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The Heterotrimeric G-protein Complex Modulates Light Sensitivity in *Arabidopsis thaliana* Seed Germination

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Abstract

Release of dormancy and induction of seed germination are complex traits finely regulated by hormonal signals and environmental cues such as temperature and light. The Red (R):Far-Red (FR) phytochrome photoreceptors mediate light regulation of seed germination. We investigated the possible involvement of heterotrimeric G-protein complex in the phytochrome signaling pathways of *Arabidopsis thaliana* seed germination. Germination rates of null mutants of the alpha (Gα) and beta (Gβ) subunits of the G-protein (At*gpa1-4* and *agb1-2*, respectively) and the double mutant (*agb1-2*/*gpa1-4*) are lower than the wildtype (WT) under continuous or pulsed light. The Gα and Gβ subunits play a role in seed germination under hourly pulses of R lower than 0.1 μmol m^{-2} s^{-1} whereas the Gβ subunit plays a role in higher R fluences. The germination of double mutants of G-protein subunits with *phyA-211* and *phyB-9* suggests that AtGPA1 seems to act as a positive regulator of phyA and probably phyB signaling pathways, while the role of AGB1 is ambiguous. The imbibition of seeds at 4°C and 35°C alters the R and FR light responsiveness of WT and G-protein mutants to a similar magnitude. Thus, Gα and Gβ subunits of the heterotrimeric G-protein complex modulate light induction of seed germination by phytochromes and are dispensable for the control of dormancy by low and high temperatures prior to irradiation. We discuss the possible indirect role of the G-protein complex on the phytochrome-regulated germination through hormonal signaling pathways.

INTRODUCTION

Several intrinsic and environmental cues are involved in the complex regulation of seed germination. Light is a crucial environmental factor regulating the release of dormancy and the induction of germination (1). The Red (R):Far-Red (FR) phytochrome photoreceptors mediate the germination responses to light in *Arabidopsis thaliana* seeds (2). Phytochromes comprise a five-member family of photochromic proteins (phyA → E), which each exist in two photoreversible forms: the Pr form efficiently absorbs R photons and the Pfr form,

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SUPPORTING INFORMATION

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which is considered the physiological active form, efficiently absorbs FR photons. Because of some spectral overlap between the Pr and Pfr forms, FR irradiation establishes a photoequilibrium of the two forms (*i.e.* Pfr:P > 0, where *P* = Pr + Pfr). The importance of phytochromes in the regulation of germination was first demonstrated by a classical experiment of R:FR photoreversibility in lettuce seeds (3). More recently, it was established that some seeds with high light sensitivity germinate by very low levels of Pfr:P established by a single FR pulse (4,5). The ecological significance of the acquisition of high light sensitivity in buried seeds of many weedy species is to be able to compete for resources sooner after germination is promoted by millisecond exposures to light during soil cultivation (6,7).

Light-inductive responses are classified into two groups depending on the established photoequilibrium of the phytochromes within the seed. The very-low-fluence response (VLFR) is induced even by a saturating pulse of FR. The low-fluence response (LFR) requires higher Pfr:P ratios to induce germination (8). PhyA is the only phytochrome member that is responsible for the VLFR (9,10) and primarily phyB, but also phyE to a lesser extent, modulate seed germination at higher Pfr:P through the LFR (11,12). Pfr increases the level of gibberellins (GAs) by transcriptional regulation of GA anabolic and catabolic genes (13–16) and by degradation of DELLA proteins (17–19). In addition, the active form of phyA and phyB inhibits PIL5, a phytochrome-interacting basic helix-loop-helix transcription factor, acting as a negative regulator of seed germination (20,21). The Pfr form of the phytochromes promotes germination through a decrease in abscisic acid (ABA), in part by transcriptional repression of ABA anabolic genes and transcriptional activation of an ABA catabolic gene (21,22).

The mechanisms by which signals are integrated to control germination remain unclear. One possible mechanism involves coupling of and cross-talk between signals by heterotrimeric G-protein because many signaling pathways controlling seed germination are compromised in Arabidopsis mutants lacking the G-protein complex (23–25). The Arabidopsis genome contains genes encoding only one canonical G-protein alpha (Gα) subunit (At*GPA1*, hereafter noted only as *GPA1*), one G-protein beta (Gβ) subunit (*AGB1*) and at least two G-protein gamma subunits (*AGG1* and *AGG2*) (26). Studies on the null alleles of *GPA1* and *AGB1* suggest that plants use heterotrimeric G-protein signaling in many growth and developmental processes (26). Germination of the *gpa1* null mutant seeds is hypersensitive to glucose, sucrose and ABA, and hyposensitive to GAs and completely insensitive to brassinolide (BR) (25). Seeds of *gcr1*, a loss-of-function of a candidate G-protein-coupled receptor, also show reduced germination in GAs and BR (23). However, additive effects in the double and triple mutants (*gcr1*, *gpa1* and *agb1*) suggest that GCR1 acts independently of the heterotrimeric G-protein promoting germination (23). Furthermore, there is no evidence to date that GCR1 controls the active state of GPA1. In accordance with these results, Arabidopsis transgenic seeds ectopically overexpressing GCR1 germinate at a higher rate than wildtype (WT) (27). Pandey *et al.* (24) concluded that AGB1, GPA1 and GCR1 all act in the same pathway of ABA and glucose-repression of germination. A yeast two-hybrid screen identified a GPA1-interacting protein as AtPirin1 (28). An *Atpirin1* T-DNA-insertion

mutant displays phenotypes similar to those of the *gpa1* mutant including reduced germination in the absence of stratification and ABA inhibition of germination (28).

Therefore, the potential participation of the G-protein complex in the phytochrome regulation of seed germination remains an open possibility. Evidence presented here demonstrates that the heterotrimeric G-protein complex modulates light induction of Arabidopsis seed germination.

MATERIALS AND METHODS

Plant material and growth conditions

Arabidopsis thaliana plants were grown in a continuous white light (WL) chamber at 22–24°C for bulking seed. For germination experiments, seeds were stored in open eppendorfs inside a closed box containing silica gel and kept in darkness at room temperature between 3 and 12 months. Seeds of *gpa1*-4, *agb1*-2 and *agb1*-2/*gpa1*-4 used in our experiments were in the Col background and were described previously (29). The *phyA-211* (CS6223) and *phyB-9* (CS6217) mutants were from the ABRC Arabidopsis Stock Center. The *gpa1-4*, *agb1-2*, *phyA-211* and *phyB-9* alleles were used for generating *gpa1-4*/*phyA-211*, *agb1-2*/*phyA-211*, *gpa1-4*/*phyB-9* and *agb1-2*/*phyB-9* double mutants. WT and mutant plants were cultivated, harvested and stored side-by-side to preclude growth condition, maternal and after-ripening effects on germination; these collections are designated as matched. Results were obtained using four matched populations to confirm genuine differences in the light responses of genotypes.

Germination experiments

Twenty-four seeds were sown in each clear plastic box (42 × 35 × 20 mm) containing 3 mL of 0.8% w/v agar (Chemit Argentina SRL, Buenos Aires). The boxes, wrapped in black plastic sheets, were incubated for 3 days at 4°C prior to irradiation (unless otherwise indicated in the text). Depending on the experiment, seeds were irradiated by continuous, repetitive hourly pulses or a single saturating pulse of light. After a pulse of light, seeds were incubated in darkness for 3 days at 25°C. Germination was scored by the presence of a protruding radical. Handling of the incubated seeds was performed under dim green light as described previously (9,11).

Light treatments

Continuous WL was provided by Philips TLD30W/54 fluorescent tubes (Phillips Electric, Inc., Brazil). Continuous R was provided by Philips PLC Electronic 11 W lamps (Phillips Electric, Inc., Holland) in combination with one yellow, one orange and one red acetate filter (La Casa del Acetato, Buenos Aires, Argentina) (9,11). Continuous FR was achieved using incandescent lamps in combination with a water filter, a red acetate filter and six 2 mm-thick blue acrylic filters (La Casa del Acetato, Buenos Aires, Argentina) (9,11). Continuous blue light (BL) was provided by Philips TLD30W/54 fluorescent tubes (Phillips Electric Inc., Brazil) in combination with two pale-blue acetate filters (La Casa del Acetato). Dose dependency experiment was performed using hourly R pulses of 3 min in combination with neutral-density filters for 3 days. A series of calculated phytochrome photoequilibria

were obtained with a single saturating light pulse. Details of the light sources for each photoequi-librium were as described earlier (9,11).

RESULTS AND DISCUSSION

G-protein complex modulates continuous and inductive light-induced germination of Arabidopsis seeds

To determine the possible participation of the heterotrimeric G-protein complex in the light induction of germination, we scored germination of WT, *gpa1-4*, *agb1-2* and *agb1-2*/*gpa1-4* seeds under continuous WL, R, BL, FR or darkness (Fig. 1). WT and *gpa1-4* seeds germinated around 25% while only 10% of *agb1-2* and *agb1-2*/*gpa1-4* seeds germinated in darkness. Seeds of *agb1-2*, *gpa1-4* and *agb1-2*/*gpa1-4* germinated at a rate that was statistically significantly less than WT under continuous BL and FR. Under continuous R, only *agb1-2*/*gpa1-4* seeds germinated significantly less than WT. No statistically significant differences between genotypes were detected under continuous WL.

Under very low fluence of pulsed R lower than 0.1 μmol m^{-2} s^{-1} (3 min h^{-1}), single and double mutant seeds of the G-protein complex germinated at a rate lower than WT (Fig. 2). At higher fluences of hourly pulsed R (between 0.1 and 5 μmol m^{-2} s^{-1}), only *agb1* seeds carrying the *agb1* null allele, regardless of the presence of a null allele of *GPA*, germinated less than WT, suggesting a different role for GPA1 and AGB1 subunits of the G-protein complex in the induction of germination by light.

To confirm the modulation of G-protein of phytochrome induction of germination, we tested whether the Gα and Gβ subunits of the heterotrimeric G-protein complex regulate the promotion of germination under different Pfr:P photoequilibria. Seeds of WT, *gpa1-4*, *agb1-2* and *agb1-2*/*gpa1-4* were irradiated with a single mixed R + FR saturated pulse that established a series of calculated phytochrome photoequilibria (11). WT seeds incubated for 1 day at 4°C germinated 30% with an FR pulse (*i.e.* Pfr:P = 3%) and 80% with an R pulse (*i.e.* Pfr:P = 87%). Intermediate and increasing values of germination rate were observed for 20%, 33% and 61% Pfr:P (Fig. 3A). A biphasic light germination response was observed for WT seeds. The reduced first phase (*i.e.* VLFR) was detected between darkness and 20%, and a significant second phase (*i.e.* LFR) between 33% and 87% Pfr:P. Seeds of *gpa1-4*, *agb1-2* and *agb1-2*/*gpa1-4* lacked the VLFR and the LFR was steeper than for WT (Fig. 3A). Increasing stratification promoted WT and mutant seeds to germinate at higher rates but with the same relative light sensitivities (Fig. 3B). The germination rate of WT seeds was higher than 80% at each established photoequilibrium indicating that chilling abrogated the light requirement for germination. However, less than 50% of mutant seeds germinated at Pfr:P photoequilibria lower than 33% and the germination rate increased to 100% at 66% Pfr:P. These results confirm that the Gα and Gβ subunits of the G-protein complex modulate Pfr:P sensitivity responses in Arabidopsis seeds.

To investigate the role of G-protein complex subunits in the signaling pathways of phyA and phyB, we generated double mutant seeds of Gα a and Gβ subunits (specifically, *gpa1-4* and *agb1-2*) and phytochromes (specifically, *phyA-211* and *phyB-9*). We incubated the seeds for 1 day at 4°C and then irradiated them with a single mixed R + FR saturated pulse to

establish different levels of Pfr:P into the seeds (Fig. 4). WT seeds displayed significant R and FR sensitivity responses: 70% of the seeds showed an R:FR response, while 25% of the seeds germinated with an FR pulse with respect to the dark control. Consistent with phyA mediation of the VLFR (9), nearly 100% of the *phyA-211* seeds germinated following an R pulse but failed to germinate with an FR pulse. *phyB*-9 seeds treated with an R pulse displayed reduced germination consistent with phyB being the principal phytochrome mediating the R:FR reversible response (11). At very low photoequilibrium of Pfr:P (*i.e.* FR pulse), *gpa1-4*/*phyA-211* and *agb1-2*/*phyA-211* seeds germinated at the same rate as *phyA-211* seeds indicating that GPA1 and AGB1 act as a positive regulator downstream of phyA signaling. The role of G-protein subunits in the phyB signaling pathway is less clear. The germination of *gpa1-4*/*phyB-9* seeds was similar to that of *phyB-9* seeds at Pfr:P ≤ 61%, however *agb1-2*/*phyB-9* seeds germinated at a rate significantly higher than that of *phyB-9* seeds at Pfr:P > 33% (Fig. 4). The results suggest that GPA1 could be operating positively downstream of phyB signaling at least at intermediate photoequilibria, while AGB1 shows a negative epistatic interaction in the range of action of the phyB.

Based on the results shown in Figs. 1 and 3 using the same populations of seeds for comparison, the inhibitory effects of continuous BL likely occur through the phytochrome signaling system. The inhibition of germination under BL shown in Fig. 1 correlates with the values obtained using R + FR mixture filters that established a Pfr:P ~40%. In Fig. 3, G-protein mutant seeds germinated, on average, between 100% and 50% less than WT at Pfr:P ~0.4 depending on the total time of stratification (26% *vs* 55% and 57% *vs* 87% for seeds chilling 1 or 3 days, respectively). Consistent with previous reports, we conclude that the induction of Arabidopsis seed germination in the BL region of the spectrum is phyA dependent (2,10).

The heterotrimeric G proteins do not mediate temperature control of seed dormancy

Dormancy can be progressively reduced by the influence of low temperatures modulating the light responses that initiate seed germination (2,30). In addition, the release of dormancy may be in certain cases a reversible process by which seeds can become dormant again when the conditions for germination are not favorable such as prolonged incubation in darkness or at a high temperature (31,32). The observation that under some light conditions, G-protein mutants have the same germination rate as WT suggests that the mutants are not impaired in germination *per se*. Rather these observations support the hypothesis that G-proteins modulate light regulation of germination specifically, and act independent of other factors controlling the dormancy status. In this context, we evaluated the possible role of Gα and Gβ subunits in the modulation of light responses of the imbibed seeds under chilling or high temperature regime. Seeds of WT, *gpa1-4*, *agb1-2* and *agb1-2*/*gpa1-4* were chilled at 4°C between 0 and 7 days before irradiation with an FR or R pulse. The germination rates were similar for WT and G-protein mutant seeds after an R pulse. On average, 70% of the seeds germinated without chilling and after an R pulse. Chilling followed by an R pulse increased germination to nearly 100% (Fig. 5). An FR pulse reduced germination to approx. 10% regardless of the amount of chilling. In darkness, the imbibition of seeds at low temperatures increased the germination rate compared with the FR pulse-treated seeds with the exception of the *agb1-2* mutant suggesting a potential role of the *AGB1* subunit in the release of

dormancy by chilling. The higher percentages of germination in darkness with respect to FR is likely to reflect a higher amount of Pfr (than those established by an FR pulse) in dry seeds originating from plants growth in WL environments characterized by a high R:FR ratio (2). Imbibition of seeds at 35°C followed by chilling for 3 days at 4°C decreased the germination of WT and G-protein mutant genotypes under R, FR or darkness (Fig. 5). These results indicate that Gα and Gβ subunits do not participate in the control of dormancy by low and high temperatures prior to light treatment in Arabidopsis seeds. However, Shikha Misra *et al.* (33) reported that transgenic tobacco seeds overexpressing Gα and Gβ subunits of *Pisum sativum* germinated at a higher rate than WT at 37 and 42°C suggesting than the effect of heat on germination is species related or that ectopic misexpression of the pea G-proteins confers a neomorphic phenotype.

The role of the G-protein complex in the light transduction pathway is dependent on the quality of light, the stage of development and the cell/tissue type. Previous and the present works demonstrate that the light regulation of the hypocotyl length in the early seedling development does not involve the G-protein complex. As shown in Supporting Information Fig. S1 and by Jones *et al.* (29), null mutations in Gα and Gβ subunit genes do not alter R and FR inhibition of hypocotyl growth. In contrast, BL regulation of early development does involve the G-protein complex. Etiolated seedlings required the putative G-protein-coupled receptor (GCR1) and the GPA1 subunit for phenylalanine production after a short single pulse of BL (34). Previous reports established that GCR1, GPA1 and AGB1 act in concert in or upon the ABA signaling pathway (24), and GPA1 could be potentiating the GA signaling pathway during seed germination (25). Light signals transduced by the active Pfr form of phytochromes are integrated into the GA biosynthesis pathways through a positive transcript regulation of *AtGA3ox1* and *AtGA3ox2* genes (14). Thus, it is plausible that GPA1 and AGB1 increase the transcript level of *AtGA3ox1 and AtGA3ox2* through the phytochrome signaling pathways. We also speculate that the nutritional state of the seed may modulate light regulation of germination as a strategy to compete for resources or to assure germination success. For example, seeds having a low carbohydrate store may require more light before germination is attempted thus reducing the chance of arrested development occurring before the seedling can reach carbon autotrophy. The heterotrimeric G-protein complex with its cognate 7-transmembrane Regulator of G Signaling Protein (AtRGS1) is clearly ensconced in the sugar sensing pathway in plant cells (35–40), thus the G-protein complex at large (G protein complex associated partners, candidate sugar receptor AtRGS1) could aptly serve to modulate the light perception machinery based on the nutritional state of the imbibed seed.

In summary, the results shown in Figs. 1–4 all indicate that both Gα and Gβ subunits or the intact heterotrimer are required to promote seed germination by light. The results of Figs. 1 and 2 demonstrate that GPA1 and AGB1 modulate germination under continuous and inductive light conditions. Data of Figs. 3 and 4 show that GPA1 and AGB1 act as a positive modulator of the phyA signaling pathway. However, conflicting data of Fig. 4 suggest that AGB1 may be a negative modulator under certain conditions, thus the role of AGB1 in PhyB signaling is less clear. It is likely that the nutrient state of the seed affects light sensitivity through the action of the G-protein complex, or that other stable phytochromes

(*i.e.* phyE) could promote the R:FR reversible promotion of germination in these conditions. New experiments using additional phytochrome mutants are required to evaluate additional interactions between the G-protein complex and other phytochromes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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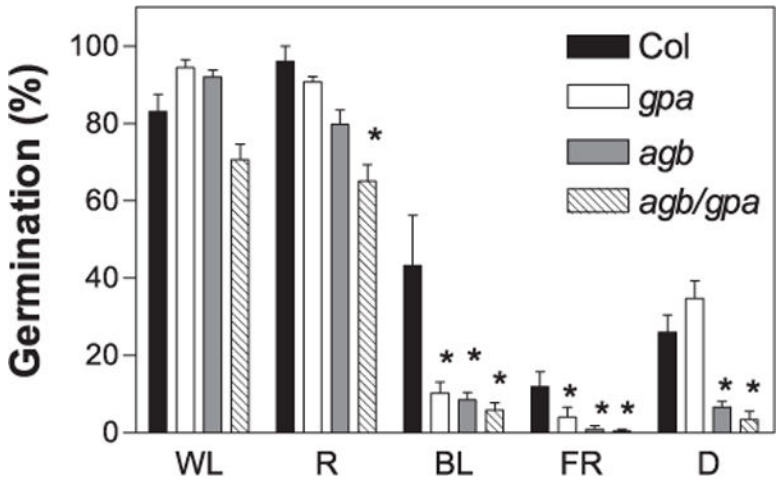

Figure 1.

Germination response for WT, *gpa1.4*, *agb1.2* and *agb1.2*/*g-pa1.4* seeds under continuous white light (WL), red (R), blue light (BL), far-red (FR) and darkness (D). Seeds were stored at 25°C for 10 months and then stratified for 3 days at 4°C before light treatments. Germination was scored at 3 days at 25°C. Asterisks indicate significant differences with respect to wildtype at $P < 0.05$ (Tukey test). Shown are the averages for percent germination ± SEM of five to eight independent samples.

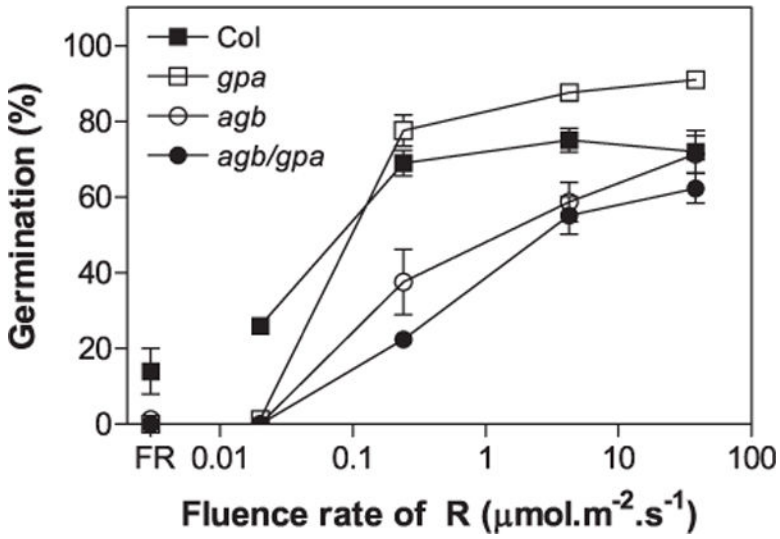

Figure 2.

Hourly red (R) fluence germination response for WT, *gpa1.4*, *agb1.2* and *agb1.2*/*gpa1.4* seeds. Seeds were stored at 25°C for 10 months and then stratified for 3 days at 4°C before hourly pulses of R at the indicated fluence rate (3 min h^{-1} for 3 days). Germination for hourly pulses of far-red (FR) is shown as control. Germination was scored at 3 days at 25°C. Shown are the averages for percent germination ± SEM of three to eight independent samples.

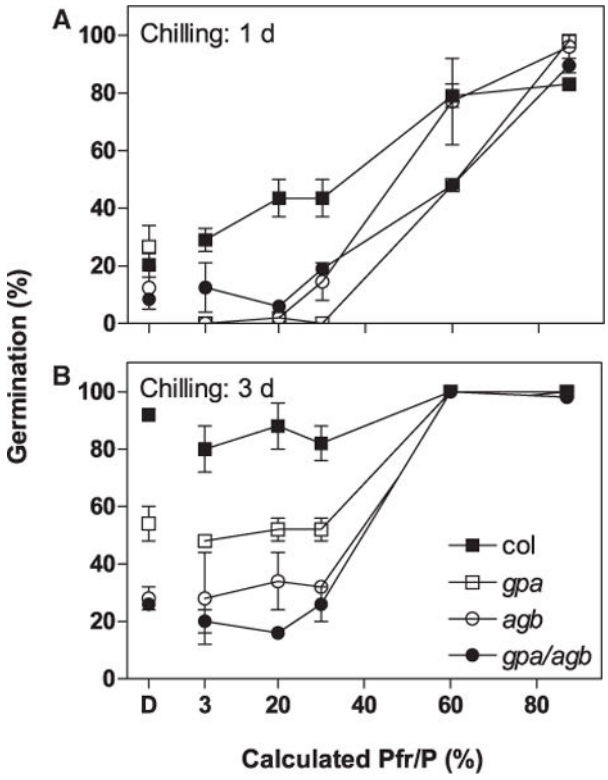

Figure 3.

Calculated Pfr/P (%) germination response for WT, *gpa1.4*, *agb1.2* and *agb1.2*/*gpa1.4* seeds. Germination in darkness is shown as control. Seeds were stored at 25°C for 14 months and then stratified for 1 (A) or 3 days (B) at 4°C as indicated. After stratification, seeds were irradiated to establish the indicated photoequilibria as described in Materials and Methods and scored for germination after 3 days at 25°C in darkness. Shown are the averages for percent germination ± SEM of three to eight independent samples.

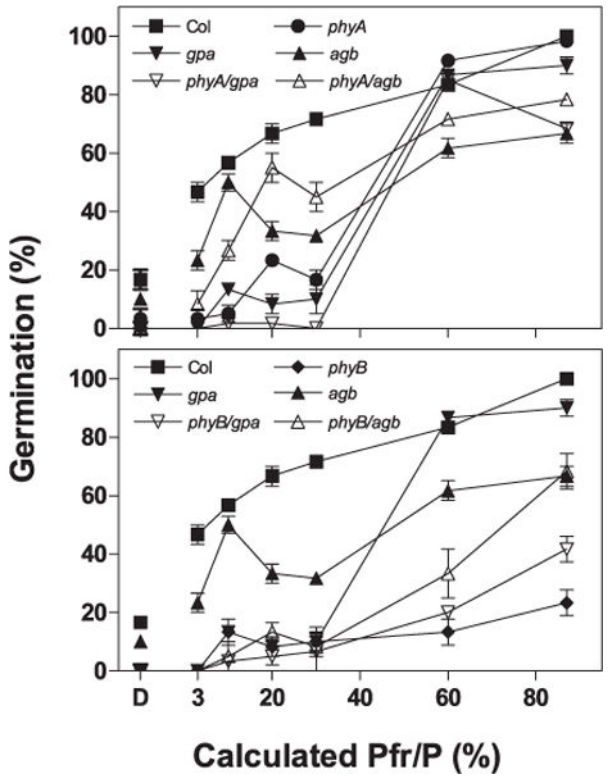

Figure 4.

Calculated Pfr/P (%) germination response for WT, *gpa1.4*, *agb1.2*, *phyA211*, *phyB9*, *phyA211*/*gpa1.4*, *phyA211*/*agb1.2*, *phyB9*/*gpa1.4* and *phyB9*/*agb1.2* seeds. Germination in darkness is shown as control. Seeds were stored at 25°C for 4 months and then stratified for 1 day at 4°C. After stratification, seeds were irradiated with a pulse of the indicated light (see Fig. 3) and scored for germination after 3 days at 25°C in darkness. Before cold imbibition, 2 h imbibed seeds were irradiated with a saturated pulse of FR (15 min) to establish a minimum photoequilibrium into the seeds. Shown are the averages for percent germination ± SEM of three independent samples.

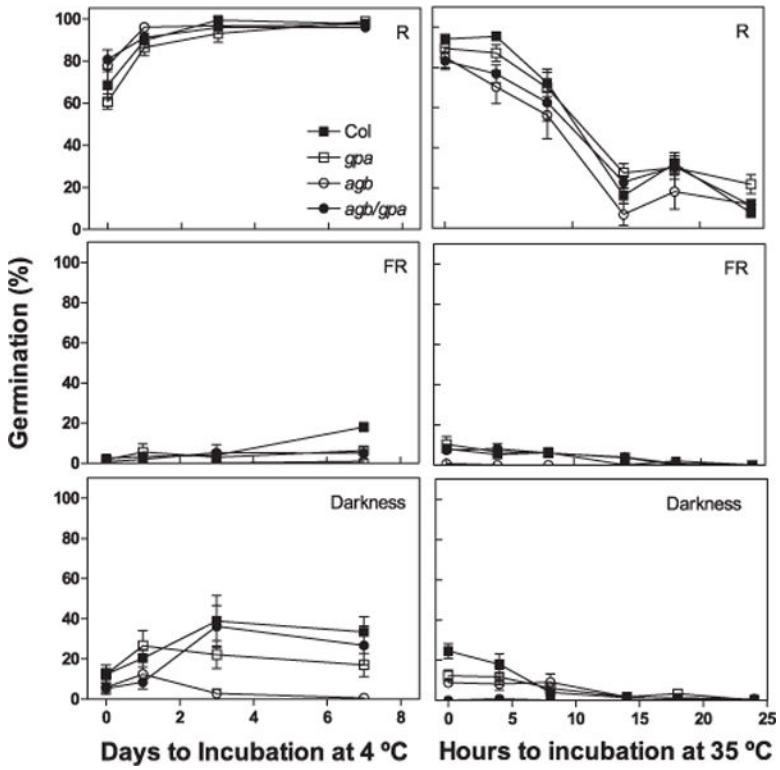

Figure 5.

Chill and heat effects on the light germination response of WT, *gpa1.4*, *agb1.2* and *agb1.2*/*gpa1.4* seeds. Seeds were stored at 25°C for 6 months and then incubated at either 4°C for 0–7 days, or at 35°C for 0–24 h following a red (R) or far-red (FR) pulse, or kept in darkness. Germination was scored after 3 days at 4°C. Shown are the averages for percent germination ± SEM of five to eight independent samples.