Late Blight Resistance of RB Transgenic
Potato Lines

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ADDITIONAL INDEX WORDS. Solanum tuberosum, Phytophthora infestans, transformation, host resistance, resistance gene pyramiding

ABSTRACT. Late blight of potato (Solanum tuberosum L.), incited by Phytophthora infestans (Mont.) de Bary, is a devastating disease affecting tuber yield and storage. Recent work has isolated a resistance gene, RB, from the wild species Solanum bulbocastanum Dun. Earlier work in Toluca, Mexico, observed significant levels of field resistance under intense disease pressure in a somatic hybrid containing RB. In this study, five transgenic RB lines were recovered from the late blight susceptible line MSE149-5Y, from the Michigan State University (MSU) potato breeding program. Transgenic lines were molecularly characterized for the RB transgene, RB transcript, and insertion number of the kanamycin resistance gene NPTII. Transgenic lines and the parent line were evaluated for resistance in field and laboratory tests. Molecular characterization alone did not predict which lines were resistant. Three of the RB transformed MSE149-5Y lines showed increased resistance under field conditions at MSU and increased resistance in detached leaf evaluations using multiple isolates individually (US-1, US-1.7, US-8, US-10, and US-14). Transfer of RB into late blight susceptible and resistant lines could provide increased protection to potato late blight. The use of the RB gene for transformation in this way creates a partially cigenic event in potato because the gene’s native promoter and terminator are used. This type of transformation provides a chance to generate greater public acceptance of engineered approaches to trait introgression in food crops.

Late blight of potato incited by Phytophthora infestans is responsible for significant annual losses in North America (Guenthner et al., 1999) and worldwide (Hijmans, 2003). The effect of climate change on potato production (Hijmans, 2003) and potato late blight (Baker et al., 2005) may result in reduced yield and increased risk of development of epidemics of potato late blight. Frequent fungicide spray intervals and rates currently used by growers to control late blight are expensive. Host resistance is an alternative control measure that is more economically and environmentally sustainable.

In the early 1900s, potato breeders successfully introgressed resistance from the Mexican wild species Solanum demissum Lindl. into cultivated potato ( Muller and Black, 1952). However, major gene resistance from S. demissum was quickly overcome by P. infestans (Wastie, 1991). A total of 11 major dominant resistance genes (R genes) were identified from S. demissum (Malcolmson, 1969; Malcolmson and Black, 1966; Muller and Black, 1952). Most S. demissum R genes have been located to potato chromosomes and R1 and R3a have already been sequenced (Ballvora et al., 2002; Bradshaw et al., 2006; Huang et al., 2005). Although these genes have been defeated by P. infestans, there is some evidence that they may be useful when combined with other sources of resistance (Stewart et al., 2003).

As races of P. infestans overcome resistance from S. demissum researchers have turned to other Solanum L. species for resistance genes. A multitude of genes have been mapped, and several have been cloned and sequenced. The resistance gene Rpi-blb1 from Solanum berthaultii Hawkes, was mapped to chromosome X (Ewing et al., 2000; Rauscher et al., 2006). Rpi-blb3, identified in Solanum mochiquense Ochoa, mapped to chromosome IX (Smilde et al., 2005). Rpi-blb1 was identified from Solanum phureja Juz. et Buk. and mapped to chromosome IX (Sliswka et al., 2006). Rpi1 from Solanum pinnatisectum Dun. was mapped to chromosome VII (Kuhl et al., 2001). A major QTL from Solanum microdontum Bitt. (Bisognin et al., 2005) was recently mapped to chromosome IV (D.S. Douches, unpublished data). Solanum bulbocastanum has yielded several resistance genes. Two alleles from a single locus on chromosome VIII were identified and cloned, RB (Song et al., 2003) and Rpi-blb1 (van der Vossen et al., 2003). Rpi-blb2 was localized to chromosome VI and subsequently cloned (van der Vossen et al., 2005). Another gene, Rpi-blb3, has been mapped to chromosome IV (Park et al., 2005).

The increasing pool of genes conveying resistance to late blight of potato raises the question of how to incorporate such resistance into cultivated lines. The cultivated potato, S. tuberosum, is tetraploid (4x) with an endosperm balance number (EBN) of 4, resulting in crossing barriers with wild Solanum species that differ in ploidy or EBN (Hammeman, 1999). Although classical methodologies have been used to overcome such barriers (Hermsen and Ramanna, 1973), the desire to incorporate more than one source of resistance presents increased challenges for the potato breeder. Plant transformation offers an efficient method to transfer genes from divergent organisms directly into the plant genome (Sharma et al., 2005). Transformation also allows both the quantity, through gene pyramiding, and timing of resistance genes to be controlled on a relatively short time scale.

Resistance genes are potentially useful control measures for potato late blight. Both RB and Rpi-blb1 have broad-spectrum resistance and conferred resistance to a range of isolates of P.
infestans carrying multiple virulence factors (Song et al., 2003; van der Vossen et al., 2003). RB has also been shown to maintain resistance in Toluca, Mexico under intense disease pressure (Helgeson et al., 1998). However, successful insertion of a transgene into the genome does not guarantee a positive phenotype. van der Vossen et al. (2003) reported that 15 of 18 Rpi-blb1 transformed potato lines were resistant and seven of nine tomato (Solanum lycopersicum L.) transformants showed resistance. Song et al. (2003) reported that of 14 RB transformed lines, 5 were highly resistant and 9 were moderately resistant, having <10% and 11% to 25% infection, respectively, in comparison with non-transformed lines. Successful transformation requires confirmation that the gene or genes are not only intact and transcribed/translated but that the desired phenotype is also recovered.

The objectives of this study were to evaluate the recovery of effectively transformed progeny plants of the potato advanced breeding line MSE149-5Y and to determine the resistance of these transformed lines to different genotypes of P. infestans in detached leaf tests and the field.

Materials and Methods

**Generation of transgenic lines.** Michigan State University breeding line MSE149-5Y was used for all transformations reported here. MSE149-5Y is not known to have any major late blight R gene. Plasmid 2.4.48pCRXLTOPO (J. Jiang, personal communication) was digested with BamHI, and the 8.59-kb fragment containing RB was isolated, including 2.5 kb upstream of the start ATG and 2.48 kb downstream of the stop codon. This fragment was ligated to pBINPLUSARS (U.S. Department of Agriculture, Albany, CA) (Garbarino and Belknap, 1994), which had been digested with BamHI, and the new construct called pSPUD69 (Fig. 1). Agrobacterium tumefaciens (Smith & Towns.) Conn.-mediated transformations using a kanamycin marker were conducted according to the manufacturer’s guidelines. cDNA was generated from total RNA using M-MLV Reverse Transcriptase (Invitrogen Corp., Carlsbad, CA) and inactivated according to the manufacturer’s guidelines. cDNA was generated from total RNA using M-MLV Reverse Transcriptase (Invitrogen Corp.) according to the manufacturer’s guidelines. Polymerase chain reaction (PCR) was conducted with REDTag DNA Polymerase (Sigma-Aldrich Co.) according to the manufacturer’s guidelines. RB and NPTII primers were the same as above. Positive control was EF1α (forward, 5’ GGTTGTTTGAAGCTGG TATCTCC 3’ and reverse, 5’ CAGTAGGGCCAAAGGTCA CA 3’), 50 °C annealing temperature. Fragments were separated on 1.0% agarose gel.

**Southern hybridizations.** Twenty micrograms of endo-restriction-digested (BamHI, EcoRI, and HindIII) DNA per sample was transferred to Amersham Hybond-N+ membrane (GE Healthcare, Piscataway, NJ) using a standard alkaline lysis procedure (Kennard et al., 1994). Isolated fragments of RB and NPTII were labeled with [α-32P]dCTP with the Random Primers DNA Labeling system (Invitrogen Corp.), and un-incorporated nucleotides were removed with Bio-Spin 30 Tris columns (Bio-Rad, Hercules, CA). Hybridizations were conducted as described in Kuhl et al. (2001). Hybridization with RB used a 1562-bp fragment, bp 1794 through bp 3356 from ATG, including the 213-bp region used to distinguish resistant and susceptible alleles. Highly stringent final wash conditions were used 0.1× SSC and 0.1% SDS for a total of 52 min at 60 °C. Hybridization with NPTII used a 700-bp fragment. The final wash condition included 2× SSC, 0.1% SDS for 30 min at 50 °C followed by 0.1× SSC, 0.1% SDS for 20 min at 60 °C.

**Detached leaf tests.** control was 38°C for 30 min. Suspensions were subsequently examined using a binocular microscope to confirm the release of zoospores, calibrated by counting with a hemocytometer and

![Figure 1](https://example.com/figure1.png)

**Fig. 1.** T-DNA region of pSPUD69. RGA2-PCR indicates the 8.59-kb BamHI fragment containing the RB gene ligated into the pBINPLUSARS base plasmid. This construct includes the NPTII gene for kanamycin resistance controlled by the ubiquitin (Ubi3) promoter (Pro) and terminator (Term). Natural promoter (2503-bp upstream) and termination sequences (2477-bp downstream) were used for RB. The region in the T-DNA where the RB primers anneal is designated by “PCR Product.”
Table 1. *Phytophthora infestans* isolates independently used in potato
detached leaf evaluations at 2.0 × 10⁴ sporangia/mL sprayed on
the abaxial leaf surface and incubated at 17 °C for 12 d.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Genotype*</th>
<th>Mating type</th>
<th>R-gene phenotype**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi95-3*</td>
<td>US-1</td>
<td>A1</td>
<td>5</td>
</tr>
<tr>
<td>Pi95-2</td>
<td>US-1.7</td>
<td>A2</td>
<td>1, 9</td>
</tr>
<tr>
<td>Pi02-007</td>
<td>US-8</td>
<td>A2</td>
<td>1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11</td>
</tr>
<tr>
<td>SR83-84</td>
<td>US-10</td>
<td>A2</td>
<td>1, 2, 4, 5, 10</td>
</tr>
<tr>
<td>Pi09-2*</td>
<td>US-14</td>
<td>A2</td>
<td>1, 5</td>
</tr>
</tbody>
</table>

*Genotypes as designated by Goodwin et al. (1995) and Young et al. (2004).

**Virulence phenotype as defined by Muller and Black (1952).

*Less than 2.0 × 10⁴ sporangia/mL for one or both detached leaf
inoculations adjusted to about 2.0 × 10⁴ sporangia/mL using SDW (Kuhl et al., 2001).

Inoculations were conducted essentially according to Kuhl et al. (2001) on 12 Dec. 2004 and replicated 3 Jan. 2005. Five plants per line of greenhouse-grown plants were used. Five fully developed leaves were selected from the upper portion of each plant and one leaf randomly assigned per *P. infestans* isolate, so that five leaves total were used per isolate. Three layers of absorbent paper towels were placed in the bottom of 150-mm-diameter petri dishes with a layer of plastic mesh on top, and 38 mL of SDW was added to the towels in each plate. Detached leaves were placed abaxial side up on the plastic mesh and then sprayed using a hand-held sprayer, once with SDW and once with *P. infestans* inoculum (0.7-mL spray volume per plate per spray). Petri dishes were wrapped with parafilm and placed into dark incubators at 17 °C with 12-h light cycle. For all experiments incubator shelves and positions were randomized. Pi95-3 (US-1) and Pi99-2 (US-14) did not produce sufficient sporangia although ~1.0 × 10⁴ sporangia/mL were used at both inoculations for Pi95-3 and ~2.0 × 10⁴ and ~1.4 × 10⁴ sporangia/mL were used for Pi99-2 at inoculations 1 and 2, respectively. Leaves were scored at 6, 9, and 12 d after inoculation (DAI) using disease severity indices (DSI) based on percentage of leaf area affected: 0 = no symptoms, 1 = 0% to 5%, 3 = 6% to 25%, 5 = 26% to 50%, 7 = 51% to 75%, 9 = 76% to 100% (Kuhl et al., 2001). Scores were converted to mean percentage values by using the midpoint value in each interval (Haynes et al., 1998). Percentages were used to calculate area under the disease progress curve (AUDPC) according to Shaner and Finney (1977) and analysis of variance conducted using XLSTAT (version 2006.5; Addinsoft, New York, NY).

**FIELD EVALUATIONS.** Inoculated fields trials were conducted in 2004 and 2005 at the Michigan Agricultural Experimental Station Muck Soils Research Farm, Laingsburg, MI. Plots were randomized in both years using an overhead sprinkler irrigation system. The zoospore suspension of mixed isolates of *P. infestans* was injected into the irrigation water feed pipeline under 0.5 kg cm⁻² of CO₂ pressure and applied at a rate of ~150 mL m⁻² of inoculum solution of trial area to give an inoculation rate of about 2.4 × 10⁶ zoospores m⁻² (2.4 × 10⁶ zoospores ha⁻¹). This amount and rate of inoculum applied were estimated from prior calibration of the irrigation system and were intended to expose all potato foliage to inoculum of *P. infestans*

As soon as late blight symptoms were detected (about 7 DAI), each plant within each plot was visually rated at 3- to 5-d intervals for percent leaf and stem (foliar) area with late blight lesions. The mean percent blighted foliar area per treatment was calculated. Evaluations continued until plots of susceptible cultivars reached 100% foliar area diseased. DAI to the time until 100% foliar area in susceptible cultivars diseased was reached was used as a key reference point for calculation of relative area under the disease progress curve [RAUDPC (Kirk et al., 2001)]. Analysis of variance was calculated using JMP (version 5.0.1; SAS Institute Inc., Cary, NC).

**RESULTS.**

**MOLECULAR EVALUATIONS.** PCR tests on total genomic DNA revealed five transformants of MSE149-5Y transformed for both RB and NPTII (Fig. 2). In addition to the two lines in Fig. 2, E69.02 and E69.10, >150 other lines tested positive for NPTII but failed to amplify the fragment from RB (data not shown). As a consequence, screening of newly transformed lines required testing for both NPTII and RB.

In an effort to determine copy number of the transformed plants, a Southern blot was hybridized with the RB fragment. The RB fragment used includes sequence before the leucine-rich repeat (LRR) region and 1113 bp of LRR, including the 213-bp region used to distinguish RB from other alleles. In anticipation that the labeled fragment would hybridize to numerous genes in *S. tuberosum*, a highly stringent final wash was used. Despite this effort, the RB fragment hybridized to many regions within the potato genome (data not shown). No bands could be identified as specific to the RB transgene. Due to the nature of the construct used (Fig. 1), NPTII could be used to approximate RB copy number.

A 700-bp fragment from NPTII was hybridized to the five RB transformed lines (Fig. 3). Four of the lines showed a single labeled fragment—E69.01, E69.03, E69.04, and E69.06—suggesting the transgene inserted into a single site in the genome. Lane 4, E69.05, contains two NPTII labeled fragments, suggesting two separate insertion events.

Reverse-transcribed PCR (RT-PCR) was used to evaluate for transcription of the RB and NPTII transgenes. Lines E69.01–E69.06 detected NPTII transcript, while RB transcript was detected in lines E69.03, E69.04, E69.05, and E69.06 (Fig. 4). Notably, RB transcript was not detected in line E69.01, even though the RB fragment amplified from total genomic DNA...
The absence of RB transcript is expected for E69.02 and E69.11, both of which lack the amplified RB fragment.

**Detached Leaf Tests.** Detached leaflet tests indicated that there were no significant differences in AUDPC among lines of MSE149-5Y transformed with the RB gene inoculated with *P. infestans* isolates Pi95-3 (US-1), Pi95-2 (US-1.7), Pi02-007 (US-8), or Pi99-2 (US-14) (Table 2). With isolate SR83-84 (US-10), lines E69.03, E69.05, and E69.06 had significantly lower AUDPC values (31–35) than lines with AUDPC values from 154 to 190 (Table 2).

**Field Data.** Foliar potato late blight developed steadily after inoculation in 2004, and the RAUDPC value in the untransformed line MSE149-5Y was 47.7 (41 DAI; Table 3). RAUDPC values of lines E69.03, E69.05, and E69.06 in 2004 ranged from 0.7 to 6.3 and were significantly lower (*P* = 0.05) values than the untransformed MSE149-5Y. Line E69.04 had a significantly greater RAUDPC value (27.8) than the three most resistant lines but significantly lower than the untransformed line (Table 3). In 2004, line E69.01 had a significantly greater RAUDPC value (38.8) than E69.04 (27.8) but significantly less than MSE149-5Y (47.7). In 2005, the epidemic progressed more slowly, and by 45 DAI the RAUDPC value in the untransformed line MSE149-5Y was 19.8 (Table 3). The RAUDPC value for E69.01 (19.5) was not significantly different from the value for MSE149-5Y (19.8). Lines E69.05 and E69.06 in 2004 had RAUDPC values from 1.8 to 4.5 and were significantly lower (*P* = 0.05) values than the untransformed MSE149-5Y.

**Discussion**

Both field and detached leaf tests detected increased levels of late blight resistance in three transformed lines, E69.03,
Table 2. Effect of transformation of potato line MSE149-5Y with RB on susceptibility to Phytophthora infestans in detached leaf tests scored at 6, 9, and 12 DAI.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MSE149-5Y</th>
<th>E69.01</th>
<th>E69.02</th>
<th>E69.03</th>
<th>E69.04</th>
<th>E69.05</th>
<th>E69.06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi95-3 2</td>
<td>0 a 3</td>
<td>168 a</td>
<td>9 a</td>
<td>24 a</td>
<td>32 a</td>
<td>161 a</td>
<td>103 a</td>
</tr>
<tr>
<td>Pi95-2</td>
<td>22 a</td>
<td>59 a</td>
<td>26 a</td>
<td>9 a</td>
<td>49 a</td>
<td>122 a</td>
<td>33 a</td>
</tr>
<tr>
<td>Po02-007</td>
<td>374 a</td>
<td>395 a</td>
<td>405 a</td>
<td>406 a</td>
<td>457 a</td>
<td>513 a</td>
<td>341 a</td>
</tr>
<tr>
<td>SR83-84</td>
<td>154 a</td>
<td>190 a</td>
<td>184 a</td>
<td>31 b</td>
<td>162 a</td>
<td>35 b</td>
<td>31 b</td>
</tr>
<tr>
<td>Pi95-2</td>
<td>22 a</td>
<td>59 a</td>
<td>26 a</td>
<td>9 a</td>
<td>49 a</td>
<td>122 a</td>
<td>33 a</td>
</tr>
</tbody>
</table>

*Area under the disease progress curve derived from measuring potato late blight lesion area from inoculation to 12 DAI.

Table 3. Effect of transformation of potato line MSE149-5Y with the RB gene on susceptibility to Phytophthora infestans in field plots at the Michigan Agricultural Experimental Station Muck Soils Research Farm at Laingsburg in 2004 and 2005.

<table>
<thead>
<tr>
<th>Yr</th>
<th>Line</th>
<th>RAUDPC ×RB transcript</th>
<th>RB transcript</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>MSE149-5Y</td>
<td>2 47.7 – –</td>
<td>47.7 – –</td>
</tr>
<tr>
<td></td>
<td>E69.01</td>
<td>2 38.8 + –</td>
<td>38.8 + –</td>
</tr>
<tr>
<td></td>
<td>E69.04</td>
<td>2 27.8 + +</td>
<td>27.8 + +</td>
</tr>
<tr>
<td></td>
<td>E69.03</td>
<td>2 6.3 + +</td>
<td>6.3 + +</td>
</tr>
<tr>
<td></td>
<td>E69.06</td>
<td>2 4.2 + +</td>
<td>4.2 + +</td>
</tr>
<tr>
<td></td>
<td>E69.05</td>
<td>2 0.7 + +</td>
<td>0.7 + +</td>
</tr>
<tr>
<td>2005</td>
<td>MSE149-5Y</td>
<td>3 19.8 – –</td>
<td>19.8 – –</td>
</tr>
<tr>
<td></td>
<td>E69.01</td>
<td>3 19.5 + –</td>
<td>19.5 + –</td>
</tr>
<tr>
<td></td>
<td>E69.06</td>
<td>3 4.5 + +</td>
<td>4.5 + +</td>
</tr>
<tr>
<td></td>
<td>E69.05</td>
<td>3 1.8 + +</td>
<td>1.8 + +</td>
</tr>
</tbody>
</table>

*Amplification of 213-bp RB fragment from total DNA extraction.

Table 4. Effect of transformation of potato line MSE149-5Y with the RB gene on susceptibility to Phytophthora infestans in detached leaf tests scored at 6, 9, and 12 DAI.

<table>
<thead>
<tr>
<th>Yr</th>
<th>Line</th>
<th>RAUDPC × RB transcript</th>
<th>RB transcript</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>MSE149-5Y</td>
<td>2 47.7 – –</td>
<td>47.7 – –</td>
</tr>
<tr>
<td></td>
<td>E69.01</td>
<td>2 38.8 + –</td>
<td>38.8 + –</td>
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<tr>
<td></td>
<td>E69.04</td>
<td>2 27.8 + +</td>
<td>27.8 + +</td>
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<tr>
<td></td>
<td>E69.03</td>
<td>2 6.3 + +</td>
<td>6.3 + +</td>
</tr>
<tr>
<td></td>
<td>E69.06</td>
<td>2 4.2 + +</td>
<td>4.2 + +</td>
</tr>
<tr>
<td></td>
<td>E69.05</td>
<td>2 0.7 + +</td>
<td>0.7 + +</td>
</tr>
<tr>
<td>2005</td>
<td>MSE149-5Y</td>
<td>3 19.8 – –</td>
<td>19.8 – –</td>
</tr>
<tr>
<td></td>
<td>E69.01</td>
<td>3 19.5 + –</td>
<td>19.5 + –</td>
</tr>
<tr>
<td></td>
<td>E69.06</td>
<td>3 4.5 + +</td>
<td>4.5 + +</td>
</tr>
<tr>
<td></td>
<td>E69.05</td>
<td>3 1.8 + +</td>
<td>1.8 + +</td>
</tr>
</tbody>
</table>

*Amplification of 213-bp RB fragment from total DNA extraction. 

In the case of E69.01 molecular data, presence of RB fragment (Fig. 2) but absence of RB transcript (Fig. 4) accurately predicted a susceptible phenotype in both laboratory and field evaluations. However, E69.04, a susceptible phenotype that contains the RB DNA fragment and transcript, would not have been detected without laboratory or field phenotypic testing. Phenotype screening is needed to accurately determine which transformed lines are worth carrying forward.

Detached leaf tests accurately predicted field resistance in lines E69.03, E69.05, and E69.06. This interpretation was only good for the US-10 isolate, SR83-84. Isolates Pi95-3 and Pi99-2 both produced suboptimum sporangia concentrations resulting in highly variable phenotypes. P. infestans US-8 isolate Pi02-007 was particularly aggressive and successfully infected all lines evaluated in detached leaf tests. This same isolate was also applied in field inoculations, but resistance was clearly observed in lines E69.03, E69.05, and E69.06. The most likely explanation is that conditions of the detached leaf test were too stringent for this isolate and that leaves were overcome by density of inoculum. Stewart (1990) found that the best inoculum concentration to distinguish resistant and susceptible phenotypes is likely to depend on the isolate. Adjustment of the inoculum concentration may help resolve susceptible and resistant phenotypes. Both RB and Rpi-blb1 are reported to contribute broad-spectrum resistance to all P. infestans races (Song et al., 2003; van der Vossen et al., 2003). However, Pi02-007 has been observed to be a highly aggressive isolate (Young et al., 2004), suggesting that lower concentration of inoculum may be required to detect resistant phenotypes. In this report, Pi02-007 was significantly different from the other isolates (P ≤ 0.05) with the highest mean AUDPC, 412 ± 120. Use of multiple isolates reported here allowed accurate detection of transgenic resistance in laboratory tests and corroborated field results.

Transformation efficiency was very low in this study. The low efficiency is unexpected with MSE149-5Y, which has proven to be particularly receptive (75% regeneration rate) to transformation (D.S. Douches, unpublished data). Studies must be conducted to understand this phenomenon so that greater numbers of lines can be generated for evaluation.

Determination of RB copy number relied on hybridization with a NPTII fragment. A single band was observed for four of five RB-containing lines, suggesting a single insertion of RB. However, this does not exclude the possibility of a closely linked tandem insertion or the possibility of additional RB insertions with the loss of NPTII. This highlights a challenge for researchers using potato genes to transform potato. One alternative approach is to use real-time PCR to estimate copy number (Ingham et al., 2001) using gene- and/or allele-specific primers. An unsuccessful effort using RB-specific primers attempted to do just that (J.C. Kuhl, unpublished data). Failure may in part be due to the low annealing temperature of the RB primers (43 °C) and the challenge to identify alternative primers that are consistent with the RB gene and allow accurate detection of RB copy number.
primers, RB, a coiled coil–nucleotide binding site–leucine-rich repeat class resistance gene (Martin et al., 2003), is expected to be homologous with other potato resistance genes, as demonstrated by hybridization with RB, making allele specific primers difficult to identify.

The 213-bp RB fragment was amplified from genomic DNA of line E69.01, but RB transcript was not detected. This could be explained by a partial deletion/rearrangement of the RB transgene, leaving intact the 213-bp DNA region, but inactivating or truncating transcription. Another possibility is post-transcriptional silencing, as observed in other transgenic lines (Felcher et al., 2003).

As transformation technologies become more efficient greater numbers of putatively transformed lines will be generated. Molecular characterization does not guarantee a resistant phenotype. Field evaluations require propagation of the desired lines and large-scale preparation and deployment of P. infestans inoculum, in addition to regular field observations throughout disease development. Therefore, it may not be desirable to take all putative transformed lines to the field. As evidenced by correlation of detached leaf assays and field results, screening of transformants in the laboratory before field evaluations will contribute to more efficient use of field space.

RB may be useful creating late blight resistant phenotypes in either susceptible or resistant cultivars of potato. Cultivar × fungicide studies, as previously conducted by Kirk et al. (2005), should be conducted to determine the best way to integrate protectant fungicides and host plant resistance to achieve late blight control for commercial conditions. These lines generated can be used for further breeding to introgress RB into additional germplasm.

The use of the RB gene from S. bulbocastanum for transformation creates a partially cisgenic event in potato because the gene’s native promoter and terminator is also used. This type of transformation creates an opportunity to generate greater public acceptance of engineered approaches to trait introgression in our food crops. Currently, the NPTII gene is also in the vector construct T-DNA region. Transformation methods exist to create marker-free transgenics in potato (Rommens et al., 2004). Further transformations in our program have created RB-transgenic lines in which RB is inserted independently from NPTII. Through crossing we may be able to identify RB-positive/NPTII-negative transgenic lines for further breeding.

### Literature Cited


