Light exposure is an important environmental factor which breaks seed dormancy in many plant species. Phytochromes have been identified as playing a crucial role in perception of the light signal that releases seed germination in Arabidopsis. Phototropins (Phot1, Phot2) are blue/UV-photoreceptors in plants which mediate phototropic responses, chloroplast relocation, hypocotyl growth inhibition and stomata opening. We studied germination under different light conditions in Arabidopsis Phot1-null and Phot2-null mutants and in a double phot1phot2 mutant. Germination of single phot1 and phot2 mutants in darkness was much lower than in wild-type (WT) seeds, whereas double phot1phot2 mutant lacking both functional phototropins germinated at frequency comparable to WT seeds, irrespective of light and temperature conditions. Light treatment of imbibed seeds was essential for effective germination of phot1, irrespective of low-temperature conditioning. In contrast, cold stratification promoted dark germination of phot2 seeds after imbibition in dim light. Low germination frequency of phot1 seeds under low light intensity suggests that the presence of functional Phot1 might be crucial for effective germination at these conditions. The lower germination frequency of phot2 seeds under continuous light suggests that Phot2 might be responsible for stimulating germination of seeds exposed to direct daylight. Thus, the phototropin system may cooperate with phytochromes regulating the germination competence of seeds under different environmental conditions.

**Key words:** Photomorphogenesis, seed germination, phototropins, light signalling, Arabidopsis thaliana.

**INTRODUCTION**

In many plant species the transition of seeds from dormancy to germination is controlled by environmental factors. In Arabidopsis, most important are light exposure and temperature decrease (Finch-Savage et al., 2006; Holdsworth et al., 2008). Phytochromes have been shown to play a crucial role in the control of germination in Arabidopsis (for reviews see: Casal and Sanchez, 1998; Bae and Choi 2008). In particular, phytochrome B has been identified as a photoreceptor involved in promotion of germination of dark-imbibed seeds by a pulse of red light, through a classical red-far red reversible low-fluence response (LFR) mode (Casal and Sanchez, 1998). Action spectroscopy experiments with mutants that lack functional phytochrome A (phyA) and phytochrome B (phyB) demonstrated that phytochrome A is a photoreceptor involved in promotion of germination via the very low fluence response (VLFR) (Shinomura et al., 1996). More recently, phytochrome E was shown to be indispensable for induction of seed germination under continuous far red (FR) light (Hennig et al., 2002), and the phytochrome-interacting basic helix-loop-helix protein PIL5 has been identified as a key negative regulator of seed germination (Oh et al., 2004).

Phototropins are blue/UV-A photoreceptors in plants, characterized recently (Briggs and Christie, 2002; Christie, 2007). Two members of the phototropin family present in Arabidopsis, Phot1 and Phot2, showing close sequence similarity, have been demonstrated to initiate the phototropic responses of hypocotyls and stems (Liscum and Briggs, 1995; Huala et al., 1997; Sakai et al., 2001) and blue-light-induced chloroplast relocation in mesophyll cells (Jarillo et al., 2001; Kagawa and Wada, 2000). Phototropins play a role in such diverse plant processes as stomata opening (Kinoshita et al., 2001), leaf expansion (Sakamoto and Briggs, 2002),...
hypocotyl growth inhibition (Folta and Spalding, 2001) and light-induced mRNA destabilization in greening cotyledons (Folta and Kaufman, 2003).

In this work we quantitatively analyzed germination under environmentally realistic light and temperature conditions in phot1 and phot2 mutants and in a double phot1phot2 mutant lacking functional phototropins. Our results suggest that phototropins 1 and 2 are involved in regulation of the transition from dormancy to germination in Arabidopsis seeds.

MATERIALS AND METHODS

PLANT MATERIAL AND SEED CULTURE

The Arabidopsis thaliana wild-type line of the Columbia ecotype (Col-0) was obtained from the Arabidopsis Biological Resource Center, Ohio State University, USA. The mutant lines used in this work were phot1 (Liscum and Briggs, 1995), phot2 (Jarillo et al., 2001) and a double phot1phot2 mutant. The seed batches used in all experiments were obtained from plants grown under standardized conditions in a conditioned growth chamber at constant 22±2°C and 65% humidity under a 12 h photoperiod (Sylvania Luxline Plus daylight fluorescent lamps, fluence 80–120 μmol m⁻²s⁻¹). At least two independent seed batches were used for germination tests.

LIGHT TREATMENT AND GERMINATION ASSAY

White light (WL, 60 μmol m⁻²s⁻¹) was delivered by Osram fluorescent tubes (36 W/20). Light fluence was measured with an SKP 215 PAR quantum sensor (Skye Instruments Ltd., UK).

For experiments including imbibition under light, wild-type and mutant seeds were sterilized for 20 min in 3% hypochlorite with 0.1% Triton X-100 added (Serva, Germany), washed thoroughly with sterile deionized water and sown in Petri dishes (typically 50–150 seeds of each tested plant line per plate) containing Murashige and Skoog medium (Sigma-Aldrich, St. Louis, USA) with 1% agar as described by Malec et al. (2002). The seeds were imbibed in dim white light (<10 μmol m⁻²s⁻¹) delivered through a Schott NG glass filter from a white fluorescent lamp for 2 h including sterilization time.

For experiments including imbibition in darkness, the seeds were either surface-sterilized and grown on 1% MS agar as above or else sown dry on wet tissue paper. The seeds were then imbibed in total darkness for 2 h. All subsequent handling of the imbibed seeds was done in total darkness. Neither surface-sterilization nor growth conditions (tissue paper vs. MS agar) had a detectable effect on germination frequency.

Imbibed seeds were directly subjected to a given light treatment (3 h or continuous white fluorescent light) or stratified at 4°C for 48 h and grown at 22°C for 4 days in darkness or irradiated with white fluorescent light (60 μmol m⁻²s⁻¹) for 3 h and grown at 22°C for 4 days in darkness.

STATISTICS

The experiments were done in at least three replicates (typically 50–150 seeds each) for each light treatment and each tested seed line. The data are presented as means ± standard error. Statistical significance was determined with Student’s t-test at p<0.05. The experiments were repeated at least five times, with consistent results.

RESULTS AND DISCUSSION

During our work with phot mutants we noted that phot1 seeds germinated poorly or not at all if the material was kept in darkness during or after imbibition, irrespective of stratification by chilling (Fig. 1a). They germinated normally if exposed to light for 3 h between the stratification period and four days of further growth in darkness (Fig. 1b). To examine the potential effect of phototropins on the dormancy-to-germination transition we measured the germination frequencies of phot1, phot2 and double phot1phot2 mutant seeds under different light and temperature treatments.

For seeds imbibed in darkness, phot1 and phot2 mutants had lower dark-germination (<2%) than wild-type (WT) seeds (~15%). Stratification for 48 h at 4°C significantly stimulated the dark-germination of WT seeds to ~60% and slightly stimulated the light-independent dark-germination of phot2, which reached ~10%. Stratification did
not stimulate dark-germination of phot1 (Fig. 2a,b, D bars).

Irradiation of dark-imbibed seeds with white light (60 μmol m⁻² s⁻¹) for 3 h prior to germination in darkness significantly enhanced the dark-germination of WT seeds to ~90%. Germination of the phot2 mutant reached ~36%; germination of phot1 (<2%) did not increase. When stratified seeds were irradiated as above, phot2 germination was comparable to that of non-irradiated dark-germinated WT seeds (63%). Germination of phot1 was below 10% under those conditions (Fig. 2a,b, WL (3 h) bars). Both phot1 and phot2 mutants showed lower germination frequencies under continuous light, with no stimulatory effect of stratification (Fig. 2a,b, WL (cont.) bars).

VLFR promotion of germination is saturated at very low concentrations of far-red-absorbing phytochrome form (Pfr) (Smith and Whitelam, 1990). To study the effect of VLFR stimulation on the germination of phototropin mutants, seeds were imbibed in dim light for 2 h and then germinated in darkness. In these conditions the dark-germination of WT seeds was boosted to 35%. Stratification further enhanced this effect to 76%. In contrast, phot1 and phot2 mutants showed less than 10% dark-germination. Stratification significantly stimulated the dark-germination of phot2 to a level close to that of WT seeds (70%) but had no such effect on phot1 seeds (<10% germination) (Fig. 2c.d, D bars).

When light-imbibed seeds were exposed to white light for 3 h prior to germination in darkness, germination of WT seeds roughly doubled (68%) versus dark-germinated material. Under these conditions the phot1 and phot2 mutants reached ~32% germination, comparable to that of dark-germinated light-imbibed WT seeds. When stratified seeds were irradiated as above, WT seed germination (82%) did not significantly differ from dark-germination of light-imbibed WT seeds (76%). Under these conditions, phot2 seeds reached 53% germination, significantly lower than the dark-germination of light-imbibed phot2 seeds (70%). In contrast, phot1 exceeded 90% germination frequency under these conditions (Fig. 2c.d, WL (3 h) bars).

When light-imbibed seeds germinated under continuous white light, germination frequency
increased in WT (75%) and phot1 (89%); phot2 germination reached 40%, comparable to that of dark-germinated WT seeds. Stratification did not significantly alter germination of WT (83%) and phot1 (94%) seeds under continuous light, and reduced germination of phot2 seeds to 31% (Fig. 2c,d, WL (cont.) bars).

Surprisingly, our analysis of light-induced germination showed that the double phot1phot2 mutant lacking both functional phototropins germinated at frequencies comparable to WT, irrespective of light and temperature conditions (Fig. 3a–d). phot1phot2 seed germination in the dark equaled that of WT, and chilling boosted it markedly (Fig. 3a,b, D bars). The only statistically significant differences were noted under continuous light. In these conditions, dark-imbibed phot1phot2 seeds had slightly lower germination than WT seeds [Fig. 3b, WL (cont.) bars], while light-imbibed non-stratified seeds of this line had slightly higher germination [Fig. 3c, WL (cont.) bars] in a manner similar to that of phot1 seeds under these conditions. These results indicate that although functional phototropins are not essential for a photoinduction of seed germination in Arabidopsis, they may modulate processes involved in the dormancy-to-germination transition.

Germination of single phot1 and single phot2 mutants in darkness was much lower than for WT. Extensive light treatment of dark-imbibed seeds was essential for effective promotion of the germination of both single mutants. Germination of the phot1 mutant was most effective under continuous light, but the maximum germination frequencies achieved by phot mutants were below the control level (WT seeds). These findings suggest that phototropins are factors modulating light-induced promotion of seed germination at low light intensities. Interestingly, a wide spectrum of light (from near UV to far-red) was found to induce VLFR-dependent germination, and the action spectrum of VLFR induction contains a broad maximum at ~400 nm (UV/blue light) (Shinomura et al., 1996). Crosstalk in photoreceptor signaling and integration of light signals with other environmental stimuli were observed in different experimental systems (Franklin et al., 2005). In Arabidopsis, co-action between phytochrome and phototropin systems has been demonstrated for regulation of hypocotyl growth inhibition (Folta and Spalding, 2001) and light-induced chloroplast relo-

Fig. 3. Induction of germination of Col 0 and phot1phot2 double mutant. (a) Imbibition in darkness for 2 h. (b) Imbibition in darkness for 2 h followed by stratification for 48 h at 4°C. (c) Imbibition in dim light (<10 μmol m⁻² s⁻¹) for 2 h. (d) Imbibition in dim light (<10 μmol m⁻² s⁻¹) for 2 h followed by stratification for 48 h at 4°C. All experimental conditions as in Figure 2.
Phototropins modulate seed germination in Arabidopsis thaliana

**Fig. 4.** Tentative scheme of the role of phototropins in control of the dormancy-to-germination transition of Arabidopsis seeds. Light sensed by the phototropin system may modulate the activity of a phytochrome-triggered signaling pathway and/or influence other processes (e.g., temperature-dependent) involved in promoting seed germination.

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