Creation and Characterization of *LRB* (*Light-Response BTB*) /*PIF* (*Phytochrome-Interacting Factor*) Mutant Lines in *Arabidopsis thaliana*

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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]. They do so by regulating the activity and levels of a family of transcription factors called Phytochrome-Interacting Factors (PIFs). In response to red light the active phys cause PIFs to be ubiquitylated and degraded, which activates expression of PIF-repressed genes. There is feedback regulation of this pathway as, in response to red light, the PIFs also induce ubiquitylation and degradation of the phys[3].

The genes *Light-Response BTB 1* and *2* [*LRB1* and *LRB2*]) are critical regulators of the phy/PIF light-response pathway[4,5]. *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)[4,5]. Plants with disruptions of the *LRB* genes have reduced light-dependent degradation of phys and, like plants with disruptions of *PIF* genes, exhibit hypersensitivity to red light [4,5,6]. The mechanism by which the LRBs modulate phy levels is not entirely clear, however it has been shown that the LRBs can bind to a complex of a PIF protein (PIF3) and a phy (phyB), leading to ubiquitylation and degradation of both (Figure 1)[5].

In order to better understand how the *LRB* and *PIF* genes interact we are taking a genetic approach, creating plants with T (transfer)-DNA disruptions of both *LRB* and *PIF* genes. Study of the phenotypes of these plants may shed light on how these two families of genes work together to regulate light responses or plant growth and development in general.

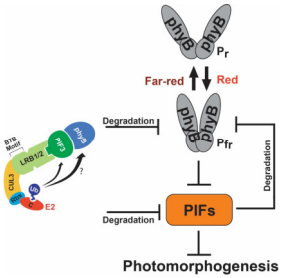


Figure 1. Structure and function of the LRB/CUL3 E3 ubiquitin ligase complex in the red light response pathway in *Arabidopsis thaliana*. Red light activates phytochrome B, which then blocks the activity of PIF proteins, which are negative regulators of red light responses. The LRB/CUL3 E3 complex mediates ubiquitylation and degradation of both PIF3 and phyB. Figure is based partly on model of LRB action presented by Ni *et. al.* (2014).

T-DNA Mutant Genotyping Strategy

T-DNAs are pieces of foreign DNA that can insert into genes. When they do so, they usually disrupt function of the genes. T-DNAs can be detected by PCR analysis.

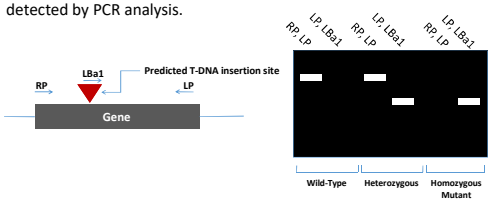
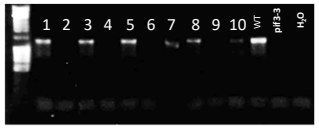


Figure 2. Strategy for identifying individuals with T-DNA insertions in a gene of interest. Genomic DNA is isolated from Arabidopsis seedlings and PCR reactions are performed with primer combinations that will produce products depending on whether or not the T-DNA is present in the gene. The PCR products are then visualized on agarose gels.

Example Genotyping Reactions

Example #1: Genotyping the *PIF3 gene* in self-cross offspring from an *lrb1-1 lrb1-1; LRB2 lrb2-1; PIF3 pif3-3; pif7-1 pif7-1* parent

Primer Combination: PIF3-3WTF, PIF3-3R3



Primer Combination: PIF3-3R3, PIF3-3DelF

Individual	Genotype for *PIF3*	Individual	Genotype for *PIF3*
1	heterozygous	6	homozygous mutant
2	homozygous mutant	7	wild-type
3	heterozygous	8	heterozygous
4	homozygous mutant	9	heterozygous
5	heterozygous	10	wild-type

Example #2: Genotyping the *LRB1* gene in self-cross offspring from an *lrb1-1 lrb1-1; LRB2 lrb2-1; PIF3 pif3-3; pif7-1 pif7-1* parent

Primer Combination: RP145146, LP145146

Primer Combination: LP145146, LBa1

Individual	Genotype for *LRB1*	Individual	Genotype for *LRB1*
1	homozygous mutant	6	homozygous mutant
2	homozygous mutant	7	homozygous mutant
3	homozygous mutant	8	homozygous mutant
4	homozygous mutant	9	homozygous mutant
5	homozygous mutant	10	homozygous mutant

Lack of Certain *pif lrb* Genotypes is Unexpected

I have genotyped 47 individuals that are self-cross offspring from an *lrb1-1 lrb1-1; LRB2 lrb2-1; PIF3 pif3-3; pif7-1 pif7-1* parent. Results are shown below.

Genotype*	Plants With This Genotype	Plants Expected with this Genotype
LRB2 LRB2; PIF3 PIF3	0	6
LRB2 LRB2; PIF3 pif3-3	18	12.1
LRB2 lrb2-1; PIF3 PIF3	4	12.1
LRB2 lrb2-1; PIF3 pif3-3	27	24.3
LRB2 LRB2; pif3-3 pif3-3	27	6
lrb2-1 lrb2-1; PIF3 PIF3	21	6
LRB2 lrb2-1; pif3-3 pif3-3	0	12.1
lrb2-1 lrb2-1; PIF3 pif3-3	0	12.1
lrb2-1 lrb2-1; pif3-3 pif3-3	0	6

We did not find plants with the following genotypes in this population:

lrb2-1 lrb2-1; pif3-3 pif3-3
LRB2 lrb2-1; pif3-3 pif3-3
lrb2-1 lrb2-1; PIF3 pif3-3
LRB2 LRB2; PIF3 PIF3

It may be that some/all of these genotypes are embryo lethal, or that certain genotypes cannot be transmitted in the gametes.

Conclusions

- The Gingerich lab has successfully generated a number of *pif lrb* genotypes, however that are certain genotypic combinations that we have been consistently unable to find in our populations.
- My analysis of self-cross offspring of an *lrb1-1 lrb1-1; LRB2 lrb2-1; PIF3 pif3-3; pif7-1 pif7-1* parent has shown that it may not be possible for a plant to be homozygous mutant for both *LRB2* and *PIF3*, or for a plant to be homozygous mutant for one of the genes while heterozygous mutant for the other (at least with the *lrb1 pif7* background).
- The absence of these genotypes may be because they are lethal to a plant during the embryonic development. Preliminary analysis of siliques, however, has failed to detect visual evidence of the aborted seeds expected if this were true.
- It may be that certain allele combinations cannot be transmitted through the male or female gametes (Figure 3).

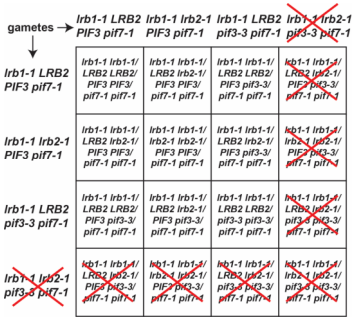


Figure 3. Expected genotypes of offspring from an *lrb1-1 lrb1-1; LRB2 lrb2-1; PIF3 pif3-3; pif7-1 pif7-1* parent, if the *lrb2-1* and *pif3-3* alleles cannot be transmitted together through the gametes. Red X's indicate the absence of plants homozygous mutant for both *LRB2* and *PIF3* or homozygous mutant for one while heterozygous for the other.

References

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