

## FINAL RESEARCH REPORT 2010

### **Project Title:**

Molecular basis for potato tuber disease resistance

### **Investigator:**

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### **Description and Objective of Research:**

Worldwide, late blight disease of potato results in multi-billion dollar yield losses and the application of millions of tons of fungicides each year. Chemical-dependent potato farming carries high production and environmental costs. One of the most economical and environmentally sound ways to reduce fungicide usage is deployment of late blight resistance genes. We have a long research history of working with *RB*, a foliar late blight resistance gene from a wild potato species. As we move towards large-scale deployment of the *RB* gene in India and other parts of the world, its impact on both foliar and tuber late blight disease must be considered.

This project utilizes “next generation”, high-throughput RNA sequencing to identify plant genes involved in resistance to late blight in foliage and tubers. Our research will enhance basic understanding in the plant sciences and will assist researchers in developing specific deployment strategies for *RB* in a no- or reduced-fungicide production setting.

This project encompasses a series of experiments aimed at testing the following hypotheses:

- Hypothesis 1: Different organs of potato (e.g., foliage vs. tubers) use the same or similar defense mechanisms against the late blight pathogen, *Phytophthora infestans*.
- Hypothesis 2: Differences in disease phenotypes in different organs within the same genotype are due to differences in timing of defense initiation.
- Hypothesis 3: High resistance gene transcript levels lead to earlier initiation of defense mechanisms in all plant organs.

### **Summary of Findings (Outputs/Outcomes):**

(1) More than 215 million RNA sequences from potato tubers infected with *P. infestans* were generated. This represents a >1,300-fold increase in data generation relative to our previous expectation of 160,000 sequence reads. This increase was achieved by capitalizing upon emerging next generation sequencing technology (Illumina Solexa).

(2) Bioinformatic comparison of tuber and foliar datasets from potato reveal that while many of the same genes are activated in both tissues when *P. infestans* attacks, overall defense response mechanisms in leaves and tubers might be substantially different. These analyses are ongoing.

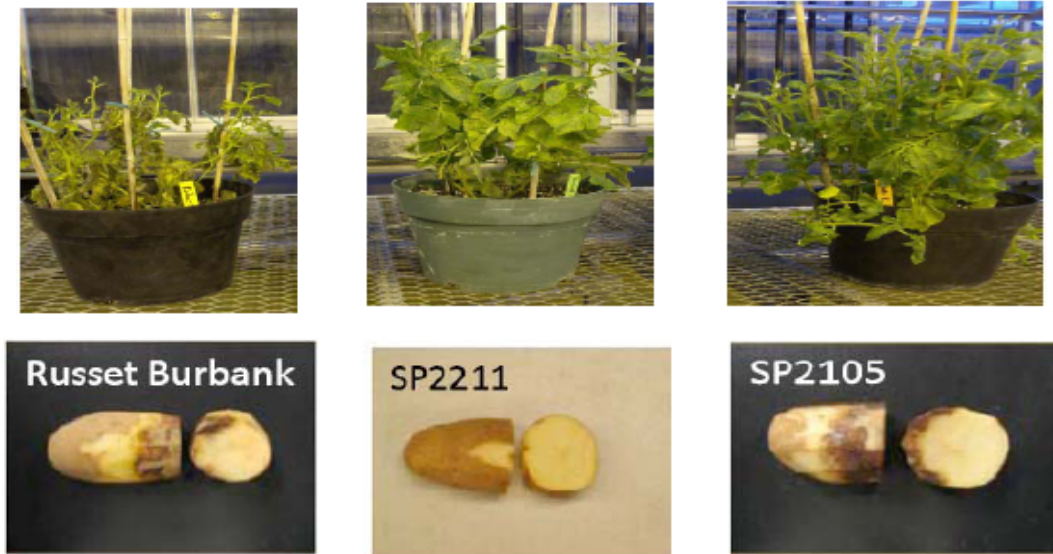
(3) We have identified a large set of genes that are responsive in the potato tuber to attack by *P. infestans*. These genes constitute candidates for genes involved in resistance mechanisms in the tuber.

(4) Although this project focused on potato gene transcription, application of newer sequencing technologies allowed us to simultaneously develop a large dataset of genes transcribed in the pathogen as well. Some of these genes may be critical in defining a microbe's ability to attack certain plant genotypes. Future analyses of these genes may reveal new disease control strategies. These genes may also be utilized in future studies as molecular markers to quantify late blight disease development.

### **Background:**

Potato is the world's fourth most important human food crop (Bradeen et al. 2008) and yields more calories per acre than any grain (Rubatzky and Yamaguchi 1997). *Phytophthora infestans*, an oomycete "fungus" that causes late blight disease, is a notorious plant destroyer with the capacity to destroy all parts of a potato plant, including foliage, stem, flower and tubers. Late blight is the most devastating disease for the world's potato production. The disease caused the Irish Potato Famine in the 1840s and still today results in multi-billion dollar losses worldwide annually (Duncan 1999; Fry 2008). Millions of tons of fungicide are applied to the world's potato crop to prevent late blight disease each year. Effective, environmentally-responsible management of late blight disease is possible with a combination of genetic resistance and reduced fungicide usage. We have a long history of research on the *RB* late blight resistance gene, a gene originating from wild potato species (Bradeen et al. 2009a; Bradeen et al. 2003; Millett and Bradeen 2007; Millett et al. 2009; Naess et al. 2000, 2001; Sanchez and Bradeen 2006; Song et al. 2003a; Song et al. 2003b). Important to this project, *RB* is the single most significant late blight resistance gene characterized to date and is scheduled to be deployed in India beginning in 2012 (K.V. Raman, *personal communication*). Our experiments were aimed at achieving the eventual goal of reducing fungicide input for potato production.

One potential challenge for employing genetic resistance for potato production is that foliar resistance to late blight does not guarantee tuber resistance to the same disease—contrasting phenotypes between tubers and foliage can be found even within the same cultivar or genotype (Figure 1)(Collins et al. 1999; Kirk et al. 2001). To effectively control late blight disease, there is a need to understand how the potato defends against the late blight pathogen in different organs (foliage vs. tubers). In order to understand these defense mechanisms, researchers worldwide have conducted a number of studies focusing on "transcriptome" dynamics of potato foliage under pathogen attack. The term "transcriptome" refers to the set of all mRNAs transcribed (from gene DNA to RNA) in a given cell type or tissue at any particular point in time. Importantly, to date, there has been no published study focusing on potato tuber disease response transcriptome dynamics. Our project aims to unravel potato defense mechanisms in the tubers, thus providing potential windows to effectively manage late blight disease both in foliage and tubers. The project is also among the first to study how resistance genes are utilized in different plant organs.



**Figure 1.** Potato late blight disease phenotypes. ‘Russet Burbank’ is a popular U.S. potato cultivar. SP2211 and SP2105 are ‘Russet Burbank’ lines with varying numbers of copies of the resistance gene “*RB*” (Song et al. 2003c). Note that ‘Russet Burbank’ is susceptible to late blight in both the foliage and the tuber; SP2105 is resistant in foliage but susceptible in tubers; SP2211 is resistant in both foliage and tubers.

### Objectives:

The purpose of this project was to support the use of genetic (rather than chemical) control of potato late blight disease by unraveling potato tuber defense mechanisms effective against the late blight pathogen and to comparing tuber and foliar defense response transcriptomics.

Our objectives tested three hypotheses:

- Hypothesis 1: Different organs of potato (e.g., foliage *vs.* tubers) use the same or similar defense mechanisms against the late blight pathogen, *P. infestans*.
- Hypothesis 2: Differences in disease phenotypes in different organs within the same genotype are due to differences in timing of defense initiation.
- Hypothesis 3: High resistance gene transcript levels lead to earlier initiation of defense mechanisms in all plant organs.

## Data, Findings, Outputs/Outcomes:

To facilitate understanding of this project report, we define several terms related to our research:

**Gene:** A gene is a segment of DNA that encodes a protein.

**EST:** Expressed Sequence Tags; RNA sequence data representing activated genes. The abundance of an EST in a dataset is a measurement of how active the corresponding gene is.

**UniGene:** Assembled from ESTs, one UniGene represents a unique gene sequence. In our analyses, UniGene may be considered as a synonym of gene.

## Summary of Approaches:

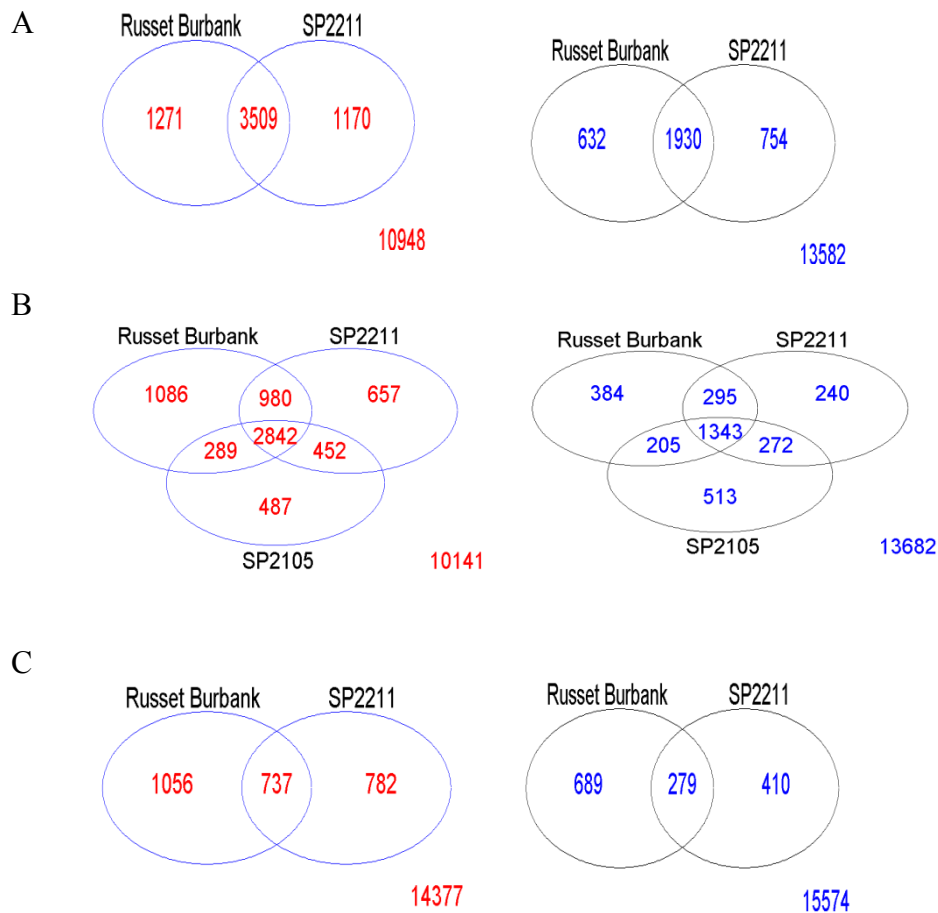
We utilized Illumina Solexa sequencing technology on eight RNA samples derived from potato tubers infected with *P. infestans* (Table 1). Included were three potato genotypes and three time points. In total, 215 million sequence reads (ESTs) were generated. More than 70 million could be directly mapped to the NCBI (National Center for Biotechnology Information) potato UniGene database using Bowtie bioinformatics alignment tool (<http://bowtie-bio.sourceforge.net/index.shtml>). This procedure allows us to predict what potato gene each EST represents. Of mapped ESTs, 18,573 out of 18,825 (98.7%) known potato UniGenes were represented. To enable cross-sample and cross-time comparisons, we normalized our mapping data using RPKM (number of mapped Reads Per Kilobase of transcript per Million total reads) approaches (Mortazavi et al. 2008).

**Table 1.** RNA samples submitted for next-generation sequencing (Illumina GA II)

Tissue	Cultivar/lines	0 hour*	24 hour	48 hour
Tuber	'Russet Burbank'	Sample 1	Sample 4	Sample 6
	SP2211	Sample 2	Sample 5	Sample 7
	SP2105	Sample 3		Sample 8

\*Times indicated are hours post pathogen inoculation

One of the major approaches for transcriptome analysis involves identifying differentially expressed genes among different times or different tissues. Through pairwise comparisons among different time points, we detected a total of 10,829 differentially expressed genes using a 2-fold change cut-off. The results are summarized in Figure 2.



**Figure 2.** Differentially expressed genes in potato cultivar ‘Russet Burbank’ and the +*RB* lines SP2105 and 2211. Red numbers within a circle indicate the number of up-regulated genes for a given potato line/cultivar; these genes are turned “on” (or at least up) when a pathogen attacks. Blue numbers within a circle indicate the number of down-regulated genes in a given potato line/cultivar; these genes are turned “off” (or at least down) when a pathogen attacks. Intersection between two circles indicates the number of regulated genes shared by the two (or three) potato lines. (A) Genes up- (red) or down- (blue) regulated at 24 hours post pathogen inoculation vs. 0 hours; (B) Genes up- (red) or down- (blue) regulated at 48 hours post pathogen inoculation vs. 0 hours; (C) Genes up- (red) or down- (blue) regulated at 48 hours vs. 24 hours post pathogen inoculation.

Several studies have investigated potato-*P. infestans* interactions in the foliage (Birch et al. 1999; Restrepo et al. 2005; Ronning et al. 2003; Tian et al. 2006). Based on these reports, we generated a list of potato genes differentially expressed (up- or down-regulated) in the foliage when *P. infestans* attacks (Table 2).

**Table 2.** Summary of published studies on potato-*P. infestans* interactions in the foliage

Tissue Examined	# Differentially Expressed Genes*	Total Genes Examined	Reference
Foliage	76	SSH**	Tian et al. 2006
Foliage	35	SSH	Birch et al. 1999
Foliage	643	7680	Restrepo et al. 2005
Foliage	567	3465	Ronning et al. 2003
Tubers	10829	18573	The Current Project

\*In the current study we used a 2-fold change to define differential expression.

\*\*SSH means the article is based on the Suppression Subtractive Hybridization process; it is not possible to estimate how many genes were examined.

Genes shown in previous studies to respond when the late blight pathogen attacks the foliage (Table 2) were matched to the NCBI potato UniGene database. Each of these genes could be matched to a UniGene with identity >90% (average is 97%). After removing redundancy, we found that defense response genes from the foliage belong to 1,003 UniGenes. These 1,003 UniGenes therefore form a dataset for comparisons with tuber data generated in our project.

**Hypothesis 1: Different organs of potato (e.g., foliage vs. tubers) use the same or similar defense mechanisms against the late blight pathogen, *P. infestans*.**

To enable transcriptome comparison between potato foliage and tubers, we examined expression dynamics of the 1,003 foliage UniGenes in our tuber samples to see if the same set of genes are differentially regulated in the tubers when *P. infestans* attacks. We summarize the results in Table 3. Our results show that 668 of the 1,003 (67%) genes that are responsive in potato foliage to pathogen attack are also responsive in the potato tubers.

**Hypothesis 2: Differences in disease phenotypes in different organs within the same genotype are due to differences in timing of defense initiation.**

Our previous research revealed that foliar and tuber responses to *P. infestans* differed in ‘Russet Burbank’ and its derived lines SP2105 and SP2211. Specifically, ‘Russet Burbank’ is susceptible to late blight disease in both the foliage and tuber; SP2105 is resistant to late blight in the foliage, but susceptible in the tuber; and SP2211 is resistant to late blight in both the foliage and tuber. Our previous research also revealed that these lines differ in the number of copies of the *RB* late resistance gene they carry: ‘Russet Burbank’ (0 *RB* copies), SP2105 (3 *RB* copies), and SP2211 (15 *RB* copies). Using these lines, in this experiment we tested if defense initiation in the tubers is faster

in potato line with higher numbers of copies of the *RB* gene (Gao and Bradeen 2010) using two approaches.

**Table 3.** Potato genes differentially expressed (DE) during infection by *P. infestans* of the tuber and foliage

	Genes Differentially Expressed in Foliage	Genes NOT Differentially Expressed in Foliage	
Genes Differentially Expressed in Tuber	668	10,191	10,859 <i>P. infestans</i> -responsive Tuber UniGenes
Genes NOT Differentially Expressed in Tuber	335	7,379	7,714
	1,003 <i>P. infestans</i> -responsive Foliar UniGenes	17,570	Fisher's exact test for count data p-value <0.001

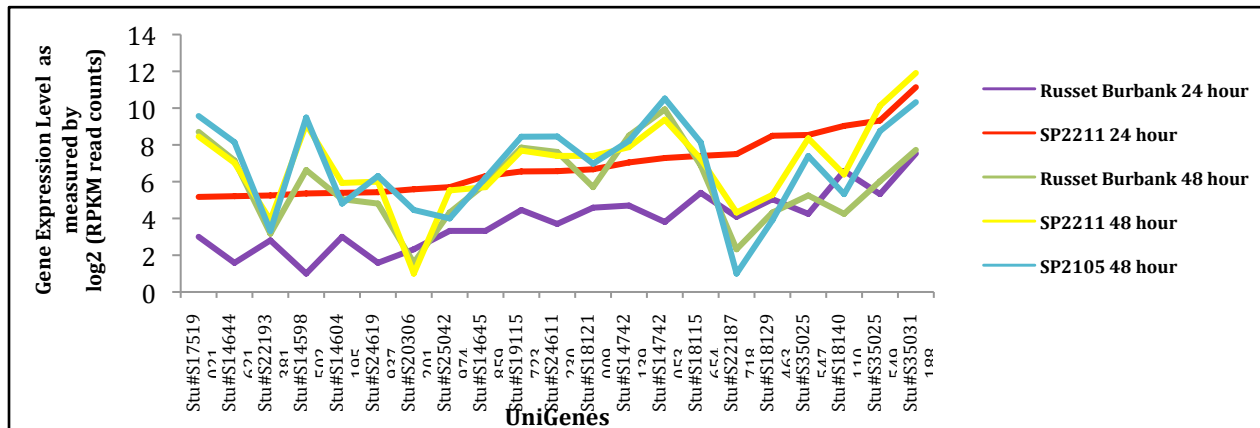
First, we employed quantitative RT-PCR to measure transcript levels of defense-related genes discovered in previous *foliar* based studies. Here, transcript levels were measured in tubers at several time points post-inoculation with *P. infestans*. Our raw data do not support our initial hypothesis; we observed no systematic bias in resistant tuber for earlier activation of the target genes. An alternative possibility, that the genes targeted in this survey (genes known to respond with *P. infestans* attacks *foliage*) may not be involved in defense of the tuber, warrants further investigation.

Second, we employed next-generation sequencing to examine genes differentially activated in the tubers of 'Russet Burbank', SP2105, and SP2211. We found 1,348 UniGenes that have a 2-fold higher expression in SP2211 than in 'Russet Burbank'. Interestingly, of these 1,348 UniGenes, only 92 are shared with those foliage responsive genes described in Table 2. This indicates that foliage and tuber might use quite different mechanisms to defense against pathogen attack and may offer an explanation for our quantitative RT-PCR results. Further studies are on-going.

**Hypothesis 3: High resistance gene transcript levels lead to earlier initiation of defense mechanisms in all plant organs.**

Previously, we demonstrated that the number of copies of *RB* in a potato line correlates with resistance gene transcript levels: 'Russet Burbank' lack *RB* and show no transcript; SP2105 has three *RB* gene copies and has moderate *RB* transcript levels; SP2211 has 15 *RB* gene copies and has high transcript levels (Bradeen et al. 2009b). This correlation holds true in both potato foliage and tuber.

We examined our next-generation sequencing dataset to determine whether high transcript levels (line SP2211) result in more rapid initiation of defense-related genes. As Figure 3 shows, we found at 24 hour post inoculation that there are >1,000 genes whose expression level in SP2211 (high *RB* copy line) is significantly higher than in ‘Russet Burbank’ (no *RB*). At 48 hours, the gene expression level among the three potato lines tend to become more similar. However, in lines containing *RB* (SP2211 and SP2105) gene induction tends to be more dramatic than in ‘Russet Burbank’ (Figure 3).



**Figure 3.** Comparison of the expression levels of 20 selected UniGenes based on Illumina Solexa sequencing results. Note that transcription of all genes at 24 hours post pathogen inoculation is higher in the high *RB* copy number line SP2211 (red line) than in ‘Russet Burbank’ (purple line), which lacks *RB*.

### Discussion:

Our project greatly exceeded expectations. Modifying our experimental approaches to capitalize upon emerging technology enabled us to generate >1,300-fold more data than originally predicted. Specifically, switching from 454 pyrosequencing to Solexa Illumina RNA sequencing technology yielded 215 million sequence reads compared to an anticipated 160,000 454 pyrosequencing reads. Our study ranks among the first potato-*P. infestans* transcriptome studies and among the first plant-pathogen interaction studies to employ Solexa Illumina technology. Our results should yield a peer-reviewed article in the near future and will serve as a roadmap for the scientific community for the application of next generation technologies to study plant-microbe interactions.

Our project revealed a large set of potato genes involved in *RB*-mediated tuber defense against the late blight pathogen. Our data suggest that the induction of pathogen-responsive genes is generally more robust in +*RB* lines SP2211 and SP2105 than in ‘Russet Burbank’, which lacks *RB* (Figure 3). Specific defense-related genes were up-regulated in our samples >10-fold in SP2211 than in ‘Russet Burbank’. These genes are candidates for further study. Our molecular analyses in combination with phenotypic assays indicate that the foliar late blight resistance gene *RB* can function in the tuber to impart tuber blight resistance, under specific conditions. Our novel approaches and

results deepen our understanding of how plants modulate defense responses in different organs (i.e. foliage vs. tubers) and have broad implications for the basic and applied plant sciences.

In addition to plant genes identified in our sequencing effort, our project generated a large data set of *P. infestans* genes up- or down-regulated when this pathogen interacts with the potato tuber. These genes are candidates for future study and may result in the development of novel disease management strategies. These same genes will be useful as molecular markers to estimate progressive disease development in future potato-*P. infestans* interaction research.

### **Conclusions:**

(1) The foliar late blight resistance gene *RB*, the most-promising genetic resource for reducing fungicide usage for potato production, can also be utilized to reduce incidence of tuber late blight disease. This finding has worldwide significance as *RB* is likely to be deployed on a large-scale. Most immediately, *RB* will be deployed in India, the third largest potato producing nation, beginning in 2012.

(2) Our study reveals basic strategies used by plants to defend against plant pathogens and suggests that defense mechanisms can be modulated in different plant tissues. This research has yielded large plant and pathogen datasets for downstream analyses.

(3) Our research is among the first to utilize next generation sequencing technologies to study plant-microbe interactions and, to our knowledge, is the very first to study the potato tuber-*P. infestans* interaction transcriptome. Our research provides a roadmap for other plant scientists. Importantly, the methodologies we tested and employed in this study can be capitalized upon to further refine our understanding of potato-*P. infestans* interactions, yielding strategies for resistance gene deployment in a no- or reduced-fungicide production setting.

### **Publications/Presentations:**

Bradeen, J.M. 2010. The genomics to potato tuber disease resistance. *Minnesota Area II Potato Research & Promotion Council 9<sup>th</sup> Annual Education Event*. Big Lake, MN

Gao, L., and J. M. Bradeen. 2010. Organ-specific disease resistance phenotypes in transgenic potato correlate with R gene dosage and transcription and defense response gene expression dynamics. *Plant and Animal Genome XVIII Conference*. San Diego, CA

### **Acknowledgements:**

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