Progress Report Cover Page

For

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Annual Progress Report
NSF Plant Genome Research Program

Overall project goals:

The overall goal of this project is to provide comprehensive structural and functional genomic resources to the scientific community for elucidation of molecular, genetic, and biochemical networks regulating potato plant growth, development, as well as responses to abiotic and biotic stress.

Specific project goals include:

Generation of a comprehensive genomic resource of Solanaceae \( R \) gene sequences (SOLAR) of wild potato, tomato and pepper genomes, and evolutionary models and bioinformatic tools for testing \( R \)-gene based strategies to identify resistance gene sequences for potential broad recognition capability and durability;

Generation of a functional genomic Solanaceae resource composed of a comprehensive set of expression profiles of significant points in growth, development and responses to abiotic and biotic stresses, and a set of functionally defined genes involved in responses to pathogens identified through RNA silencing;

An outreach and training program including \( Solanum \) gardens and associated public educational programs on plant genetic diversity, plant breeding and the impact of plant genome research on society. A training program for high school students to teach applications of bioinformatics and data mining for marker development for potato breeding for crop improvement.
A. PROJECT PROGRESS, GOALS AND ACHIEVEMENTS

Progress of each lab in terms of (1) overall goals and (2) specific goals and (3) progress for the reporting period

Barbara Baker
University of California, Berkeley

(1) Overall Goals

The work in the Baker lab is done in collaboration with all other labs in the project. The overall goals of the Baker lab are to (I) conduct structural and functional studies on Solanaceae $R$ gene regions to contribute to the (SOLAR) genomic resource and associated database; (II) conduct functional genomic studies and generate expression profiles of pathogen challenged Solanaceae and define the function of genes involved in responses to pathogens through RNA silencing to contribute to the functional genomics resource and associated databases; (III) conduct a training and education program for high school students in bioinformatics and genomics.

(2) Specific Goals

I. SOLAR Resource
   A. Assemble Solanaceae BAC contigs of disease resistance hotspots for sequencing
   B. Identify and prepare $R$ gene candidates (RGCs) for silencing and complementation analysis
   C. $R$ gene mining, identify independent populations segregating 1:1 for $P. infestans$ resistance and linked AFLP markers for each resistance trait

II. Functional Genomics
   A. Microarray analyses of pathogen-treated Solanaceae species
   B. VIGS silencing of candidate signaling pathway genes in pathogen resistance and defense.

III. Outreach
   A. Summer Genomics Workshop to train high school students
   B. Establish and augment community gardens.
(3) Progress

I. SOLAR Resource

A. Assemble Solanaceae resistance hotspot BAC contig for sequencing

Goals

We are analyzing the structure of three clusters of resistance genes in potato: the $R_1$ resistance gene cluster on chromosome 5, the major late blight resistance gene cluster located on the short arm of chromosome 11, and the $N/Rmc1$ locus on the long arm of chromosome 11. Many resistance genes, encoding both single-gene resistance and quantitative resistance, were previously mapped to these loci and several of these genes have been introgressed from *Solanum demissum*, a selfing allo-hexaploid. The goal of our project is to understand the structure, organization and evolution of these resistance gene clusters and to. Physical maps at these loci were constructed using genomic BAC clones of *S. demissum*. Selected BAC clones were sequenced and resistance gene candidate sequences and other sequences in the regions were analyzed. The data generated from this study will help to understand the role of the deduced structures on evolution of new resistance specificities and on the function of resistance genes and should facilitate cloning of additional resistance genes located at these loci.

*Progress on the Solanum Chromosome 5 R1-Nb Gene Cluster*

We have completed the structural analyses of chromosome 5 the $R_1$ cluster. The results were published in *Plant Journal* earlier this year:


*Progress on the Solanum Chromosome 11 Major Resistance Gene Cluster for Late Blight (MLB)*

The major $R$-gene cluster (MLB locus) on potato chromosome 11 harbors nine functionally distinct $R$-genes, all conferring resistance against *Phytophthora infestans*. We generated three physical maps, one for each MLB locus in the three homeologous genomes in hexaploid *Solanum demissum* (Figure 1). The MLB locus spans over 3 Mb. Approximately 800 kb were sequenced from the MLB locus.
Figure 1. Physical map of the Major Late Blight locus chromosome 11 of *S. demissum*. Physical Maps in A and B each correspond to ~2Mb of the centromeric the telomeric portions of the MLB locus respectively. The two maps are joined through marker 373T. Molecular marker locations are indicated above the thick line and those used for genetic mapping are indicated with marks that span the line. Markers in red are single copy at the MLB locus. Markers that were previously shown to be conserved in tomato and potato are in bold. Numbers below the thick line indicate the recombination events.
between markers among ~3,000 F1 progenies segregating for \textit{R3} resistance in potato (Huang et al., 2005). The names of BAC clones and their relative positions are shown in horizontal bars. BAC clones used for FISH experiments are framed in red (Figure 2). Fully sequenced clones are filled in pink and partially sequenced clones in yellow. The top three BAC contigs correspond to the MLB locus for each haplotype of \textit{S. demissum}. The non-continuous contigs shown in one panel are not necessarily from \textit{de facto} one haplotype. The small rectangles on the thin horizontal lines indicate the positions of \textit{R3a} homologues. The pink rectangles indicate homologues grouped or predicted to belong to clade 1 in Figure 4; red rectangles indicate homologues in clades 4-6; and blue rectangles indicate homologues in clades 3-4; framed rectangles indicate homologues sequenced and analyzed in this study. The last panel shows the MLB locus in tomato (Ori et al. 1997) and potato (Huang et al. 2005; Huang, S. unpublished data). The vertical dashed line separates the GP250 region from the GP185 region in \textit{S. demissum}.

Southern hybridization indicates that the MLB locus in \textit{S. demissum} contains more than 130 homologues of \textit{R3a}, a resistance gene from the MLB locus that was cloned previously and encodes coiled-coil, nucleotide binding site and leucine rich repeat (CC-NBS-LRR) domains. A total of 48 \textit{R3a} homologues (>2 kb) were sequenced. These \textit{R3a} homologues belong to six independently evolving groups (Figure 2).
Figure 2. Distance tree for all I2/R3a homologues from the MLB locus. The red dash line separates homologues in the GP250 region from those in the GP185 region. The circled numbers represent the six clades, which are supported by a bootstrap value of 100. All homologues from the GP250 region form clade 1. Homologues from the GP185 form five clades. Clades 1, 2 and 3 appear to represent three groups of fast evolving Type I genes, while clades 4, 5 and 6 represent three distinct groups of conserved Type II genes. The homologues from GP250 region in tomato are framed; homologues from S. demissum start with “Sd-”; homologues from potato R3a genotype start with “SH”; functional resistance genes I2 and R3a are shaded.

The majority of R3a homologues at the MLB locus are located in the proximal region of the loci and form a single clade in a distance tree. Frequent sequence exchanges between R-genes generated the chimeric structures found among the members of this group therefore show the evolutionary pattern of Type I R-genes. The distal region of MLB locus has three additional groups of Type I R3a homologues. Sequence exchanges occurred between homologues within each Type I group but not between homologues from different groups. Unlike the Type I homologues, two R3a homologues in the distal region of MLB locus have not undergone sequence exchanges with paralogues and are highly conserved across the three homeologous genomes in S. demissum. These two homologues therefore show an evolutionary pattern of Type II genes. Our results support the hypothesis that differentiation of Type II genes was caused by DNA sequences around the gene rather than gene function itself. Our study provides a useful reference for cloning other functional R-genes at the MLB locus in Solanaceae species. The large number of R-genes at the MLB locus also provides ideal resources for studies on R-gene evolution and gene shuffling. A manuscript summarizing the MLB locus has been prepared and is ready to submit for publication.

Progress at the N/Rmc1 locus

The N/Rmc1 locus is located on the short arm of chromosome 11. It was shown previously that NL27, an N gene homologous sequence maps near the Sen1 locus of potato (Hehl, et al. 2000) and is located in this region. NL27-related sequences were used as probes to screen the S. demissum BAC library. A total of 240 positive BAC clones were identified. These BAC clones were fingerprinted using High Throughput Fingerprinting method and proposed physical maps were verified using more than 100 PCR markers. The above analyses resulted in ~15 NL-27 contigs. Primers were designed for each contig, based on the BAC end sequences of clones assembled for each contig. These primers were genetically mapped using the segregating population of Rmc1. Two NL27 region candidate contigs, for two of the three homeologous genomes in S. demissum, were identified and shown to contain markers that cosegregate with nematode
resistance gene Rmc1 (Figure 3). Therefore, we have assembled two contigs for the N/Rmc1 locus. Three BAC clones were selected for low-pass sequencing.

Plant genomes contain large number of miniature inverted-repeat transposable elements (MITEs), however little is known about their role in gene function. We identified MITE families (MiS1-MiS22) from Solanaceae species through comparison of sequences at The R1 and MLB resistance gene loci (Table 1). We found that the alternative exon of resistance gene N of tobacco is provided by a MITE sequence, MiS1, which is indispensable for the full function of gene N. MiS1. A short coding sequence of hAT transposase is present in a MITE MiS2, providing further evidence for the hypothesis that MITEs were derived from autonomous transposons. A 355-bp insertion found in a R1 resistance gene homologue is composed of two copies of tandemly repeated MITE MiS5. MITE MiS5 elements appear individually or as a complex of two tandem repeats. The insertion of MiS5 may cause a 3-bp deletion at the target site. Members of the MiS5 family may have different sized target site duplications (TSDs) or no TSD.

Table 1 | Twenty-two MITE families from Solanaceae species

<table>
<thead>
<tr>
<th>MITE family</th>
<th>Superfamily</th>
<th>Size (bp)</th>
<th>TSD</th>
<th>TIR (size, in bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MiS1</td>
<td></td>
<td>~200</td>
<td>Unknown</td>
<td>6</td>
</tr>
<tr>
<td>MiS2</td>
<td>hAT-like</td>
<td>317-1404</td>
<td>8 bp</td>
<td>9</td>
</tr>
<tr>
<td>MiS3</td>
<td>hAT-like</td>
<td>165-177</td>
<td>8 bp</td>
<td>10</td>
</tr>
<tr>
<td>MiS4</td>
<td>hAT-like</td>
<td>798-972</td>
<td>8 bp</td>
<td>12</td>
</tr>
</tbody>
</table>

Figure 3.
Physical map of the Rmc1 locus of S. demissum.

MITEs in Solanaceae species
MiS5  ~355  None  (7)
MiS6  Mutator-like  326-935  9 bp, A/T rich  (150-250)
MiS7  Mutator-like  980-1200  9 bp, A/T rich  (84-127)
MiS8  Mutator-like  300-350  9 bp, A/T rich  (~150)
MiS9  Mutator-like  332-1320  9 bp, A/T rich  (100-180)
MiS10 Mutator-like  855-1111  9 bp, A/T rich  (260-410)
MiS11 Mutator-like  ~300  9 bp, A/T rich  (129-137)
MiS12 Stowaway-like  ~260  TA  (50)
MiS13 Stowaway-like  209-250  TA  (27)
MiS14 Stowaway-like  246-310  TA  (26)
MiS15 Stowaway-like  ~300  TA  (32)
MiS16 Stowaway-like  250-336  TA  (50)
MiS17 Stowaway-like  131-261  TA  (30)
MiS18 Tourist-like  436-1113  3 bp  (22)
MiS19 Tourist-like  190-240  3 bp  (13)
MiS20  235-508  12 bp  (10)
MiS21  717-797  10 bp  (340)
MiS22  1.6-3.4 kb  5-bp or none  TGTMAC  (6)

TSD represents target site duplication.
TIR represents terminal inverted repeat. Numbers in parenthesis refer to the length (bp) of TIR sequences

B. Identify and prepare R gene candidates (RGCs) for silencing and complementation analysis

Goals

The overall goal is to define the function of R gene candidates (RGCs) and to test for possible contributions of RGCs to disease resistance phenotypes in Solanum species using RNA silencing and complementation analyses. This year we continued work initiated last year on the Bs4-like RGC at the potato Nb-R1 locus. In collaboration with Maria Rosa we showed that the physical map of the R1 region overlapped with markers closely linked to the Nb locus for PVX resistance. The region of overlap contains a single R gene candidate very closely related to the Bs4 gene for Xanthomonas resistance in tomato. We also showed that the S. demissum Bs4 homologue, Sd-Bs4, is a slowly evolving Type II gene (highly conserved between species, with identifiable orthologs). Based on the overlapping physical and genetic maps in the Bs4-Nb regions and the identification of Sd-Bs4 as a highly structurally conserved Type II gene we hypothesized that the Bs4 homologue present in resistant Pentland Ivory S. tuberosum is a candidate Nb gene (St-Bs4 is equivalent to Nb). We used two approaches to test if the St-Bs4 sequence was the Nb gene: VIGS and a targeted candidate R gene approach. Our present
objectives are to clone the entire St-\textit{Bs4-like} gene and to test if it confers resistance to PVX strain Roth using complementation analysis.

\textit{Prior Results}

One Sd-\textit{Bs4} full-length- and one partial-length- TIR-NBS-LRR RGC were identified in \textit{S. demissum} BACs, 979H9 and 132D5, respectively. Mapping data suggested that these two BACs are located within the \textit{Nb} region. The nucleotide sequences of two of the RGCs are nearly identical to those of the tomato \textit{Bs4} gene, and are termed \textit{Sd_Bs4a} and \textit{c}, respectively. \textit{Sd_Bs4a} is a pseudogene due to a premature stop codon found within the TIR domain and the LRR domain and a frame shift within the NBS region (Figure 4).

\textbf{Figure 4.} Physical map of the \textit{S. demissum} Nb-R1 Chromosome 5 Disease Resistance Hotspot.

\textit{Progress on silencing the Bs4-like gene and homologues in potato}

A total of eight different VIGS TRV RNA2 constructs with St-\textit{Bs4} derived TIR, NBS, and LRR region sequences were constructed and transformed into Agrobacterium strain GV2260 for use in silencing of the candidate \textit{Nb} gene in potato.

To test the silencing system, the photogene desaturase (\textit{PDS}) gene was used as a visual marker of silencing. A pVOO-PDS silencing construct was used to test tuber-grown \textit{S. tuberosum} cv Pentland Ivory plantlets, along with \textit{N. benthamiana} seedlings as a positive control. The construct successfully triggered \textit{PDS} silencing in all six \textit{N. benthamiana} seedlings (Figure 4 Panels A and B, Figure 4.1), but failed to trigger silencing in the potato plants (Panels C and D, Figure 4.1). This failure is likely due to several characteristics of tuber-grown potato plants that are disadvantageous for silencing, namely the leaves are thick and contain many trichomes, which impede
infiltration. These characteristics are much less pronounced in potato plants grown from seeds, and thus seed-grown Pentland Ivory may be more conducive to PDS silencing. Tuber-grown resistant and susceptible Pentland Ivory lines were planted in the greenhouse for crossing so that seeds could be collected.

Based on our results using the VIGs silencing system in tuber grown Pentland Ivory and Katahdin F1 (segregating for Nb) true seed grown material, we chose instead to focus on cloning the entire Bs4-like from Pentland Ivory S. tuberosum using a targeted candidate R gene approach followed by complementation analyses to test if the St-Bs4-like gene is indeed the Nb gene.

Our collaborator Maria Rosa Marano previously mapped location of the Nb gene to a 0.76cM interval between CAPs markers GM339 and GM637 (Figure 4).

Figure 4.1. Silencing of PDS in N. benthamiana (panels A and B) and S. tuberosum Pentland Ivory (panel C and D).

Figure 4.2. Analysis of S. demissum, S. tuberosum cv Pentland Ivory and cv Katahdin plants with PCR-based markers GM339 and GM637 indicates that Katahdin may contain Nb gene.
In collaboration with Maria Rosa we developed two new CAPS based Sd-Bs4 sequences targeted to the TIR domain and LRR domains, respectively. After digestion with AluI or RsaI, the PCR products amplified with these primers showed polymorphism between resistant and susceptible Pentland Ivory plant lines (Figure 5).

Progress on co-expression and complementation analyses of the Bs4-like gene and homologues

Based on the initial studies showing possible co-segregation of the Bs4 markers and the Nb gene we designed new primers to amplify the full-length Bs4-like gene or its homologues (previously, we designed primers to amplify distinct regions of the gene or homologues). The cDNA from twelve S. tuberosum cv. Pentland Ivory plants were used as a template for PCR amplification. Three of the twelve Pentland Ivory plants (no. 4, no. 11, and no. 13) show a susceptible phenotype; the rest carry the Nb gene. The PCR products were cloned into pENTR1A for subsequent restriction enzyme digestion and sequencing. Based on the pattern of EcoRI and EcoRV digestion, clones were classified into eight groups. Two of the eight groups (Group1 gene and Group2 gene) encode a read-through open reading frame. The Group1 and Group2 gene sequences from Pentland Ivory have ~93% identity with the Bs4-like gene of S. demissum plants. The Group2 gene is present in both resistant plants and susceptible plants, while the Group1 gene is present in only resistant plants. These results suggest that the Group1 gene is the Nb gene. To confirm this, primers were designed to map the Group1 gene, by primers specific to the group 1 gene. Genomic DNA from 26 recombinant lines of S. tuberosum Pentland Ivory was used as template DNA. The PCR products could only be amplified from Nb positive recombinant lines (Figure 6). This result provides initial evidence that the Group1 gene is the Nb gene.

Figure 5. Polymorphism detected by Bs4 derived CAPS markers P5-P6 and P9-P10 between resistant and susceptible S. tuberosum cv. Pentland Ivory plants R, resistant; S, susceptible; Rp, R pool; Sp, S pool.
The *Group1* gene was expressed with potato mosaic virus X (PVX) strains or elicitors to support the hypothesis that the *Group1* gene is the *Nb* gene. In this assay, SR1 tobacco plants were infiltrated with a mixture of two *Agrobacterium* cultures, one expressing an elicitor (PVX-U1HB, PVX-GR106, the 25 kDa protein from PVX-ROTH1 [p25K1], the 25 kDa protein from PVX-UK3 [p25K3], or the 50 kDa protein derived from the TMV helicase protein) and the other expressing an *R* gene (*N*, *Group1* gene, or *Group2* gene) under transcriptional control of 35S promoter or the empty vector, pEarleyGate100 plasmid, as a control. PVX-U1HB and the 25 kDa protein from PVX-ROTH1 (p25K1) are able to induce *Nb*-mediated HR, but PVX-GR106 and p25K3 are not. We did not observe HR in the leaves infiltrated with elicitors and the pEarleyGate100 vector (Figure 6, 1-4). *N*-mediated HR in tobacco was only observed when *N* was co-expressed with p50 from TMV (Figure 5, 5-9). These results indicate that agro-infiltrated PVX-derived elicitors do not affect *N*-mediated HR in tobacco. HR lesions developed on leaves co-expressing the *Group1* gene and either PVX-U1HB or p25K1, but did not develop on leaves co-expressing the *Group1* gene and either PVX-GR106 or p25K3 (Figure 7, 10-13). The *Group2* gene, in contrast, did not induce HR regardless of the elicitor with which it was co-expressed (Figure 7, 14-17). These results provide strong evidence that the *Group1* gene is the *Nb* gene.

Figure 6. Analysis of genomic DNA from *S. tuberosum* Pentland Ivory with *Group1* specific primers.

Lane 1-4 and 12-22: resistant recombinant Pentland Ivory lines;
Lane 5-6 and 23-26: susceptible recombinant Pentland Ivory lines.
Figure 7. Agrobacterium-mediated co-expression of *Nb* candidate genes and different elicitors. *Agrobacterium* strains containing PVX-U1HB (1, 5, 10, 14), PVX-GR106 (2, 6, 11, 15), the 25 kDa protein of PVX-ROTH1 (3, 7, 12, 16), the 25 kDa protein of PVX-UK3 (4, 8, 13, 17), or the 50 kDa protein derived from the TMV helicase protein were infiltrated into leaves of *N. tabacum* with Agrobacterium strains containing pEarleyGate100 plasmid (1-4), *N* gene (5-9), Group1 gene (10-13), or Group2 gene (14-17).

The Group1 transcript encodes a predicted protein of 1139 amino acids with a molecular weight of 131 kDa. Among proteins with a known function, Group1 most closely resembles the TIR-NB-LRR proteins tomato B*4*, potato Y-1, and tobacco N (Figure 8).
The goals of the experiment are to identify N-pathway signaling genes leading to tomato mosaic virus (TMV) resistance and defense signaling events in Solanaceae through analysis of microarray expression profiles using the TIGR potato cDNA microarray.

**A. Functional Genomics**

**B. Microarray analyses of pathogen-treated Solanaceae**

1. Enhanced susceptibility
2. Identification of TMV-altered gene expression in resistant (N), susceptible (n), and enhanced susceptible sun1 mutant tomato

**Figure 8.** Group 1 is highly similar to the virus resistance proteins of Group 1, Group 2, tomato N, and tobacco N. Conserved amino acid residues are shaded and sequence gaps inserted by dashes.
During the previous reporting period, microarray gene expression profiles of TMV-challenged wild-type TMV-resistant tomato (N), wild-type TMV-susceptible (n), and enhanced susceptibility mutant, sun1-1, were completed at three time points (3, 9 and 27 hours post TMV challenge. 3 genotypes x 3 time points = 9 sets), augmenting the 1 hour time point generated the previous year. Last year’s report presented preliminary analysis of expression patterns at the 9 hour time point, including a list of the genes induced 1.75 fold or greater in wt resistant (N) tomato and 1.75 fold or greater in wt susceptible (n) tomato. Notably, a group of genes encoding 40s and 60s ribosomal proteins were found to show differential patterns of expression in TMV-challenged wt resistant (N) compared to wt susceptible (n) tomato.

During the present reporting period, expression patterns 27 hours post TMV challenge were analyzed using the three data points (3, 9 and 27 hours). From among the genes showing TMV-altered expression levels, seven were chosen for further analyses including functional assessment in TMV using RNAi. Four of these genes, a peroxidase, a cyclophilin, a bZIP transcription factor TGA2.1, and a glutathione S-transferase TSI-1, were identified to show greater than 1.5 fold induction in resistant (N) tomato but not in susceptible (n) or in the sun1-1 mutant tomato. Three of the genes, an abscisic acid and environmental stress-induced gene TAS14, ABP19, and a lipid transfer protein with homology to a PVR3-like gene, showed a different TMV-altered expression profile in the sun1-1 mutant tomato compared to resistant (N) and susceptible (n) tomato. Quantitative Real-time PCR confirmed the TMV-altered expression changes of the seven genes. Silencing TGA2.1, a member of a family of genes encoding TGA transcriptional activators shown to interact with SAR regulator NPR1, resulted in suppression of N function and spread of TMV-GFP in N. benthamiana (N). Additionally, TGA2.1 silenced plants were suppressed in the ability to elicit HR when Bs2 and avrBs2 were co-expressed, but the silenced plants were not suppressed in Prf/Pto-mediated cell death. Thus, TGA2.1 was determined to play a role in salicylic acid (SA)-mediated resistance pathways of N and Bs2, but not the Pto pathway.

**Progress**

*Selection of genes with reproducible differential gene expression after TMV-challenges.* Genes showing differential expression between TMV- and mock-treated tomato were identified by microarray analyses of Cy3 and Cy5-fluorescently-labeled probe pairs of TMV-challenged and mock-challenged tomato cDNA. Genes with 1.5 fold expression changes (Log2 ratio (Cy5/Cy3)>0.58) of the resistant (N), susceptible (n), and sun1-1 mutant tomato were then compared to identify (a) genes with TMV-altered gene expression in resistant (N) and sun1-1 tomato, but not susceptible (n) tomato (Table II), (b) genes uniquely induced or repressed in resistant (N) tomato (Table III), (c) genes with TMV-altered gene expression in all three genotypes (Table IV), and (d) genes with TMV-altered gene expression changes in only sun1-1 or absent in sun1-1 tomato (Table V).
Identification of genes induced by TMV in resistant (N) tomato. Comparing gene expression changes obtained from the microarrays, nine genes were induced by TMV in resistant (N) and sun1-1 but not in susceptible (n) tomato (Table II). Among the genes identified were the pathogenesis-related gene PR1b and glutathione S-transferase TSI-1, which showed induction at 9 hpi (Figure 9A and Figure 10A, respectively). Seventeen genes were identified to show greater than 1.5 fold gene expression changes in resistant (N), but not susceptible (n) or sun1-1 tomato (Table III). Among the genes identified were a peroxidase, a cyclophilin, and bZIP transcription factor TGA2.1. These genes showed clear induction at 9 hpi in resistant (N) but not susceptible (n) or sun1-1 tomato (Figure 10B-D). Table IV displays the seven genes with TMV-altered gene expression in all three genotypes. A class II chitinase, an unknown protein, an early inducible protein precursor, a pyruvate phosphate dikinase chloroplast precursor, a syringolide-induced protein and a NAC2-like protein showed induction in TMV challenged tomato. Ferredoxin-nitrite reductase was repressed in the TMV-challenged tomato. Nineteen genes showed induction in resistant (N) and susceptible (n) tomato and lacked significant TMV-altered gene expression change in sun1-1 mutant tomato, including an abscisic acid and environmental stress induced gene TAS14, a class II chitinase, and a lipid transfer protein (Table V). An auxin-binding protein ABP19 and a lipid transfer protein with homology to PVR3-like (Phaseolus vulgaris root expressed-like) gene were repressed in resistant (N) and susceptible (n) tomato (Figure 11B-C). The TMV-induced gene expression changes of several genes were assessed using quantitative Real-time PCR (Figure 12, 14).
A. Microarray gene expression of PR1b. The average Log$_2$ ratio (Cy5-labeled mock-treatment/Cy3-labeled TMV treatment) of PR1b in resistant (N), susceptible (n) and sun1-1 mutant tomato was determined for 3, 9, and 27 hours post-inoculation (hpi). TMV-challenged resistant (N) tomato had a Log$_2$ ratio of –1 at 9 hpi, which is equivalent to a two-fold induction of TMV-challenged samples compared to mock-treated samples. Standard error bars are shown for each data point.

B. PR1b gene expression assessed using quantitative Real-time PCR. TMV-altered PR1b gene expression of resistant (N), susceptible (n) and sun1-1 mutant tomato was determined for 3, 9, and 27 hpi. The y-axis shows fold-change difference in TMV-challenged tomato PR1b gene expression compared to mock-treated tomato PR1b gene expression, using ubiquitin as the
PR1b was induced three-fold in resistant (N) tomato at 9 hpi. Error bars represent the standard deviation of the technical replicates in one quantitative Real-time PCR experiment.

PR1b induced at 9 hours after TMV challenge in resistant (N) tomato. Few genes have been identified to serve as reliable markers for resistance signaling within the 27 hours of pathogen challenge. A number of induced pathogenesis-related genes were identified with 27 hours of TMV challenge, including class II chitinase (Table V), PR1b, a 1-, 3 glucanase, and a xyloglucan endotransferase (XET) (Table II). One clear example of a PR gene with early induction was PR1b isoform (Figure 9). Wt (N) tomato was clearly induced at 9 hpi of TMV, whereas susceptible (n) tomato was slightly repressed (Figure 9A). In the sun1-1 mutant, PR1b was induced to a lesser extent at 9 hours compared to resistant (N) tomato and remained induced at 27 hours in the microarray analysis (Figure 9A). Quantitative Real-time PCR confirmed a greater than 2.0 fold induction of PR1b in resistant (N) at 9 hpi (Figure 9B). The two-fold higher expression in mock-treated leaves compared to TMV-treated leaves of susceptible (n) tomato was confirmed at 27 hpi (Figure 9B). PR1b induction in TMV-challenged sun1-1 was not observed in quantitative RT-PCR experiments.
TSI-1, peroxidase, TGA2.1, and cyclophilin CYP1. Standard error bars are shown for each data point.

TMV-altered gene expression unique to resistant (N) tomato confirmed for four genes using quantitative Real-time PCR. Expression changes of four genes showing TMV-altered expression in resistant (N), but no significant changes in susceptible (n) and sun1-1 mutant tomato were assayed using quantitative Real-time PCR (Figure 10). The total RNA samples from Replicate 1 were used for quantitative Real-time PCR (Figure 12), and expression levels of TMV-challenged samples were compared to expression levels of mock-treated samples, using the ubiquitin gene as the reference. GST TSI-1 showed over two-fold induction in resistant (N) tomato at 9 hpi, and 1.6-fold induction at 27 hpi after TMV challenge, corresponding to the fold change observed in the microarray comparison (Figure 10A). Susceptible (n) tomato showed a 1.5 fold induction at 27 hpi in the quantitative Real-time PCR assay, which was not observed in the microarray analyses. Expression in sun1-1 did not change significantly at 3 hpi and 27 hpi, and a two-fold repression was observed at 9 hpi. A tomato peroxidase was confirmed to show greater than two-fold induction at 9 hpi, but no induction was observed at 3 hpi as observed in microarray comparisons (Figure 10B). However, a 1.8 fold induction of tomato peroxidase was observed at 27 hpi that was not detected in the microarray comparisons. Expression in susceptible (n) tomato did not change significantly, and sun1-1 showed an induction at 3 hpi and repression at 9 hpi and 27 hpi. Induction of bZIP transcription factor TGA2.1 in TMV-challenged resistant (N) tomato was not confirmed using Replicate 1 samples (Figure 10C), but quantitative RT-PCR of samples from a more extensive time course showed greater than two-fold induction of TGA2.1 in resistant (N) tomato at 6, 7 and 8hpi (Figure 11). Quantitative Real-time PCR confirmed induction of tomato cyclophilin, CYP1, after TMV-challenge in resistant (N) tomato (Figure 10D). However, the induction was observed at 27 hpi compared to 9 hpi observed in the microarray comparisons. Expression levels of cyclophilin in susceptible (n) tomato also were observed to increase at 27 hpi. Cyclophilin expression levels in sun1-1 did not change significantly.
Figure 11. Microarray gene expression patterns of TAS14, ABP19, and PVR3-like gene. The average Log₂ ratios (Cy5-labeled mock-treatment/Cy3-labeled TMV treatment) of the three genes in resistant (N), susceptible (n) and sun1-1 mutant tomato are shown for 3, 9, and 27 hours post-inoculation (hpi). A significantly different gene expression pattern was observed in sun1-1 compared to that of resistant (N) and susceptible (n) tomato after TMV-challenge. Standard error bars are shown for each data point. Asterisks (*) indicate that a standard error bar could not be calculated due to missing data.
Figure 12. Gene expression of TSI-1, peroxidase, TGA2.1, and cyclophilin assessed using quantitative Real-time PCR. TMV-altered gene expression of four genes was determined for 3, 9, and 27 hpi in resistant (N), susceptible (n) and sun1-1 mutant tomato. The y-axis shows fold-change difference in TMV-challenged tomato gene expression compared to mock-treated tomato gene expression, using ubiquitin and the reference. TSI-1, peroxidase, and cyclophilin were induced 1.5-fold or greater in resistant (N) tomato. TGA2.1 did not show significant induction in TMV-challenged resistant (N) tomato. Error bars represent the standard deviation in the technical replicates of one quantitative Real-time PCR experiment. The asterisks (**) indicate susceptible (n) samples from Replicate 2 were evaluated, instead of samples from Replicate 1.
Figure 13. Extensive time course of TGA2.1 gene expression was assessed using quantitative Real-time PCR. TMV-altered TGA2.1 gene expression of resistant (N), susceptible (n) and sun1-1 mutant tomato was assessed from 0 hpi to 96 hpi. The y-axis shows fold-change difference in TMV-challenged tomato PR1b gene expression compared to mock-treated tomato PR1b gene expression, using ubiquitin and the reference. TGA2.1 was induced over two-fold in TMV-challenged resistant (N) tomato at 6, 7, and 9 hpi. Error bars represent the standard deviation of the technical replicates in one quantitative Real-time PCR experiment.

Expression of genes with TMV-induced expression changes in resistant (N) and susceptible (n) but not sun1-1 tomato evaluated using quantitative Real-time PCR. Expression changes of three genes showing TMV-altered expression in resistant (N) and susceptible (n) but lacking expression changes in sun1-1 tomato were assayed using quantitative Real-time PCR. The total RNA samples from Replicate 1 were used for quantitative Real-time PCR, and expression levels of TMV-challenged samples were compared to expression levels of mock-treated samples, using the ubiquitin gene as the reference. Gene induction of TAS14, a dehydrin gene induced by abscisic acid and environmental stress, was confirmed at 3, 9, and 27 hpi after TMV-challenge in resistant (N) tomato (Figure 12A). Quantitative Real-time PCR also confirmed data from the microarray comparison showing induction of TAS14 gene expression at 9 hpi and no expression change in sun1-1. TAS14 induction in susceptible (n) tomato was not confirmed at 3 and 27 hpi. Repression of ABP19 gene expression at 3 and 9 hpi in resistant (N) tomato and induction of ABP19 in resistant (N) tomato at 27 hpi were observed in microarray analyses and by quantitative Real-time PCR (Figure 12B). ABP19 gene expression in mutant sun1-1 tomato was repressed at 3 and 9 hpi, but was highly repressed at 27 hpi. Gene expression of putative lipid transfer protein with homology to a PVR3-like gene was assessed using quantitative Real-time PCR. In the quantitative Real-time PCR assay, PVR3-like gene expression was induced greater than two-fold at 27 hpi in both resistant (N) and susceptible (n) tomato, whereas expression
was highly repressed in *sun1-1* (Figure 12C). The PVR3-like gene induction was observed at 9 hpi in TMV-challenged resistant (*N*) and susceptible (*n*) tomato in microarray comparisons, but the expression changes were not observed at 9 hpi using quantitative Real-time PCR. PVR3-like gene induction by TMV in susceptible (*n*) tomato at 3 hpi was observed in both microarray comparisons and quantitative Real-time PCR.

*Genes selected to test for involvement in N-mediated TMV resistance.* Genes identified in the microarray analyses of TMV-altered gene expression are candidate N-signaling components. A number of genes were selected to test for function in the N signaling pathway leading to TMV resistance, including TGA2.1, PVR3-like lipid transfer protein, and the tomato cyclophilin. The genes were selected on the basis of homology to known resistance signaling genes and their TMV-altered expression pattern. Functional analyses are underway for PVR3-like lipid transfer protein and tomato cyclophilin. Here, functional assessment of *TGA2.1* is presented.

*Silencing TGA2.1 suppresses N-mediated TMV resistance and Bs2/avrBs2-dependent HR, but notavrPto-dependent HR.* *TGA2.1*, a bZIP transcription factor that was found to interact with NPR1 to activate PR genes (reviewed in Durrant and Dong 2004), was silenced using a virus-induced gene silencing (VIGS) system to assess involvement in the N-mediated resistance to TMV. *N. benthamiana* expressing *N* show inhibition of TMV-GFP spread, and no virus can be detected in the upper uninoculated leaves (Jin et al. 2002). *N. benthamiana* (NN) plants silenced with pGr106::*N*, a PVX-based VIGS vector show TMV-GFP spread in the inoculated leaves and movement into the upper uninoculated leaves (Figure 13A, left). Silencing of an unrelated NB-LRR gene *Prf* does not alter TMV-GFP inhibition in *N. benthamiana* (NN) (Figure 13B, center). Silencing the *N. benthamiana* homolog of *TGA2.1* resulted in spread of TMV-GFP in local, inoculated leaves and into upper uninoculated leaves (Figure 13A, right). Efficiency of silencing and knockdown of expression levels of the genes targeted for silencing will be confirmed by quantitative PCR in studies underway. The experiment was repeated two to four times, totaling over 20 plants for each construct (Figure 13D), with 95% of the *TGA2.1*- plants showing TMV-GFP spread. Silencing of *TGA2.2*, the closest homolog to *TGA2.1*, did not suppress TMV-GFP resistance (data not shown).
Figure 14. The *N. benthamiana* homolog of *TGA2.1* was silenced using Tobacco rattle virus vector in *N. benthamiana::NN* plants.
A. TGA2.1-silenced N. benthamiana::NN plants showed TMV-GFP spread in local and systemic leaves. TMV-GFP spread locally in the inoculated leaves and long-distance into the upper leaves in both TGA2.1-silenced plants and the positive control N-silenced plants, but did not spread in N. benthamiana::NN plants silenced with Prf, a CC-NB-LRR involved in P. syringae resistance.

B. TGA2.1-silenced N. benthamiana::NN were inhibited in Bs2/avrBs2-mediated cell death. X. campestris resistance gene Bs2 and elicitor avrBs2 co-expression in N. benthamiana results in cell death (left). Expression of AvrBs2 alone did not elicit cell death (center). Bs2/avrBs2-mediated death was not observed in TGA2.1-silenced plants (right).

C. TGA2.1-silenced N. benthamiana plants are not inhibited in Pto/Prf-mediated cell death. Transient expression of an activated version of P. syringae resistance gene Pto results in cell death in the presence of Prf (left). Activated Pto-mediated cell death is inhibited in Prf-silenced N. benthamiana (center). In TGA2.1-silenced plants, and no disruption in Pto/Prf-mediated cell death was observed (right).

D. Summary of TGA2.1 silencing results in N. benthamiana::NN.

Since TGA2.1 is thought to act downstream of key SAR regulator NPR1, we reasoned it was likely to play a role in resistance mediated by other R genes. TGA2.1 silenced N. benthamiana were also tested for altered resistance mediated by other R genes. Bs2, an CC-NB-LRR pepper gene conferring resistance to Xanthomonas campestris, elicits cell death when transiently co-expressed with avrBs2 (Figure 13B, left), but not alone (center). The ability of Bs2 to elicit cell death (HR) in the presence of avrBs2 was inhibited in TGA2.1- plants (Figure 13B, right). The experiment was repeated numerous times; 9 of 9 TGA2.1- plants showed loss of Bs2/avrBs2-mediated HR.
Figure 15. Gene expression changes of TAS14, ABP19, and a PVR-like gene were assessed using quantitative Real-time PCR. TMV-altered gene expression levels of the three genes were determined for 3, 9, and 27 hpi in resistant (N), susceptible (n) and sun1-1 mutant tomato. The y-axis shows fold-change difference in TMV-challenged tomato gene expression compared to mock-treated tomato gene expression, using ubiquitin as the reference. TAS14, ABP19, and the PVR-like gene were induced 1.5-fold or greater in TMV-challenged resistant (N) and susceptible (n) tomato. These expression changes were not observed in the sun1-1 mutant. Error bars represent the standard deviation in the technical replicates of one quantitative Real-time PCR experiment. The asterisks (**) indicate susceptible (n) tomato samples from Replicate 2 were evaluated, instead of samples from Replicate 1.

Prf, an NB-LRR gene, and Pto, encoding a serine/threonine kinase, are necessary to recognize avrPto and trigger defense against Pseudomonas syringae (P. syringae) in Solanaceae (Ekengren et al. 2003). To test whether silencing TGA2.1 suppresses Prf/Pto-mediated HR, a form of Pto that constitutively activates HR and defense was expressed in TGA2.1 silenced N. benthamiana. Silencing Prf results in suppression of the activated Pto-mediated cell death (Figure 13C, center). Cell death was observed when activated Pto was expressed in TGA2.1- plants (Figure 13C, right) and in N-silenced plants (13C, left). This experiment was repeated numerous times, with six to nine plants each (Figure 13D).
TGA2.1 is induced in resistant (N) tomato after TMV challenge and is required for N-mediated TMV resistance and Bs2-mediated HR, but not Pto-mediated HR. Thus, TGA2.1 is likely to play a critical role in N- and Bs2-mediated signaling. Members of the TGA family of bZIP transcription factors were identified to interact with NPR1, a critical regulator of SA-mediated resistance signaling (Fan and Dong 2002, Subramaniam et al. 2001). NPR1 has been shown to interact with TGA1 dimers after SA-mediated reduction of the cysteine residues, and the NPR1-TGA1 interaction results in enhanced DNA binding affinity and specific binding to SA-regulatory sequences (Despres et al. 2000, Subramaniam et al. 2001, Despres et al. 2003). TGA4 also contained the cysteine residue targets for SA-mediated redox. However the other Arabidopsis TGA genes did not. A dominant-negative TGA2 construct was shown to suppress DNA binding activity of numerous TGA family proteins in addition to TGA2, inhibit SA-induced expression of two GST genes with TGA binding sites, but enhance SA-induced expression of PR genes (Pontier et al. 2001). Characterization of tobacco (NN) lines in which TGA2.1 is stably silenced will provide more information about the role of TGA2.1 in R-gene signaling.

Microarray study provides ‘screen’ to identify candidate N pathway signaling components. To our knowledge, this is the first report of microarray gene expression analysis in virus-challenged Solanaceae, and one of the few microarray studies focusing on resistance signaling within the first 27 hours of pathogen challenge. Also, this is the first demonstration of using TIGR potato cDNA microarray to successfully identify unknown signaling components of a plant cellular response. Despite variability in timing and amplitude of expression changes across replicate experiments, the microarray analysis was a successful screen for the identification of candidate signaling components of the N-mediated TMV-resistance pathway. Identification of TGA2.1 induction after TMV led to further assessment of its function in N signaling. Silencing of other genes identified in this study, including as cyclophilin and PVR3-like gene, is underway and will likely identify other signaling components. Further characterization of these genes will provide greater insight to understand the mechanism of N-mediated resistance signaling.

Gene expression profiling of Sgt1-silenced N-transgenic N. benthamiana

Goals
SGT1 is an essential component of many R gene-mediated disease resistance pathways in plants and may play a role in ubiquitination of resistance regulating proteins via a specific SCF complex. Our goal is to determine the plant genes regulated by SGT1 in the absence of TMV challenge, which will elucidate role of SGT1 in other cellular processes, and in the presence TMV, which will help us understand the mechanism of SGT1 in TMV resistance signaling and shared resistance pathways. We reported our experimental design and the back ground of this project in our last report.

Background
SGT1 is an essential component for many R gene-mediated disease resistance pathways in plants. SGT1-silencing can clearly attenuate N gene-mediated tobacco mosaic virus
(TMV) resistance in *N. benthamiana* carrying functional *N* (Jin et al., 2002). SGT1 from barley and tobacco extracts was shown to interact with SCF ubiquitin ligases and SGT1 also associates with RAR1 and COP9 signalosome (Azevedo, et al., 2002, Liu, et al., 2002). Recent reports showed that SGT1 can interact with molecular chaperone HSP90, which associates with the TMV resistance protein *N* (Liu, et al., 2004). However, the precise molecular function of SGT1 remains unknown, although these data suggest that SGT1 might play a role in ubiquitination of resistance regulating proteins via a specific SCF complex.

We have completed preparation and quality control analyses of TMV challenged and unchallenged, Sgt silenced and control vector treated *N. benthamiana* RNA for microarray analyses. A summary of our analyses follows:

TMV-resistant BN3 *N. benthamiana* plants were grown in aseptic condition. Two fully opened leave from the four weeks old *N. benthamiana* plants were used for the Sgt1 gene silencing. The *N. benthamiana* plants were either infiltrated with Agrobacteria carrying the TRV-Sgt1 construct for silencing the Sgt1 gene, or infiltrated with Agrobacteria carrying the empty vector VOO as control. After six weeks, half of the plants from either the Sgt1 silenced plants or the VOO plants were further inoculated with TMV (2 fully opened youngest leaves) and the other half were not infected. Leaf tissue, used for preparation of the RNA samples, was collected at 3, 9, 27 and 51 hours after treatment. Five replicates were completed. All leaf tissues were ground and stored at –80C. Each RNA sample was prepared from the frozen leaf tissues collected from two different plants. Total RNA of every sample was isolated using Trizol method. The total RNA samples were treated with DNase and further purified by the Qiagen purification column. The quality and quantity of RNA samples were monitored by U.V spectrometer and electrophoresis. Only those RNA samples of high quality (OD 260/280 between 1.9-2.0) were used for further analysis.

Real-time quantitative PCR experiments were used to test the efficiency of the Sgt1 gene silencing. Northern blot hybridization analyses analysis was used to determine the expression of TMV over a time course of 51 hours. RNA was also checked on agarose gels (Figure 16).

We anticipate that some genes will be identified to be up or down regulated by the Sgt1 gene via VIGS. Comparison of the gene expression profiles between the BN3 *N. benthamiana* silenced with VOO (empty vector) and the BN3 *N. benthamiana* silenced with Sgt1 will enable us to identify genes specifically induced or repressed in absence of the TMV infection. These genes might lead to the elucidation of its role in auxin signaling. While the comparison of gene expression profiles between the BN3 *N. benthamiana* silenced with VOO (empty vector) and the BN3 *N. benthamiana* silenced with Sgt1 will enable us to identify plant genes specifically induced or repressed with the TMV infection.
Figure 16.
Examples of quality control checks for samples.

A. Quantitative RT-PCR analyses of Sgt RNA following empty vector treatment (Red) and Sgt silencing (Blue). B. Northern blot analyses of uninfected and TMV infected using TMV coat protein gene as a hybridization probe. C. RNA samples run on a 1% agarose gel stained with EtBr.
B. VIGS silencing of candidate signaling pathway genes in pathogen resistance and defense

Goals

We are investigating the role of genes identified in expression profiling in disease resistance pathways. We have constructed plasmids for silencing genes identified in microarray analyses. TRV-based VIGs and plasmids for stable silencing of the PVR3, CYP1, TGA2.2 and TGA2.1 genes have been produced. Transgenic N. benthamiana plants bearing a vector for stable silencing of PVR3 (a putative lipid transfer protein) have been produced. N. tabacum and N. benthamiana transgenic plants bearing vectors for stably silenced EDS1 have also been prepared and are being analyzed for attenuation of expression of genes targeted for silencing. The EDS1 silencing constructs are also being transformed into potato. All transgenic material will be publicly available and listed on our website under Resources as expression analyses and stock preparation are completed.

III. Outreach

A. Summer Genomics Workshops to train and educate four high school students/year
B. Establish and augment community gardens

See Section F. Education, Training and Outreach.
(1) Overall Goals

Improvement of "allelic mining" methods for isolating $RB$ orthologous (allelic) and homologous (related) sequences from Solanaceous taxa, characterization of allelic diversity at the $RB$ locus, and testing of evolutionary hypotheses for $RB$. As the project has progressed, two distinct phases have emerged:

(2) Specific Goals

**Phase One:** (Year 1, 2, 3) optimization of Long Range-PCR (LR-PCR) and characterization of $RB$ alleles within *Solanum bulbocastanum*.

*Objective 1:* Technical optimization of LR-PCR

*Objective 2:* LR-PCR characterization of $RB$ alleles in *S. bulbocastanum*

**Phase Two:** (Year 3, 4, 5) application of PCR and other technologies to genome regions associated with $RB$, diversity assessment of markers near and allelic sequences at $RB$ homologous loci, and generation of $RB$ sequence data from *Solanum* species (*S. bulbocastanum, S. polyadenium*, tomato, eggplant) for SOLAR database.

*Objective 1:* Haplotype determination in *S. bulbocastanum* via EcoTilling

*Objective 2:* $RB$ homolog characterization in *Solanum* spp. via DGGE

Significant progress was made during year 3 on phase one and phase two objectives.

(3) Progress

**I. Phase One: Optimization and application of LR-PCR for recovery $RB$ orthologs (true alleles). Year 1, 2, 3.**

**Rationale:**

LR-PCR allows the amplification of fragments of up to 40kb from genomic DNA. $RB$ is an NBS-LRR gene and occurs in a cluster of related sequences of no known function. Previously we demonstrated that LR-PCR can be used to isolate intact, functional $RB$ alleles (Song et al. 2003b). In fact, the only functional $RB$ transgene associated with the NSF Potato Genome Project was isolated using LR-PCR. The transgene was amplified using CsCl-purified genomic DNA as template, primers that yielded multiple fragments in addition to the target sequence, and a user-modified amplification protocol. The approach worked well enough to allow isolation of the
A functional allele from the donor genotype PT29 but was poorly suited for multi-genotype applications.

In this phase of our research, we seek to improve upon the LR-PCR method for R gene recovery, adapting it specifically for multi-genotype applications. This allows (a) generation of sequence information that is useful in understanding how the RB locus has evolved and how it remains functional even in the Toluca Valley of Mexico, genotypic center of diversity for the late blight pathogen; and (b) isolation of intact alleles with agricultural potential. Since, like RB, more than 75% of all R genes cloned to date are of the NBS-LRR type and the majority occurs in clusters of related sequences, our modification and demonstration of LR-PCR for R gene ortholog recovery might be of widespread utility.

Objectives:
1. Technical optimization of LR-PCR for multi-genotype allelic (ortholog) mining at R gene loci: RB as a model.
2. Application of improved LR-PCR methods for characterization of RB alleles in S. bulbocastanum: testing of evolutionary hypotheses

Progress To Date:
1. Technical optimization of LR-PCR for multi-genotype allelic mining. We have completed technical optimization of LR-PCR including evaluation of (a) genomic DNA extraction methods, (b) improved LR-PCR primers for isolation of RB orthologs, (c) commercial amplification systems and protocols, and (d) cloning strategies for LR-PCR products. We have demonstrated that a consensus sequence built from three independently-generated LR-PCR clones can provide 100% sequence accuracy relative to direct cloned methods. Our findings are expected to be of widespread use for the recovery of R gene orthologs from clusters of related gene copies. Aspects of this research have been presented at the American Phytopathological Society Meeting (August 2004; Anaheim, CA) and the Solanaceae Genome Workshop (September 2005; Ischia, Italy). This work was published in the MS thesis of Maria Sanchez (UM MS, 2005) and a manuscript is currently in press in the journal Molecular Breeding (Sanchez and Bradeen in press).

2. Application of LR-PCR for multi-genotype isolation of RB alleles. Using the improved LR-PCR conditions, we have characterized RB allelic diversity in a reference collection of 44 S. bulbocastanum genotypes originating from 12 populations. The reference collection was constructed such that each of three morphologically-defined subspecies and the entire geographical distribution of the species (central Mexico through Guatemala) were represented. Each of the 12 populations included in the reference collection is represented by three or more genotypes. Thus, our approach allows us to examine how RB allelic diversity is partitioned within the species and evaluate the predictive power of subspecies classification, geographic origin, and within vs. between population relationships for observed RB allelic diversity.

In total, RB fragments were generated for 17 of 44 (~40%) genotypes in our reference collection (Fig 1). All reactions were replicated a minimum of two times and each genotype was evaluated with two independent LR-PCR primer pairs. LR-PCR
fragments were gel-purified and used as template for amplification the LRR and intron regions. The resulting LRR and intron fragments were sequenced. These regions were selected because (a) the LRR can differentiate between the RB locus (21 LRR repeats) and the RB paralogs (22 LRR repeats), (b) in other R gene systems, the LRR has been implicated in determination of pathogen specificity and observed variation in nucleotide sequence in recovered RB alleles might indicate altered in planta function, and (c) the
Figure 1: Allelic mining LR-PCR results. A sample of LR-PCR results for five *S. bulbocastanum* genotypes is shown. Primer pairs Ma3 (P1) and LRP2’(P2) amplification varies depending on the genotype. Both primer pairs amplified *RB*-fragments for genotype genblb42 and gendph6. For genotypes gendph22 and gendph8, only one primer pair, either Ma3 or LRP2’, produce a LR-PCR fragment. Neither primer pair generated a fragment for genotype genblb12. Fragments of larger than the expected size were obtained for some genotypes, e.g. gendph6, while most produced the expected fragment ~10,592bp-Ma3, e.g. gendph22, or the 8,439bp LRP2’ fragment, e.g. gendph8.
intron is phylogenetically informative, allow differentiation of *RB* alleles (Vossen et al. 2003). We have completed sequencing and analysis of 18 LRR and 18 intron fragments originating from eight genotypes. [Note that *S. bulbocastanum* is a diploid, so a maximum of two alleles per locus is expected. Consistently, our LR-PCR primers allowed recovery of one or two (never more) different alleles from several genotypes. Here we report results from eight different genotypes, so a maximum of 16 different *RB* alleles is expected. However, we used two different LR-PCR primer pairs in this survey. Some of the 18 sequences characterized originated from the same allele amplified with two LR-PCR primer pairs. Our results should not be interpreted as 18 independent *RB* alleles.]

Comparison of LR-PCR fragment end sequences (not shown) and LRR sequences (Fig 2A) with those previously reported for the *RB* locus and paralogous loci *RGA1*, *RGA3*, and *RGA 4* indicated that our LR-PCR method enabled recovery of *RB* orthologs, as expected, and not *RB* paralogs. Thus, LR-PCR can be used for multi-genotype R gene allelic mining. We noted surprisingly little variation in the LRR region of recovered alleles, with all recovered sequences being identical to previously reported *RB* (functional) or *rb* (non-functional) alleles (Fig 2A). Our LR-PCR approach to ortholog recovery may have biased our sampling toward alleles that look like *RB* or *rb*, but the use of two independent LR-PCR primer pairs should have helped to improve our sampling efficiency. This will be further tested as we continue efforts to characterize LRR sequence for the nine genotypes from which LR-PCR fragments were recovered but for which no LRR sequence has been analyzed to date. Nevertheless, our results so far indicate that previously reported *RB* and *rb* alleles occur at high frequency in natural populations. This observation suggests that selection for function may be constraining allelic diversification at the *RB* locus and *RB* alleles may play an important role in the survival of *S. bulbocastanum*. [Note: in interpreting these results, it is important to understand that the *rb* allele characterized to date has a premature stop codon upstream of the LRR. So, while there are nucleotide differences in the LRR that distinguish *RB* and *rb* alleles, the functional significance of *rb* LRR polymorphisms has not been evaluated. The high occurrence of *rb*-like LRR sequences in our samples, therefore, may not indicate a prevalence of non-functional alleles. In fact, it might suggest that the *rb* LRR is functional; this hypothesis can be tested using materials generated in this study.]

Comparison of intron sequences provides different results (Fig 2B). While most of the recovered *RB* alleles possess introns identical or highly similar to those of *RB* and *rb* alleles, intron sequences from two genotypes are more closely related to those of *RGA1*. Both of these genotypes yielded LRR sequence identical to *RB* or *rb* (Fig 2A). We have repeated the LR-PCRs at least six times for each genotype and results are the same each time. The two genotypes originated from the same population and thus share a recent evolutionary history. Our results suggest it is highly likely that a rearrangement event involving *RGA1* and the *RB* locus occurred in an ancestor of the two genotypes. Previously, we noted instability in *RB* (functional allele) BAC clones due to an *in vitro* recombination event involving *RGA1* and the *RB* locus (Song et al. 2003a). Our results reported here suggest that *in planta* inter-locus recombination might be important in the evolutionary history of the *RB* cluster, despite previous predictions of cluster stability (Song et al. 2003b). We have generated three independent LR-PCR fragments from both genotypes possessing the recombined alleles. These fragments are being sequenced to 4x
Figure 2: Neighbor Joining analysis for (A) LRR and (B) intron. Phylograms built using sequences generated in this study from eight S. bulbocastanum genotypes (18 sequences designated Ma3 or LRP2') and, for reference, previously generated RB paralog sequences (RGA1, 3, 4), and RB locus alleles RB (resistance), Rpi-blb (resistance) and rb (susceptibility). Sequences generated in this study originate from intron or LRR PCRs using Ma3 and LRP2' LR-PCR fragments as template. Intron and LRR PCRs were directly sequenced or were cloned prior to sequencing. (A) All LRR Ma3 and LRP2' LRR sequences cluster with RB (black line) or rb (gray line) clades. None of our LRR sequences cluster to RGA1 (dotted square) or any other RB paralog. Analysis of the LRR region revealed Ma3 LR-PCR template contained two alleles for gendph6 (black arrows) and genblb41 (gray arrows), both showing RB and rb LRR sequences. (B) Most Ma3 and LRP2' intron sequences cluster with the RB (gray line) or rb (black line) clades. Intron sequences from genotypes gendph6 and gendph15 cluster with the RGA1 paralog (dotted line). Gendph6 [black arrows in (A) and (B)] and gendph15, which originate from the same S. bulbocastanum population, have intron sequences more similar to the RGA1 and LRR sequences highly similar to RB locus, consistent with a structural rearrangement of the RB region in these genotypes.
depth. We anticipate these efforts will reveal the exact nature of the recombination event that led to these chimeric alleles.

We have functionally tested five of the eight characterized S. bulbocastanum genotypes for late blight resistance. Tests were conducted in our late blight nursery (Rosemount, MN) during the summer of 2005. Each genotype was tested in triplicate. Whole plants were inoculated with a local isolate of P. infestans US8 under high humidity conditions. Appropriate controls were utilized. All S. bulbocastanum genotypes tested, including one genotype carrying the recombinant RB allele, were found to be late blight resistant. However, we cannot rule out the possibility that loci other than RB are responsible for the observed resistance.

Deliverables:
Results of this research have been reported at the Plant and Animal Genome Conference (San Diego, CA; Jan, 2004) and the Solanaceae Genome Workshop (September 2005; Ischia, Italy). This work was published in the MS thesis of Maria Sanchez (UM MS, 2005), a manuscript is currently in press in Molecular Breeding (Sanchez and Bradeen in press), and a manuscript is being written for submission to Molecular Plant-Microbe Interactions. Submission is anticipated by Jan 2006.

Future Directions:
Phase one is nearing successful completion. Currently, we are working to finish sequencing efforts related to the recombinant RB allele and to nine LR-PCR products (LRR and intron only), as described above. This will proceed through the fall of 2005, with planned submission of a manuscript by Jan 2006. This will mark the end of research efforts related to phase one.

II. Phase Two: RB homolog comparisons throughout the genus Solanum. Year 3, 4, 5.

Rationale:
The genus Solanum includes potato, tomato, and eggplant—species of economic importance. The genus also includes wild potato species, including S. bulbocastanum and S. polyadenium, that are sources of genes for potato improvement (Fig 3). Although cultivated potato is a tetraploid, several wild potato species are diploid, as are tomato and eggplant. Potato species and tomato are hosts of late blight; eggplant is not. Among the diploid hosts, some species are obligate outcrossing (e.g. S. bulbocastanum) and some are inbreeding (e.g. S. polyadenium, tomato). Characterization of RB sequence from a variety of Solanum species including tomato, eggplant, and S. polyadenium, would, along with allelic mining efforts in S. bulbocastanum, provide a view of the divergence of this gene over phylogenetic distance, providing opportunity to explore allelic evolution in host vs. non-host species and in self-pollinating vs. cross-pollinating species.

LR-PCR is a useful method for the isolation of R gene orthologs, as demonstrated above, but is heavily dependent upon the availability of sequence information from regions flanking genes of interest. Our results suggest that sequences outside the coding region, and particularly sequences 3’ to the RB locus, might not be well conserved beyond species boundaries, despite previous predictions (Song et al. 2003b). This means
Figure 3: *Solanum polyadenium*—a source of genes for improvement of cultivated potato. Two *Solanum* spp. are shown growing in Grand Rapids, MN in soil infested with *Verticillium dahliae* and *Pratylenchus penetrans* (root lesion nematode), causal agents of Early Dying Syndrome. On the left, *S. schenkii*, a susceptible species, has been destroyed by disease. On the right, *S. polyadenium*, remains uninfected. Unlike its close relative, *S. bulbocastanum*, *S. polyadenium* is a self-pollinated species. In the proposed study, *RB* allelic diversity will be evaluated in *S. polyadenium* populations and other *Solanum* spp. using DGGE.
that LR-PCR primers developed for *S. bulbocastanum* may be ineffective in other *Solanum* species. BAC resources exist for eggplant, tomato, and the hexaploid potato *S. demissum*. Identification and sequencing of RB BAC clones from each species would provide information about overall RB gene cluster composition and organization and enable development of taxa-specific LR-PCR primers for ortholog recovery. However, we have been unsuccessful in procuring the needed funding for additional BAC sequencing. Instead, we seek alternative, cheaper strategies for recovery of meaningful information over phylogenetic distance. We are currently in the process of adapting and optimizing two techniques for use in R gene characterization.

The first method is EcoTilling, a method of SNP discovery that is well adapted to the characterization of single copy genome regions, including markers closely associated with R genes. EcoTilling was developed as a means of SNP discovery in self-pollinated species (Comai et al. 2004). The method requires establishment of a “reference genotype” against which other genotypes are compared. PCR fragments generated from the reference genotype and experimental genotypes are mixed, heated, and cooled, allowing DNA strands from the reference genotype to anneal with DNA strands from experimental genotypes. Any SNPs present between the annealed strands result in a local conformational change or heteroduplex, which is subsequently recognized and cleaved by a heteroduplex-specific endonuclease. The resulting products are run on an agarose gel, yielding a DNA fingerprint for each genotype relative to the reference genotype, with the relative position of SNPs in the amplicon being revealed by the size of the cleavage products. DNA fingerprints can then be used to screen multiple genotypes and classify alleles into similarity groups. Examples of each allele similarity group are then sequenced. Thus, EcoTilling can be combined with targeted sequencing to allow resource-efficient characterization of multiple genotypes. This method works well for self-pollinating species since no polymorphism is expected between the homologs of a given individual, allowing use of diploid genotypes as a reference, but adaptation is needed before the method will be of use to outcrossing, highly heterozygous species.

The second method that might be useful in the characterization of R gene sequences is denaturing gradient gel electrophoresis (DGGE), a DNA fingerprinting technique that is well suited for characterization of populations of sequences. In DGGE, PCR products are separated on polyacrylamide gels based primarily upon GC content. The method has been widely used to examine microbial diversity in soils, something our lab is currently doing. Briefly, a phylogenetically informative region (e.g., the bacterial 16S rDNA) is PCR amplified from DNA extracted from an environmental sample. For DGGE, one PCR primer is modified to include a 5’ GC rich tail or “GC clamp” of approximately 40bp. Next, the PCRs are run on a polyacrylamide gel containing an increasing gradient of DNA denaturant (e.g., urea, formamide). As they migrate, the PCR fragments begin to partially denature with GC poor species denaturing at lower denaturant concentrations (i.e. higher on the gel) and GC rich species denaturing at higher denaturant concentrations (i.e. lower on the gel). The resulting conformational change precludes further migration of the amplicons. Complete disassociation of the two stands of each amplicon is prevented by the GC clamp, which acts as a “paper clip” to hold the strands together. What results is a fingerprint of bacterial diversity for the environmental sample in which each band corresponds to one (or more) bacterial taxa. Individual fragments can be cut from the DGGE gel, reamplified, and sequenced.
We are interested in adapting DGGE for characterization of R gene allelic diversity. RB, like most cloned R genes, occurs as a cluster of related sequences. Homologs of RB also exist in at least two additional genome locations. Following the advice of the Advisory Committee (April 2005 NSF Potato Genome Meeting, St. Paul, MN), informative regions of the RB gene (e.g. LRR) will be amplified from genomic DNA. This approach will yield fragments originating from the RB locus (orthologs), RB paralogs, and RB homologs located throughout the genome—a population of sequences analogous to 16S fragments recovered from complex environmental samples. DGGE will be used to fingerprint RB alleles for each genotype followed by targeted sequencing of specific fragments.

We believe EcoTilling and DGGE, paired with targeted sequencing, will provide the needed tools to efficiently characterize RB diversity throughout the genus Solanum. Furthermore, the methods and strategies we are developing will be of widespread utility for the characterization of other R genes.

Objectives:

Progress To Date:
1. Optimization of EcoTilling for RB haplotype determination in S. bulbocastanum.

We are adapting EcoTilling for use in highly heterozygous, outcrossing species using S. bulbocastanum as a model. Our approach is to (a) locate appropriate, single copy markers associated with RB, (b) establish a cloned “reference allele” for each marker, (c) utilize the cloned reference allele for SNP detection in our set of 42 S. bulbocastanum genotypes (see above), and (d) select specific markers for targeted sequencing. Paired with RB allelic sequence generated via LR-PCR, our EcoTilling efforts will allow us to explore haplotype diversification as a function of subspecies classification, geographic origin, and within vs. between population relationships.

Associated with the RB region is more than 350kb of publicly available BAC sequence. Previously, we mapped 15 unique BAC ends to the region (Bradeen et al. 2003). Each BAC was end sequenced. We used these BAC end sequences to develop 15 PCR primer pairs, each a known distance from the RB locus. Ten of the primer pairs yielded a single amplicon in PCRs using S. bulbocastanum genomic DNA as a template (Fig 4). We are currently using an S. bulbocastanum F1 population generated by Chuck Brown to determine whether each of these amplicons maps to a single genome location (based on segregation ratios). We anticipate at least six single copy markers associated with RB will be recovered. We have also tested the EcoTilling methodology, revealing considerable nucleotide variation between homologs of a single diploid S. bulbocastanum genotype (Fig 5), demonstrating the need for establishing cloned reference alleles.
Figure 4: Single copy markers for EcoTilling. For EcoTilling, we must first identify single copy markers. BAC end sequences are a source of markers closely associated with RB. Shown is an agarose gel demonstrating PCR amplicons using 15 primer pairs developed from RB BAC end sequences. Each reaction uses genomic DNA of *S. bulbocastanum* genotype PT29 as template. First lane (left) shows 100bp ladder. In this assay, PCR primer pairs yielding multiple fragments are assumed to map to multiple genome locations and are eliminated from further study. PCR primer pairs yielding single fragments are considered “putative single copy”, an assumption that is subsequently confirmed via segregation analysis. We anticipate identification of a minimum of six single-copy markers associated with *RB* for subsequent haplotype determination using our reference set of *S. bulbocastanum* genotypes.
Figure 5: EcoTilling near the RB locus. Shown are EcoTilling results for two RB BAC-derived markers. Markers were generated from S. bulbocastanum genotype PT29 and polymorphisms observed reflect heterogeneity between homologs. Lane 1: DNA ladder. Lane 2-3: marker results for BAC marker 217D11 showing untreated (lane 3) and endonuclease treated (lane 2) amplicon. Lane 4-5: marker results for BAC marker 186A13 showing untreated (lane 5) and endonuclease treated (lane 4) amplicon. Lane 6: control reaction. DNA fingerprints resulting from endonuclease treatment (lanes 2, 4) can be used to group alleles from different genotypes into similarity classes. Representatives of each allele class can then be sequenced, reducing overall sequencing efforts and costs. EcoTilling fingerprinting will allow analysis of multiple genotypes and, when paired with LR-PCR RB allelic data, evaluation of haplotype diversity in our reference set of S. bulbocastanum genotypes.

This project is in its infancy and we welcome input from the Advisory Committee. Our approach is to develop DGGE PCR primers from informative RB regions. Using available BAC sequences, primers capable of amplifying regions from the RB locus and from RB paralogs will be designed. This strategy was suggested by the Advisory Committee during the April, 2005 meeting in St. Paul, MN. Currently, we are focusing efforts on the LRR, a region known to differ between RB paralogs, and a region in which nucleotide changes may have functional significance. Other regions of interest will include the NBS and intron.

Once developed, primers will be used to DGGE characterize allelic diversity in *S. bulbocastanum* (using genotypes from the 44 genotype reference set), *S. polyadenium* (three genotypes from each of four populations), tomato (three genotypes from each of four populations), and eggplant (three genotypes from each of four populations). As discussed above, these taxa represent the whole of genus *Solanum*, enabling a genus-wide view of RB diversity. The sampling strategy will allow examination of within vs. between population variation as it relates to late blight host (*S. bulbocastanum, S. polyadenium, tomato*) vs. non-host (eggplant), and to outcrossing (*S. bulbocastanum*) vs. inbreeding (*S. polyadenium, tomato*) species. Based on DGGE fingerprints, RB fragments will be selected for targeted sequencing. All sequence information generated will be released to the SOLAR database. This approach will enable characterization of 40 or more genotypes without requiring extensive library construction and sequencing for each. The DGGE approach may be of widespread value to other laboratories interested in characterizing R gene allelic diversity.

**Deliverables:**
Technical aspects of our EcoTilling approach have been presented at the *European Association for Potato Research Triennial Conference* (Bilbao, Spain; July 2005) and the *Solanaceae Genome Workshop* (September 2005; Ischia, Italy).

**Future Directions:**
The EcoTilling and DGGE projects are just beginning (year 3). We welcome comment from the Advisory Committee.
Charles Brown
USDA-ARS and Washington State University, Prosser

(1) Overall Goals

Fine mapping and isolation of R-mc1 locus in wild potato combined with transformation and assessment of resistance gene candidates.

(2) Specific Goals

- Develop markers linked to $R_{mc1(blb)}$
- Screen nematode resistance
- Identify contig candidates from BAC library
- Development of bridging crosses with $S. fendleri$

(3) Progress

a) Develop markers closely linked to $R_{mc1(blb)}$: the gene conferring resistance to the Columbia root-knot nematode

The major goal of this project is to map and ultimately isolate the gene conferring resistance to the Columbia root-knot nematode ($Meloidogyne chitwoodii$). This gene, $R_{mc1(blb)}$, was derived from a clone of the Mexican wild species, $Solanum bulbocastanum$ (SB22), and was located on the distal end of Chromosome 11. To facilitate the map-based cloning efforts, we are developing a high resolution map of the $R_{mc1(blb)}$ region. Previously, two AFLP markers and two CAPS markers were localized close to the gene with at least 0.5 cM genetic distance. This year, significant progress toward fine mapping $R_{mc1(blb)}$ locus was made. In addition to the four markers mapped earlier, five new STS markers co-segregated with the $R_{mc1(blb)}$ locus were developed (Figure 1).

From our earlier marker development efforts, four closely linked markers were developed by screening 576 AFLP primer combinations and 43 other type’s available markers mapped on the upper arm of Chromosome XI. The closest marker was linked at 0.5 cM genetic distance in the mapping population generated from an intraspecific cross between SB22, and a susceptible clone (PT29). Two specific CAPS markers, M33 and M39, originally developed for fine mapping resistance to potato virus Y, were localized even farther with 2.4 cM and 3.8 cM distance, respectively. A rapid and efficient approach was needed for detecting more closely linked DNA markers and should expedite plant gene isolation by positional cloning and the construction of high-density genetic and physical maps of the $R_{mc1(blb)}$ region.

By researching the distal region of potato Chromosome 11, we found that a TIR type R-gene (N-like gene) gene was located in the same region with the target $R_{mc1(blb)}$ locus and mapped earlier without associated to any function yet. Degenerate oligonucleotide probes were designed on the basis of conserved regions of $Nl$ resistance genes, and subsequently used as probes to hybridize with resistance gene analogues (RGA) on the
BAC filter. By screening the BAC library made from another wild potato species *Solanum demissum*. There were some positive cloned identified. Later, the BAC-end sequences generated from these positive BACs were used to design new primers to be used to screen and map closely linked markers in SB22xPT29 mapping population with over 250 individuals. Seven markers representing 5 BAC clones were mapped and cosegregated with $R_{Mc1(bb)}$ locus. These markers were also screened in 18 different backcross breeding lines with over 180 progenies. There was still no any recombination among these new STS markers and $R_{Mc1(bb)}$ locus. Our results indicate that it is possible to use sequence homology from conserved motifs of known resistance genes to fish candidate BACs containing RGAs and developed markers from another wild potato species.

![Linkage maps of the Columbia root-knot nematode resistance gene, $R_{Mc1(bb)}$, locus on the distal end of the upper arm of potato chromosome 11.](image)

In the last year, we made significant advances in the development new markers to solve difficult problems in fine mapping. A saturated map of the $R_{Mc1(bb)}$ locus region facilitated a map-based cloning approach of the gene responsible for the resistance to the Columbia root-knot nematode. It makes possible for us to get one more step closer to the final goal. Furthermore, these new PCR-based markers have provided an efficient alternative for screening large breeding populations for the Columbia root-knot nematode resistance. In addition, the new markers have been used as probes to screen an available BAC library derived from PT29 to locate BACs in the $R_{Mc1(bb)}$ gene region. They can be used for construction of physical maps for *Solanum bulbocastanum*. This work will enable development of BAC contigs spanning the $R_{Mc1(bb)}$ region. Furthermore, we are
continuing to enlarge the mapping population by making new crosses of SB22 x PT29, and have approximately 150 new individuals growing in tissue culture at the present time. In addition, there are some breeding lines and mapping population developed from other species available for mapping purpose.

**Marker-assisted Selection for the Resistance to the Columbia root-knot nematode**

Marker-assisted selection (MAS) on the basis of DNA markers may be a very useful and valuable tool for not only mapping efforts, but also introgressing $R_{Mc1(blb)}$ into cultivated potato. Application of the newly developed molecular markers for marker-assisted selection of $R_{Mc1(blb)}$ will provide the existing potato breeding program with new tools useful for facilitating the development of potato cultivars with improved disease resistance to the Columbia root-knot nematode. From an economical point of view, a PCR test for $R_{Mc1(blb)}$ resistance seems more economical efficient as compared to a nematode resistance greenhouse test. Additional advantages of a molecular assay are speed, accuracy, and the ability to perform a selection in the seedling stage before over 2 months long resistance test will be performed. Results from the MAS experiment showed that the markers developed for fine mapping purpose are very effective in screening disease resistance in breeding lines.

The objective of this experiment was to test the usability of five STS markers (Figure 2a and 2b) co-segregated with the $R_{Mc1(blb)}$ on the breeding lines generated for introgressing $R_{Mc1(blb)}$ into cultivated potato. Eighteen breeding families (180 individuals) were used to identify marker segregation. All clones were tested with a three replication screening. Disease resistance was evaluated by inoculated with 5,000 eggs grown for 55 days and then the eggs are extracted, root systems weighed and the eggs counted. At the same time, DNA was extracted from the same plants grown in greenhouse. All five STS markers showed polymorphism between resistant and susceptible clones. Tight linked STS markers

![Figure 2a. Segregation of STS marker 39E18F in the Backcross 5 (BC 5) breeding lines derived from the cross between a backcross resistant parent and susceptible cultivated potato Individuals with $R_{Mc1(blb)}$ nematode resistance gene contain a band at ~400bp.](image-url)
demonstrated their practicability for marker assisted breeding by differentiating between resistant and susceptible clones of a set of diverse BC breeding lines. The genotyping results of 176 individuals (out of total 180 tested) matched with their resistant phenotypes. So we can conclude there was at least 98% accuracy for these five sets of STS markers developed for marker-assisted selection of root-knot nematode resistance ($R_{Mc1(blb)}$) in potato.

This study demonstrates the utility of available markers tightly linked to $R_{Mc1(blb)}$ used for MAS purpose for breeding project. The MAS for resistance genes in potato will speed up breeding processing. The cost-efficiency of the PCR markers applied for $R_{Mc1(blb)}$ renders this approach as an attractive alternative for screening large segregating populations of potato for root-knot nematode resistance.

b) Screening Nematode Resistance: IN PROGRESS

Up until recently we regarded the resistance trait to be a block of nematode reproduction in the root. This lack of reproduction on the roots was sufficient to protect the tubers because few or no nematodes were present to attack the tubers. Some puzzling results in field screening lead to a project where a Weed Scientist examined the possibility that weeds that are hosts for *M. chitwoodi* could overcome the resistance by providing abundant nematode juveniles from their own root systems that invaded the tubers. Initially it appeared that this was generally the case. Resistant clones were overcome by weed-sources of nematodes. However, we found that one resistant breeding clone not only resisted reproduction on the roots but also blocked invasion of the tubers by nematodes that had reproduced on Hairy Nightshade (*Solanum sarrachoides*). This particular clone had been used as the parent in the advanced backcross breeding program. We noted that some of the progeny in cross appeared to be recombinant phenotypes. Several progeny that had no tuber damage showed abundant reproduction on the roots. This was the first indication we have had that there may in fact be two components to resistance.
We have been assuming there is one gene responsible for the Columbia Root-knot nematode resistance in potatoes originated from SB22. However, in our experimental fields we found that portions of resistant clones were showing damage (Figure 5a and 5b). At the same time, the latest observation from PA99N82-4 breeding families also showed that there might be another separate resistance for tuber (Table 1). Based on these hypotheses, we propose a new experiment to find out whether there is another gene(s) responsible for tuber resistance. We are also very interested to learn how far the new gene is approximately from the root resistance gene, $R_{\text{Mc1(bl)}}$.

Preliminary results from the resistance screening of PA99N82-4 breeding families showed that there were at least 2 potential recombinants (out of 50 individuals) between the root resistance and tuber resistance without co-habitant host.

Table 1. Preliminary resistance screening of PA99N82-4 breeding families on root and tuber resistance to the Columbia root-knot nematode (*Meloidogyne chitwoodi*).

<table>
<thead>
<tr>
<th>Root resistant</th>
<th>Root susceptible</th>
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<tr>
<td>Tuber resistance</td>
<td>28</td>
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<td>Tuber susceptible</td>
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c) Screening BAC filters and constructing physical maps of the target $R_{Mc1(bb)}$ locus region

In the first part of the project, a set of positive BAC clones from the Solanum demissum BAC library were identified by hybridized to NI27 probe. Later, the BAC-end sequences generated from these positive BACs were used to design primers for the fine mapping. We also have screened an available BAC library derived from PT29, the susceptible parent of SB22xPT29 mapping population (Figure 3). The identified positive clones are in progress for BAC fingerprinting to construct a physical map of the target region for the susceptible haplotype.
Figure 3. The high-density filter of the PT29 BAC library hybridized with NI27(left) EAGGMCTA213(right) probes.

We have constructed several BAC contigs containing the potential candidate \( N \) homologous genes from the \textit{Solanum demissum} BAC library. The BAC contigs were further confirmed by PCR screening from the BAC libraries using the STS markers. Figure 4 showed contigs covered over 300 kb region spanning the \( R_{Mc1(bb)} \) locus. Three BACs from these contigs were sent for sequencing at TIGR.

The anchored BAC physical map will facilitate positional cloning and elucidate the organization/distribution of resistance genes. It will provide the foundation for eventual cloning; and shed light on the nature of \( N \) gene in potato and help establish a syntenic region though which can be compared between two wild species. The data from these two genomes is being integrated and verified mutually for an efficient construction of the physical map. Chromosome walking also will be performed to establish larger BAC contigs.
Figure 4. BAC contigs developed from *Soulum dismmisum* library. Five markers developed from above contigs were found to co-segregate with the resistance gene $R_{sk-1(bib)}$ (provided by Dr. Barbara Baker’s group).

**d) Development of mapping population derived from *S. fendleri***

We made progress on the achievement of another goal, the development of population that will permit us to map the resistance to *M. chitwoodi* derived from *S. fendleri*. This species is a tetraploid, but behaves like a diploid in its crossing behavior. The development of mapping population was a circuitous affair involving crossing with cultivated diploids to generate a triploid hybrid, somatic doubling to raise ploidy and restore fertility and two backcrosses to cultivated tetraploid parents to reach a population that would be actively assorting the *S. fendleri* chromosomes among the population progeny. We were able to make these crosses, and the mapping population seed has been sown and harvested multiple tubers. These tubers have been in the cold storage and are ready for resistance screening.

![Scheme of introgression and development of the *S. fendleri* source of *M. chitwoodi* resistance](image)

**Mapping population (developed in 2005, not yet screened)**
e) Outreach (see section F)

1. Plant Bioinformatics Workshop

We organized and carried out a workshop focusing on Plant Bioinformatics. It took place August 10-12, at the Tri-Cities Campus of Washington State University. It was funded by an Outreach Grant from the National Science Foundation. Hispanic and Native American participants were vigorously recruited with excellent results. Two of the participants were high school biology teachers and one these was a Native American. Scientists from Battelle Pacific Northwest National Lab, Richland, Washington, The Institute for Genomic Research, Rockville, Maryland, Washington State University, and Plant Gene Expression Center of USDA/ARS, Albany, California were invited speakers.

The participants were physically located in computer lab. Each student had his/her own computer with a connection to the internet. The instructors used powerpoints to conduct their lessons and the students were able to obtain copies of this. They were shown how to connect to NCBI and TIGR databases and to retrieve information and perform algorithms on the information.

Each participant extracted DNA from strawberries in the cafeteria. Each participant manually constructed a model of DNA which they took home. The two teachers were given DNA model materials to take back to their classrooms. Each participant worked on a continuous journal of their experience in the class in the form of a powerpoint presentation, which they presented to the class on the last afternoon. Each participant received a certificate of participation.

Linhai Zhang, a Postdoc, in Dr. Brown’s lab was an instructor in the course. Kaleen Lions, prepared the certificates and was present at the final hours of the class. Kaleen also helped with budget planning. Kirsten Cooper assisted with the travel arrangements for the instructors and participants that required lodging. Meghan Flanagan, Director of Outreach, Albany, CA was involved in all aspects of the course, managed the budget, planned several lectures and lab exercises, served as an instructor in lecture and lab, and personally drove one of the participants to a very remote home location at the end of the course.
Camella George, a member of the Yakama Nation, receives her certificate of participation at the end of the course.

Attached to the end of this description is the class participation list, course announcement and course schedule.
Participants in the 2005 Plant Bioinformatics Workshop, WSU-Tri-Cities, Richland, Washington

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Bioinformatics is a new term applied to the sciences involved in storing genetic information in databases and the analysis that applied to the information. Analysis involves determination of relationship of specific nucleotide and derived the protein sequences to other sequences using different criteria 1) sequence, 2) function, 3) mapping position in the genome, and 4) taxonomic association. We will be exploring the databases in the National Center for Biotechnology Information, and becoming familiar with the work done by The Institute of Genomic Research.

The study of biology has changed so much in recent years, that knowledge of genetic databases and some skills in extracting information have practically become a prerequisite. The beauty of using these databases is that nothing but a computer and online access is necessary to use them. They are perfect projects for biology classes, requiring nothing in terms of budgetary resources to supply the consumables. Also, the information contained in the database is real, representing, in the case of the human genome, extremely important information about the human metabolism and disease that is constantly changing as research goes on.

This workshop will be conducted from August 10-12, 2005 at the Tri-Cities Campus of Washington State University in Richland, Washington.

Although some of the participants will be drawn from teachers and students who live locally, others will come from distant locations. Per diem, lodging and travel costs, and a stipend will be provided to these travelers. Stipends will be supplied to all participants. Accommodations will be arranged at local facilities. The workshop participants will be instructed in the keeping of Powerpoint journals of the subject areas they study and their reactions to the learning that is taking place. The powerpoints will be a good way to store information that is available in a digital format, including text, diagrams, flowcharts, and pictures. A digital camera will be available to create new photographs of classroom activities that can be incorporated into the powerpoint journal. The journals will be presented at the end of the workshop to the class. Copies of the powerpoints will be retained and the participants may use them in future communication events including teaching.

Contact:

Chuck Brown
USDA/ARS
24106 N. Bunn Rd.
2. Heritage University Project

A. Progress – Charles Brown
Under the auspices of the NSF-USDA/ARS Potato Genome, we hired Gary Pierce, Yakama Native American to continue his study of marketing of high phytonutrient potatoes. This is the third year we have hired Gary. He worked very closely with two local farmers who were growing about 50 acres of specialty potatoes. The fact that these two farmers were growing potatoes was a direct result of the three-year effort that Heritage University, USDA/ARS and NSF has carried out to promote the idea of using genetic diversity of potato to develop a value-added product and market niche. Costco has been buying 5 pound bags of purple potatoes and promoting the idea of obtaining antioxidants through potato consumption.
Brochures developed by Gary Pierce and Rich Schell to provide information for consumers about nutritional advantages of colored potatoes.

From left to right, Rosalee Harris (Heritage University, Native American Recruitment Office), Gary Pierce (Heritage University, Business Major, Class of 2006), Meghan Flanagan, Director of Outreach, Rich Schell (Owner of Schell’s Produce, Toppenish, Washington)

Red, White and Blue potatoes in clamshell container ready for shipping.
3. Distribution of Native American Potatoes to Native American Groups

Dr. Brown multiplied several thousand virus-free cuttings of Ozette, To-Le Ak and Maria’s Potato and traveled to Neah Bay and Sequim to re-distribute these potatoes for peoples gardens. The Ozette potato was entered into the somewhat bizarrely named “Slow Food Hall of Fame” by Gerry Warren of Seattle. The purpose of this organization is to identify food items with extremely high quality and taste and take measures to ensure that they are available. Due to Dr. Warren’s and my interaction we were able to involve the Makah Nation in this action. At this point in time we are looking for the opportunity to sponsor a project with Wilson Arnold (A 2005 Bioinformatics Workshop Participant and Biology Teacher in Neah, who is also a Makah Nation member) to grow the Ozette potato for sale as a high school biology project. We believe that it might be possible to set up the High School Biology Class up with tissue culture equipment so that they can have a self-sufficient project that will produce cuttings and grow the potato in local gardens on an annual basis.

During September, Dr. Brown traveled to Alaska and made contact with Tlingit and Haida Native Alaskans. He met with people involved in preserving Maria’s Potato and obtained a possibly new Native Alaskan potato from a Haida garden in Kasaan (this Haida village is near Ketchikan). Dr. Brown gave a talk at Southeastern Alaska University in Juneau and distributed various copies of the poster that Dr. Linhai Zhang made showing the relationship of Native American and Native Alaskan potatoes to other cultivated potato groups in the world. Dr Brown met with an anthropologist, Dr. Wally Olsen, who has been translating the Spanish language logs of ships that explored Alaska in the last half of the eighteenth century. Also he met with a Tlingit and Russian language specialist, Richard Dauenhauer, who is translating original Russian documents in an effort to understand the history of Russian influence (including potato issues) during the tenure of the Russian American Company (1799-1867) in Alaska. Dr. Brown learned that the Russian fleet contracted the Haida to grow potato for them as a foodstuff in the early nineteenth. This commercial relationship lead to an extensive network of potato gardens throughout the archipelagoes of southeastern Alaska. The Research Leader of the USDA/ARS unit administered out of Fairbanks, Dr. Alberto Pantoja, indicated a strong interest in initiating an outreach program with Native Alaskans with potato as a focus.
Robin Buell  
The Institute for Genomic Research (TIGR)

(1) Overall Goals

A. Expression profiling of Solanaceae species during abiotic stress and creation of a Solanaceae Gene Expression Database  
B. Sequencing of BAC clones from R gene regions in the Solanaceae

(2) Specific Goals

A. Sequence and annotate Solanaceae BACs from project participants that span disease resistance loci  
B. Develop a Solanaceae dataset of R genes and R gene candidates (SOLAR)  
C. Reamplify the potato cDNA clone amplicons  
D. Perform gene expression profiling experiments focused on abiotic stress responses in the Solanaceae  
E. Develop, expand, and make publicly available a Solanaceae Gene Expression Database  
F. Provide to the community a Solanaceae Expression Profiling Service using a potato cDNA array  
G. Provide to the community, on a cost-recovery basis, the TIGR potato cDNA array  
H. Other progress

(3) Progress

Project web site: We have continued to update our project web pages (http://www.tigr.org/tdb/potato/) as needed. Since June 2004, we have had over 49,000 access hits to our project web pages.

Solanaceae BAC sequencing: TIGR has completed the sequencing of all BACs submitted by the Baker lab. Three BACs were received in late October and these will be sequenced in November. All sequence has been sent to the Baker lab. The sequence for all BACs is available in Genbank and our website. On our website, we added a graphical annotation browser for all the BACs (http://www.tigr.org/tigr-scripts/tdb/potato/BAC_annotation/bac_display.pl). Annotation for finished BACs is also in Genbank.

Update of the SOLAR database: We have updated the SOLAR database which represents all publicly available R-genes and R-gene candidates from any species within the Solanaceae family (SOLAR: http://www.tigr.org/tdb/potato/disease_db.shtml).

Reamplify the potato cDNA array amplicons: We have prepared a new version of the
potato 10K array (10K version 4). Version 4 contains 11,412 validated clones spotted in duplicate. A total of 1100 slides of our potato cDNA array version 4 were printed.

**Functional genomics of abiotic stress responses:** To analyze abiotic stress responses in Solanaceae species, we have performed a number of expression profiling experiments using the potato microarray slides.

We have completed and published (see below) a comparative abiotic stress experiment addressing heat, cold and salt stress in potato. Potato plants were grown in hydroponic medium and subjected to heat, cold and salt stress for 3, 9 and 27 hours after stress initiation. Three biological replicates were performed. Samples were isolated from the roots and leaves. All data from this experiment is publicly available through our expression profiling service as well as through the Gene Expression Omnibus (GEO) at NCBI.

To analyze abiotic stress responses among Solanaceae, we have applied drought, heat, salt and cold stress to seven representative Solanaceae species. The goal of these experiments is to perform a comparative analysis of gene expression among these seven species. Two biological replicates have been completed and the first replicate is publicly available. The expression data are currently being analyzed to identify similarities and dissimilarities in transcriptional responses to abiotic stress. The different solanaceous species are used as a source of natural variation to identify the most important abiotic stress response genes.

Natural variation in abiotic stress tolerance is further exploited using alternative potato germplasm. A broad range of wild species of potato is available from the USDA germplasm collection that has been characterized for heat stress and freezing tolerance. We selected accessions that were characterized either as heat sensitive or heat resistant. Using comparative genomic hybridizations, we first assessed the genetic variation among ten different wild potato accessions. The same ten wild potato species were subjected to heat stress and tissue samples were collected 3, 6 and 9 days after initiation of the heat stress and hybridized to the potato cDNA microarrays. The experiment was repeated two times. Distinct expression patterns were found for heat resistant and sensitive accessions. The expression data will be integrated with the genotyping data to identify genes that are involved in heat stress tolerance or sensitivity. The same potato accessions were used to address freezing tolerance based on their database descriptors. Potato leaves were subjected to freezing and samples were collected over a temperature range of 0 °C to -12 °C. In addition to the transcriptional responses during freezing using expression profiling, ion leakage from the leaves was measured to assess the freezing damage. We have performed three biological replicates and are currently in the process of RNA isolation and performing hybridizations. The expression data will be integrated with the physiological and genotyping data.

In addition to the abiotic stress expression profiling, we have started work on an expression encyclopedia of potato during the life-cycle addressing different developmental stages and tissues. This will provide insights in the expression changes
during development as well as provide important information about tissue specific gene expression. Currently, we have isolated tissue for four biological replicates and RNA isolation and microarray hybridizations are in progress.

**Expand the Solanaceae Gene Expression Database:** In our SGED, we have 894 publicly available hybridizations from 31 studies, another 4 studies with 90 hybridizations are available internally and will be released according to the data release policy that gives users of the expression profiling service 60 days before the release. We have revised our SGED browse, search, and query web pages. These are more robust to accommodate the growing database. These pages provide information on the experiments and allow the user to download files and data. In addition to data analysis tools, we have added graphic visualization tools for the hybridizations within SGED along with pre-computed analyses of some of the studies. We have also deposited the 10K array platform to the NCBI Gene Expression Omnibus (GEO) and we now offer as a service, deposit of array studies using our platform to the GEO database.

**Solanaceae Gene Expression Profiling Service:** We are providing to the community a free expression profiling service for 12 outside scientists per year, for a total of 55 scientists for the entire period of the grant. Each user can perform up to 30 hybridizations with a minimum of 2 biological replicates per study. We have completed 7 rounds of the application process and have a total of 40 users. All hybridizations and data analysis for expression profiling users of round 5 have been completed; round 6 users hybridizations are in progress. Proposals have been accepted for round 7.

We feel the first 5 rounds of the Expression Profiling Service have been very successful. User feedback has been positive and it clearly shows that the expression profiling service makes microarrays available to labs that otherwise would not have been able to do these experiments. One user of round 2 has recently published a manuscript in Plant Physiology and we have been contacted by three other users that are preparing manuscript submissions. This illustrates both the quality as well as the usefulness of the expression data generated through our service. A list of the studies with the outside users can be viewed at:

**Provide the potato cDNA microarray to the public:** We continue to make the potato cDNA array available to outside users on a cost recovery basis. Since January 2005, we have sent out 232 slides. We will continue to provide these arrays on a cost recovery basis.

**Other progress:** We continually try to improve our methods and procedures. We have made changes to our experimental protocol for hybridizations. We have added a RNA amplification step to the labeling protocol and now perform hybridizations directly with labeled aRNA. This results in better signal intensities and reduces the amount of RNA required for hybridizations. The protocol has been posted on our website.

We have completed a computational analysis of all Solanaceae ESTs to further
document the similarity and divergence within the Solanaceae and thus document our use of the potato arrays for heterologous hybridizations. Our analyses were published in 2005. We published our work on abiotic stress in potato. A full listing of publications from the Buell group in the last year are listed below:

**Publications in 2005:**


We have also published two manuscripts from work from the first NSF Potato Functional Genomics award that began in 1999. These two new publications are listed below.


(1) Overall Goals

Develop a pepper BAC library from *Capsicum frutescens* BG2816. Identify BAC clones for sequencing by TIGR from the R gene cluster that includes the L locus for TMV resistance on pepper chromosome 11. Analyze sequence from pepper R gene cluster on chromosome 11 that is homologous to tomato and potato chromosome 11. Cooperate with subcontractors working in other genomic regions in potato, e.g., the Bradeen group, where putative homologous resistance gene clusters occur in pepper. Comparative expression studies in solanaceous species.

(2) Specific Goals

Identify and sequence BAC clones that include tomato potato R3 and tomato I2 homolog sequences. Assemble a contig using the BAC clones. Analyze sequence from the BAC clones that is I2 and R3 homologous to tomato and potato chromosome 11. Map BAC end sequences containing R3/R6/R7 and I2 or encoding genes in pepper genome. Develop potyvirus resistance strategies in potato based on results at the *pvr1/eIF4E* locus in pepper.

(3) Progress

Our original objective of this project was a comparative study between the L locus in pepper and R3/R7 and I2 loci in potato and tomato. However, the mapping of the L locus has shown the L locus is distinct from R3/R7 region. Therefore, fine mapping and physical mapping of L is a lower priority for the project, although one we are continuing to pursue through an independent collaboration. In the annual meeting of 2005, the SAC recommended that the project focus on the R3/R7 syntenic region in pepper.

1. Cloning and sequencing pepper homologs of R3/R6/R7 region

To map markers that are linked to the R3/R7 region in pepper genome we obtained 48 primer pairs from the Baker lab, based on potato BAC ends or coding sequences of genes located in the R3/R7 region. Twenty primer pairs amplified single bands of the expected size and the resulting DNA fragments were cloned and sequenced. Sequence analysis demonstrated that about 15 clones have homology with potato BAC clones, nematode resistance-like protein gene, or the Rio Grande *Pto* locus (Table 1). For example, RK-2 primers amplified a 1.2 kb DNA fragment homologous to the DNA sequence of *Solanum demissum* chromosome 11 clone PGEC542; 444D14F primers amplified a 500 bp DNA fragment homologous to the DNA sequence of *S. demissum* chromosome 11 clone PGEC591C22. Some primers, e.g., 149E11F and 26NR, amplified DNA fragments from a clone originating from *S. demissum* chromosome 5, demonstrating genomic sequences of the R1 region on chromosome 5 and the R3/R7 region on chromosome 11 may be very
similar. In order to further characterize the amplified sequences, we performed DNA blot analysis using the DNA fragments as probes. All the DNA homologs were multicopy sequences as shown Fig. 1.

Table 1. Summary of Cloning and mapping pepper homologs of R3/R6/R7 region.

<table>
<thead>
<tr>
<th>Primer Number</th>
<th>Primer Sequence</th>
<th>Primer</th>
<th>Product Size</th>
<th>Top Contig Hit in Blast (cutoff E value 1e-05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>coding R3/R7</td>
<td>RK-2</td>
<td>1.2kb</td>
<td>AC149488 Solanum demissum chromosome 11 clone PGEC542</td>
</tr>
<tr>
<td></td>
<td>coding R3/R7</td>
<td></td>
<td>1.2kb</td>
<td>AC149488 Solanum demissum chromosome 11 clone PGEC542</td>
</tr>
<tr>
<td>20</td>
<td>BAC end</td>
<td>21H19F</td>
<td>500bp</td>
<td>BT013143 Solanum lycopersicum clone 134137F</td>
</tr>
<tr>
<td>23</td>
<td>BAC end</td>
<td>26N3R</td>
<td>350bp</td>
<td>AC151957 Solanum tuberosum chromosome 5, clone PGEC13</td>
</tr>
<tr>
<td>30</td>
<td>BAC end</td>
<td>41N19R</td>
<td>350bp</td>
<td>AC 137921 Oryza sativa chromosome 3, clone OSJNba0027H16</td>
</tr>
<tr>
<td>32</td>
<td>BAC end</td>
<td></td>
<td></td>
<td>BT013143 Solanum lycopersicum clone 134137F</td>
</tr>
<tr>
<td>40</td>
<td>BAC end</td>
<td>149E11F</td>
<td>350bp</td>
<td>Solanum demissum chromosome 5, clone PGEC568H16</td>
</tr>
<tr>
<td></td>
<td>BAC end</td>
<td></td>
<td></td>
<td>Solanum tuberosum nematode resistance-like protein (Gro1-2) gene</td>
</tr>
<tr>
<td>43</td>
<td>BAC end</td>
<td>308D19F</td>
<td>450bp</td>
<td>Solanum tuberosum nematode resistance-like protein (Gro1-2) gene</td>
</tr>
<tr>
<td></td>
<td>BAC end</td>
<td></td>
<td></td>
<td>Solanum tuberosum nematode resistance-like protein (Gro1-2) gene</td>
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<tr>
<td>45</td>
<td>BAC end</td>
<td>380L15R</td>
<td>800bp</td>
<td>Panax ginseng chloroplast</td>
</tr>
<tr>
<td>49</td>
<td>BAC end</td>
<td>380B15F</td>
<td>250bp</td>
<td>Solanum pimpinellifolium Rio Grande 76R Pto locus</td>
</tr>
<tr>
<td>52</td>
<td>BAC end</td>
<td>444D14F</td>
<td>500bp</td>
<td>Solanum demissum chromosome 11, clone PGEC591C22</td>
</tr>
</tbody>
</table>

Fig. 1. DNA blot analysis of Capsicum homologs generated from primers based on the R3/R6/R7 region in Solanum. Letters on top of the figure represent DNA blot analysis of homologs of 21H19R (A), RK-2 (B), 26N3R (C), and 17G17R (D) sequences.

2. BAC library screening with I2 and contig mapping

The I2 locus in tomato confers resistance to race 2 of the soil-borne fungus Fusarium oxysporum f. sp lycopersici. I2 is a complex locus consisting of two clusters, SL8D and SL8E. The SL8D cluster contains seven CC-NBS-LRR type R gene sequences, including the I2 gene. The syntenic locus of the SL8E cluster in potato contains the late blight R3 complex. In order to identify and sequence Capsicum BAC clones that include sequences
homologous to the potato $R3/R7$ region, we screened BAC library filters containing 220,000 clones. We used the 3’ sequence of $I2C-1$ gene as a probe and obtained a total of 89 positive BAC clones.

In the $R3/R7$ region of potato, there are 17 copies of GP285, and TG105 is tightly linked to the $I2$ homolog. Therefore, we hybridized the 89 Capsicum BAC clones with GP285 and TG105 to identify BAC clones containing sequences syntenic to $R3/R7$ region. However, none of the 89 clones cross hybridized with the two RFLP markers. This demonstrates that potato and pepper have a different genomic structure in this region.

We also attempted to group the 89 clones using a BAC fingerprinting method. BAC fingerprinting was outsourced to Amplicon Express (Pullman, WA: http://www.genomex.com). After obtaining BAC fingerprints (Fig. 2A), we assembled BAC contigs with an FPC program. We used a tolerance setting of 3 and a cutoff of 1e-10, which are default parameters. All 89 clones assembled into 1 contig (Fig. 2B). The result was unexpected since there are at least five genomic locations of the $I2$ gene family in tomato. Currently, we are verifying the contig by changing parameters and looking at raw fingerprints. In addition, we are screening the BAC clones using primers provided by Baker’s group. Once we confirm BAC clones syntenic to $R3/R7$ region, we will make shotgun libraries and sequence them.
Fig. 3. BAC fingerprinting (A) and contig mapping (B).

3. Developing potyvirus resistant potato using potyvirus resistance genes from pepper

*Potato Virus Y* (PVY) infects many solanaceous crops, but the virus is especially important as a key limitation of potato production. PVY NTN, which is very closely related to PVY N, causes potato tuber necrotic ringspot spot disease (PTNRD). This necrotic strain was first identified in Europe in 1980 and has subsequently been identified in the US, Canada, the Middle East and Japan. Superficial necrotic ringspots caused by this virus make the tubers unmarketable and, in addition, infected tubers can no longer serve as seed for next year’s crop. In order to identify a possible solution to this problem, recessive resistance genes previously identified to confer potyvirus resistance in pepper have been transformed into commercial potato cultivars.

Several recessive resistance alleles in pepper have been found to confer potyvirus resistance when overexpressed in tomato. The transgenes were developed using a variant of pathogen derived resistance (PDR). Rather than overexpressing a viral gene, we used a derivation on the PDR method which relied on the overexpression of the host translation initiation factor eIF4E. eIF4E has been identified as a naturally occurring recessive resistance gene in pepper (*pvr1/2*), lettuce (*mo1*), and pea (*sbm1*). Previously, we showed that the physical interaction between eIF4E and the viral protein (VPg) of Tobacco Etch Virus is required for susceptibility. We hypothesized that expression of a mutated form of eIF4E might inhibit interaction between eIF4E and TEV-VPg and thus prevent viral replication. We transformed the dwarf tomato MicroTom with pepper *Pvr1*+ and *pvr1* sequences in both sense and antisense orientations. Overexpression of the recessive resistance allele, *pvr1*, resulted in TEV resistance. Three different *pvr1* alleles have been isolated and subsequently overexpressed in MicroTom. Each transgene tested, *pvr1, pvr1*, and *pvr1*, resulted in the same range of protection as observed in non-transformed *Capsicum* genotypes homozygous for that allele.

Unfortunately, PVY resistance in transgenic MicroTom could not be assessed because MicroTom is already resistant the virus. In order to assess resistance of these transgenes to PVY, we have initiated potato transformation with Dr. Austin-Phillips at the University of Wisconsin-Madison, who is also supported by this grant. Her lab is transforming five potato varieties differing in their susceptibility to PVY: Dark Red Norland, Superior, Russett Burbank, Katahdin and Umatilla. We are going to receive the
first batch of transgenic Russet Burbank potato plants soon. We are currently screening these varieties for susceptibility to confirm previously identified resistance status to four PVY strains: PVY O, PVY N, PVY NTN, and PVY N-O. Both non-transgenic and transgenic plants will be screened for resistance to these four PVY stains. Inoculated and systemic leaf tissue will be serologically tested by indirect enzyme-linked immunosorbent assay (ELISA) using antibodies specific to each virus strain. Additionally, tubers of inoculated plants will be examined for PTNRD symptoms.
Juming Jiang and Sandra Austin-Phillips  
University of Wisconsin, Madison

(1) Overall Goals

a. Gene expression profiling of key potato biology (polyploidy, tuberization, late blight resistance)
b. Provide services to the project for potato transformation and greenhouse late blight resistance screening.
c. Functional studies of late blight resistance gene $RB$

(2) Specific Goals

Specific goals in the 3rd year: In-depth study of gene expression modification and regulation associated with polyploidy. Microarray-based gene expression profiling using transgenic potato materials with the $RB$ gene. Services on potato transformation and greenhouse late blight resistance screening will be available to the collaborators throughout the funding period.

(3) Progress

I. MICROARRAY GENE EXPRESSION PROFILING OF POLYPLOIDY

We have conducted a series of microarray experiments to test the transcriptional differences in a synthetic autopolyploid series from *Solanum tuberosum* consisting of monoploid (1x), diploid (2x), and tetraploid (4x) plants (the P77 series). Twelve and eight microarrays were hybridized to test the affects of ploidy on growing leaves and root tips, respectively. Several gene families were consistently found to be up-regulated with ploidy in growing leaf tissues: including ribosomal protein genes, histones, and cyclins. Several other genes were also found to be up- or down-regulated with ploidy, although most gene expression changes were relatively minor. Real-time PCR and Northern blot hybridizations were performed to validate the data obtained from the microarray experiments. Most of the “ploidy-related” genes were up- or down-regulated in terms on transcript abundance across the autopolyploid series. Northern hybridization data confirmed that the expression pattern of the expansin gene STMIS17 was 2-fold down regulated from 1x to 4x series (Fig. 1). Similarly, a cyclin gene STMDE81 was 2.5-fold up-regulated from 1x to 4x ploidy series.

![Expansin and Ubiquitin Gene Expression](image-url)
Figure 1. Detection of expansin transcripts in potato plants with different ploidies by Northern blot hybridization. Equal loading of total RNA was confirmed by hybridization with an ubiquitin probe.

We have initiated a transcription analysis of the ploidy-related genes in several wild Solanum species that have different ploidies in the nature. Initially sixteen different accessions of three wild species with different ploidies were chosen. Seeds were obtained from potato Genebank at Sturgeon Bay, Wisconsin. All the plants used for expression analysis were grown in a walk-in growth chamber at UW-Madison. Morphological and cytological characterization were carried out on the wild species chosen for gene expression studies. We are currently focusing our analysis on *S. oplocense* (Fig. 2) that contains diploid, tetraploid, and hexaploid in the nature.

Real-time PCR experiments were performed using gene-specific primers in two independent ploidy series (2x, 4x and 6x) of *S. oplocense*. Primer sets for actin and ubiquitin-conjugated enzyme genes were used as controls. Real-time PCR reactions were performed for each primer pair on three different ploidies of *S. oplocense*. A minimum of two PCR reactions was performed for standard primer pairs for each biological replicate. Average expression changes and standard deviations for the two biological replicates were calculated for each primer pair.

Figure 2. Characterization of a diploid (2x), tetraploid (4x), and hexaploid (6x) series of *S. oplocense*. Top panel: leave morphology; Bottom panel: chromosome number, 2x = 24, 4x = 48, 6x = 72.

Preliminary data showed that there is an up-regulation of histone (Histone H2B and Histone H4) and ribosomal protein genes with increase of ploidy in *S. oplocense*. Except for these three genes, for most of the primer pairs, the results were not consistent or the ploidy genes do not follow the trend as was observed in the P77 series. The results were summarized in Fig. 3. The up-regulation of histone and ribosomal protein genes may be a consequence of ploidy-induced increase in cell size or may be due to greater rates of
cell division in the leaves sampled from higher ploidies. More experiments will be carried out on the wild species across the ploidies to further confirm the data with more technical and biological replicates.

Figure 3. Real-time PCR assessment of the selected ploidy-related genes in the diploid, tetraploid, and hexaploid *S. oplocense*. The error bar indicates standard deviation of two bio-replications.

II. MICROARRAY GENE EXPRESSION PROFILING OF RB-MEDIATED LATE BLIGHT RESISTANCE

1. Characterization of potato materials for microarray analysis

The materials used in the microarray study are clonally propagated T1 Katahdin plants containing the RB gene. Katahdin is highly susceptible to the late blight and does not contain any known late blight resistance genes. The transformed RB gene contains a native promoter derived from *S. bulbocastanum*. A large number of transgenic Katahdin plants were tested in the University of Wisconsin-Madison’s Biotron greenhouse facility. Normally, we use 40,000-80,000 sporangia / mL of specific *P. infestans* strains for late blight inoculation. Most of the transgenic plants showed resistant phenotype under this testing condition.

We have also tested the late blight resistance of several transgenic Katahdin lines under an “intensive inoculation condition” in which we applied 100,000 sporangia / mL. Interestingly, the resistant phenotype of some of the transgenic plants broke down under this high disease pressure, while others did not. Concurrently, we conducted Southern hybridizations to identify the copy number of RB gene in each of the resistant Katahdin plant. Interestingly, we found that resistant lines under intensive inoculation condition tend to contain more copies of the RB gene, although we currently do not have conclusive evidence on the relationship between the level of resistance and the copy numbers of the RB gene. The different resistance phenotypes under regular and intensive inoculation conditions of two specific transgenic Katahdin lines, which contain a single and multiple
copies of the *RB* gene, respectively, have been confirmed by several independent tests (Fig. 4). These two transgenic lines have been used in microarray study.

**Figure 4.** The late blight resistance phenotypes of two transgenic Katahdin lines under an intensive inoculation condition. Left Panel: Left: A transgenic Katahdin clone containing a single copy of the *RB* gene; Middle: *S. bulbocastanum* clone PT29; Right: Katahdin control. The transgenic Katahdin plant, which is resistant to late blight under regular inoculation condition, shows a susceptible phenotype. Right Panel: Left: A transgenic Katahdin clone containing multiple copies of the *RB* gene; Middle: *S. bulbocastanum* clone PT29; Right: Katahdin control. The transgenic Katahdin plant shows a resistant phenotype.

### 2. Experimental design

A set of 12 plants of the control Katahdin plant and transgenic Katahdin lines with one and multiple copy of the *RB* gene (Fig. 4) will be propagated by tissue culture. Once rooting has taken place these 36 plants will be transplanted and moved to the Biotron greenhouse. The plants are arranged in a completely randomized design. Once a week the plants are systematically rearranged in the greenhouse to minimize environmental effects. After five weeks of growth, the plants will be moved into a Biotron mist chamber, which contains ambient light and maintains 90-100% humidity. The 36 plants will again be placed in a completely randomized design that has been predetermined using the sampling feature from the statistical program R. Every plant is placed within the chamber so it will not touch another plant. This is done in order to eliminate possible cross-contamination or loss of inoculums. Each plant is watered and allowed to adjust to the mist chamber for eight hours prior to inoculation.

The *P. infestans* isolate US 940480 (US-8 genotype, A2 mating type) will be used for inoculation. Inoculums with 100,000 sporangia / mL will be prepared four hours before
inoculation. Each plant is carefully removed from its location in the Biotron and inoculated individually. Two leaves of approximately the same age are randomly chosen from each plant. Looping a portion of string around the petiole marks one leaf. The leaf marked with the string is carefully lifted up and ten 10 uL droplets of *P. infestans* are deposited in approximately the same location on either side of the leaf mid-vein. A second leaf from the same plant is inoculated in exactly the same way using water. The plant is then carefully placed back into position within the mist chamber. The time of each inoculation is recorded to identify when sampling must take place.

Samples are taken 2, 5 and 10 hours after inoculation. Sampling at each time point involves twelve plants, four from each type of the Katahdin plants. The twelve plants are carefully removed from the mist chamber one at a time. Each plant then has both of the inoculated leaves removed one at a time with scissors. A hole punch is then used to extract only the area under the water droplet. Separate hole punchers are used for each genotype and each inoculation method, water or *P. infestans*. These small leaf disks are immediately placed within an eppendorf tube and submerged in liquid nitrogen. Each tube is then stored at -80°C until RNA extraction.

We will collect 2 samples from each of the 36 plants, one sample challenged with *Phytophthora* and the other challenged with water. RNA will be extracted from the 72 samples. The samples from each of the two plants under the same time point will be pooled at the RNA level. For example, at 2 hours post inoculation we will collect 12 samples, four from each of the three different types of Katahdin plants. For each type of the three Katahdin plants there will be four challenged and four unchallenged samples. Of this, two RNA samples of the challenged or unchallenged treatment are pooled to make two total replicates or two experimental units for each treatment/genotype. Thus, for one time point, we have a total of 12 pooled RNA samples, which will reduce the total RNA samples from 72 to 36. RNA from the 36 pooled samples will be amplified using the MessageAmp aRNA amplification kit from Ambion (Ambion, Austin, Texas). Of these 36 samples, the 12 from the Katahdin clone with only one RB copy will not be used in the immediate array study (but will be saved for further use if more arrays become available). Amplified RNA (aRNA) from the other 24 mRNA samples will be converted into aminoallyl cDNA using the SuperScript III enzyme (Invitrogen, Carlsbad, CA). Aminoallyl cDNA from each experimental unit is then labeled with Cy3 or Cy5. Essentially the same protocol is followed as described on the TIGR website (http://www.tigr.org/tdb/potato/microarray_SOPs.shtml).

For each time point, we have two aminoallyl cDNA samples that were challenged with water and two aminoallyl cDNA samples that were challenged with *P. infestans* for each of the three types of Katahdin plants. These four samples will be labeled by a “dye-swap” procedure. The water challenged sample from the first two plants labeled with Cy3 and the other water challenged sample from the second two plants labeled with Cy5. The *Phytophthora* challenged sample from the first group of two plants will be then labeled with Cy5 and the second sample with Cy3. The Cy3 water challenged cDNA and the Cy5 *Phytophthora* challenged cDNA from the first set of plants are then hybridized to
the same array slide. The Cy5 water challenged cDNA and the Cy3 *Phytophthora*
challenged cDNA from the second set of plants is then hybridized to a second array.

Two array slides are then used for each set of four samples for each clone within each
time point. Thus, for each experiment, the samples from the control Katahdin plants and
the Katahdin plants with multiple copies of the *RB* gene will be hybridized to a total of 12
microarrays. This experiment will be conducted three more time for a total of four
experimental replicates, using a total of 48 array slides. Hybridization to the arrays is
performed in a moist Corning hybridization chamber overnight. Washed and dried slides
are then shipped overnight to the TIGR facility where they will be scanned on a GenePix
4000B scanner (Molecular Devices, Sunnyvale, CA).

3. Preliminary results

For the preliminary experiment described below, only differences between time and the
copy number of the *RB* gene were investigated. The first and second array experiments
were done to investigate during what time frame the most interesting transcriptome
changes were taking place. Samples were taken at 5, 10 and 25 hours post inoculation for
the first array experiment. Six arrays were hybridized with twelve samples. Six samples
were taken from the Katahdin control plants without the *RB* gene, either water and Late
light-inoculated and six samples were taken from the Katahdin plants with multiple
copies of the *RB* gene, either water or late blight-inoculated. The same sampling was
performed for array experiment two, only sampling was done at 2, 5 and 10 hours post
inoculation. Table A lists which samples were labeled with what dye and how the
samples were hybridized to the arrays.

<table>
<thead>
<tr>
<th>ARRAY EXPERIMENT 1</th>
<th>ARRAY EXPERIMENT 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Array #</strong></td>
<td><strong>Cy3</strong></td>
</tr>
<tr>
<td>1</td>
<td>Kat 5 water</td>
</tr>
<tr>
<td>2</td>
<td>Kat 15 Phy</td>
</tr>
<tr>
<td>3</td>
<td>Kat 25 water</td>
</tr>
<tr>
<td>4</td>
<td>Multi 5 Phy</td>
</tr>
<tr>
<td>5</td>
<td>Multi 15 water</td>
</tr>
<tr>
<td>6</td>
<td>Multi 25 Phy</td>
</tr>
<tr>
<td>3</td>
<td>Kat 10 Phy</td>
</tr>
<tr>
<td>4</td>
<td>Multi 2 Phy</td>
</tr>
<tr>
<td>5</td>
<td>Multi 5 Phy</td>
</tr>
<tr>
<td>6</td>
<td>Multi 10 Phy</td>
</tr>
</tbody>
</table>

Preliminary analysis of array data was performed using the GeneSpring software. The
data was imported from a text file created by the GenePix Pro 5.1.0.11 software. All data
was log transformed and normalized within and between arrays using intensity dependent
regression. All data was then filtered based on confidence using the Benjamini and
Hochberg False Discovery rate, followed by filtering on flags. Raw data was filtered
based on expression level. The minimum expression value was 50 and this expression
level had to be present in at least one of the experimental conditions tested (Fig. 5).
Figure 5. **Top Left** – Filtered data from Array 1 is shown colored by the normalized intensity ratio (log transformed) along the vertical axis. The horizontal axis represents gene expression occurring 5, 15 and 25 hours post inoculation. **Top Right** – Filtered data from Array 1 is shown colored by the normalized intensity ratio (log transformed) along the vertical axis. The horizontal axis represents changes occurring between the potatoes containing multiple copies of the RB gene on the left side and those potatoes without the RB gene on the far right side. **Bottom Left** – Filtered data from Array 2 is shown colored by the normalized intensity ratio (log transformed) along the vertical axis. The horizontal axis represents gene expression occurring 2, 5 and 10 hours post inoculation. **Bottom Right** – Filtered data from Array 2 is shown colored by the normalized intensity ratio (log transformed) along the vertical axis. The horizontal axis represents changes occurring between the potatoes containing multiple copies of the RB gene on the left side and those potatoes without the RB gene on the far right side.

The top left graph in Fig. 5 represents the filtered gene expression changes occurring 5, 10 and 25 hours post inoculation from array experiment 1. Most of the transcriptome changes are occurring between 5 and 10 hours after inoculation. There are few changes occurring between 10 and 24 hours after the plants were inoculated. Therefore, earlier time points were chosen to capture immediate changes occurring after the late blight zoospores start to encyst on the leaves at around 3 hours post inoculation. The bottom left graph in Fig. 5 represents the filtered gene expression changes occurring 2, 5 and 10 hours post inoculation from array experiment 2. The up- and down-regulated genes appear to be spread out evenly across the time points, indicating these are the appropriate sampling times to obtain the maximum amount of information regarding the transcriptome changes occurring between the plants with and without the RB gene.
When we compare the top right and bottom right graphs in Fig. 5 we see greater intensity ratios in the bottom graph. Note that the top right graph is from the plants sampled 5, 15 and 25 hours after inoculation. We can see the plants with the $RB$ gene (left side of the graph) are showing a greater difference in the number of genes up- and down-regulated compared with the plants lacking the $RB$ gene (right side of the graph). The bottom right graph is from plants sampled 2, 5 and 10 hours post inoculation. The intensity ratios are again higher in the plants containing the $RB$ gene. However, the relative increase in intensity ratios between the bottom and top right graphs look as though they are balanced between the plants with and without the $RB$ gene.

**Figure 6.** Top Left – Filtered data from Array 1 is shown colored by the normalized intensity ratio (log transformed) along the vertical axis. The horizontal axis represents the significant genes occurring 5, 15 and 25 hours post inoculation pulled out by a 1-way ANOVA. Top Right – Filtered data from Array 1 is shown colored by the normalized intensity ratio (log transformed) along the vertical axis. The horizontal axis represents the significant genes between the potatoes containing multiple copies of the $RB$ gene on the left side and those potatoes without the $RB$ gene on the far right side pulled out by a T-test. Bottom Left – Filtered data from Array 2 is shown colored by the normalized intensity ratio (log transformed) along the vertical axis. The horizontal axis represents the significant genes occurring 2, 5 and 10 hours post inoculation pulled out by a 1-way ANOVA. Bottom Right – Filtered data from Array 2 is shown colored by the normalized intensity ratio (log transformed) along the vertical axis. The horizontal axis represents the significant genes between the potatoes containing multiple copies of the $RB$ gene on the left side and those potatoes without the $RB$ gene on the far right side pulled out by a T-test.

ANOVA was performed to find genes with significant $p$-values. Images of the genes selected are shown in Fig. 6. One-way ANOVA was performed on both arrays for the
different sampling times. A t-test was performed on both arrays to compare the plants with no RB gene with those containing multiple RB genes. Table B lists those potato or Solanum genes pulled out using these analysis. It is interesting to note that array experiment 2 appeared to give a larger amount of significant potato and Solanum genes compared with array experiment 1. This may be due to the sampling times being able to better capture those genes responding to the encystment by the Phytophthora.

### TABLE B

#### 1-WAY ANOVA - ARRAY EXPERIMENT 1 - TIME

<table>
<thead>
<tr>
<th>Gene</th>
<th>FC</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>STMDT88</td>
<td>0.00552</td>
<td>precursor - potatoNADH2 dehydrogenase (ubiquinone) (chain TYKY.2</td>
</tr>
<tr>
<td>STMCR43</td>
<td>0.0404</td>
<td>STS14 protein precursor (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMHT35</td>
<td>0.0336</td>
<td>protease inhibitor II (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMIF66</td>
<td>0.0302</td>
<td>(Q84534) Short chain alcohol dehydrogenase-like protein Eukaryotic translation</td>
</tr>
<tr>
<td>STMGU62</td>
<td>0.0265</td>
<td>Translationally controlled tumor protein homolog (TCP) (P23). (Solanum tuberosum;</td>
</tr>
<tr>
<td>STMHK75</td>
<td>0.0222</td>
<td>Serine protease inhibitor S precursor (gCDI-B1). (Solanum tuberosum);</td>
</tr>
</tbody>
</table>

#### 1-WAY ANOVA - ARRAY EXPERIMENT 2 - TIME

<table>
<thead>
<tr>
<th>Gene</th>
<th>FC</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>STMCY44</td>
<td>0.0397</td>
<td>probable chaperonin 60 beta chain precursor chloroplast - potato</td>
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<tr>
<td>STMFF695</td>
<td>0.0393</td>
<td>chloride channel protein homolog CLC1 - potato (Solanum tuberosum);</td>
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<tr>
<td>STMC550</td>
<td>0.0205</td>
<td>extensin homolog - potato (fragment) (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMB346</td>
<td>0.0134</td>
<td>probable lipoygenase () (done H1) - potato (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMG781</td>
<td>0.0356</td>
<td>Mitochondrial 60S ribosomal protein L5. (Solanum tuberosum);</td>
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<tr>
<td>STMTR74</td>
<td>0.0347</td>
<td>Catalase isozyme 2 (4). (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMEV57</td>
<td>0.0118</td>
<td>Wound-induced protein 1. (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMCV38</td>
<td>0.0198</td>
<td>cytochrome P450 77A1 (Solanum melongena);</td>
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<tr>
<td>STME369</td>
<td>0.0080</td>
<td>Triose phosphate/phosphate translocator chloroplast precursor (Solanum tuberosum;</td>
</tr>
<tr>
<td>STMEV43</td>
<td>0.0092</td>
<td>Cytochrome P450 76A2 (Solanum melongena);</td>
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<tr>
<td>STMUI78</td>
<td>0.0013</td>
<td>Yippee-like protein. (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMSC50</td>
<td>0.0482</td>
<td>Eukaryotic translation initiation factor 5A-1/2 (eIF-5A 1/2) (Solanum tuberosum;</td>
</tr>
<tr>
<td>STMX62</td>
<td>0.0451</td>
<td>S-adenosylmethionine decarboxylase (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMCX28</td>
<td>0.0431</td>
<td>60S ribosomal protein L10 (EQM). (Solanum melongena);</td>
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<tr>
<td>STMDW57</td>
<td>0.0427</td>
<td>60S ribosomal protein 54. (Solanum tuberosum);</td>
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</table>

### T-TEST - ARRAY EXPERIMENT 1 - RB PRESENT OR ABSENT

<table>
<thead>
<tr>
<th>Gene</th>
<th>FC</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>STMJ33</td>
<td>0.0436</td>
<td>metallocoxy peptide inhibitor Ilia precursor [validated] - potato</td>
</tr>
<tr>
<td>STMCV33</td>
<td>0.0416</td>
<td>dna protein homolog - potato (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMCST4</td>
<td>0.0249</td>
<td>Hypothetical proteinmetallocoxy peptide inhibitor Ilia precursor - potato</td>
</tr>
<tr>
<td>STMB346</td>
<td>0.0233</td>
<td>probable lipoygenase - potato (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMMB61</td>
<td>0.0187</td>
<td>potato ubiquitin</td>
</tr>
<tr>
<td>STMH31</td>
<td>0.0151</td>
<td>membrane channel protein - potato (fragment) (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMER90</td>
<td>0.0282</td>
<td>NAD-dependent malic enzyme 59 kDa isoform mitochondrial precursor (Solanum tuberosum;</td>
</tr>
<tr>
<td>STMH694</td>
<td>0.0169</td>
<td>Actin 58. (Solanum tuberosum);</td>
</tr>
</tbody>
</table>

### T-TEST - ARRAY EXPERIMENT 2 - RB PRESENT OR ABSENT

<table>
<thead>
<tr>
<th>Gene</th>
<th>FC</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>STMFB99</td>
<td>0.0458</td>
<td>dehydrin homolog C17 - potato (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMDC26</td>
<td>0.0444</td>
<td>UDP-glucose glucosyltransferase () - potato (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMIE76</td>
<td>0.0438</td>
<td>metallocoxy peptide inhibitor Ilia precursor</td>
</tr>
<tr>
<td>STMCV39</td>
<td>0.0381</td>
<td>systemic acquired resistance-related protein SRE1a - potato (Solanum tuberosum;</td>
</tr>
<tr>
<td>STMBB92</td>
<td>0.0338</td>
<td>potato actin</td>
</tr>
<tr>
<td>STMK63</td>
<td>0.0327</td>
<td>disease resistance protein homolog 124 - potato (fragment) (Solanum tuberosum;</td>
</tr>
<tr>
<td>STMHA80</td>
<td>0.0211</td>
<td>L-lactate dehydrogenase () (chain B - potato (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMPD79</td>
<td>0.0185</td>
<td>catechol oxidase () (allele POT32) - potato (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMEI32</td>
<td>0.0162</td>
<td>ubiquinol-cytochrome c reductase Rieske iron-sulfur protein</td>
</tr>
<tr>
<td>STMDC28</td>
<td>0.0488</td>
<td>S-adenosylmethionine decarboxylase (Solanum tuberosum);</td>
</tr>
<tr>
<td>STMCV60</td>
<td>0.0453</td>
<td>Cysteine protease inhibitor 1 precursor (Solanum tuberosum);</td>
</tr>
</tbody>
</table>

### III. POTATO TRANSFORMATIONS
Our group provides potato transformation services to the entire project. Dr. Sandra Austin-Phillips at the University of Wisconsin Biotechnology Center directs this service. Our main effort has been attempting potato transformations for Molly Jahn with the goal of producing PVY resistant plants. The constructs have evidently given PVY resistance in tomato. We were sent five different constructs with the request that we produce transgenic Russett Burbank. We typically work with Katahdin or Norland since these cultivars give a high transformation rate using our established protocol but both of these have PVY resistance so weren’t suitable for these experiments. We have produced transgenic Burbank expressing RB as part of this project but at a lower transformation rate (20%). These experiments have been very frustrating. We have performed over 20 different transformations with limited success. Regeneration of Burbank is not a problem, controls are 100%, but recovery of plants on selection has been sporadic or non-existent. We will be sending the few transformants to the Jahn lab. There are two copies of each line ready for shipping November 7, 2005. The Brown lab has provided us with a different potato cultivar, Umatilla, which might be used instead of Russet Burbank. We are in the process of growing sufficient copies for setting up several transformations. When we have enough plants, we will start by putting the Jahn’s lab highest priority construct (C4) into Umatilla. We will focus on using the protocol optimized for Russett Burbank provided by Kari – and apparently for Umatilla – but we will also use our standard potato transformation protocol. We are currently seeing whether Umatilla regeneration is better than Russet Burbank regeneration on our ZIG medium. We do have concerns that the original material we were supplied with may be corrupted in some way.

We have also been working with the Jiang lab to identify genes in RB-mediated resistance pathway. We are using an advanced backcross (J101K6A6K41) from the original somatic hybrid between potato and S. bulbocastanum. We first did preliminary experiments in tobacco using two constructs to ensure that the constructs are expressed in plants. The regeneration rate for each construct (on selection) was about 50%. The same two constructs were recently (mid September) used in transformations using the backcross material. These transformations were very successful, the regeneration rate on selection is currently in excess of 50% for both constructs tested. We have already collected 11 independent potential transgenic shoots, though they are still too small for sampling. We will continue to maintain a population of J101K6A6K41 for additional transformations, this hybrid regenerates exceptionally well and very quickly on our ZIG medium.
Georgiana May  
University of Minnesota, Twin Cities

(1) Overall Goals

The goals of the May Lab are to understand the molecular and genomic mechanisms by which new resistance gene functions evolve, to translate evolutionary analyses into BioInformatic tools, and to use these data and tools to identify novel, non-host specific sources of late blight resistance in wild species. Our approach will identify genetic resources to help protect economically important Solanaceae crops (e.g., tomato, potato, eggplant and pepper) from disease.

(2) Specific Goals for 2005

A. Complete evolutionary analyses of I2-homologs from Solanaceae species.  
B. Map representative I2-homologs from potato, tomato and S. demissum onto tomato genome.  
C. BioInformatic Tools -  
   1) Develop informatic approaches to identifying candidate non-host specific resistance sequences.  
   2) Develop the SOLAR web site for display and use of project generated data and analyses.  
   3) Generate interactive and instructional web site with the goal of making evolutionary analyses accessible and data useful to broad audience.

(3) Progress for 2005

A. Previously, we obtained sequence data for more than 300 I2 homologs from potato, tomato, S. demissum, eggplant, pepper and tobacco, for the NBS and LRR protein-coding regions of the gene. Using an analysis of NBS sequences from our project and databases, we found that many gene copies are maintained in these genomes through speciation events and date as old as 12 million years. Thus, rapid gene turn-over by duplication and deletion predicted by gene-for-gene hypotheses is not supported by our data (Couch et al. 2005). To evaluate the relative importance of recombination and mutation in the evolution of novel NBS-LRR genes, we added sequence data for the LRR region of I2 homologs and low levels of recombination are also observed.

B. I2 homologs were mapped onto the tomato genome by two methods. Couch designed and generated oligonucleotide sequences distinguishing single nucleotide polymorphisms diagnostic of different I2 sequence types. Our first approach, using these oligo probes on tomato IL mapping lines, worked but was tedious and imprecise. Second, we used the oligo probes to first locate tomato BACs and then generated unique sequence from BAC
ends. BAC ends were either mapped by the AGI project or we mapped them onto IL lines. Mapping was improved by using polymorphisms in BAC ends that could be distinguished in IL lines by PCR rather than probing Southern blots (Summary: 196 BACs in 34 BAC contigs with I2 homologs; 5 BACs mapped to tomato genome, 6 BACs giving equivocal results).

C. Because 100's of NBS-LRR defense genes reside plant genomes, and relatively few have defined function, we aim to develop tools improving the search for novel resistance functions, so that conventional molecular biology approaches might be used to evaluate function. Two tools are developed:

- First, we use evolutionary analyses to describe selection acting through time and along the length of the gene. We found that only six major lineages, out of 75 diverse I2 sequences, show evidence of the positive selection expected for lineages evolving new resistance functions. Moreover, by delineating those lineages showing strong purifying selection through time, we may infer those sequences with common function but found in different species, and by definition, non-host specific resistance.
- Second, Ethy Cannon (with Brett Couch and Georgiana May) has developed software (CIVT) and modified existing software (ATV) which will allow us to directly relate I2 gene phylogenies to their location in the genome. The web tools will also allow public interactive access to the underlying data generated by our project and ultimately link to that of other labs in the project (Fig. 1).

Figure 1. Example CIVT output and genealogy of I2homologs. Sequences in boxes are examples of sequences mapped to BACs. Modifications of ATV will link these to chromosome maps and allow interactive access to evolutionary and genomic information.
B. INTERNAL DATA MANAGEMENT SYSTEMS
Progress on the status of internal data management systems.

Barbara Baker
University of California, Berkeley

This year we have extended internal project databases describing plasmid clones, seed stocks, potato tuber stocks, virus stocks, oligonucleotides for mapping, BAC end sequences, and other materials. We have also established a new virus stock database for maintaining and tracking the lab’s virus materials. Maureen Richey is the lab’s server administrator, maintaining and organizing electronic files, such as digital pictures, spreadsheets, documents, protocols, pdf reference libraries, archived materials, etc. The server and all lab computers are scheduled for back-up onto tape and back-up drives weekly. In addition, we keep an inventory of our computers and software and reassess our computing needs annually to determine necessary upgrades. We also maintain an online contacts directory and calendar from the potato genome webpage.

James Bradeen
University of Minnesota, Twin Cities

Most of our data management is accomplished using Microsoft Excel and/or Microsoft Access. The Bradeen lab is well equipped with 3 fully modern PC computers each with internet access and access to the University of Minnesota Super Computing facilities which include sequence analysis software packages such as GCG and DNAStar.

Charles Brown
USDA-ARS and Washington State University, Prosser

This year, we upgraded the hardware and software of our lab computers to improve capability and security. It made possible to backup data to the server through networking routinely. In addition to the software (such as MapManager and GeneTool) we have been using for data analysis, we continue to acquire new software, for example, NTSYS- pc for phylogenetic analysis. Furthermore, we upgraded some software to the latest versions to keep them up-to-date.

Robin Buell
The Institute for Genomic Research (TIGR)

The BAC sequence, BAC annotation, and potato gene expression databases are established. All of these are Sybase relational databases, linked for easy internal tracking of clones and sequences. The potato gene expression database can store data from multiple Solanaceae species. We will continue to maintain and add to the existing databases. We meet weekly as a group as well as on an ad hoc basis to address issues in our data processing pipeline and wet lab work.
Pepper sequences and RNA expression database development

The pepper sequence database is constructed from three resources; A) sequences obtained from a cDNA library from pepper fruit made in the Jahn lab via suppressive subtractive hybridization with pepper leaf RNA. Those sequences were annotated using BLASTX (cut off E-value E-20) and scanned for further identification with Interproscan, a tool that combines 8 different protein signature recognition methods. B) EST library made from different tissues and of different developmental stages, kindly provided by Korean collaborators. These sequences have now been submitted to Genbank and will be added into SGN after assembly and automatic annotation (BLASTX), to be released publicly upon completion. C) Publicly available pepper sequences, periodically updated and reorganized. The data and analysis of results are currently managed with a MySQL relational database.

The pepper RNA expression database is made of macroarray (nylon filter) of the clones obtained from the suppressive subtractive hybridization mentioned above, probed with RNAs extracted from leaves and through fruit development and analyzed for their relative expression. A large scale collaboration with the Giovannoni lab has generated extensive data where pepper RNAs were successfully hybridized to fruitom 2 chip. These data will soon be integrated into our database via a warehouse that should allow facile query via an expression search tool which will enable us to obtain sequence ID by function (keyword or annotation) or by their expression patterns is under construction. The tool is based on a tool developed for the tomato warehouse [http://ted.bti.cornell.edu/](http://ted.bti.cornell.edu/) by Fei Zangjun.

A stand alone tool for batch analysis of sequences, SAGA, has been developed in Jahn lab. SAGA is based on MySQL relational database and provides a friendly work environment for sequences data management. It is including NCBI's BLAST, TIGR's Assembler, the Gene Ontology database, and more. It runs stand-alone on the Windows OS, and intended for labs without full time bioinformatics support that would like to do large scale sequence analysis. SAGA is free and available to download at [http://pepperworks.plbr.cornell.edu/pepperl/](http://pepperworks.plbr.cornell.edu/pepperl/).

None.

Georgiana May
University of Minnesota, Twin Cities
The May Lab internal data management uses Excel and searchable FileMaker Pro databases. Sequence data files for the above clones are organized by the species and clone numbers and maintained in searchable FileMaker Pro databases, as are our primers sequences. The clone database, sequences, and genome locations are integrated into a single Excel spreadsheet database and information accessible through the web site as described above.
C. PUBLIC ACCESS AND DISTRIBUTION OF DATA

Progress on the status of public access to and distribution of data, biological materials and reagents, including WWW sites, databases and reagent distribution. Identify information and materials released. Include details such as how, when, and what materials were released (i.e. describe databases, web sites, educational materials).

Barbara Baker
University of California, Berkeley

We continue to maintain and improve the project web page (www.potatogenome.org), where links are available to access databases, resources, publications, research descriptions, outreach pages, contact information for project participants and project links. The site also includes a ‘participant only’ area where project participants can logon and share project related materials. This year we have changed the Databases & Resources page so that the project data maintained by TIGR appears as an integrated component of the site.

James Bradeen
University of Minnesota, Twin Cities

1. Information Release: The Bradeen lab has built and maintains its own webpage (http://ppg.coafes.umn.edu) which details our efforts, including NSF-funded projects and makes publicly available protocols and poster and oral presentations. Additionally, the Bradeen lab has contributed to the Potato Genome Project webpage (http://www.potatogenome.org) including (a) development of a Microsoft Powerpoint overview of our allelic mining efforts and (b) contribution of LR-PCR protocol. As quality sequence becomes available it will be made publicly available through Genbank for inclusion in the SOLAR database.

2. Intellectual Property: Not applicable to date.

Charles Brown
USDA-ARS and Washington State University, Prosser

Not applicable

Robin Buell
The Institute for Genomic Research (TIGR)

URL list for the NSF Potato Genome Project:

TIGR  http://www.tigr.org/tdb/potato

1) TIGR's NSF Potato Functional Genomics web page is online
(http://www.tigr.org/tdb/potato/) with information on ordering arrays, potato BAC sequence and annotation database, the *Solanum tuberosum* Gene Index, potato microarrays, and a FAQ site. Additional pages include the Expression Profiling Service and the Solanaceae Gene Expression Database. Links to the Potato NSF project page as well as several other potato-related websites are also provided.

2) Intellectual property: All gene expression studies are available to the public with the only restriction that for Expression Profiling Service the users will have a 60 day grace period to view the data prior to it becoming public.

3) We continue to provide the potato cDNA microarray slides to external researchers. A maximum of 20 slides per lab per 6 month period are available to academic researchers on a cost-recovery basis. The cost of the 10,000-clone array is $118.21. We recently became aware that we were responsible for custom taxes on these shipments and thus now require the customer to be responsible for shipping costs as well as customs taxes.

Margaret Jahn  
Cornell University

1. Information Release: The Jahn lab has built and maintains a web page (http://www.plbr.cornell.edu/psi) which contains information about our genetic and genomic resources in the lab, an up to date integrated molecular map of pepper, outreach activities related to the Public Seed Initiative and other information. Additionally, the Jahn lab has contributed to the Potato Genome Project web page (http://www.potatogenome.org) and SGN (we are currently Lab of the Month!). We have submitted our fruit library sequences to Genbank. SAGA is free and available to download at http://pepperworks.plbr.cornell.edu/pepperl/.

2. Intellectual Property: Not applicable to date.

Jiming Jiang and Sandra Austin-Phillips  
University of Wisconsin, Madison

The *RB* gene, which was cloned from *Solanum bulbocastanum* and confers broad-spectrum resistance to potato late blight, has been sent to 13 different labs, including those from Canada, Ireland, United Kingdom, India, Indonesia, China, and the international Potato Center at Peru.

Georgiana May  
University of Minnesota, Twin Cities

The May Lab has not released materials and will follow guidelines of the U. Minnesota in assigning intellectual property rights for materials produced in this project. We have no
contracts with private corporations and thus have not assigned any property rights to persons other than those involved in the NSF project.
D. PROBLEMS
Problems encountered that are likely to delay timely accomplishments of goals established in the timetable and proposed resolution. Describe how problems will be resolved.

Barbara Baker
University of California, Berkeley

Unanticipated increases in UCB postdoc salaries prevent support of 3 postdocs as described in the original proposal. We therefore did not hire a replacement for Dr. G. Brigneti when she and her family relocated to Mexico.

James Bradeen
University of Minnesota, Twin Cities

1. Problems encountered: lack of support for personnel connected with this NSF project represents a significant limitation for our laboratory.

2. Problem solving: we continue to adapt methods enabling more efficient use of resources. Examples include use of EcoTilling and DGGE fingerprinting methods to minimize overall sequencing costs.

Charles Brown
USDA-ARS and Washington State University, Prosser

Problems Encountered: The size of the mapping population limits our ability to fine mapping the $R_{Mc1(ldb)}$ locus.

Solution: We are making efforts to enlarge the mapping population SB22 x PT29 and have approximately 150 new individuals growing in tissue culture at the present time. In addition, there are some breeding lines and mapping population developed from other species available for mapping purpose.

Problems Encountered: The only genomic library available is derived from the susceptible parent.

Solution: We will explore the probability of making a Lambda DNA library if necessary.

Robin Buell
The Institute for Genomic Research (TIGR)

We foresee no problems in completing the objectives in this proposal.

D. Problems
Margaret Jahn  
Cornell University

We identified about 90 BAC clones containing the \( I2 \) homolog, indicating there are many \( I2^- \)-related sequences in pepper. Even though it will be possible to sort out the BACs by contig mapping using potato sequence information around the \( R3 \) region of potato, it will take longer than expected to find and confirm BAC clones syntenic to \( R3 \) of potato because of the repetitive nature of R genes and the unexpected sequence differences between potato and pepper as shown by results with GP250.

Jiming Jiang and Sandra Austin-Phillips  
University of Wisconsin, Madison

None.

Georgiana May  
University of Minnesota, Twin Cities

We hoped that progress in BAC contig assembly at AGI and the tomato sequencing would obviate much of the work involved in mapping \( I2 \) homologs. However, most of the BACs in which \( I2 \) homologs are located, are not yet assembled in contigs and anchored on the marker-based map, including much of chromosome 11 south. To overcome these problems, we improved efficiency of mapping BACs to IL regions using PCR-based polymorphisms in the BAC end sequences. We developed this strategy following feedback from the April 2004 project meeting. We plan to continue our effort in manually mapping the BACs onto tomato IL bins, but only to the extent needed to fully develop bioinformatics tools and for data needed to evaluate the relationship between gene evolution and chromosome rearrangement. We will communicate map locations of BACs to AGI.
E. ROLES PLAYED BY SUBCONTRACTORS AND OTHER COLLABORATORS INVOLVED

Barbara Baker  
University of California, Berkeley  
Not applicable.

James Bradeen  
University of Minnesota, Twin Cities  
Not applicable.

Charles Brown  
USDA-ARS and Washington State University, Prosser  
Not applicable.

Robin Buell  
The Institute for Genomic Research (TIGR)

<table>
<thead>
<tr>
<th>Participating Institution</th>
<th>Subcontractor/Collaborator</th>
<th>Role(s)</th>
</tr>
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<tbody>
<tr>
<td>TIGR</td>
<td>C. Robin Buell</td>
<td>Manage, supervise TIGR component (BAC sequencing, microarray fabrication, expression profiling service, gene expression profiling, data dissemination, bioinformatics)</td>
</tr>
</tbody>
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Margaret Jahn  
Cornell University

If we identify syntenic BAC clones of potato R3/R7, we will send shotgun libraries of the BAC clones to Dr. Szent-Gyorgyi at the Agricultural Biotechnology Center in Hungary. They will sequence the shotgun libraries for free. Austen Phillips will be handling 5 constructs derived from allelic variants at the pvr1/eIF4E locus in pepper that confer extreme resistance to potyviruses when expressed transgenically in tomato. Dr. Grey at Cornell will provide greenhouse space and will help to screen and evaluate transgenic potato plants.
Not applicable.

Georgiana May
University of Minnesota, Twin Cities

We contracted web site construction and tools programming with Ethy Cannon. Progress described in Section A.
F. EDUCATION, TRAINING AND OUTREACH

Educational and training activities of each post-doc, graduate student, undergraduate and high school student involved in the project, include any outreach activities they participated in over the past year.

Barbara Baker
University of California, Berkeley

1. Personnel Education And Training Activities

Principal Investigator:
Barbara Baker
Barbara Baker is the head PI on the NSF Potato Genome Project, an adjunct associate professor in the Department of Plant and Microbial Biology at the University of California, Berkeley and USDA-ARS Senior Scientist. The goal of the Baker Lab is to understand the biochemical and molecular bases of plant resistance to pathogen diseases. As the NSF Potato Project PI, Barbara helped train the summer genomics workshop students during the time spent in her lab. In addition, Barbara contributed advice, guidance and knowledge to the preparation of the high school plant biotechnology curriculum.

Staff Research Associate/ Outreach Coordinator:
Meghan Flanagan (funded by the NSF Potato Genome Project)
Meghan Flanagan joined the Baker Lab in February 2001 as a lab assistant and student at the University of California, Berkeley. In late 2001, She helped edit and write the renewal grant, particularly the training and outreach portions. Upon graduation in May 2003 from UC Berkeley, Meghan began coordination of the outreach program. As outreach coordinator, Meghan is responsible for managing all aspects of the programs associated with the Center for Plant Genomics Training and Education. She created and distributed informational documents and materials to Potato Project PIs, NSF Program Managers, Advisory Committee members, participating programs, potential collaborators and other interested parties. These include packets for the Annual Potato Genome Meeting, a program description for the NSF Potato Genome Project pamphlet and the documentation and materials necessary for outside agency grant and award applications. Meghan taught and trained the Summer Genomics Workshop students during their six-week research program, and directed the summer research of a high school student working optimizing the laboratory portion of the high school curriculum. In addition, Meghan organized and coordinated the UC Berkeley/ UC Davis Summer Research Symposium and the Brown Lab Bioinformatics Workshops.

Associated Personnel:
Jennifer White (partially funded by the NSF Potato Genome Project)
Jennifer White is the Associate Director of Education for the University of California Botanical Garden. She coordinated and managed the Summer Genomics Workshop for the Botanical Garden, and coordinated with local elementary schools to establish and
maintain community gardens. She took part in student presentations at the UC Berkeley/UC Davis Summer Research Symposium hosted at the Botanical Gardens.

**Lauri Twitchell (partially funded by the NSF Potato Genome Project)**

Lauri Twitchell is a school garden specialist at the UC Botanical Garden. She has a Bachelor of Fine Arts in painting, a Master of Fine Arts in printmaking and a Master of Arts in Landscpe Design. As the former Crops of the World Garden Coordinator at the UC Botanical Garden, Lauri taught the summer genomics workshop students about characteristic Solanaceous morphology and horticultural techniques. She is also cooperating with the elementary school teacher from the Teacher Fellowship program to integrate school gardens and associated lessons into his fourth grade curriculum.

**Beej Cronin (funded by OASIS High School)**

Beej Cronin is the biology teacher at OASIS High School, a continuation high school in Oakland, CA. He is dedicated to the achievement of his students, and works with the Center for Plant Genomics Training and Education to select underrepresented and minority students for the Summer Genomics Workshop. Students involved in the program are required to make a presentation to their peers in biology classes when they return to school in the fall.

**High School Students:**

**Alexis Cavallaro (partially funded by the NSF Potato Genome Project)**

Alexis Cavallaro is a junior at OASIS High School. She will apply to college during the fall of 2006 with the eventual goal of becoming a research geneticist working to treat skin diseases such as her own, lamellar ichthyosis. She has retained her connections with the project, presenting her summer research to project collaborators and the community at the 2005 UC Berkeley/UC Davis Summer Research Symposium. When she returns to school in the fall, she will present her research poster, teach her peers a strawberry DNA extraction and actively recruit students for the 2006 Summer Genomics Workshop.

**Paula Pereira (partially funded by the NSF Potato Genome Project)**

Paula Pereira is a senior at MetWest High School, a continuation school in Oakland, CA. She is currently applying to college and would like to become a veterinarian. Paula presented her summer research to peers, teachers and mentors at the 2005 UC Berkeley/UC Davis Summer Research Symposium, and will present her research poster in her biology class this fall with the intention of recruiting students to participate in the 2006 Summer Genomics Workshop.

**Teacher Fellows:**

**Bernie Shellam (partially funded by the NSF Potato Genome Project)**

Bernie Shellam is an AP Biology teacher at the College Preparatory High School in Piedmont, CA. In conjunction with the outreach coordinator and a professional curriculum developer at the East Bay Biotechnology Education Program, Bernie is participating in developing plant biotechnology curriculum to be used in regular and AP Biology classes, as well as at science technical high schools. He will continue to work with the project piloting and revising the curriculum. The curriculum has been accepted,
an will be presented at the October 2005 National Association of Biology Teacher’s Conference.

Shary Rosenbaum (funded by the East Bay Biotechnology Program)
Shary Rosenbaum is the Director of the Each Bay Biotechnology Program, which is an organization focused on developing biotechnology curriculum, hosting summer teacher training workshops and providing biotechnology kits and support to East Bay, CA classrooms. Shary worked directly with Bernie Shellam and Meghan Flanagan to develop the plant biotechnology curriculum. She will be involved in piloting the curriculum in classrooms in the winter of 2005, revision of the curriculum in spring 2006, and will host teacher training workshops during the summer of 2006 to reach a larger audience of East Bay biology instructors. She has also agreed to present the curriculum with Bernie at the 2005 National Association of Biology Teachers conferences.

2. Outreach Objectives Carried Out
a. Objectives:
The Center for Plant Genomics Training and Education, established in 2002 with the fundamental mission to increase the participation of underrepresented students, teachers and the public in science education, particularly in the field of plant genomics research, coordinates education and training programs that not only increase science literacy by providing public access to current information and technology, but also bring together international and domestic scientists, teachers of science at elementary and high school levels and students of all grades to help reduce the gap in United States science education.

b. Specific Achievements:
1. Summer Genomics Workshop
Objectives
The Center for Plant Genomics Training and Education hosts a Summer Genomics Workshop (SGW) in the Baker Lab and Buell Lab each summer. The purpose of the workshop is to provide high school and college students, as well as teachers with hands-on science education and training experiences via a research project that uses genetic markers and morphology to determine the origins of the Potatoes of the Northwest, and validates and determine of the utility of current potato taxonomic and genomic resources to classify unknown cultivars.

Student Selection
Two underrepresented students, Alexis Cavallaro and Paula Pereira, were recruited from OASIS High School and MetWest High School, alternative high schools located in Oakland, CA. Both students participated in the 2005 UCB-TIGR six-week summer genomics workshop. The students were selected based on high achievement in biology courses, as well as desire to pursue a career in science. As opposed to previous years participants, the science fundamentals of these students was minimal. Additional educational materials were created to provide students with the necessary background.

Schedule
**Week One:** Students learned to identify specific Solanaceae and *Solanum* morphological characteristics at the UCBG. Working with the Crops of the World Garden Coordinator, they became familiar with horticultural techniques such as transplanting and propagation, and learned about inter-cropping, soil-water relations and organic farming. They planted the four Potatoes of the Northwest (Ozette, To-Le-Ak, Maria’s Potato, Haida) and created interpretive signs for the Crops of the World Garden. Their signs focused on defining and describing the differences between subspecies, cultivars and varieties of *Solanum tuberosum*.

**Week Two:** The students spent week two at the Baker Lab preparing solutions and becoming familiarized with the project, and their summer research project. In addition to their research activities, the students participated in teaching molecular biology concepts to a Berkeley third grade class, students participating in a summer science camp and the Bay Area Science Fair award winners and their parent and teacher chaperones. Alexis and Paula hosted an activity station where students, parents, teachers and USDA scientists participated in a strawberry DNA extraction activity. Not only did Alexis and Paula coordinate and set-up their station, but they also were solely responsible for directing the extraction.

**Week Three:** During the second week of the program, Alexis and Paula traveled with the outreach coordinator to Rockville, MD for genomics and bioinformatics training at The Institute for Genomic Research (TIGR). TIGRs “Genomics Boot Camp” provided them with the opportunity to practice pipetting, DNA extraction and familiarized them with the latest genomic technology. Upon completion of the two-day course, the students received bioinformatics training in Robin Buell’s lab. They learned to identify microsatellite-containing EST sequences in TIGRs non-redundant potato EST database and design oligonucleotide primer sequences. In addition, they participated in the initial screening of some of the primers designed from the database.

**Weeks Four and Five:** In the Baker Lab, Alexis and Paula prepared solutions, harvested and extracted genomic DNA from potato leaf tissue, performed PCR, ran agarose gels and analyzed results. Their summer research project involved optimizing the laboratory portion of the high school plant biotechnology curriculum. They tested out a number of primers using different DNA extraction methods to determine the most classroom-friendly and efficient methods.

**Week Six:** During the last week, Chih Tao and Sylviah reviewed public evaluations of their interpretive signage at the UCBG, conducted follow-up research and updated their signs based on visitor suggestions. They also created a poster describing the workshop and research results for Botanical Garden Foods of the Americas festival and other public presentations.

**Follow-Up**

Following the workshop, Alexis and Paula returned to the Baker Lab to present experimental results in lab meeting and receive feedback from lab members. They also participated in the first annual Student Summer Research Symposium, a collaboration between UC Berkeley, UC Davis and Applied Biosystems (Figure 1). When the students return to school in the fall, they will present their summer research in biology classes, and will teach their peers the strawberry DNA extraction.
Generation of Teaching Aides
During the workshop, each member of the Baker Lab presented a PowerPoint lecture on ideologies and techniques fundamental to molecular biology and to the students’ summer research. Lectures included PCR and restriction digests, microsatellites, biotethics and genetically modified organisms, silencing, microarrays, RNAi and plant-pathogen co-evolution. All lectures are available online in the Resources section of http://outreach.potatogenome.org.

2. Student Summer Research Symposium
Objectives
The Center for Plant Genomics Training and Education and Partnership for Plant Genomics Education at UC Davis host underrepresented summer research interns each summer. The purpose of the workshop was to provide these high school and college students participating in summer research with a forum to discuss their research experience and results. Hosted at the UC Botanical Garden by the Center for Plant Genomics Training and Education and the Partnership for Plant Genomics Education at UC Davis, nine students presented research posters and PowerPoint presentations to their peers, scientist mentors, parents and the public (Figure 2).
3. Community Gardens

Objectives
The Center for Plant Genomics Training and Education helps establish and maintain community gardens that 1) promote increased public science literacy in the areas of biodiversity, Solanaceous crops and applications of current genomics research; 2) provide students with a venue to present research to local communities; and 3) provide teachers with a resource for hands-on plant biology education.

UC Botanical Garden (UCBG)
The Crops of the World Garden (Crop Garden) at the UC Botanical Garden in Berkeley is a forum for understanding not only a plant’s economic, but also its cultural significance. Interpretive signs created by the Summer Genomics Workshop participants inform UCBG visitors about crop nutrition, cultural significance, probable geographic origins and distribution. The annual *Foods of the Americas* festival, a three-week school outreach program, celebrates and seeks to educate the public about crops developed by native people of North America, Mexico, Central America and the Andean region of South America. Over 900 4th-6th grade students, their teachers and parent chaperones, as well as over 2,000 adults visit the exhibit each year. The research activities of the 2003, 2004 and 2005 Summer Genomics Workshop participants are permanently installed as a portion of the potato exhibit.

Makah Nation Neophyte Garden
The Makah Nation created a garden that displays local indigenous plants, particularly members of the Solanaceae family and the Makah Nation’s cultural symbol, the Ozette potato. Displays encourage visitors to the garden to learn about the cultural and historical roles of indigenous plants in Native American culture. The Makah Nation is currently undertaking a project to research the economic feasibility of marketing some of these indigenous plants for landscape and gardening efforts.

Heritage University Community Evolution Garden
Established in 2002, the Heritage University evolution garden contains native species, heirloom and commercial cultivators of tomatoes, potatoes and peppers. The garden is used as an outdoor hands-on educational tool for Heritage University classes, and is the main teaching resource for the Get Set program, which is a program designed to teach 13-17 year old foster children independent living skills. In the garden, students learned basic nutrition, plant propagation, water management and garden skills. Classes were taught to Catholic Services, Yakama Nation Tribal School, Pace Alternative School, Granger School District and Summer Science Camp for Mt. Adams School District students.

4. K-12 Teachers of Science Professional Development and Training Fellowship Program

Objectives
The objectives of the K-12 Teachers of Science Professional Development and Training Program are 1) to increase interactions between scientists and teachers of science by engaging teachers in research efforts and national scientific conferences, and 2) to utilize educational transfer plans produced by the summer fellowship teachers to host Curriculum Training Workshops for East Bay, CA teachers.

**Curriculum Development**

Summer research and training in the Baker Lab during the summer of 2004 provided Bernie Shellam, a high school AP Biology teacher with a working knowledge of recent biological advances whose applications can be translated into relevant hands-on science curricula. Piloting the curriculum developed in 2004 in his classroom in the fall of 2004, Bernie suggested a change in primers to include greater polymorphism and strengthen the curriculum. Summer Genomics Workshop students tested a variety of primers and DNA extraction methods for their research project. The updated and revised curriculum was accepted for presentation at the October 2005 National Association of Biology Teachers Conference. The curriculum will be piloted this fall in West Contra Costa Unified School District cooperating classrooms. After evaluation and modification, the curriculum will be released nationally online at http://outreach.potatogenome.org and http://babec.org. Following online curriculum release, the East Bay Biotechnology Education Program (EBBEP) will host training workshops to familiarize East Bay teachers with the curriculum components, as well as the associated scientific techniques. EBBEP maintains a list of teachers interested in professional development, but the Center will also focus on engaging teachers unaware of such programs. Teachers who enroll in the course are qualified to borrow EBBEPs biotechnology loan kits for use in their classrooms throughout the academic year.

5. **Bioinformatics Workshop**

**Objectives**

The objective of the Bioinformatics Workshop is to tailor the bioinformatics resources of the Brown Lab, TIGR, National Center for Biotechnology Information (NCBI), Washington State University-Tri-Cities and Batelle Pacific Northwest Laboratory into an education and training workshop accessible to underrepresented high school students and teachers in the Prosser/ Tri-Cities Washington region.

**Student Selection**

Thirteen Hispanic high school students from Sunnyside High School, one Native American high school student from Neah Bay High School and three biology majors from Heritage University were selected to participate in the three day workshop based on their academic motivation and interest in science. In addition, one Native American and one Hispanic high school biology teacher attended the workshop with the intention of creating lesson plans that can be translated to classroom use.

**Workshop Curriculum**

The workshops intensive three-day curriculum included lectures and associated activities that engaged students at all levels of biology education. Presentations by staff researchers at TIGR, NCBI, USDA/ARS, UC Berkeley and Batelle Pacific summarized
the use of bioinformatics data in current research, providing a relevant context for workshop participants. Lectures included Introductory Genetics and Gene Structure, Nucleic Acids and the Genetic Code, Genomics, Introduction to the Potato Genome Project and the Institute for Genomic Research (TIGR), Genomic Sequencing and Annotation, Microarrays, as well as Bioinformatics and Bioethics. Hands-on lessons accompanied lectures, and students had the opportunity to build DNA models, make paper models of recombinant DNA, use TIGR databases and extract strawberry DNA (Figure 3).

**Figure 3.** High school students and teachers, as well as college students participated in an intensive 3-day workshop that introduced students to bioinformatics and genomics.

### Generation of Teaching Aides

During the workshop, each student was required to create a PowerPoint diary and present it to peers and instructors on the last day of the workshop. The two teachers who attended the workshop created PowerPoints in addition to writing up tentative lesson plans for their biology courses. Materials to build DNA models and extract strawberry DNA were provided to each of the teachers for use in their classrooms. All lectures and PowerPoints are available online in the Resources section of http://outreach.potatogenome.org.

### 6. Small-Scale Potato Marketing Study

**Objectives**

The goal of this research project was to employ a member of the Yakama Nation interested in pursuing a study of the commercial value of both cultivated and niche market potatoes for potential inclusion in a Yakama Nation economic development project.

**Background**
The Yakama Nation, located in the Yakima Valley, WA, is interested in incorporating small-scale specialty potato production into their economic development program. Gary Pierce, a Heritage College Business student, in conjunction with Chair of the Business Department and a local farmer, conducted analyses on the marketability of specialty potatoes with high levels of antioxidants. Door-to-door surveys collected in three Yakima Valley towns on quantities purchased, potato substitutes, consumer perception of the ease of cooking a meal containing potatoes and common motivators to buy potatoes sought to characterize common conceptions about the nutritional benefits of potato. A local vegetable grower pilot marketed three varieties of red, purple and yellow-fleshed potatoes at his roadside vegetable stand. Images and fact sheets accompanied the three-pound potato bags, explaining beneficial health properties of the potatoes high concentration of anthocyanin and carotenoid antioxidants. A preliminary summary of survey and pilot marketing results were presented to Potato Extension Specialist, Dr. Robert Thornton, of WSU and Dr. Joe Guenthner, Agricultural Economist from the University of Idaho. They advised that Gary grow specialty potatoes on a larger scale and pilot market them at Yakama Nation-owned vegetable stands.

Gary presented his data to local farmers and businessmen interested in the results of his study report. Based on the data, a local farmer agreed to plant 70 acres of red, purple and yellow-fleshed niche market potatoes, and is currently marketing them to Costco and other high-end retailers. Gary and his peers at Heritage University competed in the annual Students in Free Enterprise (SIFE) competition where they were awarded top honors.

![Figure 4. Yakama Nation business students, Meghan Flanagan and local farmer Richard Schell at Schell Produce stand displaying new marketing materials for the colored potatoes.](image)

7. Awards/ Recognition Events
USDA/ ARS Pacific West Area Research Apprenticeship
The ARS Research Apprenticeship Program is a student outreach program aimed at diversifying the workforce and including high school students in current scientific research. The program is designed to provide students with summer lab work experience and encourage students to continue to pursue careers in science. The Plant Genomics Training and Education Program was awarded with apprenticeships for summers 2003, 2004 and 2005 to support their summer genomics workshop student interns.

James Bradeen
University of Minnesota, Twin Cities

1. Personnel:

Postdocs: no postdoc is currently associated with this project

Graduate Students: no graduate student is currently funded by this project

(a) Maria Sanchez
MS student from Peru, joined my lab in the fall of 2002 and completed her MS degree in Plant Pathology in 2005. Maria was directly involved with the goals of the RB allelic mining project, specifically optimizing LR-PCR as a method for RB allele recovery, demonstrating the effectiveness of the method in a core collection of S. bulbocastanum genotypes, and evaluating evolutionary hypotheses presented in Song et al. (2003). Maria was an outstanding student and a valuable asset to the RB allelic mining project.

(b) Ben Millett
MS student, joined my lab in the fall of 2003. Ben was initially funded by an endowed competitive fellowships by the Department of Plant Pathology but has since been awarded an NSF Graduate Fellowship. Ben is characterizing the expression and phenotypic effect of the RB gene in wild and cultivated potato. Although not funded by the NSF Potato Genome Project, Ben’s research is closely aligned with the Project’s efforts.

(c) Ed Quirin
MS student, joined my lab in the fall of 2003. Ed has a BS in Biology from Cornell University and worked for two years as a lab technician in the laboratory of Dr. Molly Jahn. Ed’s experiences include marker development and application in pepper with specific emphasis on disease resistance regions. Ed’s research, supported by a grant from the University of Minnesota Graduate School,
includes the construction of an RGA library for the wild potato *S. bulbocastanum* and generation of integrated physical and genetic maps for RGAs. Ed’s research will not be supported directly by this NSF project.

(d) Riccardo Aversano

Riccardo is a PhD student in Plant Breeding & Genetics at the University of Naples, Italy. He works in the laboratory of my collaborator Dr. Domenico Carputo. From February, 2004 through June 2005, Riccardo was in my laboratory completing research and training in plant molecular genetics. Riccardo’s research included development of the EcoTilling approach for characterization of R gene haplotypes. These efforts, collaborative between our laboratory and those of Domenico Carputo, continue. Riccardo’s research was not supported by this NSF project.

(e) Ryan Syverson

Ryan Syverson has been in my lab since the summer of 2002, first as an undergraduate researcher and then, post graduation, as a junior scientist. In May 2004, Ryan joined my program as an MS student in molecular plant pathology. Ryan’s research entails construction of linkage maps in the wild potato *S. bulbocastanum*. Ryan’s research is not supported by this NSF project.

(f) Adriana Telias

Adriana Telias, a PhD student of Hispanic origin, is co-advised by myself and Dr. Emily Hoover (Department of Horticultural Science, UM). Adriana’s research focuses on the epigenetic control of anthocyanin production in the apple peel, including differential phenotype observed in ‘Honeycrisp’ apple. Adriana’s research is not supported by this NSF project.

(g) Steven McKay

Steven McKay, a PhD student, is co-advised by myself and Dr. Jim Luby (Department of Horticultural Science, UM). Steve’s research focuses on genetic mapping of genes imparting the “crispness” texture to fruit of the ‘Honeycrisp’ apple. Steve’s research is not supported by this NSF project.

_Undergraduate Students:_ no undergraduate student has been associated with my program during this funding year.

_High School Students:_ no high school student is currently associated with this project.

2. Planned activities
Graduate student Ed Quirin will complete his MS degree in February, 2006. Ed is currently seeking PhD opportunities. Other personnel changes are not anticipated. Graduate students Ben Millett and Ed Quirin will attend and present research results at the Plant and Animal Genome Conference in Jan 2006.

3. Outreach activities:

None.

Charles Brown
USDA-ARS and Washington State University, Prosser

1. Personnel education and training activities

Post Doc

Linhai Zhang has involved in marker development, linkage analysis, Marker-assisted selection screening, BAC library screening and contig construction for the \( R_{Mc1(bhh)} \) locus region. He also has helped nematode resistance screening.

Technician

Hassan Mojtahedi has engaged in the conduct of nematode resistance screening. He has introduced our entire mapping population \( \textit{in vitro} \). He has compared histologically the resistance reactions of the three species. He conducted the experiment that elucidated the systemic acquired resistance cross-protection interaction between sequential reactions of races 1 and 2.

Richard Quick is a USDA/ARS technician. He has a Bachelor Degree in Biology minor in Chemistry. He has been assisting all aspects of this project.

2. Activities planned for the coming year

We will conduct a Bioinformatics workshop if extra funding is made available. We will provide cuttings of Native American Potatoes to Neah Bay and other Native American groups in May of 2006.

3. Outreach activities carried out

In 2005 we carried out the third Bioinformatics Workshop. We had a strong representation of Hispanic Students and three Native Americans. At twenty participants it was the highest participation yet. Willem Rensink of TIGR provided excellent instruction as did David Culley of Pacific Northwest National Laboratories and Meghan Flanagan of the Albany lab. Dr Brown contacted groups that facilitate educational opportunities for Hispanics and contacted high school science teachers of High Schools with high percentages of Hispanic enrollment. Much of his recruiting was done through
extensive telephone communication after establishing key contacts. He also went to high schools and made presentations about the workshop. A number of Native Americans needed to travel long distances. Dr. Brown set the Workshop up so that long-distance travelers could be lodged locally. Staff in the USDA/ARS Prosser Unit traveled long distances to pick up some participants and return them to their homes, due to lack of resources in some households. The workshop took place over five days. Students listened to lecture style presentations and engaged in computer exercises in accessing genetic databases and performing analyses on complex genetic information. By the end of the workshop students were very skilled in the use of BLAST analyses to compare DNA sequences within the database. Dr. Brown designed the workshop so that feedback information useful for USDA/ARS and National Science Foundation would emerge from this. Each participant developed a biography and a powerpoint document as a daily journal and note-taking instrument which was submitted at the end of the course and made available to the sponsors, including the National Science Foundation “Potato Genomics Project” manager.

During the Summer 2005 a student studying Business at Heritage University in Toppenish Washington was supported as an NSF Outreach Activity. Gary Pierce, the student in question, developed educational brochures around the idea of using the genetic variation in phytonutrients, an underexploited resource in North America, to market potatoes differently than is done at present. Gary and his Professor Mentor, Len Black, took highly pigmented potatoes developed in Dr. Brown’s program and presented them for their phytonutrient characteristics to various large buyers. Costco indicated an interest in selling red and purple flesh potatoes paying a very high per pound price for them. Since the Native American Group to which Gary belongs is engaged in an Economic Revitalization Effort, the ideas that Gary, a Yakama Native American, is coming up with has real potential to effect the destiny of his people. Two
local growers undertook a fifty acre field of red, blue and yellow flesh potatoes. Gary’s brochures, developed in consultation with the growers were printed en masse and distributed along with the potatoes sold to Costco as educational materials.

Dr. Brown produced about 1,000 cuttings of virus-free Ozette, to-Le-Ak and Maria’s and took them to the Makah Nation on May 19, 2005. Yvonne Wilkie, a member of the Makah Nation arranged for a group of community members to meet and discuss the potato. Many of the questions centered around the proper culture and fertilization of the potato. On this visit he also recruited high school students to participate in the Bioinformatics Workshop. Also, five copies of a poster that Linhai Zhang had developed were distributed to participants, the cultural center, and the High School. One of the participants in the first bioinformatics workshop, Cassandra Smith, came to the meeting and upon seeing the poster with her name as one of the authors described the experience as “being carried away with emotion.” Cassandra has an abiding interest in science, and although her first attempt at living away from home at a college campus was not successful, she will try again. I told her we would help her in any way possible.
Robin Buell
The Institute for Genomic Research (TIGR)

Willem Rensink, a Staff Scientist on the project, presented a series of lectures on bioinformatics at a workshop in Summer 2005 hosted by Chuck Brown. In the summer of 2005, TIGR hosted for one week, two minority high schools students from the Oakland, CA area as part of the outreach component of the project. The students were given instruction in genomics, specifically in potato ESTs and their uses in genotyping. Willem Rensink presented a catalyst seminar at the University of Maryland about the Solanaceae genomic research at TIGR to prospective students. Over the summer of 2005 TIGR has hosted a summer intern (Nate Pumplin) to participate in the ongoing research and work on the verification of computationally predicted SSRs in Solanaceae species. We also hosted a second intern (Leslie Kaleikau) for a two-week period to receive research experience as part of her Scholar’s program at the University of Maryland.

In November-December 2005, Buell will host Yasmina Jaufeerrally-Fakim, a visiting Fulbright scholar from Mauritius. Dr. Jaufeerrally-Fakim will be trained in microarray methodologies and expression data analyses methods.

coll Buell presented a poster on the project progress at the 2nd Solanaceae Genome Workshop in Ischia, Italy and presented a talk at the Potato Satellite meeting. TIGR Staff Scientist Rensink attended the American Society for Plant Biology meeting in Seattle, WA in July 2005 and presented a talk on the heat stress response in potato as well as presented two posters about the progress of the project. At the Plant Genomic European Meeting 2005 in Amsterdam, the Netherlands, Rensink presented a poster on the Solanaceae functional genomic resources generated through the project.

Margaret Jahn
Cornell University

1. Personnel education and training activities

Postdoc
Dr. Arnon Ben-Chaim
Dr. Ben Chaim’s responsibilities have included leading the interaction with TIGR, building our digital platform for data analysis and management of results, genetic and genomic resources and analysis of pepper expression data. He attended a Keystone meeting in March, 2004 to present our comparative expression analyses. He has attended several bioinformatic trainings and workshops and established strong ties with our consulting statisticians for array analysis and with our CS department for database development and tool design.

Graduate Students:
Kari Perez
Kari Perez is a third year student currently funded by a supplement to this grant. Kari’s thesis research will focus on the SOLAR effort (Solanaceae dataset of R genes and R gene candidates) on two separate projects, \( L \) locus contig assembly and PVY resistance in potato.

**Charles Stewart**

Charles Stewart, a fifth year student, is now contributing to comparative expression analysis studies and other work related to comparative genetics and genomics in the Solanaceae. Charles has been involved in optimizing RNA extraction protocols and in generating expression profile data for pepper fruit genes via arrays and northerns. Charles won first prize in the student competition for his oral presentation at the annual national meeting in 2003 of the organization, MANNRS, Minorities in Agriculture, Natural Resources and Related Sciences. He is active in a number of organizations that promote diversity on campus and encourage younger students to pursue advanced education in science.

**Michael Mazourek**

Michael Mazourek is a fourth year student in the lab, funded on an NIH training grant, who is working on R gene evolution, R gene sequence analysis and synteny relationships. We plan a submission to the journal Genetics in early 2004. Michael has mentored the undergraduate student, Liz Cirulli.

**Undergraduate Students:**

**Elizabeth Cirulli**

Biology undergraduate major who has worked in our lab since Spring, 2002. Liz has developed the data base of R genes, has identified modern software to reannotate domains, and has contacted corresponding authors on numerous studies to obtain additional sequence data not in Genbank or obtain corrected sequence. Liz graduated in May 2005 and is now enrolled as a graduate student at Duke University, NC.

### 2. Planned outreach activities

In 2003, we turned our Public Seed Initiative outreach effort towards potato. This activity has been a highly successful mechanism to enhance delivery of improved germplasm in various crops to the private sector via seed distributors or farmer breeders. We have enlisted the cooperation of the potato breeder at Cornell, Walter de Jong, our potato seed specialist, Prof. Keith Perry, Dept. of Plant Pathology, Cornell, and a potato extension specialist, Prof. Don Halseth, Dept. of Horticulture. We will be using linkages we have established with the non-profit group, Northeast Organic Farming Association, to get Cornell varieties into participatory trials on organic ground throughout New York State during the field season, 2004. In November, 2004, several roundtables for organic growers have been held in Pennsylvania (11/10/04), NY (11/17/04) and Maine (11/24/04) to identify potato breeding and trialing objectives of importance to this community, which is small but growing very rapidly. In 2005, extensive trials were conducted for potato based on farms throughout the Northeast. Results are due from farmers November 11, and will be tabulated and reported as soon as possible.
3. Outreach activities

Our research has been communicated within Cornell University at weekly group meetings, and at Journal Club meetings and Departmental Seminars. In the past year or so, Jahn has given two seminars at University of California, Davis, University of South Carolina, University of Minnesota (grad-organized symposium), Purdue University, University of Florida (grad student-invited), New Mexico State (recipient of the Lowenstein lectureship which requires both a popular lecture and an academic seminar) and USDA. She has given presentations at the ASPB Plant Genetics meeting in Utah and PAG in San Diego in the NSF/USDA sponsored workshop on Outreach.

As part of our PSI, we now have more than 30 commercial licenses in force for germplasm developed in our breeding programs. We have recently received additional funding from the USDA Organic Research and Education Initiative to extend these activities more earnestly to the organic ag./sustainable ag. community. This award supports participatory trialing of disease-resistant breeding lines developed with marker-assisted genetics on organic ground managed by NY farmers. This allows us to demonstrate the benefits of investments in crop genomics to the alternative agriculture community in the Northeast through strong alliances we have developed a network of cooperating non-profit groups active in our region with interests in organic agriculture, crop genetic diversity, underserved or specialty markets, and rural livelihoods. We have been invited to make presentations about our work and this approach to extension outreach at seed conferences around the country from Maine to Washington state. We have conducted workshops in farmer-based breeding, seed production techniques and demonstrations at large fairs throughout the northeast, reaching hundreds of people through the workshops and thousands through the fairs.

Jiming Jiang and Sandra Austin-Phillips
University of Wisconsin, Madison

1. Personnel Education and training activities:

Graduate Students

Robert Stupar
Bob graduated in March, 2005 and now is a postdoctoral associate at the University of Minnesota. Bob has traveled to TIGR twice to collect and analyze microarray data with Dr C. R. Buell and her associates. Bob has also worked extensively with Prof. Brian Yandell’s group in the Department of Statistics, UW-Madison, for microarray data analysis. Bob’s first paper that was supported by this potato functional genomics award is accepted by Genetics and his second manuscript on microarray analysis of potato polyploidy will be ready for submission with 1-2 months.

Lara Colton
New Ph.D. student joined the Jiang lab in August, 2003. Lara is currently using a microarray-based approach to study the functional mechanisms of the RB-mediated late
blight resistance. Lara’s first paper on marker-assisted breeding for RB-mediated late blight resistance has been accepted for publication by *Crop Science*.

**Bala Pudota**

New Ph.D. student joined the Jiang lab in October, 2003. Bala is continuing Bob Stupar’s effort on gene expression analysis associated with potato polyploidy. He is currently confirming the “polyploidy-associated” genes identified by microarray using real-time PCR and Northern blot hybridization.

2. Outreach Activities

Dr. Austin-Phillips is the Director of the Plant Biotechnology Laboratory, which is part of the University of Wisconsin Biotechnology Center. The Plant Biotechnology Laboratory specializes in all aspects of plant tissue culture with emphasis on the genetic transformation of crop plants (particularly potato and alfalfa) using A. tumefaciens. Transformation services are offered to both academic and industrial researchers with a fee for service operation. Several faculty at UW have used the facility to produce transgenic plants. Having a centralized facility allows researchers in other disciplines to produce transgenic plants and more importantly, allows for the training of their graduate and postdoctoral associates in this area.

Working with the UW Biotechnology Center Outreach staff the Plant Biotechnology Laboratory also offers opportunities for outreach activities for K through 12 students. Middle and high school students visit the laboratory on a regular basis for hands-on plant tissue culture activities during the school year. There are also opportunities for minority students to participate in summer programs at the Biotechnology Center, which may involve plant tissue culture.

**Georgiana May**

**University of Minnesota, Twin Cities**

Educational/training activities of each postdoc, graduate student, undergraduate, and high school student involved in the project, including any outreach activities they participated in over the past year.

**Postdoctoral Research Assistants**

Dr. Brett Couch  
Activities above  
December 2003 - present

**Graduate Students**

David Schladt  
rotation student  
August - September 2005

**Undergraduate Students**

Karla Sorenson  
undergrad research assistant  
June 2005 - August 2005
• Karla was involved in developing markers of potential use for determining the evolutionary history of the NW Native American potatoes.

**Publications supported or partially supported by the potato genome project, to date:**


Couch, B., and May, G. 2006. Recombination does not constantly lead to rearranged NBS-LRR genes in the Solanaceae. *in prep* *J. Mol. Evol.*

**Software**
Modification of ATV (All Taxa Viewer), Ethy Cannon and C. Zmasek. Modification of ATV allows user to call up data files, and groups of sequence files from a phylogenetic display.
Related Work (not funded under PGI)


• collaborative research, assistance in data analysis, writing instruction, editing.


• provided ideas using SNPs in mapping, data analysis, writing.


Outreach activities:

Georgiana May has frequent, informal interactions with a potato breeder (C. Theill, Agronomy) and potato pathologist, Jim Bradeen (Plant Pathology). As we develop methods of search for new sources late blight resistance, these will be communicated to these potential users.
G. STATUS OF DIVERSITY

(1) Status of diversity among the project personnel, including all undergraduates, graduate students, and postdoctoral fellows supported by the project. (2) Reports on proactive steps taken by individuals within the project and/or the project as a whole to increase the role of members of under-represented groups in the activities of the project.

Barbara Baker  
University of California, Berkeley

1. Status of diversity among the project personnel, including all undergraduates, graduate students, and postdoctoral fellows supported by the project.

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Underrepresented Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexis Cavallaro</td>
<td>High School Student, Summer Genomics Workshop Participant</td>
<td>Female</td>
</tr>
<tr>
<td>April Dobbs</td>
<td>UCB Undergrad, Assistant II (work study)</td>
<td>Female</td>
</tr>
<tr>
<td>Meghan Flanagan</td>
<td>Staff Research Associate II, Outreach Coordinator</td>
<td>Female</td>
</tr>
<tr>
<td>An Hoang</td>
<td>UCB Undergrad, Assistant II</td>
<td>Asian Male</td>
</tr>
<tr>
<td>Hanhui Kuang</td>
<td>Associate Specialist</td>
<td>Asian Male</td>
</tr>
<tr>
<td>Wing Yee Lau</td>
<td>UCB Undergrad, Assistant II</td>
<td>Asian Female</td>
</tr>
<tr>
<td>Hajie Liu</td>
<td>Staff Research Associate I</td>
<td>Asian Female</td>
</tr>
<tr>
<td>Jianzhong Liu</td>
<td>Associate Specialist</td>
<td>Asian Male</td>
</tr>
<tr>
<td>KwangChul Oh</td>
<td>Postdoctoral Fellow</td>
<td>Asian Male</td>
</tr>
<tr>
<td>Sharimen Newaz</td>
<td>UCB Undergrad, Assistant II (work study)</td>
<td>Asian Female (Bangladesh)</td>
</tr>
<tr>
<td>Paula Pereira</td>
<td>High School Student, Summer Genomics Workshop Participant</td>
<td>Hispanic Female</td>
</tr>
<tr>
<td>Mathilda Regan</td>
<td>Assistant II</td>
<td>Female</td>
</tr>
<tr>
<td>Maureen Richey</td>
<td>Staff Research Associate III, Lab Manager</td>
<td>Female</td>
</tr>
<tr>
<td>Bernie Shellam</td>
<td>Teacher Fellow (part-time supporting outreach)</td>
<td>African-American Male</td>
</tr>
<tr>
<td>Laurie Twitchell</td>
<td>UCB Grad Student (part-time supporting outreach)</td>
<td>Female</td>
</tr>
<tr>
<td>Jennifer White</td>
<td>UCB Grad Student (part-time supporting outreach)</td>
<td>Female</td>
</tr>
<tr>
<td>Ayako Kamei Yamamoto</td>
<td>Visiting Postdoctoral Researcher</td>
<td>Asian Female</td>
</tr>
</tbody>
</table>
2. Proactive steps to increase the role of members of under-represented groups in the activities of the project

Each year, we identify two underrepresented minority high school students to participate in the Summer Genomics Workshop. During summer 2005, Paula Pereira and Alexis Cavallaro were recruited to the program. During the summer of 2004, we also recruited a minority elementary school teacher to participate in our first Teachers of Science Summer Fellowship Program. He continues to work with the project in curriculum development efforts. In addition, as employment opportunities become available they are advertised via UC Berkeley's web page and the potatogenome.org web page.

James Bradeen
University of Minnesota, Twin Cities

Statement of general policy: The Bradeen lab is acutely and actively dedicated to providing a dynamic, diverse work environment that is free of discrimination and representative of the scientific and University communities at large. Although small (currently a total of 7 researchers, including the PI), our laboratory includes a diversity of sexes, races, sexual orientations, nationalities, and religious backgrounds/beliefs. We are fully compliant with and supportive of the stated University of Minnesota non-discrimination policy.

1. Personnel (Note: no Bradeen Lab members are funded by this NSF project)
   Maria Sanchez (Graduate Student) Hispanic, female
   Adriana Telias (Graduate Student) Hispanic, female

2. Efforts to enhance participation:
The University of Minnesota Life Sciences Summer Undergraduate Research Program (LSSURP; www.cbs.umn.edu/main/summer_research) facilitates training opportunities for 50 undergraduates each year. Approximately 50% of participants are from underrepresented minority groups and 25% of participants are first generation college students. Participants in LSSURP are from geographically diverse parts of the US and its territories, including from non-research institutions. LSSURP has formal recruitment collaborations with Fort Valley State University (Fort Valley, GA; an “1890s” school primarily serving the African American community) and with The University of Puerto Rico—Cayey. LSSURP students spend 10 weeks on the UM-Twin Cities campus conducting research. Since July 2004, I have been in contact with LSSURP staff to identify an appropriate student to participate in training activities in my lab during the summer 2006.

Charles Brown
USDA-ARS and Washington State University, Prosser
1. Status of diversity among the project personnel, including all undergraduates, graduate students, and postdoctoral fellows supported by the project.

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Underrepresented Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linhai Zhang</td>
<td>Post-doctoral scientist</td>
<td>Asian</td>
</tr>
<tr>
<td>Candelaria Moreno</td>
<td>Helper</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Encarnación Rivera</td>
<td>Technician</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Ricarda Castañeda</td>
<td>Helper</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Alex Moreno</td>
<td>Helper</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Hassan Mojtabahi</td>
<td>Technician</td>
<td>Iranian</td>
</tr>
<tr>
<td>Gary Pierce</td>
<td>Outreach Heritage University Student</td>
<td>Yakama</td>
</tr>
<tr>
<td>Veronica Almeida</td>
<td>Outreach High School Student and Workshop Participant</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Teri Alvarez-Ziegler</td>
<td>Outreach High School Science Teacher and Workshop Participant</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Wilson Arnold</td>
<td>Outreach High School Science Teacher and Workshop Participant</td>
<td>Makah Native American</td>
</tr>
<tr>
<td>Eldonna Beal</td>
<td>Outreach Heritage University Student and Workshop Participant</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Josephina Chavez</td>
<td>Outreach High School Student and Workshop Participant</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Sarah Ehmer</td>
<td>Outreach Heritage University Instructor and Workshop Participant</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Elida Farias</td>
<td>Outreach High School Student and Workshop Participant</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Celia Fernandez</td>
<td>Outreach High School Student and Workshop Participant</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Jessica Galvan</td>
<td>Outreach High School Student and Workshop Participant</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Maria Garibay</td>
<td>Outreach High School Student and Workshop Participant</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Camella George</td>
<td>Outreach Heritage University Student and Workshop Participant</td>
<td>Yakama Native American</td>
</tr>
<tr>
<td>Ymelda Guizar</td>
<td>Outreach High School Student and Workshop Participant</td>
<td>Hispanic</td>
</tr>
<tr>
<td>Addy Hernandez</td>
<td>Outreach High School Student and Workshop Participant</td>
<td>Hispanic</td>
</tr>
</tbody>
</table>
2. Proactive steps to increase the role of members of under-represented groups in the activities of the project

There is an ongoing effort to recruit Hispanics from the local population which constitutes 40% of the population.

Robin Buell  
The Institute for Genomic Research (TIGR)

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Underrepresented Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Hart</td>
<td>Technician</td>
<td>Asian, Female</td>
</tr>
<tr>
<td>K. Rehfeld</td>
<td>Technician</td>
<td>Caucasian, Female</td>
</tr>
<tr>
<td>J. Liu</td>
<td>Bioinformatic Analyst</td>
<td>Asian, Female</td>
</tr>
<tr>
<td>E. Ly</td>
<td>Bioinformatic Engineer</td>
<td>Asian, Male</td>
</tr>
<tr>
<td>W. Rensink</td>
<td>Staff Scientist</td>
<td>Caucasian, Male</td>
</tr>
<tr>
<td>J. Zaborsky</td>
<td>Technician</td>
<td>Caucasian, Female</td>
</tr>
<tr>
<td>R. Buell</td>
<td>Faculty</td>
<td>Caucasian, Female</td>
</tr>
<tr>
<td>A. Cowles</td>
<td>Technician</td>
<td>African-American, Female</td>
</tr>
</tbody>
</table>

Margaret Jahn  
Cornell University

1. Status of diversity among the project personnel, including all undergraduates, graduate students, and postdoctoral fellows supported by the project.

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Underrepresented Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mary Kreitinger</td>
<td>Lab Manager</td>
<td>Female</td>
</tr>
<tr>
<td>Sherry Roof</td>
<td>Research Technician</td>
<td></td>
</tr>
<tr>
<td>Nicole Moskal</td>
<td>Undergrad</td>
<td></td>
</tr>
<tr>
<td>Liz Cirulli</td>
<td>Undergrad</td>
<td></td>
</tr>
<tr>
<td>Shanna Moore</td>
<td>Postdoc</td>
<td></td>
</tr>
</tbody>
</table>
Maryann Fink  
Brynda Beeman  
Research Technician  
Research Aide

Kari Perez  
Graduate Student  
Hispanic, female

B.C. Kang  
Inhwa Yeam  
Research Associate  
Grad Fellow  
Asian male  
Asian female

Charles Stewart  
Grad Fellow  
African-American male

2. Proactive steps to increase the role of members of under-represented groups in the activities of the project

Statement of general policy: The Jahn lab is actively dedicated to providing a dynamic, diverse work environment that is free of discrimination and representative of the scientific and University communities at large. We actively enhance participation of minorities and others whose backgrounds might not typically lead them into research through targeted recruitment, word-of-mouth, the MANNRS organization, and contacts at 1890 schools including active collaborations with Alcorn State U., Alcorn, MS, West Virginia State University, and schools with strong Hispanic presence including University of Texas at Kingsville, Cal State Fresno and New Mexico State University.

Georgiana May  
University of Minnesota, Twin Cities

(1) Brett Couch is Canadian

(2) Funding was requested by G. May for David Schladt who is interested in a teaching career. Undergraduate students have been identified through courses and through the College of Biological Sciences undergrad research programs for participation in the project.
### H, I, J, J’. STATUS OF COLLABORATORS WITH PGRP PARTICIPANTS AND OTHER COLLABORATORS

**Barbara Baker**  
**University of California, Berkeley**

<table>
<thead>
<tr>
<th>Partnership</th>
<th>Description</th>
<th>PGRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradeen Lab</td>
<td>Possible collaboration for long range PCR on the R7 candidate from R7 region BAC</td>
<td>PGRP-Continuing</td>
</tr>
<tr>
<td>Brown Lab</td>
<td>Physical mapping of the Rmc1 region in <em>S. demissum</em> and <em>S. bulbocastanum</em>. Provided seed from wild Solanum species for UCBG for summer genomic program. Development of the outreach project’s goals and curriculum</td>
<td>PGRP-Continuing</td>
</tr>
<tr>
<td>Buell Lab</td>
<td>Assistance with BAC contig assembly, sequencing. BAC annotation and analysis. Provides all necessary information and materials for microarray analysis. Organized ‘genomics camp’ for training El Cerrito High School students.</td>
<td>PGRP-Continuing</td>
</tr>
<tr>
<td>Jones/Brigneti Lab</td>
<td>R gene mining. Provided Baker lab with silencing protocols.</td>
<td>PGRP-Continuing</td>
</tr>
<tr>
<td>Jiang Lab</td>
<td>Provided excellent help on physical mapping in <em>S. demissum</em> by FISH analyses using BACs and <em>S. demissum</em> seeds provided by the Baker lab.</td>
<td>PGRP-Continuing</td>
</tr>
<tr>
<td>UC Davis</td>
<td>David M. Tricoli provides plant transformation services for all tomato, <em>N. benthamiana</em> and including all those transformations requiring hygromycin selection. MingCheng Luo and Frank You provided fingerprinting services and browser based data viewing and analysis increasingly user friendly and accessible. Provides the Baker lab with latest software upgrades for fingerprinting analyses.</td>
<td>Governmental New</td>
</tr>
<tr>
<td>University of Arizona</td>
<td>Distribution of potato cDNA clones</td>
<td>Governmental New</td>
</tr>
<tr>
<td>USDA/ARS</td>
<td>Olin Anderson, Yong Qu and Devin Derr have provided use of the Q-bot for replication of PT29 <em>S. bulbocastanum</em> BAC library. Dave Hane of the Anderson lab has provided computer advice</td>
<td>Governmental Continuing</td>
</tr>
</tbody>
</table>
Maria Rosa Marano, Universidad de Rosario, Argentina

Provided key markers to assist in the assembly of a BAC contig spanning the Nb region.

Wageningen University & Research Centre

Collaboration with Herman VanEck and Richard Vissar on physical and genetic mapping in the R3, R6 and R7 regions. Provides markers and information on progress in mapping.

James Bradeen
University of Minnesota, Twin Cities

H. Collaborations with PGRP awardees: (1) Continuing:

University of Wisconsin

The Bradeen lab continues a long and fruitful collaboration with the Jiang and Austin-Phillips labs (University of Wisconsin) including sharing of research materials (in vitro plants, tubers, BAC libraries, PCR primers, etc.), protocols, and late blight screening data. During 2004, Austin-Phillips and I submitted a joint USDA-NRI proposal.

University of Minnesota

The Bradeen lab also continues collaborations with the May lab (University of Minnesota) that includes frequent face-to-face meetings, emails, and phone calls. These interactions have been enhanced following the hiring of Brett Couch by the May lab during 2004. The May lab has expressed support for inclusion of mapping data generated by my lab (not funded by this NSF project) into their burgeoning database on Solanaceae R genes.

USDA—ARS

The Brown lab (USDA—ARS) supplied my program with an S. bulbocastanum F1 mapping population. This population is currently being used by the Brown lab to map Columbia root knot nematode resistance and by our lab to construct a genome wide scaffold map for R genes (not funded by this NSF proposal), and validation of markers for EcoTilling. AFLP data generated by my lab is to be shared with the Brown lab in its entirety.

I. Collaborations with private industry: None

J. Collaborations with international community: (1) Continuing: Dr. Domenico Carputo (University of Naples, Italy) sent graduate student Riccardo Aversano to my laboratory for 15 months (Feb 2004 – June 2005). Riccardo will receive his PhD from the
University of Naples (Dec 2005), but completed a portion of his research in molecular plant genetics in my lab. Riccardo’s research includes the isolation of R gene homologs from the wild potato *S. bulbocastanum*, construction of integrated physical and genetic maps for these R genes, and EcoTilling. Riccardo’s research is not supported by this NSF project. (2) New: none

**Charles Brown**  
**USDA-ARS and Washington State University, Prosser**

<table>
<thead>
<tr>
<th>Partnership</th>
<th>Description</th>
<th>PGRP Private International Governmental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barbara Baker</td>
<td>I have collaborated with Barbara Baker of USDA/ARS, Albany, CA.</td>
<td>PGRP Continuing</td>
</tr>
<tr>
<td>Sandra Austin-Phillips</td>
<td>Sandra Austin-Phillips is transforming potato varieties economically important to the Columbia Basin with Pepper pvr1/eIF4E factor, with the intent of testing for resistance to diverse strains of potato virus Y (PVY). We provided cultures of these cultivars and protocols for transformation to Dr. Austin Phillips and Kari Perez</td>
<td>PGRP continuing</td>
</tr>
<tr>
<td>Jiming Jiang</td>
<td>We screened protoplast fusion hybrids with PT29 got resistance to Green Peach Aphid</td>
<td>PGRP continuing</td>
</tr>
<tr>
<td>Washington State Potato Commission</td>
<td>I have received grants for 17 years from the Washington State Potato Commission to develop resistance to Columbia Root-Knot nematode and corky ringspot. A high performing clone has been in trials for two years in the Tri-State Variety Trials and was promoted to the Western US Regional Trial for 2006. The Washington State Potato Commission has a real stake in our research on RMc1(blb) as it is a genetic factor controlling a gene which when incorporated into new potato varieties will provide a true host resistance based non-pesticidal control of the most significant soil-borne pest of the Columbia Basin.</td>
<td>Governmental Continuing</td>
</tr>
</tbody>
</table>

**Robin Buell**  
**The Institute for Genomic Research (TIGR)**
### Partnership Description

<table>
<thead>
<tr>
<th>Partnership</th>
<th>Description</th>
<th>PGRP Private International Governmental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various</td>
<td>The TIGR personnel routinely interact with other PGRP awardees at TIGR, including the pine EST and microarray project, the rice oligo array project, and the maize oligoarray project. Through our Expression Profiling Service, we have established collaborations with a range of scientists throughout the world.</td>
<td>Various</td>
</tr>
</tbody>
</table>
| Baker       | USDA PGEC/U. California  
Baker and Jahn have had a long association focused on comparative structural and functional analysis of dominant and recessive genes for disease resistance in the Solanaceae. Paran and Van Eck are also contributing to active exchanges of markers required for assembly of the pepper contig for chromosome 11. | PGRP                                    |
| Bradeen     | University of Minnesota  
We will collaborate to identify pepper homologs and homologous genomic regions                                                                                                  | PGRP                                    |
| Austen Phillips | University of Wisconsin  
We sent constructs for potato transformation in late November, 2004 and hope to receive transformants back by late 2005.                                                                 | PGRP                                    |
| Giovannoni, Rose, | Cornell University  
Jahn directs a USDA IFAFS funded project on Capsicum genomics with a number of collaborators                                                                                                                                 | PGRP Completed                         |

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**Margaret Jahn**  
**Cornell University**
The Jahn lab has numerous collaborators worldwide on projects that relate to or support work or exchange of personnel on this project including Ilan Paran, Israel; B.D. Kim, Korea; I.G. Mok Korea; A. Palloix and C. Caranta, France; Paul Gennai, AVRDC, Taiwan; Umesh Reddy, WVSU; Ravi Reddy, Alcorn State University, Alcorn, MS, John Murphy, Auburn U, Alabama, Mary O’Connell, New Mexico State University and collaborations with many seed companies around the world.

Jiming Jiang and Sandra Austin-Phillips
University of Wisconsin, Madison

<table>
<thead>
<tr>
<th>Partnership</th>
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<th>PGRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toluca, Mexico</td>
<td>Jiang/Austin-Phillips group has sent materials to Toluca, Mexico for late blight resistance evaluation</td>
<td>International</td>
</tr>
<tr>
<td>Brown Lab, Bradeen Lab</td>
<td>Jiang/Austin-Phillips group has sent transgenic potato lines containing the RB gene for field late blight resistance evaluation</td>
<td>PGRP</td>
</tr>
<tr>
<td>Baker Lab</td>
<td>Dr. Jiang’s group has helped Dr. Baker’s group with FISH analysis on R7-related BAC clones</td>
<td>PGRP</td>
</tr>
</tbody>
</table>

Georgiana May
University of Minnesota, Twin Cities

h. Status of Collaboration With Other PGRP Awardees: Steve Cannon and Nevin Young (Plant Pathology; Medicago PGRP) and the May Lab are developing models for evolution of resistance genes and collaborating on web tools and programming for SOLAR.
K. FEEDBACK FROM THE USER COMMUNITY

Barbara Baker
University of California, Berkeley

We have had positive feedback from the community on our outreach program. Several colleagues have provided positive feedback on our website.

James Bradeen
University of Minnesota, Twin Cities

None to date.

Charles Brown
USDA-ARS and Washington State University, Prosser

Our collaborators at Heritage College have expressed great enthusiasm for the Garden and Potato marketing Project. They feel this will facilitate their interest in strengthening life science and agricultural production curriculum at the College. The Makah Nation expressed tremendous satisfaction that genetic fingerprinting has elucidated the relationship of the Ozette potato to other cultivated potatoes in the world. They also were quite happy to see the poster describing the work and were happy that members of their Nation had participated in the work. Native Alaskans and Non-Natives expressed tremendous enthusiasm for the information concerning Maria’s potato and its relationship to other cultivated potatoes. They wanted to set up a Native Alaskan Potato Research Project and search for other, previously uncollected Native varieties.

Robin Buell
The Institute for Genomic Research (TIGR)

At TIGR, we frequently receive e-mail regarding the potato web page and have incorporated several remarks to improve the web page design. We have expanded our FAQ page to provide the users more available information on our resources, especially the microarrays and the Solanaceae Gene Expression Database.

Margaret Jahn
Cornell University

None to date.

Jiming Jiang and Sandra Austin-Phillips
University of Wisconsin, Madison
Several groups have already developed late blight resistant potato using the $RB$ gene. We have received numerous praises from the research and industry communities on our effort of cloning the $RB$ gene.

Georgiana May  
University of Minnesota, Twin Cities

None to date.
L. WORK PLAN FOR THE UPCOMING YEAR

Barbara Baker  
University of California, Berkeley

We will complete structural analyses of \( R_{Mc1} \) region in \( S. demissum \), complete phylogenetic analyses of MITE 1 in Nicotiana species and assess its role in evolution of TMV resistance. We will complete comparative analyses of MITEs in the Solanaceae. We will complete expression profiling and analyses of Sgt, TMV- challenged \( N. benthamiana \). We will complete analyses and make publicly available all transgenic material and corresponding vectors. We will submit three papers one the MLB locus (nearly complete), N-MITE 1 analyses and characterization of other MITE sequences identified in the Solanaceae (draft nearly complete).

James Bradeen  
University of Minnesota, Twin Cities

During year 4, we will continue efforts to adapt EcoTilling and DGGE for fingerprinting \( RB \) related genome regions from throughout the genus \( Solanum \), including \( S. bulbocastanum, S. polyadenium, \) tomato, and eggplant. As outlined above, our sampling will include several genotypes from multiple populations for each taxon. Paired with targeted sequencing and with completed LR-PCR sequencing efforts, our planned research will enable diversity assessment of \( RB \) haplotypes (EcoTilling project) and \( RB \) homologous alleles (DGGE project) in diploid \( Solanum \) spp. as a function of (a) classification, (b) geographic origin, (c) within vs. between population relationships, (d) late blight host vs. non-host, and (e) inbreeding vs. outcrossing mating habit. We anticipate that, beyond providing sequence data for the SOLAR database and elucidating the evolutionary history of \( RB \) (goals of this project), the EcoTilling and DGGE protocols that will results will be of widespread utility.

Charles Brown  
USDA-ARS and Washington State University, Prosser

1. Determine linkage relationship between genetic factor(s) for tuber resistance and \( R_{mc1(bh)} \)
2. Construct contig using PT29 BACs.
3. Send out BAC candidates for sequencing at TIGR.
4. Determine inheritance of resistance to \( M. chitwoodi \) in the \( S. fendleri \) population

Robin Buell  
The Institute for Genomic Research (TIGR)

1. We will sequence and annotate BACs from the project participants per year.
2. We will continue with our in-house abiotic stress experiments.
3. We will continue to improve and update our Solanaceae Gene Expression Database and associated web pages so that the users can maximally benefit.
4. We will complete studies for approved users of the Expression Profiling Service and begin the next round.
5. We will continue to make the potato cDNA array available to the public on a cost recovery basis.

Margaret Jahn  
Cornell University

No activities planned for upcoming year.

Jiming Jiang and Sandra Austin-Phillips  
University of Wisconsin, Madison

1. Late blight disease resistance testing of 150 plants per year
2. Maintenance of key in vitro plant germplasm
3. Transformations with 10 candidate disease resistance genes/year
4. Complete microarray studies of RB-mediated late blight resistance
5. In-depth analysis of candidate genes associated with polyploidy and late blight resistance

Georgiana May  
University of Minnesota, Twin Cities

1. Complete mapping of representative \(I2\)-homologous sequences in tomato mapping populations by identifying BACs carrying \(I2-h\) and mapping unique flanking sequence.
2. Further develop informational and interactive website with Ethy Cannon.
M. PERSONNEL AND FINANCIAL REPORT

Barbara Baker
University of California, Berkeley

(1)

<table>
<thead>
<tr>
<th>Name</th>
<th>Funding</th>
<th>Role</th>
<th>Technical Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gianinna Brigneti</td>
<td>NSF 01/01/03 to 10/05</td>
<td>Post-doctoral Researcher in collaborators lab (J. Jones Lab)</td>
<td>Gianinna conducted <em>P. infestans</em> R gene mining. She has studied disease resistance in potatoes since 1993 and has extensive experience with late blight tests and mining late blight genes.</td>
</tr>
<tr>
<td>April Dobbs</td>
<td>NSF 5/23/05 to 8/18/05</td>
<td>UCB Undergrad, Assistant II</td>
<td>April Dobbs is a UCB undergraduate majoring in plant and microbial biology. She assisted several members of the lab with preparation of media, plant propagation, and molecular biology techniques over summer 2005.</td>
</tr>
<tr>
<td>Megan Flanagan</td>
<td>NSF 10/01/03 to present</td>
<td>Staff Research Associate II (Outreach Coordinator)</td>
<td>Meghan directly supervised high school students, coordinating all garden activities and outreach activities and accompanied students on their visit to TIGR.</td>
</tr>
<tr>
<td>An Hoang</td>
<td>NSF (50%) 11/7/05 to present</td>
<td>UCB Undergrad, Assistant II (work-study)</td>
<td>An is a UCB undergrad. He is assisting several members of the lab with preparation of media, plant propagation, and molecular biology techniques.</td>
</tr>
<tr>
<td>Hanhui Kuang</td>
<td>NSF 8/1/03 to present</td>
<td>Associate Specialist</td>
<td>Dr. Kuang received his PhD in genetics from the University of Georgia, Athens. Hanhui joined the lab after postdocs in Israel and at UC Davis where he studied resistance genes in several species. He is currently working on the evolution and organization of resistance gene clusters in potato.</td>
</tr>
<tr>
<td>Haijie Liu</td>
<td>NSF To present</td>
<td>Staff Research Associate I</td>
<td>Haijie has a MA in Food Microbiology from Kansas State University. She is working on microarray expression profiling of Sgt1-silenced <em>N</em>-transgenic <em>N. benthamiana</em> and other functional genomics projects.</td>
</tr>
<tr>
<td>Jianzhong Liu</td>
<td>NSF 9/28/05</td>
<td>Associate Specialist</td>
<td>Dr. Liu received his PhD in Genetics from the Institute of Genetics at the Chinese</td>
</tr>
<tr>
<td>Name</td>
<td>Position</td>
<td>Department/Location</td>
<td>Description</td>
</tr>
<tr>
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<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Dr. Liu</td>
<td>to present</td>
<td>Academy of Sciences</td>
<td>Dr. Liu completed worked as a post-doc in Hong-Kong, Singapore and most recently the Noble Foundation before joining the lab. He is currently working on functional analysis of genes involved in Solanaceae disease resistance.</td>
</tr>
<tr>
<td>Kwangchul Oh</td>
<td>NSF 10/15/04 to present</td>
<td>Visiting Post-doctoral Researcher</td>
<td>Dr. Oh received his PhD in Plant Physiology from Oregon State University. He joined the lab after postdoc at University of North Carolina where he studied heterotrimeric G-protein mediated signal transduction pathways in Arabidopsis. He is currently working on the functional analysis of resistance genes in Solanaceae.</td>
</tr>
<tr>
<td>Maureen Richey</td>
<td>NSF 09/23/04 to present</td>
<td>Staff Research Associate III (Lab Manager)</td>
<td>Maureen has a B.A. in Molecular and Cell Biology from UCB and worked in the lab previously as a staff research associate (2002-2003). Maureen has taken over management and administrative aspects of the project.</td>
</tr>
<tr>
<td>Wai Shum</td>
<td>NSF 2/1/04 to 8/31/05</td>
<td>Assistant II</td>
<td>Wai is a recent UCB graduate in bioengineering and is applying to graduate school in bioinformatics. He assisted Dr. Hanhui Kuang in studying the organization and evolution of resistance gene clusters in potato.</td>
</tr>
<tr>
<td>Laurie Twitchell</td>
<td>NSF 01/01/03 to present</td>
<td>UCBG grad student (part time supporting outreach)</td>
<td>Laurie is a graduate student at the UC Botanical Gardens. She is assisting with the Solanaceae diversity exhibit and teaching outreach participants about gardening and plant biology.</td>
</tr>
<tr>
<td>Jennifer White</td>
<td>NSF Asst. Director of Education at UCBG 5%</td>
<td>Dr. White is directing the UCBG’s outreach responsibilities. She is supervising grad student Twitchell and coordinating</td>
<td></td>
</tr>
<tr>
<td>Ayako Kamei Yamamoto</td>
<td>NSF 4/1/05 to present</td>
<td>Visiting Post-doctoral Researcher</td>
<td>Dr. Kamei received her PhD in bacteriology from the University of Tokyo, Japan. Ayako joined the lab after a postdoc at RIKEN (Japan) where she studied abiotic stress inducible transcription factor in Arabidopsis in the Shinozaki lab. She is currently working on the isolation and functional analysis of resistance genes in potato.</td>
</tr>
</tbody>
</table>
Miki Yamamoto 01/01/03 to 9/14/05 Graduate Student Researcher Miki completed her PhD in PMB at UCB this year. She conducted functional genomic studies on pathogen challenged Solanaceae using microarray-based expression profiling.

Zhong-Lin Zhang NSF 8/1/04 to 8/31/05 Assistant Specialist Dr. Zhang received a PhD in both biomedical engineering and cellular and molecular biology from Zhejiang University in China. Zhong-Lin joined our lab after a postdoc at the University of Nevada. He investigated the function of resistance gene candidates through virus induced gene silencing.

(2) Cost sharing: None
(3) Carryover: None

James Bradeen
University of Minnesota, Twin Cities

(1) Personnel Financial Report (see attached)
(2) Cost Sharing: None
(3) Carryover: None

Charles Brown
USDA-ARS and Washington State University, Prosser

(1) Dr. Linhai Zhang is a Postdoc involving in mapping the $R_{Mc1/bb}$ locus. He also has helped nematode resistance screening. He received his Ph.D. from Clemson University in Genetics.

Dr. Hassan Mojtahedi, a Nematologist, is employed by the USDA/ARS, as a technician. He is a well-known Nematologist with an extensive publication record. He received his Ph. D. from UC Davis in 1974. He has been screening our mapping population for resistance. He has also screened the $S. fendleri$ introgression population, carried out the successful demonstration of type of “cross-protection” in root knot nematode materials. He is instrumental in discovering the dissection of resistance into root and tuber components.

Richard Quick is a USDA/ARS technician. He has a Bachelor Degree in Biology minor in Chemistry. He has been assisting all aspects of this project.
Encarnacion Rivera is USDA/ARS technician. He has a GED exam equivalent. He has been involved in all aspects of greenhouse management of plant culture. He has considerable experience in establishing plantings in the field, has excellent people skills.

(2) Cost Sharing: None

(3) Carryover: None

Robin Buell
The Institute for Genomic Research (TIGR)

(1)

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<tr>
<td>C.Robin Buell</td>
<td>25 %</td>
<td>CoPI</td>
<td>Technical expertise in high throughput sequencing and microarray hybridization. Responsible for management of all aspects of the TIGR component of the project including BAC sequencing and annotation, microarray fabrication, and microarray hybridization.</td>
</tr>
<tr>
<td>Amy Hart</td>
<td>100 %</td>
<td>Research Associate</td>
<td>Technical expertise in microarray fabrication and hybridization. Responsible for microarray fabrication, hybridization and data analysis.</td>
</tr>
<tr>
<td>Jia Liu</td>
<td>50 %</td>
<td>Bioinformatic</td>
<td>Expertise in bioinformatics. Responsible for BAC annotation and microarray data processing pipeline.</td>
</tr>
<tr>
<td>Kimberly Rehfeld</td>
<td>100 %</td>
<td>Research Associate</td>
<td>Technical expertise in microarray fabrication and hybridization. Responsible for microarray fabrication, hybridization and data analysis.</td>
</tr>
<tr>
<td>Kimberly Rehfeld</td>
<td>100 %</td>
<td>Research Associate</td>
<td>Technical expertise in microarray fabrication and hybridization. Responsible for microarray fabrication, hybridization and data analysis.</td>
</tr>
<tr>
<td>Kimberly Rehfeld</td>
<td>100 %</td>
<td>Research Associate</td>
<td>Technical expertise in microarray fabrication and hybridization. Responsible for microarray fabrication, hybridization and data analysis.</td>
</tr>
<tr>
<td>Willem Rensink</td>
<td>90 %</td>
<td>Staff Scientist</td>
<td>Responsible for experimental design, data analysis, and manuscript preparation.</td>
</tr>
<tr>
<td>Closure Research Associate</td>
<td>20 %</td>
<td>Research Associate</td>
<td>Expertise in closure techniques. Responsible for performing closure work on the potato BACs.</td>
</tr>
</tbody>
</table>

(2) and (3)

It is too early to estimate carryover of funds. If there are funds, these will be
used for reagent costs for microarrays not anticipated in our original budget.

Margaret Jahn
Cornell University

(1) See attached form
(2) Cost Sharing: None
(3) Carryover: None

Jiming Jiang and Sandra Austin-Phillips
University of Wisconsin, Madison

Robert Stupar
Ph.D. student (100% time). Bob graduated in March of 2005.

Lara Colton
Ph.D. student (100% time). Ms. Colton joined the Jiang in the fall of 2003. Lara is currently using a microarray-based approach to study the functional mechanisms of the RB-mediated late blight resistance.

Bala Pudota
Ph.D. student (100% time). Mr. Pudota joined the Jiang in the fall of 2003. He is currently confirming the “polyploidy-associated” genes identified by microarray using real-time PCR and Northern blot hybridization.

Susan Wielgus
Technician (75% time). Ms. Wielgus has extensive experience on evaluating late blight resistance and conducting DNA marker-based analysis of transgenic potato. She is responsible for the late blight evaluation service, which is available to all Co-PIs within the project.

John Raasch
Technician (50% time). Mr. Raasch has extensive experience on Agrobacterium-mediated potato transformation. He is responsible for the potato transformation service, which is available to all Co-PIs within the project.

2. Cost Sharing: None

Georgiana May
University of Minnesota, Twin Cities

(1)
David Schladt (graduate student) U. Minnesota
Ethyl Cannon (web tools)
Karla Sorenson (undergraduate) U. Minnesota

(2) Cost sharing: None
(3) Carryover: None
NSF POTATO GENOME PROJECT MEETING, February 10, 2006, Berkeley, CA
SCIENTIFIC ADVISORY COMMITTEE REPORT

Four members of the Scientific Advisory Committee (SAC) (R. Innes, S. Kamoun, S. Hulbert, and R. Michelmore) attended the 2006 annual meeting of the project, which was at the University of California, Berkeley Plant Gene Expression Center on February 10, 2006.