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Phytochromes are Involved in Elongation of Seminal Roots and Accumulation of Dry Substances in Rice Seedlings

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Abstract: Phytochromes have been reported to play important roles in seedling de-etiolation and flowering in rice. To identify the roles of phytochromes in regulating root growth and accumulation of dry substances, the lengths of seminal roots and the dry weights of seedlings were measured in the wild type as well as the *phytochrome A* (*phyA*) and *phytochrome B* (*phyB*) mutants grown under different conditions. When the whole seedlings were exposed to white light, the elongation of the seminal roots was significantly photoinhibited in the wild type, whereas this inhibitory effect was clearly reduced in the *phyA* and *phyB* mutants. When the roots of the seedlings were blocked from white light, the *phyA* and *phyB* mutants exhibited significantly longer seminal roots than the wild type. These results suggest that both the root-localized and shoot-localized PHYA and PHYB are involved in the photoinhibition of seminal root elongation in rice seedlings. By measuring the dry weights of roots and shoots, it is revealed that PHYB positively regulates the accumulation of dry substances in shoots, however, PHYA exerts the contrary effects on the accumulation of dry substances in roots and shoots of rice seedlings.
Key words: rice; phytochrome; seminal root; dry substance

Light is one of the most important environmental stimuli and plays a pivotal role in the regulation of plant growth, development and metabolic activities. The perception of environmental light by plants is achieved by a family of plant photoreceptors that includes phytochromes, cryptochromes, phototropin and several others (Briggs and Huala, 1999; Neff et al, 2000; Franklin and Quail, 2010). The rice (*Oryza sativa*) phytochrome gene family is composed of three members: *PHYTOCHROME A* (*PHYA*), *PHYB* and *PHYC* (Kay et al, 1989; Dehesh et al, 1991; Basu et al, 2000; Takano et al, 2001, 2005). In recent years, single mutants of each phytochrome, as well as all the possible combinations of double and triple mutants have been isolated. Based on the photomorphogenic characteristics of these mutants, the perception of the three phytochromes to red (R) and far-red (FR) light as well as their roles in rice photomorphogenesis were reported (Takano et al, 2005, 2009; Osugi et al, 2011). Until now, most research on the rice phytochromes has focused on their roles in seedling de-etiolation and the determination of floral initiation.

Shoots and roots both respond to their light environment and modulate their growth and development. In *Arabidopsis*, some observation has suggested that light irradiation affects the rate and direction of root growth and the development of root hairs (Okada and Shimura, 1992; Kurata and Yamamoto, 1997; De Simone et al, 2000; Kiss et al, 2002; Correll and Kiss, 2005). For more than 40 years, the growth of rice seminal roots has been known to be inhibited by light irradiation (Ohno and Fujiwara, 1967). Recently, root-localized PHYA and PHYB were found to function in the photoinhibition of seminal roots in rice (Shimizu et al, 2009). The similar observation was also reported in *Arabidopsis* (Correll and Kiss, 2005). In both reports, light signals perceived by shoot-localized phytochrome proteins were suggested to make weak contribution to photoinhibition of root elongation (Correll and Kiss, 2005; Shimizu et al, 2009).

Jumtee et al (2009) observed distinct accumulation of amino acids, organic acids, sugars, sugar phosphates and nucleotides in the leaf blades of *phyAphyBphyC* triple mutants compared with those in the wild type in

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rice by metabolomics approach. Thus, we speculate that phytochromes probably affect accumulation and distribution of dry substances (otherwise known as dry matter), all cell constituents excluding water, in rice.

We investigated the seminal root length, as well as the accumulation and distribution of dry substances in roots and shoots of phytochrome mutants and wild type in this study. We identified new functions for rice phytochromes in the photoinhibition of the seminal root elongation. Moreover, the involvement of rice phytochromes in the accumulation and allocation of dry substances was revealed. Our findings provide additional insights into the roles of phytochromes in coordinating shoot and root growth in rice.

MATERIALS AND METHODS

Plant material and growth conditions

Two phytochrome-deficient mutants, *phyA* and *phyB*, and parental wild type rice (*Oryza sativa* L., cv. Nipponbare) were used, and the previously described *phyA4* and *phyB1* mutants (Takano et al, 2001, 2005) were used as the respective *phyA* and *phyB* mutants.

Rice seeds were surface sterilized in 70% ethanol for 30 s and placed in 5% NaClO for 20 min. The seeds were then rinsed six times in sterile double-distilled water, placed on 0.5% agar (containing agar and double-distilled water) in glass pots and grown for 10 d at 28 °C in an artificial climate box (RXZ-280B; Jiangnan Company, Ningbo, China). The seedlings were incubated for 10 d under the following three conditions: dark (the seedlings were grown under complete darkness), light (the whole seedlings were exposed to white light), and partial light (the seedling roots were blocked from white light). In the experiments with the roots blocked from light, the sterilized seeds were placed on medium, and then covered with sheets of sterile vermiculite. Furthermore, the medium in the glass pots was completely blocked from light by being trapped into vermiculite. White light was supplied at an irradiance of 49.5 μmol/(m^2·s) by white fluorescent tubes (FL20W-B, Hitachi, Tokyo, Japan).

Immunoblot analysis

One gram of seven-day-old seedlings were homogenized with 2 mL of protein extraction buffer (100 mmol/L Tris-HCl, pH 8.3, 5 mmol/L EDTA, 0.2% 2-mercaptoethanol and protease inhibitor cocktail). Homogenates were centrifuged at 12 000 × *g* for 30 min at 4 °C; the supernatant was precipitated with 66% saturated ammonium sulfate (Nagatani et al, 1993). The pellet was resuspended in 0.1 mL of protein extraction buffer, and the protein concentrations were determined by the Coomassie PLUS Protein Assay Reagent (Pierce, Rockford, IL). Sixty micrograms of protein were size-fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in a 10% gel and then blotted onto a PVDF membrane (Millipore, Billerica, MA). Immuno-chemical detection was performed using PHYA- and PHYB-specific antibodies as described by Takano et al (2005).

Measurements of seminal root length and dry weight of seedlings

The lengths of the seminal roots in the wild type, *phyA* and *phyB* mutant seedlings grown in different light conditions were measured. The relative length of seminal roots was calculated using their seminal root length grown under darkness as 100%, respectively. To determine the relative dry weights of roots, shoots and seedlings, the roots and shoots in the wild type, *phyA* and *phyB* mutant seedlings grown in different light conditions were separately harvested, and dried in a drying oven (Jinghong Company, Shanghai, China). The roots and shoots were dried at 100 °C for 30 min and then at 80 °C for 4 d. The dry weights of roots and shoots were measured by a precision electronic balance with an accuracy of 0.0001 g (Sartorius, Germany). The dry weight of seedlings in each material was calculated based on that of roots and shoots. The relative dry weights of roots, shoots, or seedlings were calculated using their dry weight grown under darkness as 100%, respectively. All experiments were repeated at least three times, and the values are reported as the mean ± *SE*. Statistical differences in the data were determined by the Student's *t* test.

RESULTS

Expression of PHYA and PHYB proteins in different organs of rice seedlings

To determine the effects of PHYA and PHYB on rice seedling growth, we initially examined the levels of phytochrome proteins in the roots and shoots of wild type seedlings by immunoblot detection. As shown in Fig. 1, both PHYA and PHYB proteins were detected in the roots and shoots of rice seedlings grown under dark conditions. The levels of the PHYA proteins were negligible in the shoots and roots under continuous white light irradiation. In contrast, the levels of the