

A new role for phytochromes in temperature-dependent germination

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Summary

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- Germination timing is a fundamental life-history trait, as seedling establishment predicates realized fitness in the wild. Light and temperature are two important cues by which seeds sense the proper season of germination. Using *Arabidopsis thaliana*, we provide evidence that phytochrome-mediated germination pathways simultaneously respond to light and temperature cues in ways that affect germination.
- Phytochrome mutant seeds were sown on agar plates and allowed to germinate in lit, growth chambers across a range of temperatures (7°C to 28°C).
- *phyA* had an important role in promoting germination at warmer temperatures, *phyE* was important to germination at colder temperatures and *phyB* was important to germination across a range of temperatures.
- Different phytochromes were required for germination at different temperatures, indicating a restriction or even a potential specialization of individual phytochrome activity as a function of temperature. This temperature-dependent activity of particular phytochromes reveals a potentially novel role for phytochrome pathways in regulating the seasonal timing of germination.

Key words: *Arabidopsis thaliana*, ecology, germination, life history, phytochrome, temperature.

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Introduction

The timing of germination determines the environment experienced by plants throughout their lives (Donohue, 2003, 2005). Therefore, accurate assessment of a seasonal environment by seeds is crucial to the lifetime fitness of plants. Light and temperature are two major cues through which seeds sense the proper season of germination (Spalding & Folta, 2005), but little is known about the integration of light and temperature information to elicit germination (Fielding *et al.*, 1992; Yamaguchi & Kamiya, 2000; Steckel *et al.*, 2004). In particular, the genetic basis of germination responses to temperature is poorly understood. Temperature varies geographically and is also predicted to change with shifts in the global climate. Therefore, appropriate germination responses to temperature are necessary

for successful establishment after long-distance dispersal and could be crucial for preserving adaptive life-history phenology under a changing climate. Understanding the genetic basis of germination sensitivity to temperature is fundamental for dissecting the interactions between ecology and germination and predicting responses to climate change scenarios. Using *Arabidopsis thaliana*, we demonstrate that phytochrome-mediated germination pathways are sensitive to both light and temperature, revealing a potentially novel role for phytochrome pathways in regulating the seasonal timing of germination.

Arabidopsis thaliana (Brassicaceae) is a highly selfing, weedy annual. The typical life history displayed by *A. thaliana* is a 'winter annual' where seeds germinate in the autumn, overwinter as rosettes, and reproduce in the spring. Some populations display a 'spring annual' life history in which seeds germinate

in the early spring and reproduce later in the spring. An 'autumn annual' life history has also been observed in some populations, in which plants germinate and flower in the autumn (Thompson, 1994; Griffith *et al.*, 2004). The mechanism determining these different life history strategies in *A. thaliana* is influenced by both germination timing and vernalization requirements for flowering (Napp-Zinn, 1976; Nordborg & Bergelson, 1999). Therefore germination is a key determinant of life-history strategy and predates realized fitness in the wild.

Global climate models predict changes to the environment that would directly alter the conditions that influence germination. Global climate change is predicted to influence temperature and precipitation and the variation of these factors (Watson *et al.*, 1997). This study focuses particularly on the predicted changes in temperature, because temperature represents one of the most important germination cues for seeds (Pons, 2000). Germination cueing to temperature has the potential to influence successful climate tracking and thereby influence species distributions. Since phytochrome genes have known importance to germination and have been shown to have temperature-dependent effects on flowering time (Halliday & Whitelam, 2003), phytochromes are likely to be important influences on phenological changes accompanying climate change and range expansion.

Phytochromes regulate germination responses to light. In *A. thaliana*, the genes *PHYA*, *PHYB*, *PHYC*, *PHYD* and *PHYE* encode five distinct phytochromes, which are photoreversible biliproteins. Phytochromes are synthesized in the inactive red-absorbing (Pr) form (Casal & Sanchez, 1998; Franklin & Whitelam, 2004), and red light converts them to the bioactive far-red absorbing (Pfr) isomer that is involved in light-stimulated germination (Whitelam & Devlin, 1997); Pfr reverts to Pr in the dark. *PHYA* encodes light-labile phyA whereas the other phytochrome genes (*PHYB-E*) encode apoproteins that are more light-stable upon photoconversion to Pfr (Whitelam & Devlin, 1997). Active phytochrome regulates the synthesis of gibberellin (GA), which promotes germination (Toyomasu *et al.*, 1998; Yamaguchi *et al.*, 1998; Yamaguchi & Kamiya, 2000; Garcia-Martinez & Gil, 2002; Peng & Harberd, 2002; Ogawa *et al.*, 2003). Because temperature affects GA concentration, sensitivity to GA (Derckx & Karssen, 1993; Yamaguchi *et al.*, 2004), rate of dark reversion (Kristie & Fielding, 1994; Hennig & Schafer, 2001), and possibly the rate of phytochrome photoconversion (Pons, 1986), phytochromes may have temperature-dependent effects on germination. Therefore, we examined the relative contributions of different phytochromes to germination across a range of temperatures, focusing on phyA, phyB, and phyE after preliminary observations. We first assessed the germination frequencies of single and multiple loss-of-function phytochrome mutants at 10°C and 22°C. We then assessed their germination at a range of temperatures (7°C, 13°C, 16°C, 19°C, 25°C and 28°C). The range of temperatures used in the experiment spans that of

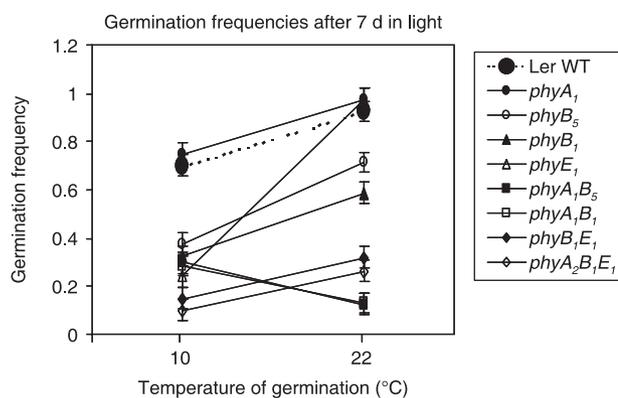


Fig. 1 Germination frequency after 7 d in the light for *Arabidopsis thaliana* single and multiple phytochrome loss-of-function mutants compared with wild type (Ler WT). The data represent mean values pooled across blocks in Expt 1. Large closed circles, *Landsberg erecta*; small filled circles, *phyA-201*, stock #CS6219; open circles, *phyB-5*, #CS6213; filled squares, *phyA-201/phyB-5*, #CS6224; filled triangles, *phyB-1*; open squares, *phyA-201/phyB-1*; open triangles, *phyE-1*; closed diamonds, *phyB-1/phyE-1*; open diamonds, *phyA-2/phyB-1/phyE-1*.

different seasonal temperatures across a range of latitude. The temperature intervals were chosen to represent the degree of temperature alterations expected based on models of global climate change. The study therefore aims to investigate the changing role of different phytochromes in responding to changing environmental temperatures due to global climate change and range expansion.

Materials and Methods

Plant material

Phytochrome mutants were generated by chemical mutagenesis and gamma radiation of the *Landsberg erecta* (henceforth Ler) background of *Arabidopsis thaliana* (L.) Heynh. (Brassicaceae) (see Fig. 1 for mutant list). Phytochrome mutant lines on the Ler background were supplied from three sources. Lines obtained from TAIR (The *Arabidopsis* Information Resource) included the monogenic *phyA* and *phyB* mutants as well as a double *phyA/phyB* knockout. These TAIR line mutations were created through chemical mutagenesis (Nagatani *et al.*, 1993). Lines generated by the R. A. Sharrock laboratory represented the monogenic *phyA* and *phyB*, and the double mutant *phyA/phyB* (Fig. 1). Mutants with a *phyE* deficiency (*phyE*, *phyB/phyE* and *phyA/phyB/phyE*) were generated by the G. C. Whitelam laboratory (Fig. 1). The *PHYA* allele in the triple mutant differs from the *PHYA* allele in the other lines.

Experimental conditions

All lines were grown at 22°C and 12 h of light to generate experimental seeds. The seeds from these maternal plants were

first used to assess germination frequency at two temperatures (10°C and 22°C: Expt 1), and then at six temperatures (7°C, 13°C, 16°C, 19°C, 25°C, and 28°C: Expt 2). Twelve seeds per line were individually placed onto replicate Petri plates containing 0.5% agar, dark- and wet-stratified at 4°C for 5 d to break dormancy and then transferred to light.

For the first experiment, seeds were transferred after cold stratification to either 10°C or 22°C in Percival germination incubators (Percival Scientific, Inc., Perry, IA, USA). Seeds were given a 12-h photoperiod of white fluorescent light with a photon flux density (PFD) of approx. 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The germination experiment was conducted during December 2003, with eight replicate plates per line per temperature treatment. Germination was scored after 0 d and 7 d in the light and seed viability was determined after 7 d.

For the second experiment, seeds were transferred after cold stratification to 7°C, 13°C, 16°C, 19°C, 25°C, or 28°C in Conviron E7/2 growth chambers (Controlled Environment Ltd., Winnipeg, Manitoba, Canada). Seeds were given a 12-h photoperiod of white fluorescent light set to half intensity, with a photon flux density (PFD) of approx. 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$, such that light quality and quantity were comparable across the two experiments. The experiment was conducted in two temporal blocks, during August and September 2004. Each temporal block had 14 lines and four replicate plates per temperature treatment for a total of 672 plates. At each temperature, germination was scored after 0, 7, and 10 d in the light. Viability of seeds was assessed after 7 and 10 d in the light.

Data analysis

All statistical analyses were performed with the Statistical Analysis System (version 8.2; SAS Institute, Cary, NC, USA). The frequency of germination in the light was calculated as the number of germinants at 7 d, for example, minus the number of 0-d germinants, divided by the total number of viable seeds (the total number of seeds minus the number of dead seeds). Dead seeds were assessed by testing the firmness of hydrated seeds with microforceps; seeds without active embryos become less turgid as the endosperm degrades with time. To test whether the response of each mutant to temperature differed significantly from that of the wild type (Ler), we conducted a series of ANOVAs that included one mutant and the wild type in a model, with fixed factors of block, line, and temperature and the interaction between line and temperature. A significant interaction between line and temperature would indicate that the response of the mutant differed from that of the wild type. We next tested across blocks for a significant response to temperature for each line separately with a two-factor ANOVA (treatment effect was significant at $P=0.05$ across all lines, results not presented), and then tested for significant differences between the mutant and wild type at each temperature separately using nonparametric Kruskal–Wallis tests. We also used Kruskal–Wallis nonparametric contrasts to test for

significant differences in germination between specific pairs of mutants in each temperature treatment. To test whether *PHYA*, *PHYB*, and *PHYE* allelic effects were additive, we scored wild type, single mutants, and double mutants for functionality in the three phytochromes using dummy variables, with 1 being a functional state, and 0 being a null state. Using ANOVA, we tested for significant interactions between allelic states of the different phytochromes, which would indicate nonadditive contributions of different phytochromes to germination – namely that the effect of functionality of a given phytochrome depends on the functional state of the other phytochrome. Thus, we defined additive effects as independent effects of phytochrome alleles on germination. We tested for these interactions at both temperatures (10°C, 22°C) in Expt 1 and at the extreme temperatures (7°C, 28°C) in Expt 2 (the temperatures in which the mutational effects were most pronounced).

Results

Expt 1: 10°C and 22°C

The only mutants that did not differ from wild type were the *phyA* single mutant at both temperatures and the *phyE* single mutant at 22°C (Fig. 1, see the Supplementary Material, Table S1). All lines, including the wild type, responded significantly to temperature ($P < 0.05$ in all cases), with most lines germinating to higher proportions at 22°C than at 10°C. However, both *phyA/phyB* double mutants had lower germination at 22°C than at 10°C. The *phyE* single mutant had strongly reduced germination at 10°C, and therefore had a greater response to temperature than the wild type.

At 22°C, disrupting *phyB* function significantly reduced germination, as indicated by the significantly lower germination of both *phyB* single mutants. While the single *phyA* mutant did not have reduced germination compared with wild type, both *phyA/phyB* double mutants had lower germination than the single *phyB* mutant (see the Supplementary Material, Table S2), indicating that *phyA* also contributed to germination at 22°C. The *phyA/phyB* double mutants had up to 80% reduced germination at 22°C compared with the wild type (Fig. 1), and this difference was statistically significant (Table S1). The contributions of *PHYA* and *PHYB* to germination at 22°C were nonadditive (Table 1), with the contribution of *PHYA* being much more pronounced on a *phyB* background. However, germination of the *phyA/phyB/phyE* triple mutant was the same as that of the *phyB/phyE* double mutant, indicating that disruption of *phyA* in addition to both *phyB* and *phyE* did not significantly reduce germination further.

Germination was most strongly influenced by *PHYB* at 22°C, followed by nonadditive contributions by *PHYA*, and then *PHYE*. Disruption of *phyE* function alone did not influence germination at 22°C (Table S1; Fig. 1), but disrupting it on a *phyB* background did significantly reduce germination,

Table 1 Tests of phytochrome additive gene contributions to germination at different temperatures

Source	7°C	10°C	22°C	28°C
<i>PHYA</i> ₁	28.4816***	0.0074	39.1784***	705.6000***
<i>PHYB</i> ₁	19.3251***	54.1101***	329.3414***	705.6000***
<i>PHYA</i> ₁ × <i>PHYB</i> ₁	26.5087***	0.6234	60.7350***	739.6000***
<i>PHYA</i> ₁	0.1415	3.8321 ⁺	59.1005***	142.0201***
<i>PHYB</i> ₅	17.1180***	95.8029***	230.9361***	219.0258***
<i>PHYA</i> ₁ × <i>PHYB</i> ₅	0.0509	9.0097**	83.1370***	150.7371***
<i>PHYB</i> ₁	0.5158	21.8211***	145.5002***	15.1317**
<i>PHYE</i> ₁	164.4737***	40.1909***	7.0217*	15.1317**
<i>PHYB</i> ₁ × <i>PHYE</i> ₁	0.0105	8.0437**	14.9333***	18.4850**

Results are given from both temperatures from Expt 1 (10°C and 22°C) and temperature extremes from Expt 2 (7°C and 28°C). The upper portion tests for interactions between *PHYA* and *PHYB*. The lower portion tests for interactions between *PHYB* and *PHYE*. *F*-values from ANOVA models run at each temperature are presented.

***, **, *, +, Significant at $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P < 0.10$, respectively.

as indicated by the reduced germination of the *phyB/phyE* double mutant compared with the *phyB* single mutant (Table S2). Thus the contributions of *PHYB* and *PHYE* to germination were nonadditive (Table 1), such that the role of phyE might be conditionally redundant with phyB. The effect of disrupting phyE was not as strong as the effect of disrupting phyA, as indicated by the significantly lower germination of the *phyA/phyB* mutant than the *phyB/phyE* mutant. Interestingly, the germination frequency of the *phyA/phyB/phyE* triple mutant was greater than that of the *phyA/phyB* double mutant, suggesting that disruption of phyE on backgrounds with disrupted phyA and phyB activity may actually increase germination. However, the *PHYA* allele differed between the double and triple mutant, so this increased germination may be due to a weaker effect of the particular *PHYA* allele in the triple mutant.

At 10°C, all mutants with disrupted function of phyE (*phyE*, *phyBE* and *phyABE*) had the lowest germination frequencies, with 45% to 55% reduced germination compared with the wild type (Fig. 1), and these differences were statistically significant (Table S1). Disruption of phyB alone also reduced germination, suggesting additive function (Table 1), but it did not disrupt germination significantly on the *phyE* background, as indicated by equivalent germination of the *phyE* single mutant and the *phyB/phyE* double mutant (Table S2). Thus the contributions of *PHYE* and *PHYB* to germination were potentially nonadditive, with phyB having a larger effect on germination in the background with functional phyE.

Functional phyA is not critical for germination at 10°C; disruption of phyA had no effect on germination at 10°C, even on a *phyB* background, as indicated by the equivalent germination of *phyB* and *phyA/phyB* (Table S2). Also, disruption of phyA had no effect on a *phyB/phyE* background, as indicated by the equivalent germination of the *phyB/phyE* and *phyA/phyB/phyE* (Table S2). Thus, at 10°C, phyE had the strongest contribution to germination, followed by phyB, with no detectable contribution of phyA.

Expt 2: temperature range

All the mutants responded significantly to temperature treatment, but the wild type, *phyA* and one of the *phyB* mutants had consistently high germination across the entire temperature range (Fig. 2, Table S1). Many lines responded to temperature by having decreased germination at the highest and/or lowest temperatures, but incremental changes in temperature of only 3°C significantly changed the germination frequency of most mutants, especially after 7 d in the light. Mutant germination responses to warm and cold conditions were consistent with the direction of responses in the first experiment, but differences in the degree of germination responses were present; these differences were most likely attributable to growth condition differences between the two experiments. All lines exhibited their maximum germination at 19°C, and only the *phyA/phyB* double mutants had germination that was significantly lower than wild type at this temperature. Thus, phytochrome contributions to germination are maximally redundant at intermediate temperatures.

At higher temperatures such as 28°C, mutants with disrupted phyB function (*phyB* single mutant, *phyA/phyB* double mutants, *phyB/phyE* double mutant and the triple mutant) had significantly reduced germination, as in Expt 1 (Table S1; Fig. 2). Also, as in Expt 1, the contribution of phyA to germination was contingent upon the loss of phyB function, since the *phyA* and wild type had statistically equivalent germination (Table S1), but the germination of *phyA/phyB* double mutants was significantly lower than that of the *phyB* single mutants (see the Supplementary Material, Table S3). Thus the contribution of *PHYA* and *PHYB* to germination at high temperature was nonadditive (Table 1). Moreover, the germination frequency of *phyA/phyB* was significantly lower than that of *phyB/phyE* at 28°C (Fig. 2; Table S3), indicating a larger contribution of phyA to germination at high temperature than phyE. Therefore, phyB had the largest contribution to

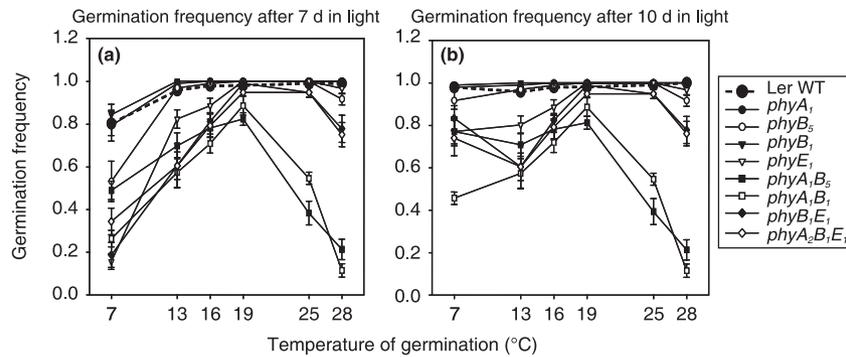


Fig. 2 Germination frequency after 7 d (a) and 10 d (b) in the light for *Arabidopsis thaliana* single and multiple phytochrome loss-of-function mutants relative to wild-type (Ler WT). The data represent mean values pooled across blocks in Expt 2. The germination frequency of each mutant line responded significantly to temperature across blocks (two-factor ANOVA, significant treatment effect, $P = 0.05$). Large closed circles, Landsberg *erecta*; small filled circles, *phyA-201*, stock #CS6219; open circles, *phyB-5*, #CS6213; filled squares, *phyA-201/phyB-5*, #CS6224; filled triangles, *phyB-1*; open squares, *phyA-201/phyB-1*; open triangles, *phyE-1*; closed diamonds, *phyB-1/phyE-1*; open diamonds, *phyA-2/phyB-1/phyE-1*.

germination at the highest temperature, followed by *phyA*, then *phyE*, as in Expt 1.

At the coldest temperature, mutants deficient in *phyE* function (*phyE*, *phyB/phyE* and *phyA/phyB/phyE*) had strongly reduced germination (Table S1; Fig. 2), indicating a strong contribution of *phyE* to germination at low temperature. The *phyE* single mutant had a lower germination frequency than single *phyA* and *phyB* mutants, indicating a greater contribution of *phyE* to germination at low temperature than *phyA* or *phyB* (Fig. 2; Table 1). *phyB* also contributed to germination at low temperature, as indicated by the significant reduction of germination in one *phyB* single mutant and in the *phyA/phyB* double mutants relative to wild type (Table S1). Interestingly, disrupting *phyB* function reduced germination more strongly at low temperature in Expt 1 than Expt 2. Nonetheless, as in Expt 1, disrupting *phyB* function had no effect on a *phyE* background, since germination of *phyE* was already very low (Table S3), leading to potentially nonadditive contributions of *PHYB* and *PHYE* to germination at low temperature. It was found that *phyA* did not strongly contribute to germination at low temperature, even on a *phyB* or *phyB/phyE* background, as indicated by the equivalent germination of the *phyB₅* and *phyA1/phyB₅* mutants and equivalent germination of the *phyB/phyE* and *phyA/phyB/phyE* mutants (Table S3). However, *phyA* did affect germination frequency at 7°C in the *phyB1* background (Table S3), indicating that the effect of *PHYA* at low temperature is variable, depending on the particular *PHYB* allele present. This result explains why there were significant nonadditive and additive effects of *PHYA* and *PHYB* at low temperature (Table 1).

After 10 d (in contrast to 7 d) in the light at 7°C, germination percentages of *phyE* and *phyBE* increased, indicating a reduced contribution of *phyE* to germination in cold temperatures as time proceeds. After 10 d, mutants deficient in both *phyA* and *phyB* had the lowest germination. Germination of the *phyA/phyB* double mutants was significantly lower than

the single *phyB* mutants, and germination of the triple *phyA/phyB/phyE* mutant was significantly lower than that of the *phyB/phyE* double mutant (Table S3), indicating that *phyA* can contribute to germination at low temperature after prolonged exposure to cold.

Discussion

Germination frequencies of the phytochrome mutants depended on temperature, indicating a novel ability of phytochrome to respond to both light and temperature during germination. Specifically, at warm temperatures, *phyB* contributed the most to germination, followed by *phyA* then *phyE*. At cool temperatures, *phyE* contributed most strongly, followed by *phyB*, with *phyA* contributing only after prolonged cold. Thus, *phyA* contributes to germination primarily in warm temperatures, *phyE* contributes primarily in cold temperatures, and *phyB* contributes across a broad range of temperature.

The results indicate that *phyB* and *phyA* would be important for germination in late spring or early summer conditions, whereas *phyE* and *phyB* would be most important for germination in late autumn or early spring, with *phyE* being most important in cooler seasons. A study by Hennig *et al.* (2002) indicates that *phyE* can also contribute to germination in warm conditions on a *phyA/phyB* background (i.e. in the *phyA/phyB/phyE* mutant) under light and stratification conditions different from those used in this study, suggesting that the contribution of *phyE* to germination also depends on light and/or stratification conditions. Most significantly, the very strong contribution of *phyE* to germination at cool temperatures indicates that it has an ecologically important function that has not been previously recognized.

The contribution of *phyB* and *phyE* to germination diminished with prolonged exposure to cold. This effect may be because abscisic acid (ABA), which represses germination, is degraded at temperatures below 13°C (Ali-Rachedi *et al.*, 2004);

three additional days of degrading ABA at 7°C may have been enough to promote greater germination for *phyE* mutants after 10 d. Therefore, *phyE*-mediation of germination may involve ABA.

An intermediate optimum germination temperature of 19°C existed for nearly all of the mutant lines. A common optimum value indicates that phytochrome function can be redundant and that *phyA*, *phyB* and *phyE* play a minimal role in promoting germination at intermediate temperatures. In fact, at 19°C, loss of all three of these phytochromes was not enough to reduce germination significantly. Moreover, high germination of *phyA/phyB/phyE* at intermediate temperatures indicates that *phyC* and *phyD* may be involved with germination at this temperature, or that germination does not depend strongly on phytochrome at 19°C. Since phytochromes are thought to regulate germination via gibberellic acid (GA), this suggests a new potential role for *phyC* and/or *phyD* in affecting GA concentration or sensitivity (Yamaguchi *et al.*, 2004). Interestingly, recent evidence from natural populations of *Arabidopsis* indicates that allelic variants of *phyC* affect GA-mediated hypocotyl responses (Balasubramanian *et al.*, 2006).

In addition to hormonal mechanisms, the effect of temperature on phytochrome-mediated processes may result from the influence of temperature on the threshold level of Pfr needed for dormancy breakage, the reversion rate of Pfr in darkness, or a combination of these (Kristie & Fielding, 1994; Pons, 2000). Temperature has been shown to affect the response of seeds to the ratio of red to far-red light through the effect on the Pfr threshold required for germination (Pons, 1986; Senden *et al.*, 1986; van der Toorn & Pons, 1988). Furthermore, a study by Fielding *et al.* (1992) suggests that Pfr levels in seeds influence germination by affecting the upper temperature limit for germination. Our data indicate that this upper temperature limit may be established by Pfr levels of *phyA* and *phyB*, and that a temperature threshold may exist around 25°C.

Overall, our experiments demonstrated that *phyA* has a role in promoting germination at high temperatures, *phyE* has a previously unknown role in promoting germination at low temperatures, and *phyB* is important to germination across a wide range of temperatures. Even temperature changes as small as 3°C had significant effects on phytochrome contributions to germination, especially at intermediate and high temperatures. At different temperatures, different phytochromes contribute significantly more to germination than others, indicating a restriction, or even a potential specialization of individual phytochrome activity as a function of temperature (see also Halliday & Whitelam, 2003). Moreover, the phytochromes were maximally functionally redundant near 19°C. This temperature occurs during the peak season of germination in the field, suggesting that functional redundancy may be greatest under favorable conditions for germination, and that the loss of function of some phytochromes would still not prevent germination under favorable conditions. A temperature of 19°C is also similar to the temperature at which circadian

clock mutants are maximally redundant (Gould *et al.*, 2006), suggesting that disruption of diurnal and seasonal affectors of germination timing may be less important at intermediate temperatures.

Conclusion

Our data suggest a new role for phytochrome in temperature-dependent germination; the activities of different phytochromes are sensitive to both temperature and light – two very different environmental cues. In particular, three phytochrome genes, *PHYA*, *PHYB* and *PHYE* may provide an efficient and flexible system that allows a seed to sense different seasonal cues with the same set of proteins. Specifically, the downregulation or inactivation of particular phytochromes or their pathways can cause temperature-dependent germination. Even small changes in temperature had a large effect on the contribution of particular phytochromes to germination. Thus, the changes in temperature that may accompany global climate change or long-distance dispersal have the potential to alter which of the three phytochromes may be most important in regulating germination. Generally, by affecting seasonal germination timing, these three phytochromes may influence the selective environment experienced in the wild.

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Supplementary Material

The following supplementary material is available for this article online:

Table S1 The χ^2 values and *P*-values for Kruskal–Wallis tests for significant differences between mutant and wild-type genotypes for germination frequency after 7 d in light and 10 d in light

Table S2 The χ^2 values for Kruskal–Wallis tests comparing germination frequency of different mutants after 7 d in the light in Expt 1

Table S3 The χ^2 values for Kruskal–Wallis tests comparing germination frequency of different mutants after 7 d in the light in Expt 2

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