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 dry weights of seedlings were measured in wild type as well as 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. Moreover, we reveal that phyA plays the reverse effects on the accumulation of dry substances in roots and shoots in rice seedlings.

Key words: rice; phytochromes; 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 light (R) and far-red (FR) 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 change 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 (Yoshikazu and Fujiwara, 1967). Recently, root-localized phyA and phyB were found to function in the photo inhibition 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 contributions to photoinhibition of root elongation (Correll and Kiss, 2005; Shimizu et al, 2009). However, phyA and phyB mutant seedlings had longer roots when being grown in the conditions with roots blocked from white light than they did when being grown in darkness. Thus, our result indicates the role of shoot-localized phytochrome proteins in inhibiting the root elongation; this contradicts the observation described in both Arabidopsis and rice (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 phyA phyB phyC triple mutants compared with those in the wild type in rice by metabolomics approach. Thus, we speculate that phytochromes probably affect accumulation and
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 role 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 the 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 irradiation of the shoots (the seedling roots were blocked from white light). In experiments with the roots blocked from the 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 the 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).

Immunoblotting analysis

Seven-day-old seedlings of one gram were homogenized with 2 mL 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 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). Immunochemical 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 mutant 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 mutant 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 means ± 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 level of the PHYA protein was 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
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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. We measured the lengths of the seminal roots in the wild type, phyA and phyB mutant seedlings 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 mutants (Supplemental Fig. 1). To dissect the effects of light perceived by phytochromes on seminal root growth, the relative lengths of the wild type, phyA and phyB mutants were calculated with the root length of the dark-grown seedlings set as 100%.

When the 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 this response under this growth condition.

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 root, shoots and seedlings were not identical in the wild type, phyA and phyB mutants (Supplemental Fig. 3). To dissect the effects of light perceived by phytochromes on dry substances, the dry substances of the wild type, phyA and phyB mutants

![Fig. 1. Immunoblot analysis of PHYA and PHYB proteins in rice seedlings.](image1)

![Fig. 2. Effect of light on root elongation in wild-type, phyA and phyB mutants grown under different conditions.](image2)
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 the roots was clearly increased in the wild type, phyA and phyB mutants compared to the seedlings grown in the dark (Fig. 3-A). This result indicates that white light enhances the accumulation of dry substances in roots of rice seedlings. However, the dry weight of the roots in the phyA mutant was higher than that of the wild type and phyB mutant (Fig. 3-A), suggesting that phyA negatively regulates the accumulation of dry substances of roots. When the seedlings were grown with only partial irradiation of the shoots, the same tendency were observed (Fig. 3-A). When comparing the dry weight of the roots in seedlings grown in the two different light conditions, it was found that dry weight of roots in seedlings grown with only partial irradiation of the roots was significantly higher than those grown under white light. Thus, it was deduced that light irradiation to the roots reduced the accumulation of the dry substances, probably due to the light-triggered, energy-consuming metabolism in roots. However, phyB mutants have the same dry weight of roots as the wild type regardless of light conditions (Fig. 3-A), indicating that phyB did not affect the accumulation of the dry substances of roots.

When whole seedlings were exposed to white light, the dry weight of shoots was increased in the wild-type compared to dark-grown seedlings (Fig 3-B), suggesting that white light enhances the accumulation of dry substances in shoots of rice seedlings. However, phyA and phyB mutant 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 with only partial irradiation of the shoots, the dry weight of shoots was significantly decreased in phyA mutants relative to that of wild type seedlings (Fig. 3-B). By comparison with wild type seedlings, phyB mutants have lower dry weight of shoots, but the difference is not significant (Fig. 3-B). These results suggest that phyA positively regulates the accumulation of dry substances in shoots, while the role of phyB is negligible. Thus, we hypothesize that phyA plays the reverse 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 weight of seedlings grown under different conditions, the dry weight of seedlings grown in the light was much higher than that of seedlings 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 phyA mutants. 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 phenotype than in the phyA mutant, indicating a prominent role for phyB in this response. This result is consistent with a recently reported observation by Shimizu et al (2009). Furthermore, Correll and Kiss et al (2005) also linked the inhibition of root elongation induced by red light to phytochromes in Arabidopsis. In the present study, the partial irradiation of shoots 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 (Correl 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 then do phytochromes regulate the growth and development of roots? Recently, Salisbury et al (2007) revealed that root-localized phy-GFP shows 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 of 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 of the shoots did not inhibit seminal roots elongation in the wild type. On the contrary, the promotive effects of partial irradiation of the shoots 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 wave-lengths 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 of the shoots. 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 data 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 phytochrome regulates a subset of auxin-responsive genes and components of the complex auxin transport machinery, including IAA1, IAA3/SHY2 (Tian et al, 2002; Devlin et al, 2003), PIN3 and PIN7 (Sidler et al, 1998; Tian et al, 2002; Devlin et al, 2003; Lin and Wang, 2005). 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 for ABA in root elongation in maize. Recently, Iwamoto et al (2011) confirmed that rice phytochromes control the internode elongation by regulating the expression of gibberellins (GA) and ethylene biosynthesis genes in rice. In addition, several reports reveal that there is a close
link between jasmonate (JA) and phytochromes in rice (Riemann et al, 2003; Syvatyna and Riemann, 2012). Our group also observed that phytochromes influences 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 from seedlings grown under the partial irradiation of shoots 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 promotes 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 the accumulation of dry substances in rice**

In this study, we determined that phyB is involved in the accumulation of dry substances in shoots, but not in roots when whole seedlings are exposed to white light (Fig. 3-A and -B). However, phyA plays the reverse 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. Juntee 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 transport or a combination of the latter in rice. However, when the seedlings were grown under the partial irradiation of shoots, the phyB mutant showed the same dry weight as the wild type, a situation contrary to that observed when 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 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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**REFERENCES**


Lin R C, Wang H Y. 2005. Two homologous ATP-binding cassette transporter proteins, AtMDR1 and AtPGP1, regulate *Arabidopsis* photomorphogenesis and root development by mediating polar...


