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Photosynthetic Characteristics of Flag Leaves in Rice White Stripe Mutant 6001 During Senescence Process

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Abstract: Physiological, biochemical and electron microscopy analyses were used to investigate the photosynthetic performance of flag leaves in rice white stripe mutant 6001 during the senescence process. Results showed that the chlorophyll content at the heading and milk-ripe stages in rice mutant 6001 were about 34.78% and 3.00% less than those in wild type 6028, respectively. However, the chlorophyll content at the fully-ripe stage in rice mutant 6001 was higher than that in wild type 6028. At the heading stage, the net photosynthetic rate (P_n) in rice mutant 6001 was lower than that in wild type 6028. Rice mutant 6001 also exhibited a significantly slower decrease rate of P_n than wild type 6028 during the senescence progress, especially at the later stage. Furthermore, Ca^{2+}-ATPase, Mg^{2+}-ATPase and photophosphorylation activities exhibited the similar trends as the P_n. During the senescence process, the 68 kDa polypeptide concentrations in the thylakoid membrane proteins exhibited a significant change, which was one of the critical factors that contributed to the observed change in photosynthesis. We also observed that the chloroplasts of rice mutant 6001 exhibited higher integrity than those of wild type 6028, and the chloroplast membrane of rice mutant 6001 disintegrated more slow during the senescence process. In general, rice mutant 6001 had a relatively slower senescence rate than wild type 6028, and exhibited anti-senescence properties.

Key words: chlorophyll content; fluorescence emission spectrum; net photosynthetic rate; polypeptide component; photophosphorylation; rice mutant; ultrastructure

Leaf color mutation is a significant phenotypic characteristic that is excellent for investigating plant photosynthesis and gene function (Hansson et al, 1999; Fambrini et al, 2004). White stripe mutations, which are common and accessible mutation of leaf color, are first found by Nagamatsu and Omura (1962). These mutations, caused by changes of nuclear or cytoplasmic genes, are ideal material for investigating nuclear-cytoplasmic interactions (Tilney-Bassett, 1975; Aluru and Rodermel, 2004). Leaf color mutants are found in many plants, such as *Arabidopsis thaliana* and *Oryza sativa* (Cheng et al, 2013). Chloroplasts are specific semi-autonomous plant organelles for the synthesis of chlorophyll, starch, lipids and amino acids (Klimyuk et al, 1999; Chen et al, 2005). Chlorophyll is the main photosynthetic pigment in plants. If one or more of the synthesis processes of chlorophyll are disrupted, the equilibrium between chlorophyll and its intermediate products is changed, which results in the reduction of chlorophyll content in the leaf color mutants. Previous studies suggested that leaf color mutants, caused by chlorophyll deficiency, can lead to the absorption of light energy reduced (Melis and Thielen, 1980; Ghirardi and Melis, 1988; Greene et al, 1988). However, some studies demonstrated that leaf color mutants have relatively higher photosynthetic ability and photoinhibition tolerance than their wild type counterparts (Zhou et al, 2006). In general, leaf color mutant is closely related to rice yield, and is a research hotspot in agronomy and related fields.

Rice white stripe mutant 6001 was resulted from a spontaneous mutation of indica rice variety 6028. At the two-leaf stage, the leaf is albinistic and gradually develops white-green interphase stripes after the three-leaf stage. The edge and vein of the leaf exhibit a white-green interphase arrangement from the edge to

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the leaf vein. Furthermore, the leaf sheath is also striped (Liu et al, 2012). The photosynthetic characteristic of rice mutant 6001 has not been reported. Therefore, in this study, we determined the anti-senescence properties of rice mutant 6001 by comparing the photosynthetic characteristic with its wild type 6028 during the senescence process, and provided certain theoretical knowledge for application of the leaf color as a genetic marker in cross-breeding.

MATERIALS AND METHODS

Rice materials

Rice white stripe mutant 6001 and its wild type 6028 were provided by the Grain Crops Research Institute, Academy of Agricultural Sciences, Jiangsu Province, China. The seeds were sown in early May 2012, and the management was administered as a conventional rule for rice. Samples were obtained at the heading (15 August, 2012), milk-ripe (5 September, 2012), and fully-ripe (25 September, 2012) stages. All analyses were repeated three times.

Measurements

Chlorophyll content

Chlorophyll content was measured according to the method of Arnon (1949). In brief, 0.1 g of leaves, excluding the veins, was ground using a mortar and pestle, and precooled 80% acetone, quartz sand and $CaCO_3$ were added. The mixture was then ground into a slurry and freeze-centrifuged at 3 000 × *g* for 10 min, and the supernatant was collected. The residue was dissolved with 80% acetone and centrifuged again under the aforementioned conditions. The supernatant of the second centrifugation was mixed with the initially collected supernatant, and the volume was maintained at 10 mL. The absorbance was measured at 645 and 663 nm using an ultraviolet spectrophotometer (Cintra-1010, GBC, Australia), and the chlorophyll content was calculated.

Net photosynthetic rate

The net photosynthetic rate (P_n) was measured by a portable photosynthesis system (CIRAS-2, PP Systems, Hitchin, UK) from 9:00–10:00 am. The measurement conditions were as follows: (360 ± 2) mol/L of CO_2 in the leaf chamber, the same temperature and humidity with environment, and a light intensity of 1 000 μmol/(m^2·s). Six successive automated recordings were carried out at 1.6 s intervals when the fluctuation range of P_n was no more than 0.5 μmol/(m^2·s). The interval of different light intensities was 120 s.

Preparation of chloroplast, and measurements of photophosphorylation, Ca^{2+}- and Mg^{2+}-ATPase activities

Chloroplasts were prepared according to the method of Ketcham et al (1984). Leaves (5 g) without veins were ground in a mortar and pestle with precooled extracting solution [50 mmol/L Tris-HCl (pH 7.6), 5 mmol/L $MgCl_2$, 10 mmol/L NaCl, 0.4 mol/L sucrose, and 0.1% BSA (Albumin from bovine serum)] and filtered through four layers of gauze. The filtrate was centrifuged for 2 min at 200 × *g*, and the supernatant was centrifuged for 5 min at 2 000 × *g*. The sediment was suspended in extracting solution, and the liquid was used as the chloroplast suspension (final concentration, 100 μg/mL). The photophosphorylation of chloroplasts (10 μg) was then determined by a light photometer (FG-300, Institute of Plant Physiology and Ecology, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences, Shanghai, China). Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were measured using an ultraviolet spectrophotometer according to the method of Vallejos et al (1983).

Emission spectrum of thylakoid membrane

Thylakoid membranes were prepared according to a modified method of Dunahay et al (1984). Leaves (2 g) without veins were ground in a mortar and pestle, mixed with precooled B_1 buffer solution [0.4 mol/L sucrose, 2 mmol/L $MgCl_2$, 0.2% BSA, 20 mmol/L tricine (pH 8.0)], and filtered through four layers of gauze. The filtrate was centrifuged at 300 × *g* for 2 min, and the supernatant was centrifuged again at 4 000 × *g* for 10 min. The residue was suspended in B_2 buffer solution [0.15 mol/L sucrose, 5 mmol/L $MgCl_2$, 0.2% BSA, and 20 mmol/L tricine (pH 8.0)] and centrifuged at 4 000 × *g* for 10 min. The pellet was then suspended in B_3 buffer solution {15 mol/L NaCl, 5 mmol/L $MgCl_2$, and 20 mmol/L MES [2-(N-Morpholino) ethanesulfonic acid] (pH 6.5)}. The resultant suspension was used as the thylakoid membrane suspension. Emission spectra of the thylakoid membrane were measured according to the method of Liu et al (2004). The fluorescence emission spectra (λx = 480 nm) were obtained using a luminescence spectrometer (LS50B, Perkin Elmer, USA) with the chlorophyll content as 4 μg/mL.

Polypeptide in thylakoid membrane

Polypeptides in the thylakoid membranes were