Enhancement of Tomato Fruit Quality: Maximizing Lycopene and Solids Contents and Minimizing Yellow Shoulder Disorder

Report of a research supported by:

The Pennsylvania Vegetable Marketing and Research Program

December 2010

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

Develop tomato cultivars with improved fruit quality, disease resistance, and adaptation to PA conditions (e.g. high yield).

Specific objectives of this year project as outlined in the proposal:

1) *To develop processing and fresh-market tomatoes with enhanced fruit quality characteristics, in particular high fruit lycopene content.*

2) *To develop processing tomatoes with reduced ripening disorders (yellow shoulders, etc.)*

3) *To develop high yielding tomato cultivars adapted to PA conditions.*

Funds Received in 2010 From PVMRP: $2,300

2010 Research Progress:

(Please note that the research described below is a summary of the research conducted during 2010 (and some prior to 2010) in the tomato genetics and breeding program at Penn State with funds received from different sources, including the $2,300 from the PVMRP. While funds from PVMRP have been very helpful, not all of the research described below was the result of such funds.)

We conducted laboratory, greenhouse and field experiments toward our goals of understanding the genetic bases of various fruit quality characteristics in tomato, in particular fruit lycopene content, and transferring controlling factors to different tomato genetic backgrounds. For example, as to the fine mapping of two previously-identified QTLs for high fruit lycopene content (one QTL on each of chromosomes 7 and 12), near-isogenic lines (NILs) were developed

and analyzed resulting in a better understanding of the location of at least the QTL on chromosome 12. This project involved significant amount of research work conducted in the laboratory (i.e, molecular marker analysis as well as fruit lycopene measurement via HPLC), greenhouse (growing plants and making self- and cross-pollination), field (growing plants to maturity and evaluate them for fruit lycopene content) and office (data analysis and manuscript preparation). Furthermore, during summer 2010, we grew approximately 8 acres of experimental tomatoes at the Russell E. Larson Agricultural Research Center at Rock Springs, PA, which were evaluated for different characteristics and hundreds of selections were made. Selected plants were advanced to next generations for further studies. In below, a brief summary of some of the experiments conducted and results obtained in 2010 is provided.

Identification and mapping of QTLs for fruit quality characteristics in the recombinant inbred line (RIL) population

As described in previous reports, we have developed a new recombinant inbred line (RIL) population of tomato from a cross between an advanced tomato breeding line (NCEBR-1) and a highly desirable accession (PSLP125) of tomato wild species *Solanum pimpinellifolium*. The parental wild accession (PSLP125) of this RIL population harbors many desirable horticultural characteristics, including superior fruit quality such as high lycopene content. The RIL population was grown in replicated trials under field conditions in several years and evaluated for various horticultural characteristics, including growth habit, plant type, plant size, genetic variation within families, earliness in maturity, fruit size, fruit lycopene content, fruit soluble solids content, fruit Ph, etc.. The fruit lycopene content, for example, was estimated/measured through three different methods, including colorimeter, spectrophotometer and HPLC (high performance liquid chromatography). Using data obtained from the various lycopene assays, the molecular linkage map of the population, and the various method of genetic mapping (including simple, composite and multiple interval genetic mapping), we identified several QTLs controlling high fruit lycopene content in the RIL population. In particular, two QTLs were identified on chromosomes 7 and 12 with strong and stable effects across generations and years. These QTLs will be useful for transferring of high lycopene trait to our tomato breeding lines. In 2010, we spent major efforts to prepare a manuscript describing our finding in the RIL population as to these new QTLs for high fruit lycopene content. This manuscript is in the final stage before submitting to a refereed journal. When published, the final article can be provided to PVMRP upon request.

Fine mapping of genes (QTLs) identified for fruit lycopene content and development of near-isogenic lines (NILs) for each QTL

This has been a multi-year project and a rather complete background of this research was provided in our previous research reports. However, briefly, a new RIL population was developed (described above), grown and evaluated for several fruit quality characteristics, including fruit lycopene content, for a few years. Based on these evaluations, we identified and verified two QTLs, on chromosome 7 (referred to as *lyc7*) and 12 (referred to as *lyc12*), with significant effects on fruit lycopene content. The high fruit quality characteristic of the wild *S. pimpinellifolium* accession has been transferred to our processing and fresh-market tomatoes via

traditional breeding, as described in below (under the title "*Transfer of genes for fruit quality characteristics…..*). However, a comprehensive research was initiated to fine map (i.e., clearly identify the chromosomal location of) the two QTLs on chromosomes 7 and 12 and develop near-isogenic lines (NILs) containing each of the QTLs in a cultivated tomato genetic background. As described in our previous research reports, from the QTL mapping analysis of the RIL population, we had identified one RIL which had both QTLs, exhibited high fruit lycopene content, and had minimum genetic background from the wild *S. pimpinellifolium* accession. This RIL was used for repeated backcrossing to the recurrent parent (NCEBR-1), along with phenotypic and markers assisted selection (MAS), to develop NILs with *lyc12* and or *lyc7*.

Various backcrossing and QTL analysis experiments confirmed the phenotypic effect and genetic position of *lyc12*. The *Lyc7* did not appear to have a phenotypic effect in the heterozygous condition; it is likely that *lyc7* is recessive and thus not expressing its effect in a heterozygous conditions. However, the marker-assisted backcross program was continued for both QTLs with the goal of producing NILs for the two regions of the genome. As described in previous research reports, extensive research was done in the BC_2 population to fine map the locations of *lyc12* and *lyc7* and identify individuals in this population with minimum unwanted genomic regions from the donor parent, PSLP125. Consequently, one BC_2 individual was identified which exhibited homozygous alleles for NCEBR-1 (the recurrent parent) at every background genomic location, except for one small region of chromosome 12 (i.e. including *lyc12*). Based on this information, we accelerated the return of the recurrent parent genotype to approximately 95% of the background loci (approximately double the rate as backcrossing without using MAS). In late December 2008, 189 BC_3 lines were produced by crossing selected BC_2 lines (including the individual with *lyc12* only) with the recurrent parent, NCEBR1. The selected BC_2 lines all contained *lyc7* and/or *lyc12*, as well as a very small amount of wild genomic background intervals. In early 2009, BC_3 individuals descended from 3 specific BC_2 × NCEBR1 crosses (so the background genotypes were known) were germinated in the greenhouse, and genomic DNA was collected from each individual at the seedling stage. Each individual was genotyped for 4 markers flanking the *lyc7* and *lyc12* intervals. BC_3 individuals containing these intervals were then genotyped for any segregating background genomic intervals. Any individual that contained *lyc7* and *lyc12*, and had eliminated all wild genomic background, was allowed to self-fertilize and seed was collected. To be sure that enough self-seed was collected, individuals containing the two QTLs and one other background interval were also allowed to self. This produced a BC_3S_1 population (similar to an F_2), segregating for the two QTL regions, which would enable fine mapping of the QTLs.

At this point in the project, it was decided that *lyc12* would be the primary target for fine mapping and positional cloning in the future. This decision was made based on the fact that BC_2 individuals harboring *lyc12* had ~70% more lycopene than NCEBR1, and there were many more markers in the *lyc12* interval with which to fine map the QTL. However, more markers were still needed to achieve success in fine mapping. Fortunately, tomato BAC sequence data from the *lyc12* area was deposited on Solanaceae Genome Network (SGN). Thus, these sequences were downloaded and searched for SSR motifs, primers were designed for putative SSR-containing amplicons, and each amplicon was surveyed for polymorphism between a BC_2 individual and NCEBR1. Eight new polymorphic SSR markers within the *lyc12* interval were developed using

this approach. Another advantage of using this approach is that the precise physical locations of these markers are known, which will aid the positional cloning process. We have identified and mapped 20 markers inside the 9.5 cM *lyc12* interval, although 6 of these are RFLPs, which are less friendly to use.

In order to fine map *lyc12* and precisely delineate its position, 1500 BC_3S_1 individuals segregating for the *lyc12* region were germinated in the greenhouse in April 2009. Genomic DNA was extracted from each individual using the same quick DNA preparation used in previous experiments, but was scaled up to a 96-well plate format. This allowed for the 1500 DNA samples to be completed in less than 4 days. The 1500 BC_3S_1 individuals were transplanted in the field in mid-June 2009. This entire population was genotyped for markers flanking the *lyc12* interval. A total of 265 recombinant individuals within this interval were identified, and further genotyped with 10 markers residing within the *lyc12* interval. Due to high pressure of LB disease during the season, 28 recombinants were lost in the field. The remaining recombinants, as well as 30 individuals from each *lyc12* genotypic class (homozygous for *lyc12*, heterozygous, or homozygous for NCEBR-1) (for a total of 297 BC_3S_1), were analyzed for lycopene content using reverse-phase HPLC. Recombinant individuals homozygous or heterozygous for *lyc12* were statistically indistinguishable from each other as to LYC content, while both of these classes were significantly higher in LYC than NCEBR-1 homozygotes. This indicated that *lyc12* was a dominant genetic factor affecting tomato fruit LYC content. Further marker and phenotypic analysis of the BC_3S_1 plants, using recombinants with different introgression size and additional SSR markers, indicated that *lyc12* was delimited to a 1.5 cM region between two new SSR markers. BLAST searches using end sequences from the corresponding BACs revealed that *lyc12* resides on a contiguous genome sequence scaffold of 1.5 Mb.

At the end of 2009 field season, BC_3S_2 seed were collected from recombinant BC_3S_1 plants. During spring 2010, BC_3S_2 families from recombinant BC_3S_1 plants were grown in the greenhouse. All plants were genotyped with different SSR markers and individuals homozygous for different portions (segments) of *lyc12* regions were identified (several individuals from each of the following families: 140, 544, 901, 1132, 1139, 1189, 1382, 1438, 1447 and 1495). These plants along with plants homozygous for the entire 9.5 cM *lyc12* interval (including several individuals from families 1425, 1432,1433, 1436, 1437, 1446, 1460, 1471, 1472, 1475, 1493, 1494) as well as the negative control (NCEBR-1; no *lyc12*) were planted in the field. We visually phenotyped these plants (i.e. high or low lycopene) in the field, and harvested fruit for these families. We extracted lycopene from these families, which will be used for HPLC analysis and determination of the exact amount of LYC in each family. Coupled with genotypic data, this analysis will determine the smallest segment (amongst the available recombinants) within the *lyc12* interval which would contain the QTL (may be to less than 1 cM). These new sub-NILs in homozygous conditions could be used for further backcrossing with NCEBR-1 to further breaking of the *lyc12* region. Based on the identification of the *lyc12*-containing interval, and the exact physical location of the previously-identified SSR markers (as well as future identification of more SSR markers) within the *lyc12* interval, this data can be used to identify all possible genes underlying *lyc12* when compared to the recently released tomato genome sequence. This process sets the stage for candidate gene identification through bioinformatic analyses, followed by candidate gene verification via transformation of low-lycopene genotypes with PSLP125 alleles.

This project has produced lines that will be useful for several purposes, including the following:

1. The near isogenic lines of NCEBR1 can be useful as high lycopene content lines and could potentially be useful for production of commercial hybrids.

2. The NILs can be used as a source of high lycopene to transfer the QTL to other desirable tomato genetic backgrounds. Note that it is much easier to transfer QTLs for desirable traits from within the cultivated species rather than from the wild type. Given that markers closely flanking *lyc12* are now available, breeders can easily incorporate this QTL into germplasm with minimal linkage drag from the original wild *S. pimpinellifolium* accession (PSLP125) using conventional MAS protocols.

3. The NILs can be used for further fine mapping toward map-based cloning of the desirable QTLs or genes. Once cloned, the desirable gene(s) can be transferred to different genetic backgrounds via genetic transformation.

It should be noted that we are in the process of preparing a manuscript describing the fine mapping of the new QTL on chromosome 12, for publication in a refereed journal. Once the manuscript is published, the paper can be shared with PDA upon request.

Transfer of genes for fruit quality characteristics from S. pimpinellifolium to various processing and fresh-market tomato lines via traditional protocols of plant genetics and 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 genes for fruit quality and other desirable horticultural characteristics (such as disease resistance) to our tomato breeding lines. This includes transfer of desirable 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 2009 field season, where we had grown about 8 acres of tomato breeding materials in the field, plants were evaluated for many characteristics, including fruit quality, disease resistance, 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 were selected with LB resistance, including those with *Ph-5* or *Ph-3*/*Ph-2*. A goal of this project is to combine high fruit quality (e.g., high fruit lycopene content) with disease resistance. Thus upon further evaluation and analysis of the field data, a limited number of PSU tomatoes with high fruit lycopene content were selected for crosses with our LB resistance materials. In early 2010, a total of 93 combination crosses were made involving different types of fresh-market and processing tomatoes with the purpose of improving different characteristics, including fruit quality. During the field season, self-progeny

of selections from 2009 field as well as all new crosses were planted in the field. During the growing season in 2010, the following materials were planted in the field:

A. From 2009 Field
262 plots of processing lines
190 plots of large size fresh-market lines
51 plots of plum fresh-market lines
25 plots of cherry fresh-market lines
29 plots of grape fresh-market lines

B. From 2009 Crosses (Experimental Hybrids)

84 plots of plum fresh market hybrids
60 plots of cherry fresh market hybrids
73 plots of grape fresh market hybrids

C. From 2010 Crosses (Transferring LB Resistance to High Fruit Quality Backgrounds)
9 plots of large size fresh-market tomatoes segregating for *Ph-5* LB resistance
3 plots of processing tomatoes segregating for *Ph-5* LB resistance

7 plots of large size fresh-market tomatoes segregating for *Ph-3* LB resistance

3 LB-resistant cherry lines (*Ph-5* resistance)
3 LB-resistant grape lines (*Ph-5* resistance)

6 plots of new processing hybrids developed from a *Ph-5*-resistant line and 6 PSU lines
5 plots of new fresh-market hybrids developed from a *Ph-5*-resistant line and 5 PSU lines

12 plots of new processing hybrids developed from two *Ph-3*-resistant pools and 6 PSU lines
20 plots of new fresh-market hybrids developed from four *Ph-3*-resistant lines and 5 PSU lines

D. Commercial Breeding Lines and Hybrids
41 plots of commercial fresh market lines/hybrids
18 plots of commercial fresh market lines/hybrids

During the field 2010, all of these materials were evaluated for various horticultural characteristics, including fruit quality, and the following selections were made:

Processing Tomatoes: total of 128 selections; about 10 lines were identified for release; several lines were identified for further crosses, including development of experimental hybrids or combining different traits such as disease resistance and high fruit quality.

Large Size Fresh Market Tomatoes: total of 101 selections; about 21 lines were identified with different characteristics for release; several lines were identified for further crosses, including development of experimental hybrids or combining different traits such as disease resistance and high fruit quality.

Plum Fresh Market Tomatoes: total of 42 selections; several lines were identified for further crosses, including development of experimental hybrids or combining different traits such as disease resistance and high fruit quality.

Cherry Fresh Market Tomatoes: total of 27 selections; a few lines were identified for further crosses, including development of experimental hybrids or combining different traits such as disease resistance and high fruit quality.

Grape Fresh Market Tomatoes: total of 34 selections; several lines were identified for further crosses, including development of experimental hybrids or combining different traits such as disease resistance and high fruit quality.

All of the selected materials were self-pollinated and advanced to the next generation. A goal of this project is to combine high fruit quality (e.g., high fruit lycopene content) with disease resistance and other desirable horticultural characteristics. Thus upon further evaluation and analysis of the field data, several PSU tomatoes with high fruit lycopene content were selected for crosses with our LB resistance materials. In general, a total of 134 crosses are planned involving different types of fresh market (large, plum, cherry and grape) and processing tomatoes. At the time of writing this report, these plants are grown in the greenhouse to be used for making crosses. It should also be noted that, following 2010 field evaluations, we also have identified several tomatoes of each class for release in early 2011. These lines have different desirable horticultural characteristics, including high fruit lycopene content. In summary, this multi-year project has resulted in the development of advanced processing and fresh-market tomato breeding lines with numerous desirable horticultural characteristics, including disease resistance (mainly early blight and late blight), fruit quality (in particular high fruit lycopene content), high yield, early maturity and otherwise adapted to PA conditions.

Conclusions

Over the years, we have 1) identified accessions within the related wild species of tomato with agriculturally desirable fruit quality characteristics, 2) examined the genetic bases of and identified and mapped genes for such traits, 3) developed genetic and breeding populations segregating for various fruit quality traits, 4) fine mapped genes for high fruit lycopene content and developed NILs, 5) developed new tomato germplasm, including inbred lines and experimental hybrids, with improved fruit quality and other desirable horticultural characteristics, and 6) released several lines with high fruit lycopene content, disease resistance and other desirable horticultural characteristics. We are in the process of combining high fruit lycopene content with resistance to late blight.

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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 are the *approximate* expenditures in this project:

Wages (including fringe benefits)	$1,500
Greenhouse and field expenses	$800
TOTAL	$2,300

Duration of Project: 8 months (April 1, 2010 to November 30, 2010)