Genetics of Disease Resistance in *Arabidopsis* to Crop Pathogens

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Abstract

Arabidopsis is universally resistant as a species to many crop pathogens, including examples from other crucifers such as *Albugo candida* and *Hyaloperonospora parasitica* from *Brassica oleracea*. This species level trait could potentially provide a source of durable disease resistance in crops if examples can be found which are amenable to molecular genetic characterization. Our research has developed from the observation that null mutation in *Arabidopsis* of a defense regulatory gene *EDS1* (**e**nhanced **d**isease **s**usceptibility) is susceptible to isolates of *A. candida* and *H. parasitica* from brassica. *EDS1* is required by a major structural class of R-genes to confer resistance in *Arabidopsis*. We have therefore focused on identifying *R*-genes in *Arabidopsis* that are responsible for conferring resistance to brassica pathogens.

Keywords: *Arabidopsis thaliana*; *Brassica oleracea*; Albugo; white rust; *Hyaloperonospora*; downy mildew; species level disease resistance; non-host resistance

INTRODUCTION

A slogan posted above the entrance to our office reads, "Arabidopsis, a weed with a purpose". It was the title banner from an exhibit presented at the UK Royal Agricultural Show in 1994, designed for farmers and the general public to illustrate a frontier in plant biology and the promising opportunities for crop improvement that could arise from fundamental research of a common, inconspicuous wildflower. The slogan was coined with the pursuit of knowledge firmly in mind. A pursuit driven in numerous research laboratories around the world by fundamental questions about the mechanisms of disease resistance in plants and the excitement of contributing to a rapidly expanding Arabidopsis research community. Crop improvement was not an immediate focal point, but instead, thought of as something that would eventually "spin-off" from academic research.

Eight years hence, and Arabidopsis research has come of age as a dominating focal point for advances reported in professional plant journals. As described below, disease resistance in particular has provided a fertile subject for investigation. The spin-off from such research for crop improvement, is still in its infancy but there is certainly enormous potential especially if *Arabidopsis* is considered from the perspective of plant breeding. The purpose of this article is to describe how we have begun to use *Arabidopsis* as a wild relative of crops and a tertiary gene pool, much as a plant breeder would consider any wild species. A major advantage, however, is the wealth of detailed knowledge of genotype specific resistance in the donor species, and the opportunity to marry traditional breeding and pathology methods with more advanced molecular tools and bioinformatics.

Exploiting natural genetic variation of *Arabidopsis* for gene discovery

Molecular genetic investigation of disease resistance has been at the vanguard in Arabidopsis research of

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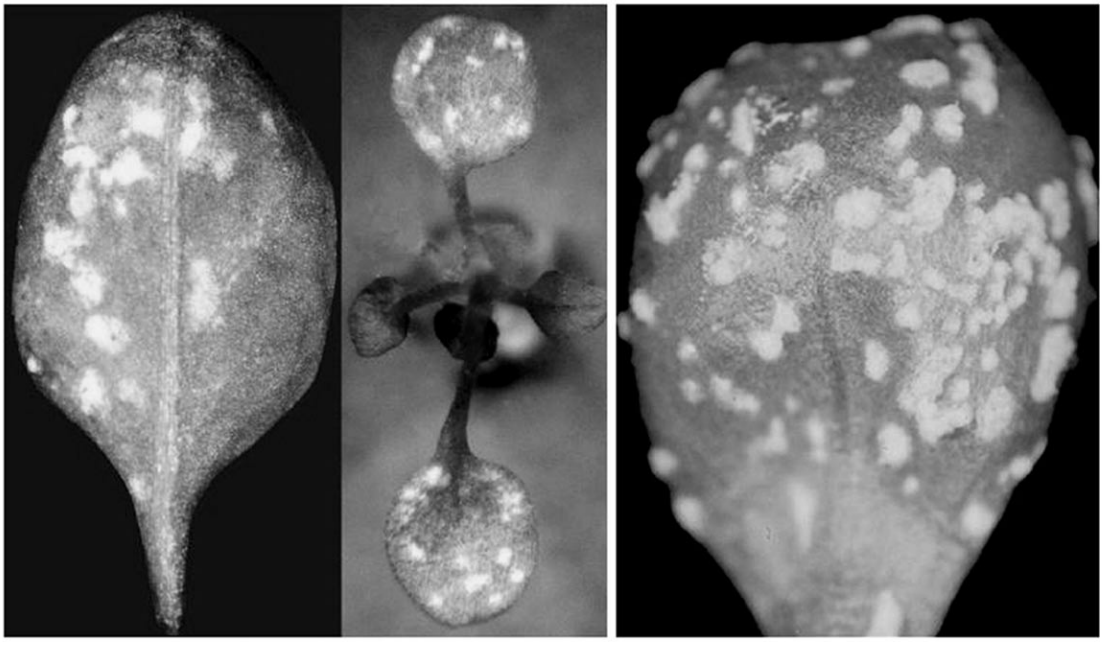
utilizing natural genetic variation. Numerous disease resistance genes (so-called ***R*-genes**) have been revealed that confer genotype or race specific resistance to a wide assortment of pathogens, a subject reviewed elsewhere (DANGL & JONES 2001; HOLUB 2001). A majority of these genes are of the same structural class, now widely known by the abbreviated name of NB-LRR (**n**ucleotide **b**inding site and **l**eucine **r**ich **r**epeat domains). Genes of this class have also been described in a wide diversity of crops such as maize, lettuce, sugar beet, tomato and tobacco. An obvious spin-off, therefore would be to use DNA sequence from NB-LRR genes and other known *R*-gene classes to develop more effective genetic markers for traditional breeding of resistance in crops (AARTS *et al*. 1998a,b; SPEULMAN *et al*. 1998; VICENTE & KING 2001; WANG & XIAO 2002). The prospect of using *Arabidopsis R*-genes themselves to confer resistance in crops has also been demonstrated (XIAO *et al*. 2002).

Gene discovery from artificial genetic variation

Most *Arabidopsis* research has been advanced using genetic variation that was derived artificially from mutagenesis induced chemically, by irradiation, or by transposon or T-DNA insertion. For investigation of disease resistance, numerous mutants classified broadly as ones that either enhance susceptibility to avirulent pathogens or else enhance resistance to virulent pathogens, have been molecularly characterized (for example, DANGL & JONES 2001; SHAPIRO 2000). A complex impression of defense signaling has emerged from these mutational analyses, and consequently redefines the frontline for Arabidopsis pathology as an effort to identify the intermediate components of defense regulation and dynamic relationships among distinct responses.

The underlying defense responses appear to be widely conserved among plant species. In some cases, genes have been conserved across Kingdoms, such as *SGT1* which has recently been identified as a major regulator of powdery mildew resistance in barley and downy mildew resistance in *Arabidopsis*, having first been described as a critical gene for cell cycling and proteolysis in yeast (AZEVEDO *et al*. 2002; TOR *et al*. 2002). Disease resistance can potentially be improved in crops by altering the expression of defense regulation, as suggested by broad spectrum resistance that was achieved by over-expression in *Arabidopsis* of *NPR1,* a regulator of acquired disease resistance (CAO *et al*. 1998).

White rust caused by *Albugo candida* f.sp. *brassica*



Arabidopsis thaliana (Ws-*EDS1.1*) *Brassica oleracea*

Figure 1. White rust caused by the same isolate of *Albugo candida* subsp. *brassica* in the *EDS1* (**e**nhanced **d**isease **s**usceptibility) mutant of *Arabidopsis*, and in its natural host *Brassica oleracea*. Wild-type *Arabidopsis* exhibits species level resistance to this same isolate

Species level ("non-host") disease resistance

Wild plant species represent a gene pool that can potentially provide a rich source of durable, broad spectrum disease resistance for use in crops. Species that are inter-fertile with a particular crop have often been used for transferring monogenic resistance that is often genotype or race specific. Unfortunately, such resistance derived from either crop or wild germplasm often lacks durability when deployed in field production because of selection it imposes that forces the pathogen to adapt and overcome the resistance. Non-durable resistance is potentially still useful, however, plant breeders are usually challenged to continue the search for new sources of resistance (PINK 2002).

Broad spectrum resistance from wild germplasm, which may be polygenic and inherently more difficult to transfer and re-construct in a crop, will be increasingly more accessible as a result of recent advances in our knowledge of candidate resistance genes. The underlying basis for this resistance is that the pathogen as a taxonomic group (species or subspecies) is highly specialized to cause disease in the crop, but not in the wild crop relative. Broad spectrum resistance that occurs universally in the wild relative is referred to below as *species level ("non-host") resistance* to the pathogen.

Some of the recent advances demonstrate clearly that signaling mechanisms governing genotype specific resistance can also govern species level resistance. For example, mutations in one of the defense regulators discovered in *Arabidopsis*, designated *EDS1* (enhanced disease susceptibility), exhibit broad spectrum susceptibility to several pathogens including isolates of *Albugo candida* (white rust) (Figure 1) and *Hyaloperonospora parasitica* (downy mildew) (CONSTANTINESCU & FATEHI 2002) from *Brassica oleracea* (PARKER *et al.* 1996). Isolates of both pathogens collected from either brassica or Arabidopsis represent distinct subspecies, based on their restrictive host specialization and molecular divergence (REHMANY *et al.* 2000). Therefore, *EDS1* is an essential regulator of species level resistance to at least two brassica pathogens. The highly conserved protein, *SGT1* has also recently been reported as a key regulator for species level resistance (PEART *et al.* 2002).

Two bacterial *R*-genes, *RPS4* and *RPS5,* have been described in Arabidopsis which demonstrate that NB-LRR genes can provide necessary components of species level resistance (GASSMAN *et al.* 1999; WARREN *et al.* 1998). Both of these genes were characterized using corresponding avirulence determinants that were derived from legume isolates of *Pseudomonas syringae*. Arabidopsis exhibits species level resistance, at least in symptomology, to these pathogens. The avirulence determinants, however, were introduced into an Arabidopsis-virulent isolate of *Ps. syringae* to facilitate molecular characterization of the resistance genes.

Transient suppression of species level resistance has also been observed in *Arabidopsis*. For example, *Bremia lactuca* (lettuce downy mildew) can reproduce asexually in cotyledons or true leaves following pre-infection with *A. candida* (COOPER *et al.* 2002).

Future discovery of genes that contribute to species level resistance

EDS1 has been shown to regulate disease resistance conferred by some but not all *R*-genes, and particularly for a subclass of NB-LRR genes that contain a third TIR domain (Toll/interleukin-like receptor). Enhanced susceptibility of the *EDS1* mutant to the brassica subspecies of *H. parasitica* is only partial, suggesting that the residual resistance is conferred by non-*EDS1* dependent *R*-gene(s). *R*-genes have been shown to differ in their capability of signaling via alternative defense responses (AARTS *et al.* 1998a,b; MCDOWELL *et al.* 2000; TOR *et al.* 2002).

It is therefore reasonable to propose that species level resistance can be achieved in individual plants by "layering" of pathogen recognition via multiple *R*-genes and alternative defense responses. This hypothesis only considers inducible defense, and therefore would apply, perhaps exclusively, to pathogens that are capable of infecting a host (as observed microscopically) but invariably succumb to the induced defense soon after penetrating the host (as observed using the same isolates to inoculate a geographic diverse collection of host accessions). Species level resistance in *Arabidopsis* to the brassica subspecies of *A. candida* provides a useful model for further research with a focus on crop improvement. Practical application for the work could be targeted at white rust in vegetable brassica (*B. oleracea*) and oilseed *B. juncea*. This oilseed crop is favored in Asian countries because it has greater drought tolerance and a shorter life cycle than oilseed *B. napus*. However, the disease complex of white rust and downy mildew causes major annual losses. Similarly, white rust is a major factor that limits the use of oilseed *B. juncea* in North America.

Our current goal is to identify at least three white rust resistance genes from *Arabidopsis* that can confer resistance in brassica, before initiating studies to as-

sess different strategies for deploying the resistance in a crop. Two "proof of concept" genes (both NB-LRR class) have already been identified in collaboration with Hossain Borhan and Roger Rimmer (Agri. Canada-Saskatoon), and *Agrobacterium*-mediated transfer into *B. oleracea* is currently underway to determine whether they confer resistance in a crop. A third gene, designated *RAC5,* has been mapped in Arabidopsis to a region that does not contain NB-LRR genes, and exhibits a phenotype that does not appear to involve hypersensitive cell death. The avirulent pathogen can colonize a *RAC5* resistant plant but is unable to reproduce asexually. Additional genes will be sought by systematic testing of *R*-gene homologues from *Arabidopsis* in *B. oleracea*. A pilot project is underway in a co-ordinated initiative with Jonathan Jones (Sainsbury Lab), Brian Staskawicz (UC-Berkeley) and Richard Michelmore (UC-Davis). A survey of how these genes are spatially distributed in wild populations of *Arabidopsis* is also being investigated, to provide insight for how best to deploy such genes in crops.

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