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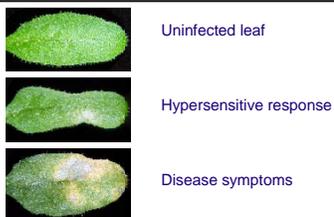


# Identifying the genetic components of plant disease resistance

## Overview

Due to the heavy use of pesticides, an estimated 12% of total 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 increase 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 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 throughout the whole plant (Fig.1).

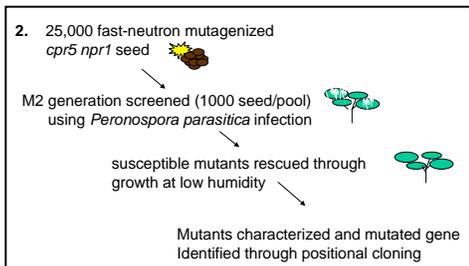


Recent experiments have shown that the over-expression of genes regulating disease resistance can provide resistance to virulent pathogens. Cao et al. have found that increasing the expression of a gene, *NPR1*, whose product mediates the induction of SAR, enhances the plants ability to resist virulent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

Identification of novel genes could enhance resistance to plant pathogens. Mutants of *Arabidopsis thaliana* that are altered in SAR signaling have been identified through screens for constitutive expressors of pathogenesis-related (PR) genes (*cpr5*) and non expressors of PR genes (*npr1*). The *cpr5* mutant spontaneously develops lesions that mimic the HR and 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* dependent resistance.

## Scientific Approach

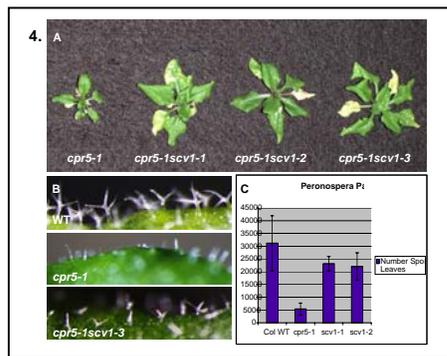
**Hypothesis: A genetic screen in the *cpr5npr1* mutant background will identify novel positive regulators of disease resistance**



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 *cpr5* or *scv*.

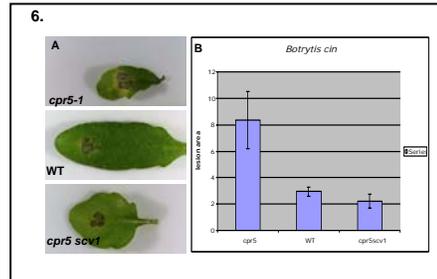
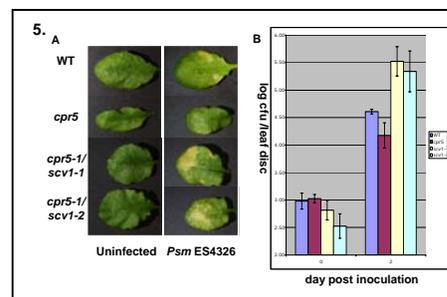


The screen for suppressors of *cpr5* identified three alleles of *scv1* on the basis of restored size and susceptibility to *Peronospora parasitica* NOCO2. One allele of *scv1* was selected from the M1 generation on the basis of partially restored growth and trichome development (Fig.4A&B). Complementation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



*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 to *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).



*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 achieve increased resistance to bacterial and fungal pathogens.

### Acknowledgements and Literature cited

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