Using genomic tools to foster innovation and competitiveness in Canada's processing tomato industry: Tomato breeding research report to the Ontario Tomato Research Institute, November 2017.

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1. Brief review of program background

Between 1984 and 2002 at AAFC-GPCRC Harrow, modern cultivated varieties of tomato were hybridized with wild species of tomato. These crosses represent a source of new genetic variation to increase genetic diversity of breeding lines and Ontario processing tomato cultivars developed from them. Work at Ridgetown continues to focus on backcrossing and selection to combine this new genetic variation with commercially adapted traits for Ontario.

The OTRI succeeded in obtaining matching research funding from the Agriculture and Agri-Food Canada Agricultural Innovation Program to support this breeding effort. This project brings together two important goals. While continuing to support the work to increase genetic diversity in processing tomato lines adapted for Ontario production, this project enables the adoption of applied genomic technologies and breeding techniques to achieve this goal.

2. Summary of objectives

These objectives are summarized from the Growing Forward 2 project AIP-P119:

a) To release between 15 and 20 breeding lines annually to private sector breeders. These breeding lines will be developed and selected based on field performance.

b) To extend the harvest season by gathering data on earliness of individual breeding lines (date of 80% red ripe) and the ability of breeding lines to hold fruit quality once fruit are ripe (number of weeks fruit quality is retained following 80% red ripe maturity), to inform decisions for selection.

c) To advance two projects on enhancing the nutritional value of processing tomatoes. The high pigment, and similar genes provide one strategy to significantly increase lycopene levels, but these genes are associated with several field production challenges. One gene (*Aft*) from *S. chilense* combined with another gene (*atv*) from *S. cheesmaniae* results in fruit with high levels of anthocyanins and these compounds also show promise for enhancing the nutritional benefits of tomatoes.

d) To identify 20 breeding lines as candidates for genotyping to expand our knowledge base of how the genetic sequence affects field performance of lines developed in our program.

e) To prepare datasets to enable us to investigate the genetic diversity represented in our breeding collection and to enable the implementation of genome wide selection as a breeding approach to augment our field selection approach.

3. Release of breeding lines

Twenty F_7 generation breeding lines, selected in fall 2016, were released in time for the 2017 field planting. Most of these breeding lines built on the work done at AAFC-GPCRC, Harrow. Our focus remained on increasing genetic diversity in processing tomato and our current work attempts to combine backgrounds of multiple wild species in individual lines as illustrated in Table 1.

Table 1. Number of breeding lines released in 2017 with wild tomato species, either alone or in combination, in the recent pedigree.

Species in the recent pedigree	Number of lines released in 2017
Solanum habrochaites	3
S. pennellii	1
S. habrochaites and S. pennellii	2
S. habrochaites and S. corneliomulleri	1
S. habrochaites and S. peruvianum	1
S. peruvianum and S. pennellii	1
S. habrochaites and S. chilense	1
S. pimpinellifolium and S. lycopersicoides	3
S. habrochaites, S. peruvianum and S. chilense	1
S. habrochaites, S. chilense and S. pennellii	1
S. corneliomulleri, S. habrochaites, S. peruvianum and S. chilense	1

4. Extending the harvest season: Maturity dates and field-holding ability

In 2017 we collected 80%-red-ripe maturity dates on all selections. When a selected plant reached 80% red-ripe maturity, we began counting the number of weeks that fruit quality held in the field. We had 4 selections that reached 80% red ripe maturity in 86 days after transplanting in 2017, and only a very few of some quite wild lines that did not reach 80% red-ripe maturity prior to our September 29 cut-off date.

Out of 769 total selections, 2 selections held fruit quality in the field for 6 weeks (compared to 2016: 4; 2015: 6), 41 selections held fruit quality for 5 weeks (compared to 2016: 38; 2015: 40), 200 selections held quality for 4 weeks (compared to 2016: 303; 2015: 111) and 203 selections held quality for 3 weeks (compared to 2016: 159; 2015: 176). There were 60 late maturing selections that held fruit quality in the field for 3 weeks or longer (compared to 2016: 47; 2015: 13). In 2017, we did not observe the over-maturity that we saw in 2016 due to the weather patterns that year.

Numbers from prior years are provided to give a general context only. While there is some overlap in lines or pedigrees from year to year, caution should be used when comparing across years since there is some change in the specific pedigrees comprising each annual cohort.

5. Summary of field selections 2017

Nine acres of breeding plots were established on a farm on Selton Line, northwest of Ridgetown. There were 843 breeding lines from F_2 to F_6 generations planted (compared to 2016: 584; 2015: 662). The F_6 to F_3 generations originated partly from selections made at Ridgetown during fall 2016, and partly from selections made in 2012 but that were temporarily shelved. A total of 769 selections were made in fall 2017. Field selection work began on August 28 (2016 August 29; 2015 August 31) and was completed on September 28 (2016 Sept 22; 2015 Sept 25).

6. Enhancing the nutritional value of tomatoes, I: Lycopene

We are continuing to work with breeding lines having high-pigment genes and attempting to overcome some of the defects associated with these genes. They provide a means to increase lycopene and beta-carotene significantly compared to normal tomatoes. In 2017, we completed a replicated yield trial at 1 location to help determine if yields are adequate for introduction. We have been making additional crosses in some cases to attempt to increase yield, and improve earliness still further. In other cases, we need to eliminate defects like green shoulders or jointed stems.

7. Enhancing the nutritional value of tomatoes II: Anthocyanins

We are continuing to make selections to improve the fruit size and earliness on the high anthocyanin breeding lines we have. There are 29 lines at F5 or F4 generations and a small number of these are now classed in our midseason maturity group. Our goal is to develop high-anthocyanin processing tomatoes as a potential niche product with enhanced nutritional value.

8. Applied genomics tools I: Genotyping to expand the knowledge of our breeding collection

The work leading up to the publication of the tomato genome sequence in 2012 fostered the development of single nucleotide polymorphism microarrays ("SNiP chips"). For relatively low cost per data point, this tool allows rapid genetic sequencing of tomatoes. While we have no difficulty isolating DNA from tomato plants, we have struggled to get a high-enough concentration of DNA for our

downstream, genotyping service providers, from a small percentage of our breeding lines. For these more difficult lines, we now use either a SpeedVac concentrator, or do multiple extractions with reduced volume and then combine them.

We completed our first SNiP genotyping job on the SolCAP consortium SNiP microarray (7,720 markers) and our service provider commented on the good quality of the DNA our lab provided. We are preparing another set of samples to do a second genotyping job in November 2017.

Our goal is to build this knowledge base over time to enable us to answer different kinds of questions. This kind of information can guide the choice of parent lines when designing future crosses to help us accomplish our objectives in a more targeted way. Combining genetic sequence information with field performance data will enable us to use genetic sequencing as a tool to enhance our field selection work.

9. Applied genomics tools II: Investigating the use of genome wide selection

A relatively new breeding approach being discussed among plant breeders is genome wide selection (GWS). Very briefly GWS requires genetic sequence data on some breeding lines, and field performance data on these same breeding lines. This group of breeding lines is called the training population. By creating a mathematical model that accounts for the entire genetic sequence (not just a few markers) and relating this to field performance of important traits, we can then predict the breeding value of a line, for all important traits simultaneously. While the approach holds great promise to make breeding progress based on genetic sequence information, especially on traits that are difficult or expensive to measure, it also has some weaknesses. Direct measurement of the important traits in the field remains the most reliable way to make progress.

We have a data set for a training population based on 99 of our own breeding lines. In 2016 and 2017 we gathered standardized field data on another set of 72 lines to expand our dataset from which we can build our model for GWS to augment our field selection program.

10. Plans for the future

This current project, which ends in December 2017, has accomplished all of our objectives. In addition to breeding for extended field holding ability, and enhanced nutritional qualities like elevated lycopene and anthocyanins, we have succeeded in developing expertise in some of the basic techniques, and in data analysis procedures to take advantage of genomics technologies in our field-based breeding program. We anticipate using several new procedures to assist with: (a) managing our germplasm collection, (b) identifying potential markers associated with various traits within our diverse germplasm collection, (c) designing crosses for new parental lines with greater precision, and (d) augmenting decision-making both for selections, and final breeding line release.