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Processing Tomato Germplasm with Improved Resistance to Bacterial Spot

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Bacterial spot is a devastating disease affecting all market classes of field-grown tomato (*Solanum lycopersicum*) produced in humid regions. Breeding for resistance to this disease is complicated because bacterial spot is caused by four species of *Xanthomonas* with multiple physiological races recognized for some species. To address the challenge of breeding for resistance, we developed four processing tomato near-isogenic lines (NILs) with improved disease resistance to bacterial spot using a marker-assisted backcross breeding scheme. The four NILs, OH813A, OH804A, OH802B, and OH807C, contain different resistance quantitative trait loci (QTL) in combination with major resistance loci. NILs were evaluated for disease resistance, horticultural characteristics, and genetic similarity. Disease severity was evaluated in the field during Summer 2017 and Summer 2018 against *Xanthomonas euvesicatoria*, *Xanthomonas gardneri*, and *Xanthomonas perforans*. In addition, we measured yield (kg), soluble solids (Brix), pH, color uniformity, and other quality traits for each NIL and the susceptible recurrent parent, OH88119, in conventional trials. The four NILs and OH88119 were genotyped with 371 single-nucleotide polymorphism (SNP) markers spread across the genome. The NILs provided effective resistance to multiple *Xanthomonas* species. The resistant lines displayed production traits comparable to OH88119 and are suitable for agricultural use. Genotyping data indicated that the genetic background of these tomato lines are between 93% and 99%, similar to OH88119. The Ohio State University Crop Variety Release and Distribution Committee approved the release of this germplasm, and it will be deposited into a suitable germplasm repository.

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Origin and Development

Hawaii 7998 (Yang et al., 2005), PI 114490 (Scott et al., 2003), and LA2533 (Liabeuf et al., 2015) served as three distinct sources of quantitative resistance to bacterial spot that mapped to the centromeric region of chromosome 11. The inbred lines OH08-7663, 01-BR-7087, and FG12-433E-43 were used as the donor parents for resistance originally derived from Hawaii 7998, PI 114490, and LA 2533, respectively (Bernal et al., 2020). Each donor parent was crossed to OH88119 (Berry et al., 1995), which served as the susceptible recurrent parent in the development of backcross populations. Each population and later tomato near-isogenic lines (NILs) were designated with a unique tag: "A" refers to QTL-11A from Hawaii 7998; "B" refers to QTL-11B from PI 114490; and "C" refers to QTL-11C from LA 2533 (Bernal et al., 2020). The tomato NILs OH813A, OH804A, OH802B, and OH807C resulted from a backcross-breeding scheme in which marker-assisted selection (MAS) was applied to select for the centromeric region of chromosome 11 (Bernal et al., 2020). This region was selected by DNA-based molecular markers Sli_1831 (forward primer: 5′-GGAAGCTTGGATTAA-AGGGG-3′; reverse primer: 5′-CAGTCGCT-TAGGAAACCGAG-3′) and Sli_1901 (forward primer: 5′-CGCGTTTCATCTTTTTCCTC-3′; reverse primer: 5′-TCACCTGATAGCAGT-GACGTAG-3′), with physical positions at 20.0 Mb and 46.6 Mb, respectively, on the tomato reference genome (SL 4.0). The indel marker PCC12 (forward primer: 5′-TCCACATCAA-ATGCGTTTCT-3′; reverse primer: 5′-TTCCAATCCTTTCCATTTCG-3′) was used to select for *Xv3/Rx4*, a gene that recognizes the effector *avrXv3* in *X. perforans race* T3 (Pei et al., 2012). OH813A contains QTL-11A and *Xv3/Rx4* in the coupling phase, whereas OH804A contains only QTL-11A. At the BC_1 stage, populations were screened with SNP markers distributed across the genome to select for the recurrent parent, OH88119 allele (Bernal et al., 2020). Genotyping was repeated at the BC_3S_5 stage.

The performance of specific lines selected from populations and families have been described previously (Bernal et al., 2020) and were evaluated in independently inoculated fields against *X. perforans*, *X. euvesicatoria*, and *X. gardneri*. The *X. gardneri* isolates SM230-10 and SM174-10, *X. euvesicatoria* isolates *Xcv*110c and *Xcv*767, and *X. perforans* race T3 isolate *Xcv*761 were used for the inoculations. Trials were arranged as randomized complete block designs (RCBDs) with three blocks per trial in 2018 and four blocks per trial in 2019, respectively. Plant symptoms were assessed for disease severity on a per-plot (10 plants per plot) basis using the Horsfall-Barratt scale (Horsfall and Barratt 1945).

In parallel, we evaluated NILs in non-inoculated fields and measured the yield, Brix, pH, color parameters, fruit index, and fruit weight as described previously (Merk et al., 2012). Yield trials were arranged as an RCBD with two blocks. In 2017, the total yield was measured by collecting fruits from the three most inner plants within 10-ft. plots; however, in 2018, five plants were collected from 20-ft. plots. The total yield was calculated on a per-plant basis. Individual fruit weight was measured during both years. In 2017 and 2018, 10 and 25 fruit were weighed from each plot per block, respectively. Fruit weight was calculated on a per-fruit basis. The fruit shape index, pH, and color of NILs were measured by taking nine fruit per NIL from each block, cutting the fruit into longitudinal halves, and placing the cut side down on a scanner (Merk et al., 2012). Color uniformity was quantified by removing the peel at the stem-scar end and imaging the flesh. The percentage of the surface area that was yellow (hue 70–100) provided an estimate of yellow shoulder disorder (Merk et al., 2012). The perimeter, area, and fruit shape index I were calculated for each individual fruit using tomato analyzer software (Brewer et al., 2006; Darrigues et al., 2008). The cut fruit were subsequently blended, and the puree was used to quantify the pH and sugar content (Brix).

In 2019, the BC_3S_5 selections were genotyped with 381 SNP markers to confirm the percentage of the genomic background relative to OH88119. Leaf tissue was collected from seedlings and genomic DNA was extracted using a CTAB method (Bernal et al., 2020). Genotyping was performed at Agriplex Genomics (Cleveland, OH) using their PlexSeq™ platform. On average, each chromosome contained 30 SNP markers. The highest number of markers was on chromosome 4 (with 65) and the lowest number was on chromosomes 6 and 7 (both with 18 each).

Description of NILs

Hypocotyls of OH813A, OH804A, OH802B, and OH807C seedlings are

Table 1. Horticultural and quality characteristics of tomato near-isogenic lines (NILs) in 2017 and 2018.

Genotype	Yield[z] (kg)[y]	Fruit wt (g)[x]	Fruit shape index I	Brix	Avg hue[w]	Avg L[w]	%YSD[w]	pH
OH813A	3.48 a	52.85 ab	1.24 b	4.63 a	52.44 ab	40.13 b	24.86 ab	4.00 a
OH804A	2.54 b	51.94 ab	1.19 c	4.55 a	52.82 ab	39.68 b	26.95 ab	4.00 a
OH802B	3.15 ab	52.62 ab	1.26 b	4.43 a	52.85 ab	41.53 a	28.40 ab	4.05 a
OH807C	4.04 a	48.99 b	1.32 a	4.88 a	50.82 b	39.26 b	22.24 b	4.05 a
OH88119	2.64 b	56.54 a	1.20 c	4.48 a	54.23 a	40.27 ab	30.74 a	3.95 a

[z]Mean separation based on Fisher's least significant difference at $\alpha = 0.05$. The table summarizes fruit characteristics that were measured for the NILS. Means in the same column with the same letter are not significantly different.
[y]Yield is measured as kilograms per plant.
[x]Fruit weight is measured as grams per fruit.
[w]Hue is a measure of color and L a measure of lightness-darkness in the L*a*b* color space; YSD is yellow shoulder disorder and is measured as a percentage of pixels showing yellow tissue (hue >60°).

Table 2. Horsfall-Barrat disease rating of near-isogenic lines inoculated with different *Xanthomonas* species in 2017 and 2018.

		X. perforans		*X. euvesicatoria*		*X. gardneri*	
Effect	df[z]	F statistic	*P*[y]	F statistic	*P*	F statistic	*P*
Genotype	4	21.42	<0.0001	23.18	<0.0001	22.30	<0.0001
Year	1	0.94	0.34	11.87	0.002	24.27	<0.0001
Block:year	5	0.29	0.91	2.93	0.03	0.35	0.88

[z]df = degrees of freedom.
[y]*P* indicates the significance for the disease rating for each *Xanthomonas* species. The disease rating was measured when fruit in plots were 80% mature (ripe fruit stage).

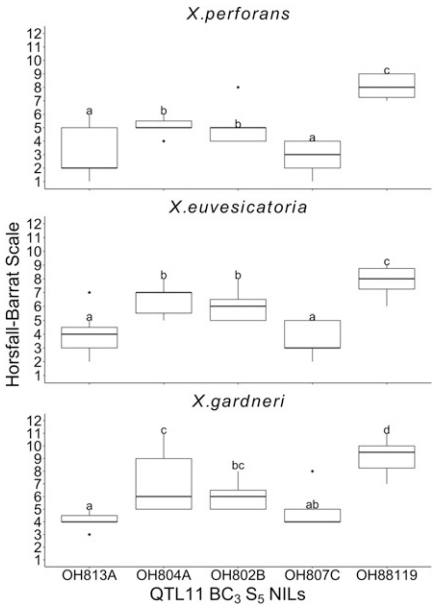

Fig. 1. Boxplots represent the disease rating using the Horsfall-Barrat Scale for *Xanthomonas perforans*, *Xanthomonas euvesicatoria*, and *Xanthomonas gardneri*. Letters above each boxplot represent mean separations using Fisher's least significant difference at $\alpha = 0.05$.

pigmented by anthocyanins. Mature vines for all four are determinate and compact. Plants exhibit profuse branching with shallowly scalloped leaves. Inflorescences are simple and occasionally forked, with seven to nine flowers. Fruit are attached by a jointless pedicle, are blocky to ellipsoid plum-shaped, and contain two to three locules. Fruit are uniform and ripen with red flesh and gel, and the skin is pigmented.

The average fruit size of NILs was 51.6 g. OH813A, OH804A, and OH802B did not differ significantly from the recurrent parent, OH88119. OH807C had smaller fruit (Table 1). Chromosome 11 contains a known QTL, FW 11.2, between positions 53.48 and 54.56 Mb in the tomato genome (Li et al., 2019). The NIL OH807C was developed from a progeny line with a recombination event separating QTL11-C from the *Rx4/Xv3* locus (53.88 Mb). Markers between 49.2 and 68.7 Mb are inherited from OH88119, and the decrease in fruit size is therefore thought to be from effects closer to the centromere. The perimeters of fruit for OH813A, OH802B, and OH807C were not significantly different from each other. However, the fruit height-to-width ratio describing fruit shape index I (Merk et al., 2012) differed between these NILs and OH88119, with the NILs having longer fruit. Brix was not significantly different between the four NILs and the recurrent parent. Color, as measured by hue, was also not significantly different among OH813A, OH804A, OH802B, and OH88119, but it differed for OH807C, with this NIL being more red. Yellow shoulder disorder (YSD) was also measured as a percent of the tissue that was not red for NILs and OH88119. OH807C displayed a lower percent of YSD and lower hue (more red). These color attributes were significantly improved relative to OH88119. Higher yields were observed for OH813A and OH807C relative to OH88119 (Table 1).

The disease severity was analyzed using a fixed-effect linear model for the analysis of variance (Table 2). Significant differences in genotypes were detected against all *Xanthomonas* species; in all cases, NILs displayed less disease severity compared with OH88119. The most effective NILs against multiple *Xanthomonas* species were OH813A and OH807C (Fig. 1).

The proportion of the OH88119 background genome was estimated based on 381 SNPs using either the percentage of SNPs identical to OH88119 or the physical size of genomic regions between contiguous SNPs (Cambiaso et al., 2020). Both methods provided similar estimates and suggested that OH813A, OH804A, OH802B, and OH807C contain 0.981, 0.976, 0.978, and 0.938 of the OH88119 genome, respectively.

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