Characterizing a TIR-only/CC-NBS-LRR Dual Receptor System for Plant Pathogen Recognition

BIOLOGY
COLORADO STATE UNIVERSITY

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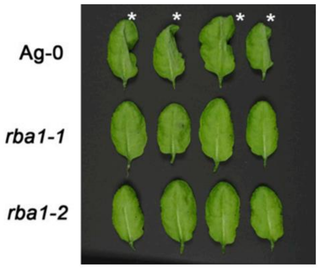
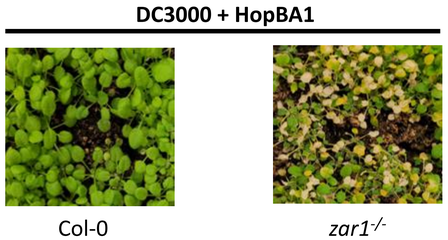
ABSTRACT

RBA1 is an Arabidopsis TIR-only immune receptor responsible for the recognition of the *Pseudomonas syringae* type III effector, HopBA1. RBA1 is unusual, lacking C-terminal domains that regulate canonical TIR-NBS-LRR (TNL) immune receptors. In the Arabidopsis accession Ag-0, recognition of HopBA1 by RBA1 triggers cell death. In other Arabidopsis accessions (e.g. Col-0), HopBA1 is instead recognized by ZAR1, a CC-NBS-NLR (CNL), to trigger disease resistance without cell death. It is currently unclear if RBA1 and ZAR1 immune responses are independent or if one regulates the other. To determine the genetic requirements for cell death and disease resistance in Ag-0 we have generated *rba1*, *zar1*, and *rba1/zar1* Cas9 mutants. These mutants reveal the hypersensitive response to HopBA1 in Ag-0 previously attributed to RBA1 also requires ZAR1. ZAR1 is known to be activated upon recognition of modified PBL kinases. We hypothesize that HopBA1 targets one or more PBL kinases, leading to both ZAR1 and RBA1 activation. To identity putative HopBA1 virulence targets, we have screened the Arabidopsis PBL kinase protein family for physical interactions with HopBA1. The biological relevance of these interactions is being assessed using reverse genetics in Arabidopsis. The HopBA1/RBA1/ZAR1 system will help explain how structurally diverse receptors can work together to form an immune system that appropriately responds to pathogens to trigger disease resistance and, in some cases, cell death.

BACKGROUND



HopBA1-induced cell death in Ag-0 *Arabidopsis* depends on the truncated TIR immune receptor, RBA1. Adapted from Nishimura *et. al* (2017).



***Pseudomonas syringae* pv. DC3000 carrying HopBA1 are growth-restricted on WT Col-0 *Arabidopsis* and display reduced symptoms. Recognition by the host is dependent on the full-length CNL plant immune receptor, ZAR1.** (Unpublished, see also Laflamme *et. al* (2020))

HYPOTHESES AND MODEL

Hypothesis 1: HopBA1 targets one or more PBL kinases to suppress plant immunity.

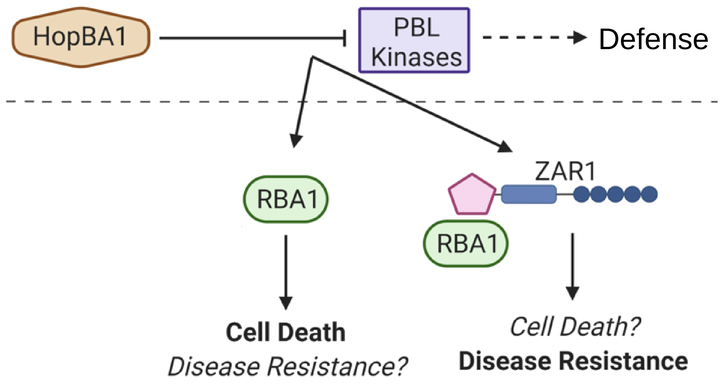
Hypothesis 2: RBA1 acts as an enhancer of the plant immune response to HopBA1.

KEY

HopBA1: Type III Secreted Effector (*Pseudomonas syringae*)

RBA1: TIR-only NLR

ZAR1: CC-NBS-LRR NLR



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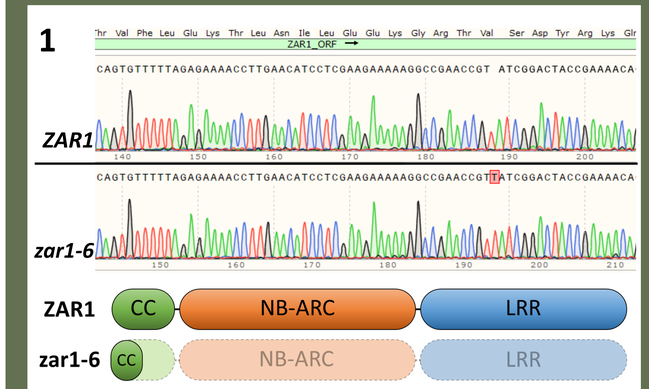

***zar1-6* CAS9 mutant in Ag-0 Arabidopsis. ZAR1 mutants were made to assess the contributions of RBA1 and ZAR1 to HopBA1-induced cell death and disease resistance.**

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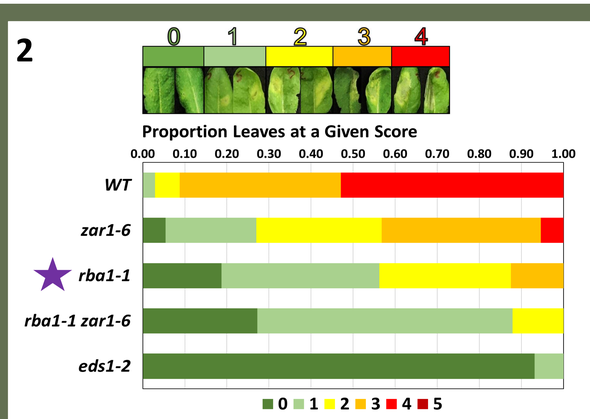

RBA1 and ZAR1 both contribute to HopBA1-triggered HR in Ag-0 Arabidopsis. Pf-0 carrying HopBA1 was used to assay HR in Ag-0 mutants

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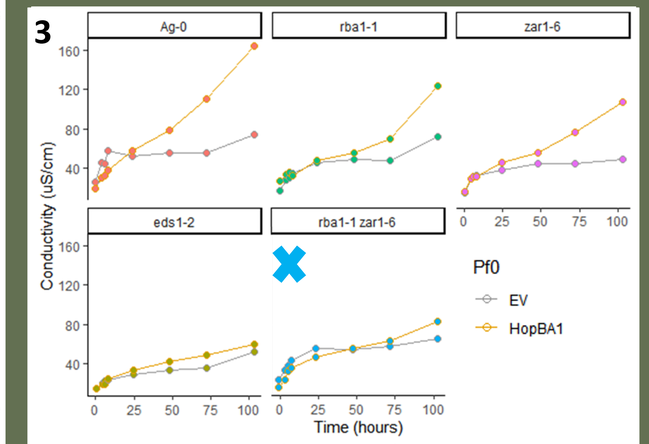

***rba1-1 zar1-6* Ag-0 double mutants lose the hypersensitive response to HopBA1 as measured by ion leakage.** Pf-0 carrying EV (promoterless GFP) or HopBA1 was used to assay HR in Ag-0 mutants

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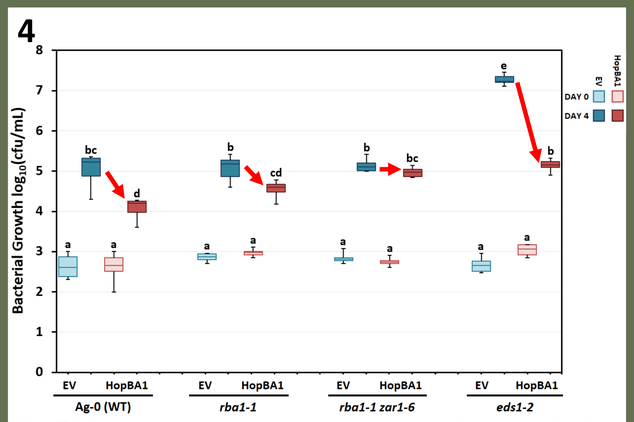

HopBA1-induced growth restriction is partially lost in Ag-0 *rba1-1* and is fully lost in Ag-0 *rba1-1 zar1-6*. The *eds1-2* mutant is presumably blocked for TIR-pathway RBA1-mediated growth restriction, but not ZAR1-mediated growth restriction.

ONGOING EXPERIMENTS

Arabidopsis PBL kinases have been screened for interaction with HopBA1 via yeast-2-hybrid assays. SALK T-DNA knockout lines for putative HopBA1-PBL interactors are being screened for increased pathogen susceptibility.

Yeast-3-hybrid approaches will be taken to further validate yeast-2-hybrid results and assess if HopBA1 activity on its virulence targets increases interaction strength with downstream signaling components. Previous studies have found that effector induced modifications to PBL kinases increase PBL-ZRK association strength.

Characterize genetic interactions between ZAR1 and RBA1. Defense assays on mutant Ag-0 *Arabidopsis* in *zar1*, *rba1* and *rba1/zar1* backgrounds will reveal genetic requirements for HopBA1 recognition.

Identify physical interactions between immune signaling components. Co-IP with signaling components will reveal protein complexes formed during defense responses to HopBA1.

Characterize HopBA1's biochemical activity. Mass spectrometry analysis of HopBA1-interacting proteins will identify any post-translational modifications made by HopBA1.

Identify downstream signaling components required for HR and growth restriction responses to HopBA1. Like other TIR proteins, RBA1 requires EDS1, however which helper NLRs are required for defense and other possible TNLs involved in the response to HopBA1 have yet to be identified.

CONCLUSIONS

★ **RBA1 and ZAR1 appear to additively contribute to HopBA1-triggered cell death in HR assays.**

✖ **Cell death response to HopBA1 as measured by ion leakage assay is lost in the *rba1-1 zar1-6* double mutant.**

↘ **HopBA1-triggered disease resistance is fully lost in the *rba1-1 zar1-6* double mutant.**

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