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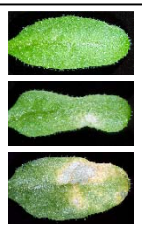
# Identifying the genetic components of plant disease resistance

## Overview

**Despite the heavy use of pesticides, an estimated 12% of potential global crop production is lost to fungal and bacterial pathogens** (James et al., 1990). Therefore, genetic engineering of crop plants should be explored as a means to improve yield, and reduce modern agriculture's dependence on pesticides, which pose a potential threat to human health.

**Current strategies focus on modifying the plants' innate defense capacity to achieve enhanced resistance or greater response to pathogens upon infection.** Two major defense mechanisms in plants are the hypersensitive response (HR) and systemic acquired resistance (SAR). The HR is a type of rapid localized programmed cell death at the site of a primary infection that isolates the pathogen and initiates SAR, a state of heightened resistance to a broad spectrum of pathogens through out the whole plant (Fig.1).

1.



Uninfected leaf

Hypersensitive response

Disease symptoms

**Previous experiments have shown that the over-expression of genes regulating disease resistance can provide resistance to virulent pathogens.** Cao et al. have found that increasing the expression of a gene, *NPR1*, whose product regulates the induction of SAR, enhances the plants ability to resist virulent pathogens (1998).

**Identification of novel genes could enhance resistance to other pathogens.** Mutants of *Arabidopsis thaliana* that are affected in SAR signaling have been identified through screens for constitutive expressors of pathogenesis-related (PR) genes (the *cpr*s) and non expressors of PR genes (*npr1*). The *cpr5* mutant spontaneously develops lesions that mimic the HR and has constitutive resistance to the virulent oomycete pathogen, *Peronospora parasitica* Noco2 that is independent of NPR1. This suggests that there are other signaling pathways in addition to the one regulated by NPR1 that contribute to disease resistance. We carried out a screen in the *cpr5npr1* double mutant background to identify genes involved in NPR1 independent resistance.

## Scientific Approach

**Hypothesis: A genetic screen in the *cpr5npr1* mutant background will identify novel positive regulators of disease resistance**

2. 25,000 fast-neutron mutagenized *cpr5 npr1* seed

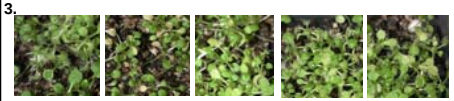
M2 generation screened (1000 seed/pool) using *Peronospora parasitica* infection

susceptible mutants rescued through growth at low humidity

Mutants characterized and mutated gene Identified through positional cloning

**The *cpr5npr1* double mutant is completely resistant to *P. parasitica*, so any growth of the pathogen is due to a mutation in a gene that regulates disease resistance.** A population of 25,000 *cpr5npr1* seeds was mutagenized by fast-neutron bombardment (dose 60Gy) and infected with *P. parasitica* Noco2 (Fig. 2). Sixty-six independent lines were confirmed to be susceptible to *P.parasitica* (examples in Fig. 3). Because these lines have a mutation which suppresses the constitutive disease resistance phenotype of cpr5, they were named *suppressors of cprV* or *scv.*

3.



ColWT  *cpr5 npr1*  *#42*  *#216*  *#95*

**The screen for suppressors of *cpr5* identified three alleles of *scv1* on the basis of restored size and susceptibility to *Peronospora parasitica* NOCO2.** One allele of scv1 was selected from the M1 generation on the basis of partially restored growth and trichome development (Fig.4A&B). Complementation tests established that the three dominant *scv1* lines were allelic.

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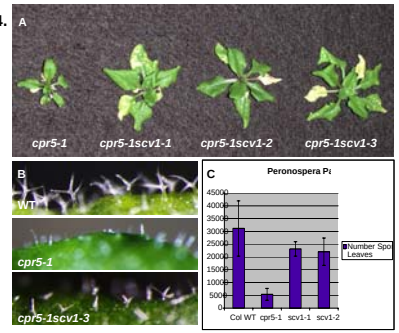


***scv1* completely restores *cpr5* to wild-type levels of susceptibility to virulent strains of *Peronospora parasitica*.** *scv1-1* and *scv1-2* were identified by their susceptibility *P.parasitica* NOCO2 and show pathogen growth similar to wild-type, as quantified by spore development 7 days post infection (Fig.4C).

***scv1* blocks resistance to the bacterial pathogen *Pseudomonas syringae.*** To test whether the disruption of *cpr5* mediated resistance extended to other pathogens, we tested the *in planta* growth of the virulent bacterial pathogen *P. syringae* ES4326. Two days post inoculation the *cpr5scv1* lines showed greater symptom development (Fig.5A) which correlated with greater bacterial growth (Fig.5B).

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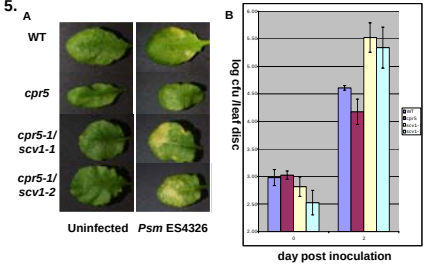


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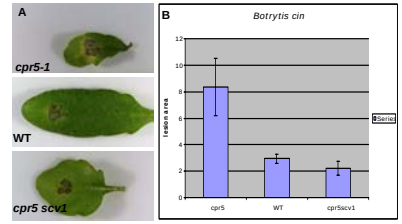


***scv1* restores resistance to the fungal pathogen *Botrytis cinerea*.** This suggests that the bacterial resistance and fungal resistance pathways may be antagonistic. Future work will look at gene expression downstream of each signaling pathway.

***SCV1* is located between genetic markers g4026 and m305, on the bottom arm of chromosome 1.** We are in the process of identifying the *SCV1* locus through positional cloning (Lukowitz, 2000)

## Impact

**We have identified a gene that is required for disease resistance.**

**The dominant mutation in this gene decreases resistance to bacterial pathogens, but increases resistance to fungal pathogens.** Thus, the cloning of *SCV1* will increase our understanding of how bacterial and fungal resistance mechanisms differ.

***SCV1* will be a potential target for genetic engineering in plants to acheive increased resistance to bacterial and fungal pathogens.**

**Acknowledgements and Literature cited**

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