Mapping Campbell 1943 Stem Early Blight Resistance and Adding an Additional Source of Foliar Early Blight Resistance to Cornell Fungal Resistant Tomato Line

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Background on early blight resistance:

Prior work at Cornell University (CU) produced “triple resistant” fresh market tomato lines combining strong resistance to late blight and Septoria leaf spot and resistance/partial resistance to early blight. These tomato lines enabled the creation of blight resistant commercial hybrids such as Iron Lady, Stellar, Brandywise and Summer Sweetheart.

The current form of early blight resistance, derived from Campbell 1943, provides good control of the disease on the stems and peduncles (protecting the fruit), but provides less protection for foliage, though it does reduce the frequency of sprays required for control of leaf blighting. Although this form of early blight resistance has been used by public and private breeding programs, the location and genetic architecture of this form of resistance to early blight was previously unknown, and markers have not be available.

As a side product of the project transferring bacterial speck and bacterial spot resistances to the Cornell lines with multiple fungal resistances, we surveyed early blight response in the parental lines. As expected, the Ohio (OH) line (which is the source of the bacterial speck and bacterial spot resistance genes) was susceptible to early blight on its stems. This allowed us to use the breeding populations created to transfer bacterial resistances from the Ohio processing tomatoes to our fresh market tomatoes to also map the early blight stem resistance.

A serendipitous discovery from these screens was that one Ohio line has a foliar early blight resistance that hitherto was not identified. We believe this resistance could complement the Campbell 1943 resistance already present in the Cornell lines, and so are working to combine the two forms of resistance in the Cornell tomato background to achieve improved control of early blight on the foliage as well as the stems of tomato.

Summer 2017 inoculated early blight mapping experiments:

The inoculated early blight field trial in 2017 included an F2 population from the cross of the triple blight resistant fresh market line CU151095-146 and the bacterial speck and spot resistant processing tomato line OH7663. Joint analysis of the disease and genotype data putatively identified three or four chromosome regions (also known as quantitative trait loci or QTL) associated with early blight resistance. Two of the QTL were from the CU parent that reduced disease on stems, foliage or both, and one QTL was from the OH parent that reduced disease on foliage. Encouragingly, several F2 plants which were homozygous for all three of the QTL displayed exceptional early blight resistance, while other plants homozygous for the lack of all three of the QTL were extremely susceptible. This information was the basis for the work in 2018.

Summer 2018 inoculated early blight trials:

Since single plant data from individual F2 plants is not always reliable, during the 2018 field season we attempted to confirm the locations and effects of the putative QTL. We

established an inoculated replicated field trial in which we tested the self-progenies of selected F2 plants (i.e. replicated F2:3 families) with the early blight pathogen. Families were chosen solely based on their genotypes for all three putative QTL, using the best information about the locations of the QTL available at that time. The F3 families included in this replicated trial were intended to be homozygous for either the presence or absence of the resistance alleles at all three of these QTL. However, after subsequent genotype data became available, the locations of some QTL shifted slightly within a chromosome, and the QTL on chromosome 1 was divided into two distinct QTL, revealing some of the selections to be heterozygous at markers near the resistances.

Since this was an inoculated trial, all plants were infected simultaneously and uniformly with the goal of reducing the experimental error inherent to naturally infected experiments. Environmental conditions were excellent for disease development, and disease pressure in the trial was severe, resulting in excellent data. As in prior years, we collected early blight disease data on stems using a 1 to 5 visual rating scale, and on foliage using a 0-100% visual rating scale.

Analysis of the 2018 replicated trial validated the action of the strongest putative QTL predicted from the 2017 F2 data. These QTL were confirmed to be on chromosomes 1, 5, and 9. Limited details follow, as formal descriptions and additional validation of these QTL will be available presently in an upcoming publication.

Chr. 1 QTL: The data indicate that this CU-parent derived region might include two adjacent QTL rather than of one QTL, a proximal QTL protecting the foliage and a distal QTL protecting stems. Recombinant plants are being selected this winter to fine map this region and determine whether stem and foliage resistances are indeed two distinct closely linked QTL, and to determine the best markers for use in a marker-assisted breeding. The QTL confidence intervals for the two QTL are about 8 Mbp and 4 Mbp, respectively.

Chr. 5 QTL: The data to date for this OH-parent derived QTL are consistent with the action of one QTL protecting only the foliage. The 95% confidence region for the QTL is only 1 Mb, and several markers for use in selection of this QTL are currently being tested to select the best one for use in marker-assisted breeding.

Chr. 9 QTL: The data to date for this CU-parent derived QTL are consistent with the action of one major QTL protecting the stems. This QTL also provides significant, but lesser, reduction in foliar death. The 95% confidence region for this QTL is 5.4 Mb. Current populations are being used to further reduce this region and select the best marker(s) for use in selecting this QTL in breeding populations.

Results of the replicated trial confirmed that F2:3 families that had two or more partial resistance QTL against early blight defoliation had lower rates of defoliation relative to those with fewer resistance loci (See Table 1). The two families that combined all three QTL in the homozygous state (179188-1 and 179190-5) were among the most resistant in the field, although they were not statistically separable from most of the families homozygous for just two QTL. Conversely, the family without any of the QTL was the most susceptible to defoliation, also being the first among the 15 families tested to be completely defoliated.

While the small sample size of this experiment and the inability to control for segregating regions throughout rest of the tomato genome limits our ability to draw sweeping conclusions regarding the relative impacts of these QTL on foliar resistance, our experience over the last two years suggests, somewhat counter-intuitively, that the QTL on chromosomes 5 and 9 are the primary drivers of foliar resistance, but that having either the chromosome 1 or chromosome 5 foliar resistance QTL in combination with the stem resistance on chromosome 9 provides a high level of resistance. This can also be seen in Table 1, where several of the most resistant families were homozygous for the QTL on chromosome 5 and 9. While it is possible that the same mechanism which confers stem resistance is also responsible for foliar resistance, it is also possible that this phenomenon occurs because the collapse of stems accelerates the death of the tomato foliage. We can see this effect local to the primary infection site, as an elongating stem lesion that reaches the base of a petiole, which may cause the entire compound tomato leaf to rapidly wilt and die. Damage to stems may also accelerate the death of the plant globally by reducing the flow of nutrients throughout the plant vasculature, thereby weakening the plant and emboldening the necrotrophic fungal pathogen that causes early blight disease.

Table 1. Mean early blight foliage AUDPC for the F2:3 families and controls (italics) in the 2018 inoculated early blight trial.

Tomato Family ID	Genotypes for three QTL			Foliar AUDPC	
	Chr1 QTL	Chr5 QTL	Chr9 QTL		
179197-4	- -	++	++	260.5	a
179188-1	++	++	++	263.6	ab
CU151095-146	++	- -	++	284.4	ab
179190-5	++	++	++	289.0	ab
179174-3	- -	++	++	302.5	ab
179167-2	- -	++	++	306.8	ab
179169-6	++	++	+/-	372.8	abc
179206-3	++	++	- -	379.8	abc
179202-5	++	+/-	++	399.6	bcd
OH7663	- -	++	- -	427.8	bcde
179178-6	++	- -	- -	464.6	cde
179179-6	- -	++	- -	517.5	def
179214-4	++	+/-	- -	561.1	efg
179205-2	- -	++	- -	622.6	fg
179183-6	- -	++	- -	627.4	fg
179193-6	- -	- -	++	690.5	gh
179190-2	- -	- -	- -	818.8	h

+ +: homozygous for presence for region of QTL; - -: homozygous for absence for region of QTL
+/-: heterozygous for region of QTL
The presence/absence of the three focal QTL in the F2 parent for each F2:3 family are indicated at the center of the table.
Estimated means are statistically separated by Tukey HSD ($\alpha = 0.05$).
Overall ANOVA indicates highly significant differences $P << 0.001$.

The level of stem resistance is perfectly associated with the genotype at the chromosome 9 QTL (See Table 2). Families that had the CU151095-146 allele on chromosome 9 had highly resistant stems with lesions that did not elongate substantially like those without the resistance alleles. We also confirmed that the gene action is largely additive, as the heterozygote was mid-parent for stem resistance, as predicted by the 2017 trial data. Unfortunately, because we updated the location (and therefore the genotype) of the QTL for stem resistance on chromosome 1 subsequent to the test, we could not test the full set of pairwise contrasts, and it is therefore difficult to separate the impact of the QTL on chromosome 1. The data do not refute our hypothesis that this QTL enhances early blight stem resistance, though it does suggest that it's effect may be low in the presence of the strong chromosome 9 resistance.

Table 2. Mean early blight stem AUDPC for the F2:3 families and controls (italics) in the 2018 inoculated early blight trial.

Tomato Family ID	Genotypes for two QTL		Stem AUDPC	
	Chr1 QTL	Chr9 QTL		
179202-5	+/-	++	0.28	a
179190-5	+/-	++	0.41	a
179188-1	+/-	++	0.44	a
179167-2	+/-	++	0.47	a
CU151095-146	++	++	0.50	a
179174-3	++	++	0.53	a
179197-4	+/-	++	0.59	a
179193-6	++	++	0.65	a
179169-6	- -	+/-	1.75	b
OH7663	- -	- -	2.66	c
179205-2	+/-	- -	2.69	c
179206-3	++	- -	2.84	cd
179190-2	- -	- -	2.97	cd
179178-6	- -	- -	2.97	cd
179179-6	- -	- -	3.03	cd
179214-4	- -	- -	3.44	d
179183-6	- -	- -	3.50	d

+ +: homozygous for presence for region of QTL
- -: homozygous for absence for region of QTL
+/-: heterozygous for region of QTL
The presence/absence of the two focal QTL in the F2 parent for each F2:3 family are indicated at the center of the table.
Estimated means are statistically separated by Tukey HSD ($\alpha = 0.05$).
Overall ANOVA indicates highly significant differences $P << 0.001$.

Summary and future availability of germplasm: To illustrate the effectiveness of the resistance to early blight available in the Cornell fresh market tomato breeding program, we have included images of a few inoculated tomato families in Figure 1. Here we can see the contrasts between tomatoes which lack early blight resistances and are largely susceptible and those that have two or three resistance genes/QTL and are therefore largely resistant.

In addition to the F2:F3 families used for the QTL analysis discussed above, we are close to completing new fresh market lines that add the chromosome 5 QTL for foliar early blight resistance to the fungal resistant background of CU151095-146. These lines will also carry the bacterial spot resistance genes Rx3 and Rx4, as well as the broad-spectrum bacterial spot QTL-11, and so would possess the widest range of fungal and bacterial resistances. We are currently selecting for the last segregating regions in greenhouse populations, and hope to release the new lines, along with the genetic markers needed to transfer each of the three QTL, late in 2019.

Figure 1. Different levels of early blight disease on foliage and stems of entries homozygous for different combinations of early blight QTL in the 2018 inoculated replicated field trial in Freeville, NY.

A. plants lacking all three early blight resistance QTL showing full susceptibility that is greater than that of either of the two parental lines

B. plants homozygous for the chromosome 5 QTL of OH7663, showing modest control of early blight disease on the foliage.

C. plant homozygous for both the chromosome 1 and 9 QTL from the Cornell parent (CU151095-146), showing strong control of stem and moderate control of foliar early blight disease.

D. plants homozygous for all three of the early blight QTL showing strong control of stem disease and enhanced foliar control over the Cornell parent.