

Foliar blight resistance transgene *RB* transcription levels correlated with blight resistance in tubers

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Abstract

Late blight of potato caused by *Phytophthora infestans* is an important disease, resulting in multibillion dollar losses annually worldwide. Recent efforts have led to the identification and cloning of multiple late blight resistance (R) genes. One of the most promising genes, *RB*, identified from wild potato *Solanum bulbocastanum*, imparts broad spectrum foliar blight resistance. Previous studies have noted that foliar and tuber resistance to late blight are not necessarily controlled by the same R genes. The cloning of *RB* has provided an opportunity to directly examine this phenomenon. Tubers of eleven *RB* transformed cultivated potato lines were used in whole tuber phenotypic assays. Although all eleven lines are foliar blight resistant, all but two lines were as susceptible to tuber blight as nontransgenic controls. Two lines displayed enhanced tuber resistance. Quantitative Real-Time RT-PCR results confirmed that the *RB* transgene is transcribed in tubers of all lines at all time points. Interestingly, a weak correlation between *RB* transcription and tuber blight resistance was noted, despite the two transgenic lines with enhanced tuber resistance showing the highest levels of transcription. This general correlation is in contrast to results observed in foliar assays conducted by our laboratory. However, the two anomalous transgenic lines suggest that manipulation of *RB* transgene transcription may be an effective means of enhancing both foliar and tuber blight resistance.

Importance

- Potato is the fourth most important food crop in the world and is the single most important dicot food crop (<http://apps.fao.org>)
- Late blight (caused by *Phytophthora infestans*) is responsible for multibillion dollar losses worldwide annually
- Phytophthora infestans* attacks both foliage (Fig. 1b, c) & tuber
- R gene *RB*, recently cloned from the wild potato species *Solanum bulbocastanum* (genotype PT29; Fig. 1a), imparts broad-spectrum foliar blight resistance to all known races of *P. infestans* (PNAS, 100:9128)



Figure 1. Potato grown in a late blight nursery. a. Resistant *S. bulbocastanum* PT29. b. Susceptible cultivated potato. c. Resistant transgenic Katahdin+*RB* grown next to susceptible cultivated potato (arrow).

Results: Tuber blight



Figure 2. *Phytophthora infestans*-inoculated 'Russet Burbank' tubers. Tubers of untransformed and *RB* transgene transformed (lines SP2105, SP2182, SP2211, and SP2213) 'Russet Burbank' plants were removed from cold-storage at one, six, and nineteen weeks post-harvest and inoculated with *P. infestans*. Two weeks post-inoculation, tubers were assessed for blighted tissue. Tubers pictured are representative of three replicates at each tuber age. Water-inoculated samples from the week six trial shown here are representative of the water-inoculated samples at all tuber ages. The length, width, and depth of diseased tissue were measured to calculate disease volume, as described in Materials and Methods. Inoculated tubers of 'Katahdin' and 'Dark Red Norland' exhibited similar phenotypes to those of 'Russet Burbank' untransformed and SP2105 and SP2182.

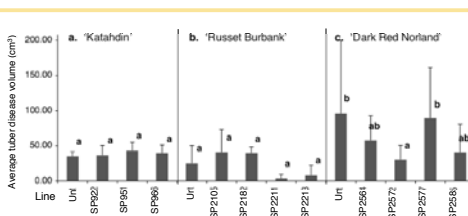


Figure 3. Tuber blight development is not significantly different between most tested lines. Calculated volumes of tuber blight development were averaged across ages and replicates within each line tested. Large disease volume variations within a line were observed, as indicated by standard deviation bars. This is especially notable for 'Dark Red Norland' lines, which on average developed more tuber blight disease. Tukey significant ($p < 0.05$) differences between lines within a cultivar are indicated by differing letters. Additional Tukey significant ($p < 0.05$) differences in amount of tuber disease were found between the cultivar Dark Red Norland and both 'Katahdin' and 'Russet Burbank'.

Results: Tuber blight, cont.

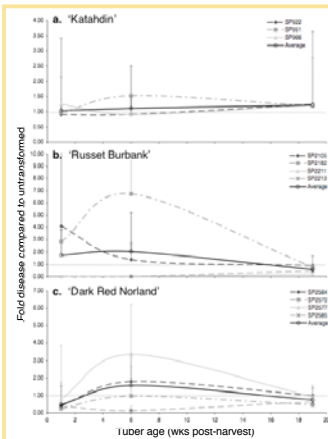


Figure 4. Amount of blighted tuber tissue across tuber age. Volume of blighted tuber tissue was calculated and normalized to the volume of disease in tubers of the corresponding untransformed cultivar. Values greater than one indicate greater amounts of disease compared to untransformed, while values less than one indicate lower amounts of disease. Adjusted disease volumes were plotted relative to tuber age (weeks post-harvest). a. 'Katahdin'. All transformed lines exhibited disease levels similar to the untransformed line. b. 'Russet Burbank'. Transformed lines SP2105 and SP2182 had much higher levels of disease at earlier ages. At those same early ages, lines SP2211 and SP2213 exhibited very low levels of disease.

Results: qRT-PCR

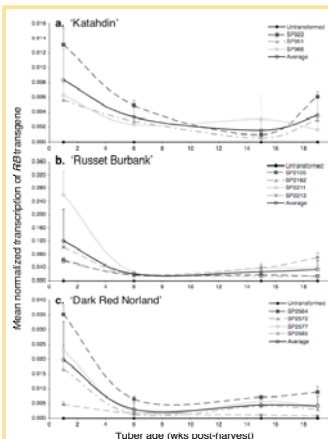


Figure 5. Transcription of *RB* transgene varies across tuber age. RNA was collected from tubers at one, six, fifteen, and nineteen weeks post-harvest and evaluated for *RB* transgene and *EF1*. *RB* transgene transcription levels were normalized to those of *EF1* from corresponding RNA samples. All *RB* transgenic lines exhibit higher transcription levels at week one with lower levels at later ages. As expected, all untransformed lines had *RB* transgene transcription values of 0.000. a. 'Katahdin'. b. 'Russet Burbank'. c. 'Dark Red Norland'. For 'Katahdin' and 'Dark Red Norland', there is no significant ($p > 0.05$) difference between individual transformed lines for *RB* transgene transcript levels. However, SP2211 ('Russet Burbank') differs significantly ($p < 0.05$) in *RB* transgene transcription from all other tested 'Russet Burbank' lines, according to Tukey HSD. Average *RB* transgene transcription in 'Russet Burbank' is significantly ($p < 0.05$) different from that of 'Katahdin' and 'Dark Red Norland'. Note: Y-axes are at different scales.

Results: Correlation

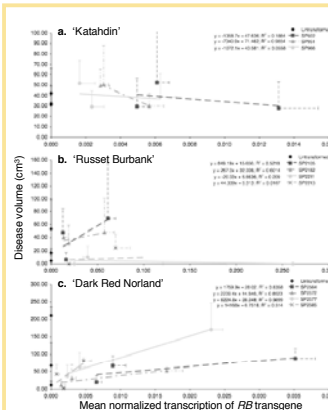


Figure 6. Correlation of *RB* transgene transcription in the tuber with tuber blight disease volume. Levels of *RB* transgene transcription (normalized to control gene *EF1*) were compared to amount of tuber blight disease (normalized to water-inoculated controls) in tubers of corresponding lines. Three independent replicates of each line were averaged for both *RB* transgene transcript levels and disease volume at one, six, and nineteen weeks. X-axis error bars reflect variance of the *RB* transgene transcript within the line at each age. Y-axis error bars indicate disease volume variation within a line at each age. a. 'Katahdin'. All *RB* transgenic lines exhibit an inverse correlation of transcript levels with disease. Line SP951 exhibits a strong correlation ($R^2 = 0.9854$), while the other two lines are quite weakly correlated (both $R^2 < 0.2$). b. 'Russet Burbank'. Only line SP2211 has an inverse correlation, although the correlation is relatively weak ($R^2 = 0.209$). The other three lines have a direct correlation, ranging from $R^2 = 0.0167$ (SP2213) to $R^2 = 0.0014$ (SP2182). c. 'Dark Red Norland'. Transcript levels are directly correlated with tuber blight development. SP2577 has the strongest correlation ($R^2 = 0.9899$) and SP2585 the weakest ($R^2 = 0.514$).

Results: ANOVA

Table 1. ANOVA: the effect of age, cultivar, transgene presence, and line on tuber blight development				Table 2. ANOVA: the effect of age, cultivar, transgene presence, and line on RB transgene transcription			
	df	Mean Sq	F		df	Mean Sq	F
Age	1	680 ns		Age	1	0.0169 ***	
Age ²	1	3015 ***		Age ²	1	0.0176 ***	
Cultivar	2	11684 ***		Cultivar (y/n)	2	0.02811 ***	
Transgene (y/n)	1	3668 ns		Transgene (y/n)	1	0.01412 ***	
Line within (Cultivar x Transgene (y/n))	8	3649 ***		Line within (Cultivar x Transgene (y/n))	8	0.00329 ***	
Cultivar x Transgene (y/n)	2	4420 *		Cultivar x Transgene (y/n)	2	0.00725 ***	
Age x Cultivar	2	9194 ***		Age x Cultivar	2	0.00791 ***	
Age ² x Cultivar	2	17219 ***		Age ² x Cultivar	2	0.00954 ***	
Age x Cultivar x Transgene (y/n)	3	2751 *		Age x Transgene (y/n)	1	0.00412 *	
Age ² x Cultivar x Transgene (y/n)	3	4304 **		Age ² x Transgene (y/n)	1	0.00493 *	
Error	100	1004		Age x Line within (Cultivar x Transgene (y/n))	8	0.00554 ***	
				Age ² x Line within (Cultivar x Transgene (y/n))	8	0.00331 ***	
				Age ² x Cultivar x Transgene (y/n)	2	0.00240 *	
				Degrees of freedom			
				Error	128	0.00074	

Discussion and summary

- Disease at all tuber ages
- nonsignificant ($p > 0.05$) transgene effect
- Least variable disease amounts at 19 wph
- Transcript detected at all ages
- Highest at 1 wph
- Cultivar and its interactions with age had highly significant ($p < 0.001$) effects
- Highly significant ($p < 0.001$) differences from line effects
 - 'Russet Burbank' SP2211 and SP2213 - no significant differences from untransformed but had virtually no disease at 1 & 6 wph
- RB* transcription correlated with resistance in apparently line-dependent manner

Overall conclusions

- RB* transgene does not enhance resistance to tuber blight in almost all cases
- RB* transgene is transcribed in tubers at all time points assessed, but highest at 1 wph

Next steps

- Assess transcription / function of downstream defense signaling pathway components
- Additional assessment of 'Russet Burbank' SP2211 and SP2213
 - Effect of transgene dosage
- Put *RB* under control of
 - constitutive CaMV 35S promoter
 - tuber-specific Patatin promoter

Materials and methods

Plant material

- Field grown tubers of *S. tuberosum* cultivars indicated below were stored at 4°C after harvest for 1, 6, 15, or 19 weeks
 - 'Katahdin' (Untransformed and *RB* transgenic lines SP922, SP951, & SP966)
 - 'Russet Burbank' (Untransformed and *RB* transgenic lines SP2105, SP2182, SP2211, & SP2213)
 - 'Dark Red Norland' (Untransformed and *RB* transgenic lines SP2564, SP2572, SP2577, & SP2585)

- Whole tuber assays (APS Biol. & Cult. Tests, 17: PT03)
 - Wound tuber & add 10 μ L *P. infestans* inoculum (7000 sporangia/mL H₂O, treated 6' 4°C, 30' RT)
 - Maintained at 22°C, ~95% RH, dark for 34 hrs followed by 13°C, ~90%RH, dark for 11 days
 - Measure depth and radii of infection to calculate volume

RT-PCR

- RNA extractions
 - SV Total RNA Isolation (Promega, Madison, WI)
 - Tissue core (1 x 1 x 2.5 cm³ in size) removed from center lengthwise of tuber (inoculated) on day of whole tuber inoculations)
- qRT-PCR
 - SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR (Invitrogen, Carlsbad, CA)
 - Total RNA: 100 ng
 - 25 μ L reactions
 - ABI 7500 Real Time PCR machine
- RB* reactions
 - Primers [Fig. 2; Millett and Bradeen (2007) Theor Appl Genet 114:501-513]
 - Forward primer (2MAMA3): 200 μ M
 - Reverse primer (2MAMA31): 900 μ M
 - Parameters: 50°C 30', 95°C 2', 40x[95°C 15', 57°C 28', 72°C 32"]
 - Elongation Factor 1 (*EF1*) control reactions
 - Primers [Nicot et al. (2005) J Exp Bot 56:2907-2914]: 400 μ M each
 - Parameters: 50°C 30', 95°C 2', 40x[95°C 15', 60°C 60"]
 - Cr values processed with Visual Basic software Q-Gene [Muller et al. (2002) BioTechniques 32:1372-1379] and subsequently analyzed by ANOVA and Tukey HSD using R for Mac OS X

Acknowledgments

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