Several Downy Mildew Resistance Genes in *Arabidopsis* Require Signaling via a Homologue of Yeast *SGT1*

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Abstract

A fast neutron mutant in *Arabidopsis* (Columbia) was identified that exhibits **e**nhanced **d**owny **m**ildew (*edm1*) susceptibility to several *Peronospora parasitica* isolates, including the RPP7-diagnostic isolate Hiks1. The mutation was mapped to chr.4 and physically characterised as a 35kb deletion spanning seven genes. One of these genes restored wild-type resistance to all of the *P. parasitica* isolates. This gene (*AtSGT1b*) encodes a predicted protein that is orthologous to yeast *SGT1*, originally described as a key regulatory protein in centromere function and ubiquitin-mediated proteolysis. *AtSGT1b* contains three tatratrico-peptide repeats at the N terminus followed by a bipartite "CS" (CHORD containing Sgt) domain and an SGT specific (SGTS) domain at the carboxyl terminus. Altered expression of this gene is being investigated in Arabidopsis and *Brassica olarecea* to determine its potential use for crop improvement.

Keywords: *Brassica*; downy mildew resistance; defence signalling; *SGT1*

INTRODUCTION

In their natural environments, plants are challenged routinely by parasites including viruses, bacteria, fungi, nematodes and insects, all of which have the molecular capability to manipulate host plants for their own sustenance. To detect these foreign invaders and defend themselves, plants have an innate immune system, which involves a complex array of constitutively expressed *R* genes. Several *R* genes have already been cloned from model plants as well as crop plants and their primary structures have been determined.

In the model cruciferous plant *Arabidopsis thaliana*, the resistance gene Col-*RPP7* recognises the Hiks1 isolate of *Peronospora parasitica* and the defence response is not altered in any of the key Columbia mutants that enhance susceptibility (suppress resistance) to other isolates of *Peronospora* including *ndr1, coi1, jar1, ein2, npr1, pbs1, pbs2, pbs3, pad1, pad2, pad4 and pad5* (MCDOWELL *et al.* 2000). When Col::NahG plants were tested with Hiks1, no alteration in the wild-type RPP7 function was observed. In view of all the data and extensive studies, we concluded that RPP7-mediated signalling involves an, as yet undetermined, novel signalling response.

To gain further information about this signalling pathway, we took a mutational approach to identify genes that are required for resistance-mediated by the *RPP7* gene. Here we report that in one of these mutants, Col-*edm1*, the *SGT1b* gene restores wild-type resistance.

RESULTS AND DISCUSSION

The **e**nhanced **d**owny **m**ildew (*edm1*) susceptibility mutant was selected from screening fast neutron treated Col-5 using Cala2, a different *Peronospora* isolate that is recognised by *RPP2*. This mutant supported enhanced sporulation following infection with seven Col-incompatible isolates that are each recognised by different *RPP* specificities (Table 1). The level of susceptibility (measured by the amount of sporulation) varied for each isolate, ranging from heavy (more than 20 sporangiophores per cotyledon, defining full

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Table 1. Interaction phenotypes of seven isolates of *Peronospora parasitica* (downy mildew) in *Arabidopsis thaliana* (wild-type Columbia and Col-*edm1*)

Isolate	*RPP* locus[a]	*R*-gene class[b]	Col-0 IP[c]	Col-*edm1* IP
Cala2	RPP2- *IV*	TIR-NB-LRR	R	H
Emoy2	*RPP4*-IV	TIR-NB-LRR	L2	M
Wela3	*RPP6*-I	unknown	N	L6
Hiks1	*RPP7*-I	non-TIR	N	M
Wand1	*RPP28*-II	unknown	R	L6
Cand5	nd-polygenic	unknown	R	M
Hind4	nd-II	unknown	L2	M

[a] **R**ecognition of ***P**eronospora **p**arasitica*. Chromosome indicated by roman numeral nd = not designated

[b] TIR = **T**oll/**I**nterleucin **R**eceptor-like domain; NB = **N**ucleotide **B**inding site; LRR = **L**eucine **R**ich **R**epeats

[c] Interaction phenotype interpreted from quantitative data: N = no sporulation; R = rare sporangiophore (< 1 per cotyledon); L = low (1–10 sporangiophores); M = medium (11–16); H = heavy (> 16)

susceptibility) produced by Cala2, to low level (less than ten sporangiophores per cotyledon) produced by Wand1 and Wela3. The other four isolates, including Hiks1, produced moderate sporulation (ca. 15 sporangiophores per cotyledon) in the mutant.

A total of 310 Hiks1-susceptible F_2 Col-*edm1* × Ler-0 seedlings were used to map *EDM1* within an interval spanned by the markers F17A8 and SC5. New markers were generated from the sequence information available and the *EDM1* region was mapped onto two BACs, T22B4 and F8L21, with one and four flanking recombinants, respectively. Further attempts to generate markers within this interval revealed a 35 kb deletion spanning seven genes, determined by lack of PCR amplification and Southern hybridisation of locus specific sequences from seven predicted genes. Three of the deleted genes in this region are shown in Figure 1.

Col-*edm1* plants were transformed with seven constructs that contained a single gene from the deleted region. The construct that carries the *AtSGT1b* gene (At4g11260) restored H_2O_2 production and wild-type resistance to all isolates (Figure 2) (TÖR *et al*. 2002). *Arabidopsis* has another homolog, *AtSGT1a*, and the possible role of this gene in disease resistance has yet to be determined. Both *SGT1a* and S*GT1b* have homology to the yeast *SGT1* gene, which is involved in protein degradation and kinetochore function (KITAGAWA *et al*. 1999). The protein encoded by the *SGT1b* has three TPR (Tetratricopeptide repeat) motifs at the N-terminal region, a bipartite CS (CHORD containing Sgt – SHIRASU *et al*. 1999) domain in the

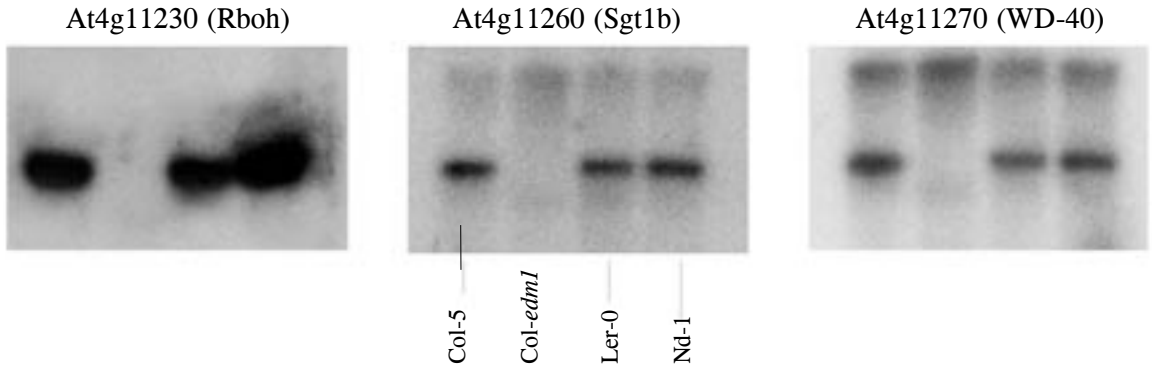

Figure 1. Southern hybridisation of Col-*edm1* and wild type plants. Genomic DNAs from wild-type plants, Col-5, Ler-0 and Nd-1, and from Col-*edm1* were digested with Bgl II, separated by agarose gel electrophoresis and transferred to a nylon membrane. The membranes were hybridised with ^{32}P labelled gene specific probes generated from the genes in the locus. Only three of seven membranes are shown. Rboh, respiratory burst oxidase, WD-40, repeat motif of the gene

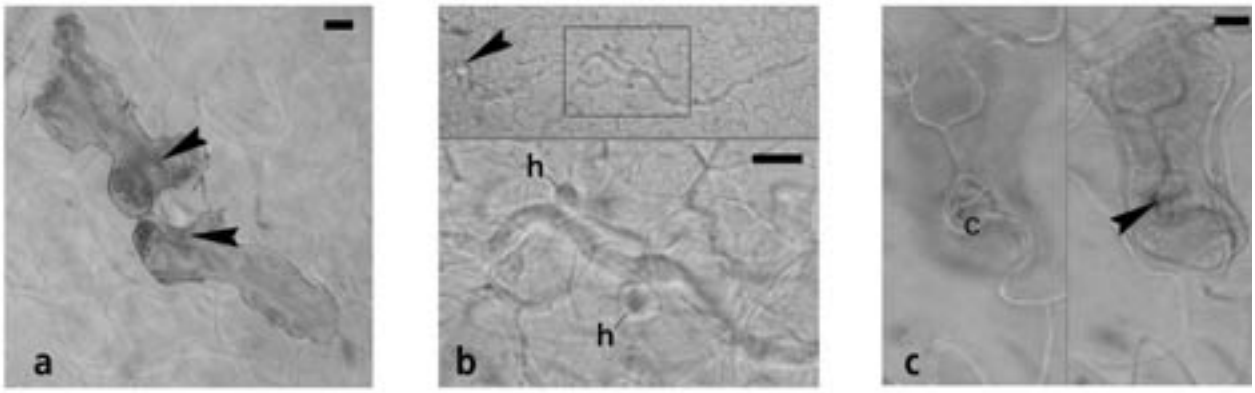

Figure 2. Detection of H_2O_2 accumulation in infected mesophilly cells. Cotyledons of Col-5 (a), Col-*edm1* (b) and Col-*edm1*::*SGT1b* (c) were stained with DAB 1 day after inoculation with the *Peronospora* isolate Hiks1

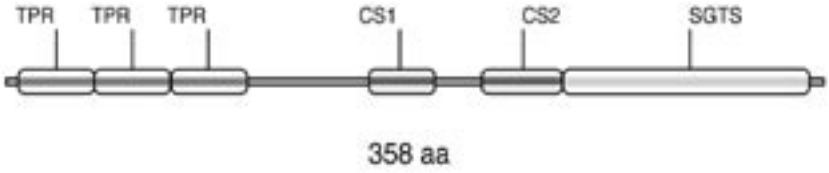

Figure 3. Structural domains of AtSGT1b. TPR-**T**etratrico**p**eptide **R**epeat; CS-**C**HORD containing **S**gt; SGTS-SGT specific

central region and an SGTS (SGT-Specific) domain at the C-terminal region (Figure 3).

Recently, AZEVEDO *et al*. (2002) reported that *Arabidopsis SGT1a* and *SGT1b* genes interact with Rar1, a gene that regulates several downy mildew and bacterial resistance genes (TORNERO *et al*. 2002) through the CS domain. In addition, they showed that the barley *SGT1* gene co-immunoprecipitates with the components of the SCF-complex, indicating that the *SGT1* gene interacts with SKP1 through the SGTS domain.

This provides clear evidence that the *SGT1b* plays a pivotal role in defence signalling pathway activated by R-Avr interaction and the SCF-complex. To date, we have identified at least two *Brassica* homolog of *SGT1b*. Interestingly, we have not found an *SGT1a* homolog in *Brassica* crop plants. This may be an indication of that crop plants may have only one copy of *SGT1* gene. Further investigations and over and under expression studies with *SGT1b* are underway to exploit the function of this gene in *Brassica* crop plants.

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