Progress Towards Identifying *lrb1 lrb2* Enhancer Mutations in the Plant *Arabidopsis thaliana* by Next-Generation Genome Sequencing and Mapping

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Introduction

Light is vital to plant survival and thus plants have developed sophisticated pathways to respond properly to their light environments[1]. Plants sense specific wavelengths of light via photoreceptors, one family of which are the red (R)/far-red (FR)-absorbing phytochromes (phys). Absorption of red light activates the phys, which causes their translocation from the cytosol to the nucleus where they modulate gene expression[2]. Work by our lab and others has implicated two genes (called *Light-Response BTB 1* and *2* [*LRB1* and *LRB2*]) as critical regulators of the phy light-response pathway[3,4]. *LRB1* and *LRB2* encode BTB (Bric-a-Brac, Tramtrack, Broad Complex) domain-containing proteins that act as target adapters in E3 ubiquitin-ligase complexes (Figure 1)[3,4]. Plants with disruptions of the *LRB1* and *2* genes (*lrb1 lrb2* mutants) have reduced light-dependent degradation of phys and exhibit hypersensitivity to red light [3,4].

To identify additional genes involved in light responses, our lab used these *lrb1 lrb2* mutants as the basis for a genetic screen. We mutagenized *lrb1 lrb2* plants, and then looked for individuals where the red light hypersensitivity was suppressed or enhanced. These plants likely carry a mutation in a gene involved, like *LRB1* and *2*, in light responses. We successfully identified numerous suppressor and enhancer mutants, two of which are discussed here. Our current focus is to map the mutations in these lines, with the hope of identifying new genes not previously implicated in light responses. To identify the mutations, we are using a whole-genome sequencing and next-generation sequencing strategy, described here.

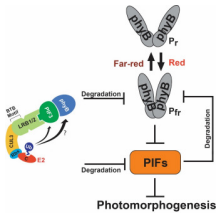


Figure 1. Structure and function of the LRB/CUL3 E3 ubiquitin ligase complex in the red light response pathway in *Arabidopsis thaliana*. Red light activates phytochrome B, which then blocks the activity of PIF proteins, which are negative regulators of red light responses. The LRB/CUL3 E3 complex mediates ubiquitylation and degradation of both PIF3 and phyB. Figure is based partly on model of LRB action presented by Ni *et. al.*[4]

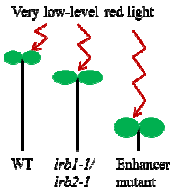
Screen to Identify Enhancer Mutants

Mutagenzie population of *lrb1-1 lrb2-1* seed with ethylmethanesulfonate (EMS)

Germinate seeds and grow plants (10 plants/pot), 2000 individuals total

Collect seed from these individuals

Germinate and grow this next generation under red filtered light/ identifity individuals that have reduced or increased red light sensitivity compared to the *lrb1-1 lrb2-1* double mutants.



Red Light Response of the E2-1-2 and E11-6-5 Enhancer mutants

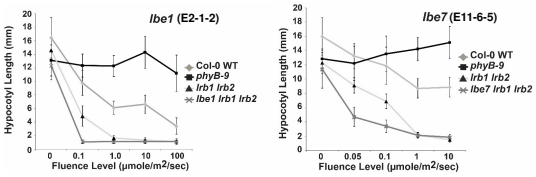


Figure 2. Hypocotyl elongation responses to red light of the E2-1-2 (*lbe1*) and E11-6-5 (*lbe7*) mutants. Hypocotyl lengths of wild-type (WT), *lrb1 lrb2*, *phyB-9*, and enhancer mutants grown in darkness or various levels of red light are shown. Seeds were germinated on MS media without supplemental sucrose under white light and then grown in the dark or under continuous red light for 4 days. Error bars indicate ± SD.

Strategy to Map Enhancer Mutants by Whole Genome Sequencing

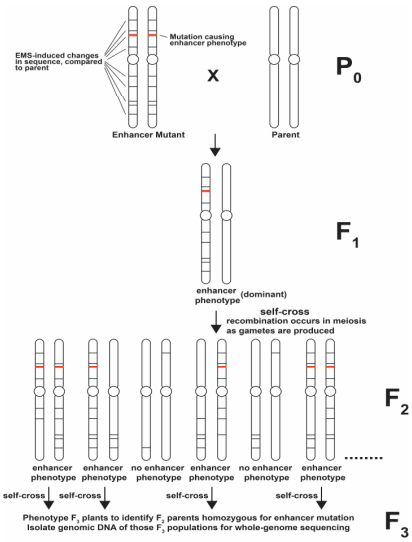
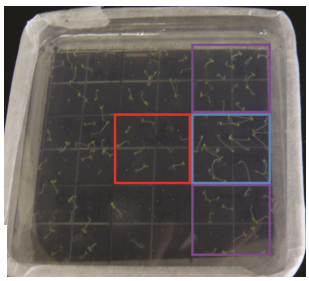
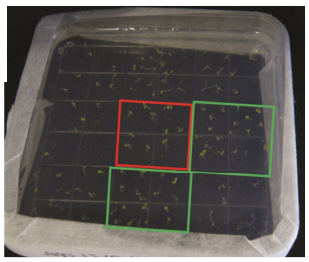


Figure 3. Generation of plants for whole-genome sequencing. *lrb1-1 lrb2-1* double mutant parent is crossed with original enhancer mutant, which also contains the *lrb1-1/2-1* double mutation (not shown). All F1 populations express the short hypocotyl phenotype, since the enhancer mutation is dominantly inherited. F1 individuals self-cross and a 3:1 enhancer phenotype is expected in the F2 generation. F2 individuals showing the dominant phenotype self-cross and F3 offspring from these are analyzed to determine which F2 individuals were homozygous for the enhancer mutation. F3 plants from 9-14 F2 parents are pooled for isolation of genomic DNA. This DNA is whole-genome sequenced, along with genomic DNA isolated from the *lrb1-1/lrb2-1* parent. Comparison of these sequences allows us to narrow the region of the genome containing the enhancer mutation and possibly identify that mutation.

Analysis of F3 Populations



BLUE: *lrb1-1/2-1* double mutant control, with all long hypocotyls
RED: E11-6-5 control, with all short hypocotyls
PURPLE: F3 populations with all short hypocotyls (enhancer phenotype). F2 parent was homozygous for enhancer mutation. These populations will have genomic DNA isolated for sequencing



RED: E2-1-2 control, with all short hypocotyls
GREEN: F3 populations with all short hypocotyls (enhancer phenotype). F2 parent was homozygous for enhancer mutation. These populations will have genomic DNA isolated for sequencing

The E2-1-2 and E11-6-5 Enhancer Mutations are Dominant

lbe/lrb1-1/lrb2-1 — Phenotype under very low-level RL: short hypocotyl

X

lrb1-1/lrb2-1 — Phenotype under very low-level RL: long hypocotyl

↓

F1 ***lbe+/-/lrb1-1/lrb2-1*** — Phenotype under very low-level RL: short hypocotyl

↓⊗

F2 offspring phenotypes:

	lbe1 (E2-1-2)	*lbe7* (E11-6-5)
short hypocotyl # individuals	557	203
long hypocotyl # individuals	181	47

DNA Isolation for Whole Genome Sequencing

- From the F3 population analysis we determined that 14 E2-1-2 F2 individuals were homozygous for the enhancer mutation, while 9 E11-6-5 individuals were homozygous for the enhancer mutation. F3 offspring from these F2 individuals were pooled for DNA preparations for sequencing.

DNA yield from E2-1-2 F3 DNA prep: 126.6 µg of DNA
DNA yield from E11-6-5 F3 DNA prep: 181.3 µg of DNA

- DNA from these preps were recently sent to Dr. Lucia Strader at Washington University, who is leading a consortium of Arabidopsis labs that pool DNA samples for whole genome sequencing. The Genome Technology Access Center at Washington University will perform this analysis.

References

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