Breeding Tomatoes for Early Blight and Late Blight Resistance and Other Desirable Traits for Production in PA

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The Pennsylvania Vegetable Marketing and Research Program

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

Develop breeding lines and F_1 hybrid cultivars of tomato with disease resistance, in particular early blight (EB) and late blight (LB), improved fruit quality, and adaptation to PA conditions (e.g. high yield and early maturity).

Research Activities Pursued in 2016 Included the Followings:

Objective 1. Field evaluation and advancement of Penn fresh-market tomato breeding lines

Objective 2. Field evaluation and advancement of Penn State processing tomato breeding lines

Objective 3. Transfer of late blight resistance genes *Ph-3* and *Ph-5* to PSU fresh-market tomato breeding lines

Objective 4. Transfer of late blight resistance genes *Ph-3* and *Ph-5* to PSU processing tomato breeding lines

Objective 5. Development and evaluation of a new recombinant inbred line (RIL) population of tomato segregating for LB resistance

Objective 6. Genetic characterization of new sources of LB resistance and breeding for LB resistance using new sources

Objective 7. Development and evaluation of experimental F_1 hybrids with EB resistance and other desirable horticultural characteristics

Objective 8. Evaluation of inbred lines and hybrid cultivars in collaboration with a seed company

Objective 9. Evaluation of commercial inbred lines and hybrid cultivars and comparison with Penn State material

Duration of the Project: 8 months (April 1, 2016 to November 30, 2016)

Funds Received in 2016 From PVMRP: $12,000

2016 Research Progress:

(Disclaimer: Please note that the research described here is a summary of work conducted during 2016 in the tomato genetics and breeding program at Penn State with funds received from different sources, including the $12,000 from the PVMRP. While funds from PVMRP have been very helpful, not all of the research described below was the result of such funds.)

Summary

In 2016, we grew in the field a total of 649 tomato entries (including inbred lines, F_1 hybrids, families, etc.) and evaluated them over the season for numerous characteristics, including yield, disease resistance, fruit quality, etc. From among the PSU inbred lines and families, a total of 291 lines were selected for advancement, and further evaluation and characterization. The experimental hybrids were evaluated for potential commercialization. Throughout the year, we also conducted several experiments in the greenhouse evaluating numerous PSU lines and families for late blight (LB) resistance. Also, thousands of cross hybridizations were made in the greenhouse to develop experimental hybrids. Further, laboratory experiments were conducted throughout the year for molecular (DNA) genotyping and characterization of various tomato germplasm.

Abbreviations: *EB: early blight; FM: fresh-market; GH (greenhouse); LB: late blight; RILs: recombinant inbred lines.*

Objective 1. Field evaluation and advancement of Penn fresh-market tomato breeding lines

Over the years, we have developed many fresh-market (FM) tomato inbred lines (including large round, plum, cherry and grape tomatoes) with desirable horticultural characteristics, including high yield, disease resistance (in particular resistance to EB), fruit quality (including size, color, firmness, taste/flavor, tolerance to physiological disorders, etc.) and adaptation to PA condition. Every year during our field season we grow these inbred lines for further evaluation and selection. The purpose of this research is to develop elite breeding lines of FM tomato with multiple desirable horticultural characteristics, which could be used for development of commercial processing F_1 hybrids. Briefly, in 2016, we grew and evaluated 36 large-size and 12 plum FM lines, and at the end of season we selected a total of 48 lines. Almost all selected lines within the different categories are of high quality and can potentially be used to develop experimental F_1 hybrids. In 2017, a major focus will be development of experimental hybrids of large-size FM tomatoes using our inbred lines. Experiments have been designed to develop a total of 102 large-size FM tomato experimental hybrids. These hybrids will be evaluated during field season in 2017. Several hybrids (~10) will also be evaluated under farmers' conditions in PA, in collaboration with Extension Agent Tim Elkner. Also, several of the hybrids will be evaluated in other states in collaboration with other public and private-sector tomato breeders.

Objective 2. Field evaluation and advancement of Penn State processing tomato breeding lines

Over the years, we have developed many tomato processing inbred lines with desirable horticultural characteristics, including high yield, disease resistance (in particular resistance to early blight, EB), fruit quality (including color, firmness, tolerance to physiological disorders, etc.) and adaptation to PA condition. Every year during our field season we grow these inbred lines and further evaluate them and make additional selection. The purpose of this research is to develop elite breeding lines of processing tomato with multiple desirable horticultural characteristics, which could be used for development of commercial processing F_1 hybrids. Briefly, in 2016, we grew and evaluated 44 PSU processing lines and at the end of season we selected a total of 34 lines. Almost all selected lines are of high quality and can potentially be used to develop experimental F_1 hybrids [from crosses among PSU lines or (preferably) from crosses between PSU lines and elite lines from other tomato breeding programs]. In 2017, for example, we will develop a total of 30 experimental F_1 hybrids, from crosses among our processing lines (5 males and 6 females). These hybrids will be grown and evaluated under field conditions in 2017.

Objective 3. Transfer of late blight resistance genes Ph-3 and Ph-5 to PSU fresh-market tomato breeding lines

Ph-3 was previously identified as a LB resistance gene by other researchers and has been incorporated into several commercial FM tomato breeding lines and hybrid cultivars. *Ph-5* was recently identified as a new LB-resistance gene at Penn State (Reference: *Merk HL, H Ashrafi and MR Foolad. 2012. Selective genotyping to identify late blight resistance genes in an accession of the tomato wild species Solanum pimpinellifolium. Euphytica: 187:63-75*). We have been conducting research to transfer *Ph-3* and *Ph-5* resistance genes to our FM tomato-breeding lines. During winter (February) 2016, we grew and evaluated for LB resistance all of our 30 FM families/lines with *Ph-3* (17) and *Ph-5* (13) resistance genes under GH conditions. From each family, two most resistant plants were selected, which were grown to maturity and advanced to the next generation. The progeny of these resistant families were subsequently grown in the GH and seedlings were transplanted into the field in late-June 2016. During the field season, the 30 FM tomato families with *Ph-3* (17 families) or *Ph-5* resistance genes (13 families) were evaluated for various horticultural characteristics. From among the 17 families with *Ph-3* resistance, 7 families were eliminated and 10 plants (one from each of the other 10 families) were selected. Similarly, from among the 13 families with *Ph-5* resistance, 5 families were eliminated and 8 plants (one from each of the other 8 families) were selected. The total of 18 plants with LB resistance (10 with *Ph-3* and 8 with *Ph-5*) were grown to maturity under field conditions and advanced to the next generation. During the winter and spring of 2017, the selected families (18) will be evaluated for LB resistance under GH conditions, the most resistant plants will be identified, grown to maturity and advanced to the next generation. These progeny will be grown under field conditions in 2017 for evaluation of other desirable horticultural characteristics. By the end of field season in 2017, we expect that we will have advanced FM breeding lines with LB resistance (*Ph-3* or *Ph-5*) and other desirable horticultural characteristics. During the winter and spring 2017, we also will make crosses between a *Ph-3* FM LB-resistant line and a *Ph-5* FM LB-resistant line to develop an experimental hybrid with combined *Ph-3* and *Ph-5* resistance genes. We expect that the combination of these two sources of resistance will

results in tomatoes with stronger and more durable LB-resistance. In 2017, the one experimental hybrid will be evaluated for LB resistance under GH conditions and also grown and evaluated for horticultural characteristics under field conditions.

Objective 4. Transfer of late blight resistance genes Ph-3 and Ph-5 to PSU processing tomato breeding lines

As described above, *Ph-3* was previously identified as a LB resistance gene by other researchers and *Ph-5* was recently identified as a new LB resistance gene in the *Tomato Genetics and Breeding Program at Penn State*. We have been conducting research to transfer these two resistance genes to our processing tomato-breeding lines. During winter (February) 2016, we grew and evaluated for LB resistance all of our 30 processing families/lines with *Ph-3* (15) and *Ph-5* (15) resistance genes under GH conditions. From each family, two most resistant plants were selected, which were grown to maturity and advanced to the next generation. The progeny of these resistant families were subsequently grown in the GH and seedlings were transplanted into the field in late-June 2016. During the field season, the 30 processing tomato families with *Ph-3* (15 families) or *Ph-5* resistance genes (15 families) were evaluated for various horticultural characteristics. From among the 15 families with *Ph-3* resistance, 6 families were eliminated and 9 plants (one from each of the other 10 families) were selected. Similarly, from among the 15 families with *Ph-5* resistance, 5 families were eliminated and 10 plants (one from each of the other 10 families) were selected. The total of 19 plants with LB resistance (9 with *Ph-3* and 10 with *Ph-5*) were grown to maturity under field conditions and advanced to the next generation. During the winter and spring 2017, the 19 selected families will be evaluated for LB resistance under GH conditions, the most resistant plants will be identified, grown to maturity and advanced to the next generation. These progeny will be grown under field conditions in 2017 for evaluation of other desirable horticultural characteristics. By the end of field season in 2017, we expect that we will have advanced processing breeding lines with LB resistance (*Ph-3* or *Ph-5*) and other desirable horticultural characteristics.

Objective 5. Development and evaluation of a new recombinant inbred line (RIL) population of tomato segregating for LB resistance

Previously, we had identified an accession (PSLP 153 = PI 270443) within tomato wild species *Solanum pimpinellifolium* with high level of resistance to LB. Subsequently, we investigated the genetic control of LB resistance in this accession, including the inheritance of resistance (*Merk HL and MR Foolad, 2012. Parent-offspring correlation estimate of heritability for late blight resistance conferred by an accession of the tomato wild species Solanum pimpinellifolium. Plant Breeding: 131: 203-210*) and identification and mapping of resistance genes (*Merk HL, H Ashrafi and MR Foolad. 2012. Selective genotyping to identify late blight resistance genes in an accession of the tomato wild species Solanum pimpinellifolium. Euphytica: 187:63-75*). We also used this accession for breeding tomatoes with LB resistance, as described above (*Ph-5* resistance gene). The resistance in this accession has been determined to overcome situations when the *Ph-2 + Ph-3* combination may fail (personal observation, as well as results obtained by a collaborator at NC State). Therefore, this accession can be very valuable in tomato breeding for developing stronger and more durable resistance to LB. With this goal in mind, a few years ago we initiated a project to develop a recombinant inbred line (RIL) population from crosses between PI 270443 and a LB-susceptible tomato breeding line, NC EBR-2 to further characterize the genetic basis of LB resistance in this accession. During winter 2016, we grew the F_6 generation of this population

(167 lines) in the GH and advanced it to F_7 generation, which was subsequently grown under field conditions in the summer of 2016. During the field season the population was evaluated for disease resistance (EB) and various horticultural characteristics, and the population was advanced to F_8 generation. During fall 2016, the F_8 RILs (167 lines) was grown in the GH and evaluated for LB resistance. The F_8 generation was also grown in the GH for generation advancement, DNA collection and genetic map construction (currently going on). In December 2016, the DNAs of the 167 lines were shipped to a sequencing facility for genetic marker development. Once the markers are generated within the next few months, we will develop a genetic map, which will be used for mapping LB resistance genes in this population. The genetic mapping will be based on LB resistance data in F_8, F_9 and F_{10} generations. The purpose of this project is to confirm the LB resistance genes in this population and work toward their fine mapping. Once the resistance genes are mapped and fine-mapped, they would be used for breeding purposes and development of LB-resistant breeding lines and hybrid cultivars.

Objective 6. Genetic characterization of new sources of LB resistance and breeding for LB resistance using new sources

In addition to the identification and characterization of LB-resistant *S. pimpinellifolium* accession PSLP 153 (PI 270443) (described above), we have been investigating the genetic basis of LB resistance in four other new accessions of *S. pimpinellifolium*, namely PSLP 150 (PI 270441), PSLP 151 (PI 270442), PSLP 142 (PI 163245) and PSLP 144 (PI 224710), which were identified in a recent disease screening study (*Foolad et al. 2014. Response of Accessions within Tomato Wild Species, Solanum pimpinellifolium to Late Blight. Plant Breeding 133:401-411*). In 2016, we continued our research on two of these accessions, namely PSLP 142 (PI 163245) and PSLP 144 (PI 224710). The research included: a) determining the genetic basis of resistance in these accessions, including inheritance of resistance; b) identifying and mapping underlying resistance genes (QTLs), and c) transferring resistance genes from the wild accessions into the cultivated tomato genetic background. Briefly, the inheritance studies indicated that the LB-resistance in both accessions was heritable and could be transferred to other genetic backgrounds by cross-hybridization and phenotypic selection. In 2016, the results of the inheritance studies was published for accession PI 163245 in a refereed journal (*Ohlson, EW and Foolad, MR. 2016. Genetic analysis of resistance to tomato late blight in Solanum pimpinellifolium accession PI 163245. Plant Breeding 135: 391-398*). Below is the abstract of this publication:

Genetic analysis of resistance to tomato late blight in *Solanum pimpinellifolium* accession PI 163245

ERIK W. OHLSON AND MAJID R. FOOLAD

Abstract. *Late blight (LB), caused by Phytophthora infestans, is one of the most devastating diseases of tomato (Solanum lycopersicum) worldwide. Due to the emergence of new and aggressive P. infestans isolates, identifying new genetic resistance to LB is a priority in tomato breeding. Recently we reported the identification of several S. pimpinellifolium accessions with strong LB resistance. In this study we investigated the utility of resistant-accession PI 163245 for tomato breeding by examining heritability (h^2) of resistance and the response to selection for resistance. Estimates of h^2 based on F_2:F_3 and F_3:F_4 parent:offspring correlation analyses averaged 0.79 and 0.94, respectively, suggesting the heritable nature of LB resistance in PI 163245. Analysis of response to*

selection for resistance from F_2 to F_4 generations indicated a realized h^2 of 0.63, confirming the utility of this resistance in tomato breeding. Two methods of estimating the minimum number of loci involved indicated the presence of one major resistance locus. Currently genetic mapping and breeding efforts are underway to further confirm the viability of this accession for improving tomato LB resistance.

In 2016, we also conducted further genetic studies of these two accessions, including the followings:

PSLP 142 (PI 163245; yellow) Project: During spring 2016, we evaluated a BC1S1 population from PSLP142 for LB resistance in GH J, and selected 21 LB resistant plants, which were transplanted into the field in summer 2016. During the field season, we evaluated the plants for horticultural characteristics and selected a total of 4 plants, two with red fruit (R-1 and R-2) and 2 with yellow fruit (Y-1 and Y-2). These plants were grown to maturity and harvested BC1S2 seed. The four BC1S2 families were grown in the GH during fall/winter 2016, and we have been making crosses to develop FM tomatoes (yellow grape and red slicer) with LB resistance conferred from this accession. For further mapping the two BC1S2 families with red fruit will be evaluated for LB resistance and the most resistant plants will be used for backcrossing to the cultivated tomato to develop mapping population for verifying and fine mapping LB resistance genes.

PSLP 144 (PI 224710; red) Project: During spring 2016, we evaluated a BC2S1 population from PSLP 144 for LB resistance in GH J, and selected 16 LB resistant plants, which were transplanted into the field in summer 2016. During the field season, we evaluated the plants for horticultural characteristics and selected a total of 2 plants (both red). These selected plants, which are in BC2S2 generation, will be used for breeding purposes. These plants will be backcrossed to the cultivated tomato breeding lines to develop LB-resistant breeding lines derived from this accession. For mapping LB-resistance genes, we will develop a new F2 population from a cross between a recently purified PSLP 144 and a breeding line and will conduct a trait-based marker analysis to identify resistance genes. Results of this research will be described in the 2017 research report.

Regarding accessions PSLP 150 (PI 270441) and PSLP 151 (PI 270442), currently we are developing manuscripts reporting our heritability and genetic mapping studies. More detailed report on these two accessions will be provided in our 2017 research report.

Objective 7. Development and evaluation of experimental F_1 hybrids with EB resistance and other desirable horticultural characteristics

The ultimate goal of a tomato-breeding program is to develop F_1 hybrids from crosses between advanced inbred lines, and evaluate them for commercial production. During the past several years, we have developed many advanced breeding lines of processing and FM (cherry, grape, plum, large size) tomatoes with numerous desirable characteristics. Many of these lines are ready to be used as parents to develop experimental hybrids. During the winter and spring 2016, we developed a total of 180 new FM experimental hybrids, all of which were large-size (slicer/beef) tomatoes. During the summer 2016, these hybrids, along with their control parental lines, were

grown under field conditions and evaluated throughout the season. These hybrids were grown side-by-side with many commercial tomato hybrid cultivars. In general, the field conditions in 2016 were not optimal (for various reasons), so most hybrids did not perform to their full genetic potential. However, many of the Penn State hybrids performed well and looked comparable or superior to commercial cultivars we trialed in the same field. A representative from one seed company, with whom we have collaboration, also visited our field at Rock Springs. They have interest in several of our hybrids, which they have trialed in their locations as well, and are interested in trialing them again in 2017. Based on 2016 experimental hybrid evaluations, we have set out experiments to develop a total of 102 large-size FM experimental hybrids during the winter/spring of 2017, some of which are repeats of 2016 and some are new. As to the new ones, for example, we will be making crosses between our EB and LB resistant lines to combine the two resistance traits in our hybrids. In 2017, for the first time, we also will be making crosses among some of our processing tomato breeding lines to develop processing experimental hybrids.

Objective 8. Evaluation of inbred lines and hybrid cultivars in collaboration with a seed company

We have extended our collaboration with one major seed company, and as a part of this collaboration, in 2016 we grew and evaluated a total of 50 inbred lines and hybrids sent from this company at Penn State farm. The idea is to look for company tomato germplasm, which could be complementary to Penn State and we could develop co-hybrids for potential commercialization. This project is ongoing and in 2017 we will be making a total of 35 new experimental hybrids from crosses between 7 PSU elite lines and 5 lines from the company. We plan to trial these hybrids in multi-locations in 2017.

Objective 9. Evaluation of commercial inbred lines and hybrid cultivars and comparison with Penn State material

For comparison with PSU tomato germplasm, in 2016 we grew and evaluated for various horticultural characteristics a total of 9 processing and 55 FM inbred lines and hybrid cultivars.

Conclusions

We continued our research toward understanding the genetic bases of early blight (EB) and late blight (LB) resistance and developing tomato breeding lines and experimental F_1 hybrids with disease resistance, high fruit quality, and adaptation to PA conditions. We have identified new genetic sources of desirable traits in wild species of tomato, characterized the genetic basis of various traits and incorporated desirable traits into our tomato germplasm. The 2016 field trial clearly indicated that we do have inbred lines and experimental hybrids, which are comparable or superior to many commercial varieties. The goal is to commercialize these material, in particular for use by our tomato growers in PA.

Budget:

The actual expenses for the projects described in this report were much higher than what was provided by the PVMRP. However, financial support from growers is highly critical and very much appreciated; it is essential to the continuation of this long-term breeding research. Below are the *approximate* expenditures in this project:

Budget:	Wages and benefits	$7,500
	Greenhouse and field supplies	$2,000
	Greenhouse and field rent	$2,500
	TOTAL	$12,000

Duration of Project: Eight months (April 1, 2016 to November 30, 2016)

2016 Publication:

Ohlson, EW and Foolad, MR. 2016. Genetic analysis of resistance to tomato late blight in Solanum pimpinellifolium accession PI 163245. Plant Breeding 135: 391-398.

Foolad MR. 2016. Tomato breeding at Penn State. In: Proc. Mid-Atlantic Fruit and Vegetable Convention, February 2 – 5, Hershey, PA, pp 78-81.