

Novel Fresh Market and Processing Tomatoes with Additional Resistances to Bacterial Spot, Bacterial Speck, and Early Blight

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Summary: This report summarizes work accomplished to date and availability of tomato inbreds possessing multiple fungal/oomycete and bacterial disease resistances. Effective resistances to bacterial speck and bacterial spot were previously characterized, allowing us to apply new genomics tools to rapidly backcross these resistances into our best-performing fungal/oomycete resistant fresh market tomatoes. Recently, we genetically mapped early blight disease resistance quantitative trait loci (QTL), facilitating the creation of new lines with enhanced early blight resistance. The current and upcoming fresh market tomato releases contrast primarily for their disease resistance packages but are in the same tomato background. One line being released is a processing tomato. Practical considerations regarding introgression impacts on horticultural type and harvest profile are also reported.

Background on diseases and impact

Bacterial spot of tomato is caused by a species complex that includes *Xanthomonas euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri*. Maximum infection occurs under warm moist conditions. Due in part to changes in the climate, this disease has begun to appear in the Northeast (NE), a region that until recently was thought too cool for serious bacterial spot infections.

Bacterial speck, caused by *Pseudomonas syringae*, can cause up to 25% loss of crop under cool rainy conditions, and has been intermittently problematic in NE tomato growing regions. Control of bacterial speck and spot is difficult due to broad distribution of copper-insensitive pathogen populations.

Early blight is a widespread and problematic disease affecting tomatoes caused by the fungal pathogen *A. linariae* (syn. *A. tomatophila*). Symptoms include damaged tomato stems, fruits, and foliage, resulting in yield losses. Infections are worst in humid growing regions such as the NE.

Disease resistance genes and sources

Bacterial spot: *Rx-3*, derived from Hawaii 7998, provides strong (R-gene mediated hypersensitive response) field resistance to T1 strains of *X. euvesicatoria*. *Rx-3* is on the long arm of chromosome 5. *Rx4* (syn. *Xv3*) from *S. pimpinellifolium* PI 128216 provides strong resistance to T3 strains of *X. perforans* and resides on chromosome 11. Broad spectrum resistance to all races has been conferred through the action of several quantitative trait loci. The most important of these QTL is QTL-11, located on the long arm of chromosome 11. Dr. Francis (at OSU) found the combination of *Rx-3*, *Rx-4* and QTL-11 provides partial but effective resistance to bacterial spot species for which there are currently no R-genes, such as *X. gardneri*.

Bacterial speck: The *Pto* gene (R-gene mediated hypersensitive response), derived from *S. pimpinellifolium* PI 370093, controls *P. syringae* pv tomato, race 0. *Pto* is on the short arm of chromosome 5 and has been cloned and sequenced, providing reliable markers.

Early blight: Complete resistance to early blight has never been observed in tomato. Yet several commercially adapted breeding lines have valuable levels of early blight resistance. Low trait heritability and a poor understanding of the genetic underpinnings of this resistance have historically made it difficult to maintain and increase resistance to early blight. To address this challenge, we mapped QTL associated with early blight resistance in cultivated tomatoes. QTL were subsequently validated and characterized in inoculated field trials, facilitating the identification of the four most promising loci: a chromosome 9 QTL, which we named *EB-9*, underlies strong stem (68.7% variance explained (VE)) and moderate (14.9% VE) foliar resistance from C1943. A large effect chromosome 5 QTL, named *EB-5*, offers significant (40.0% VE) foliar blight resistance. Because *EB-5* was obtained from OH08-7663, we hypothesize a *S. pimpinellifolium* source. Two linked QTL of unknown origin and smaller resistance effects (typically < 10% PVE) were identified on the long arm of chromosome 1. Dubbed *EB-1.1* and *EB-1.2*, these QTL are associated with early blight stem and foliar resistance, respectively.

Breeding progress and methodology

Genome-wide marker-assisted selection was used to rapidly backcross introgressions carrying disease resistance genes into a commercially adapted background. The background was the fresh market inbred CU151095-146, which has resistance to early blight (*EB-1.1/1.2*, *EB-9*), late blight (*Ph-2*, *Ph-3*), *Septoria* leaf spot (*SLS-2*), *Verticillium* (*Ve*) and *Fusarium* (*I*, *I-2*).

Detailed breeding methodology was provided in prior years' VBI reports (available upon request). We provide a summary here: Two OSU processing lines were used as sources of large introgressions on chromosome 5 (containing a coupling linkage of *Pto* and *Rx-3*) and on chromosome 11 (containing *Rx-4* + *QTL-11*). We separately transferred the chromosome 5 and the chromosome 11 introgressions to an elite late blight/early blight/*Septoria* leaf spot resistant fresh market line, creating mono-introgression lines. These mono-introgression lines were intermated and progenies were genotypically selected to combine all of these resistances in a series of di-introgression sibling lines (NILs). NILs with different combinations of these introgressions facilitated the study of the effects of these introgressions on horticultural type. For detailed disease resistance data, see the 2018 VBI report and an upcoming publication (2020).

Lines with enhanced early blight resistance were created by using marker based selection to pyramid the *EB-1.1/EB-1.2*, *EB-5*, and *EB-9* QTL for early blight resistance into the backgrounds of two separate inbreds from different market classes: fresh market/slicer and processing/plum tomato. Both backgrounds have bacterial spot, *Verticillium* and *Fusarium* resistance, and the fresh market line additionally has *Septoria* and late blight resistance.

Summary of 2019 field trials and results

A. Horticultural trial I

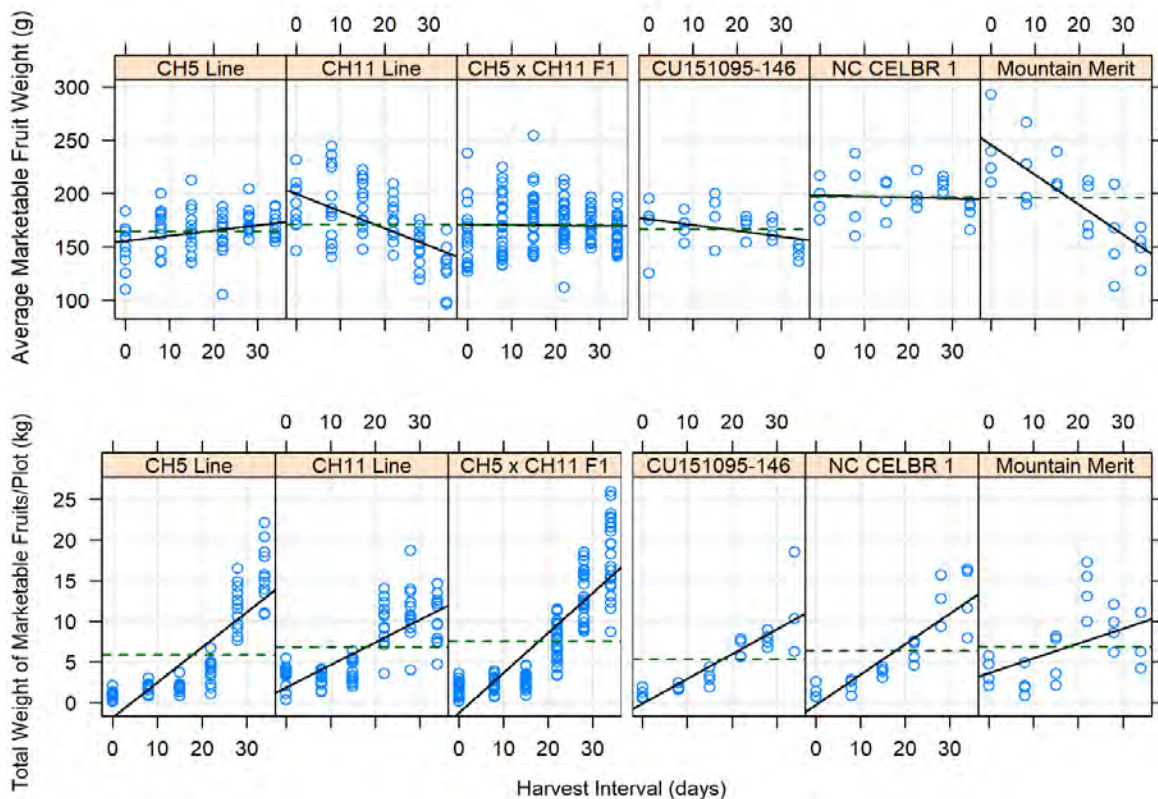
- **Entries:** The entries in this trial were the CH5 mono-introgression and CH11 mono-introgression lines, CH5 x CH11 F₁ hybrids (heterozygous for both the CH5 and CH11 introgressions), the inbred controls CU151095-146 and NC CELBR 1, and the commercial hybrid control “Mountain Merit”.
- **Maturity:** Tabulation of early season yields (Table 1), and harvest interval yield profiles (bottom panel Fig. 1) reveals that lines with the chromosome 5 resistance introgression were later maturing (1-2 weeks) than lines with the chromosome 11 introgression, while F₁ hybrids had intermediate maturity.
- **Growth habit:** Lines with the chromosome 11 introgression had reduced plant height (probably due to shorter internodes) and concentrated fruit set. Lines with the chromosome 5 introgression exhibited increased vine size and vigor relative to CU151095-146. This was not due to the *self-pruning* (*sp*) locus; all lines are determinate. Dr. Francis observed similar increases in vine size and vigor in processing tomatoes with the chromosome 5 introgression for *Pto* and *Rx-3*.
- **Yield:** There were no significant differences in yield among the inbreds (Table 1). The late maturity of the CH5 lines resulted in about 1 more harvests’ worth of fruit remaining on the vine beyond the harvest interval. In a location with a longer season, that would have raised the total harvest possible.
- **Fruit size:** There were no differences in fruit size among the CU entries (Table 1).
- **Marketability Ratio:** Field trials in 2019 were extremely wet, causing bacterial canker disease pressure and reduced marketability ratios. Marketability ratios were highest for lines possessing the chromosome 5 introgression, likely due to later maturity and comparatively diffuse fruit set on vigorous vines.

Table 1 Effect of bacterial disease resistance introgressions on horticultural performance in a 2019 uninoculated field trial. The chromosome 5 or chromosome 11 mono-introgression lines and their F₁ hybrid are compared. For context, means of three commercial benchmarks are shown at bottom

	Marketable Yield (kg/6-plant plot)		Marketable Fruit Size (g)	% Marketable
	Early Yield	Total Yield		
CH5 Line	2.56 ^c	35.5 ^b	169 ^{bc}	79.6 ^a
CH11 Line	6.06 ^a	41.5 ^{ab}	164 ^c	67.7 ^c
F ₁ : CH5 x CH11	3.97 ^{bc}	46.6 ^a	169 ^{bc}	76.9 ^{ab}
CU151095-146	2.98 ^c	31.9 ^b	163 ^{bc}	67.9 ^c
NC CELBR 1	3.69 ^{abc}	41.8 ^{ab}	197 ^a	70.3 ^{abc}
Mountain Merit	6.28 ^{ab}	41.3 ^{ab}	190 ^{ab}	69.1 ^{bc}

There were 24 plots/entry, combining within genotypic class, for the CH5, CH11, and F₁ entries and 4 plots/entry for commercial benchmarks. Fruit were harvested weekly for 6 weeks. Early yields are aggregate weights from the first two harvests. Statistics: one-way ANOVA on genotype class, letter groups designated by Tukey honest significant difference procedure ($\alpha=.05$)

Fig. 1 Harvest profiles over a 6-wk harvest interval for CH5 or CH11 disease resistant mono-introgression lines and their F₁ (24 plots/entry combined within class) from a 2019 field trial. For context, the harvest profiles of three commercial benchmarks (4 plots/entry), CU151095-146, NC CELBR 1, and Mountain Merit, are shown at right. Solid lines are least-squares regression lines, while dashed lines are means



B. Horticultural trial II

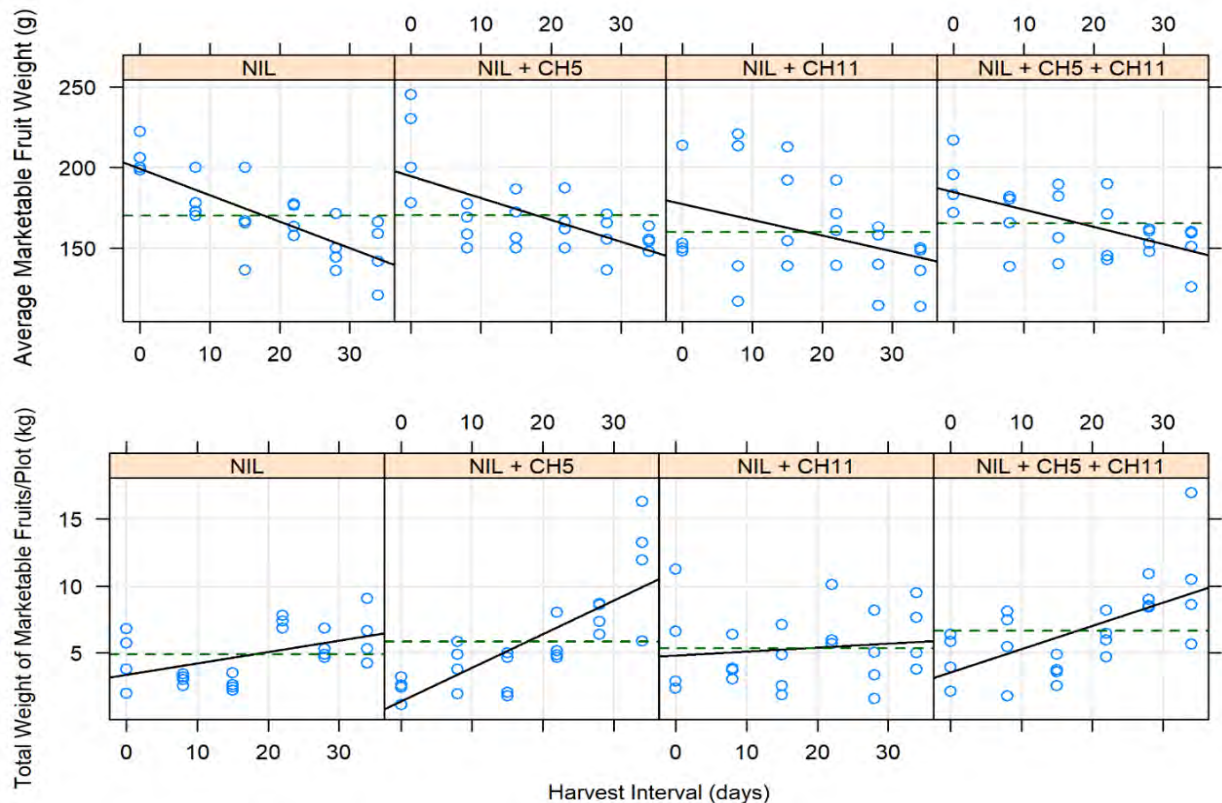
- **Entries:** The entries in this trial were the set of full-sib NILs with different combinations of the CH5 and CH11 introgressions: none, mono or di-introgressions conferring distinct combinations of bacterial disease resistances.
- **Maturity:** Harvest interval yield profiles (bottom panel Fig 2) confirmed later maturity among NILs with the chromosome 5 introgression, and greater early season yields (Table 3) among NILs with the chromosome 11 introgression. Di-introgression NILs showed intermediate maturity.
- **Growth habit:** The CH5 NILs exhibited increased vine size and vigor. The CH11 NILs had reduced plant height (probably due to shorter internodes), consistent with the first experiment (See section A, above). When these introgressions were combined in the di-introgression (CH5 + CH11 NILs) lines, their effects were effectively cancelled, resulting in growth habits similar to CU151095-146.
- **Yield:** There were no significant differences in yield among the NILs.
- **Fruit size:** There were no significant differences in fruit size among the NILs.
- **Marketability Ratio:** Marketability ratios were highest for lines possessing the chromosome 5 introgression, likely due to later maturity.

Table 2 Effect of bacterial disease resistance introgressions on horticultural performance in a 2019 uninoculated field trial. NILs containing zero, one, or two introgressions conferring bacterial disease resistance are compared

	Marketable Yield (kg/6-plant plot)		Marketable Fruit Size (g)	% Marketable
	Early Yield	Total Yield		
NIL	8.09 ^a	31.0 ^a	172 ^a	44.1 ^a
NIL + CH5	7.30 ^a	38.6 ^a	164 ^a	64.6 ^b
NIL + CH11	10.52 ^a	33.3 ^a	158 ^a	50.3 ^a
NIL + CH5 + CH11	10.57 ^a	41.5 ^a	162 ^a	72.9 ^b

There were 4 plots/entry for all entries. Fruit were harvested weekly for 6 weeks. Early yields are aggregate weights from the first two harvests. Statistics: one-way ANOVA on genotype class, letter groups designated by Tukey honest significant difference procedure ($\alpha=.05$)

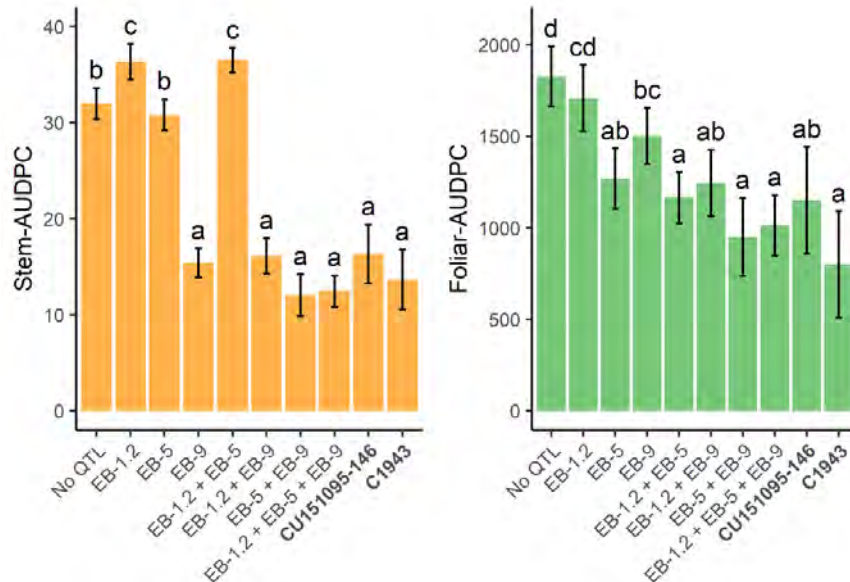
Fig. 2 Harvest profiles of near-isogenic breeding lines with different combinations of disease resistance introgressions (4 plots per entry). The first column shows the no QTL control line, which is approximately equivalent to the recurrent parent CU151095-146. The second and third columns show the chromosome 5 (*Pto* + *Rx-3*) or chromosome 11 (*Rx-4* + *QTL-11*) mono-introgression lines. The fourth column shows the di-introgression lines (chromosome 5 (*Pto* + *Rx-3*) plus chromosome 11 (*Rx-4* + *QTL-11*) lines) with the complete set of bacterial speck and spot resistance genes. Solid lines are least-squares regression lines, while horizontal dashed lines are the season means.



C. Early blight inoculated disease trial

- **Entries:** Near-isogenic lines in the background of CU151095-146 were placed in a replicated inoculated disease trial in Freeville, NY. These NILs contained distinct combinations of the early blight resistance QTL *EB-1.2*, *EB-5*, and *EB-9*.
- **Growth habit:** These NILs all have the chromosome 11 introgression containing the bacterial spot resistance genes *Rx-4* and *QTL-11* that also reduces vine vigor. Such growth habits result in greater susceptibility to early blight. This illustrates the value of early blight resistance QTL in lines with the chromosome 11 introgression.
- **Early blight resistance**
 - Lines with *EB-9* exhibited stem lesion and collar rot resistance equivalent to the putative resistance donor, C1943 (Fig. 3).
 - NILs with the novel combination of *EB-5* and *EB-9* were among the most resistant to early blight defoliation.
 - Interestingly, *EB-1.2*'s effect was negligible in our fresh market background, despite repeatedly demonstrating efficacy in other populations. We suspect a market class dependency for this QTL's effect (this will be tested in 2020).
 - All QTL exhibited incomplete dominance, indicating that hybrids heterozygous for early blight resistance QTL would require multiple resistance QTL to acquire valuable levels of early blight resistance.
 - As QTL were pyramided in increasingly resistant inbred backgrounds, QTL allele substitution effects diminished. This is consistent with a resource-limiting resistance model typical of necrotrophic pathosystems, such as early blight.

Fig. 3 Comparisons of early blight stem lesions (left) and defoliation (right) among the NILs homozygous for zero (susceptible control), one, two, or three early blight QTL reveal the effects of QTL pyramiding. For context, CU151095-146 and C1943 are shown at right (bolded). Error bars depict 95% confidence intervals, letters show significant differences at the Tukey-adjusted 95% confidence level.



Ongoing work

Our current leading early blight resistant (EBR) fresh market inbred has late blight resistance from *Ph-3* but may lack *Ph-2* due to a recombination on chromosome 10. Underlying this uncertainty is the fact that the precise location of *Ph-2* is unknown; *Ph-2* has not been cloned. Work is ongoing to reliably incorporate *Ph-2* into this elite early blight resistant inbred by replacing its recombinant chromosome 10 with a non-recombinant one from a sibling line; this work should be completed by Dec 2020.

As described above, preliminary data suggests the original large *EB-5* introgression might be associated with a delay in maturity. In the planned 2020 horticultural field trials, we will test our new EBR lines, with much smaller *EB-5* introgressions, to determine whether the reduction in introgression length eliminates the association with delayed maturity.

Releases

Inbred releases for 2020 are described in the tables below. Information on disease resistance packages and horticultural performance (if applicable) can also be found in the release tables.

Four of the five releases below are fresh market lines, which are all very close to their common recurrent parent, CU151095-146, which is one of the “triple resistant” lines which possess combined resistances to late blight, early blight, and *Septoria* leaf spot. This line was released several years ago is not listed below but can still be requested; it would be a good control for testing the new releases. The fifth line is a processing tomato in the background of OSU line OH08-7663, which possesses *Rx-3*, *Rx-4*, and *QTL-11*.

We can send out seed now for the three lines listed in Table 3, however we must increase seed of the two lines in Table 4 in the greenhouse this spring to have enough seed to fill requests. The seed of the two lines in Table 4 would be available ca. June 2020. Requests can include both lines in both Table 3 and Table 4; we will send out the available lines immediately, and the delayed lines as soon as the seed is in hand. NOTE: since the processing tomato line is in the background of an OSU line, it is a joint release. OSU and Cornell IP offices are arranging for proper management of this line, using a slightly different MTA.

We have intermittently observed that both our original “triple resistant” lines and some lines created in the “triple resistant” background (such as the fresh market offerings below) are more successful when crosses are made in one direction rather than its reciprocal. Therefore, we recommend that initial crosses using these lines are made reciprocally to test if the phenomenon occurs.

Table 3 Current Releases (early 2020)

Inbred ID*	Availability	Disease Resistances
<p>CU191357-A and CU191357-B</p> <p><i>A member of class: "EB-1.2 + EB-5 + EB-9"</i></p>	<p><i>New release, available now</i></p>	<p>Bacterial spot: Rx-3, Rx-4, QTL-11 Early blight: EB-1.1/1.2, EB-5, EB-9 Late blight: Ph-3 (possibly Ph-2) Septoria leaf spot: SLS-2 Verticillium wilt: Ve Fusarium wilt: I, I-2</p>
<p>Market Class: Determinate fresh market / slicing tomato Average marketable fruit size: Not yet tested Maturity: Not yet tested Product summary: These new inbreds uniquely combine enhanced early blight and bacterial spot resistances with resistances to <i>Septoria</i>, late blight, <i>Verticillium</i> and <i>Fusarium</i>. The prior generation had the best early blight stem resistance among the 66 entries in the 2019 inoculated field trial in Freeville, NY, and ranked 11th for resistance to early blight defoliation. Horticultural performance data of these lines and test hybrids will be determined from field trials Summer 2020.</p>		
<p>CU181245</p> <p><i>A member of class: "CH5 Line"</i></p>	<p><i>New release, available now</i></p>	<p>Bacterial spot: Rx-3 Bacterial speck: Pto Early blight: EB-1.1, EB-9 Late blight: Ph-2, Ph-3 Verticillium wilt: Ve Fusarium wilt: I, I-2</p>
<p>Market Class: Determinate fresh market / slicing tomato Average marketable fruit size: 171 ± 5.7 grams (2019 trial) Maturity: Late Product summary: This line, which has the chromosome 5 resistances <i>Pto</i> and <i>Rx-3</i>, retains the fruit size and yield of its CU151095-146 recurrent parent but has later maturity and a significantly increased vine size.</p>		
<p>CU181055-A and CU181055-B</p> <p><i>A member of class: "CH11 Line"</i></p>	<p><i>Originally released 2019, available now</i></p>	<p>Bacterial spot: Rx-4, QTL-11 Early blight: EB-1.1, EB-9 Late blight: Ph-2, Ph-3 Septoria leaf spot: SLS-2 Verticillium wilt: Ve Fusarium wilt: I, I-2</p>
<p>Market Class: Determinate fresh market / slicing tomato Average marketable fruit size: <i>CU181055-A:</i> 173 ± 5.7 grams (2019), 175 ± 6.2 grams (2018) <i>CU181055-B:</i> 164 ± 5.7 grams (2019), 167 ± 6.2 grams (2018) Maturity: Intermediate, slightly earlier for CU151055-B Product summary: Combined bacterial and fungal disease resistance with a more compact vine type, earlier maturity, and higher yields than the recurrent parent. Yields comparable to NC CELBR 1, as well as the hybrid Mountain Merit. The compact vine size and earlier maturity are associated with the chromosome 11 introgression carrying <i>Rx-4</i> and <i>QTL-11</i>.</p>		

Table 4 Upcoming releases (mid-2020)

Inbred ID*	Availability	Disease Resistances
<p>Names TBA after final seed produced</p> <p><i>A member of class: "NIL + CH5 + CH11"</i></p>	<p><i>Available mid-2020 after seed increase</i></p>	<p>Bacterial spot: <i>Rx-3, Rx-4, QTL-11</i> Bacterial speck: <i>Pto</i> Early blight: <i>EB-1.1, EB-9</i> Late blight: <i>Ph-2, Ph-3</i> Verticillium wilt: <i>Ve</i> Fusarium wilt: <i>I, I-2</i></p>
<p>Market Class: Determinate fresh market / slicing tomato Average marketable fruit size: 162 ± 7.1 grams (2019) Maturity: late – intermediate Product summary: The culmination of our recent efforts to unify bacterial and fungal disease resistances in a Northeast-adapted fresh market lines. This di-introgression (chromosome 5 + chromosome 11) line possesses all the bacterial speck and bacterial spot genes/QTL discussed above. The line has a similar vine size to CU151095-146. The presence of both introgressions results in slightly later maturity</p>		
<p>Names TBA after final seed produced</p>	<p><i>Available mid 2020 after seed increase</i></p>	<p>Bacterial spot: <i>Rx-3, Rx-4, QTL-11</i> Early blight: <i>EB-1.1/1.2, EB-5, EB-9</i> Late blight: <i>Ph-3</i> Verticillium wilt: <i>Ve</i> Fusarium wilt: <i>I</i></p>
<p>Market Class: Processing / plum tomato Average marketable fruit size: NA Maturity: NA Product summary: This new processing tomato inbred brings late blight and enhanced early blight resistance into the background of a spot-resistant OSU processing tomato with commercial adaptability. This inbred has a 92% genetic similarity to OH08-7663, suggesting it could have similar horticultural performance. This line is a joint release between Cornell University & The Ohio State University</p>		