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
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/(m2·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). Immunochemoical 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
PHYB proteins were slightly reduced by white light in the shoots and roots. These results suggest that PHYA and PHYB are present in all organs of rice seedlings and the level of PHYB is predominant in light-grown rice seedlings.

Involvement of PHYA and PHYB in photoinhibition of seminal root elongation in rice seedlings

Because the PHYA and PHYB proteins were detected in the roots, we investigated the roles of the phytochromes in regulating the elongation of the seminal roots. Therefore, we measured the lengths of the seminal roots in the wild type, phyA and phyB mutants grown under different light conditions.

When the seedlings were grown in the dark, the length of the seminal roots was not completely same in the wild type, phyA and phyB mutant seedlings grown under different light conditions.

When the whole seedlings were exposed to white light, the seminal roots in the wild type were inhibited to about 43% of the relative length compared to 59% and 69% of the relative lengths in the phyA and phyB mutants, respectively (Fig. 2). Thus, it was deduced that the light signals perceived by both PHYA and PHYB inhibited the elongation of seminal roots. Moreover, PHYB is the primary photoreceptor for the response under this growth condition.

To determine whether the reduced elongation in the roots was caused by light signals perceived and transmitted by the shoots, we conducted the experiments under partial irradiation. We observed that the length of the seminal roots in the wild type under partial irradiation was similar to that of seedlings grown in the dark (Fig. 2). However, the phyA and phyB mutants under partial irradiation exhibited much longer seminal roots than those in the dark. The values calculated were 127% and 126% of the relative length of the dark-grown seedlings in the phyA and phyB mutants, respectively (Fig. 2). These results suggest that the light signals perceived by the shoot-localized PHYA and PHYB inhibited the elongation of the seminal roots. However, whether the shoot-localized PHYA and PHYB are directly involved in the inhibition of root elongation remain elusive. Nonetheless, when comparing the relative length of light-grown seedlings to those grown under partial light, it was clear that the light signals perceived by the root-localized PHYA and PHYB inhibited the elongation of the seminal roots in rice seedlings.

Involvement of PHYA and PHYB in accumulation and allocation of dry substances in rice seedlings

To identify the roles of phytochromes in accumulating dry substances in rice seedlings, we measured the dry weights of the roots and shoots in the wild type, phyA and phyB mutants grown under different light conditions.
When the seedlings were grown in the dark, the dry substances of roots, shoots and seedlings were not identical in the wild type, phyA and phyB mutants (Supplemental Fig. 2). To dissect the effects of light perceived by phytochromes on dry substances, the dry substances of the wild type, phyA and phyB mutants were calculated with that of the dark-grown seedlings set as 100%.

When the whole seedlings were exposed to white light, the dry weight of roots was clearly increased in the wild type, the phyA and phyB mutants compared to those grown in the dark (Fig. 3-A). This result indicates that white light enhances the accumulation of dry substances in the roots of rice seedlings. However, the dry weight of the roots in the phyA mutant was higher than those in the wild type and the phyB mutant (Fig. 3-A), suggesting that PHYA negatively regulates the accumulation of dry substances of roots. When the seedlings were grown under partial light, similar tendency was observed (Fig. 3-A). When comparing the dry weight of the roots in seedlings grown under the two different light conditions, we found that the dry weight of roots in seedlings grown under partial light was significantly higher than those grown under white light. Thus, it was deduced that light irradiation to the roots reduced the accumulation of dry substances, probably due to the light-triggered, energy-consuming metabolism in roots. However, the phyB mutant had the dry weight of roots similar to the wild type regardless of light conditions (Fig. 3-A), indicating that PHYB did not affect the accumulation of dry substances in roots.

When the whole seedlings were exposed to white light, the dry weight of shoots was increased in the wild type seedlings compared to dark-grown seedlings (Fig 3-B), suggesting that white light enhances the accumulation of dry substances in shoots of rice seedlings. However, the phyA and phyB mutants had statistically lower dry weight of shoots than the wild type (Fig. 3-B). These results indicate that PHYA and PHYB play important roles in the accumulation of dry substances in shoots. When the seedlings were grown under partial light, the dry weight of shoots was significantly decreased in the phyA mutant relative to that of wild type seedlings (Fig. 3-B). By comparison with the wild type seedlings, the phyB mutant had lower dry weight of shoots, but the difference was not significant (Fig. 3-B). These results suggest that PHYA positively regulates the accumulation of dry substances in shoots, whereas the role of PHYB is negligible.

Thus, we hypothesize that PHYA plays the contrary roles in regulating the accumulation of dry substances in the shoots and roots. However, we can not explain why PHYB does not obviously affect the dry weight of shoots in seedlings with partial irradiation at present.

By calculating the dry weights of seedlings grown under different conditions, the dry weight of seedlings grown in the light was much higher than those grown in the dark (Fig. 3-C). The phyA seedlings had similar dry weights to those of wild type, due to the combined effect of increased dry weight of roots and decreased dry weight of shoots in the phyA mutant. However, the phyB seedlings had reduced dry weight (Fig. 3-C), mainly resulting from the decreased dry weight of
shoots when whole seedlings were exposed to white light.

**DISCUSSION**

**Root-localized phytochrome proteins are involved in photoinhibition of seminal root elongation**

We determined the contribution of PHYA and PHYB in the photoinhibition of seminal root elongation. In the present study, the elongation of seminal roots was inhibited by white light, and the photoinhibitory effects were weakened significantly in the phyB and phyA mutants relative to those in the wild type (Fig. 2). These results suggest that the photoinhibitory responses induced by white light are mediated by PHYA and PHYB. Moreover, the inhibitory phenotype was less severe in the phyB mutant than in the phyA mutant, indicating a prominent role for PHYB in this response. This result is consistent with a recent observation reported by Shimizu et al (2009). Furthermore, Correll and Kiss (2005) also linked the inhibition of root elongation induced by red light to phytochromes in *Arabidopsis*. In the present study, the partial irradiation did not inhibit root elongation, in contrast to the inhibitory responses observed when both the roots and shoots were irradiated with white light (Fig. 2). Thus, we concluded that root-localized phytochromes have primary roles in regulating root elongation in rice seedlings. This conclusion is also supported by both of the reports referenced above (Correll and Kiss, 2005; Shimizu et al, 2009). Noticeably, a very small amount of PHYA protein were only detected in the roots, not in the shoots, of light-grown seedlings, which probably make a contribution to the stronger inhibition of the root elongation in the wild type compared to the phyA mutant (Fig. 2). On the contrary, although PHYA protein could not be detected in shoots (Fig. 1), the wild type seedlings had shorter coleoptile than the phyA mutants grown in the white light (Supplemental Fig. 3).

How do phytochromes regulate the growth and development of roots? Recently, Salisbury et al (2007) revealed that root-localized PHY-GFP showed light-regulated nuclear translocation characteristics similar to those described for shoot phytochromes. Nuclear translocation is thought to be necessary for phytochrome-mediated responses (Nagatani, 2004). Several factors acting the downstream of phytochromes in light signaling in roots have previously been characterized in shoots in *Arabidopsis* (Molas et al, 2006), suggesting that the molecular mechanism for phytochrome-mediated responses is somehow conserved in roots and shoots. Indeed, models of PHYA and PHYB in the photoinhibition of seminal roots are essentially the same as those in the photoinhibition of coleoptile growth in rice (Xie et al, 2007; Shimizu et al, 2009, 2010).

**Shoot-localized phytochrome proteins contribute to photoinhibition of seminal root elongation**

As shown in Fig. 2, the partial irradiation did not inhibit the seminal roots elongation in the wild type. On the contrary, the promotive effects of partial irradiation on root elongation were significant in the phyA and phyB mutants relative to those in the wild type, indicating that PHYA and PHYB play important roles in inhibiting root elongation even when the roots were blocked from the light.

How does the light signal intercepted from above-ground portions influence the phytochrome-mediated inhibition of root elongation? Previous work has confirmed that light is conducted axially from the shoots to the roots via the vascular tissue, with wavelengths in the 710–940 nm range being transmitted with the greatest efficiency (Sun et al, 2003). Given the photoperception properties of PHYA to FR light, we speculate that the root-localized PHYA is the main photoreceptor to inhibit root elongation in the seedlings grown under partial irradiation. However, our data suggest important roles for PHYB in inhibiting seminal root growth (Fig. 2). Thus, we deduced that the PHYA and PHYB localized in the shoots perceive light signals to modulate root elongation. Our deduction was supported by Salisbury et al (2007). In soil-grown *Arabidopsis* seedlings, PHYB activation by axially conducted light is unlikely to play a significant role in controlling root development based on the nuclear translocation characteristics of PHYB-GFP (Salisbury et al, 2007). Taken together, these results support the speculation that the shoot-localized phytochromes exert their control on the roots through a long distance signal.

How do shoot-localized phytochromes function in inhibiting root elongation? It is well established that auxin exerts a major influence on root growth and development. Several reports have shown that phytochromes regulate a subset of auxin-responsive genes and genes encoding components of the complex auxin transport machinery, including *IAA1, SHY2/IAA3* (Tian et al, 2002; Devlin et al, 2003), *PIN3* and *PIN7* (Sidler et al, 1998; Tian et al, 2002; Devlin et al, 2003; Lin...
Therefore, it is possible that the roles of the shoot-localized phytochromes regulate root elongation by controlling auxin transport and auxin responses. However, phytochromes were recently reported to be involved in the metabolism and signaling of abscisic acid (ABA) (Seo et al, 2006; Chen et al, 2008; Oh et al, 2009). Saab et al (1990) reported a role of ABA in root elongation in maize. Recently, Iwamoto et al (2011) confirmed that phytochromes controlled the internode elongation by regulating the expression of gibberellins (GA) and ethylene biosynthesis genes in rice. In addition, several reports revealed that there was a close link between jasmonate (JA) and phytochromes in rice (Riemann et al, 2003; Syvatyna and Riemann, 2012). Our group also observed that phytochromes influenced the transcript levels of ABA, GA, JA, ethylene metabolite and signaling genes in rice (Liu et al, 2010; Xie et al, 2011). Thus, the shoot-localized phytochromes may control root elongation via combined effects of diverse hormone-mediated mechanism in rice seedlings.

Because the shoot-localized phytochromes act to inhibit seminal root elongation, it is not clear why seminal roots of seedlings grown under partial irradiation were as long as those grown in the dark in the wild type (Fig. 2). Kurata and Yamamoto (1997) suggested that white-light irradiation of the whole seedling promoted root growth primarily by photosynthetic activity. In this context, it is hypothesized that the promotive effect of photosynthetic activity counteracts the inhibitory effect of the phytochrome-perceived light signal.

**Phytochromes are involved in regulating accumulation of dry substances in rice**

In this study, we determined that PHYB was involved in the accumulation of dry substances in shoots, but not in roots when the whole seedlings were exposed to white light (Fig. 3-A and -B). However, PHYA plays the contrary roles in the accumulation of dry substances in roots and shoots (Fig. 3-A and -B). These results led us to conclude that phytochromes play important roles in rice photosynthesis. Jumtee et al (2009) investigated the relationship between rice phytochrome signaling and metabolism and revealed that phytochromes play crucial roles in sugar metabolism, carbon partitioning, sugar transportation or a combination of the latter in rice. However, when the seedlings were grown under partial irradiation, the dry weight of seedlings in the phyB mutant is similar to that in the wild type, which differed from the observation when the whole seedlings were exposed to light. The latter phenomenon may result from the root-localized PHYB perceiving light signals and regulating energy-consuming metabolites in both roots and shoots.

Our findings in this study provide insights into the function of phytochromes in the coordination between the above-ground parts and underground parts in rice seedlings. However, whether our experimental results in rice seedlings represent the situation at other developmental stages of rice should be further investigated. Based on previous reports, we hypothesize that phytochromes may coordinate shoot and root development by regulating hormone metabolism and signaling. However, the molecular basis of this control remains to be explored.

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**SUPPLEMENTAL DATA**

The following materials are available in the online version of this article at http://www.sciencedirect.com/science/journal/16726308; http://www.ricescience.org.

Supplemental Fig. 1. Effects of light on root lengths in wild type, phyA and phyB mutants grown under different conditions.

Supplemental Fig. 2. Effects of white light on dry weights in wild type, phyA and phyB mutants grown under different conditions.

Supplemental Fig. 3. Effects of light on coleoptile length in wild type, phyA and phyB mutants grown under different conditions.

**REFERENCES**


