

Albugo candida (White Rust) Suppresses Resistance to Downy Mildew Pathogens in *Arabidopsis thaliana*

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Abstract

Arabidopsis thaliana accessions were inoculated with incompatible isolates of downy mildews, following pre-inoculation with compatible *Albugo candida*. Three isolates of *Hyaloperonospora parasitica* subsp. *A. thaliana*, an isolate of *H. parasitica* subsp. *Brassica oleracea* and one *Bremia lactucae* (lettuce) isolate were included. All downy mildews sporulated on *A. thaliana*, suggesting *A. candida* suppresses broad-spectrum downy mildew resistance. The white rust resistance gene, *RAC5*, is being investigated. The resistance phenotype associated with *RAC5* seems not to involve a hypersensitive response. *RAC5* has been mapped telomeric of *nga106* on chromosome 5, in a region lacking NB-LRR genes, the most common structural class of resistance genes known in *A. thaliana*.

Keywords: *Arabidopsis thaliana*; *Albugo candida*; downy mildew; *Hyaloperonospora parasitica*; *Bremia lactucae*; host cell death; compatibility

INTRODUCTION

A. candida (Pers. ex Fr.) O. Kuntze (white rust) is an obligate biotroph, which infects a variety of crucifers, including the wildflower *A. thaliana* (Thale cress) and economically important *Brassica* species. Another oomycete pathogen, *Hyaloperonospora parasitica* (Downy mildew), is commonly found growing in close association with *A. candida* under natural conditions, even though it is compatible at low frequencies on its own. For example, *A. candida* was observed to appear first in combined infections with *H. parasitica* on *Brassica juncea* (BAINS & JHOOTY 1985), similar observations have been made with *A. thaliana* (HOLUB *et al.* 1995). RIMMER *et al.* (2000) noted that incompatible isolates of *A. candida* are capable of growing on *Brassica rapa* and *B. juncea* previously colonised by a compatible isolate. The authors also found evidence for sexual recombination between the different pathotypes of *A. candida*. These observations suggested that compatible isolates of *A. candida* are

capable of suppressing the hypersensitive response, which usually entails host cell death.

The most curious example of host cell death suppression by *A. candida* involves a lesion mimic mutant of *A. thaliana* designated *lsd 1* (Lesion Simulating Disease) (HOLUB & BEYNON 1997). When this mutant was inoculated with incompatible pathogens, including incompatible *A. candida* and other compatible pathogens, rapidly expanding necrosis was observed. This mutant is therefore “hyper” responsive to cell death initiators and is unable to limit the extent of cell death (DIETRICH *et al.* 1994). However, when *A. thaliana lsd 1* is inoculated with compatible *A. candida*, no host cell death was observed and the pathogen grew and reproduced, without restriction, similar to wild type interactions (HOLUB & BEYNON 1997).

MATERIALS AND METHODS

A. thaliana was dual inoculated, firstly with a compatible isolate of *A. candida* followed by incompatible

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downy mildew pathogens. The number of sporophores were counted to quantify the suppression of downy mildew resistance. Incompatible isolates of *H. parasitica* from *A. thaliana* and *B. oleracea* and *Bremia lactucae* from *Lactuca sativa* were used in these studies.

RESULTS

All incompatible downy mildew pathogens sporulated in the presence of *A. candida* and levels of sporulation in each experiment were significant compared to the control (Figure 1).

Downy mildew pathogens sporulated at lower levels than they would on their natural hosts. For example, the mean sporulation of *B. lactucae* on dual infected *A. thaliana* was 0.83 (sporophores per cotyledon), whereas on lettuce the pathogen typically sporulates 20 times or more per cotyledon. Sporulation of *B. lactucae* on *A. thaliana* *Ws-eds1* was doubled compared to *Ws-0*.

DISCUSSION

A. candida suppressed resistance effected by several different defence signalling pathways, including *SGT1b* (TÖR *et al.* 2002), *EDS1* (PARKER *et al.* 1996) and *NDR1* (CENTURY *et al.* 1995). Other unknown defence signalling pathways were also suppressed. Increased levels of *B. lactucae* sporulation on *A. thaliana* *Ws-eds1* suggest that *EDS1* is involved in resistance signalling towards this pathogen, however the mutant alone is unable to support *B. lactucae*.

Further investigation of the limits of *A. candida* mediated host cell death suppression should include experiments to determine whether *A. candida* could enhance resistance of *A. thaliana* to necrotrophic pathogens. Other research supports this hypothesis; DICKMAN *et al.* (2001) transformed tobacco to express animal antiapoptotic genes (*Bcl-2*, *Bcl-xl*, *CED-9* and *Op-IAP*), they found that these transgenes conferred heritable resistance towards several necrotrophs. This

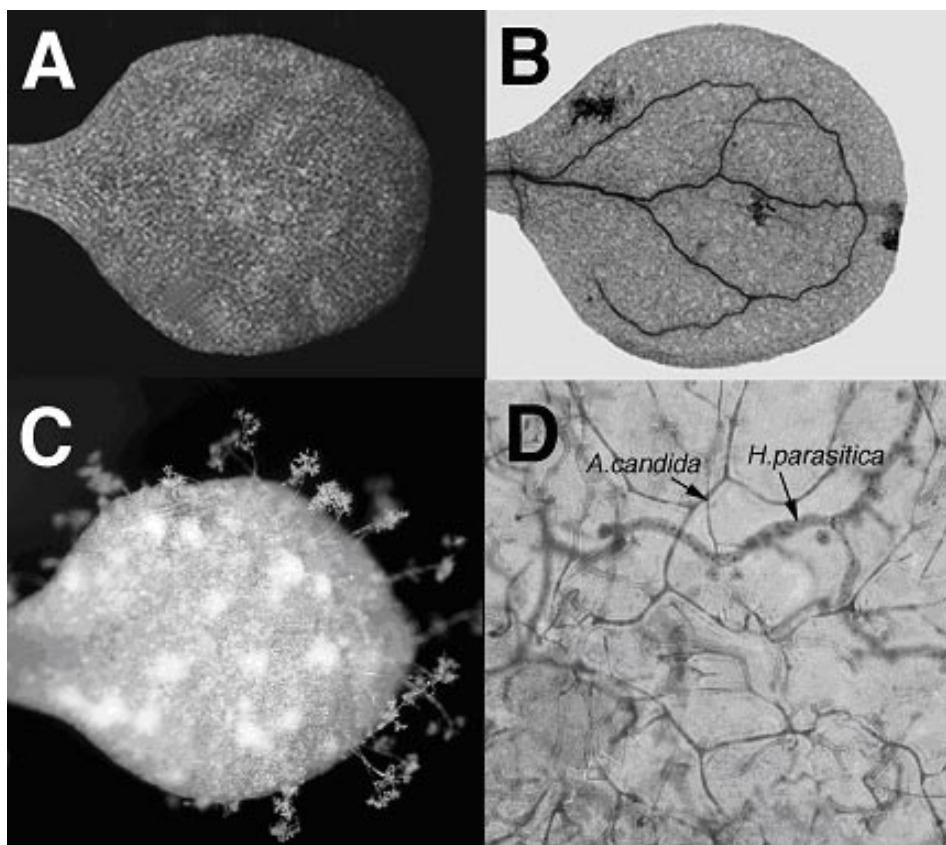


Figure 1. A shows *Arabidopsis thaliana* cotyledon seven days after inoculation with incompatible *Hyaloperonospora parasitica*, B shows a trypan blue stained whole cotyledon seven days after inoculation with incompatible *H. parasitica* $\times 100$, C shows a cotyledon seven days after inoculation with incompatible *H. parasitica* and nine days after inoculation with *A. candida*, D shows a trypan blue stained cotyledon supporting the growth of compatible *A. candida* and incompatible *H. parasitica*

work suggests that necrotrophic pathogens utilise host cell death pathways to facilitate disease.

Secondly, it will be important to determine whether *A. candida* can suppress resistance to biotrophic pathogens from other Kingdoms including bacteria and viruses. Research by YARWOOD (1951) found a positive association between *Uromyces phaseoli* (bean rust) on *Phaseolus vulgaris* (bean) with five viruses including: *Tobacco mosaic virus*, *Tobacco ringspot virus*, *Tobacco necrosis virus*, *Alfalfa mosaic virus* and *Cucumber mosaic virus*, the first two viruses are also associated with *Puccinia helianthi* (sunflower rust) on *Helianthus annuus* (sunflower). *Tobacco ringspot virus* was also found to be associated with snapdragon rust (*Puccinia antirrhini* on *Antirrhinum majus*) (YARWOOD 1951).

RICHAEL *et al.* (2001) co-infiltrated caspase inhibitors (caspases are involved in promoting apoptosis) and virulent *Pseudomonas syringae* pv. *tabaci* into susceptible tobacco. This resulted in reduced plant cell death and a “virtual cessation in bacterial growth”, it is therefore possible that *A. candida* could alter bacterial growth within *A. thaliana*.

Our interest is focused on identifying host genes that are vulnerable to *A. candida* mediated suppression. For example, we are mapping *RAC5* (resistance to *Albugo candida*) from *A. thaliana*, for the purpose of cloning. This gene is probably required for the growth and reproduction of *A. candida*, in its wild host, *Capsella bursa-pastoris* (shepherd’s purse).

At the moment *RAC5* has been mapped to an interval of eleven BACs (bacterial artificial chromosomes) telomeric of nga106 on chromosome 5, in a region that lacks typical resistance (*R*) genes (those that contain leucine rich repeats LRRs, nucleotide binding domains NB). Initial microscopy suggests that *A. candida* can grow partially in accessions homozygous for *RAC5*, despite minimal tissue collapse. It seems that *A. candida* is capable of limited reproduction in heterozygotes, which suggests a dosage effect. These phenotypic observations are analogous to *A. thaliana pmr* (enhanced powdery mildew resistant) mutants, which can not support the normal growth of *Erysiphe cichoracearum* (VOGEL & SOMERVILLE 2000) but do not exhibit elevated levels of hallmark defence genes. Molecular isolation and characterisation of *RAC5* will help further our understanding of the types of gene which are vulnerable to exploitation by a biotrophic pathogen.

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