | W1                                | 26.6 ab          | 17.3 a          | 1.34 a          | 1.20 a          |
|      |          | W2        | 28.8 a                           | 17.9 a           | 1.44 a          | 1.28 a          |
|      |          | W3        | 24.5 b                           | 14.9 a           | 1.19 b          | 0.90 b          |

W1, W2 and W3 were different water treatments (W1, no stress; W2, mild water stress; W3, severe water stress). The values followed by a different lowercase letter within a column for the same cultivar are significantly different at \( P = 0.05 \).

| Cultivar | Treatment | SSC (mg g\(^{-1}\) FW) | APC (\( \mu \text{g g}^{-1} \text{FW} \)) | MDA content (nmol g\(^{-1}\)) | CAT (mg g\(^{-1}\) min\(^{-1}\) FW) | POD (U g\(^{-1}\) min\(^{-1}\) FW) |
|----------|-----------|------------------------|---------------------------------|-----------------|-----------------|-----------------|
| Zhejing22| W1        | 122.3 a                | 42.5 a                          | 0.274 b         | 14.50 a         | 152.6 a         |
|          | W2        | 126.7 a                | 45.2 a                          | 0.292 b         | 15.85 a         | 160.1 a         |
|          | W3        | 105.2 b                | 28.7 b                          | 0.405 a         | 11.44 b         | 124.7 b         |
| Ilyou7954| W1        | 110.2 a                | 35.9 a                          | 0.169 b         | 15.84 a         | 143.9 a         |
|          | W2        | 102.5 a                | 39.3 a                          | 0.184 b         | 17.23 a         | 152.2 a         |
|          | W3        | 85.0 b                 | 21.4 b                          | 0.267 a         | 12.04 b         | 120.3 b         |

W1, W2 and W3 were different water treatments (W1, no stress; W2, mild water stress; W3, severe water stress). SSC, Soluble sugar content; APC, Amino proline content; FW, Fresh weight. The values were measured on the 7 day after the heading of rice. The values followed by a different letter within a column for the same cultivar are significantly different at \( P = 0.05 \).
stomatal conductance ($C_s$) were highest in W2 and lowest in W3, and the values in W3 were significantly lower than those in W1 and W2.

3. **Physiological characteristics of flag leaf**

The contents of soluble sugar and free proline, and the MDA, CAT, and POD activities showed no significant differences among the water stress treatments at 7 d after heading (Table 3). The photosynthetic ability was higher in W2, yielding a higher soluble sugar in the flag leaf. There was a significant difference in soluble sugar content between the plants in W2 and W3 ($P<0.05$). The free proline content was significantly higher in W2 than in W3. MDA is a membrane lipid peroxidation product, and it can represent the degree of membrane lipid peroxidation and membrane injury. The MDA content of the flag leaf in W3 was significantly higher than that in either W1 or W2 at the level of 0.05 ($P<0.05$). As a result, the membrane system was seriously injured in W3. CAT and POD are protective antioxidant enzymes, and the performance of these two enzymes was significantly lower in W3 than in W1 and W2. The accumulation of reactive oxygen species exceeded the scavenging activity to protect the enzymes. The antioxidant enzyme system was thus damaged, resulting in a decline in activity in W3.

4. **Effect of water stress on panicle and leaf temperature**

In the fine weather during the 7 d after heading in 2008 and 2009, panicle and leaf temperature were determined at 1300–1330. Table 4 shows the temperatures of the panicle and leaf. Under similar air temperature and relative humidity conditions, the panicle and leaf temperature of rice rose with the increase of water stress. The panicle and leaf temperature under severe water stress (W3) was significantly higher than that in W1 and W2, while there was no difference between W1 and W2 in both years. This showed that the severe water stress reduced the photosynthetic rate, transpiration rate and stomatal conductance. The reduced transpiration raises the leaf temperature, and probably panicle temperature indirectly through heat diffusion via air, or due to peduncle stomata closure. Therefore, the physiological reasons for lower leaf temperature in W2 and W1 might be the higher transpiration rate, stomatal conductance, and photosynthetic rate. Lower panicle and leaf temperatures in W2 and W1 were the substantial cause of its higher spikelet fertility. Similar results were observed in the four cultivars in both years.

5. **Effect of water stress on canopy temperature**

Canopy temperature in rice plots under different water stresses were recorded thermographically, and we obtained a different infrared image of canopy (Fig. 1), which showed the effect of water stress on the canopy temperature. The blue and red colors indicate the lower and higher temperatures respectively. Canopy temperature increased with the increase in water stress (Table 5), namely W3> W2> W1. The temperature difference was up to 3°C. Thus, the severer the water stress, the higher the canopy temperature. The canopy temperature in W3 was significantly higher than that in either W1 or W2, but the canopy temperature in W2 was not significantly higher than that in W1. Therefore, the plants in W2 and W1 had higher photosynthetic ability and transpiration rate. Compared with W3, W2 and W1 reduced panicle, leaf and canopy temperatures, and this enhanced the ability of rice to resist heat injury.

### Table 4. Effect of water treatments on the panicle and the leaf temperature of rice.

| Year | Cultivar | Treatment | Panicle | Flag leaf | Second leaf | Third leaf |
|------|----------|-----------|---------|-----------|------------|-----------|
|      |          |           | Organ temperature (ºC) |           |            |           |
| 2008 | 2401     | W1        | 30.53 b | 30.16 b | 29.43 b | 28.73 b |
|      |          | W2        | 31.00 b | 30.62 b | 30.02 b | 29.17 b |
|      |          | W3        | 32.14 a | 31.69 a | 31.07 a | 30.38 a |
|      | Ilyou107  | W1        | 30.99 b | 30.74 b | 30.18 b | 29.22 b |
|      |          | W2        | 31.40 b | 31.03 b | 30.34 b | 29.52 b |
|      |          | W3        | 32.62 a | 32.17 a | 31.78 a | 30.81 a |
| 2009 | Zhejing22 | W1        | 29.95 b | 29.64 b | 29.05 b | 28.30 b |
|      |          | W2        | 30.31 b | 29.93 b | 29.45 b | 28.85 b |
|      |          | W3        | 31.53 a | 30.89 a | 30.39 a | 29.82 a |
|      | Ilyou7954 | W1        | 30.52 b | 30.14 b | 29.65 b | 28.96 b |
|      |          | W2        | 30.97 b | 30.61 b | 30.03 b | 29.34 b |
|      |          | W3        | 32.20 a | 31.77 a | 31.20 a | 30.33 a |

W1, W2 and W3 were different water treatments (W1, no stress; W2, mild water stress; W3, severe water stress). The values followed by a different letter within a column for the same cultivar are significantly different at $P<0.05$. 

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Table 4. Effect of water treatments on the panicle and the leaf temperature of rice.
The test results indicated that severe water stress (W3) had a negative influence on the seed setting rate and 1,000 grain weight, but mild water stress (W2) did not. The yield in W3 was significantly lower than that in W1 and W2 \( (P<0.05) \); and there were no significant differences between W1 and W2 in either the yield, seed setting rate or 1,000 grain weight. W2 had no negative impact on the yield. This is because the soil moisture in W2 fully meets the needs of the growth of rice. Mild water stress would be conducive to accumulation of photosynthetic products and high grain yield. However, severe water stress damaged the photosynthetic chloroplast, accelerating the decomposition of chlorophyll and leaf senescence. Thus, it decreased the net photosynthetic rate, transpiration rate, and stomatal conductance reducing the accumulation of photosynthetic products and finally resulting in a decreased yield. Similar results were observed in the four cultivars tested in both years.

Under water stress, plants accumulate soluble substances for osmotic adjustment, which is an important physiological mechanism for adapting to water stress (Hare et al., 1998). These osmotic substances include soluble sugar, proline, etc., and the larger the accumulation, the stronger the drought resistance (Ashraf and Iram, 2005; Chaves and Oliveira, 2005; Martino et al., 2003). However, under severe water stress, these osmotic substances will decrease (Patakas et al., 2002; Pinheiro et al., 2001). The results of this study showed that soluble sugar and proline contents were highest under mild water stress, although MDA content under severe water stress was significantly higher than that under mild or no water stress. The membrane system may be seriously injured under severe water stress, resulting in metabolic disorders in the cell, as suggested by Zhang et al. (2008). Under severe water stress, CAT and POD activity was reduced and the accumulation of reactive oxygen species exceeded the scavenging capability of the protective enzymes. The antioxidant enzyme system was thus damaged, resulting in a decline in its activity. The photosynthetic rate was the

### Table 5. Effect of water stress on canopy temperature.

| Year | Cultivar  | Treatment | Canopy temperature (°C) |
|------|-----------|-----------|-------------------------|
| 2008 | 2401      | W1        | 28.81 ± b               |
|      |           | W2        | 29.27 ± b               |
|      |           | W3        | 30.51 ± a               |
|      | Ilyou107  | W1        | 29.94 ± b               |
|      |           | W2        | 30.32 ± b               |
|      |           | W3        | 31.65 ± a               |
| 2009 | Zhejing22 | W1        | 29.37 ± b               |
|      |           | W2        | 29.61 ± b               |
|      |           | W3        | 30.72 ± a               |
|      | Ilyou7954 | W1        | 29.82 ± b               |
|      |           | W2        | 30.19 ± b               |
|      |           | W3        | 32.13 ± a               |

W1, W2 and W3 were different water treatments (W1, no stress; W2, mild water stress; W3, severe water stress). We measured the canopy temperature of rice on clear days within 7 d after heading. The values followed by a different lowercase letter within a column for the same cultivar are significantly different at \( P = 0.05 \).
lowest under severe water stress, in agreement with Hu et al. (2004). Compared with severe water stress, mild water stress gave a higher net photosynthetic rate; which was propitious to accumulation of the photosynthetic products as the ‘source’ for grain filling.

Crop canopy temperature is closely related to water stress. Use of the crop canopy temperature to increase the profit and crop water status may lead to a new way to diagnose crop water status. Turner et al. (1986) researched the correlation of the degree of water stress with canopy temperature and growth, and found that with a decline of soil moisture, the temperature difference between the canopy and the air increased, while the accumulation of dry matter decreased. The grain yield and seed setting rate were negatively correlated with canopy temperature at the heading stage. The present experiments showed that at the same temperature and humidity, the panicle and leaf temperature of rice was increased by water stress, significantly under severe water stress. The canopy temperature under severe water stress was significantly higher than that under no stress or mild water stress. The maximum temperature difference was 3°C. Compared with severe water stress, mild water stress increased the photosynthetic ability and transpiration rate, and reduced panicle, leaf and canopy temperatures. This enhanced the ability of rice to avoid heat injury. Furthermore, compared with no water stress, we saved 13.3 L water per day per square meter under mild water stress during the 7-day period.

Conclusion
There was a significant impact of water stress on flag leaf physiological characteristics, organ temperature and canopy temperature. Compared with the rice plants under severe water stress, the plants under mild water stress and no stress had better photosynthetic and physiological characteristics resulting in a higher yield. Panicle, leaf and canopy temperatures of rice were significantly increased under severe water stress, but not under mild water stress. A similar trend was observed in the four cultivars tested. Mild water stress enhanced the ability of rice to avoid heat injury, and was propitious to saving of water resources for rice cultivation.

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