Molecular Genetic Characterization of the *RB* Late Blight Resistance Response

Zhenyu Liu[1], Yu Chen[1], and Dennis Halterman[1,2]
[1]Department of Plant Pathology
[2]Vegetable Crops Research Unit, USDA-ARS,
University of Wisconsin-Madison, Madison, Wisconsin 53706-1598, USA

Introduction

Late blight, caused by the oomycete pathogen *Phytophthora infestans*, continues to be one of the most devastating diseases of potato. A key long-term management strategy for combating potato diseases is to develop cultivars with high levels of resistance through identification and integration of major resistance (*R*) genes. The *RB* gene, cloned from the Mexican diploid potato species *Solanum bulbocastanum*, confers broad-spectrum resistance to potato late blight. The full-length gene coding sequence of *RB*, including the open reading frame and promoter, has been integrated into cultivated potato (*S. tuberosum*) using *Agrobacterium*-mediated transformation. All transgenic lines containing *RB* exhibit strong foliar resistance. We examined the interactions between *P. infestans* and transgenic *RB*-containing potato SP951 (*S. tuberosum* cv. Katahdin plus *RB*) and susceptible Katahdin to better understand the resistance mechanism mediated by *RB*. We used trypan blue staining to visualize hypersensitive cell death (HR) mediated by *RB*, and quantitative real-time reverse transcription PCR to monitor the transcription of pathogenesis related (*PR*) genes. Additionally, two years of field trials were used to ascertain whether the presence of *RB* had any effect on tuber yield.

We have determined that functional *RB*-like genes are present in other wild species of potato. We investigated the late blight resistance phenotypes of eight accessions of *S. verrucosum*, another wild diploid potato species, using greenhouse inoculations and discovered variability among the accessions. Transcribed orthologs of the *RB* gene from these eight *S. verrucosum* accessions were cloned using a homology-based PCR approach. Stable introduction of the *RB* ortholog from late blight resistant *S. verrucosum* into susceptible *S. tuberosum* confers resistance to *P. infestans*. Allele mining for *RB*-like genes in other wild species will be a useful approach for identifying related *R* genes that might also combat late blight in potato.

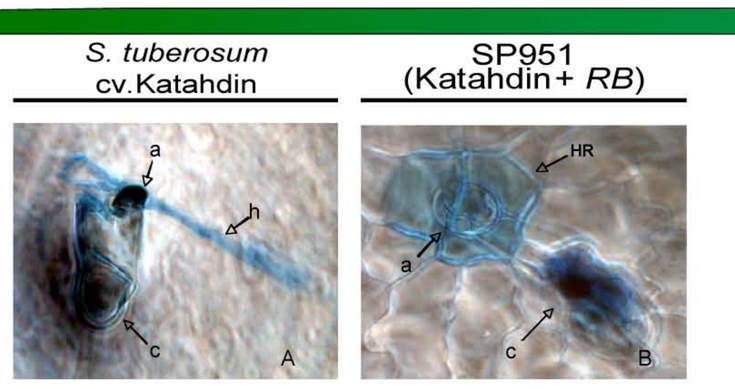

Figure 1. Microscopic characterization of *RB* resistance response. Both the leaves of susceptible potato cultivar Katahdin (A) and resistant transgenic potato SP951 containing the *RB* resistance gene (B) were inoculated with *Phytophthora infestans*. Inoculated leaves were stained with lactophenol trypan blue. c, cyst; a, appressorium; h, haustorium; HR, hypersensitive response; p, penetration site. Pictures were taken 8 hours post inoculation.

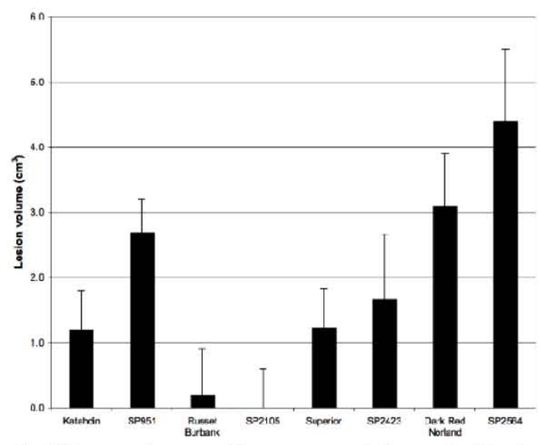

Figure 4. *RB* gene does not increase resistance to *Phytophthora infestans* in tubers.
Three tubers from each *Solanum tuberosum* cultivar and the corresponding transgenic lines were inoculated with *P. infestans*. The lesion volume was calculated from the width and depth of each lesion 10 days after inoculation.

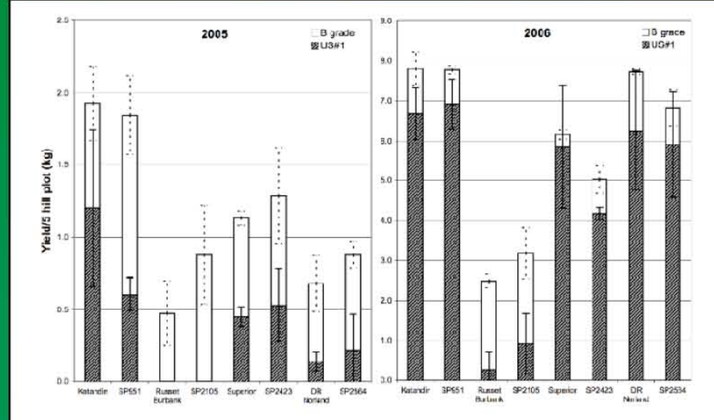

Figure 5. Comparison of yield in potato cultivars transformed and not transformed with the *RB* transgene for resistance to late blight.
Three replicates of five hill plots were planted with *RB*-transgenic Katahdin (SP951), Russet Burbank (SP2105), Superior (SP2423), and Dark Red Norland (SP2564) as well as nontransgenic controls in **A,** 2005 and **B,** 2006. Tubers were hand graded and divided into US#1 (dark gray bars) and B (light grey bars) grades. Data shown are the mean yields of each plot. There were no significant differences between the yield of the transgenic and control potato plants according to *t* tests at $P = 0.05$.

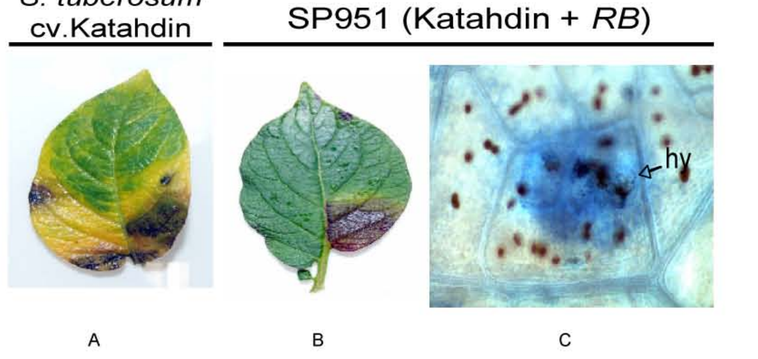

Figure 2. *RB*-mediated HR does not stop pathogen spread.

A and B were taken 5 days post inoculation. C was taken 24 hours post inoculation. hy, hypha.

Accession	Late blight score	
	US 940480	MX 980085
PI 161173	6.6 ± 1.1	7.9 ± 0.1
PI 275256	7.0 ± 0.7	7.2 ± 0.7
PI 275258	6.8 ± 0.8	4.8 ± 6.0
PI 275260	7.4 ± 0.5	7.8 ± 0.1
PI 310966	6.2 ± 1.1	4.0 ± 5.8
PI 365404	6.6 ± 0.5	7.6 ± 0.3
PI 558485	6.6 ± 1.1	7.4 ± 0.8
PI 570643	3.2 ± 1.6	1.3 ± 0.3

0 = 100% diseased tissue
8 = < 10% diseased tissue

Table 1. Testing of *Solanum verrucosum* acccesions for resistance to late blight.
Six week old seedlings were placed in a misting chamber (100% humidity, 18˚C) and spray inoculated with sporangia from the US 940480 and MX 980085 strains of *P. infestans*, respectively. Plants were scored 10 days after inoculation. The late blight score was calculated based on observation of diseased leaf tissue.

Plants	Late blight score
Katahdin	4.0 ± 1.0
SP951	8.0 ± 0.0
SP2808	6.0 ± 2.7
SP2824	6.5 ± 0.5
SP2829	4.7 ± 2.1
SP2906	7.0 ± 1.0

0 = 100% diseased tissue
8 = < 10% diseased tissue

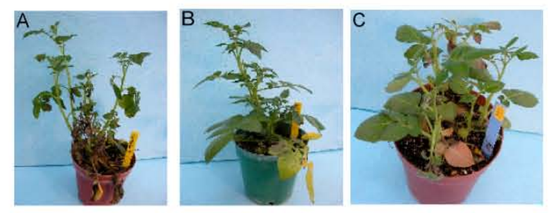

Figure 6. RB^{ver} gene isolated rom resistant *S. verrucosum* accession PI 275260 confers partial late blight resistance.
Disease symptoms were recorded 10 days after inoculation. Susceptible *S. tuberosum cv. Katahdin* (A) and resistant RB^{blb}-transgenic plant SP951 (B) were used as controls. SP2906 (C) was one of the four transgenic RB^{ver} Katahdin plants showing rate-limiting resistance to *P. infestans* (refer to the table above).

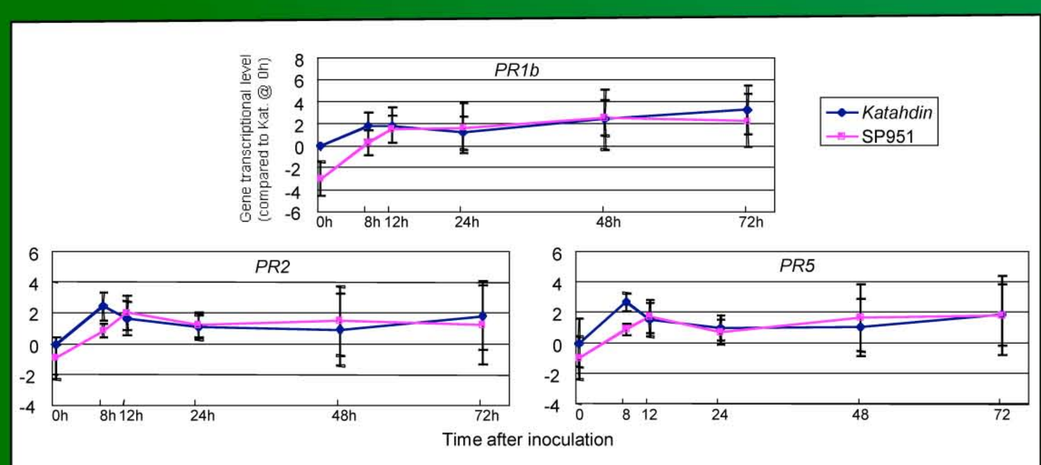

Figure 3. Real time RT-PCR quantification of *PR1B*, *PR2*, *PR5* transcription 0, 8, 12, 24, 48, 72 hours after inoculation.
Transcript levels were estimated using quantitative real-time reverse transcription PCR (QRT-PCR). Fold change was calculated from the QRT-PCR efficiencies and the threshold cycle. The fold change represents the means of three replicates. The fold change ratio of control (0 hour) was always 1.

Summary

1) HR mediated by *RB* does not stop pathogen growth completely.
2) Preliminary data suggests that *PR* gene induction is delayed or suppressed even when *RB* is present.
3) In contrast to the foliar resistance phenotype, *RB*-containing tubers do not exhibit increased resistance.
4) Two years of field trails revealed that there is no significant effect on tuber size or yield after addition of the resistance gene *RB* to several *S. tuberosum* cultivars.
5) While most *S. verrucosum* accessions are resistant, one accession PI 570643 was notably more susceptible.
6) Transcribed orthologs of the *RB* gene from the eight *S. verrucosum* accessions were cloned using a homology-based PCR approach.
7) Sequence analysis revealed that the RB^{ver} orthologs share up to 83.5% nucleotide identity with RB^{blb}.
8) Stable introduction of the *RB* ortholog from late blight resistant *S. verrucosum* PI 275260 into susceptible *S. tuberosum* confers resistance to *P. infestans*.
9) This functional RB^{ver} ortholog differs from a non-functional ortholog at only four amino acid residues.

For further information e-mail dah@plantpath.wisc.edu or visit http://www.plantpath.wisc.edu/fac/dah.