Development of Early Blight and Late Blight Resistant Tomatoes

Report of a research supported by:

The Pennsylvania Vegetable Marketing and Research Program

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Long-range goal of the Penn State tomato genetics and breeding program:

Develop tomato cultivars with strong disease resistance (in particular early blight and late blight), improved fruit quality, and adaptation to PA conditions (e.g. high yield).

Specific objectives of 2009 project:

1. Fine mapping and characterization of *Ph-5*, a new late blight resistance gene identified at Penn State.

2. Transfer of *Ph-5* to our processing and fresh-market tomatoes, using a combination of traditional breeding (phenotypic selection, PS) and marker-assisted breeding (marker-assisted selection, MAS).

3. Transfer of *Ph-2* and to *Ph-3* to our processing and fresh-market tomatoes, using *a combination of PS and MAS.*

4. To develop tomato breeding lines with disease resistance (early blight and late blight) and other desirable horticultural characteristics.

2009 Research Progress:

(Please note that the research described below is a summary of research done during 2009 in the tomato genetics and breeding program at Penn State with funds received from different sources, including PVMRP/PVGA. While funds from PVMRP/PVGA have been very helpful, not all of the research described below was the result of such funds.)

We continued our research toward understanding the genetic bases of early blight (EB) and late blight (LB) resistance and developing tomatoes with disease resistance, high fruit quality, and adaptation to PA conditions. The work included laboratory research (e.g. identifying polymorphic genetic markers, genetic mapping of new markers, determining genomic location of

genes or QTLs), greenhouse research (e.g. growing plants for cross hybridization, disease resistance evaluation, preparing seedlings for field trials), and field research. In summer 2009, for example, we grew approximately 8 acres of experimental tomatoes at the Russell E. Larson Agricultural Research Center at Rock Springs, PA, which were evaluated for disease resistance and many other characteristics and resulted in the selection of close to 600 entries for further evaluation or cross-hybridization (including development of experimental hybrids and new breeding populations). A brief description of some of the experiments conducted in 2009 is provided below.

Identification, mapping and fine mapping of new late blight resistance genes

This has been a multi-year project and a complete background information on this project was provided in our previous research reports. Briefly, we had identified a few accessions of the wild species of tomato *Solanum pimpinellifoium* L. with strong resistance to multiple isolates of *Phytophthora infestans*, the causal agent of tomato late blight. We had chosen one accession (PSLP153) for genetic mapping and identification of gene(s) conferring resistance. A project was initiated to identify and map gene(s) conferring LB resistance in accession PSLP153. A part of this mapping project has been identifying molecular markers polymorophic between PSLP153 and cultivated parent (NCEBR-2) of the mapping population. This has been proved to be very challenging due to close phylogenetic relationship between PSLP153 and NCEBR-2. Thus, major efforts were devoted to this task and fortunately we have been able to identify sufficient number of polymorphic DNA markers. The mapping project, conducted in an F2 population, has indicated the presence of possibly 2 resistance genes, one on chromosome 1, named Ph-5, and one on chromosome 10, yet unnamed. Several generations of marker-assisted selection (MAS) and breeding as well as phenotypic selection resulted in the identification of one F4BC2 plant, highly similar to the recurrent parent, NCEBR-2 (i.e., with minimal background contribution from the wild type parent, PSLP153), LB resistant, and heterozygote at the two target (resistance) regions on chromosomes 1 and 10 (as determined by 3 molecular markers).

The selected F4BC2 plant was hybridized with the recurrent parent, NCEBR-2, and produced F4BC3 seed. A total of 300 F4BC3 progeny were grown, which were phenotypically evaluated for LB resistance and also genotyped with the 3 molecular markers associated with resistance genes on chromosomes 1 and 10. Individuals exhibiting resistance to LB and carrying the resistance-lined markers were subsequently genotyped with other background molecular markers to identify individuals with minimum background genetics from PSPL153. We selected a LB-resistant individual with minimum genetic background from PSLP153 and heterozygous at the three marker loci in the target (foreground) region. This individual was self-pollinated to produce F4BC3S1 and also crossed with NCEBR-2 to produce F4BC4. Currently we are growing 1116 F4BC4/F4BC3S1 individuals to narrow down the resistance regions. Actually, all these individuals are considered near-isogenic lines (NILs), which are lines that are genetically identical to each other and to the recurrent parent, except for small regions at the vicinity of the resistance genes. These individuals will be screened for LB resistance and the resistant lines will be genotyped to identify the best NILs for further analyses. The identified NILs will be useful for transferring the LB resistance to different genetic backgrounds without having to use the original resistant wild parent (PSLP153). The NILs will also be useful for determining the individual

effects of each new gene identified as well as their effects in homozygous or heterozygous conditions. Furthermore, the NILs will facilitate further fine mapping and possibly cloning the new resistance genes. In addition to determining the exact locations the new resistance genes and developing NILs, a major goal of this project has been transferring of these genes to our tomato breeding lines *via* marker-assisted selection (MAS), as described below.

It should be noted that during 2009 significant progress was made in identifying polymorphic molecular markers and mapping them in the F2 populations. Currently, we do have a total of 176 DNA markers mapped in the F2 population.

Breeding processing and large-size fresh-market tomatoes for late blight resistance via transferring of Ph-5

As was indicated in the previous section, our recent research indicates that there might be two resistance genes in the PSLP153, one on chromosome 1 (*Ph-5*) and one on chromosome 10 (yet unnamed). Thus, our goal has been to transfer these genes to our large size fresh market and processing tomatoes. A complete background of this multi-year breeding project was given in our 2008 research report to PVMRP, and thus the reader is referred to that report. Briefly, in 2008 we developed 8 BC2 families (6 fresh-market and 2 processing). During the summer and fall 2008, 6 plants of each of the 8 BC2 families were grown in the greenhouse and self-pollinated to produce a total of 48 BC2S1 families (36 FM and 12 Processing families). In early 2009, 12 plants of 47 BC2S1 families (35 FM and 12 Processing families) were grown in a greenhouse and evaluated for LB resistance. All susceptible plants were eliminated. All resistant plants were subsequently genotyped with molecular markers associated with resistance genes on chromosome 1 (1 marker) and chromosome 10 (2 markers). Thus all plants that were either homozygous or heterozygous for resistance at both regions on chromosomes 1 and 10 were maintained, and the rest were discarded. Most plants were heterozygous at both regions, however, a few were homo for one and even fewer were homo for both regions. All resistant plants, including 92 FM and 35 processing individuals, were planted in the 2009 field. During the season, plants were evaluated for horticulture characteristics and a total of 12 fresh-market and 3 processing type plants were selected. Plants were selected only if they had sufficient desirable horticultural characteristics. Some plants were highly undesirable in horticultural, and thus were eliminated. Most selected plants were heterozygous for both regions, with a few exceptions. An important observation from field 2009 was that most of these plants needed significant improvement in horticultural characteristics, thus the need for making 2 or more generation of backcrosses with desirable Penn State tomato materials. Recently, upon further evaluation of the field data only two plants selected for further backcrossing, one fresh-market and one processing plant (both homozygous for resistance regions on chromosomes 1 and 10). Currently, these plants are grown in the greenhouse for crossing with Penn State breeding lines with several desirable horticultural characteristics. For example, the LB-resistant fresh market plant with be hybridized with five Penn State FM lines and the LB-resistant processing plant will be hybridized with six Penn State processing lines. Further crossing, disease screening and horticultural evaluation are needed before identifying inbred lines with LB resistance and other desirable horticultural characteristics.

Breeding fresh-market cherry and grape tomatoes for late blight resistance via transferring of Ph-5

A major goal of this project has been to develop cherry and grape tomatoes with late blight (LB) resistance, using *Ph-5* gene. A complete background of this multi-year breeding project was given in our 2008 research report to PVMRP, and thus the reader is referred to that report. Following field evaluation in 2008, six LB-resistant inbred lines (3 cherry and 3 grape) were selected and named as PSCHLB-1, PSCHLB-2, PSCHLB-3, PSGRLB-1, PSGRLB-2 and PSGRLB-3. Selected lines were self-pollinated and produced progeny for the next cycle of field evaluation. During spring 2009 several new experimental hybrids were produced from crosses between these lines and several Penn State cherry and grape lines with high lycopene and other desirable characteristics. However, as the hybrid seeds did not become available before field season, they were not included in the 2009 field, but they will be included in the 2010 field. However, during the 2009 field season, the six lines were again grown under field conditions and evaluated for various horticultural characteristics. From these evaluations, two lines, namely PSCHLB-2 and PSGRLB-3 were selected for developing experimental hybrids. Currently these lines are grown under greenhouse conditions for crossing with five Penn State cherry and nine Penn State grape tomatoes. The resulting hybrids will be evaluated for commercial use during field 2010, and will be released as appropriate. We expect that new cherry and grape hybrids should have LB resistance plus numerous other horticultural characteristics. The new crosses will also be used for selection and breeding to develop new inbred lines with LB resistance and other desirable horticultural characteristics.

Breeding processing and large-size fresh-market tomatoes for late blight resistance via transferring of Ph-2 and *Ph-3*

A goal of this project has been to incorporate the previously-identified LB resistance genes, *Ph-2* and *Ph-3*, into our fresh-market and processing tomatoes. A complete background of this multi-year breeding project was given in our 2008 research report to PVMRP, and thus the reader is referred to that report. In below, the progress to develop FM and processing tomatoes with *Ph-2* and *Ph-3* resistance is described:

Development of FM tomatoes with Ph-2 and Ph-3 resistance: As indicated in previous research reports, a North Carolina breeding line (NC03220) was used as the source of Ph-2 and Ph-3 resistance. Originally, several Penn State tomatoes were hybridized with NC03220 and produced F3 (from crosses in 2007) and F4 (from crosses in 2006) families. Selected F4 individuals (i.e., LB resistant and homozygous for Ph-3) were hybridized with a Florida line or a NC hybrid cultivar and produced F4BC1 families. A total of 37 F4BC1 families were developed, which were grown in the greenhouse, self-pollinated and produced BC1S1 seed. Similarly, the F3 families were both self-pollinated to produce "new" F4 families and crossed with a Florida lines to produce F3BC1 families. During the spring 2009, the following four sets of families were grown in the greenhouse, evaluated for LB resistance and genotyped for Ph-3 resistance. Only plants that were either homozygous or heterozygous for Ph-3 were planted in the field as follow:

F4BC1S1 (FL): 19 families, each 6 or more individuals; all HOMO for Ph-3
F4BC1S1 (NC): 15 families, each 6 or more individuals; all HOMO for Ph-3
F4 NEW: 18 families, each 6 or more individuals; all HOMO for Ph-3
F3BC1 (FL): 18 families, each 6 or more individuals; all HET for Ph-3

During the 2009 field season, plants were evaluated for various horticultural characteristics and several plants were selected from each family. Specifically, 13, 5, 9 and 4 individual selections were made from each of F4BC1S1 (FL), F4BC1S1 (NC), F4 NEW and F3BC1 (FL) families, respectively. These individuals were self-pollinated and advanced to the next generation. It appeared that these plants still needed further improvement in their horticultural characteristics. Thus, upon further evaluation of the results, four selections were picked for further crosses, including 2 from F4BC1S1 (FL) and one from each of F4BC1S1 (NC) and F4 NEW families. Progeny of these four selections were recently grown in the greenhouse, which will be hybridized with five Penn State FM tomato lines (thus will be producing a total of 20 new families with LB resistance and other desirable horticultural characteristics). All new families (BC2) will be heterozygous for Ph-3 and possibly for Ph-2. We will Grow the new BC2 progeny in 2010 field for field evaluation and further improvement. We do not know at this time whether further crosses would be needed, but expect to have plants with Ph-2 + Ph-3 late blight resistance within a few years. Once, we will have those lines, they will be released and also hybridized with our Ph-5 late blight resistant lines to produce stronger and more durable resistance to LB (i.e. due to multiple disease resistance genes).

Development of processing tomatoes with Ph-3 resistance: A goal of this project was to transfer a previously-identified LB resistance gene, Ph-3, into Penn State processing breeding lines. Thus, in 2006 a processing tomato line from Cornell University, supposedly containing *Ph-3* in homozygous condition (CULBPt-04-5; hereafter noted as CN04-5), was hybridized with several of our breeding selections and the progeny families were grown under field conditions during summer 2006. Unfortunately, most families did not look desirable, as they were more like FM tomatoes with small round fruits. No selection was made from 6 families and only 5 individuals were selected from 3 families. The 5 families were grown in the greenhouse during late 2006 and evaluated for LB resistance in January 2007. Most plants within families exhibited LB susceptibility. Evaluations resulted in the selection of 10 plants with apparent resistance. These 10 plants were grown to maturity and marker genotyped for *Ph-3* markers. The 10 plants were also hybridized with a processing tomato genotype. A total of 8 BC_1 progeny families were developed (did not get BC_1 progeny from two of the crosses). The 8 BC_1 families were grown under field conditions in 2007 and were evaluated for horticultural characteristics. We selected a total of 9 plants from 4 different families. Plants were selected only from families in which the parental PSU parents were homozygous for *Ph-3* markers. Thus, it could be assumed that these BC1 families were heterozygous for *Ph-3* marker. The *BC1S1* seeds were collected from the selected BC1 families in the field. In spring 2008, the 9 BC1S1 families (collected from field 2007) were evaluated for LB resistance in the greenhouse. Of these, 4 families were dropped as they were susceptible. From each of the remaining 5 BC1S1 families, 2-5 resistant plants were grown to maturity. The plants were genotyped for Ph-3 markers. From these 5 families, a total of 11 BC1S1 plants were found to be homozygous for Ph-3. Subsequently, each of these 11 plants were hybridized with two desirable processing genotypes. BC2 seeds were obtained from 11

crosses with one and 10 crosses with the other processing parent. So we developed a total of 21 new BC2 families. In addition to these crosses, pollens were pooled from individuals coming from each of the 5 BC1S1 families and hybridized with each of the two desirable processing genotypes, and thus developed another 10 new BC2 families. Thus, altogether we developed a total of 31 BC2 families, all of which were expected to be heterozygous for Ph-3. The BC2 families were grown under greenhouse conditions and evaluated for LB resistance. Only highly resistant families were maintained, and self-pollinated to produce BC2S1 families. A few families were lost due to unforeseen situations and a few families did not show sufficient resistance. Thus, there remained 20 BC2S1 families for further research activities. Currently, these families are grown under greenhouse conditions (12 plants of each family, for a total of 240 plants). These families will be evaluated for LB resistance, and the resistant individuals will be genotyped for Ph-3. We will identify individuals homozygous for Ph-3. Briefly, we will identify 12 plant with high resistance to LB and homozygous at Ph-3. These plants will be used to cross with 6 selected Penn State processing tomato breeding lines to develop advanced lines with good horticultural characteristics and LB resistance. Ultimately Ph-3 resistance processing lines will be hybridized with Ph-5 resistance materials to develop tomato breeding lines and cultivars with strong and durable resistance to LB. Further progress in this research will be reported in our future research reports.

Construction of a new molecular linkage map of tomato based on a RIL population of a S. lycopersicum × S. pimpinellifolium cross

As described in previous reports, we developed a new recombinant inbred line (RIL) population from a cross between an advanced tomato breeding line and an accession of *S. pimpinellifolium*. The wild *S. pimpinellifolium* accession has several desirable characteristics, including disease resistance and high fruit quality. To take advantage of the superior characteristics of this wild accession, we developed a permanent RIL population, which will be highly useful for various research purposes, including identification and mapping of genes for disease resistance and high fruit quality as well as breeding purposes. To achieve such goals, we developed a molecular linkage map of this population including 294 molecular markers. In 2008, extensive efforts were made to finalize this map and develop a manuscript describing the map. In 2009 we submitted the manuscript for publication in GENOME, an internationally known high quality journal in the area of plant genetics and genomics. After a few rounds of review and revision, the manuscript was accepted for publication in GENOME and was published in November 2009. Below is the published abstract of the paper. The full paper citation is shown below. A pdf copy of the final paper can be forwarded to the Research Board upon request.

Ashrafi H, MP Kinkade and **MR Foolad**. 2009. A new genetic linkage map of tomato based on a *Solanum lycopersicum* × *S. pimpinellifolium* RIL population displaying locations of candidate resistance ESTs. *Genome* 52: 935-956.

Abstract: The narrow genetic base of the cultivated tomato, *Solanum lycopersicum* L., necessitates introgression of new variation from related species. Tomato wild species represent a rich source of useful genes and traits. Exploitation of genetic variation within wild species can be facilitated by the use of molecular markers and genetic maps.

Recently we identified an accession (LA2093) within the red-fruited tomato wild species *Solanum pimpinellifolium* L. with exceptionally desirable characteristics, including disease resistance, abiotic stress tolerance and high fruit lycopene content. To facilitate genetic characterization of such traits and their exploitation in tomato crop improvement, we developed a new recombinant inbred line (RIL) population from a cross between LA2093 and an advanced tomato breeding line (NCEBR-1). Furthermore, we constructed a medium-density molecular linkage map of this population using 294 polymorphic markers, including standard RFLPs, EST sequences (used as RFLP probes), CAPS and SSRs. The map spanned 1091 cM of the tomato genome with an average marker distance of 3.7 cM. A majority of the EST sequences, which were mainly chosen based on the putative role of their unigenes in disease resistance, defense-related response or fruit quality, were mapped onto the tomato chromosomes for the first time. Co-localizations of relevant EST sequences with known disease-resistance genes in tomato were also examined. This map will facilitate identification, genetic exploitation and positional cloning of important genes or QTLs in LA2093. It also will allow the elucidation of the molecular mechanism underlying important traits segregating in the RIL population. The map may further facilitate characterization and exploitation of genetic variation in other *S. pimpinellifolium* accessions as well as in modern cultivars of tomato.

Development of processing and fresh-market tomato lines via traditional protocols of plant breeding

This has been an ongoing research for several years. Each year many large-scale greenhouse and field experiments are conducted with the aim of transferring resistance genes and other desirable horticultural characteristics to our tomato breeding lines. This includes transfer of resistance genes to both processing and fresh-market tomato lines. We have been advancing hundreds of lines and developing hundreds of new breeding populations each year and evaluating them under field conditions. For example, during the field season in 2008, we grew and evaluated about 12 acres of experimental tomatoes at Rock Springs. The breeding populations, families or lines grown in the field were of different generations and levels of advancement. They were also of both fresh-market and processing types. The fresh-market types included cherry, grape, plum-shape and large-size round tomatoes. During the field season in 2008, plants were evaluated for many characteristics, including disease resistance, fruit quality, maturity, yield, plant type, etc. and a total of 986+ selections were made based on different evaluations. These included 555 processing selections, 425 fresh market selections (314 large and medium round tomatoes, 65 plum tomatoes, 23 cherry tomatoes, 22 grape tomatoes, 1 brachytic tomato, and 6 cherry and grape tomatoes with *Ph-5* LB resistance gene). All these selections were self-pollinated and advanced to next generation. In the area of fresh market tomatoes, several lines of cherry, grape and plum tomatoes were considered final (reached homozygosity) and could be parents for production of hybrid cultivars. During early 2009 we grew in the greenhouse 22 plum, 14 grape and 12 cherry breeding lines to develop a total of 217 new experimental hybrids, including 84 plum, 73 grape and 60 cherry hybrids. Crosses with most, but not all, hybrids were successful, however, the hybrid seeds were not ready for the 2009 field plantation. These hybrids will be planted in the 2010 field for evaluation. However, in the 2009 field we grew the self-progeny of

all the 2008 field selections (i.e. 986+ selections) plus many commercial lines and hybrid cultivars for evaluation. We had a total of 8 acres of experimental tomatoes.

During the field season in 2009, plants were evaluated for many characteristics, including disease resistance, fruit quality, maturity, yield, plant type, etc. and a total of 580+ selections were made based on different evaluations. These included 226 processing selections, 302 fresh market selections, including 190 large and medium round tomatoes, 51 plum tomatoes, 25 cherry tomatoes (NOT including 3 *Ph-5* LB resistant cherry), 29 grape tomatoes (NOT including 3 *Ph-5* LB resistant grape) and one brachytic tomato. In addition, 52 new FM or processing plants/lines were selected with LB resistance. These included the followings:

Large size FM tomatoes with Ph-5: 12 selections
Processing tomatoes with Ph-5: 3 selections;
Cherry tomatoes with Ph-5: 3 selections;
Grape tomatoes with Ph-5: 3 selections;
Large size FM tomatoes with Ph-2/Ph-3: 31 selections.

Note that the processing tomatoes with Ph-3 LB resistance did not become ready for field evaluation in 2009, but are currently being grown for further crosses, as described above under "*Development of processing tomatoes with Ph-3 resistance*".

Further evaluation and analysis of the field data resulted in the selection of a limited number of PSU tomatoes for further crosses as follow:

6 PSU processing lines to be crossed with one Ph-5 resistance processing and one Ph-2/Ph-3 resistant source (actually pooled pollen from 12 selected BC2S1 plants with Ph-2/Ph-3 resistance);

5 PSU large size FM lines to be crossed with one Ph-5 resistance FM and 4 Ph-2/Ph-3 resistance;

5 PSU cherry lines to be crossed with one Ph-5 resistance cherry, one Ph-5 resistance grape, NC1 grape and NC 2 grape;

9 PSU grape lines to be crossed with one Ph-5 resistance cherry, one Ph-5 resistance grape, NC1 grape and NC 2 grape;

Altogether, this winter/spring we will be developing a total of 93 new populations/hybrids. We expect that some of these crosses will result in new experimental hybrids, which can be released for commercial evaluation. Other crosses will result in populations which will be used for further breeding to derive new inbred lines with disease resistance and high fruit quality. Also, it should be noted that in early 2009, we release 13 fresh-market tomato breeding lines, including 3 plum, 5 grape and 5 cherry tomatoes, were released via Material Transfer Agreement (MTA) and several seed companies are currently evaluating these materials for potential commercial use.

Conclusions

We have continued our research toward genetic characterization of EB and LB resistance in tomato and development of new tomato inbred lines and experimental hybrids with improved disease resistance and other desirable horticultural characteristics, including high fruit lycopene content. Specifically, we have 1) identified resistant wild accessions of tomato with resistance to EB and LB, 2) developed several interspecific populations of tomato segregating for disease resistance, 3) characterized genetic controls of EB and LB resistance including the identification of QTLs controlling EB resistance, 4) developed new tomato germplasm, including inbred lines and experimental hybrids, with improved disease resistance and other desirable horticultural characteristics, 5) have released 13 fresh market plum, cherry and grape tomatoes with improved EB resistance and high fruit lycopene content, and 6) will releasing more lines and hybrids within the next few years with resistance to LB as well as other desirable horticultural characteristics.

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Budget:

The actual expenses for the projects described above were much higher than what was provided by the Pennsylvania Vegetable Marketing and Research Program. However, financial support from growers is highly appreciated and it is valuable and important to the continuation of this long-term research. Below is an approximate description of the expenditures:

Wages (including fringe benefits)	$3,750
Greenhouse and field rent	$750
TOTAL	$4,500

Duration of Project: 8 months (April 1, 2009 to November 30, 2009)