Potato Tuber Blight Resistance Phenotypes Correlate with *RB* Transgene Transcript Levels in an Age-Dependent Manner

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ABSTRACT

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Plants have evolved strategies and mechanisms to detect and respond to pathogen attack. Different organs of the same plant may be subjected to different environments (e.g., aboveground versus belowground) and pathogens with different lifestyles. Accordingly, plants commonly need to tailor defense strategies in an organ-specific manner. *Phytophthora infestans*, causal agent of potato late blight disease, infects both aboveground foliage and belowground tubers. We examined the efficacy

of transgene *RB* (known for conferring foliar late blight resistance) in defending against tuber late blight disease. Our results indicate that the presence of the transgene has a positive yet only marginally significant effect on tuber disease resistance on average. However, a significant association between transgene transcript levels and tuber resistance was established for specific transformed lines in an age-dependent manner, with higher transcript levels indicating enhanced tuber resistance. Thus, *RB* has potential to function in both foliage and tuber to impart late blight resistance. Our data suggest that organ-specific resistance might result directly from transcriptional regulation of the resistance gene itself.

Plants have evolved sophisticated systems to monitor for and respond to pathogen attack. Disease resistance (*R*)-gene-mediated defense responses have been well characterized at the molecular level in recent years (Bernoux et al. 2011; Collier and Moffett 2009; Dodds and Rathjen 2010; Elmore et al. 2011; Lukasik and Takken 2009; Takken and Tameling 2009) and *R* genes have been widely used for crop protection. The prominent nucleotide-binding site leucine-rich repeat (NBS-LRR) class of *R* genes has been particularly well studied. NBS-LRR proteins function as both cellular sentinels and cellular switches that activate defense responses when needed. One hallmark of NBS-LRR gene-mediated defense is the hypersensitive response (HR), programmed host cell death at the site of infection that also kills the invading pathogen. However, not all pathogens are the same. Some pathogens rely on living plant tissue for sustenance. These biotrophic pathogens are effectively targeted by the HR because destruction of adjacent host cells is detrimental to their existence. In contrast, other pathogens live on dead and dying plant materials. These necrotrophic pathogens would theoretically benefit from the HR. Hemibiotrophic pathogens have adopted a lifestyle intermediate to that of biotrophs and necrotrophs, requiring living plant tissue early in the infection process but later benefiting from host death. Although there are exceptions, experimental evidence mostly indicates that plants employ HR defenses against biotrophs and hemibiotrophs and non-HR defenses against necrotrophs (Glazebrook 2005). Most known root pathogens are necrotrophic fungi, against which HR is ineffective (Okubara and Paulitz 2005).

Several researchers have contrasted molecular resistance mechanisms in different organs of the same plant in response to attack by different pathogens (Adomas and Asiegbu 2006; van Esse et al. 2009). Others have examined the developmental regulation of plant disease resistance in a single organ at different time points throughout plant development (Chintamanani et al. 2008; Goggin et al. 2004; McDowell et al. 2005; Millett et al. 2009). Schreiber et al. (2011) concluded that organ specificity plays a role in regulating nonhost *Arabidopsis* responses to the fungal pathogen *Magnaporthe oryzae*, although specific mechanisms were not proposed. Surprisingly few studies have examined the effect of a specific *R* gene in different organs in response to the same pathogen.

Hermanns et al. (2003) studied the role of the *R* gene *RPP1* in regulating defense responses in *Arabidopsis* leaves and roots to *Hyaloperonospora parasitica*, a hemibiotrophic pathogen that initially infects through the roots but only causes disease in aboveground organs. These researchers demonstrated that, although *RPP1* is transcribed in *Arabidopsis* leaves and roots, the *RPP1* protein functions to trigger resistance responses only in the leaves. In contrast, Cabrera Poch et al. (2006) developed an experimental system in which potato cyst nematodes, which normally infect the tomato root, could be induced to infect tomato foliage. Using this system, these authors demonstrated that the nematode *R* gene *Hero* is both transcribed and functional in tomato leaves as well as roots. These studies have contributed significantly to our current understanding of the regulatory mechanisms behind organ-specific functional regulation of *R* gene resistance but knowledge gaps and questions of applicability to other pathosystems remain.

Late blight is one of the most destructive diseases of potato (*Solanum tuberosum* L.), causing annual multibillion dollar losses to farmers worldwide (Garelik 2002; Kamoun 2001). The causal agent of late blight, *Phytophthora infestans*, an oomycete hemibiotroph, is particularly troublesome because it can infect and cause

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disease in both foliage and tubers. The fact that two distinct potato organs—aboveground foliage and belowground tuber—are targets of infection by *P. infestans* makes the potato-*P. infestans* pathosystem ideal for the study of the organ-specific regulation of defense response.

Historically, a primary aim of potato improvement research has been identifying durable sources of genetic foliar blight resistance in wild potato species. In recent years, four late blight resistance genes have been cloned from *S. bulbocastanum* Dun. (Lokossou et al. 2009; Oosumi et al. 2009; Song et al. 2003; Van Der Vossen et al. 2003; van der Vossen et al. 2005), a Mexican diploid species sexually isolated from the cultivated potato. Each of these is an NBS-LRR gene and each imparts foliar blight resistance. Among these genes, *RB* (also referred to as *Rpi-blb1*) imparts broad-spectrum, nonpathogen-referenced, agriculturally meaningful levels of foliar blight resistance in the absence of fungicide sprays (Bradeen et al. 2009).

Tuber blight resistance has not been as extensively studied as foliar blight resistance. The initial line of defense of tubers against *P. infestans* is the periderm (Toxopeus 1961). The tuber periderm can mechanically block the pathogen, forcing it to infect through lenticels, buds, or wounds, while the adjacent cell layer, the outer cells of the cortex, is thought to slow pathogen growth (Lacey 1967; Pathak and Clarke 1987). *R* genes play an important role in defending against *P. infestans* in the medulla of the tuber (Flier et al. 2001). Therefore, *R* genes are more important when tubers are wounded or cut.

The genetic relationship between foliar late blight resistance and tuber blight resistance is complex. Toxopeus (1958) observed a positive correlation between foliar and tuber resistance, as did Stewart et al. (1994). In contrast, others have found only weak correlation between foliar and tuber resistance (Dorrance and Inglis 1998; Kirk et al. 2001; Stewart et al. 1992). The effects of specific *R* genes are equally varied. Lines containing the foliar blight *R* gene *R1* and foliar resistance QTLs derived from *S. microdontum* and *S. demissum* have demonstrated a positive correlation between foliar and tuber resistance but no significant correlation between foliar and tuber resistance was found in lines containing the *R* genes *R3a* or *Rpi-abpt* (Bradshaw et al. 2006; Park et al. 2005; Roer and Toxopeus 1961). Potato lines containing the foliar blight resistance gene *RPi-ber* were tuber blight resistant to both foliar-compatible and incompatible *P. infestans* isolates (Mayton et al. 2011), implying the existence of additional *R* genes linked to *RPi-ber* or alteration of *RPi-ber* pathogen specificity in an organ-specific manner. Based on small-scale field assessment of four transgenic lines, each carrying the *RB* transgene, Halterman et al. (2008) concluded that *RB* functions in the leaves but not the tubers to impart late blight resistance.

In this study, we extend analyses of the role of *RB* in preventing tuber blight infection. We conducted field and postharvest experiments to measure tuber blight disease severity of three potato cultivars and their *RB* transgenic lines. We further examined *RB* gene transcription levels across genetic backgrounds, independently transformed lines, and tuber age (poststorage). We analyzed phenotypic potato tuber disease data and *RB* gene transcript data, and we explored the relationship between *RB* transcription and tuber disease levels. Importantly, our data indicate a correlation between *R* gene transcript levels and disease resistance phenotypic effect in potato tubers, suggesting that the regulation of organ-specific function of *RB* may be achieved through transcriptional regulation of the *R* gene itself.

MATERIALS AND METHODS

Plant material. *S. bulbocastanum* Dun. genotype PT29, cultivated potato carrying the *RB* transgene (*S. tuberosum* L. ‘Katahdin’, lines SP922, SP951, and SP966; ‘Russet Burbank’, lines SP2105, SP2182, SP2211, and SP2213; and ‘Dark Red

Norland’, lines SP2564, 2572, 2577, and 2585), and untransformed cultivated potato cultivars (*S. tuberosum* Katahdin, Russet Burbank, and Dark Red Norland) were kind gifts from John Helgeson (United States Department of Agriculture–Agricultural Research Service and University of Wisconsin-Madison) and Sandra Austin-Phillips (University of Wisconsin-Madison) and have been previously described (Bradeen et al. 2009). All plant materials were produced in greenhouses on the University of Minnesota-St. Paul campus or in field plots at the University of Minnesota Sand Plain Research Farm, Becker, using standard cultural practices. For RNA isolation, plant tissue (the third fully expanded leaf below the crown of the plant and a tuber core of 1 by 1 by 2.5 cm taken halfway between the stolon and apical ends) was collected prior to inoculation, frozen in liquid nitrogen, and stored at -80°C .

Whole-tuber assay. Tuber blight resistance was tested as previously described (James et al. 2002), with slight modifications, in 2006 and 2008. Inoculum (7,000 sporangia/ml of H_2O) was prepared from *P. infestans* (Mont.) de Bary US-8 (isolate 940480 [A2]) sporangia using established protocols (Millett et al. 2009). Whole, unwounded, field-grown tubers were stored at 4°C after harvest until 1, 4, 6, 15, or 19 weeks postharvest, when five tubers per line per tuber age were hand washed and air dried at room temperature 24 h prior to inoculation. Three of the five tubers were inoculated with *P. infestans* as described below, one was water inoculated, and one was reserved for RNA extraction. For inoculation, a wound was made in each tuber halfway between the stolon and apical ends using a flat end screw (2 mm in diameter) that extended 2 mm through a wooden handle, yielding a wound approximately 6.3 mm^3 in size. Prepared inoculum (10 μl) or sterile H_2O (for the control tuber) were pipetted into each wound. Inoculated tubers were then maintained in the dark, first at 22°C and approximately 95% relative humidity (RH) for 72 h, then at 13°C and approximately 90% RH for 11 days. Inoculated tubers were peeled around the inoculation site at the termination of the incubation period and cut in half across the longitudinal axis through the inoculation site to measure radii and depth of diseased tissue in order to calculate volume of diseased tissue. Blight data were not collected in week 15 in either year, for week 4 in 2006, or for week 1 in 2008. Additionally, transgenic lines SP966 (Katahdin) and SP2213 (Russet Burbank) were not evaluated in 2008. Phenotypic data were analyzed by analysis of variance (ANOVA) and Tukey’s honestly significant difference (HSD) or Dunnett’s test using R statistical packages (The R Foundation for Statistical Computing).

Quantitative reverse-transcription polymerase chain reaction methods. Primer design, total RNA isolation, and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) methodologies have been previously described (Bradeen et al. 2009; Millett and Bradeen 2007; Millett et al. 2009). Briefly, *RB* transgene-specific primers 2MAMA5’3 and 2MAMA3’1 and elongation factor 1 α (*EF1* α) primers developed by Nicot et al. (2005) (EF1-f and EF1-r) were utilized in 25 μl total volume (5 μl (7 to 50 μM) of template) of qRT-PCR using SuperScript III Platinum SYBR Green One-Step qRT-PCR kit (Invitrogen). *RB* and *EF1* α fragments were amplified in independent reaction tubes and each sample was tested in triplicate. Results were evaluated using Sequence Detection Software (v. 1.4.0.25; Applied Biosystems). The threshold value was adjusted against control reactions for consistent cycle threshold (CT) values across multiple experimental plates. CT values of *RB* transgene and *EF1* α were then processed with the Visual Basic software Q-Gene (Muller et al. 2002) in Microsoft Excel (v11.3.7; Microsoft Corporation) and subsequently analyzed by ANOVA and Tukey’s HSD using R statistical packages.

Logistic regression method. Relationships between tuber blight disease incidence and *RB* transcript levels were analyzed using a logistic regression model in R. In these analyses, *RB* transcript levels from 2006 and 2008 were scaled using the upper

quartile values (Bullard et al. 2010). In the logistic regression model, logit (probability of observing a resistant tuber) = $\alpha + \beta$ (transgene transcription levels). Logistic models have been successfully used to study lettuce *Verticillium* wilt disease incidence (Wu and Subbarao 2014) and *Fusarium* head blight epidemics (Shah et al. 2014) and in numerous other applications (Agresti 2002).

RESULTS

Phenotypic tuber blight assays. In field tests, variation in inoculum levels produced on foliage can lead to highly variable and inaccurate tuber blight assessments (Platt and Tai 1998). Furthermore, field tests can only assess the ability of the pathogen to circumvent the defensive barrier of the periderm whereas *R* genes are predominantly thought to play a role in reducing blight development in the inner tissue of a tuber (Flier et al. 2001). To circumvent these difficulties, we employed a whole-tuber assay that entailed wounding of the periderm and introducing the pathogen to the medulla.

On average, the presence of the transgene has a positive (i.e., transgenic lines are associated with less disease) yet only marginally significant ($0.05 < P < 0.1$) (Table 1) effect (8.47 cm³) on tuber resistance. However, at certain tuber ages, some transgenic lines are significantly ($P < 0.05$) more resistant than corresponding untransformed lines, even after *P* values were corrected based on multiple test comparisons. These trends are most notable for Russet Burbank lines SP2211 and SP2213.

Visual inspection of diseased tubers provides a good indication of patterns of *RB* function across cultivars and tuber ages. Although all transformed lines of Katahdin (Fig. 1A) and Dark Red Norland (Fig. 1C) appear similar to the corresponding untransformed controls in tuber blight development, the transformed Russet Burbank lines SP2211 and SP2213 at weeks 1 and 6 appear to have far less tuber blight development than the untransformed controls (Fig. 1B). Disease resistance phenotypes suggest that the effect of the *RB* transgene on tuber blight development varies with cultivar, transformed line, and tuber age.

In an attempt to better understand the nature of observed variation in phenotypic tuber blight resistance, we next examined *RB* transcript levels in uninfected tubers of different cultivars, transformed lines, and ages.

***RB* transcript accumulation patterns.** *RB* transcript levels were measured in uninfected tubers of each line at discrete time points throughout the experiment. As expected, untransformed tubers lacked *RB* transcript in all cases. Across all transformed lines, *RB* transcript data from 2006 and 2008 are well correlated (Pearson’s correlation, $r = 0.8$) and reveal common trends (Fig. 2). *RB* transcript levels are highest in young (usually 1 week postharvest) tubers and decline as the tubers age in storage (Fig. 2). In most cases, *RB* transcript levels increase slightly in week 19. This is likely due to physiological changes in tubers associated with breaking of dormancy and the initiation of sprouting. The data indicate that cultivar has substantial impact on *RB* transcript levels, with average scaled week 1 *RB* transcript of transformed Russet Burbank lines (0.0274) being nearly an order of magnitude greater

Fig. 1. Tuber blight resistance phenotypes. Tubers of each genotype were removed from cold storage at 1, 6, and 19 weeks postharvest (as indicated) and inoculated with *Phytophthora infestans* US-8 (isolate 940480). Two weeks postinoculation, tubers were assessed for blighted tissue and photographed. Tubers pictured were tested in 2006 and are representative of three replicates at each tuber age. Water-inoculated samples from the week 6 trial shown here are representative of the water-inoculated samples at all tuber ages. Tubers of week 4 (2008 data) were visually similar to those of week 1 (2006 data) (not shown). **A**, Tubers of untransformed and *RB* transgene-transformed (lines SP922, SP951, and SP966) Katahdin plants. **B**, Tubers of untransformed and *RB* transgene-transformed (lines SP2105, SP2182, SP2211, and SP2213) Russet Burbank plants. **C**, Tubers of untransformed and *RB* transgene-transformed (lines SP2564, SP2572, SP2577, and SP2585) Dark Red Norland plants.

TABLE 1. Effect of age, cultivar, and transgene presence on tuber blight development

Variables	DF	Sum Sq	Mean Sq	<i>F</i>	Pr ($> F$)	Significant ^a
Trial	1	37581	37581	39.1	2.07E-09	***
Cultivar	2	26687	13343	13.88	2.10E-06	***
Age	3	35604	11868	12.35	1.70E-07	***
Transgene	1	3307	3307	3.44	0.065	.
Cultivar:Age	6	60236	10039	10.44	3.46E-10	***

^a Significance levels: ***, **, *, and . indicate significant at 0.001, 0.01, 0.05, and 0.1, respectively.

than those of Katahdin (0.0028) and more than six times those of Dark Red Norland (0.0041). The magnitude of the difference in transcript levels between Russet Burbank and the other cultivars is generally maintained across tuber age. The effect of cultivar on *RB* transcript levels is significant ($P < 0.01$).

Importantly, transformed Russet Burbank lines SP2211 and SP2213, which were more tuber blight resistant than untransformed Russet Burbank at 1 and 6 weeks postharvest (Fig. 1B), had the highest *RB* transcript levels of any other lines in our study at 1 week after harvest (Fig. 2). *RB* transcript in these lines, like those of other transformed lines, decreased as the tubers aged in storage. Significant ($P < 0.05$) differences in *RB* transgene transcription were evident between both Russet Burbank transformed lines SP2211 and SP2213 and all other tested Russet Burbank lines. Variation in *RB* transcript levels between lines within a cultivar mirrors observations that *RB* transcript levels in the potato foliage also vary across independently transformed lines (Bradeen et al. 2009). In all transformed lines, transcription of the *RB* transgene is driven by its native promoter. Although transgene copy number is

positively correlated with transcript levels, *RB* transcript levels in foliage vary even among transformed lines with the same number of transgene copies, suggesting that positional effects may influence *RB* transcription (Bradeen et al. 2009).

Tuber resistance levels correlates with *RB* transcript levels. Across all cultivars, transformed lines, and tuber ages, disease resistance levels are dependent on *RB* transcript levels ($P = 0.01$) (Fig. 3). Disease resistance levels averaged across all cultivars and transformed lines are also significantly dependent on *RB* transcript levels 1 week ($P < 0.001$), 6 weeks ($P = 0.050$), and even 19 weeks ($P = 0.099$) after harvest. Finally, considering only Russet Burbank transformed lines averaged across all tuber ages, *RB* transcript levels significantly ($P = 0.016$) affected observed resistance levels. Thus, tuber blight susceptibility is associated with comparatively low *RB* transcript levels while enhanced tuber blight resistance, as demonstrated in SP2211 and SP2213, is associated with comparatively high *RB* transcript levels. Together, these observations document a positive association between levels of *RB* transgene transcript accumulation and phenotypic disease resistance function in tubers of transgenic potato.

DISCUSSION

The potato-*P. infestans* pathosystem is ideal for further study of organ-specific *R* gene function. *P. infestans* actively infects and causes disease in both aboveground foliage and belowground tubers. *P. infestans* is a hemibiotroph, displaying a lifestyle dependent on living plant material in early infection stages followed by consumption of dead and dying host tissue.

All lines tested for tuber blight resistance in the current study were previously demonstrated to be foliar late blight resistant (Bradeen et al. 2009). Here, we demonstrate that tuber blight resistance in *RB*-transformed lines is influenced by genetic background, transformed line, and tuber age. Consistent with the conclusions of Halterman et al. (2008), 9 of the 11 tested transformed lines in the Russet Burbank, Dark Red Norland, and Katahdin backgrounds fail to display visually obvious enhanced tuber blight resistance relative to untransformed controls, regardless of tuber age (Fig. 1). However, Russet Burbank lines SP2211 and SP2213 demonstrate enhanced tuber blight resistance in an age-specific manner, with young tubers being most resistant and old tubers being phenotypically indistinguishable from untransformed controls (Fig. 1). We note that neither SP2211 nor SP2213 was analyzed by Halterman et al. (2008). Consistent with our observed

Fig. 2. Transcription of the *RB* transgene varies across tuber age. Summarized are *RB* transcript data from 2006 samples. RNA was collected from uninoculated tubers at 1, 6, 15, and 19 weeks postharvest and evaluated for *RB* transgene and *EF1α*. *RB* transgene transcript levels were normalized to those of *EF1α* from corresponding RNA samples. All *RB* transgenic lines exhibit higher transcript levels at week 1, with lower levels at later ages. As expected, all untransformed lines had *RB* transgene transcription values of 0.00. Results are shown for untransformed and transformed lines of **A**, Katahdin; **B**, Russet Burbank; and **C**, Dark Red Norland. Note that Y-axes are presented at different scales.

Fig. 3. Tuber late blight resistance correlates with *RB* transcript levels in the tuber. X-axis indicates *RB* transcript levels measured in replicated, uninoculated potato tubers. Y-axis indicates tuber late blight disease resistance measured as the proportion of nondiseased tubers. Katahdin transgenic (+*RB*) lines are represented as red open circles, Russet Burbank transgenic lines are represented as blue open circles, and Dark Red Norland transgenic lines are represented as black open circles. Within each genetic background, data generated across all transgenic lines, all replicates, all tuber ages, and 2 years are represented. The dashed line indicates the fitted logistic curve based on a generalized linear model. The association between *RB* transcript levels in untransformed tubers and observed tuber blight resistance is significant (P value = 0.01).

line-to-line variation, we speculate that the conclusions of Halterman et al. (2008) are valid for the specific transformed lines they tested and are not inconsistent with the data presented in the current study.

In this study, we further demonstrated that *RB* transcript level in tubers, like phenotypic resistance, is also influenced by genetic background, transformed line, and tuber age. On average, Russet Burbank lines have higher *RB* transcript levels than lines of Dark Red Norland and Katahdin (Fig. 2). Transformed Russet Burbank lines SP2211 and SP2213 demonstrate unusually high levels of *RB* transcript in 1-week-old tubers, with *RB* transcript levels declining as the tuber ages in storage (Fig. 2). In potato foliage, we previously demonstrated that *RB* transcript levels vary as a function of genetic background and transformed line (Bradeen et al. 2009) but not plant age (Millett et al. 2009). Importantly, foliar levels of the *RB* transcript were 7 to 12 times higher than were observed in potato tubers of the same line (data not shown), demonstrating transcriptional regulation of *RB* in an organ-specific manner.

Together, our phenotypic and transcriptional data document variability in both *R* gene function and transcriptional regulation across different genetic backgrounds, independent transformation events, and tuber ages. Yet careful analysis of SP2211 and SP2213 provides insight into the relationship between *RB* transcript levels and tuber blight resistance. Both lines have unusually high *RB* transcript levels in 1-week-old tubers (Fig. 2) and in foliage (SP2211 and SP2213 had the second and eighth highest *RB* transcript levels, respectively, of 40 lines tested) (Bradeen et al. 2009). At a phenotypic level, both SP2211 and SP2213 display high levels of tuber blight resistance (Fig. 1, week 1) and foliar blight resistance (SP2211 and SP2213 were ranked as third and fourth most resistant of 56 lines tested for foliar blight resistance) (Bradeen et al. 2009) relative to other tested lines and untransformed controls. Thus, high *RB* transcript levels in both the tuber and the foliage correlate with enhanced late blight resistance. However, in the current study, *RB* transcript levels were shown to decline as tubers age in storage. This trend was evident across all genetic backgrounds and transformed lines (Fig. 2). Indeed, whereas tubers of SP2211 and SP2213 displayed high *RB* transcript levels at week 1, with transcript levels 1.7- to 5.1-fold greater than those of other transformed Russet Burbank lines, by week 6, transcript levels of these lines were indistinguishable from those of other Russet Burbank transformed lines (Fig. 2). For SP2211 and SP2213, we showed a concomitant decline in tuber blight resistance, with 1-week-old tubers being highly resistant and 19-week-old tubers being phenotypically indistinguishable from untransformed controls (Fig. 1). We speculate that 6-week-old tubers of SP2211 and SP2213 retain functional *RB* protein despite low *RB* transcript levels, thus remaining temporarily more tuber blight resistant than untransformed controls. We recently explored late blight resistance mechanisms in 6-week-old SP2211 potato tubers using an RNA-seq approach (Gao et al. 2013). Our results indicate that *RB* triggers an HR response to *P. infestans* not only in foliage but also in tubers (Gao et al. 2013)—thus, the *RB* protein functions in a similar manner in both leaves and tubers. Further analysis of *RB* protein dynamics in potato tubers is warranted.

Few models to explain the organ-specific phenotypic function of *R* genes have been put forth. Hermanns et al. (2003) demonstrated that transcription of the *R* gene *RPP1* in *Arabidopsis* roots was insufficient to mount defense responses against *H. parasitica*. These authors postulated that organ-specific *R* gene function may be dependent upon an unspecified “enabler of defense responses” that is expressed in an organ-specific manner. This interpretation was favored by McDowell et al. (2005). In contrast, Cabrera Poch et al. (2006) demonstrated that the *R* gene *Hero*, which imparts potato cyst nematode resistance function in tomato roots, is both expressed and functional in the foliage, a nontarget organ for potato cyst nematodes. In that study, *Hero* alleles demonstrated identical race specificity in both root and foliage. Together, the observations of Cabrera Poch

et al. (2006) suggest that no overarching organ-specific regulator of *R* gene function exists in the examined pathosystem.

Consistent with Cabrera Poch et al. (2006), our data suggest a simple model for the organ-specific regulation of *R* gene function. We hypothesize that the observed correlation between *RB* transcript levels and phenotypic function in both tubers (this study) and foliage (Bradeen et al. 2009) are indicative of a direct cause-and-effect relationship. Thus, on average, enhanced *R* gene transcript levels result in enhanced phenotypic resistance. This hypothesis offers immediate insight into how plants regulate *R* gene function in different tissues or organs. Specifically, transcriptional regulation, achieved through differential expression of transcription factors in a tissue- or organ-specific manner, leads to quantitative variation in *R*-gene transcript levels that, in turn, leads to a differential phenotypic effect. Further analysis, including analysis of transcription factor accumulation in potato foliage and tubers and artificial manipulation of organ-specific expression of the *RB* transgene through modification of its promotor, is needed to test this possibility.

Worldwide, late blight disease of potato is the leading production constraint for this crop. Several late blight *R* genes have been cloned in recent years (Bradeen 2011), including *RB*, a gene with significant deployment potential (Song et al. 2003). Indeed, in addition to field tests already conducted in the United States (Bradeen et al. 2009), field trials have been or are being conducted in Europe, India, and other regions with the intention of eventual transgene deployment. In foliage, *RB* imparts delayed but not eliminated *P. infestans* sporulation (Song et al. 2003). Spore production in the field may expose tubers to late blight disease. Here, we demonstrate that tuber resistance varies as a function of *RB* transcript levels, providing an efficient strategy for selecting lines with enhanced tuber resistance. We propose that a simple model of regulation of *R* gene transcript levels is sufficient to explain organ-specific regulation of *RB* late blight resistance function.

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