Response of Osmotic Regulation Substance Content and Protective Enzyme Activities to Shading in Leaves of Different Rice Genotypes

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Abstract: Effects of shading at the flowering and grain filling stages on osmotic regulation substance content and protective enzyme activities in the leaves of different rice genotypes (e.g., Ilyou 498, Gangyou 188, Dexiang 4103, Gangyou 527 and Chuanxiang 9838) were investigated. The results showed that the malondialdehyde content of Gangyou 188 significantly increased under shading, whereas those of Ilyou 498, Dexiang 4103, Gangyou 527 and Chuanxiang 9838 showed no significant differences compared to the control. The soluble sugar content significantly decreased in Ilyou 498, Gangyou 188, Dexiang 4103 and Chuanxiang 9838, whereas it did not significantly increase in Gangyou 527 under the weak light conditions (shading). Moreover, the soluble protein content in the leaves of different genotypes tended to decrease under shading. Further, the protective enzyme activities in the leaves varied in different rice genotypes under the shading treatment. Based on the osmotic regulation substance content and the protective enzyme activities in the leaves of different rice genotypes, it was concluded that Gangyou 188 and Gangyou 527 had strong abilities to adapt to the low light conditions. In addition, the mechanism of damage to rice leaves in different genotypes under shading was discussed.

Key words: malondialdehyde content; osmotic regulation substance content; protective enzyme; rice genotype; shading

Environmental factors such as light, temperature and air, play important roles in crop growth. Long periods of low temperature, rain, and lack of sunlight (i.e., temperature and sunshine levels < 2 and precipitation level > 2) have significantly affected the rice crop cultivation in Sichuan Basin of China (Peng and Yang, 2001). Much research has been conducted to study the influence of shading on crop yields and its physiological characteristics. The results from these studies reveal that the protein content in rice grains significantly increased with the light density (Ren et al, 2003). Further, shading reduced crop yields (Kato, 1986; Murty and Sahu, 1987; Kumudini et al, 2008; Ding and Su, 2010; Lazaro et al, 2010). Likewise, leaf width, leaf length and relatively dry matter increased under the weak light conditions (Ren et al, 2002). The decline in enzyme activities was directly proportional to the decrease of starch components in rice under shading (Li et al, 2005), and the soluble protein content per unit leaf area reduced as the illumination intensity weakened (Wang et al, 1998). Both the superoxide dismutase (SOD) and malondialdehyde (MDA) contents decreased in the leaves of Sabina davurica under shading, however, the peroxidase (POD) activity increased at first and then decreased under shading (Jia et al, 2010). The shading after heading extended the growth duration of flag leaves, but reduced the POD activity (Okada and Katoh, 1998; Ding et al, 2004). Little research has been done on the effects of shading on osmotic regulation substance content and protective enzyme activities in hybrid rice genotypes. In this study, we used a randomized block design and five rice genotypes such as Ilyou 498, Gangyou 188, Dexiang 4103, Gangyou 527 and Chuanxiang 9838 for testing. The aims of this study were: (1) to determine the rules affecting the osmotic regulation substance content and the protective enzyme activities under shading, (2) to understand the ecophysiological mechanisms for the adaptive capacity of rice against the harm caused by shadow, and (3) to help in shade-tolerance cultivation and rice variety selection in low light areas.
MATERIALS AND METHODS

Experimental site and conditions

The experiments were conducted at the experimental farm of Sichuan Agriculture University, Ya’an City, Sichuan Province, China in 2010 as pot experiment using heavy loam soils, and the chemical properties in the soils were as follows: organic matter 19.74 g/kg, total nitrogen (N) 2.14 g/kg, total phosphorus (P) 0.24 g/kg, total potassium (K) 27.6 g/kg, available N 161.47 mg/kg, available P 82.24 mg/kg, and available K 97.61 mg/kg. A pH of 5.93 was obtained in an aqueous solution of the soil.

Experimental design and methods

Five different hybrid rice genotypes such as Ilyou 498 (II-32A × Suhui 498), Gangyou 188 (Gang 46A × Lehui 188), Dexiang 4103 (Dexiang 074A × Luhui H103), Gangyou 527 (Gang 46A × Suhui 527) and Chuanxiang 9838 (Chuanxiang 29A × Fuhui 838) were applied. The treatments were arranged in a randomized block design with three replications. A total of 30 plots with each plot area of 3.6 m × 4.0 m were used. Two methods for lighting, shading and natural light (CK), were performed. A gauze with 0.50 mm pore size was hung above 1.5 m high from the ground for shading from the initial heading (5 August) to maturity stages (26 September), with a light transmittance of 47%. Seeds were sown on 8 May and raised on a dry land bed. Seedlings were transplanted on 15 May with two seedlings per hill at a row spacing of 33.3 cm × 20.0 cm.

Fertilizers used were: urea for N, single superphosphate for P, and potassium chloride for K with doses of 180 kg/hm², 90 kg/hm² and 180 kg/hm², respectively. N was split-applied: 75.6 kg/hm² at basal, 32.4 kg/hm² at mid-tillering, and 72.0 kg/hm² at panicle initiation. P was applied at basal. K was split equally at basal and panicle initiation. Local rainfall was 2 042.1 mm in 2010, annual average temperature was 16.4 °C, and annual sunshine was 955.8 h. The local average daily temperature and sunshine were 23.7 °C and 3.0 h, respectively, during the rice growth period from heading to maturity.

Measuring items and methods

Sampling method

Samples were collected after shading at an interval of 10 d. The flag leaves were taken from two hills in each plot and rinsed with distilled water. The veins were removed by shears. The samples were blended, weighed and wrapped in aluminum foils, and frozen in liquid nitrogen, then placed into a sealed plastic bag and stored at -80 °C.

Osmotic regulation substance and MDA contents

The selected leaves (0.5 g) after being sheared and blended were ground to homogenate using a mortar and pestle. After centrifugation, the supernatant was taken and the osmotic regulation substance and MDA contents were measured. Soluble sugar (SS) and soluble protein (SP) contents were determined by using the anthraquinone colorimetric and coomassie brilliant blue G-250 methods, respectively (Li, 2002; Xiong, 2003). The MDA content was measured by thio-barbituric acid (Xiong, 2003).

Protective enzyme activity

Rice leaves (0.5 g) were taken in a precooled mortar in a cooling bath cooled by liquid nitrogen, phosphoric acid buffer was added, and then ground into a homogenate. After centrifugation, the supernatant solution (SOD coarse extraction liquid) was set aside at low temperature. Guaiacol colorimetric (Xiong, 2003), permanganate titration (Xiong, 2003), and nitroblue tetrazolium (Xiong, 2003; Liu et al, 2008) methods were used to measure peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activities, respectively.

Data processing

Microsoft Excel was used for data processing. The DPS V7.05 statistical analysis software was used for variance analysis.

RESULTS

Shading on MDA content in different hybrid rice genotypes

MDA, the product of lipid peroxidation, can strongly react with various components in cells, and cause serious damage to enzymes and membranes. It destroys membrane structure and its physiological function, therefore, the increased MDA content in plants leads to its fast aging (Guan et al, 2003). Fig. 1 showed that the effects of shading on the MDA contents in different hybrid rice genotypes were similar to the control.
experiments in the same variety. Therefore, the change in MDA content was probably related to the different hybrid rice genotypes. The MDA contents in IIyou 498 under both shading and normal light conditions (CK) showed a rising trend as the time of stress (shading days) increased. During the whole shading period, the MDA content in Dexiang 4103 under shading was higher than that in the normal light treatment, whereas the MDA content in Gangyou 527 under shading was lower than that in the normal light treatment. Compared to the control experiment, the MDA content in Chuanxiang 9838 did not have a noticeable difference under shading. At 40 d after shading, the MDA content in Gangyou 188 under shading was higher than that in the normal light treatment, whereas the MDA contents of IIyou 498, Dexiang 4103, Gangyou 527 and Chuanxiang 9838 did not have noticeable differences between the shading and normal light treatments.

**Shading on osmotic regulation substance content in different hybrid rice genotypes**

Soluble sugar (SS) is one of the important organic matters in plants, and is closely related to the plant resistance. Fig. 2 showed that the shading treatment decreased the SS content in the leaves of rice genotypes IIyou 498, Gangyou 188 and Dexiang 4103, and these changes were similar to that in the control experiments in the same genotype. However, the differences in SS content and amplitude of variation were observed in the leaves of different rice genotypes. The results of variance analysis showed that the SS contents in Gangyou 188 and Dexiang 4103 in the shading treatment (40 d) were significantly lower than those in the control treatment, and the SS content in IIyou 498 did not have noticeable difference between the shading and control treatments. The SS content in Gangyou 527 in the shading treatment (10 d) was significantly lower than that in the control treatment. After that, the difference became small between the shading and control treatments, and at 40 d after shading, the SS content in the shading treatment was higher than that in the control treatment, although the difference was not significant. The SS content in Chuanxiang 9838 was a bit higher under shading than that in the control treatment at the initial stage (10 d), but it declined under shading. The results of variance analysis showed that the SS content in Chuanxiang 9838 significantly declined at 40 d after shading.

Soluble protein (SP) plays an important role in studying the nitrogen metabolism of plants. Fig. 2 showed that the SP contents in different hybrid rice genotypes decreased during the elongation growth. The SP contents in different hybrid rice genotypes were lower than those in the normal light treatment after shading for 10 d, but higher than CK after shading for 20 d. The SP contents in IIyou 498, Dexiang 4103 and Gangyou 527 in the shading treatment were lower than those in the normal light treatment. There was a noticeable difference between the shading and normal treatments after shading for 40 d. The SP content in Gangyou 188 was lowered by 37.90% at 30 d after shading compared to that in the normal light treatment, but the difference became small at 40 d after shading. The SP content in Chuanxiang 9838 at 30 d after shading was higher than that in the normal light treatment, and then decreased fast. At 40 d after shading, the SP content in Chuanxiang 9838 in the shading treatment
was lowered by 17.90% compared to CK.

**Shading on protective enzyme activities in different hybrid rice genotypes**

**SOD activity**
SOD is an antioxidant enzyme that defends against the damage caused by superoxide radicals to the cells in plants, and the SOD activity is a good indicator of plant resistance and senility. As shown in Fig. 3, compared to the control treatment, the SOD activity in IIyou 498 was significantly higher after shading for 10 d, and then decreased. The SOD activities in Gangyou 188 and Dexiang 4103 were lower than those in the control treatment during the whole period of shading, but there existed a genotypical difference in the decline range of the SOD activity. In addition, the SOD activity in Gangyou 527 was significantly higher than that in the control treatment during the whole period of shading. The SOD activity in Chuanxiang 9838 decreased to the minimum value at 20 d after shading in the normal light treatment, whereas it decreased to the minimum value at 30 d after shading in the shading treatment. After shading for 40 d, the SOD activities in IIyou 498, Gangyou 188, Dexiang 4103 and Chuanxiang 9838 were significantly lower than those in the control treatment.

**CAT activity**
CAT is one of the important protective enzymes in plants and helps to remove hydrogen peroxide from physiological systems (Zhou et al, 2010). A certain relationship exists between the CAT activity and plant characteristics such as metabolism strength, anti-aging, cold-resistance ability, and disease-resistant ability. As shown in Fig. 3, the CAT activities in IIyou 498 and Chuanxiang 9838 were higher than those in the control treatment during the whole period of shading. The difference in CAT activities between the shading and normal light treatments in IIyou 498 increased with the time of shading. The trend of CAT activity in Chuanxiang 9838 showed fluctuations in the shading treatment, whereas it showed a ‘single-peak curve’ in the normal light treatment. The CAT activity in Gangyou 188 increased significantly in the shading
treatment at the later stage of development. The trend of CAT activity in Dexiang 4103 in the shading treatment was similar to that in the normal light treatment. The CAT activity in Dexiang 4103 decreased with the time of shading, and it was lower than that in the normal light treatment during the whole period of shading. The CAT activity in Gangyou 527 was relatively stable in both the shading and normal light treatments. After shading for 40 d, the CAT activities in IIyou 498, Gangyou 188 and Chuanxiang 9838 in the shading treatment were significantly higher, but the CAT activity in Dexiang 4103 was significantly lower than those in the normal light treatment.

**POD activity**

POD is another kind of enzyme in the active oxygen scavenging system that helps to remove reactive...
Effects of shading on osmotic regulation substance content and protective enzyme activities in rice leaves

Osmotic regulation is an important physiological mechanism that helps to produce protective response to adversity stress in plants, and the response of osmotic regulation substances to the adversity stress differs in crops (Zhu et al, 2003; Cai et al, 2008; Hou et al, 2009). Cai et al (2008) observed that drought stress could increase the osmotic regulation substance content. However, Zhu et al (2003) found that drought stress could increase the soluble protein content while reduce the soluble sugar content. Hou et al (2009) reported that low temperature and poor light conditions were responsible for the decrease in soluble protein and soluble sugar contents. The results of this study indicated that the osmotic regulation substance content under shading stress followed a different direction. The reduction of SS contents in Ilyou 498, Gangyou 188, Dexiang 4103 and Chuanxiang 9838 is shading treatment may be significant to the photosynthetic capacity of flag leaves. The stomatal density reduced after shading, which in turn reduced the light absorption in leaves, photochemical reaction rate, and the fixed rate of carbon dioxide uptake resulting in the decline in the photosynthetic reaction rate and the accumulation of photosynthesis products. An increase in SS content indicates that the plant would accumulate a few small molecular osmotic regulation substances (e.g., soluble sugar) against adversity under shading (Cai et al, 2008). Protein is an important functional material in plant’s life processes, and its metabolism is influenced and regulated by many factors (Longstreth, 1980; Yang et al, 2010). Boardman et al (1977) reported that the synthesis of soluble protein in leaves was controlled by light, and the soluble protein content in leaves under shade was usually lower than those under normal light conditions. This study also confirmed that the soluble protein contents in Ilyou 498, Dexiang 4103, Gangyou 527 and Chuanxiang 9838 were reduced under shading, and there was no noticeable difference in soluble protein content in Gangyou 188 between the shading and normal light treatments.

Reactive oxygen species can be produced by many pathways in plants, and SOD, POD, CAT, etc. are important protective enzymes for the active removal of these species (Pan et al, 2003). Adversity stress destroys the dynamic balance between the generated oxygen free radicals and their scavenging (Liu et al, 2008). Active oxygen species begin to accumulate in plants and the activities of the corresponding protective enzymes increase at the later shading. When the amount of reactive oxygen species exceeds the amount that the protective enzymes could remove, it leads to the damage of membrane systems, and the MDA content in plants increases (Huang et al, 2008). The increase in MDA content affects the physiological and biochemical functions in plants, and the plant suffers from a fast aging process. This study showed that, compared to the normal light treatment, the MDA contents in Gangyou 188, Dexiang 4103 and Gangyou 527 increased, but those in Ilyou 498 and Chuanxiang 9838 decreased in the shading treatment. Dhinsa et al (1981) reported that the protective enzyme activities in the strong resistance varieties maintained a high level under adversity conditions to benefit for free radical scavenging and reduce the level of membrane lipid peroxidation and the degree of membrane damage. The differences in the SOD, POD and CAT activities in the different hybrid rice genotypes under the weak light stress indicate that there exists different shade tolerance in rice varieties, and there may be more...
than one way for various rice varieties to overcome the influence of shading. The SOD activities in Ilyou 498 and Chuanxiang 9838 reduced significantly in the shading treatment, both mostly through the increased CAT activity to remove the reactive oxygen species in the plants. Gangyou 188 showed enhanced adaptability to shading owing to the increased combined CAT and POD activities. The active oxygen scavenging system in Dexiang 4103 was destroyed under shading since the SOD, POD and CAT activities were significantly reduced. The SOD activity in Gangyou 527 was significantly increased while the POD and CAT activities were significantly reduced.

**Genotype differences and physiological mechanism for rice adapting to shading**

Plants in the long evolution form two types of protection systems: enzymatic and non-enzymatic pathways, to remove reactive oxygen species such as protective enzymes and osmotic regulation substances (Xia et al., 2000). Dong and Liu (2009) reported that the relations between protective enzymes and osmotic regulation substances were significant. Sugar is a low molecular weight substance involved in osmotic regulation, and the content of soluble sugar increases under adversity situations in plants. Part of these sugars work as osmotic regulation substances while another part provides carbon skeletons for the synthesis of new proteins (Shi et al., 2007). The soluble protein in plants constitutes most of the enzymes involved in all kinds of metabolism processes (Xu et al., 2008). The results of this study showed different protection systems of resistance against shading in different hybrid rice genotypes. With the combined enzymatic and non-enzymatic systems, reactive oxygen species (oxidative radicals) were removed in Gangyou 188 and Gangyou 527 in the shading treatment, but both membrane and lipid peroxidation were aggravated, indicating that the accumulation of MDA could increase the protective enzyme activities and the osmotic regulation substance content (Dong and Liu, 2009). Ilyou 498 and Chuanxiang 9838 overcame the influence of shading mainly through the enzymatic systems, and the changes in the defense capability in the protective enzymatic systems mainly depended on the comprehensive coordination of several enzymes (Xia et al., 2000). Both the enzymatic and non-enzymatic systems of Dexiang 4103 were destroyed by low light conditions, and it might be due to the fact that the synthesizes of soluble proteins were blocked or decomposed. This led to the decreased enzyme activity in plants, and membrane lipid peroxidation was aggravated. The lower MDA content in the parochial bound, higher contents of soluble sugar and soluble protein, and the SOD, CAT and POD activities formed a physiological basis in the shade-tolerance of hybrid rice genotypes. Because of the differences in these physiological indexes, Gangyou 188 and Gangyou 527 exhibited strong shade-resistance, Ilyou 498 and Chuanxiang 9838 exhibited medium shade-resistance, and Dexiang 4103 exhibited weak shade-resistance.

The effects of shading in the low light areas in later rice growth period might be due to the coordination of osmotic regulation substance content and protective enzyme activities in different hybrid rice genotypes, therefore, the yield and quality of rice crop were affected. These differences in the shade-resistant rice genotypes could provide a theoretical reference for breeders in variety selection. It needs further exploration the influence on protective enzyme gene expression systems and whether synthesizing new proteins in plants under low light stress conditions.

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