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

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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 lycopene, sugar and vitamin contents.

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

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

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 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 appropriate 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), BC3 and BC3S1 populations 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 in the laboratory (i.e, molecular marker analysis as well as fruit lycopene measurement via HPLC), greenhouse

(growing plants and making many self and cross pollination) and under field conditions (growing plants to maturity to evaluate fruit lycopene content). Furthermore, during summer 2009, 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, some of the experiments conducted and results obtained in 2009 are briefly described.

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.

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. Briefly, a new RIL population was developed (described above), which was 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 (hereafter referred to as *lyc7*) and 12 (hereafter 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 from S. pimpinellifolium to…..")*. However, a research was initiated to fine map (i.e., clearly identify the chromosomal location of) the two QTLs 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 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 (BC-ing) to the recurrent parent (NCEBR-1), along with phenotypic and markers assisted selection (MAS), to develop NILs with *lyc12* and or *lyc7*.

In 2008, a BC_2 experiment was conducted that confirmed the phenotypic effect and position of *lyc12*. *Lyc7* did not appear to have a phenotypic effect in the heterozygous condition (note that all BC2 individuals were heterozygous for both *lyc12* and or *lyc7*); however, *lyc7* may be recessive and thus not expressing its effect in a heterozygous condition. Thus, the marker-assisted backcross program initiated in 2008 was continued for both QTLs with the goal of producing NILs for the two regions of the genome. As described in the last year research report for this project, extensive research was done on the BC2 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 BC2 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*). We were very fortunate to have identified this individual. Based on this information, we accelerated the return of 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 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-pollinate. 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. Tomato BAC sequence data from the lyc12 area has been deposited on Solanaceae Genome Network (SGN); 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. There are now 20 markers mapped inside the 9.5 cM lyc12 interval, although 6 of these are RFLPs, which will only be used if absolutely necessary.

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 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 late blight disease during the season, approximately 40 recombinants were lost in the field. The remaining recombinants, as well as 30 individuals from each lyc12 genotypic class (homozygous for lyc12, heterozygous, and homozygous for NCEBR-1), have been analyzed for lycopene content using reverse-phase HPLC. The lycopene data is currently being analyzed, and coupled with genotypic data from each individual, these data will enable the fine mapping of lyc12 to a small (< 1 cM) interval. In addition, self-seed from all remaining recombinants was collected, in order to produce a homozygous sub-NIL population with which one could repeat the previous experiment.

Based on the identification of the lyc12-containing interval, and the exact physical location of the previously-identified SSR markers within the lyc12 interval, our 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, which will be conducted early in 2010, followed by candidate gene verification via transformation of low-lycopene genotypes with PSLP125 alleles.

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 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 LB resistance). 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. Among these, 13 lines, including 3 plum, 5 cherry and 5 grape tomatoes were released via MTA to several seed companies for trials.

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 2009 field season, 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/lines 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. The parental plants for these crosses are currently being grown in a greenhouse. These crosses will result in the development of 93 new populations or

hybrids. We expect that some of the new experimental hybrids will 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.

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 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)