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Phytochrome E Plays a Role in the Suppression of Germination in Far-Red Light in Tomato

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ABSTRACT

As photoautotrophs, plants use light not only as a source of energy but also as cues for directing growth and development. Phytochromes comprise a small gene family of plant specific light receptors that absorb mostly in the red/far-red portion of the electromagnetic spectrum. These light receptors are well-studied in the model species *Arabidopsis thaliana,* yet much less is known about their functions in other species. We have generated CRISPR-induced mutations in *SlPHYTOCHROME E* (*SlPHYE*) and *SlPHYF,* produced higher order mutants, and characterized some of their physiological functions in tomato (*Solanum lycopersicum*). We report that SlphyE plays a major role in detecting far-red light, repressing germination when light conditions are unfavorable for establishing a new seedling. While SlphyE functions on its own, it also synergistically works with another phytochrome, SlphyB1, which by itself only plays a minor role in germination control. Aside from its role in far-red light detection, SlPhyE is also involved in perceiving red light, leading to the repression of hypocotyl elongation and the promotion of light avoidance growth in the roots. SlPhyF acts synergistically with phyB1 during photomorphogenesis but it is not involved in far-red light detection during germination.

1 | Introduction

The likelihood of success for a seed to develop into a strong, fertile plant depends in large part on the time and location of its germination. For example, if the seed germinates in dry or cold conditions, the fragile seedling may be at danger from frost or desiccation. Likewise, if the seed germinates in a place where it is shaded by a larger plant, lack of adequate lighting may not allow it to establish a robust seedling.

To sense their environment, plants have evolved the use of a light receptor called phytochrome, which is able to sense both temperature and light by undergoing a change in its chemical conformation in changing environmental conditions. Phytochromes belong to a family of specialized proteins that absorb light mostly in the red (R) and far-red (FR) part of the electromagnetic spectrum and in turn exert numerous effects on plant growth and development from germination through adulthood and the formation of fruits and new seeds (Kami et al. 2010; Chen and Chory 2011; Xu et al. 2015). Indeed, it is the ratio of R:FR that determines the magnitude of many of these responses (Smith 2000).

Phytochrome action has been well-studied in Arabidopsis, where five members of the gene family (*AtPHYTOCHROME A [AtPHYA]* through *AtPHYE*) are known (Clack et al. 1994; Mathews and Sharrock 1997). These genes fall into two main categories: the highly light-labile phyA (type I), which controls

many FR responses, and the more light-stable phytochromes B-E (type II), which mediate for example many responses to continuous R. Phytochromes appear to exert their function either as homodimers or heterodimers, where type I phytochrome (phyA) only homodimerizes, while type II phytochromes can exist as homodimers or heterodimers in many combinations (Sharrock and Clack 2004).

Despite the enormous importance that phytochromes play for plants, comparatively little work has been done on this light receptor in species outside the Brassicaceae. In solanaceous plants, such as tomato (*Solanum lycopersicum*), gene duplication during evolution has led to two subfunctionalized copies of *PHYB*, *SlPHYB1*, and *SlPHYB2* (Carlson et al. 2020), while the other three *PHY* genes are more closely related to their homologs in Arabidopsis (Hauser et al. 1995; Alba et al. 2000).

Mutants for SlPHYA, SlPHYB1, and SlPHYB2 have been available for some time (van Tuinen et al. 1995b; van Tuinen et al. 1995a; Lazarova et al. 1998), and mutants for SlPHYF were recently developed (Balderrama et al. 2023). Additionally, using RNA interference, a partially down-regulated artificial miRNA mutant for phyE (*SlPHYE amiRNA*) has also been reported (Schrager-Lavelle et al. 2016), however, a stable knock out version of this gene has been elusive until now.

Here we report the generation and initial analysis of two CRISPR-induced knock-out alleles in *SlPHYE*. Our data show roles for PHYE in seedling development, root growth, and the repression of seed germination under unfavorable light conditions.

2 | Materials and Methods

2.1 | Mutant Construction

We used established methods to create *phyE* mutants employing a CRISPR-Cas9 meditated mutagenesis approach (Brooks et al. 2014). All vectors used and described in Brooks et al. 2014 were purchased from Addgene (www.addgene.com) Briefly, with the help of the Van Eck group at the Boyce Thompson Institute, *Agrobacterium tumefaciens* strain LBA4404, carrying a vector with two guide RNAs (Table S1) designed to match the target sequence, was used to transform tomato cells of the variety Moneymaker. Successful transformation was validated in the first generation (T0) by PCR using primers against the 35S promoter and the Cas9 sequence (Figure 1 and Table S1), and the *PHYE* gene was amplified with PCR, cloned, and sequenced (Table S1) to identify mutants. Only plants transformed with Cas9 and guide RNAs (gRNAs), but

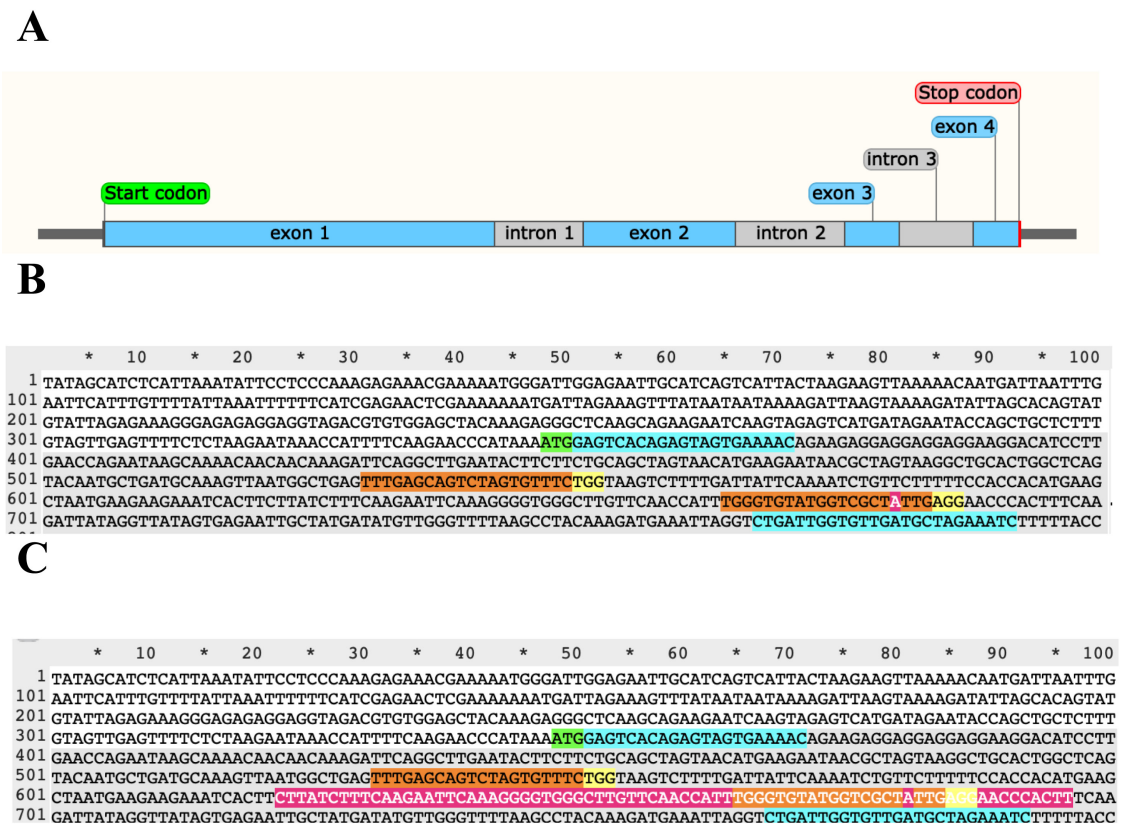

FIGURE 1 | CRISPR-induced *phyE* mutations create likely loss of function alleles. (A) PhyE gene model indicating exons (blue) and introns (gray), translational start (green) and stop (red) codons, and overall transcript length including 5′ and 3′ ends (thin, dark gray line). CRISPR was used to generate two alleles. (B) Nucleotide-level detail of allele *phyE-5*. C: Nucleotide-level detail of allele *phyE-8*. Sequences include: 5′ untranslated region (white background, nt 1–347), the beginning of exon 1 (starting at nt 348), start codon (nt 348–350, same green background as in A), left and right sequencing primers (light blue), PAM sequences (yellow), the two guide RNAs (orange background) and the mutated sequences in magenta with white lettering. The phyE-5 allele has a single base pair deletion at nt 681, while the *phyE-8* mutation has a deletion of 75 bp from nt 622–696. Primer sequences can also be found in Table S1.

not empty vector control transformants, showed mutations in the target genes. T0 plants were allowed to flower, self-fertilize, and each T1 offspring was screened for the presence of the T-DNA construct. Those without evidence of the T-DNA were genotyped for their targeted mutations. Two *phyE* lines (*phyE-5, phyE-8*) were identified without the T-DNA construct and with targeted mutations (Figure 1). Both mutations lead to predicted truncated proteins due to early stop codons (*phyE-5*) or a large, 75 bp deletion (*phyE-8*). Both alleles were used for initial experiments and seemed to respond similarly after which only the *SlphyE-8* allele was used for the experiments shown here and the construction of higher order mutants due to the ease of genotyping its large deletion. Confirmed mutant lines were propagated by selfing and subsequently seeds were bulked. The higher order mutants were constructed by hand-pollination followed by Mendelian segregation and PCR genotyping using the same primers (Figure 1). Seeds from *S. lycopersicum phyA, phyB1, phyB2*, and *phyB1B2* mutants (cultivar Moneymaker) were originally obtained from the Tomato Genome Resource Center (TGRC, Davis, CA, USA). Higher order mutants containing mutations in PHYF were constructed as described earlier (Balderrama et al. 2023).

2.2 | Analysis of Hypocotyl Growth in Continuous Red Light

Seeds were sterilized in 50% bleach under green light for 15 min. Seeds were then washed in DI water sufficiently long to ensure that all traces of bleach were removed. Rinsed seeds were placed in light-excluding boxes with 10 layers of water-saturated paper towels. Boxes were placed in a dark incubator at 25°C for ~4 days. Individuals ~2 cm tall were removed from the dark box in green safe light and placed in 7.6 × 7.6 × 10.2 cm clear Magenta Jars containing 50–70 mL of 1% agar medium supplemented with 1.17 g/L (0.5X) Murashige and Skoog (MS) salts (Sigma) pH 5.8, and sealed with a lid. The jars were then placed in either continuous R at ~15 $\mu mol \cdot m^{-2} \cdot s^{-1}$, or, for dark grown conditions, the jars were wrapped in aluminum foil. After three additional days the seedlings were removed from the jars, photographed, and measured using ImageJ (https://imagej.nih.gov/ij/).

2.3 | Germination Assay

For each replicate experiment, ~15 seeds were surface sterilized and placed in clear Magenta Jars with 50 mL of 1% (w/v) agar growth medium as described above. For ABA treatment, 500 nM ABA was added to the medium. Seeds were then kept in a growth chamber for 7 days in continuous FR at ~10.5 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 25°C. After 7 days the number of individuals that had germinated were recorded and removed. To determine if a lack of germination was due to FR treatment or due to a lack of seed viability, the remaining seeds were then transferred to a growth chamber with continuous R at 25°C and allowed to germinate. After 7 days in R, the number of individuals that had germinated were recorded and the rate of germination in FR determined as number of seeds germinated after 7d in FR/number of seeds germinated after 7d in FR + number of seeds germinated after 7d of additional R.

2.4 | Root Assays

For root growth experiments, seeds were sterilized in 50% bleach under green light for 15 min, rinsed and placed in light-excluding boxes as described above. Boxes were then placed in a dark incubator at 25°C for ~2 days. Using visual inspection, seeds with emerged radicals (~0.5 cm ± 0.25 cm) were placed on 22.8 × 22.8 cm agar plates containing ~200 mL of 2% 0.5 X MS agar medium. For dark treatment, plates were wrapped in aluminum foil. Plates were wrapped around the edges with parafilm to avoid desiccation. Both sets of plates were placed in an incubator at 25°C with continuous R with a fluence rate of ~15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level for 7 days before they were photographed and analyzed using ImageJ (https://imagej.nih.gov/ij/).

2.5 | Light Conditions

R conditions were created using light from a 25 W red LED bulb (ABI LED lighting, abilights.com) with an emission spectrum maximum of 660 nm and a total fluence rate of ~15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level. To create FR conditions, we used a 16-W FR LED bulb (Agromax, www.htgsupply.com) with an emission spectrum maximum of 730 nm and a fluence rate of ~10–12 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at plant level and a R/FR ratio of 0.009. An SS-110 field spectroradiometer (Apogee Instruments, www.apogeeinstruments.com) was used to verify fluence rates and spectra.

For bulking, adult phenotyping (Figure 2), and higher order mutant construction, plants were grown in a greenhouse under lighting conditions that varied throughout the year, but days were extended to 16 h with sodium lamps and R/B/W LED arrays (BESTVA 2000 W, LM301B). Spectral profiles of the light sources used can be found in Balderrama et al. (2023).

2.6 | Statistical Analysis

All statistical analyses were performed using R (R Core Team 2023) version 3.5.0 or higher. Scripts used for the analyses can be found on https://github.com/amadlung/Barnwell_etal_2025.

3 | Results

3.1 | Construction and Verification of CRISPR Mutants

Using CRISPR technology, we generated *SlphyE* knockout mutants isolating two separate alleles (*SlphyE-5* and *SlphyE-8*), which contained independent mutations, and had lost the Cas9 transgene during the segregation process (Figure 1). Sanger sequencing showed that the *SlphyE-5* allele has a 1-bp deletion while the *SlphyE-8* allele has a 75-bp deletion (Figure 1). The *SlphyE-5* allele is a nonsense mutation resulting in the loss of the proposed chromophore attachment region, the dimerization domain, and the nuclear localization domain. It thus seems highly likely that this mutation results

FIGURE 2 | Phenotypes of 4-week-old phytochrome mutants. Seedlings were grown in 10 × 10 cm pots in potting soil without additional fertilizer. Scale: pots are 10 cm in width. Plants were grown in triplicate in the greenhouse and one representative individual is shown.

in a loss of function of the PHYE protein. The *SlphyE-8* allele results in an in-frame 75-bp deletion in the same general location of the gene, and the loss of 25 amino acids in exon 1 also likely leads to a null mutation.

3.2 | Single phy Mutants Show Only Subtle Phenotypic Variation in 5-Week-Old Seedlings

Visual phenotypic inspection of 5-week-old single mutant seedlings shows only slight differences from the WT (Figure 2 and Table S2) with the *phyB1* mutant being somewhat taller than WT as reported previously (van Tuinen et al. 1995b). Double mutants between *SlphyE* and either *SlphyB1* or *SlphyB2* did not greatly alter the phenotype compared to the single mutants (Figure 2) while the *SlphyB1/SlphyB2* double mutant is significantly taller than either *SlphyB1* or *SlphyB2* (Figure 2 and Table S2, Weller et al. 2000). The triple *SlphyB1/SlphyB2/SlphyE* mutant displayed highly elongated internodes (Figure 2 and Table S2) as previously also reported for the same genotype but using a partial knock-down *phyE amiRNA* allele (Schrager-Lavelle et al. 2016) and was noticeably lighter in color than the double or the single mutants. Interestingly, the quadruple knock-out mutant carrying lesions in *phyA, phyB1, phyB2,* and *phyF* and thus having phyE as its only functional phy was less elongated than the *phyB1/phyB2/phyE* triple knockout (Figure 2 and Table S2).

3.3 | PhyE Is Involved in Hypocotyl Elongation During Photomorphogenesis

Phytochromes typically are involved in regulating responses to R during early seedling morphogenesis. We germinated seeds in the dark, selected synchronized seedlings of similar height, exposed them to R, and subsequently measured hypocotyl lengths. It is well-known, and our data confirmed this, that the wild type response under these experimental conditions leads to a reduction of hypocotyl elongation growth in R compared to seedlings grown in darkness (e.g., Weller et al. 2000). Loss of SlphyB1 results in statistically significantly longer hypocotyls of mutant seedlings grown in R when compared to the WT, although the *SlphyB1* mutant maintains a significant hypocotyl shortening response to R (Figure 3). Single mutants in SlPHYE or SlPHYF, as well as the *SlphyE/SlphyF* double mutant showed a vigorous, statistically significant, response to R although, as in *SlphyB1*, the single mutants had slightly taller hypocotyls than the WT. This response was statistically significant in *SlphyE* but only in one of the two *SlphyF* alleles we tested. Only the loss of both SlphyB1 and SlphyE, or SlphyB1 and SlphyF, or SlphyB1 and SlphyB2 together (Figure 3) resulted in the complete loss of responsiveness to R suggesting that SlphyB1 and either SlphyB2, SlphyE, or SlphyF together are required to regulate R perception during seedling establishment in tomato. These data are also consistent with the notion that SlphyB1and SlphyE, as well as SlphyB1 and SlphyF can form functional heterodimers. It is of interest to note that some dark-grown genotypes with a mutation in SlphyB1 (e.g., *SlphyB1* or *SlphyB1E*), as well as the *SlphyB2F* mutant also were statistically significantly taller than WT seedlings grown in the dark, a somewhat surprising observation for a light receptor, but one which has also been previously reported for *SlphyA* (Carlson et al. 2019).

3.4 | Inhibition of Germination in Shade Conditions Is Mediated Individually and Synergistically by SlphyE, SlphyB1, and SlphyB2

Phytochromes in Arabidopsis have been shown to play a role in the regulation of germination, and a partial knock-down

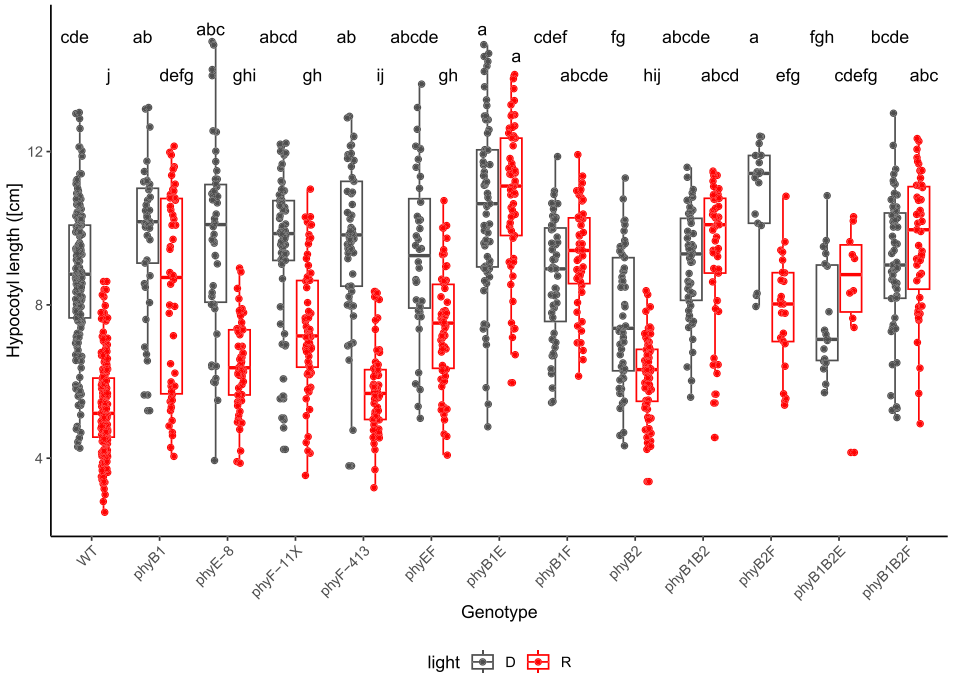

FIGURE 3 | Both phyB1 and phyE play critical roles in the repression of hypocotyl elongation in R. Seedlings were germinated for 4 days in the dark and then grown for an additional 3 days either in darkness or R. Three-way ANOVA (type I) followed by Tukey analysis was performed using the software package R. Genotypes/treatments that are not connected by the same letter are statistically significantly different from each other. The box plot shows the 25–75 percentile values between the bottom and the top of the box. The whiskers extend to maximally 1.5* IQR (inter quartile range). The bold line indicates the median. $N = 1674$ total.

phyE amiRNA mutant in tomato has been used to propose a role for SlphyE in the epicotyl and internode shade avoidance response (Schrager-Lavelle et al. 2016). Since the shade avoidance response requires the sensing of the R/FR ratio, we asked if SlphyE was required to sense FR light and if so, whether or not such ability would enable tomato seeds to use this information to time its germination based on optimal light conditions.

The typical response of tomato seeds to irradiation with FR is an almost complete lack of germination, which can be reversed by subsequent irradiation with R (Appenroth et al. 2006). To ascertain that any recorded lack of germination in FR light was really due to a specific FR effect and did not simply indicate reduced seed viability, we incubated imbibed seeds first in continuous FR and, after determining the germination rate over a week, irradiated the seeds with germination-inducing continuous R, before calculating the FR germination rate of viable, R-responsive seeds only. We confirmed that WT tomato seedlings were strongly inhibited in their ability to germinate in FR conditions (Figure 4). Mutations in SlphyB2 or SlphyF had no statistically significant effect on lifting the germination-repressing response to FR while both *SlphyB1* and *SlphyE* mutants showed a statistically significant increase in germination rates under those conditions. Interestingly, the combination of *SlphyB1* and *SlphyE* mutations almost completely released the seeds from germination inhibition under FR light. Double mutants with lesions in either SlphyB1/SlphyB2, or in SlphyE/SlphyF, or in SlphyB1/SlphyF had smaller, but statistically significant effects on germination when compared to the WT response. Our data suggest that SlphyB1 and SlphyE can act alone or synergistically together, while SlphyB2 and SlphyF act only in conjunction with SlphyB1 to prevent germination under unfavorable light conditions and even then, only at a comparatively smaller magnitude.

3.5 | The Inhibition of Germination via ABA Is Synergistically Mediated by the Action of SlphyB1 and SlphyE

ABA is well-known to act as an inhibitor of germination, while gibberellin (GA) promotes it (Finkelstein et al. 2008). We asked if phytochromes play a role in mediating ABA's inhibitory effect in various light conditions. We first tested the promoting effect of GA under germination-repressive FR. On 10 μM GA-supplemented medium, WT seeds remained dormant in FR, yet germinated, as expected, after 7 days of illumination with R (Figure S1). In FR hardly any differences were observed between GA-treated and control seeds (Figure S1).

Treatment with 500 nM ABA resulted in the abolishment of significant germination in all genotypes, except the *SlphyB1/SlphyE* double mutant (Figure 4), which was able to germinate in FR on ABA, although germination rates were decreased by approximately half when compared to the rates without ABA. These results show that although SlphyB1, SlphyB2, and SlphyE mediate germination in FR on ABA-free medium (Figure 4), only the combination of lesions in both SlphyB1 and SlphyE together lead to partial removal of FR-induced repression of germination, even in the presence of ABA in the growth medium. These data are consistent with the notion that both ABA and the FR-activated SlphyB1 and SlphyE signal can independently but additively repress germination.

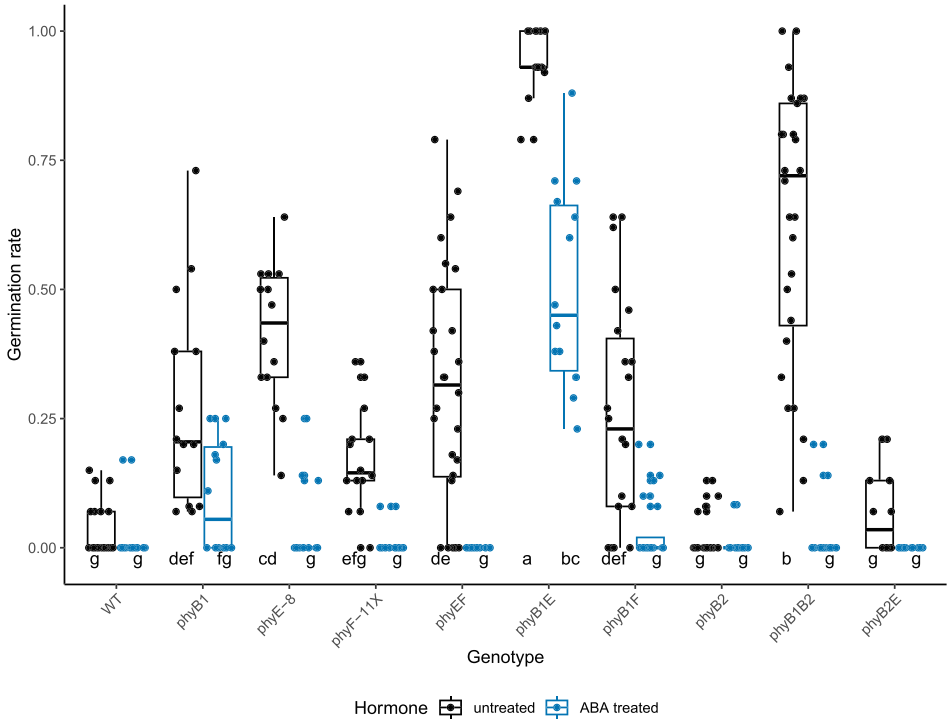

FIGURE 4 | Phytochrome E plays a critical role in preventing germination in FR light. Batches of seeds were allowed to germinate in FR light either on 500 nM ABA-supplemented or non-supplemented MS agar (see Section 2 for details). Three-way ANOVA (type I) followed by Tukey analysis was performed using the software package R. Genotypes/treatments that are not connected by the same letter are statistically significantly different from each other. Each data point ($N = 350$) represents one batch of 15 seeds. Box plot parameters are the same as in Figure 3.

3.6 | Root Growth Is Mediated by the Interaction of Red Light and ABA

In WT tomato, R leads to increased root growth compared to roots growing in the dark (Figure 5). To test if any phytochrome is the mediator of this response, we grew our mutant collection on large agar plates either in darkness or continuous R. Single mutations in SlphyB2, SlphyE, and SlphyF did not result in a loss of responsiveness; however, the lesion in the *SlphyB1* allele led to a complete inability of the roots to accelerate growth to escape light. Roots of double mutants not containing *SlphyB1*, such as *SlphyB2/SlphyE* and *SlphyB2/SlphyF,* were able to increase growth statistically significantly in R, but all tested combinations containing the *SlphyB1* allele, as expected, were non-responsive to R. It is of note that *SlphyB1* roots were consistently statistically significantly longer than WT roots in the dark, an observation that also held true in *SlphyB1/SlphyE,* but not the other double mutants containing the *phyB1* mutant allele (Figure 5).

Root elongation in response to light is mediated by the stress hormone ABA and reactive oxygen species (ROS) (Gil et al. 2018). To test if in tomato R signaling also utilized ABA for signal transduction, we tested a subset of the mutants on agar plates supplemented with 500 nM ABA. We observed that despite supplementation of the medium with ABA, the WT and *SlphyE* still responded to the light stimulus with root elongation. The addition of ABA effectively restored R responsiveness to the *SlphyB1* mutant, similar to levels seen in the WT. The lack of sensitivity seen in *phyB1/phyB2* without ABA (Figure 5) was phenocopied in the *SlphyB1/SlphyE* double mutant on ABA (Figure 6), albeit with the result that *SlphyB1/SlphyE* roots in both light conditions were significantly shorter than without ABA in the medium (Figure 6). These data suggest that ABA does not require SlphyB1 or SlphyE action to repress root growth and that SlphyB1 and SlphyE can circumvent ABA repression of root growth when light conditions warrant increased light avoidance growth (Figure 6). Interestingly, treatment with ABA in the dark resulted in root growth reduction in *SlphyB1* down to WT levels. Taken together, our results suggest that phytochrome and ABA separately regulate and fine-tune germination (Figure 4) and light-induced root growth (Figure 6) in tomato.

4 | Discussion

Despite the enormous importance of light signals in the development of plants, and the well-known significance of the role that phytochrome plays in the transduction of the light signal into actionable molecular information, surprisingly little is known about this light receptor's functions in species other than in the model organism *A. thaliana*. With the construction of a stable, CRISPR-induced mutant in phytochrome *SlphyE,* we have added an important tool to the study of phytochromes in the Solanaceae and have begun to characterize the gene's function in tomato.

4.1 | The Role of SlphyE in Tomato Is Different From Its Role in Arabidopsis

In *A. thaliana*, AtPHYE is most closely related to AtPHYB and AtPHYD. Those three phytochromes represent one subclade and stand in contrast to the sister sub-clade consisting of AtPHYA and AtPHYC. While SlPHYE is a close homolog to

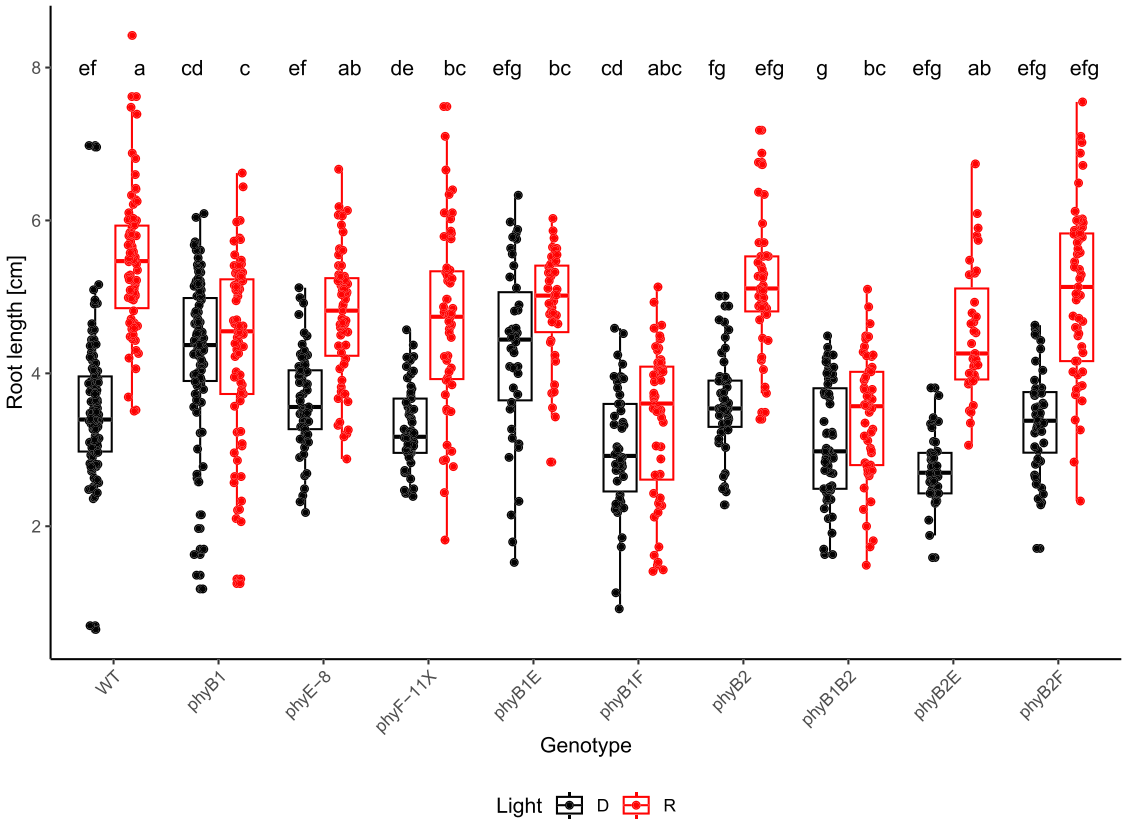

FIGURE 5 | The light signal for roots to elongate is mostly mediated by phyB1, with phyE, and phyF playing minor roles. Nine-day-old seedlings were grown on agar plates, and either exposed to continuous R or darkness. Three-way ANOVA (Type I) followed by Tukey analysis was performed using the software package R. Genotypes/treatments that are not connected by the same letter are statistically significantly different from each other. $N = 1118$ total. Box plot parameters are the same as in Figure 3.

AtPHYE, the AtPHYB/D genes separated from the SlPHYB1/B2 genes prior to the separation of the AtPHYB/D and SlPHYB1/B2 genes from each other (Figure S2) and might thus be expected to display a relatively larger amount of subfunctionalization than for example the phyE homologs in tomato and Arabidopsis (Carlson et al. 2020). It is against this background that we compared the functions of SlphyE with those known for AtphyE.

While single mutant seedlings of *AtphyE* have been described as phenotypically essentially indistinguishable from the WT (Devlin et al. 1998; Hennig et al. 2002), we note several, in some cases small, in other cases large, yet always statistically significant differences between the Moneymaker WT cultivar and monogenic *SlphyE* seedlings (Figures 3–6). These observations suggest that phyE in tomato plays a larger overall role in early plant development than in Arabidopsis. It is interesting that combinatorial mutants between *AtphyE* and *AtphyB* in Arabidopsis and *SlphyE* and *SlphyB1* in tomato showed similarly synergistic effects with respect to their effect on germination in continuous FR (Figure 4, Hennig et al. 2002), yet differences during the hypocotyl elongation inhibition response (Figure 3, Hennig et al. 1999). Specifically, in tomato the *SlphyB1/SlphyE* double mutant showed a complete loss of growth inhibition in continuous R (Figure 3), while the relative hypocotyl elongation inhibition to R pulses versus FR pulses in Arabidopsis showed the same somewhat reduced response in *AtphyB1* single as well as in *AtphyB1/AtphyE* double mutants (Hennig et al. 1999). These comparisons show that interactions between phytochromes are different in the Solanaceae versus the Brassicaceae and further corroborates the notion that SlphyE in tomato has distinct functions from AtphyE in Arabidopsis.

4.2 | Light-Mediated Root Growth in Tomato Is Mostly Regulated by SlphyB

Light is a powerful signal for plant growth. On the one hand, plants interpret light with a higher R to FR ratio as an indication that sunlight is plentiful leading to a prioritization of leaf expansion over stem elongation. On the other hand, a low R:FR ratio is read as a signal that the plant is shaded by the canopy of another plant, resulting in the initiation of a growth spurt to avoid growing in the shade (Casal and Fankhauser 2023). In both Arabidopsis and tomato, phyE has been implicated in the shade avoidance response (Devlin et al. 1998; Schrager-Lavelle et al. 2016). By contrast, light avoidance root growth, or "escape tropism" as it has also been called (Yokawa et al. 2011), has less frequently been studied.

Despite usually growing underground, roots can sense light in three ways: first, via molecular signal transduction, second, via light channeling through the tissue, and third by sensing light in the roots themselves (for review, see Lee et al. 2017). Possibly due to the use of differing methodologies and light

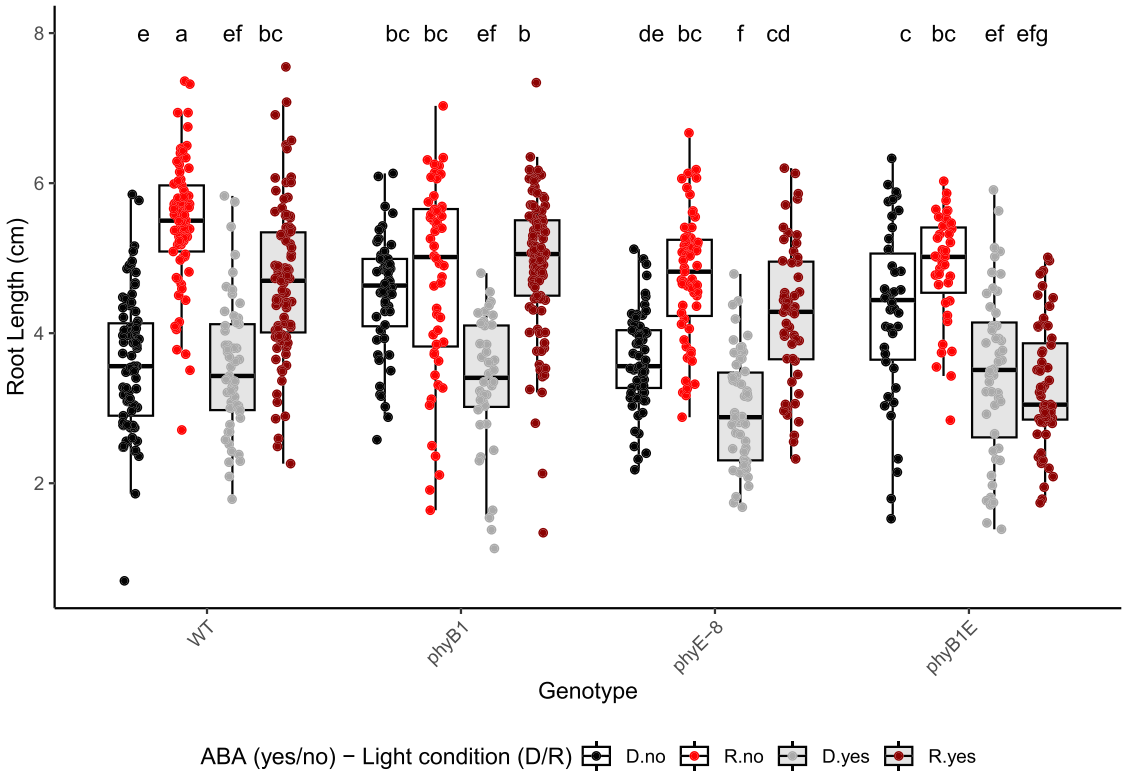

FIGURE 6 | Phytochrome E together with phyB1 mediates the light-induced root growth response. Nine-day-old seedlings were either grown in R or darkness on MS medium either supplemented with 500 Sq nM ABA or grown on non-supplemented medium. Three-way ANOVA (Type I) followed by Tukey analysis was performed using the software package R. Genotypes/treatments that are not connected by the same letter are statistically significantly different from each other. $N = 955$ total. Box plot parameters are as in Figure 3. Black: no ABA in darkness, red: no ABA in R, gray: ABA in darkness, dark red: ABA in R.

conditions, surprisingly there is considerable confusion and apparent disagreement found in the literature about the effects of light on root growth. Roots of Arabidopsis seedlings have for example been reported to be longer in light than in dark conditions (e.g., Laxmi et al. 2008; Dyachok et al. 2011; Yokawa et al. 2011; van Gelderen et al. 2018), shorter in the light than in darkness (Silva-Navas et al. 2015; Spaninks and Offringa 2023), or unaffected by light (Ha et al. 2018). In one report, roots of Arabidopsis seedlings grown in R were significantly longer than those grown in white light and about the same length as roots in the dark (Silva-Navas et al. 2015), while no such effect was seen in R-grown roots when compared to dark grown roots in another report (Spaninks and Offringa 2023). Conflicting results were also reported in those two studies with respect to the effect of blue light on root growth (Silva-Navas et al. 2015; Spaninks and Offringa 2023). In both published cases, as well as in our own results, there is a high degree of sample-to-sample variation within each genotype and treatment, shedding a degree of uncertainty on the findings and highlighting the need for large sample sizes to attain reliable results. One possible reason for the discrepancy with respect to the effect of light on root growth in published data is that the involvement of reactive oxygen species (ROS) in light-mediated root growth can both promote or reduce root growth, with short term exposure leading to a temporary increase, and long term exposure to a decrease in root growth (Gil et al. 2018). In this context, in Arabidopsis shoots AtphyB appears to stimulate ABA production, which can act as a mobile signal to mediate ROS levels in the root (Gil et al. 2018; Ha et al. 2018).

Our data, accumulated from measuring over 1100 seedlings (on average >55 per genotype and condition), show that tomato roots grown in continuous R have a robust escape response that is strongly mediated by SlphyB1 and unaffected by SlphyB2 (Figure 5). This is potentially in contrast to Arabidopsis *AtphyB* mutants, which were shown to be shorter than WT when grown in full-spectrum white, not in red, light (Ha et al. 2018). It should be noted here with caution that white light, containing light of both blue and red wavelengths, also affects changes in morphology mediated by blue-light receptors, such as cryptochrome and phototropins (Briggs and Christie 2002; Wu and Spalding 2007).

Monogenic mutations in *SlPHYE* and *SlPHYF* show less, yet statistically significant attenuation of the R response (Figure 5). It is interesting to note that the double mutants of *SlphyB1* with *SlphyF* markedly reduce absolute root length in R compared to the *SlphyB1* single mutant, while roots remain at the length of *SlphyB1* in the combination of this mutation with *SlphyE* (Figure 5), suggesting that SlphyE and SlphyF have different functions when combined with SlphyB1. These results, as well as those from the measurements on hypocotyl elongation repression and germination (Figures 3 and 4) also are consistent with the notion that SlphyB1 and SlphyE, as well as SlphyB1 and SlphyF can dimerize to achieve their functions and supports

our previous speculations on SlphyB1/SlphyF dimerization (Balderrama et al. 2023).

4.3 | SlPhyB1 and SlPhyE act Synergistically With Each Other and in Antagonism to ABA to Fine-Tune Root Growth Responses to Light

We show that SlphyB1 and SlphyE mediate R-induced root growth in tomato (Figure 5). In Arabidopsis, shoots, but not roots of *AtphyB*, contain reduced levels of ABA in comparison to WT, and treatment of *AtphyB* seedlings grown in white light with exogenous ABA at physiological concentrations leads to an increase in root growth in the mutant, restoring it to WT levels (Ha et al. 2018). From these and other experiments Ha et al. (2018) proposed that in Arabidopsis, AtphyB enhances ABA biosynthesis, explaining the change in root growth during light treatment. More recently, exogenous ABA has been shown to affect root growth in water-replete Arabidopsis in a concentration-dependent manner, with low concentrations (0.1 μM) slightly promoting and at higher concentrations strongly reducing root growth (Miao et al. 2021).

In tomato, low concentration (0.5 μM) ABA treatment had no effect in darkness and in R shortened the primary root in the WT (Figure 6). Interestingly, despite ABA treatment the WT, *SlphyB1* and *SlphyE* roots remained sensitive to the growth enhancing effects of R illumination, but loss of SlphyB1 and SlphyE together, just like growth in dark conditions, resulted in the synergistic loss of light-induced growth (Figure 6). These data, taken together, are consistent with a model (Figure 7) in which R-responsive SlphyB1/SlphyE and ABA (at 0.5 μM) act antagonistically on root growth in tomato (Figure 6), while the same phytochromes act additively with ABA in the process of repressing germination in FR conditions (Figure 4).

It is interesting to note that root elongation in the *phyB1/phyF* and the *phyB1/phyB2* double mutants in the absence of supplied ABA (Figure 5) is significantly less compared to elongation in WT and *phyB1/phyE*. Yet root length is at about the same absolute level as the *phyB1/phyE* mutant when treated with 500 nM ABA (Figure 6). Given that ABA inhibits post-germination seedling development (Yadukrishnan and Datta 2021), that phytochrome de-represses HY5 (Chen and Chory 2011), which in turn activates ABI5 expression, and which then directly activates ABA-response genes (Yadukrishnan and Datta 2021), it can thus be speculated that it is the lack of functional phyB1 plus either phyB2 or phyF that constitutes the light signal mediator in the root light-escape growth response in tomato.

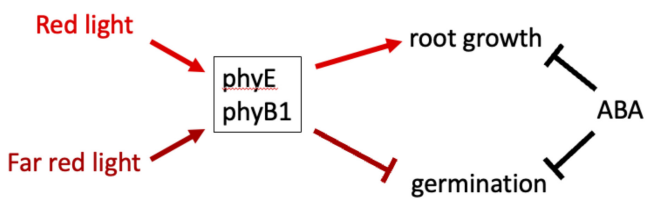

FIGURE 7 | Model illustrating how light quality effects the role of PhyB1/E in coordination with ABA to fine-tune growth and developmental responses in tomato.

It remains curious that mutation in SlphyB1 leads to statistically significantly longer roots in darkness than what is seen in WT. One possibility for a reason for this observation is that phytochrome activation might have occurred previous to germination and is carried over trans-developmentally from developing seed to germinating seedling as has been proposed for Arabidopsis previously (Mazzella et al. 2005).

5 | Conclusions and Outlook

Creating a new set of mutants allowed us to begin to show the roles that phyE in tomato plays in early photomorphogenesis, and during seed germination. Ongoing work is currently directed at better understanding the interaction between phytochromes and ABA in tomato and the coordination of these responses.

Author Contributions

A. M. conceived the original research experiments. K. C. designed and created the CRISPR mutants. K. C., D. B, S. B., S. P., T. T., and A. M. constructed and verified the higher order mutants. S. B. and A. M. performed the physiological experiments and analyzed the data. S. B. and A. M. wrote the article with input from all.

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Peer Review

The peer review history for this article is available in the Supporting Information for this article.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.