



Characterization of an *Arabidopsis thaliana* Mutant Identified in a Genetic Screen for Altered Red Light Responses

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Introduction

Perception of light is crucial for a plant's survival. Plants can perceive red (~670nm) and far-red (~730nm) wavelengths of light via cellular light receptors known as phytochromes. The Gingerich lab has observed the participation two genes, *LRB* (*Light-Regulating BTB*) 1 and *LRB*2, in the red-light signaling pathway. Disruption of both genes (in an *lrb1/lrb2* mutant) in the model dicotyledonous plant *Arabidopsis thaliana* produces a red hypersensitive phenotype, one outcome of which is to make the plants shade-tolerant. The proteins encoded by these genes are part of large protein family known as the Bric-a-Brac, Tramtrack, and Broad Complex (BTB) proteins. BTB proteins are part of protein complexes known as BTB/CUL3 E3 ubiquitin ligases, which select target proteins for destruction (Figure 1). BTB proteins function in these complexes by binding the proteins to be degraded (Pintard, 2004).

To better understand the function of the *LRB1* and *LRB2* genes and the red light signaling pathway, we conducted a genetic suppressor screen to identify other genes which participate in the red light pathway. This screen identified mutations which reduce the red-hypersensitive phenotype conferred by the *lrb1/lrb2* mutations. Here we present one of the mutants identified in this screen and describe our work mapping the mutation in the genome and identifying the gene affected by the suppressor mutation.

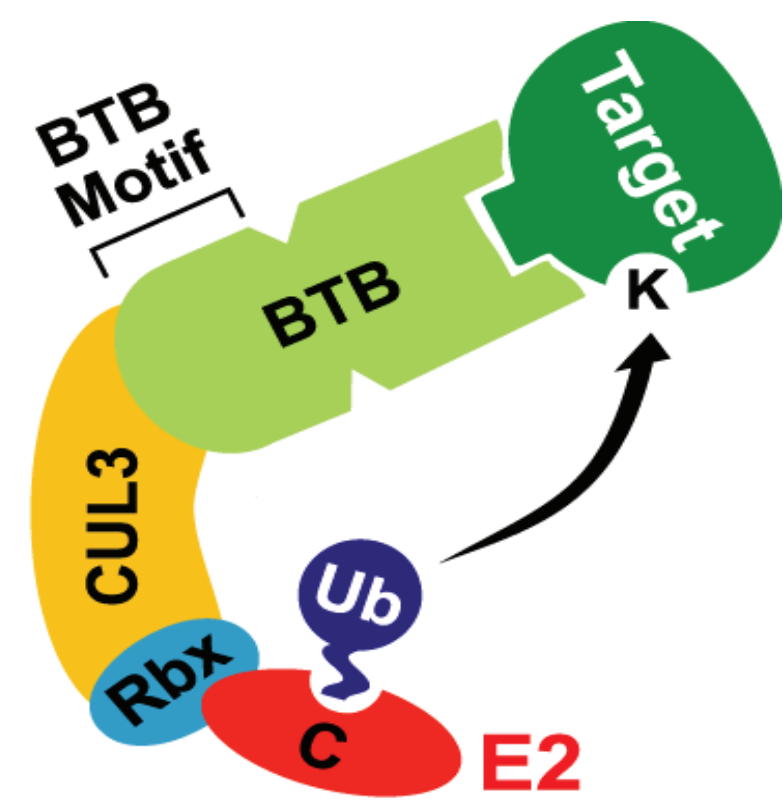


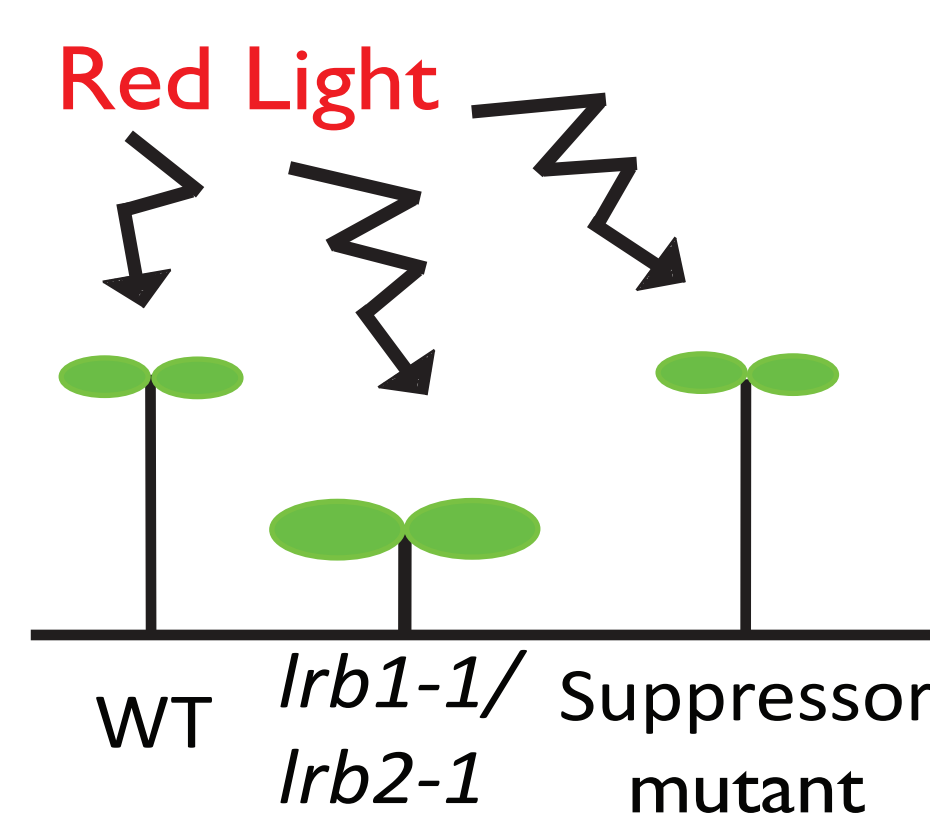
Figure 1. Structure of the BTB/CUL3 E3 ubiquitin ligase complexes. The E3 binds the target protein and ubiquitinates it. This ubiquitination leads to degradation of the target.

Suppressor Screening

A population of *lrb1/lrb2* double mutants (in the Col-0 ecotype background) seeds were exposed to ethyl methanesulphonate (EMS), a chemical which causes point mutations throughout the genome. These M1 seeds were germinated and the plants self-fertilized to produce M2 seed. This M2 seed was then screened under red light, and individuals with reduced red sensitivity were compared to the *lrb1/lrb2* double mutants were identified.

Strategy:

- Mutagenize population of *lrb1-1/lrb2-1* seeds with ethylmethanesulphonate (EMS).
- Germinate seeds and grow plants (10 plants/pot), 2000 individuals total.
- Collect seed from these individuals.
- Germinate and grow this next generation (M2) under red filtered light; identify individuals that have reduced red light sensitivity compared to the *lrb1-1/lrb2-1* double mutants.



Result: Line S3-5-2 was one of the mutants identified in this screen.

S3-5-2 Phenotype

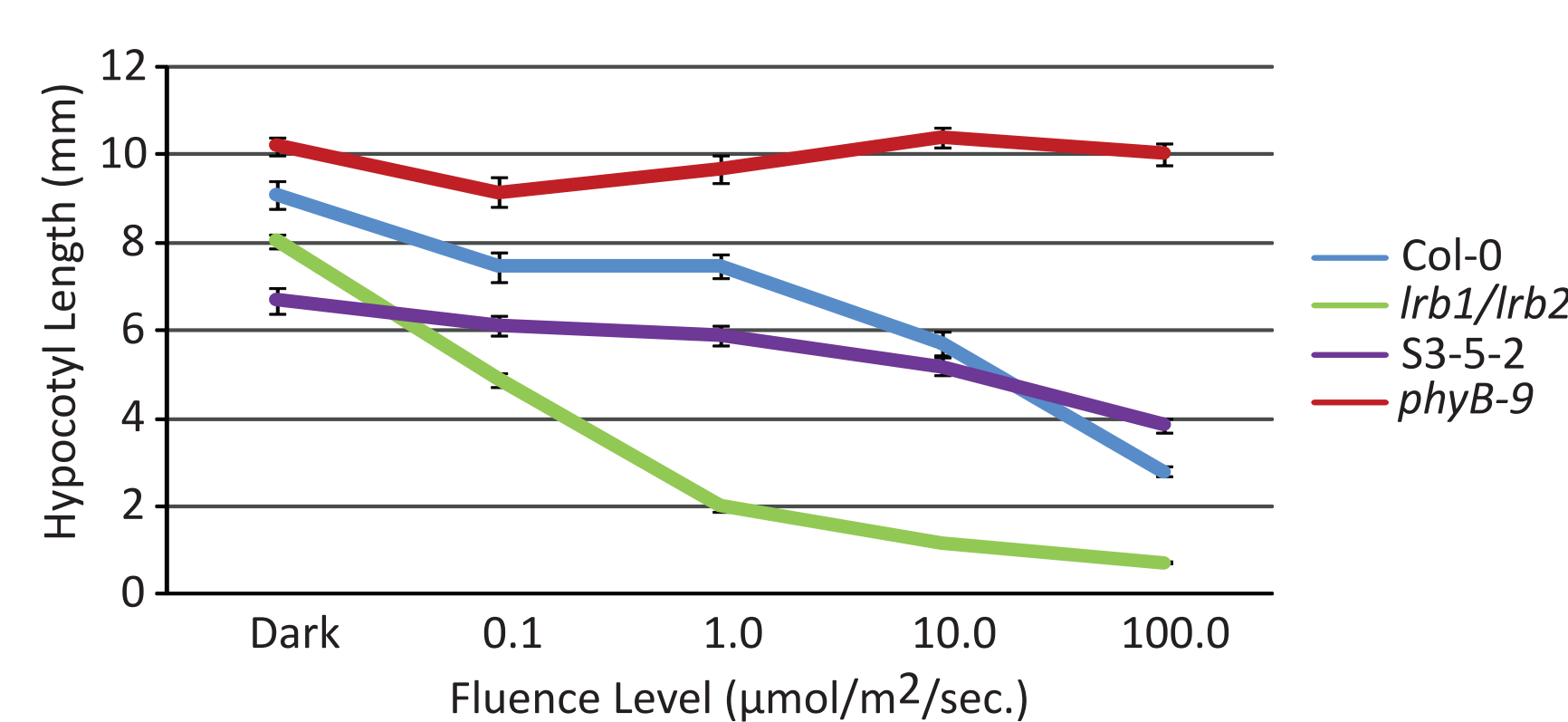


Figure 2. Mean hypocotyl length for line S3-5-2 and controls at 4 red light fluence levels and in the dark. The seeds were sterilized and plated on 1/2 MS media; cold treated at 4°C for four days in the dark; then germination was induced with an 8 hour white light treatment. Following this the seedlings were dark treated for 16 hours prior to transfer to red light for four days. Hypocotyl length was measured at the end of the red light treatment. Standard error bars are shown.

S3-5-2 Phenotype cont.

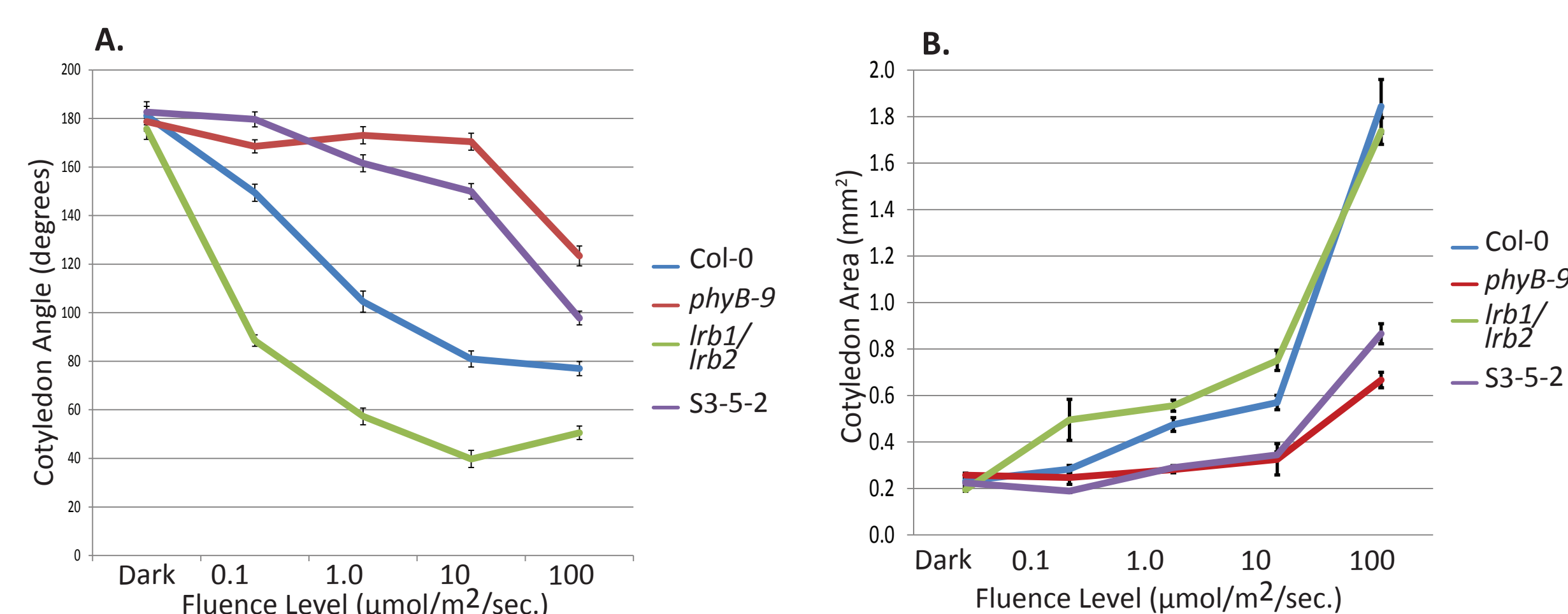


Figure 3 A. Mean cotyledon angle of S3-5-2 and controls at 4 fluence levels and dark. Seeds were sterilized and grown on 1/2 MS media; cold treated at 4°C for 4 days in the dark; then germination was induced with an 8 hour white light treatment. Following this the seedlings were dark treated for 16 hours prior to transfer to dark 0.1, 1.0, 10, or 100 μmol/m²/sec red light for four days. Standard error bars are shown. **B.** Mean cotyledon areas of S3-5-2 and controls at four red light levels and dark. The seedlings were grown as above. Standard error bars are shown.

Result: Mutant S3-5-2 from the suppressor screen displayed a strong red light insensitive response and was selected for genetic mapping.

Mapping

- Cross line S3-5-2 (in the Col-0 ecotype background), with wild-type Landsberg (Ler) ecotype plants to generate triple *lrb1/lrb2/suppressor* heterozygous individuals.
- Self-cross *lrb1/lrb2/suppressor* heterozygous individuals to produce F2 seed.
- Germinate and grow F2 individuals under red light. F2 individuals that display the phenotype characteristic of the suppressor mutation (long hypocotyl and small cotyledon area) are selected from this population.
- Isolate DNA from >150 F2 plants with the suppressor phenotype.
- PCR genotyping is employed on subsets of these plants to analyze single-sequence length polymorphism (SSLP) and Insertion/Deletion (InDel) markers scattered throughout the Arabidopsis genome.

Because of genetic linkage, the region in the genome near the mutation in most or all of the F2 individuals should consist of Col-0 sequence, not Ler sequence.

Examples of Agarose Gel Analysis of PCR Genotyping Reactions

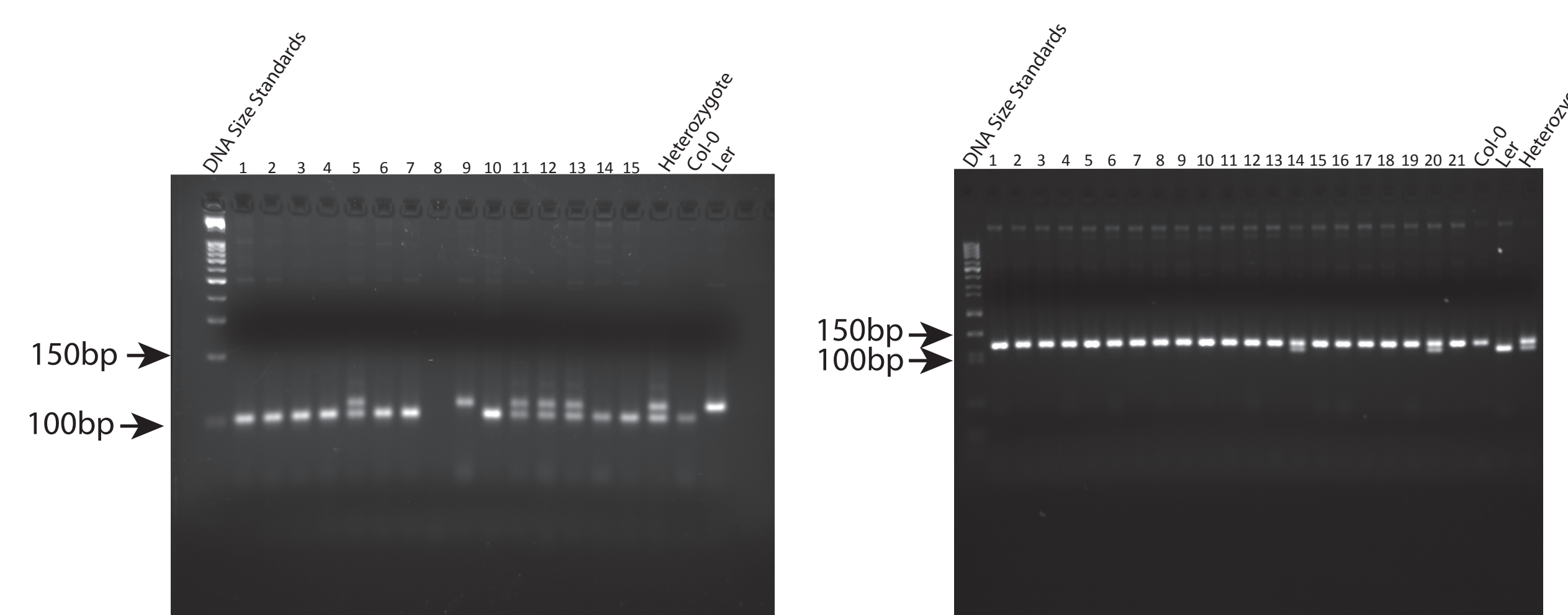


Figure 4. PCR analysis of 15 F2 individuals and controls using marker nga361, which is located on chromosome 2. The PCR product from a Col-0 version of the nga361 marker should be 114bp. The PCR product from a Ler version of the marker should be 120bp.

Figure 5. PCR analysis of 21 F2 individuals and controls using marker CER458641, which is on chromosome 2. The PCR product from a Col-0 version of the CER458641 marker should be 123bp. The PCR product from a Ler version of the marker should be 112bp.

Result: The suppressor mutation is closer to marker CER458641 than marker nga361

Rough Mapping

Marker	Marker Type	Chromosome	Ler Alleles/Total Alleles
CIW12	SSLP	1	15/34
LRB1	InDel	2	16/34
nga361	SSLP	2	14/36
CIW3	SSLP	2	3/36
CIW2	SSLP	2	6/36
CIW11	SSLP	3	13/30
LRB2	InDel	3	19/36
nga1107	SSLP	4	20/36
CTR1.2	SSLP	5	16/36
PHYC.3	SSLP	5	16/36

Table 1. The suppressor mutation in line S3-5-2 is linked to markers CIW3 and CIW2 on chromosome 2 in the Arabidopsis genome. Markers tested by PCR in initial rough-mapping experiments using plants from the F2 mapping population are shown. Marker type (simple-sequence length polymorphism [SSLP], and insertion/deletion [InDel]), chromosome location, and number of Ler ecotype alleles out of total assayed are shown. If a marker is genetically linked to the suppressor mutation than few Ler alleles should be found in the mapping population.

Mapping (cont.)

Fine Mapping

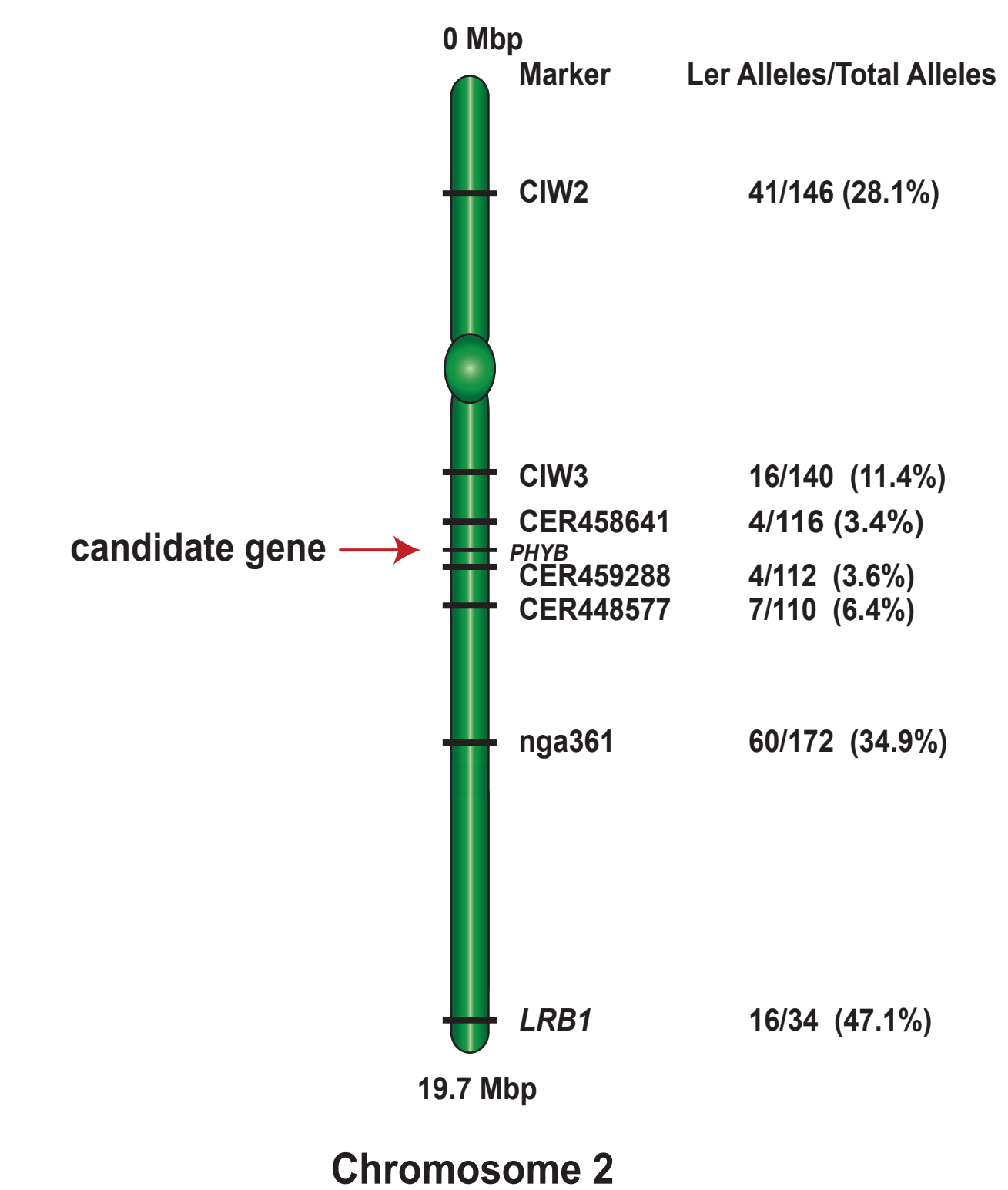


Figure 5. Fine-scale mapping on chromosome 2 was done using additional InDel markers on this chromosome. This analysis showed that the mutation is near markers CER459288 and CER458641, as very few Ler versions of these markers were found in the F2 plants with the suppressor phenotype. *PHYB* was considered a likely candidate gene because it encodes the primary red light photoreceptor.

Complementation Analysis

To determine if the suppressor mutation in line S3-5-2 is in the *PHYB* gene, a genetic complementation analysis was performed. Line S3-5-2 (with the *lrb1/lrb2* background) was crossed with the *phyB-9 lrb1/lrb2* mutant. If the F1 offspring display the S3-5-2 and/or *phyB* phenotype then the S3-5-2 mutation is in the *PHYB* gene.

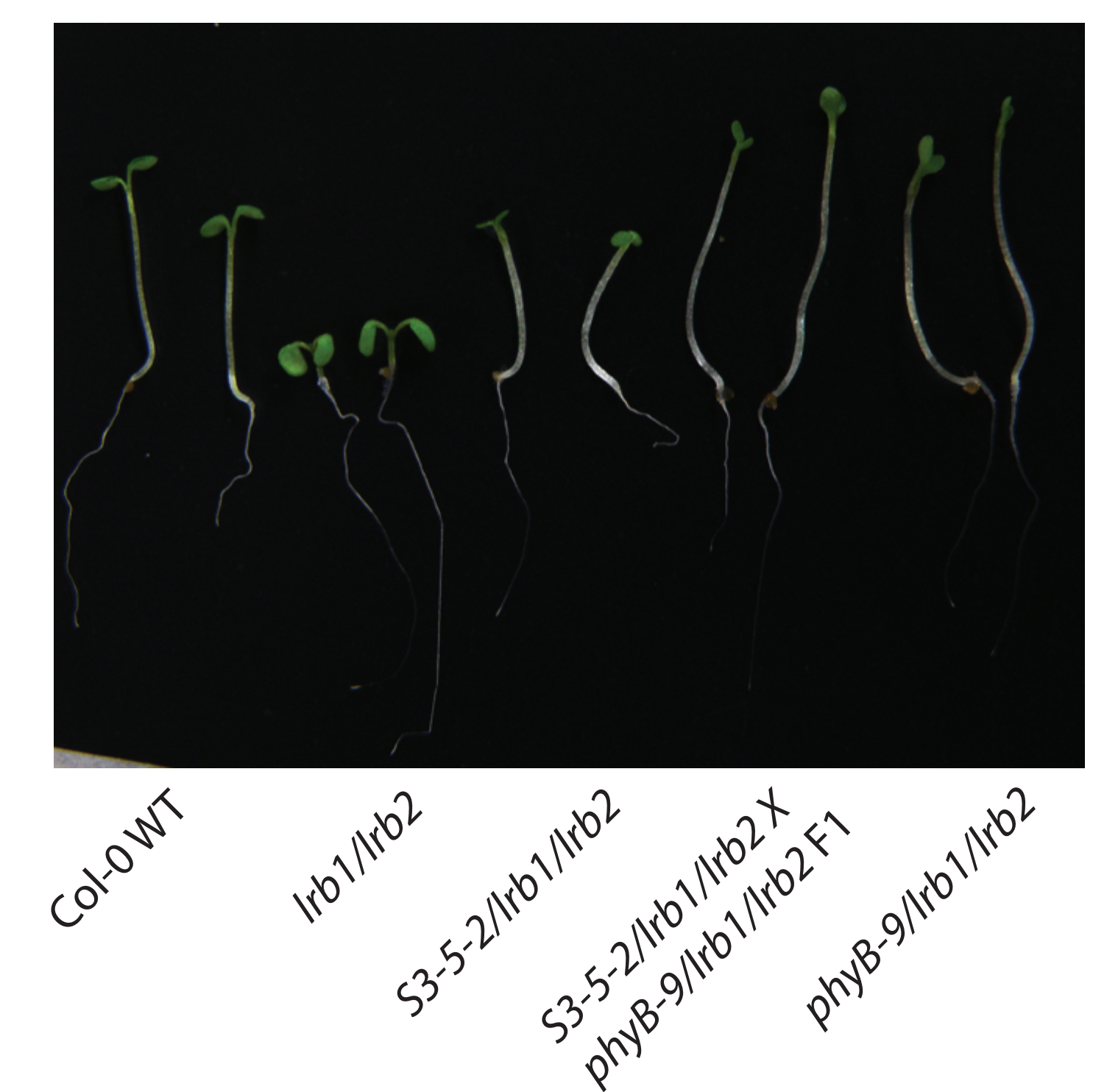


Figure 6. Phenotypic analysis of S3-5-2 *lrb1 lrb2* x *phyB-9 lrb1 lrb2* F1 progeny and controls. The seedlings were germinated and grown under 10 μmol/m²/sec red light for 4 days.

Conclusions

- lrb1 lrb2* suppressor mutant S3-5-2 displays strong insensitivity to red light
- Mapping and complementation testing show the mutation responsible for this response in mutant S3-5-2 is located near or possibly in the *PHYB* gene on chromosome 2
- PHYB* encodes the primary red light photoreceptor in Arabidopsis
- Genetic sequencing is needed to confirm the S3-5-2 mutation in the *PHYB* gene

References

Pintard, L., Willems, A., & Peter, M. (2004). Cullin-based ubiquitin ligases: Cul3-BTB complexes join the family. *Embo Journal*, 24(5), 1092-1092.

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